COMORBIDITY AND AUTISM SPECTRUM DISORDER

EDITED BY: Manuel Fernando Casanova, Emily L. Casanova, Richard Eugene Frye and Christopher Gillberg
PUBLISHED IN: Frontiers in Psychiatry and Frontiers in Pediatrics

frontiers Research Topics

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

COMORBIDITY AND AUTISM SPECTRUM DISORDER

Topic Editors:
Manuel Fernando Casanova, University of South Carolina, United States
Emily L. Casanova, University of South Carolina, United States
Richard Eugene Frye, Phoenix Children's Hospital, United States
Christopher Gillberg, University of Gothenburg, Sweden

Table of Contents

Editorial: Comorbidity and Autism Spectrum Disorder

Manuel F. Casanova [1]*, Richard E. Frye [2], Christopher Gillberg [3] and Emily L. Casanova [1]

[1] University of South Carolina School of Medicine Greenville, Greenville, SC, United States, [2] Phoenix Children Hospital, Phoenix, AZ, United States, [3] University of Gothenburg, Sahlgrenska Academy, Gothenburg, Sweden

Editorial on the Research Topic

Comorbidity and Autism Spectrum Disorder

This Research Topic consists of 32 articles, contributed by 283 authors, focusing on recent understanding regarding the impact and management of comorbidities associated with autism spectrum disorder (ASD). This Research Topic sought to answer questions such as: Are standard screening instruments capable of delineating the full range of impairment in ASD without considering comorbidities? Given the difficulties in communication for many ASD patients, what red flags point toward the presence of comorbidities? How do comorbid conditions relate to maladaptive behaviors? How do health care providers grapple with the juxtaposition of intellectual disability (ID), non-verbal clinical care, and comorbidities in ASD? This Research Topic attempted to answer these and other questions while raising awareness on how comorbid conditions increase both mortality and morbidity in ASD.

The presence of a chronic disorder that co-occurs, at the same time or in tandem, with a primary disease is a comorbidity. Defining the presence and natural history of comorbidities is important as it serves to better inform treatment and prognosis (1). For many individuals, comorbidities worsen the way they feel, behave, and think about themselves. Indeed, comorbidities add a multidimensional component to both diagnosis and treatment that colors the expectations of patients as well as their treating physicians.

By themselves, comorbidities are associated with worse outcomes and increased health needs. The presence of co-occurring medical conditions confers those affected with a higher level of morbidity, increased risk of depression, and reduced level of social well-being (2). These patients require more frequent and targeted clinical management (e.g., to avoid dangers of polypharmacy), services planning (e.g., coordination between various health care professionals), and financing. The more precise our understanding of a disease and its comorbidities the better the clinical care and health services that can be provided. It should come as no surprise that treatment of comorbidities generally improves the quality of life of affected patients.

The abundance of chronic conditions (e.g., diabetes, arthritis, obesity, depression) in modern medicine makes comorbidity the norm rather than the exception. In 2014, approximately 42% of adult Americans had multiple chronic conditions (3). Indeed, almost 77% of Medicare spending goes to people with multimorbid conditions; that is, the presence of 2 or more chronic disorders (4). A population-based survey in Sweden revealed that half of individuals with ASD (9-year-old twins born between 1992 and 2001) had four or more coexisting disorders while only 4% did not have a concomitant disorder (5). Considering that ASD is a lifelong condition, affecting 1 of every 54 children, the subject of comorbidities is of great importance for patient-centered health research, especially so as the mean age of our population increases (6–8).

In ASD, comorbidities do not occur evenly but tend to cluster into different subgroups. A recent study reported that ASD individuals can be subtyped according to whether they exhibit seizures, multisystem disorders, psychiatric disorders, or whether they lack a defined comorbidity (9). External validation of the different clusters was derived from within group commonalities in medical trajectories and an inability to generalize results among the different subgroups. The study demonstrates the usefulness of thinking about ASD as being divided into "essential or uncomplicated" and "complex" subgroups each with a different outcome and recurrence risk [e.g., (10)].

Comorbidities in ASD manifest as impairments outside of its core diagnostic features; the latter being deficits in social communication/interaction, and restricted, repetitive patterns of behavior, interests, or activities, including sensory processing difficulties (11). Epilepsy, psychiatric/behavioral complaints, and gastrointestinal (GI) disorders are common comorbidities of ASD, especially in subjects with intellectual disability (ID) (12). Comorbidities frequently manifest in preschool years (Muratori et al.) and their presence may be the best predictor of maladaptive behavior, respective of ASD symptom severity, presence of ID or limitations in adaptive functioning (Skwerer et al.).

The prevalence of comorbidities varies widely, from study to study, probably pointing to the fact that ASD is not a unitary disorder but a spectrum involving different pathophysiologies. Lumping patients into a single group tends to average out significant differences within a study population. Conclusions from these prevalence studies should be taken cautiously as research designs tend to exhibit multiple limitations. Indeed, when interpreting results, one must consider the variability ingrained in different ascertainment methods and the lack of available specialized consultative care in some study centers. In addition, many prevalence studies on comorbidities use small patient samples and/or fail to address the impact of demographic, socioeconomic, ethnic, and geographical factors on diagnosis.

In ASD, the expression of comorbidities stemming from any given organ system can take multiple forms. For GI comorbidities this may involve gastroesophageal reflux (GERD), constipation, diarrhea, food allergies, colitis, ulcers, and inflammatory bowel disease (13). These are all common disorders, readily observed in the general population and frequently associated with ID, sleep problems, behavioral disorders, and even connective tissue disorders like Ehlers-Danlos syndrome (EDS), which are also comorbid with ASD (Penzol et al.; Baeza-Velasco et al.). Associated behaviors may exhibit varying relationships to GI symptoms at different ages, a fact having important implications for medical care (Cawthrope; Ferguson et al.). In effect, treating GI disorders may help alleviate a broad array of disorders (e.g., mental, behavioral, sleep) in the multimorbid ASD patient (Neuhaus et al.).

Symptoms of comorbidities in ASD may be atypical and are often difficult to recognize (14). A major culprit propitiating these diagnostic difficulties is communication problems. In ASD, 25–50% of individuals are unable to speak (15). Furthermore, 90% of ASD toddlers are unable to point protodeclaratively or protoimperatively (16). Indeed, many individuals with ASD are incapable of pointing to the source of their discomfort, find it difficult to attend to or detect bodily sensations (17), have poor integration of body scheme representation, and have atypical sensory perceptions or reports of pain (18). This inability to communicate pain or discomfort to other people may propitiate the enactment of inappropriate behaviors as a way for patients to express themselves and attract attention to their plight.

Changes in behavior, even maladaptive behaviors, may indicate an underlying comorbidity. It is common for maladaptive behaviors to mask comorbidities and for these conducts to be dismissed as being either "autistic behaviors" (diagnostic overshadowing) or the result of environmental/sensory stressors. Changes in behaviors may manifest at home, at school, as alterations in every-day situations or as problems in already established personal relationships. Curiously, on some occasions, these behaviors may serve to mask an ASD diagnosis, especially in women. Females with ASD are the great chameleons of diagnostic medicine. They often gain social acceptance through rehearsed mimicry. Those that successfully camouflage their ASD are often misdiagnosed as having a borderline personality disorder, being bipolar or as suffering from major depression [see (19)]. In these cases, a putative comorbidity draws attention away from the primary diagnosis.

It is important to be cautious when facing unexplained multi-system involvement in a patient with ASD. Think of an underlying comorbidity if: there are unexplained signs and symptoms (including self-injurious and hetero-aggressive behaviors), in the case of maladaptive behaviors that cannot be contextualized during psycho-educational behavioral assessments (e.g., functional analysis), there are changes from baseline (including regression of skills—especially after 3 years of age), the patient is not responding as expected to therapeutic interventions, has a history of frequent visits to the emergency room, has a history of perinatal complications, or is taking multiple drugs along with over-the-counter (OTC) medications and dietary supplements (20–23). It is mandatory in these patients to screen for comorbidities by first taking a structured review of systems. It does not cost anything to ask questions! A review of systems is less expensive and more illuminating than many laboratory tests (24). It is surprising to us how many times a patient's symptoms are not reported because they are not considered relevant. In those occasions when parents do report symptoms, trust them, especially when they make comparisons to their other children.

Aging is a non-modifiable risk factor for comorbidity that makes apparent the need for comprehensive health care targeting multiple conditions. There are differences in the strength of association between symptoms across age strata that suggests a plausible temporal mechanism (Cawthrope). Gender is another non-modifiable factor that may help identify specific comorbidities (e.g., anorexia nervosa, see Margari et al.) or act through sex-related perinatal complications (25). Lack of exercise, improper diet (caloric dense and nutrient-poor), the use of certain medications (e.g., neuroleptics), food selectivity and genetic makeup may predispose individuals with ASD to obesity and poor nutrition (Nor et al.). This modifiable comorbidity, obesity, predisposes an individual to insulin resistance and type

2 diabetes mellitus, hypertension, dyslipidemia, cardiovascular disease, stroke, sleep apnea, gallbladder disease, hyperuricemia, gout, and osteoarthritis. Obesity has emerged as an epidemic among our ASD children who are plagued by both risk factors and an "obesogenic" environment (26). Prevention of excess weight gain is therefore a major target of personalized medicine meant to improve physical fitness in children with ASD (27–29).

This Research Topic can be divided into five subtopics that include: (1) comorbidities and psychiatric manifestations, (2) anatomical correlates, (3) specific examples, (4) intellectual disabilities, and (5) mechanisms. In the paragraphs below we will summarize each article within its subtopic.

1) Comorbidities and psychiatric manifestations: According to Druss and Walker (30) "having a mental disorder is a risk factor for developing a chronic condition, and having a chronic condition is a risk factor for developing a mental disorder." Indeed, the pathways leading to the co-occurrence of medical and mental disorders are complex and bidirectional, often espousing common risk factors.

Using cross-sectional regression analyses (Avni et al.) found that an ASD diagnosis was associated with poorer adaptive functioning skills in a large and well-characterized sample of 260 young people with ADHD/ anxiety symptoms. The study also found that ADHD and anxiety symptoms predicted poorer adaptive functioning skills over and above IQ scores. Diagnosing these comorbidities early on is of importance in improving outcome.

Guerrera et al. recruited 37,500 children and adolescents and assessed psychopathological comorbidities using the Child Behavior Checklist (CBCL). Results showed that approximately 30% of the subjects exhibited internalizing problems as compared to the 6% that manifested externalizing problems. There was no correlation between CBCL scores and indices of ASD severity. The authors suggest that detection of distinct behavioral and emotional problems in ASD may lead to more specific and individualized treatments.

Muratori et al. completed a detailed assessment of the incidence of psychiatric comorbidity (PC) in 989 preschool age children with ASD using the DSM-Oriented Scales of an age-specific version of the CBCL 1.5–5. They compared IQ measures and other characteristics of the children with ASD only and ASD + PC, both mono-comorbid vs. multi-comorbid PC. They reported that 37.8% of the sample had at least one PC in addition to ASD, and that such subjects tended to exhibit lower overall performance IQ and higher ADOS calibrated severity scores. The higher rate of PC was reflective of increased Affective Problems, ADHD Problems, Anxiety Problems, and to a lesser extent Oppositional Problems. When considered together their data suggests that greater consideration of PC should be undertaken when evaluating and developing individual treatment or intervention plans for children with ASD.

Roberts et al. followed a large sample of males ($n = 74$) with fragile $\times$ syndrome (F $\times$ S) for their developmental and temporal profile of social avoidance during early childhood. Parameters for increased social avoidance predicted elevated ASD severity

but reduced ADHD and anxiety within the study population. ASD, ADHD and anxiety symptoms related inconsistently to social avoidance behaviors. The results provide new insights into ongoing debates as to the independence or overlap among these disorders in F $\times$ S.

Skwerer et al. characterized the profile of co-morbid symptoms of psychopathology and emotion dysregulation in people with ASD with minimally verbal abilities. Correlations were found among ratings of emotional dysregulation, severity scores on co-morbidities and maladaptive behavior ratings, but none of these measures were correlated with ASD symptom severity scores. The number of clinically significant psychiatric symptoms was found to be the main predictor of maladaptive behaviors.

The study by Top et al. had three main aims. Firstly, the study reports data on self-report measures of sensory processing, anxious apprehension, and intolerance of uncertainty in three groups; (a) adults with ASD (AUT), (b) individuals with high levels of anxiety (ANX), and (c) neurotypical adults (NT). Adults with ASD scored higher than both other groups on Sensation Avoiding, Low Registration, and Sensory Seeking and scored similar to the ANX group on Sensory Sensitivity and Intolerance of Uncertainty. Anxious participants scored higher than both other groups on anxious apprehension. Significant correlations were reported between sensory sensitivity, anxious apprehension, and intolerance of uncertainty. Secondly, the study reports baseline physiological arousal in each of the three groups through measurements of baseline pupillary size. The AUT group had larger baseline pupil size than the NT group but similar size to the ANX group. However, the ANX group demonstrated decreasing pupil size throughout the experiment, which was not demonstrated by the AUT group. Thirdly and lastly, pupil size did not differ between groups in response to auditory stimuli or in habituation to stimuli over time.

Weiss and Fardella studied prior experiences with victimization and perpetration in adults with ASD ($n = 45$) and a healthy control group ($n = 42$). The participants filled out questionnaire measures, and the adults with ASD reported higher levels of negative experiences with victimization than the healthy control group. These group differences are not better explained by self-reported abilities in sociocommunicative abilities or emotion regulation difficulties. However, no group difference appeared in relation to experiences with perpetration. Previous studies have tended to focus on specific types of victimization and not perpetration.

2) Anatomical correlates: Form and function complement our understanding of each other. This is especially true for multimodal imaging classifiers that combine anatomical, neurochemical and white matter integrity measures in systems-biology-based models that enable health practitioners to better address the underlying causes of disease.

Cai et al. investigated structural differences between patients with High Functioning Autism (HFA) vs. Low Functioning Autism (LFA). Using an unbiased whole brain VBM-DARTEL method and a 3T MRI system to assess neuroanatomical differences

in Gray Matter Volume (GMV) in various regions of interest (ROIs). They found that LFA and HFA had the same abnormal brain regions, but LFA had more abnormal brain anatomy regions than HFA. Increased GMV in the left Inferior Temporal Gyrus (ITG) were found in both HFA and LFA and increased GMV of the Left Medial Temporal Gyrus (MTG) was found only in the LFA group. Significant negative correlation between Gray Matter Volume of Left Inferior Temporal Gyrus and the score of repetitive behavior was also found, at least among HFA subjects.

Dekhil et al. reported on a computer-aided detection and diagnosis system (CAD) for ASD based on anatomical and functional indices of connectivity in brain regions that are commonly abnormal in ASD. The accuracy of a diagnosis was 75% when using fMRI data, 79% when using structural MRI data, and 81% when using a combination of both. By parcellating the data according to brain regions, a personalized brain map can be created that can help target therapeutic interventions.

Using functional magnetic resonance imaging (fMRI) (Lukito et al.) compared the neurofunctional correlates of duration discrimination between young adult males with ASD ($n = 23$), ADHD ($n = 25$), the comorbid condition of ASD+ADHD ($n = 24$), and those with typical development (TD, $n = 26$). By means of fMRI the authors aimed to study duration discrimination deficits and to test whether those deficits are underpinned by the same or different processes. Using both ROI and whole brain analyses the comorbid ASD + ADHD demonstrated significant under-activation in the right inferior frontal cortex compared to the other groups. The findings suggest that in adulthood, the comorbid ADHD + ASD, but not the pure conditions, demonstrate deficits in a brain region responsible for duration discrimination.

Nickel et al. reported a comparison of MRI findings in males with ASD and comorbid ADHD to those with ADHD only and neurotypical controls. A significant (after correction) decrease in volume of the left inferior frontal gyrus (pars orbitalis region) was found to be driven by ADHD, which was categorical rather than correlative with severity. ADHD alone, ASD alone, and ASDxADHD were each tied to trends in mean curvature changes in multiple different brain regions. The study suggested that the left inferior frontal gyrus might play a role in modulating symptoms of inattention and/or impulsivity in ADHD. Other observations based on cortical thickness and mean curvature, require further attention in studies using a larger sample population.

3) Specific examples: Feinstein first defined a comorbidity as, "Any distinct additional entity that has existed or may occur during the clinical course of a patient who has the index disease under study" (31). This terminology is imprecise and provides little guidance to health care professionals who may not find it self-evident as to which is the designated index condition and which is the comorbid condition (32). In the primary care setting index these labels are often neither obvious nor helpful, "and may vary in relation to the research question, the disease that prompted a particular episode of care, or of the specialty of the attending physician" [(32), p. 358]. Comorbidities challenge the single-disease framework upon

which most of our health care system and medical education is configured (33).

Baeza-Velasco et al. described the current knowledge on possible links between ASD and joint-hypermobility related disorders. Joint hypermobility occurs in isolation or associated with genetic syndromes especially in collagen related disorders. Connective tissue diseases are frequently a source of joint pain and multisystem involvement. Individuals with ASD have social communication defects that may conceal pain conditions. The authors suggest keeping in mind joint hypermobility and/or collagen related disorders in people with ASD as a source of pain that can, in turn, exacerbate behavioral disturbances.

Cawthorpe screened for associations between ASD and a wide range of ICD diagnoses in a pediatric population. Analyses showed differences in terms of strength of associations across age strata suggesting plausible temporal mechanisms. Levels of GI symptoms accounted for unique variance in psychiatric outcomes over and above other factors, linking increased GI problems with increased psychiatric symptoms in children with ASD.

De Crescenzo et al. performed a meta-analysis of the literature on ASD symptoms in individuals with schizophrenia. Both disorders share impairments in reciprocal social interaction and social withdrawal, as well as other cognitive symptoms. Furthermore, childhood onset schizophrenia has a high comorbidity with ASD. This study compared ASD scores on the Autism Spectrum Quotient and subscores from 13 studies involving patients with Schizophrenia. Other ASD assessments were also included such as SRS, ADOS-2, CARS, and ASSQ. The evidence from 1958 patients supported the finding of increased ASD symptoms in patients compared to control samples, but fewer symptoms compared to those with ASD diagnosis. This suggest some shared psychiatric vulnerability, which is not surprising given some common genetic underpinnings.

Dy and Tanchanco provide the first case report of co-occurring achondroplasia and ASD. Both conditions are common on their own and their coincidental co-occurrence is to be expected. Achondroplasia typically presents with musculoskeletal impairments; however, the presence of delays in other domains of development, particularly social communication, should raise the suspicion of ASD. Recognition is important as early intervention is critical toward addressing the unique needs of such a patient.

Ferguson, Dovgan, Severns et al. studied the relationship between nutritional intake and gastrointestinal symptomatology in children with ASD ($n = 120$). The study found no significant associations between GI symptoms, omega-3 fatty acids and/or other micro- and macronutrients in the diet. The findings suggest a disconnect between dietary changes and GI symptoms in ASD individuals.

Ferguson, Hamlin, Lantz et al. examined the relationship between GI disorders, problem behaviors and internalizing symptoms in a sample of 340 children and adolescents with ASD. Externalizing problem behaviors and internalizing symptoms associated with GI problems differ between young and older children with ASD. The results suggest a different relationship

of GI symptoms at different ages, a difference which may have implications for the longitudinal follow-up of patients as well as for their treatment.

Nor et al. designed and conducted a cross sectional study to assess the prevalence of obesity and overweight among Malaysian ASD children and adolescents. The methods were predominantly psychometric in nature and completed by the children's parents. The reported prevalence in ASD was higher than in the general population matched for age and gender, with higher risk for the older age ones, high maternal BMI, older paternal age, low physical activity, food refusal, and food selectivity. Feeding difficulties, limited variety, and a lack of physical activity was related to higher weight gain.

Lindor et al. studied whether the link between greater ASD symptom severity and problem behaviors is moderated by sleep disturbance. The authors reported a positive relationship between ASD severity and problem behaviors for those ASD individuals with mild or no sleep disturbance. By way of contrast, there was no significant relationship between ASD symptom severity and problem behavior among those ASD individuals with moderate-to-severe sleep disturbance. Significant problem behaviors were apparent across all individuals with moderate-to-severe sleep disturbances regardless of the severity of ASD symptoms. The study highlights how profiling of ASD individuals based on their sleeping habits may help identify those prone to clinically significant problem behaviors.

While sex-differences in ASD has been associated with IQ and cognitive function, Margari et al. examined whether male and female high functioning autism (HFA) patients might develop specific comorbidities phenotypes in this retrospective study. The study evaluated 159 HFA patients (100 male and 59 female) for the presence/absence, type and gender distribution of psychopathological comorbidities (Attention Deficit Hyperactivity Disorder, Anxiety Disorders, Depressive Disorders, Bipolar Disorder, Obsessive-Compulsive Disorder, and Anorexia Nervosa), according to DSM-5 diagnostic criteria. The study found a bias, favoring females, in Anorexia Nervosa among HF ASD patients.

Neuhaus et al. studied the records of 2,800 patients with ASD and found that parent-reported GI concerns are present in approximately one third of patients, and that GI symptoms are an independent predictor of internalizing, externalizing, and self-injurious behaviors. They document an increased prevalence of comorbid psychiatric problems in those patients with GI symptoms.

In a retrospective study Penzol et al. reviewed the prevalence of GI disorders in ASD and described their clinical correlates. The study included all patients ($n = 845$) with documented information regarding GI disorders (GID) admitted to a general hospital during a span of 3 years. GIDs were present in 30.5% of the patients, the most common complaint being constipation. GIDs were significantly associated with ID, sleep disorders and treatment with psychopharmacological drugs.

4) Intellectual disabilities: There is a large degree of clinical heterogeneity in both ID and ASD. Their complex interactions and expression may negate the generalizability

of observational findings to the general population. Still, this area should be prioritized in research. Their multi-morbidity burden is greater, occurs at a much earlier age, and they are at the receiving end of health service inequalities.

Casanova et al. investigated genotype-phenotype correlations in ID. The study selected from a database of patients with ID and comorbidities of ASD, epilepsy, and a group without comorbidities (neither ASD nor epilepsy). These groups were further characterized by secondary manifestations of complex vs. simple facial dysmorphia (CFD/SFD) and neurodegenerative-like features (NLF). Phenotypic analysis showed high frequency of CFD in ID with ASD and NLF in ID with epilepsy. Gene covariation analyses were conducted and revealed ID networks with functional enrichment in relation to CFD and NLF. These findings suggest that clinical features related to ID are predictive of underlying genotype.

Miot et al. studied 63 adults with ASD-ID with detailed clinical examinations and screening for comorbidities. Investigators found a large range of comorbidities with a burden similar to those of older geriatric patients. This "premature aging" in adults with ASD-ID positively correlated with age, decreased autonomy, and polypharmacy. The results highlight the need for personalized medical care in this patient population.

Paulais et al. aimed to investigate possible cross-cultural differences and, on an individual level, the degree of heterogeneity in the cognitive and socio-emotional levels and severity of ASD symptoms. Developmental profiles of children with ASD + ID across countries showed extremely high heterogeneity in comparison with those of developmentally matched TD children. Profiles of ASD + ID children across countries were also similar to one another. ASD + ID children tended to have their lowest scores in language and vocal imitation. Heterogeneity within profiles was unrelated to country, but a few differences were found between countries in correlations of heterogeneity index with developmental level or developmental quotient. Algerian children had significantly greater differences between cognitive and socioemotional heterogeneity indices. Heterogeneity of profile was not related to age but was negatively related to overall developmental level and positively to severity of ASD.

5) Mechanisms: Many comorbidities represent intertwined conditions linked by shared risk factors (e.g., disease associated genes, biological pathways) and complications. The health profile of affected individuals is compounded by the duration, severity, and presence of other superimposed health conditions.

Ashwood reports that soluble TNFR2 levels are significantly decreased in ASD compared to TD controls following PHA stimulation, suggesting, lower TNF-alpha antagonism. Likewise, following PHA stimulation, cell surface TNFR2 was increased compared to controls. Overall, this work suggests that the TNF-alpha pathways, particularly that downstream of TNFR2, may be dysregulated in ASD.

Ferguson, Dovgan, Takahashi et al. sampled electrodermal activity (EDA) in eight individuals with severe ASD at a residential facility. The investigators found an anticipatory rise in EDA prior to problem behaviors. EDA may help anticipate and manage problem behaviors while also monitoring the return to baseline.

Hirosawa et al. reported higher rates of interictal epileptiform discharges (IEDs) in individuals with ASD compared to TD children. In addition, while IEDs have been reported to negatively impact cognition in TD children, little is known regarding the impact of IEDs in children with ASD. The study included 163 TD children and 107 children with ASD for which 10 min of MEG recording was conducted as well as an assessment of cognition with the Kaufman Assessment Battery for Children (K-ABC). Linear regression was employed to examine the effect of IED frequency on cognitive function. In the TD group, there was a significant negative relationship between mental processing (MPS) and IED frequency, while in the ASD group, a significant positive relationship was found between MPS scores and IED frequency. The authors suggest that the higher frequency of IEDs in ASD implies a local excitatory/inhibitory imbalance in the brain. However, the authors further suggest that this might not be pathogenetic in children with ASD, but instead might reflect epiphenomenal or compensatory processes.

Hughes and Ashwood report that antibodies that target *Candida albicans*, were positive in 36% of children with ASD and 14% of TD controls and GI dysfunction in half of the children with positive findings. The study draws attention to the fungal microbiota of individuals with ASD and the possibility of significant alterations (dysbiosis) in the same. The study provides suggestive evidence of a new microbial risk factor for ASD.

Sokol et al. review findings suggesting how secreted APPα and the ADAM family of α-secretases (non-amyloidogenic pathway of βAPP processing) may increase white matter volume in ASD.

Recognition of those pathological pathway may lead to new treatment interventions.

Tye et al. considered those comorbid conditions that have been repeatedly associated with ASD; epilepsy, sleep, gastrointestinal, and immune functioning. The authors discuss research into potential etiological mechanisms and potential interactions between each comorbidity and ASD. The networks derived from the analysis of abnormal biological profiles may help define potential subgroups and personalize targets for interventions.

In conclusion, the presence of comorbidities delineates subgroups within ASD that allow us to better define underlying mechanisms, etiologies, and possible genetic/environmental contributions. Comorbidities provide additive risk factors for morbidity and mortality (Tye et al.) which demand special monitoring and long-term follow-up by primary care physicians. If health care providers are to improve outcome, it is imperative to reorient services in such a way so as to better recognize the presence of comorbidities and to refer patients to appropriate professionals. In order to diminish the burden of comorbidities in ASD, management requires a multidisciplinary team approach comprised by parents, special educators, psychologists, and medical specialists (34, 35). We also advocate for the implementation of a standardized review of systems, development of interpretative guidelines for polypharmacy peculiar to this patient population, execution of targeted assessments for specific psychiatric and GI disorders, and the enactment of preventative strategies for any existing modifiable risk factor (e.g., obesity).

AUTHOR CONTRIBUTIONS

REFERENCES

1. Gillberg C, Billstedt E. Autism and Asperger syndrome: coexistence with other clinical disorders. *Acta Psychiatr Scand.* (2000) 102:321–30. doi: 10.1034/j.1600-0447.2000.102005321.x

2. Davis JW, Chung R, Juarez DT. Prevalence of comorbid conditions with aging among patients with diabetes and cardiovascular disease. *Hawaii Med J.* (2011) 70:209–13.

3. Buttorff C, Ruder T, Bauman M. *Multiple Chronic Conditions in the United States.* Santa Monica, CA: RAND Corporation (2017). doi: 10.7249/TL221

4. MaCurdy T, Bhattacharya J. *Challenges in Controlling Medicare Spending: Treating Highly Complex Patients.* Burlingame, CA: Acumen, LLC (2014).

5. Lundström S, Reichenberg A, Melke J, Råstam M, Kerekes N, Lichtenstein P, et al. Autism spectrum disorders and coexisting disorders in a nationwide Swedish twin study. *J Child Psychol Psychiatry.* (2015) 56:702–10. doi: 10.1111/jcpp.12329

6. Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, et al. Prevalence of autism spectrum disorder among children 8 years- Autism and Developmental Disabilities Monitoring Sites, United States, 2014. *MMWR Surveill Summ.* (2018) 67:1–23. doi: 10.15585/mmwr.ss6706a1

7. Rydzewska E, Hughes-McCormack LA, Gillberg C, Henderson A, MacIntyre C, Rintoul J, et al. Prevalence of long-term health conditions in adults with autism: observational study of a whole country population. *BMJ Open.* (2018) 8:e023945. doi: 10.1136/bmjopen-2018-023945

8. Maenner MJ, Shaw KA, Baio J, Washington A, Patrick M, DiRienzo M, et al. Prevalence of autism spectrum disorder among children aged 8 years- autism and developmental disabilities monitoring network, 11 sites, United States, 2016. *MMWR Surveill Summ.* (2020) 69:1–12. doi: 10.15585/mmwr.ss6904a1

9. Doshi-Velez F, Ge Y, Kohane I. Comorbidity clusters in autism spectrum disorders: an electronic health record time-series analysis. *Pediatrics.* (2014) 133:e54–e63. doi: 10.1542/peds.2013-0819

10. Miles JH, Takahashi TN, Bagby S, Sahota PK, Vaslow DF, Wang CH, et al. Essential versus complex autism: definition of fundamental prognostic subtypes. *Am J Med Genet A.* (2005) 135:171–80. doi: 10.1002/ajmg.a.30590

11. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders.* 5th ed. Washington, DC (2013). doi: 10.1176/appi.books.9780890425596

12. Mpaka DM, Okitundu DL, Ndjukendi AO, N'situ AM, Kinsala SY, et al. Prevalence and comorbidities of autism among children referred to the outpatient clinic for neurodevelopmental disorders. *Pan Afr Med J.* (2016) 25:82. doi: 10.11604/pamj.2016.25.82.4151

13. Buie T, Campbell DB. Fuchs GJ III, Furuta GT, Levy J, Vandewater J, et al. Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. *Pediatrics.* (2010) 125:S1–18. doi: 10.1542/peds.2009-1878C

14. Bellardinelli C, Raza M, Taneli T. Comorbid behavioral problems and psychiatric disorders in autism spectrum disorders. *J Childhood Dev Dis.* (2016) 2:11. doi: 10.4172/2472-1786.100019

15. Patten E, Ausderau K, Watson LR, Baranek G. Sensory response patterns in nonverbal children with ASD. *Autism Res Treat.* (2013) 2013:436286. doi: 10.1155/2013/436286

16. Gernsbacher MA, Stevenson JL, Stevenson JL, Khandakar S, Hill Goldsmith H Why does joint attention look atypical in autism? *Child Dev Perspect.* (2008) 2:38–45. doi: 10.1111/j.1750-8606.2008.00039.x

17. DuBois D, Ameis SH, Lai MC, Casanova MF, Desarkar P. Interoception in autism spectrum disorder: a review. *Int J Dev Neurosci.* (2016) 52:104–11. doi: 10.1016/j.ijdevneu.2016.05.001

18. Whitney DG, Shapiro DN. National prevalence of pain among children and adolescents with autism spectrum disorders. *JAMA Pediatr.* (2019) 173:1203–5. doi: 10.1001/jamapediatrics.2019.3826

19. Takara K, Knodo T, Kuba T. How and why is autism spectrum disorder misdiagnosed in adult patients: -from diagnostic problem to management for adjustment. *Mental Health Family Med.* (2015) 11:73–88. doi: 10.25149/1756-8358.1102011

20. Huffman LC, Sucliff TL, Tanner IS, Feldman HM. Management of symptoms in children with autism spectrum disorders: a comprehensive review of pharmacologic and complementary-alternative medicine treatment. *J Dev Behav Pediatr.* (2011) 32:56–68. doi: 10.1097/DBP.0b013e3182040acf

21. Owens EB, Hinshaw SP. Perinatal problems and psychiatric comorbidities among children with ADHD. *J Clin Child Adolesc Psychol.* (2013) 42:762–8. doi: 10.1080/15374416.2013.785359

22. Frye RE. Autism spectrum disorder in the emergency department: looking beyond behavior. *Int J Emergency Mental Health Hum Resil.* (2015) 17:661–3. doi: 10.4172/1522-4821.1000253

23. Londoño A, Ballester Navarro P, Martínez E, Cawthorpe A. Comorbidities as predictor of polypharmacy in autism spectrum disorder. *Eur Neuropsychopharmacol.* (2016) 26:S730–1. doi: 10.1016/S0924-977X(16)31881-8

24. Guinchat V, Cravero C, Lefèvre-Utile J, Cohen D. Multidisciplinary treatment plan for challenging behaviors in neurodevelopmental disorders. *Handb Clin Neurol.* (2020) 174:301–21. doi: 10.1016/B978-0-444-64148-9.00022-3

25. Cawthorpe D. Comprehensive description of comorbidity for autism spectrum disorder in a general population. *Perm J.* (2016) 21:16-088. doi: 10.7812/TPP/16-088

26. Diane A, Pierce WD, Kellly SE, Sokolik S, Borthwick F, Jacome-Sosa M, et al. Mechanisms of comorbidities associated with the metabolic syndrome: insights from the JCR: LA-cp corpulent rat strain. *Front Nutr.* (2016) 3:44. doi: 10.3389/fnut.2016.00044

27. Srinivasan SM, Pescatello LS, Bhat AN. Current perspectives on physical activity and exercise recommendations for children and adolescents with autism spectrum disorders. *Phys Ther.* (2014) 94:875–89. doi: 10.2522/ptj.20130157

28. Healy S, Pascanowsli CR, Williams R. Weight management interventions for youth with autism spectrum disorder: a systematic review. *Int J Obes.* (2019) 43:1–12. doi: 10.1038/s41366-018-0233-8

29. Levy SE, Pinto-Martin JA, Bradley CB, Chittams J, Johnson SL, Pandey J, et al. Relationship of weight outcomes, co-occurring conditions, and severity of autism spectrum disorder in the study to explore early development. *J Pediatr.* (2019) 205:202–9. doi: 10.1016/j.jpeds.2018.09.003

30. Walker ER, Druss BG. Mental disorders and medical comorbidity. *Synth Proj Res Synth Rep.* (2011) 1–26.

31. Feinstein AR. Pre-therapeutic classification of co-morbidity in chronic disease. *J Chronic Dis.* (1970) 23:455–68. doi: 10.1016/0021-9681(70)90054-8

32. Valderas JM, Starfield B, Sibbald B, Salisbury C, Roland M. Defining comorbidity: implications for understanding health and health services. *Ann Fam Med.* (2009) 7:357–63. doi: 10.1370/afm.983

33. Barnett K, Mercer SW, Norbury M, Watt G, Wyke S, Guthrie B. Epidemiology of multimorbidity and implications for health care, research, and medical education: a cross-sectional study. *Lancet.* (2012) 380:37–43. doi: 10.1016/S0140-6736(12)60240-2

34. Gillberg C. The essence in child psychiatry: early symptomatic syndromes eliciting neurodevelopmental clinical examinations. *Res Dev Disabil.* (2010) 31:1543–51. doi: 10.1016/j.ridd.2010.06.002

35. Kohane IS, McMurry A, Weber G, MacFadden D, Rappaport L, Kunkel L, et al. The co-morbidity burden of children and young adults with autism spectrum disorders. *PLoS ONE.* (2012) 7:e33224 doi: 10.1371/journal.pone.0033224

Check for
updates

Victimization and Perpetration Experiences of Adults With Autism

Jonathan A. Weiss and Michelle A. Fardella*

Department of Psychology, York University, Toronto, ON, Canada

This study aimed to describe the self-reported experiences of childhood and adult victimization and perpetration in adults with autism spectrum conditions (ASC) compared to a matched sample, and how victimization and perpetration are associated with autism-related difficulties. Forty-five adults with ASC and 42 adults without ASC completed questionnaires regarding violence victimization and perpetration, emotion regulation, and sociocommunicative competence. Participants with ASC reported experiencing, as children, more overall victimization; specifically, more property crime, maltreatment, teasing/emotional bullying, and sexual assault by peers, compared to participants without ASC. Participants with ASC also reported experiencing more teasing/emotional bullying in adulthood and greater sexual contact victimization. No significant differences were found between groups on perpetration. Sociocommunicative ability and emotion regulation deficits did not explain the heightened risk for victimization. Individuals with ASC have an increased vulnerability to violence victimization, which speaks to the need for interventions, and proactive prevention strategies.

Keywords: autism, emotion regulation, social skills, victimization, perpetration, bullying, adults, maltreatment

INTRODUCTION

Adults with autism spectrum conditions (ASC) may be at considerable risk for interpersonal violence victimization, which refers to violence and abuse that occurs between people, including child maltreatment, intimate partner violence, adolescent dating violence, and bullying (1). Individuals with ASC have a number of impairments in social communication and social interaction across multiple contexts, and exhibit restricted interests and/or repetitive body movements and behaviors (2). The current estimated prevalence of ASC is ~1 in 68, with ~44% having average to above average intellectual abilities (3, 4).

There is a paucity of research examining discrete experiences of interpersonal violence in those with ASC, although what does exist points to an increased risk for child maltreatment, bullying, and sexual victimization (5–7). In children, having an autism diagnosis is associated with an increased chance of physical, emotional, or sexual abuse compared to peers without disabilities (8). More recent research interviewing 182 parents of children with ASC found high rates of reported physical abuse (18.5%), sexual abuse (12.2%), or both kinds (4.4%), though no information on the sources of this abuse was noted (7). Studies have also found high rates of peer victimization in children [65–77%; (6, 9)]. Studies of adults with ASC have largely focused exclusively on sexual victimization. In a college sample, students with ASC were twice as likely to report unwanted sexual contact compared to students without ASC (10). In an online survey, 70% of adults with ASC reported experiencing some form of sexual victimization after age 14 and into adulthood, compared

to 45% of those without ASC (5). Authors have suggested that increased risks of bullying, physical, and emotional abuse may also be present in adults with ASC due to heightened social vulnerability (11, 12).

Research has begun to move from an understanding of experiences of interpersonal violence in isolation to understanding the co-occurrence and interconnections between experiences of interpersonal violence, known as polyvictimization, and polyperpetration (13). Too often forms of violence are studied in isolation, and some authors state that focusing on specific forms in isolation may mask the important information that would be gained by studying the complex, varied patterns of traumas (14). Research has yet to examine the broader interpersonal violence experiences of adults with ASC beyond sexual violence victimization, or to look at interpersonal violence perpetration in adults in the community, though what does exist on this latter question suggests no clear association between ASC and violent crime (15–17). Additional research is needed to understand the context of violence across a number of different kinds of acts in adults with ASC.

It is critical to understand the mechanisms that are associated with heightened risk for interpersonal violence (18). Deficits in sociocommunicative competence may be a particular set of risk factors for violence victimization and perpetration in adults with ASC (19, 20). It is well known that individuals with ASC can have challenges with social reasoning, are literal thinkers, and may miss contextual cues (21, 22), and authors have suggested that such sociocommunicative difficulties may be related to an increased risk of sexual abuse (23) and bullying in children with ASC (6). With regard to perpetration, social naivety and misinterpretation of social cues may inadvertently lead to criminal behavior in individuals with ASC, though not specifically to interpersonal violence (24–26). For instance, authors have noted that individuals with ASC inadvertently engage in stalking behaviors when they seek out contact with others for friendship or intimacy [e.g., (27–29)]. No study has tested whether sociocommunicative difficulties explain an increased risk of violence for adults with ASC.

Emotion regulation deficits have also been linked with violence victimization and perpetration in adults in general [e.g., (30)], and may be a particularly salient factor for adults with ASC. In children and adults without ASC, maladaptive emotion regulation is a risk factor for chronic victimization (31, 32). For perpetration, the ability to regulate one's negative emotions may be a factor that helps individuals refrain from initiating violence (33). While difficulties in emotion regulation, emotional expression, and emotion processing have been widely discussed in the ASC literature (34), its link to violence in this population has only been briefly explored, with one study reporting an association among emotion dysregulation and bullying perpetration and victimization in youth (35).

The negative effects of violence are well known in the non-ASC literature (36), and additional efforts to understand the prevalence, characteristics, and causes in adults with ASC are needed. The current study aimed to identify (1) patterns of violence victimization and perpetration in adults with and without ASC across many types, (2) differences in self-reported polyvictimization and polyperpetration, and (3) whether impairments in the areas of sociocommunicative competence and emotion regulation mediate the expected higher rates of violence victimization and perpetration. Self-report was used to gain a reliable estimate of violence victimization and perpetration experiences in adults with ASC living in the community.

METHODS

Recruitment

Participants with ASC were recruited through study notices distributed by community-based programs and organizations that support those with ASC, online ASC communities, several colleges/universities academic support services, and from study participants to others at their discretion. The comparison group was recruited through postings within the University setting and on community message boards. Advertisements indicated that this was a research project on interpersonal violence in adults that aimed to understand the experiences of interpersonal violence, and that any adult could participate, even if they did not experience violence themselves. Identical recruitment and consent materials were used for both groups. Eligible participants with ASC were required to (a) have a diagnosis of an ASC (e.g., Autism, Asperger Syndrome, Autism Spectrum Disorder, PDD-NOS) according to self-report, which was verified by administering the Autism Diagnostic Observation Schedule−2nd Edition (37), (b) be 18 years of age or older, and (c) have an estimated IQ above 80 on the Wechsler Abbreviated Scale of Intelligence (38). Participants without ASC had to meet criterion b and c. Equal numbers of men and women with ASC responded to the study advertisements.

Participants

The sample included 45 adults with ASC between 18-53 years of age ($M = 30.00$, $SD = 1.48$) and 42 adults without ASC, matched on mean chronological age, between 19 and 54 years ($M = 32.12$, $SD = 8.62$). Groups did not significantly differ with respect to the percentage of men (42.5% ASC; 50% non-ASC) or on self-identified minority status (15.6% ASC; 31% non-ASC). Participants were also similar in IQ estimates (non-ASC $M = 113.33$, $SD = 16.10$, Range 87–146; ASC $M = 110.22$, $SD = 13.19$, Range 81–134; $t_{(85)} = -0.98$, $p = 0.36$), and in the percentage who obtained at least a college degree (85% ASC; 95% non-ASC). All participants lived in the Greater Toronto Area. Participants in the ASC group reported a diagnosis of ASC and met the clinical cut-off on the ADOS-2 Module 4 (37). Participants without ASC reported that they did not identify with being on the autism spectrum and had never received a diagnosis of an ASC (e.g., autism, Asperger's Syndrome, etc...).

Measures
Autism Diagnostic Observation Scale- 2nd Edition (ADOS-2)
The ADOS-2 (37) is a semi-structured observational measure that examines social and communicative behaviors, and was used to verify ASC status for the ASC group. The ADOS has been

found to have good test-retest reliability and excellent internal consistency (37).

Wechsler Abbreviated Scale of Intelligence (WASI)

The four-subset WASI (38) was administered to obtain a general estimate of intellectual functioning (Full Scale IQ). This measure has been shown to have adequate to high test-retest reliability ($r = 0.72–0.95$) depending on the subtest, and high internal consistency across groups and subtests (Cronbach's alpha = 0.87–0.98). The WASI has been used in adults with ASC as a brief measure of IQ (39).

Juvenile Victimization Questionnaire-Adult Retrospective Questionnaire (JVQ-AR)

The JVQ-AR was used as a measure of childhood victimization, adult victimization, and adult perpetration. The original child victimization version is a 34-item self-report questionnaire that collects information on several forms of childhood victimization (40). The questionnaire assesses the frequency of 34 discrete forms of childhood victimization, scored as a dichotomy (1 = *experienced*; 0 = not *experienced*). For *childhood victimization*, participants are asked about any experiences from birth up until the 18th birthday (0 through 17 years 12 months). The 34 questions fall within six categories: property crime, physical assault, child maltreatment, peer/sibling victimization, sexual victimization, and witnessed/indirect victimization. For *adult victimization*, a modified version of JVQ-AR was used where participants reported on any of 29 victimization experiences across the same 6 categories, which occurred from their 18th birthday onward. Items that pertained to childhood experiences were removed. Scores are provided for each individual item and each aggregate category. Polyvictimization was computed by summing the endorsed victimization items, with scores ranging from 0 to 34 for childhood victimization, and 0 to 29 for adult victimization [as recommended by (41)], with higher scores indicating a greater number of discrete victimization experiences.

For *adult perpetration,* a modified version of the JVQ-AR was used where participants were asked about perpetration experiences that occurred from age 18 on. Items pertaining to witnessing violence (e.g., witnessing domestic violence) and child maltreatment (e.g., being bullied by peers) were removed, since the focus of this questionnaire was adulthood and perpetration experiences (i.e., acts committed by the individual during adulthood). Polyperpetration was computed by summing the endorsed perpetration items, with scores ranging from 0 to 19, with higher scores indicating a greater number of discrete acts of violence.

Difficulties in Emotion Regulation Scale (DERS)

The DERS (42) is a 36-item self-report measure of emotion regulation ability. Subscales assess six dimensions of difficulties: Nonacceptance, Goals, Impulse, Awareness, Strategies, and Clarity. Participants rate how often statements apply to them on a Likert scale with answer categories: 1 = *almost never* to 5 = *almost always*. An overall score was used for the current study. Higher scores indicate greater difficulty with emotion

regulation. The DERS has been shown to have good internal consistency, test-retest reliability, and construct validity (42–44), and was recently used in a sample of young adults with ASC (45). Internal consistency for the DERS across the whole sample and individual groups demonstrated good to excellent reliability (whole sample α = 0.95, ASC group α = 0.89, no ASC group α = 0.94).

Multidimensional Social Competence Scale (MSCS)

Sociocommunicative competence was measured utilizing the self-report version of the MSCS (46). The MSCS is designed to assess social competence among adolescents and adults with ASC. Psychometric evidence provided preliminary support for the reliability and validity of the scale [Cronbach's alpha reliabilities for domain, subscale, and total scores were all above 0.84; (46)]. The MSCS measures seven domains of social competence: social motivation, social inferencing, demonstrating empathic concern, social knowledge, verbal conversation skills, nonverbal sending skills, and emotion regulation. Participants rated how statements applied to them, where 1 = *not true or almost never true,* to 5 = *very true or almost always true.* An overall score was used for the current study, without including emotion regulation (given the use of the DERS). Overall Cronbach's alpha within both groups demonstrated excellent internal consistency (no ASC group α = 0.95, ASC group α = 0.93).

Procedure

All participants met in person with a trained graduate student. Informed consent was obtained, IQ was assessed, and for those with ASC, the ADOS-2 was completed. Participants were then provided with a laptop computer to complete questionnaires on the online Qualtrics data system (www.qualtrics.com). The University ethics board approved this research. Participants with and without ASC received a gift card to an online retailer for their participation. The informed consent articulated the limits of confidentiality and that participants may have experienced feelings of discomfort generated by the content of the questions asked. A list of support resources were provided to all participants, and they were informed that if they experienced any emotional distress and wanted to speak with a counselor, the researcher would facilitate. One participant with ASC requested this information.

Data Analysis

Chi-square analyses and odds ratios were used to examine whether there were differences in the self-report of victimization and perpetration between groups. Due to non-normal data, the Mann Whitney test was calculated to compare groups on self-reported breadth of victimization and perpetration, on sociocommunicative competence, and emotion regulation abilities. Preliminary analyses revealed no differences when comparing men to women in either of the two groups (e.g., men with ASC vs. women with ASC, or men without ASC to women without ASC) on overall polyvictimization or polyperpetration, and on aggregate scores, within either the ASC group or non-ASC group (all p's >.10).

To establish whether self-reported polyvictimization and polyperpetration experiences would be mediated by deficits in sociocommunicative competence and emotion regulation, a test of multiple mediation was run using SPSS INDIRECT macro script for testing multiple mediator models with bootstrapping (47). Given the large age range and concerns that men and women could differ in terms of their experiences of victimization or perpetration, all mediation analyses entered age and sex as control variables (the same analyses were run without these controls and no differences emerged in the pattern of results).

RESULTS

Childhood Victimization

As shown in **Table 1**, during their childhood, participants with ASC were 6.7 times more likely to report experiencing a form of property crime, largely the result of being more likely to have been robbed than peers without ASC. Those with ASC were 4 times more likely to report experiencing a form of child maltreatment, including physical abuse, and psychological or emotional abuse from adults, 27.1 times more likely to endorse teasing from peers, 3.7 times more likely to endorse bullying from peers, and 7.3 times more likely to endorse sexual assault by a peer, compared to adults without ASC. Participants without ASC were 4.4 times more likely to endorse having sexual relations with someone over 18 than participants with ASC. Participants with ASC reported significantly higher polyvictimization than those without ASC ($U = 1204$, $p = 0.03$; ASC $M = 12.62$, $SD = 5.45$; no ASC $M = 10.05$, $SD = 7.12$).

Adult Victimization

As shown in **Table 2**, participants with ASC were 2.7 times more likely to endorse that they had experienced teasing during adulthood. There was a trend toward those with ASC being more likely to report sexual assault from a known adult, attempted or complete rape, and dating violence. Sexual victimization was further examined in order to separate contact victimization versus noncontact victimization. Sexual assault (by a known adult or unknown adult), and rape (attempted or completed) were summed (resulting in a score of 0–3). Individuals with ASC had significantly higher scores on this composite score than those without ASC ($U = 1148.5$, $p = 0.03$; ASC group $M = 0.67$, $SD = 0.93$; no ASC group $M = 0.29$, $SD = 0.71$) Participants without ASC were 4.4 times more likely to endorse assault with a weapon during adulthood. Participants with ASC did not report greater polyvictimization in adulthood than those without ASC ($U = 894$, $p = 0.66$; ASC group $M = 6.16$, $SD = 5.52$; no ASC $M = 5.95$, $SD = 4.22$).

Adult Perpetration

Table 3 presents the frequencies of endorsing each type and category of perpetration, and the comparisons across groups. No significant differences were found between groups on any form of perpetration, with very low rates reported. Groups did not differ on their polyperpetration score ($U = 1006$, $p = 0.59$, ASC group mean = 2.40, $SD = 3.02$; no ASC group $M = 1.90$, $SD = 2.09$).

Mediators of Victimization and Perpetration

As expected, the ASC group reported less developed sociocommunicative competence (ASC $M = 3.32$, $SD = 0.40$; no ASC $M = 4.05$, $SD = 0.40$; $U = 200.00$, $p < 0.001$) and poorer emotion regulation abilities (ASC $M = 2.72$, $SD = 0.57$; no ASC $M = 1.88$, $SD = 0.51$; $U = 200.00$, $p < 0.001$) compared to the comparison group. Neither sociocommunicative competence or emotion regulation were significantly correlated with childhood polyvictimization or adult polyvictimization in the ASC group or the non ASC group (all p's > 0.05). Multiple mediation analyses were used in order to further examine whether emotion regulation and sociocommunicative competence were related to the group differences found in childhood polyvictimization experiences. **Table 4** shows the unstandardized coefficients of each pathway, the confidence intervals, and the bootstrapping results based on 1,000 resamples. The total direct effect (path c) of ASC status approached significance before entering the mediator variables, $z = 1.95$, $p = 0.05$. The relationship between ASC diagnosis and polyvictimization in childhood was not mediated by sociocommunicative status or emotion regulation. The direction of estimates in the mediator pathways (path a) indicated that having ASC was associated with lower sociocommunicative competence ($t = -8.30$, $p < 0.001$), and poorer emotion regulation ($t = 7.27$, $p < 0.001$). The total indirect effects did not suggest the presence of mediation, as emotion regulation and sociocommunicative competence were not related to polyvictimization (path b). Mediation analyses were not computed for adult polyvictimization or polyperpetration, as no significant differences were found between groups.

DISCUSSION

Victimization

This is the first study to explore self-reported experiences of many forms of victimization and perpetration in adults with ASC compared to those without. ∼90% of participants with and without ASC reported experiencing at least one form of victimization in childhood, and approximately the same number reported victimization in adulthood. Using the same measure of childhood victimization, other research has found that 97% of college age women (48) and 80% of young adult men and women who had been identified as "at risk for high school drop out" endorse experiencing at least one form of victimization in childhood (36). It appears that using a broad measure of violence experiences, in a broader range of adult ages, results in similar high rates.

Adults with ASC reported a greater breadth of victimization during childhood compared to adults without ASC, matched on sex, IQ, and age. Adults with ASC were more likely to report that as a child, they experienced physical abuse, psychological/emotional abuse from an adult, peer/sibling victimization, various forms of bullying from peers, robbery, and sexual assault by a peer than respondents without ASC. The current research also shows that they are at risk for

TABLE 1 | Frequency Table for the 34 types of childhood victimization on the JVQ-AR as reported by adults with and without ASC.

Victimization Type	ASC n (%)	No ASC n (%)	Chi-square/Fisher's exact
34 types of victimization, at least one type	45 (100)	41 (97.6)	$\chi^2_{(1)} = 1.08$, $p = 0.30$
Property Crime aggregate (at least one type)	43 (95.6)	32 (76.2)	Fisher's exact $p = 0.01$; OR = 6.7
Robbery	40 (90.9)	22 (47.6)	Fisher's exact $p < 0.0001$; OR = 9.1
Theft	31 (68.9)	25 (59.5)	$\chi^2_{(1)} = 0.83$, $p = 0.36$
Vandalism	30 (68.2)	23 (54.8)	$\chi^2_{(1)} = 1.64$, $p = 0.20$
Physical Assault aggregate (at least one type)	43 (95.6)	37 (88.1)	Fisher's exact $p = 0.26$
Assault with a weapon	24 (53.5)	19 (45.2)	$\chi^2_{(1)} = 0.57$, $p = 0.45$
Assault without a weapon	37 (82.2)	28 (66.7)	$\chi^2_{(1)} = 2.78$, $p = 0.09$; OR = 2.31
Attempted assault	22 (48.9)	13 (31)	$\chi^2_{(1)} = 2.91$, $p = 0.09$; OR = 2.13
Kidnap, attempted, or completed	5 (11.1)	3 (7.1)	Fisher's exact $p = 0.71$
Bias attack	7 (15.6)	8 (19)	$\chi^2_{(1)} = 0.19$, $p = 0.67$
Physical abuse (not spanking)	26 (57.8)	11 (26.2)	$\chi^2_{(1)} = 8.87$, $p = 0.003$; OR = 3.9
Assault by group or gang of peers	23 (51.1)	14 (33.3)	$\chi^2_{(1)} = 2.81$, $p = 0.09$; OR = 2.09
Peer/sibling assault	35 (77.8)	32 (76.2)	$\chi^2_{(1)} = 0.03$, $p = 0.86$
Genital assault	21 (46.7)	15 (35.7)	$\chi^2_{(1)} = 1.07$, $p = 0.30$
Dating violence	3 (6.7)	5 (11.9)	Fisher's exact $p = 0.48$
Child maltreatment	36 (80)	21 (51)	$\chi^2_{(1)} = 8.65$, $p = 0.003$; OR = 4.0
Physical abuse (not spanking)	26 (57.8)	11 (26.2)	$\chi^2_{(1)} = 8.87$, $p = 0.003$; OR = 3.9
Psychological or emotional abuse	28 (62.2)	15 (35.7)	$\chi^2_{(1)} = 6.11$, $p = 0.01$; OR = 3.4
Neglect	9 (20)	6 (14.3)	$\chi^2_{(1)} = 49$, $p = 0.48$
Custodial interference or family abduction	5 (11.1)	5 (11.9)	Fisher's exact $p = 1.0$
Peer/sibling victimization aggregate (at least one type**)**	44 (97.8)	36 (85.7)	Fisher's exact $p = 0.05$; OR = 7.33
Assault by group or gang	23 (51.1)	14 (33.3)	$\chi^2_{(1)} = 2.81$, $p = 0.09$; OR = 2.09
Peer/sibling assault	35 (77.8)	32 (76.2)	$\chi^2_{(1)} = 0.03$, $p = 0.86$
Genital assault	21 (46.7)	15 (35.7)	$\chi^2_{(1)} = 1.07$, $p = 0.30$
Bullying	34 (75.6)	19 (45.2)	$\chi^2_{(1)} = 8.39$, $p = 0.004$; OR = 3.7
Teasing, emotional bullying	44 (97.8)	26 (61.9)	Fisher's exact $p < 0.001$;OR = 27.1
Dating violence	3 (6.7)	5 (11.9)	Fisher's exact $p = 0.48$s
Witnessed/indirect victimization aggregate (at least one type)	35 (77.8)	31 (73.8)	$\chi^2_{(1)} = 0.19$, $p = 0.67$
Witness domestic violence	8 (17.8)	9 (21.4)	$\chi^2_{(1)} = 0.18$, $p = 0.67$
Witness physical abuse	10 (22.2)	8 (22.2)	$\chi^2_{(1)} = 0.10$, $p = 0.76$
Witness assault with a weapon	17 (37.8)	18 (42.9)	$\chi^2_{(1)} = 0.23$, $p = 0.63$
Witness assault without a weapon	26 (59.1)	25 (61)	$\chi^2_{(1)} = 0.03$, $p = 0.86$
Household theft	22 (50)	16 (39)	$\chi^2_{(1)} = 1.03$, $p = 0.31$
Someone close murdered	0 (0)	4 (9.5)	Fisher's exact $p = 0.05$
Witness murder	1 (2.3)	2 (4.9)	Fisher's exact $p = 0.61$
Exposure to shooting, bombs, riots	4 (9.1)	10 (23.8)	Fisher's exact $p = 0.08$; OR = 3.13
Sexual victimization aggregate (at least one type)	25 (55.6)	21 (50)	$\chi^2_{(1)} = 0.27$, $p = 0.60$
Sexual assault, known adult	7 (15.6)	7 (16.7)	$\chi^2_{(1)} = 0.02$, $p = 0.89$
Sexual assault, unknown adult	3 (6.7)	2 (4.8)	Fisher's exact $p = 1.00$
Sexual assault, with peer	12 (26.7)	2 (4.8)	Fisher's exact $p = 0.007$; OR = 7.3
Rape, attempted or completed	6 (13.3)	5 (11.9)	Fisher's exact $p = 1.00$
Flashing or sexual exposure	9 (20)	6 (14.3)	$\chi^2_{(1)} = 0.50$, $p = 0.48$
Sexual harassment	16 (35.6)	11 (26.2)	$\chi^2_{(1)} = 0.89$, $p = 0.35$
Sexual interactions with someone over 18	3 (6.7)	10 (23.8)	Fisher's exact $p = 0.04$; OR = 4.4

violence victimization more broadly in childhood. Although the short and long-term impact of victimization, or trauma more broadly, on individuals with ASC is relatively unknown, peer victimization in youth with ASC has been related to internalizing and externalizing symptoms (6, 49), and maltreatment among youth with ASC has been related to externalizing behavior, suicide attempts, conduct and academic problems (7). It is important that childhood victimization in various contexts (home, school, and community) be addressed in order to keep this vulnerable group of youth safe. There is emerging evidence

TABLE 2 | Frequency table for the 29 types of adulthood victimization on the modified JVQ-AR as reported by adults with and without ASC.

Victimization Type	ASC n (%)	No ASC n (%)	Chi-square/Fisher's exact
29 types of victimization, at least one type	41 (91.1)	39 (92.8)	$\chi^2_{(1)} = 0.09, p = 0.77$
Property Crime aggregate (at least one type)	25 (55.6)	28 (66.7)	$\chi^2_{(1)} = 1.13, p = 0.29$
Robbery	9 (20)	9 (21.4)	$\chi^2_{(1)} = 0.03, p = 0.87$
Theft	23 (51.1)	22 (52.4)	$\chi^2_{(1)} = 0.01, p = 0.91$
Vandalism	8 (17.8)	15 (35.7)	$\chi^2_{(1)} = 3.59, p = 0.06; OR = 2.57$
Physical Assault aggregate (at least one type)	27 (60)	25 (59.5)	$\chi^2_{(1)} = 0.02, p = 0.96$
Assault with a weapon	3 (6.7)	10 (23.8)	Fisher's exact $p = 0.04; OR = 4.4$
Assault without a weapon	20 (44.4)	16 (38.1)	$\chi^2_{(1)} = 0.36, p = 0.55$
Attempted assault	8 (17.8)	9 (21.4)	$\chi^2_{(1)} = 0.18, p = 0.67$
Kidnap, attempted or completed	0 (0)	2 (4.4)	Fisher's exact $p = 1.00$
Bias attack	2 (4.4)	2 (4.8)	Fisher's exact $p = 1.00$
Physical abuse	18 (40)	12 (28.6)	$\chi^2_{(1)} = 1.26, p = 0.26$
Assault by group or gang of peers	3 (6.7)	4 (9.5)	Fisher's exact $p = 0.71$
Genital assault	2 (4.4)	3 (7.1)	Fisher's exact $p = 0.67$
Dating violence	12 (26.7)	10 (23.8)	$\chi^2_{(1)} = 0.09, p = 0.76$
Maltreatment in Adulthood	29 (64.4)	21 (50)	$\chi^2_{(1)} = 1.85, p = 0.17$
Physical abuse	18 (40)	12 (28.6)	$\chi^2_{(1)} = 1.26, p = 0.26$
Psychological or emotional abuse	16 (38.1)	25 (55.6)	$\chi^2_{(1)} = 2.66, p = 0.10; OR = 2.03$
Peer/Coworker victimization aggregate (at least one type)	27 (60)	23 (54.8)	$\chi^2_{(1)} = 0.24, p = 0.62$
Assault by group or gang of peers	3 (6.7)	4 (9.5)	Fisher's exact $p = 0.71$
Genital assault	2 (4.4)	3 (7.1)	Fisher's exact $p = 0.67$
Bullying	12 (26.7)	11 (11.9)	Fisher's exact $p = 0.11$
Teasing, emotional bullying	27 (60)	15 (35.7)	$\chi^2_{(1)} = 5.13, p = 0.02; OR = 2.7$
Dating violence	12 (26.7)	10 (23.8)	$\chi^2_{(1)} = 0.09, p = 0.09; OR = 1.16$
Witnessed/indirect victimization aggregate (at least one type)	26 (57.8)	32 (76)	$\chi^2_{(1)} = 3.31, p = 0.07$
Witness domestic violence	4 (8.9)	4 (9.5)	Fisher's exact $p = 1.00$
Witness physical abuse	3 (6.7)	3 (7.3)	Fisher's exact $p = 1.00$
Witness assault with a weapon	6 (13.3)	10 (26.3)	$\chi^2_{(1)} = 2.23, p = 0.14$
Witness assault without a weapon	16 (35.6)	19 (46.3)	$\chi^2_{(1)} = 1.03, p = 0.31$
Household theft	10 (22.2)	15 (36.6)	$\chi^2_{(1)} = 2.45, p = 0.14$
Someone close murdered	5 (11.1)	2 (4.8)	Fisher's exact $p = 0.44$
Witness murder	3 (6.7)	3 (7.3)	Fisher's exact $p = 1.00$
Exposure to shooting, bombs, riots	6 (13.3)	12 (29.3)	$\chi^2_{(1)} = 3.29, p = 0.07; OR = 2.69$
Sexual victimization aggregate (endorsed at least one type)	21 (46.7)	17 (40.5)	$\chi^2_{(1)} = 0.34, p = 0.56$
Sexual assault, known adult	11 (24.4)	4 (9.5)	Fisher's exact $p = 0.09; OR = 3.07$
Sexual assault, unknown adult	6 (13.3)	3 (7.1)	Fisher's exact $p = 0.49$
Rape, attempted, or completed	13 (28.9)	5 (11.9)	Fisher's exact $p = 0.07; OR = 3.01$
Flashing or sexual exposure	8 (17.8)	14 (33.3)	$\chi^2_{(1)} = 2.78, p = 0.10; OR = 2.31$
Sexual harassment	12 (26.7)	8 (19.0)	$\chi^2_{(1)} = 0.71, p = 0.40$

for strength-based school programming to reduce experiences of victimization in general (50), and these programs could be examined for their utility in decreasing victimization for those with ASC.

No differences were found between groups on polyvictimization in adulthood, though differences did emerge in specific kinds. Individuals with ASC were more likely to report experiencing teasing/emotional bullying from other adults, which speaks to a continued risk for interpersonal difficulties with peers across the lifespan. Adults with ASC, whether in the role as an employee or with peers in the community, may

benefit from specific training on what constitutes bullying and harassment and how to effectively manage those situations (51). Adults with ASC were also more likely to endorse experiencing some form of sexual victimization that involved contact, including sexual assault and rape, in line with previous research (5). There has been some research advocating for interventions targeting the risk of sexual victimization of individuals with developmental disabilities (52), focusing often on addressing self-protection and assertiveness [e.g., (53)], and education on sexual abuse for support workers (54). In considering how to best reduce the risk of interpersonal violence victimization for adults

TABLE 3 | Frequency table for the 19 types of adulthood perpetration on the modified JVQ-AR as reported by adults with and without ASC.

Victimization Type	ASC n (%)	No ASC n (%)	Chi-square/Fisher's exact
19 types of perpetration, endorsed at least one type	32 (71)	25 (59.5)	$\chi2_{(1)} = 1.29$, $p = 0.26$
Property Crime aggregate (at least one type)	25 (55.6)	28 (66.7)	$\chi2_{(1)} = 1.13$, $p = 0.29$
Robbery	7 (15.6)	7 (16.7)	$\chi2_{(1)} = 0.02$, $p = 0.88$
Theft	9 (20.5)	4 (9.5)	Fisher's exact $p = 0.23$
Vandalism	8 (19)	8 (18.2)	$\chi2_{(1)} = 0.01$, $p = 0.91$
Physical Assault aggregate (at least one type)	27 (60)	25 (59.5)	$\chi2_{(1)} = 0.002$, $p = 0.96$
Assault with a weapon	3 (6.8)	2 (4.8)	Fisher's exact $p = 1.00$
Assault without a weapon	14 (31.8)	15 (35.7)	$\chi2_{(1)} = 0.15$, $p = 0.70$
Attempted assault	8 (18.2)	3 (7.1)	Fisher's exact $p = 0.20$
Kidnap, attempted or completed	0 (0)	0 (0)	–
Bias attack	0 (0)	0 (0)	–
Physical abuse of other adults	13 (29.5)	12 (28.6)	$\chi2_{(1)} = 0.01$, $p = 0.92$
Committing assault with a group or gang of peers	1 (2.3)	1 (2.4)	Fisher's exact $p = 1.00$
Genital assault	3 (6.8)	2 (4.8)	Fisher's exact $p = 1.00$
Dating violence	5 (11.9)	10 (22.7)	Fisher's exact $p = 0.26$
Emotional abuse/bullying aggregate (at least one type)	19 (43.2)	16 (38.1)	$\chi2_{(1)} = 0.23$, $p = 0.63$
Psychological or emotional abuse	19 (43.2)	15 (35.7)	$\chi2_{(1)} = 0.50$, $p = 0.48$
Bullying	5 (11.4)	2 (4.8)	Fisher's exact $p = 0.43$
Sexual victimization aggregate (at least one type)	3 (7.1)	4 (9.1)	Fisher's exact $p = 1.00$
Sexual assault, known adult	1 (2.3)	0 (0)	Fisher's exact $p = 1.00$
Sexual assault, unknown adult	1 (2.3)	0 (0)	Fisher's exact $p = 1.00$
Rape, attempted, or completed	1 (2.3)	0 (0)	Fisher's exact $p = 1.00$
Flashing or sexual exposure	2 (4.5)	2 (4.8)	Fisher's exact $p = 1.00$
Sexual harassment	3 (6.8)	2 (4.8)	Fisher's exact $p = 1.00$

TABLE 4 | Multiple mediation analysis results for the mediating effect of sociocommunicative competence and emotion regulation on the relationship between group and childhood polyvictimization after controlling for sex and age.

IV, Mediators, and Control	Path	B	SE	Bootstrapping for Indirect Results		95% CI	Lower	Upper
				Point estimate				
				z/t	p			
Sex	Control	0.81	1.41	0.57	0.57			
Age	Control	0.08	0.08	1.03	0.30			
Group	C	2.68	1.37	1.95	0.05	−0.17	−3.9	4.55
	C'	2.84	2.01	1.42	0.16			
Emotion Regulation	A	0.85	0.12	7.27	<0.001	1.17	−1.95	3.88
	B	1.37	1.36	1.01	0.32			
Sociocommunicative Competence	A	−0.77	0.09	−8.30	<0.001	−1.34	−4.05	2.12
	B	1.73	1.71	1.01	0.32			

with ASC, proactive and accessible programming that promotes inclusion and healthy relationships within relevant contexts (including the home, school, workplace, and community levels) are needed.

Perpetration

Groups had similar rates across all forms of perpetration, categories of perpetration, and on polyperpetration, largely due to the equally low endorsements. Low rates were found for both severe and more minor occurrences of violence

perpetration. These results map onto the existing reviews finding low rates of perpetration in individuals with ASC and no clear association with violent crime (15, 16). While other studies have examined inpatients, file reviews of incarcerated individuals, or parent/caregiver report, the current study is the first to compare two matched community samples. Researchers and clinicians have cautioned that the sensational and unusual nature of some criminal incidents with individuals with ASC may garner media attention, and perpetuate the notion that individuals with ASC are more violent that individuals without ASC, which is not

the case (55). It may be the case that perpetrators with ASC present differently than perpetrators without ASC, with authors describing the links between the symptomology of ASC and offending behaviors (56). These differences will not emerge in examining rates *per se*, but in the nuances of how perpetration is expressed and the contexts that underlie these behaviors.

Mediators of Victimization

Contrary to expectations, sociocommunicative ability and emotion regulation deficits in adults with ASC did not explain a heightened risk for victimization. In fact, neither polyvictimization in childhood or adulthood was correlated with either variable, in either group. In the typical population, many additional factors have been associated with discrete types of victimization (e.g., bullying) and with overall risk, including age, gender, childhood experiences of victimization (emotional/physical/sexual abuse), and mental and physical health problems [e.g., (57–62)], which could be examined in future research. As well, models of victimization largely underscore the important of context, and the dynamics among individual and contextual factors (13). Researchers have begun to study the interplay, and differential impact, of individual and contextual factors, and some have found that contextual factors, such as dangerous neighborhoods, play an important role in adult repeat victimization (63). This study did not consider contextual risk factors for interpersonal violence (e.g., SES, education, family relationships etc.), which may provide a more comprehensive understanding of polyvictimization experiences.

Limitations and Future Directions

The present study is based on retrospective reporting, which limits any discussion of causality and directionality. Longitudinal design could be used to further examine the pathways that lead to violence victimization and perpetration. Participation was not anonymous, questions were answered in the presence of a researcher, and we did not measure social desirability, making it difficult to know whether participants in either group were under reporting their experiences. We also did not attempt to substantiate reports with other informants, as we sought to understand and value self-reported experiences. Future research could examine both self- and informant-report to examine how responses may be correlated. It is possible that this sample represents a more well-adjusted and functional group of individuals with ASC, and it is unclear whether these results generalize to those who have greater difficulties, as the link between level of functioning and the violence experiences of those with ASC is not well understood. We also did not employ the ADOS-2 to ensure that the comparison group did not have significant symptoms of ASC, though none reported identifying as on the spectrum or being diagnosed with ASC.

This study has both statistical and psychometric limitations. This study was aimed to describe different kinds of victimization and perpetration, and was the first study to apply the JVQ-AR with an adult focus and with respondents with ASC. Alternative measures of violence that are psychometrically validated could provide different results, and are an important endeavor given the current pattern of reported polyvictimization. Additionally,

our study had a small sample size and relatively low power for low frequency occurring kinds of victimization or perpetration. There multiple exploratory comparisons do increase the risk of Type I error, and we did not correct for this as a result of the exploratory nature of these comparisons and the relatively small, but important, clinical sample. This remains an important first step to inform future investigations. Finally, this sample of participants had proportionally more women than expected in ASC research, and it is likely that this does not reflect the gender distribution in the population. While the two groups were matched on gender, education level, ethnicity status, age, and IQ, we did not collect or match on other demographics which may differ between groups or be associated with victimization (e.g., employment status, poverty).

CONCLUSION

Participants with ASC are at considerable risk for experiencing polyvictimization in childhood and for bullying and sexual contact victimization in adulthood. This increased vulnerability to victimization, especially in childhood, highlights the need for intervention and proactive prevention strategies to decrease vulnerability and impact. These findings have serious implications for how we discuss violence victimization, and suggest that understanding interpersonal violence more broadly is critical to ensuring that we identify and target factors that may place people with ASC at risk for many kinds of negative experience.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Canadian Tri-Council Policy Statement Ethical Conduct for Research Involving Human Participants, and the York University Senate Policy, Research Involving Human Participants. The protocol was approved by the York University Human Participants Review (Ethics) Sub-Committee of the Office of Research Ethics. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

ACKNOWLEDGMENTS

REFERENCES

1. Hamby S, Grych JH. *The Web of Violence Exploring Connections Among Different Forms of Interpersonal Violence and Abuse*. Dordrecht; New York, NY: Springer (2013).

2. American Psychiatric Association (2013). *Diagnostic and Statistical Manual of Mental Disorders, 5th Edn.* Arlington, VA: American Psychiatric Publishing.

3. Centers for Disease Control and Prevention. Prevalence of autism spectrum disorders: autism and developmental disabilities monitoring network, 14 sites, United States, 2008. *MMWR Surveill Summ.* (2012) 61:1–19.

4. Centers for Disease Control and Prevention. Prevalence and characteristics of autism spectrum disorder among children aged 8 Years — autism and developmental disabilities monitoring network, 11 sites, United States, 2012. *Surveill Summ.* (2016) 65:1–23.

5. Brown-Lavoie SM, Viecili MA, Weiss JA. Sexual knowledge and victimization in adults with autism spectrum disorders. *J Autism Dev Disord.* (2014) 44:2185–96. doi: 10.1007/s10803-014-2093-y

6. Cappadocia MC, Weiss JA, Pepler D. Bullying experiences among children and youth with autism spectrum disorders. *J Autism Dev Disord.* (2012) 42:266–77. doi: 10.1007/s10803-011-1241-x

7. Mandell DS, Walrath CM, Manteuffel B, Sgro G, Pinto-Martin JA. The prevalence and correlates of abuse among children with autism served in comprehensive community-based mental health settings. *Child Abuse Neglect* (2005) 29:1359–72. doi: 10.1016/j.chiabu.2005.06.006

8. Sullivan PM, Knutson JF. Maltreatment and disabilities: a population-based epidemiological study. *Child Abuse Neglect* (2000) 24:1257–73. doi: 10.1016/S0145-2134(00)00190-3

9. Carter S. Bullying of students with Asperger syndrome. *Issues Compr Pediatr Nurs.* (2009) 32:145–4. doi: 10.1080/01460860903062782

10. Brown KR, Peña EV, Rankin S. Unwanted sexual contact: students with autism and other disabilities at greater risk. *J Coll Stud Dev.* (2017) 58:771–6. doi: 10.1353/csd.2017.0059

11. Fisher MH, Moskowitz AL, Hodapp RM. Differences in social vulnerability among individuals with autism spectrum disorder, Williams syndrome, and Down syndrome. *Res Autism Spectr. Disord.* (2013). 7:931–7. doi: 10.1016/j.rasd.2013.04.009

12. Jawaid A, Riby DM, Owens J, White SW, Tarar T, Schulz PE. 'Too withdrawn' or 'too friendly': considering social vulnerability in two neurodevelopmental disorders. *J Intellect Disabil Res.* (2012) 56:335–50. doi: 10.1111/j.1365-2788.2011.01452.x

13. Finkelhor D, Ormrod R, Turner H, Holt M. Pathways to poly-victimization. *Child Maltreat.* (2009) 14:316–29. doi: 10.1177/1077559509347012

14. Kazdin, AE. Conceptualizing the challenge of reducing interpersonal violence. *Psychol Viol.* (2011). 1:166–87. doi: 10.1037/a0022990

15. Bjørkly S. Risk and dynamics of violence in Asperger's syndrome a systematic review of the literature. *Aggress Violent Behav.* (2009) 14:306–12. doi: 10.1016/j.avb.2009.04.003

16. Ghaziuddin M, Tsai L, Ghaziuddin N. Brief report: violence in asperger syndrome, a critique. *J Autism Dev Disord.* (1991) 21:349–54. doi: 10.1007/BF02207331

17. King C, Murphy GH. A systematic review of people with autism spectrum disorder and the criminal justice system. *J Autism Dev Disord.* (2014) 44:2717–33. doi: 10.1007/s10803-014-2046-5

18. Sevlever M, Roth ME, Gillis JM. Sexual abuse and offending in Autism Spectrum Disorders. *Sex Disabil.* (2013) 31:189–200. doi: 10.1007/s11195-013-9286-8

19. Barnhill GP. Outcomes in adults with Asperger syndrome. *Focus Autism Other Dev Disabl.* (2007) 22:116–26. doi: 10.1177/10883576070220020301

20. Howlin P. Outcome in adult life for more able individuals with autism or Asperger syndrome. *Autism* (2000) 4:63–83. doi: 10.1177/1362361300004001005

21. Happe FGE. An advanced test of theory of mind: understanding of story characters' thoughts and feelings by able autistic, mentally handicapped, and normal children and adults. *J Autism Dev Disord.* (1994) 24:129–54. doi: 10.1007/BF02172093

22. Jolliffe T, Baron-Cohen S. A test of central coherence theory: linguistic processing in high-functioning adults with autism or Asperger

23. syndrome: is local coherence impaired? *Cognition* (1999) 71:149–85. doi: 10.1016/S0010-0277(99)00022-0

23. Howlin P, Clements J. Is it possible to assess the impact of abuse on children with pervasive developmental disorders? *J Autism Dev Disord.* (1995) 25:337–54. doi: 10.1007/BF02179372

24. Haskins BG, Silva JA. Asperger's disorder and criminal behavior: Forensic-Psychiatric considerations. *J Am Acad Psychiatry Law* (2006) 34:374–84.

25. Murrie DC, Warren JI, Kristiansson M, Dietz PE. Asperger's syndrome in forensic settings. *Int J Forensic Ment Health* (2002) 1:59–70. doi: 10.1080/14999013.2002.10471161

26. Palermo MT. Pervasive developmental disorders, psychiatric comorbidities, and the law. *Int J Offender Ther Comp Criminol.* (2004) 48:40–8. doi: 10.1177/0306624X03257713

27. Church C, Alisanski S, Amanullah S. The social, behavioral and academic experiences of children with Asperger syndrome. *Focus Autism Other Dev Disabl.* (2000) 15:12–20. doi: 10.1177/108835760001500102

28. Stokes M, Newton N, Kaur A. Autistic spectrum disorders and stalking. *Autism* (2004) 8:337–9. doi: 10.1177/1362361304045220

29. Stokes M, Newton N, Kaur A. Stalking, and social and romantic functioning among adolescents and adults with autism spectrum disorder. *J Autism Dev Disord.* (2007) 37:1969–86. doi: 10.1007/s10803-006-0344-2

30. Gratz KL, Paulson A, Jakupcak M, Tull MT. Exploring the relationship between childhood maltreatment and intimate partner abuse: Gender differences in the mediating role of emotion dysregulation. *Violence Vict.* (2009) 24:68–82. doi: 10.1891/0886-6708.24.1.68

31. Mahady-Wilton M, Craig WM, Pepler DJ. Emotional regulation and display in classroom bullying: characteristic expressions of affect, coping styles and relevant contextual factors. *Soc Dev.* (2000) 9:226–45. doi: 10.1111/1467-9507.00121

32. Walsh K, DiLillo D, Messman-Moore T. Lifetime sexual victimization and poor risk perception: does emotion dysregulation account for the links?. *J Interpers Violence* (2012) 27:3054–71. doi: 10.1177/0886260512441081

33. McNulty JK, Hellmuth JC. Emotion regulation and intimate partner violence in newlyweds. *J Family Psychol.* (2008) 22:794–7. doi: 10.1037/a0013516

34. Mazefsky CA, Herrington J, Siegel M, Scarpa A, Maddox BB, Scahill L, et al. The role of emotion regulation in autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry* (2013) 52:679–88. doi: 10.1016/j.jaac.2013.05.006

35. Rieffe C, Camodeca M, Pouw LBC, Lange AMC, Stockmann L. Don't anger me! Bullying, victimization, and emotion dysregulation in young adolescents with ASD. *Eur J Dev Psychol.* (2012) 9:351–70. doi: 10.1080/17405629.2012.680302

36. Hooven C, Nurius PS, Logan-Greene P, Thompson EA. Childhood violence exposure: Cumulative and specific effects on adult mental health. *J Fam Violence* (2012) 27:511–22. doi: 10.1007/s10896-012-9438-0

37. Lord C, Rutter M, DiLavore PC, Risi S, Gotham K, Bishop S. (2012). *Autism Diagnostic Observation Schedule: ADOS-2.* Torrance: Western Psychological Services.

38. Wechsler D. *Wechsler Abbreviated Scale of Intelligence.* San Antonio, TX: Psychological Corporation (1999).

39. Kandalaft MR, Didehbani N, Krawczyk DC, Allen TT, Chapman SB. Virtual reality social cognition training for young adults with high-functioning autism. *J Autism Dev Disord.* (2012) 43:34–44. doi: 10.1007/s10803-012-1544-6

40. Hamby SL, Finkelhor D, Ormrod RK, Turner HA. (2004). *The Juvenile Victimization Questionnaire (JVQ): Administration and Scoring Manual.* Durham, NH: Crimes Against Children Research Center.

41. Finkelhor D, Ormrod R, Turner H, Hamby SL. The victimization of children and youth: a comprehensive, national survey. *Child Maltreat.* (2005) 10:5–25. doi: 10.1177/1077559504271287

42. Gratz KL, Roemer L. Multidimensional assessment of emotion regulation and dysregulation: development, factor structure, and initial validation of the difficulties in emotion regulation scale. *J Psychopathol Behav Assess.* (2004) 26:41–54. doi: 10.1023/B:JOBA.0000007455.08539.94

43. Gratz KL, Gunderson JG. Preliminary data on an acceptance-based emotion regulation group intervention for deliberate self-harm among women with borderline personality disorder. *Behav Ther.* (2006) 37:25–35. doi: 10.1016/j.beth.2005.03.002

44. Tull MT, Barrett HM, McMillan ES, Roemer L. A preliminary investigation of the relationship between emotion regulation difficulties and posttraumatic stress symptoms. *Behav Ther.* (2007) **38**:303–13. doi: 10.1016/j.beth.2006.10.001

45. Swain D, Scarpa A, White S, Laugeson E. Emotion dysregulation and anxiety in adults with ASD: does social motivation play a role? *J Autism Dev Disord.* (2015) **45**:3971–7. doi: 10.1007/s10803-015-2567-6

46. Yager J, Iarocci G. The development of the Multidimensional Social Competence Scale: a standardized measure of social competence in autism spectrum disorders. *Autism Res.* (2013) **6**:631–41. doi: 10.1002/aur.1331

47. Preacher KJ, Hayes AF. SPSS and SAS procedures for estimating indirect effects in simple mediation models. *Behav Res Methods Instrum Comput.* (2004) **36**:717–31. doi: 10.3758/BF03206553

48. Richmond JM, Elliott AN, Pierce TW, Aspelmeier JE, Alexander AA. Polyvictimization, childhood victimization, and psychological distress in college women. *Child Maltreat.* (2009) **14**:1270147. doi: 10.1177/1077559508326357

49. Storch EA, Larson MJ, Ehrenreich-May J, Arnold EB, Jones AM, Renno P, et al. Peer victimization in youth with autism spectrum disorders and co-occurring anxiety: relations with psychopathology and loneliness. *J Dev Phys Disabil.* (2012) **24**:575–90. doi: 10.1007/s10882-012-9290-4

50. Rawana JS, Norwood SJ, Whitley J. A mixed-method evaluation of a strength-based bullying prevention program. *Can J Sch Psychol.* (2011) **26**:283–300. doi: 10.1177/0829573511423741

51. McGrath L, Jones R, Hastings RP. Outcomes of anti-bullying intervention for adults with intellectual disabilities. *Res Dev Disabil.* (2010) **31**:376–80. doi: 10.1016/j.ridd.2009.10.006

52. McEachern AG. Sexual abuse of individuals with disabilities: prevention strategies for clinical practice. *J Child Sex Abus.* (2012) **21**:386–98. doi: 10.1080/10538712.2012.675425

53. Bruder C, Kroese BS. The efficacy of interventions designed to prevent and protect people with intellectual disabilities from sexual abuse: a review of the literature. *J Adult Protect.* (2005) **7**:13–27. doi: 10.1108/14668203200500009

54. Bowman RA, Scotti JR, Morris TL. Sexual abuse prevention: a training program for developmental disabilities service providers. *J Child Sex Abus.* (2010) **19**:119–127. doi: 10.1080/10538711003614718

55. Ghaziuddin M. Violent behavior in autism spectrum disorder: Is it a fact, or fiction? *Curr Psychiatr.* (2013) **12**:23.

56. Mogavero MC. Autism, sexual offending, and the criminal justice system. *J Intell Disab Off Behav.* (2016) **7**:116–126. doi: 10.1108/JIDOB-02-2016-0004

57. Brown J, Cohen P, Johnson JG, Salzinger S. A longitudinal analysis of risk factors for child maltreatment: findings of a 17-year prospective study of officially recorded and self-reported child abuse and neglect. *Child Abuse Negl.* (1998) **22**:1065–78. doi: 10.1016/S0145-2134(98)00087-8

58. Darves-Bornoz JM, Lemperiere T, Degiovanni A, Gaillard P. Sexual victimization in women with schizophrenia and bipolar disorder. *Soc Psychiatry Psychiatr Epidemiol.* (1995) **30**:78–84. doi: 10.1007/BF00794947

59. Moffitt TE, Robins RW, Caspi A. A couples analysis of partner abuse with implications for abuse prevention policy. *Criminol Public Policy* (2001) **1**:5–36. doi: 10.1111/j.1745-9133.2001.tb00075.x

60. Schumacher JA, Feldbau-Kohn S, Smith Slep AM, Heyman RE. Risk factors for male-to-female partner physical abuse. *Aggress Violent Behav.* (2001) **6**:281–352. doi: 10.1016/S1359-1789(00)00027-6

61. Vicary JR, Klingaman LR, Harkness,W. L. Risk factors associated with date rape and sexual assault of adolescent girls. *J Adolesc.* (1995) **18**:289–306. doi: 10.1006/jado.1995.1020

62. Widom CS, White HR. Problem behaviours in abused and neglected children grown up: prevalence and co-occurrence of substance abuse, crime and violence. *Crim Behav Mental Health* (1997) **7**:287–310. doi: 10.1002/cbm.191

63. Outlaw M, Ruback B, Britt C. Repeat and multiple victimizations: the role of individual and contextual factors. *Violence Vict.* (2002) **17**:187–204. doi: 10.1891/vivi.17.2.187.33648

Check for updates

Gastrointestinal and Psychiatric Symptoms Among Children and Adolescents With Autism Spectrum Disorder

Emily Neuhaus [1,2*], Raphael A. Bernier [2,3], See Wan Tham [1,4] and Sara J. Webb [1,3]

[1] Center on Child Health, Behavior, and Development, Seattle Children's Research Institute, Seattle, WA, United States, [2] Autism Center, Seattle Children's Hospital, Seattle, WA, United States, [3] Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA, United States, [4] Seattle Children's Hospital, University of Washington School of Medicine, Seattle, WA, United States

Individuals with autism spectrum disorder (ASD) are at heightened risk of psychiatric comorbidities across the lifespan, including elevated rates of internalizing, externalizing, and self-injurious behaviors. Identification of medical comorbidities that contribute to these concerns may elucidate mechanisms through which psychiatric concerns arise, as well as offer additional avenues for intervention. Gastrointestinal (GI) conditions are of particular interest, as they are prevalent among those with ASD, may share genetic or neurobiological etiologies with the core features of ASD, and are linked with psychiatric difficulties in the general population. In this paper, we draw on data from nearly 2,800 children and adolescents with ASD within the Simons Simplex Collection to characterize the unique contributions of (1) autism symptoms, (2) psychosocial factors (child's age, sex, verbal and nonverbal IQ, adaptive behavior, race, and household income), and (3) GI concerns with respect to multiple psychiatric outcomes. Multiple regression models revealed unique contributions of ASD symptoms and multiple psychosocial factors such as verbal IQ, adaptive behavior, and family income to internalizing, externalizing, and self-injurious behavior. In general, higher levels of psychiatric symptoms were associated with more ASD symptoms, higher verbal IQ, lower adaptive behavior skills, and lower family income. Furthermore, levels of GI symptoms accounted for unique variance in psychiatric outcomes over and above these other factors, linking increased GI problems with increased psychiatric symptoms in children with ASD. Taken together, results indicate that the presence and quantity of GI symptoms should be considered when evaluating psychiatric and behavioral concerns among children with ASD, and that treatment of GI conditions may be an important component in alleviating a broad array of mental health concerns in this group.

Keywords: autism, gastrointestinal, comorbidity, internalizing, externalizing, self-injury

INTRODUCTION

Despite their absence from diagnostic criteria, internalizing and externalizing symptoms are frequent and pervasive among individuals with autism spectrum disorder (ASD). As early as toddlerhood, children later diagnosed with ASD evidence diminished positive affect and heightened negative affect relative to children without ASD (1, 2). During childhood, rates of anxiety, depression, aggression, and self-injurious behaviors are elevated relative to peers without ASD (3–5), and psychiatric symptoms often persist into adulthood (6). Compounding these symptoms, significant internalizing and externalizing difficulties often occur concurrently (7), with long lasting consequences with regard to quality of life, educational and vocational outcomes, and clinical service utilization throughout the lifespan (8).

Along with significant psychiatric symptoms, ASD is often characterized by a number of medical comorbidities, including seizure disorders (9), sleep difficulties (10), metabolic concerns (11), and immune system dysfunction (11). Rates of these concerns exceed not only those observed in the general population (11), but also those observed among individuals with other neurodevelopmental diagnoses such as ADHD (12). Furthermore, reviews of medical records indicate that medical comorbidities may cluster together among individuals with ASD (13), such that experiencing one concern (e.g., gastrointestinal disorder) can indicate a heightened likelihood of additional concerns [e.g., sleep disorders; (10, 14)].

Among medical comorbidities, gastrointestinal symptoms are particularly prominent among individuals with ASD, occurring nearly four times as frequently as comparison groups without ASD (15). Prevalence rates for GI concerns in ASD vary considerably depending on sample characteristics and methodological approach (16). Lower estimates place the frequency of broadly defined GI symptoms around 15–20% [e.g., (17)], whereas other researchers estimate that up to 90% of children with ASD may experience significant GI difficulties (18, 19). Differences in data collection strategies likely account, in part, for this sizeable range, as parent questionnaires appear to yield higher prevalence rates [e.g., (19)] in comparison to direct evaluation by a medical provider [e.g., (17)]. Within the broader construct of GI concerns, specific symptoms reported by parents and other caregivers often include abdominal pain, chronic constipation, frequent vomiting, and gastro-esophageal reflux [see (16) for review]. Similar variability characterizes prevalence rates and relative distributions of specific symptoms, with differing findings as to the most prevalent GI symptom in ASD [e.g., constipation (17), diarrhea (19)]. Regardless of this variability, it is clear that GI difficulties affect an appreciable proportion of individuals with ASD, and such symptoms likely have considerable effects on children's educational participation, family functioning, and quality of life.

The cause(s) of such pervasive GI concerns in ASD are not fully understood, but several pathways are plausible. First, it may be that shared genetic substrates underlie both ASD and GI dysfunction, at least for some individuals with ASD. Increasingly understood to stem from complex genetic bases,

ASD has thus far been associated with familial and *de novo* genetic events across hundreds of genes (20), many of which contribute to GI function as well. For example, mutations and polymorphisms in genes such as CHD8 and MET have been associated with ASD phenotypes with comorbid GI complaints [CHD8, Bernier et al. (21); MET, Campbell et al. (22)]. Downstream, comorbidity between GI concerns and ASD may reflect in part the core sensory-related symptoms often observed among affected individuals (23). For example, many children with ASD experience heightened awareness of their sensory experiences, including tactile and vestibular sensations, likely amplifying subjective experiences of GI discomfort.

The cumulative data to date support the co-occurring relationship between psychiatric and GI concerns in children with ASD. As early as preschool age, children with ASD with significant GI symptoms demonstrate higher levels of internalizing, aggressive, and repetitive behavior, with positive correlations between GI and behavioral symptoms (24). Among older children and adolescents with ASD, increased GI symptoms appear to be associated with general affective problems (25), as well as with more specific psychiatric symptoms such as anxiety (26), depression (27), irritability (26), and self-injurious behavior (28). Thus, links between GI and psychiatric concerns span both internalizing and externalizing spectrums.

To some extent, these findings echo relations observed in the general population, as children without ASD also tend to display increased GI concerns in the context of both externalizing disorders such as ADHD (29) as well as internalizing symptoms such as anxiety and depression (30). However, interactions between psychiatric and GI symptoms are particularly pertinent in ASD, as the core symptoms of ASD appear to affect these associations. Compulsive and repetitive behaviors, key diagnostic criteria for ASD, correlate with GI symptoms among children and adolescents with ASD (31). Similarly, sensory over-responsivity and anxiety provide unique contributions in the prediction of GI concerns among children and adolescents with ASD (32). Finally, core impairments in communication appear to relate to the presence and expression of GI concerns. Not only do poor expressive language skills predict nearly 12-fold increase in risk for particular GI symptoms (33), but Buie et al. (16) suggest that "problem behavior in patients with ASDs may be the primary or sole symptom of the underlying medical condition, including some gastrointestinal disorder" (p. S1).

Our overarching goal in this paper was to explore the role of gastrointestinal concerns as they relate to psychiatric symptoms among children and adolescents with ASD. Within this goal, we pursued two aims. First, we sought to document the prevalence and variety of GI concerns within a large, well-characterized sample of children and adolescents with ASD. Second, we sought to understand relationships between ASD symptoms and GI concerns over and above the effects of psychosocial factors. Given the prevalence and pervasiveness of psychiatric and gastrointestinal comorbidities in ASD, better understanding of the relations between these components may offer additional avenues for intervention for children and adults with ASD, as well as inform our understanding of the multisystemic nature and mechanisms of ASD.

METHOD

Participants

Data for the current analyses were obtained as part of the Simons Simplex Collection [SSC; (34)], a collaboration across 12 research sites within the United States in which families participated in comprehensive and rigorous phenotypic assessment. In total, the SSC includes nearly 3,000 families with exactly one child with ASD between the ages of 4 and 18 years. Following protocols approved by each site's human subjects division, research reliable clinicians evaluated children using the Autism Diagnostic Observation Schedule [ADOS; (35)] with revised algorithm scoring (36), Autism Diagnostic Interview–Revised [ADI-R; (37)], and expert clinical judgment. All participating children and adolescents met diagnostic criteria established by the Collaborative Programs of Excellence in Autism [CPEA; (38)] for autism spectrum disorder. The sample had a mean calibrated severity score of 7.44 ($SD = 1.7$; Range $= 4$–10) according to the guidelines developed by Gotham et al. (39), in which scores can range from 1 to 10 and scores 4 or higher fall above the diagnostic threshold. In addition, children and their parents were carefully screened for a wide range of medical and familial factors. Exclusionary criteria included the following: any family history of suspected or diagnosed ASD; a nonverbal mental age below 18 months; presence of known genetic conditions, neurological disease, or head injury; significant sensory or motor impairment; extensive perinatal complications; gestational age below 36 weeks at birth; birth weight below 2,000 g; and a primary language other than English.

The resulting sample included 2,756 children (13.6% female, 86.4% male) with the following parent-reported racial/ethnic backgrounds: African American (4.0%), Asian (4.0%), Native American or Hawaiian (0.3%), more than one race (7.8%), other than listed (4.5%), and white (78.6%). **Table 1** displays descriptive statistics for the families included in the current analyses.

Measures

Autism Spectrum Disorder Symptoms

The presence and degree of symptoms associated with ASD were assessed using the total score from the age-appropriate ADI-R Current Behavior algorithm, consistent with the procedure described in Neuhaus et al. (40). This approach yields a single total score that summarizes parent-report of both social-communication and restricted/repetitive domains of symptoms at the time of data collection.

Gastrointestinal Concerns

Parents completed extensive interviews regarding the child's medical history, including the presence/absence of 7 distinct GI symptoms. Symptoms were considered to be present if they were recurrent, not attributed to known acute illnesses (e.g., food poisoning), and caused "significant bother" for the family. We computed a summary variable tallying the number of symptoms reported for each child with ASD: constipation, diarrhea, severe abdominal pain, gastro-esophageal reflux, vomiting, excessive gas, and bloating. We included data of reported symptoms that were present beyond the age of 36 months, in order to exclude symptoms (e.g., physiologic reflux) that may be benign during infancy and very early childhood, with expected resolution over time.

Psychiatric Symptoms

We identified three psychiatric symptom clusters of interest. Parent-reported internalizing and externalizing symptoms were measured via the age-appropriate versions of the Child Behavior Checklist [CBCL; (41)]. Internalizing symptoms were indexed with T-scores from the Anxious/Depressed subscale, which contains items relating to worries, fear, sadness, and negative cognitions and self-image, without items related to somatic symptoms to avoid inflating associations with our GI measure. Externalizing symptoms were reflected in the Externalizing Problems broadband T-score, which incorporates items relating to aggression and rule-breaking. In addition to these, the Self-Injurious Behavior subscale of the Repetitive Behavior Scale-Revised [RBS-R; (42)] was used as a measure of self-directed injury (e.g., biting self, hitting self with objects). As noted earlier, symptoms in all of these areas are elevated among individuals with ASD.

Psychosocial Characteristics

A number of child and family characteristics relevant to the emergence of psychiatric symptoms were extracted from the background and clinical information available in the SSC dataset. These included child age, biological sex, verbal and nonverbal IQ scores as assessed with age-appropriate standardized measures (43–45), child's adaptive behavior composite score (46), the family's annual household income (dichotomized as below/above $80,000), and the child's race/ethnicity (sample demographics permitted analysis of African American, Asian, and white backgrounds).

Analytic Approach

In order to understand relations between child/family factors and psychiatric outcomes, we assessed direct contributions of these factors to each of our three psychiatric symptom areas (internalizing, externalizing, self-injurious behavior). Within the two age groups corresponding to ADI-R scoring algorithms (4 through 9 years of age; 10+ years of age), we used SPSS version 19 to create separate three-level multiple regression models predicting each psychiatric measure. Within each model, missing data were handled with pairwise deletion.

- At Level 1 of the models, we entered ASD symptoms to quantify the variance in psychiatric outcomes attributable to core features of ASD.
- At Level 2, we entered psychosocial factors hypothesized to account for additional variance in psychiatric concerns. These consisted of child's age, child's biological sex, child's verbal and nonverbal IQ scores, child's adaptive behavior, family's annual income, and child's race (African American, Asian, or white).
- At Level 3, we entered GI concerns present after the age of 36 months to assess contribution of these concerns over and above ASD symptoms and psychosocial factors.

RESULTS

Prevalence of Parent-Report GI Concerns

Consistent with previous literature, families in the SSC frequently reported that their child with ASD had significant GI symptoms. Over one third of the sample (37.7%) experienced at least one symptom, with a mean of 0.61 ($SD = 0.98$; Range = 0–6) conditions endorsed. In all, 23.7% of participants experienced one GI symptom, 8.3% experienced two symptoms, 3.0% experienced three symptoms, and 2.6% experienced four or more symptoms. **Table 2** provides rates of specific parent-reported GI concerns. Rates of symptoms ranged from 4.2% (vomiting) up to 24.1% (constipation) of the SSC sample. As shown, the most frequently reported symptoms were constipation, diarrhea, and excessive gas.

Factors Associated With Psychiatric Outcomes

Internalizing Symptoms

The sample as a whole had a mean Anxious/Depressed T-score of 58.36 ($SD = 8.9$, Range = 50.0–98.0), with 24.7% of participants' scores falling at or above 65, indicating borderline or clinical level of concern. Within the younger group (ages 4:0 to 9:11), ASD symptoms did not account for significant variance in internalizing symptoms when entered alone [$F_{(1, 1681)} = 0.25$, $p = 0.62$]. The addition of psychosocial factors accounted for an additional 15.7% of the variance in internalizing, a significant increase over ASD symptoms alone [$F_{(9, 1672)} = 35.80$, $p < 0.001$]. GI symptoms accounted for 1.0% of variance over and above

autism symptoms and psychosocial factors [$F_{(1, 1671)} = 17.94$, $p < 0.001$], such that the combined model accounted for 16.5% of the variance in internalizing symptoms among the younger age group [$F_{(11, 1682)} = 31.24$, $p < 0.001$]. Within the combined model, there were unique contributions from ASD symptoms, child age, verbal IQ, adaptive behavior, family income, and GI symptoms. For younger children, higher levels of internalizing symptoms were associated with higher levels of ASD symptoms, older age, higher verbal IQ, lower adaptive behavior, lower family income, and more GI concerns. See **Table 3**.

Among the older children and adolescents (ages 10:0 and older), ASD symptoms entered alone did not account for significant variance [$F_{(1, 907)} = 1.17$, $p = 0.28$]. However, psychosocial factors contributed 14.9% of variance [$F_{(9, 898)} = 17.52$, $p < 0.001$], and GI concerns accounted for an additional 1.1% [$F_{(1, 897)} = 11.45$, $p < 0.001$]. As a whole, the combined model accounted for 15.1% of the variance in internalizing symptoms [$F_{(11, 908)} = 15.67$, $p < 0.001$], with unique contributions from ASD symptoms, verbal IQ, family income, and GI symptoms. Again, higher levels of internalizing symptoms were associated with more ASD symptoms, higher verbal IQ, lower family income, and more GI symptoms.

Externalizing Symptoms

A similar set of findings emerged with respect to externalizing outcomes. The sample as a whole had a mean Externalizing T-score of 56.6 ($SD = 10.6$, Range = 32.0–97.0), with 22.8% of participants' scores falling at or above 65. Within the younger age group, significant variance was accounted for at each level of the regression model, with unique variance accounted for by ASD symptoms [3.8% of variance, $F_{(1, 1681)} = 68.13$, $p < 0.001$], psychosocial factors [6.0% of variance, $F_{(9, 1672)} = 12.34$, $p < 0.001$], and GI concerns [1% of variance, $F_{(1, 1671)} = 16.42$,

TABLE 1 | Demographic and clinical characteristics for participants.

	Mean (SD)	Range
Age (years)	9.03 (3.6)	4–18
ADI-R Current Behavior total score	27.47 (10.1)	1–60
ADOS Calibrated Severity Score	7.44 (1.7)	4–10
Verbal IQ standard score	78.04 (31.3)	5–167
Nonverbal IQ standard score	84.52 (26.2)	9–161
Vineland-2 Composite standard score	73.13 (12.1)	27–115
CBCL Anxious/Depressed T-Score	58.36 (8.9)	50–98
CBCL Externalizing T-Score	56.58 (10.6)	32–97
RBS-R Self Injurious	2.09 (2.9)	0–21

TABLE 2 | Prevalence of parent-reported GI concerns.

	Endorsed beyond 36 months of age
Bloating	4.6%
Constipation	24.1%
Diarrhea	10.6%
Excessive Gas	6.9%
Reflux	5.5%
Severe Abdominal Pain	5.1%
Vomiting	4.2%

TABLE 3 | Unique contributions of child and family factors to internalizing symptoms.

	Younger age group (4 years < 10 years)		Older age group (10 yrs < 18 years)	
	β	t	β	t
ASD symptoms	0.11	4.24***	0.13	3.68***
Age	0.24	10.71***	−0.01	−0.18
Child sex	−0.04	−1.57	0.04	1.38
Verbal IQ	0.43	10.06***	0.41	6.64***
Nonverbal IQ	−0.06	−1.55	−0.01	−0.21
Adaptive behavior	−0.09	−2.57*	−0.03	−0.68
Household income	−0.09	−4.07***	−0.07	−2.26**
RACE/ETHNICITY				
African American	−0.03	−1.04	−0.04	−1.26
Asian	−0.02	−0.77	−0.05	−1.47
White	−0.04	−1.61	0.05	1.26
GI symptoms	0.10	4.24***	0.10	3.83***

*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$. Internalizing symptoms assessed via Child Behavior Checklist Anxious/Depressed subscale T-scores.

$p < 0.001$]. In total, the combined model accounted for 10.2% of the variance in externalizing symptoms [$F_{(11, 1682)} = 18.31$, $p < 0.001$]. As shown in **Table 4**, there were unique contributions from ASD symptoms, verbal IQ, adaptive behavior, family income, and GI symptoms. Externalizing behavior was higher when children had higher levels of ASD symptoms, higher verbal IQ, lower adaptive behavior, lower income, and more GI concerns.

For older children and adolescents, there were again significant contributions from each level of the model [ASD symptoms: 5.3% of variance, $F_{(1, 908)} = 52.35$, $p < 0.001$; Psychosocial factors: 9.7% of variance, $F_{(9, 899)} = 11.44$, $p < 0.001$; GI symptoms: 1.4% of variance, $F_{(1, 898)} = 14.64$, $p < 0.001$], and the combined model accounted for 15.5% of the variance in externalizing symptoms [$F_{(11, 909)} = 16.16$, $p < 0.001$]. However, the relative contributions of individual predictors within the combined model were somewhat different, with significant contributions from ASD symptoms, child's age, verbal IQ, nonverbal IQ, adaptive behavior, family income, child's ethnicity, and GI symptoms. Externalizing behavior was higher when children were younger, and had higher levels of ASD symptoms, higher verbal IQ, lower nonverbal IQ, lower adaptive behavior, lower income, parent-reported white ethnicity, and more GI concerns.

Self-Injurious Behavior

Among the younger children in the sample, the regression model predicting self-injurious behaviors revealed significant unique variance associated with each level of the model, including ASD symptoms alone [4.6%, $F_{(1, 1681)} = 82.89$, $p < 0.001$], psychosocial factors [4.4%, $F_{(9, 1672)} = 9.02$, $p < 0.001$], and GI concerns [1.1%, $F_{(1, 1671)} = 21.31$, $p < 0.001$]. In total, the combined model accounted for 9.7% of the variance in self-injurious behaviors [$F_{(11, 1682)} = 17.36$, $p < 0.001$]. Unique contributions were associated with ASD symptoms, adaptive behavior, family income, and GI concerns. Self-injurious behaviors were higher for children with more ASD symptoms, lower adaptive behavior, lower income, and more GI concerns. See **Table 5**.

Finally, for the older children and adolescents, the regression model predicting self-injurious behavior revealed a more distinctive set of findings. As before, ASD symptoms and psychosocial factors each accounted for unique variance [ASD: 6.3%, $F_{(1, 909)} = 60.73$, $p < 0.001$; psychosocial: 3.6%, $F_{(9, 900)} = 3.94$, $p < 0.001$]. However, the addition of GI symptoms at the third level of the model did not account for significant variance over and above these [$F_{(1, 899)} = 1.94$, $p = 0.16$]. Taken together, the combined model accounted for 8.9% of the variance in self-injurious behavior [$F_{(11, 910)} = 9.09$, $p < 0.001$], with significant contributions from ASD symptoms, nonverbal IQ, family income, and child race. Older children and adolescents displayed higher levels of self-injurious behavior when they had higher levels of ASD symptoms, lower nonverbal IQ, lower income, and did not identify as African American. Unlike the models described to this point, the presence of GI concerns did not appear to contribute to level of self-injurious behaviors by parent report.

TABLE 4 | Unique contributions of child and family factors to externalizing symptoms.

	Younger age group (4 years < 10 years)		Older age group (10 years < 18 years)	
	β	t	β	t
ASD symptoms	0.12	4.55***	0.20	5.54***
Age	0.02	−0.98	−0.17	−5.37***
Child sex	0.00	0.14	0.06	1.95
Verbal IQ	0.32	7.19***	0.45	7.30***
Nonverbal IQ	−0.07	−1.55	−0.22	−3.59***
Adaptive behavior	−0.28	−7.46***	−0.18	−3.56***
Household income	−0.11	−4.65***	−0.11	−3.51***
RACE/ETHNICITY				
African American	−0.01	−0.56	−0.03	−0.70
Asian	−0.04	−1.37	0.02	0.50
White	−0.03	−0.92	0.10	2.62**
GI symptoms	0.09	4.05***	0.12	3.83***

$^{**}p < 0.01$. $^{***}p < 0.001$. Externalizing symptoms assessed via Child Behavior Checklist Externalizing T-scores.

TABLE 5 | Unique contributions of child and family factors to self-injurious symptoms.

	Younger age group (4 years < 10 years)		Older age group (10 years < 18 years)	
	β	t	β	t
ASD symptoms	0.13	4.59***	0.17	4.65***
Age	0.04	1.52	−0.04	−1.20
Child sex	0.00	0.00	0.06	1.82
Verbal IQ	0.03	0.66	0.09	1.42
Nonverbal IQ	0.00	0.08	−0.16	−2.54*
Adaptive behavior	−0.18	−4.75***	−0.09	−1.66
Household income	−0.12	−5.20***	−0.07	−2.18*
RACE/ETHNICITY				
African American	−0.01	−0.30	−0.08	−2.15*
Asian	−0.04	−1.69	−0.03	−0.80
White	−0.02	−0.84	−0.03	−0.71
GI symptoms	0.11	4.62***	0.04	1.39

$^{*}p < 0.05$. $^{***}p < 0.001$. Self-injurious behavior assessed via the Self-Injurious Behavior subscale of the Repetitive Behavior Scale- Revised.

DISCUSSION

Our findings underscore the intertwined roles of psychiatric and gastrointestinal symptoms among children and adolescents with ASD. Although individuals with ASD are at elevated risk for both psychiatric and medical comorbidities across the lifespan (3, 5, 47), these constructs have not been fully investigated in tandem despite increasing recognition of ASD as a disorder with implications across neurobiological symptoms. In this paper, we found evidence of unique variance associated with GI symptoms across all three measures of psychiatric symptoms

we examined, including internalizing, externalizing, and self-injurious behaviors. In all but one of the regression models described above, the inclusion of parent-reported GI symptoms significantly increased the statistical variance explained in psychiatric outcomes, over and above the contributions of psychosocial variables.

Beyond GI concerns, our analyses also identified a number of other child and family factors that corresponded to increased psychiatric symptoms in this sample. For both internalizing and externalizing symptoms, we found increased psychiatric difficulties when children had more ASD symptoms, higher verbal IQ scores, lower adaptive behavior skills, and lower household income. These findings fit with previous literature linking increased internalizing concerns with stronger cognitive skills (48, 49) and lower family income (50) in ASD. Patterns of findings were more distinct for older participants (age 10 years and older) with regard to externalizing and self-injurious behaviors. For both of these outcomes in our older participants, nonverbal IQ and race/ethnicity emerged as significant predictors. Specifically, higher levels of externalizing and self-injurious behavior were both associated with having a lower nonverbal IQ. Higher levels of externalizing were associated with being younger and being of white race/ethnicity, whereas lower levels of self-injurious behavior were associated with African American race/ethnicity. This overall picture—in which similar predictors hold for younger children across psychiatric outcomes while differences emerge for older children across psychiatric outcomes—might suggest a developmental trend in which psychiatric difficulties earlier in life are related to a common set of factors, whereas contributing factors begin to diverge and demonstrate more specificity in their links to outcomes as children move toward adolescence.

With regard to the prevalence of GI symptoms among individuals with ASD, our findings suggest marked GI symptoms among approximately one third of children and adolescents in this sample. As discussed earlier, estimates of prevalence span a wide range among published studies on GI function in ASD, likely due to methodological differences between them (16). Higher estimates of GI concerns may be associated with data collection approaches such as questionnaire measures and medical record review, whereas medical history data in the Simons Simplex Collection result from a standardized parent interview. In addition, participants in the SSC met strict and tightly defined diagnostic criteria for ASD, and this procedure may have excluded some children who would have been included in community-based clinic samples. As we discuss in more detail later, the SSC is unique in its recruitment purely of simplex families, and this feature likely also affects the phenotype described here.

Taken together, our findings have implications for assessment and intervention across disciplines. With regard to mental health providers, our findings indicate a need to assess for GI symptoms even when those are not the presenting complaint, as they may serve to contribute to the behavioral concerns for which a family is seeking services. Although GI symptoms accounted for relatively small proportions of variance in psychiatric symptoms in our analyses, they were nonetheless significantly associated with mental/behavioral health. Moreover, effects of GI symptoms were not limited to a single psychiatric symptom area but rather applied across three different measures. As such, despite the limited variance in some models, appropriate treatment of GI symptoms may be an important part of reducing a broad array of mental health symptomatology in this population, and may be critical in improving quality of life and overall functioning. Such recommendations are bolstered by observations that the presence of significant GI conditions among children with ASD may moderate response to psychopharmacological treatment for behavior problems (51).

Conversely, with respect to medical providers, our findings suggest that families seeking treatment for GI symptoms may benefit from a comprehensive assessment of possible "downstream" behavioral or psychological effects. Use of brief, standardized, broad-based questionnaire measures such as those included in the analyses presented here [e.g., Child Behavior Checklist; (41)] can provide medical providers with a broad overview of a child's well-being, and can assist in exploration as to whether medical treatment should be augmented with behavioral intervention (e.g., parent support, psychotherapy) to address effects in those domains.

Limitations of the Current Study

As always, conclusions from the current findings should be considered within the context of the sample and measures with which they were observed. By design, the Simons Simplex Collection comprises children and adolescents with ASD with minimal familial, perinatal, or historical risk factors for autism, with the goal of enriching possible *de novo* genetic contributions to ASD (34). Given this approach, it may be that our results are most applicable to individuals with ASD who have similar familial and genetic backgrounds (i.e., simplex status), and may be less applicable to individuals with ASD in the context of positive family history for ASD, significant perinatal complications, or other identified ASD risk factors. While many occurrences of ASD do appear to be spontaneous or idiopathic in nature (34), the current approach does leave a proportion of individuals with ASD for whom the SSC may not be fully representative and for whom prevalence of GI concerns and relations between GI and psychiatric symptoms may be different than observed here.

Similarly, given the nature and goals of the SSC sample and procedures, we cannot speak to the generalizability of our findings to a broader population of individuals without ASD, such as those with other neurodevelopmental or psychiatric diagnoses. For instance, individuals with intellectual disabilities may also experience heightened prevalence of psychiatric comorbidities and gastrointestinal concerns when compared to the general population (52), but our results cannot clarify whether links between those psychiatric and GI symptoms parallel those observed in our findings. The same is true with regard to links between psychiatric and GI symptoms for children in the general population, for whom psychosocial and environmental factors are associated with GI symptoms [e.g., (53)], as our analyses do not include data from typically-developing children and adolescents.

With regard to measurement, the measures used in the current study carry both advantages and limitations. Our measures of GI (parent-reported symptoms present after the age of 36 months) and psychiatric symptoms (standardized parent-report questionnaires) rely upon parent report. A medical evaluation based on standard diagnostic criteria (ROME IV; DSM 5) and comprehensive testing (e.g., imaging, GI studies, direct assessment) may determine the etiology of symptoms, and extend our understanding of potential mechanisms underlying the gastroenterology-psychiatry relationship. Our approach also cannot clarify whether the GI concerns assessed are reported by caregivers with equal reliability, as some may be more apparent to parents (e.g., vomiting) while others (e.g., nausea, pain) may yield fewer observable signs and rely more on children's ability to understand and communicate their internal state. In addition, the nature of our GI variable does not include evaluation of the severity and impact on quality of life. Severity, rather than quantity, of GI symptoms may have greater impact on psychiatric well-being, and symptom severity may show stronger associations between measures of GI and psychiatric health. For example, a child with severe abdominal pain may experience greater negative impact, with reduced participation in physical activities and increased school absenteeism, compared to a child with multiple but less severe GI symptoms who may continue in their normative roles. The summary variable in the current study cannot capture the severity of GI concerns, and such questions will be important aspects for future work. Finally, as GI symptoms are aggregated into a composite score, we cannot identify whether and how specific GI concerns have more fine-grained or unique associations with particular psychiatric symptoms.

Despite these considerations, this approach likely mirrors typical clinical situations encountered by medical and mental health providers, in which families present for medical or psychiatric issues and clinicians must rely upon parent report or questionnaire methods to gain information, with limited access to prior diagnostic evaluations and/or referrals to specialty evaluation. It is also promising to note that parents' reports of children's GI symptoms tend to be relatively strong indicators of true GI conditions (33), suggesting that the GI variables included in the SSC dataset stand as a reasonable proxy for these purposes.

Future Directions

Moving forward, conclusions from our analyses and others [e.g., (24, 25, 27)] regarding the links between psychiatric and GI comorbidities among individuals with ASD would be strengthened by use of longitudinal data that could inform questions of causality and the direction of effects between symptoms. Our findings are consistent with interpretations positing a causal influence such that the presence of GI symptoms are associated with increased likelihood of mental health symptoms, either as a result of ongoing physical discomfort and/or as a behavioral expression of that discomfort. However, while these interpretations are both plausible and consistent with previous thinking [e.g., (16)], the current data cannot confirm that direction of effect due to the cross-sectional nature of the SSC dataset.

Future research should also explore how comorbidities between ASD, behavioral, and medical (including GI) concerns relate to the biological mechanisms and genetic substrates of ASD. A number of candidate genes in which *de novo* changes can increase likelihood of ASD also appear to carry effects on GI function, both in people with ASD and in animal models of analogous genetic changes. For example, protein truncating mutations to *CHD8*, a chromatin remodeling gene expressed both in the central and enteric nervous systems, are among the most common disruptive mutations identified in ASD through exome sequencing (21). What is more, these *de novo* changes also correspond to significant GI dysfunction (primarily constipation) for 83% of affected individuals with ASD, and to disrupted gut motility in zebrafish models of those same *de novo* changes (21). Delineation of mechanistic pathways through which genetic events result in an ASD phenotype will be critical, and better understanding of intertwined medical and psychiatric comorbidities may suggest candidate systems in those pathways as well as identify additional systems influencing phenotype (e.g., regulation of serotonin, implicated in both gut and brain function).

Together, findings presented here reinforce the need for conceptualizing ASD as a diagnosis affecting multiple neurobiological systems. With this perspective comes the potential of (1) identifying comprehensive research questions to clarify ASD etiology and mechanism, (2) exploring meaningful subgroups within the larger ASD diagnosis to guide that research, and (3) offering multiple points of intervention through which to support affected individuals and their families. In light of the current findings, ties between psychiatric and gastrointestinal comorbidities may be particularly well-suited for these purposes and will continue to be an important avenue for investigation.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the University of Washington Human Subjects Committee with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the University of Washington Human Subjects Committee.

AUTHOR CONTRIBUTIONS

The authors made substantial contributions to the data collection (RB), conception and design (EN, SW, and ST), and analysis (EN). All authors participated in interpretation of the data and manuscript drafting, revising, and approval.

FUNDING

ACKNOWLEDGMENTS

REFERENCES

1. Filliter JH, Longard J, Lawrence MA, Zwaigenbaum L, Brian J, Garon N, et al. Positive affect in infant siblings of children diagnosed with autism spectrum disorder. *J Abnorm Child Psychol.* (2015) 43:567–75. doi: 10.1007/s10802-014-9921-6

2. Garon N, Bryson SE, Zwaigenbaum L, Smith IM, Brian J, Roberts W, et al. Temperament and its relationship to autistic symptoms in a high-risk infant sib cohort. *J Abnorm Child Psychol.* (2009) 37:59–78. doi: 10.1007/s10802-008-9258-0

3. Muris P, Steerneman P, Merckelbach H, Holdrinet I, Meesters C. Comorbid anxiety symptoms in children with pervasive developmental disorders. *J Anxiety Disord.* (1998) 12:387–93. doi: 10.1016/S0887-6185(98)00022-X

4. Neuhaus E, Bernier RA, Beauchaine TP. Social skills, internalizing and externalizing symptoms, and respiratory sinus arrhythmia in autism. *J Autism Dev Disord.* (2014) 44:730–7. doi: 10.1007/s10803-013-1923-7

5. Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G. Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. *J Am Acad Child Adolesc Psychiatr.* (2008) 47:921–9. doi: 10.1097/CHI.0b013e318179964f

6. Lever AG, Geurts HM. Psychiatric co-occurring symptoms and disorders in young, middle-aged, and older adults with autism spectrum disorder. *J Autism Dev Disord.* (2016) 46:1916–30. doi: 10.1007/s10803-016-2722-8

7. Vaillancourt T, Haltigan JD, Smith I, Zwaigenbaum L, Szatmari P, Fombonne E, et al. Joint trajectories of internalizing and externalizing problems in preschool children with autism spectrum disorder. *Dev Psychopathol.* (2017) 29:203–14. doi: 10.1017/S0954579416000043

8. Ballaban-Gil K, Rapin I, Tuchman R, Shinnar S. Longitudinal examination of the behavioral, language, and social changes in a population of adolescents and young adults with autistic disorder. *Pediatr Neurol.* (1996) 15:217–23. doi: 10.1016/S0887-8994(96)00219-6

9. Minshew NJ, Sweeney, JA, Bauman ML. Neurologic aspects of autism. In: Cohen DJ and Volkmar FR, editors. *Handbook of Autism and Pervasive Developmental Disorders.* 2nd Edn. New York, NY: Wiley (1997). p. 344–369.

10. McCue LM, Flick LH, Twyman KA, Xian H. Gastrointestinal dysfunctions as a risk factor for sleep disorders in children with idiopathic autism spectrum disorder: a retrospective cohort study. *Autism* (2017) 21:1010–20. doi: 10.1177/1362361316667061

11. Croen LA, Zerbo O, Qian Y, Massolo ML, Rich S, Sidney S, et al. The health status of adults on the autism spectrum. *Autism* (2015) 19:814–23. doi: 10.1177/1362361315577517

12. Davignon MN, Qian Y, Massolo M, Croen LA. Psychiatric and medical conditions in transition-aged indivduals with ASD. *Pediatrics* (2018) 141:S335–45. doi: 10.1542/peds.2016-4300K

13. Doshi-Velez F, Ge Y, Kohane I. Comorbidity clusters in autism spectrum disorders: an electronic health record time-series analysis. *Pediatrics* (2014) 133:e54–63. doi: 10.1542/peds.2013-0819

14. Aldinger KA, Lane CJ, Veenstra-VanderWeele J, Levitt P. Patterns of risk for multiple co-occurring medical conditions replicate across distinct cohorts of children with autism spectrum disorder. *Autism Res.* (2015) 8:771–81. doi: 10.1002/aur.1492

15. McElhanon BO, McCracken C, Karpen S, Sharp WG. Gastrointestinal symptoms in autism spectrum disorder: a meta-analysis. *Pediatrics* (2014) 133:872–83. doi: 10.1542/peds.2013-3995

16. Buie T, Campbell DB, Fuchs GJ, Furuta GT, Levy J, VandeWater J, et al. Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. *Pediatrics* (2010) 125:S1–18. doi: 10.1542/peds.2009-1878C

17. Fombonne E, Chakrabarti S. No evidence for a new variant of measles-mumps-rubella-induced autism. *Pediatrics* (2001) 108:e58. doi: 10.1542/peds.108.4.e58

18. Horvath K, Perman JA. Autism and gastrointestinal symptoms. *Curr Gastroenterol Rep.* (2002) 4:251–8. doi: 10.1007/s11894-002-0071-6

19. Parracho HMRT, Bingham MO, Gibson GR, McCartney AL. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *Indian J Med Microbiol.* (2005) 54:987–91. doi: 10.1099/jmm.0.46101-0

20. Ronemus M, Iossifov I, Levy D, Wigler M. The role of de novo mutations in the genetics of autism spectrum disorders. *Nat Rev Genet.* (2014) 15:133–41. doi: 10.1038/nrg3585

21. Bernier R, Golzio C, Xiong B, Stessman HA, Coe BP, Penn O, et al. Disruptive CHD8 mutations define a subtype of autism early in development. *Cell* (2014) 158:263–76. doi: 10.1016/j.cell.2014.06.017

22. Campbell DB, Buie TM, Winter H, Bauman M, Sutcliffe JS, Perrin JM, et al. Distinct genetic risk based on association of MET in families with co-occurring autism and gastrointestinal conditions. *Pediatrics* (2009)123:1018–24. doi: 10.1542/peds.2008-0819

23. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders.* 5th Edn. Arlington, VA: American Psychiatric Association (2013).

24. Prosperi M, Santocchi E, Balboni G, Narzisi A, Bozza M, Fulceri F, et al. Behavioral phenotype of ASD preschoolers with gastrointestinal symptoms or food selectivity. *J. Autism Dev Disord.* (2017) 47:3574–88. doi: 10.1007/s10803-017-3271-5

25. Mazefsky CA, Schreiber DR, Olino TM, Minshew NJ. The association between emotional and behavioral problems and gastrointestinal symptoms among children with high-functioning autism. *Autism* (2014) 18:493–501. doi: 10.1177/1362361313485164

26. Ferguson BJ, Marler S, Altstein LL, Lee EB, Akers J, Sohl K, et al. Psychophysiological associations with gastrointestinal symptomatology in autism spectrum disorder. *Autism Res.* (2017). 10:276–88. doi: 10.1002/aur.1646

27. Greenlee JL, Mosley AS, Shui AM, Veenstra-VanderWeele J, Gotham KO. Medical and behavioral correlates of depression history in children and adolescents with autism spectrum disorder. *Pediatrics* (2016) 137:S105–14. doi: 10.1542/peds.2015-2851I

28. Soke GN, Rosenberg SA, Rosenberg CR, Vasa RA, Lee LC, DiGuiseppi C. Self-injurious behaviors in children with autism spectrum disorder enrolled in the study to explore early development. *Autism* (2018) 22:625–35. doi: 10.1177/1362361316689330

29. Jameson ND, Sheppard BK, Lateef TM, Vande Voort JL, He JP, Merikangas KR. Medical comorbidity of attention-deficit/hyperactivity disorder in US adolescents. *J Child Neurol.* (2016) 31:1282–9. doi: 10.1177/0883073816653782

30. von Gontard A, Moritz AM, Thome-Granz S, Equit M. Abdominal pain symptoms are associated with anxiety and depression in young children. *Acta Paediatrica* (2015) 104:1156–63. doi: 10.1111/apa.13134

31. Peters B, Williams KC, Gorrindo P, Rosenberg D, Lee EB, Levitt P, et al. Rigid-compulsive behaviors are associated with mixed bowel symptoms in autism spectrum disorder. *J Autism Dev Disord.* (2014) 44:1425–32. doi: 10.1007/s10803-013-2009-2

32. Mazurek MO, Vasa RA, Kalb LG, Kanne SM, Rosenberg D, Keefer A, et al. Anxiety, sensory over-responsivity, and gastrointestinal problems in children

with autism spectrum disorder. *J Abnorm Child Psychol.* (2013) 41:165–76. doi: 10.1007/s10802-012-9668-x

33. Gorrindo P, Williams KC, Lee EB, Walker LS, McGrew SG, Levitt P. Gastrointestinal dysfunction in autism: parental report, clinical evaluation, and associated factors. *Autism Res.* (2012) 5:101–8. doi: 10.1002/aur.237

34. Fischbach GD, Lord C. The Simons simplex collection: a resource for identification of autism genetic risk factors. *Neuron* (2010) 68:192–5. doi: 10.1016/j.neuron.2010.10.006

35. Lord C, Rutter M, DiLavore PC, Risi S. *Autism Diagnostic Observation Schedule Manual*. Los Angeles, CA: Western Psychological Services (2003).

36. Gotham K, Risi S, Pickles A, Lord C. The autism diagnostic observation schedule: revised algorithms for improved diagnostic validity. *J Autism Dev Disord.* (2007) 37:613–27. doi: 10.1007/s10803-006-0280-1

37. Lord C, Rutter M, Le Couteur A. Autism diagnostic interview - revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord.* (1994) 24:659–85. doi: 10.1007/BF02172145

38. Lainhart JE, Bigler ED, Bocian M, Coon H, Dinh E, Dawson G, et al. Head circumference and height in autism: a study by the collaborative program of excellence in autism. *Am J Med Genet A.* (2006) 140A:2257–74. doi: 10.1002/ajmg.a.31465

39. Gotham K, Pickles A, Lord C. Standardizing ADOS scores for a measure of severity in autism spectrum disorders. *J Autism Dev Disord.* (2009) 39:693–705. doi: 10.1007/s10803-008-0674-3

40. Neuhaus E, Beauchaine TP, Bernier RA, Webb SJ. Child and family characteristics moderate agreement between caregiver and clinician reports of autism symptoms. *Autism Res.* (2018) 11:476–87. doi: 10.1002/aur.1907

41. Achenbach TM. *Manual for the Child Behavior Checklist/4-18 and 1991 Profile*. Burlington, VT: University of Vermont Department of Psychiatry (1991).

42. Bodfish JW, Symons FJ, Parker DE, Lewis MH. Varieties of repetitive behavior in autism: comparisons to mental retardation. *J Autism Dev Disord.* (2000) 30:237–43. doi: 10.1023/A:1005596502855

43. Mullen EM. *Mullen Scales of Early Learning*. Los Angeles, CA: Western Psychological Services. (1997).

44. Wechsler D. *Wechsler Abbreviated Scale of Intelligence*. San Antonio, TX: The Psychological Corporation (1999).

45. Elliott CD. *Differential Ability Scales: Introductory and Technical Handbook*. 2nd Edn. San Antonio, TX: The Psychological Corporation (2007).

46. Sparrow S, Cicchetti D, Balla D. *Vineland Adaptive Behavior Scales*. 2nd ed. Minneapolis, MN: Pearson Assessments (2005).

47. Bauman ML. Medical comorbidities in autism: challenges to diagnosis and treatment. *Neurotherapeutics* (2010) 7:320–7. doi: 10.1016/j.nurt.2010.06.001

48. Gotham K, Brunwasser SM, Lord C. Depressive and anxiety symptom trajectories from school-age through young adulthood in samples with autism spectrum disorder and developmental delay. *J Am Acad Child Adolesc Psychiatr.* (2017) 54:369–76. doi: 10.1016/j.jaac.2015.02.005

49. van Steensel FJA, Heeman EJ. Anxiety levels in children with autism spectrum disorder: a meta-analysis. *J Child Family Stud.* (2017) 26:1753–67. doi: 10.1007/s10826-017-0687-7

50. Taylor JL, Seltzer MM. Changes in the autism behavioral phenotype during the transition to adulthood. *J Autism Dev Dis.* (2010) 40:1431–46. doi: 10.1007/s10803-010-1005-z

51. Nikolov RN, Bearss KE, Lettinga J, Erickson C, Rodowski M, Aman MG, et al. Gastrointestinal symptoms in a sample of children with pervasive developmental disorders. *J Autism Dev Disord.* (2009) 39:405–13. doi: 10.1007/s10803-008-0637-8

52. Charlot L, Abend S, Ravin P, Mastis K, Hunt A, Deutsch C. Non-psychiatric health problems among psychiatric inpatients with intellectual disabilities. *J Intellect Disabil Res.* (2011) 55:199–209. doi: 10.1111/j.1365-2788.2010.01294.x

53. Koppen IJN, Vriesman MH, Saps M, Rajindrajith S, Shi X, van Etten-Jamaludin FS, et al. Prevalence of functional defecation disorders in children: a systematic review and meta-analysis. *J Pediatr.* (2018) 198:121–30. doi: 10.1016/j.jpeds.2018.02.029

Inferior Frontal Gyrus Volume Loss Distinguishes Between Autism and (Comorbid) Attention-Deficit/Hyperactivity Disorder—A FreeSurfer Analysis in Children

Correspondence:
Kathrin Nickel
kathrin.nickel@uniklinik-freiburg.de

[†] *These authors share senior*
authorship

Kathrin Nickel[1]*, Ludger Tebartz van Elst[1], Jacek Manko[2], Josef Unterrainer[3], Reinhold Rauh[2], Christoph Klein[2], Dominique Endres[1], Christoph P. Kaller[4], Irina Mader[5], Andreas Riedel[1], Monica Biscaldi[2†] and Simon Maier[1†]

[1] *Department of Psychiatry and Psychotherapy, Faculty of Medicine, Medical Center – University of Freiburg, Freiburg, Germany,* [2] *Department of Child and Adolescent Psychiatry, Psychotherapy and Psychosomatics, Faculty of Medicine, Medical Center – University of Freiburg, Freiburg, Germany,* [3] *Medical Psychology and Medical Sociology, Faculty of Medicine, University of Freiburg, Freiburg, Germany,* [4] *Department of Neurology, Faculty of Medicine, Medical Center – University of Freiburg, Freiburg, Germany,* [5] *Department of Neuroradiology, Faculty of Medicine, Medical Center – University of Freiburg, Freiburg, Germany*

Objective: Autism spectrum (ASD) and attention-deficit/hyperactivity disorder (ADHD) are neurodevelopmental disorders with a high rate of comorbidity. To date, diagnosis is based on clinical presentation and distinct reliable biomarkers have been identified neither for ASD nor ADHD. Most previous neuroimaging studies investigated ASD and ADHD separately.

Method: To address the question of structural brain differences between ASD and ADHD, we performed FreeSurfer analysis in a sample of children with ADHD ($n = 30$), with high-functioning ASD ($n = 14$), with comorbid high-functioning ASD and ADHD ($n = 15$), and of typically developed controls (TD; $n = 36$). With FreeSurfer, an automated brain imaging processing and analyzing suite, we reconstructed the cerebral cortex and calculated gray matter volumes as well as cortical surface parameters in terms of cortical thickness and mean curvature.

Results: A significant main effect of the factor ADHD was detected for the left inferior frontal gyrus (Pars orbitalis) volume, with the ADHD group exhibiting smaller Pars orbitalis volumes. Dimensional measures of autism (SRS total raw score) and ADHD (DISYPS-II FBB-ADHD score) had no significant influence on the left Pars orbitalis volume. Both, ASD and ADHD tended to have an effect on cortical thickness or mean curvature, which did not survive correction for multiple comparisons.

Conclusion: Our results underline that ADHD rather than ASD is associated with volume loss in the left inferior frontal gyrus (Pars orbitalis). This area might play a relevant role in modulating symptoms of inattention and/or impulsivity in ADHD. The effect of comorbid ADHD in ASD samples and vice versa, on cortical thickness and mean curvature, requires further investigation in larger samples.

Keywords: autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), FreeSurfer, cortical thickness, mean curvature

INTRODUCTION

Previous studies mainly investigated autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD) in isolation. The question to what extent there is a clinical overlap between ADHD and ASD, whether they represent distinct diagnostic categories or form a continuous disorder with a continuum of brain dysfunction remains open to discussion (1, 2). To identify endophenotypes across diagnostic categories, it is important to further investigate the overlap between ADHD and ASD on a behavioral, neurocognitive, and neurobiological level (3, 4).

Co-occurrence of ASD and ADHD

ASD is characterized by impairment in social communication and interaction as well as by a range of stereotypic behaviors whereas deficits in attention, hyperactivity, impulsiveness, disorganization and affective instability represent the core symptoms of ADHD (5). As ASD is a frequent comorbid condition in ADHD and vice versa (1), there is the possibility of dual diagnosis according to DSM-5 (5). The interpretation of studies prior to 2013 is limited by DSM-IV guidelines not permitting the dual diagnosis of ASD and ADHD (6).

Studies report frequent co-occurrence with 30-50% of individuals with ASD manifesting ADHD symptoms (particularly at pre-school age) and two-thirds of individuals with ADHD showing features of ASD (7). Social difficulties in ADHD are often interpreted as part of ADHD symptoms rather than reflecting impairments in social communication being characteristic for ASD (8). Both disorders are highly heritable (9).

Previous Volumetric Studies in ASD and ADHD

Previous structural neuroimaging studies encompassing children and adolescents with both, ADHD and ASD, are scarce and show heterogeneous results (**Table 1**). There is only one previous FreeSurfer study on ASD children with and without comorbid ADHD, but without a separate ADHD group (12). Mahajan et al. (12) found that gray matter (GM) volume and surface area (SA) were increased in the left postcentral and the right precentral gyrus which in this study was specific for ASD children without ADHD, whereas an increase in the left precentral gyrus was specific for children with ASD and comorbid ADHD. Regardless

of ADHD comorbidity, all children with ASD showed increases in GM volume and SA in the left inferior parietal cortex (12).

Voxel based morphometry (VBM) studies display a heterogeneous picture. The most recent VBM study suggested GM reduction in the right posterior cerebellum to be disorder-specific for ADHD relative to ASD. GM enlargement in the middle/superior temporal gyrus, on the other hand, was reported to be disorder-specific for ASD relative to ADHD (11). An earlier VBM study pointed toward shared GM volume reduction within the medial temporal and higher GM in the inferior parietal cortex (3). Further, increased GM volume of the supramarginal gyrus was reported in ASD, but not ADHD, relative to controls (3). In the largest VBM study so far an increasing ASD score was associated with greater global GM volume (10).

In ASD it has frequently been reported that after having a normal (13) or smaller (14) brain size at birth, there is a period of early brain overgrowth prior to 4 years of age (14–16). The pathophysiology of such alterations is unknown, but it is proposed to result from deviant neuronal proliferation and axonal growth during fetal development that in turn leads to an aberrant developmental pruning (17). In contrast, in children with ADHD, smaller whole brain volumes (18–20) and lower GM volumes have been described (21). It is hypothesized that ADHD children show a delayed brain maturation process (22).

Despite similarities in clinical presentation as well as mutual comorbidity rates in ASD and ADHD, these disorders present a rather different neuroanatomical profile. Most studies report subcortical temporal structures such as the amygdala to be enlarged in young children (at ages 2–4) with ASD (23) with a normalization in late childhood and adolescence (24). Amygdala volumes in adults with ADHD have been found to be relatively normal (25, 26) or smaller than in controls (27). Basal ganglia are reported to be enlarged (15) in ASD and smaller in ADHD (1). With regard to the corpus callosum, thalamus and cerebellum, however, in many studies ASD and ADHD show a volume reduction (1).

Previous Cortical Surface Parameter Studies in ASD and ADHD

Studies focusing on cortical surface parameters in ASD also reported mixed results. When investigating cortical surface parameters, it has to be reflected that cortical development in ASD varies across developmental stages or brain regions. Three different phases have been proposed: accelerated expansion in early childhood, accelerated thinning in later childhood and adolescence, and decelerated thinning in early adulthood (28).

TABLE 1 | Previous volumetric studies comparing children with ADHD and ASD.

Study	n (ADHD/ASD/TD)	Age in years (ADHD/ASD/TD) (Mean ± SD)	IQ	Methods	Region(s) and results
1. Brieber et al. (3)	15 ADHD 15 ASD 15 TD	13.13 ± 1.4 14.2 ± 1.9 13.3 ± 1.8 (10–16 y)	104.1 ± 15.8 106.8 ± 21.4 107.7 ± 12.7	VBM	Smaller GM in left medial temporal lobe and higher GM volume in left inferior parietal cortex in ADHD and ASD vs. TD Increased GM volume in right supramarginal gyrus in ASD vs. ADHD and TD
2. O'Dwyer et al. (10)	180 ADHD 140 TD 124 unaffected siblings	16.2 ± 3.7 16.8 ± 3.6 16.9 ± 4.0 (7.4–28.5 y)	98.8 ± 14.9 106.6 ± 13.2 101.8 ± 14.2	VBM	Increasing ASD score is associated with greater GM volume
3. Lim et al. (11)	44 ADHD 19 ASD 33 TD	13.6 ± 1.87 14.9 ± 1.86 14.3 ± 2.52	92.2 ± 11.7 113 ± 15.7 110 ± 11.5	VBM	Smaller right posterior cerebellar GM volume in ADHD vs. ASD and TD Larger left middle/superior temporal gyrus GM volume in ASD vs. ADHD and TD
4. Mahajan et al. (12)	30 ASD- 33 ASD+ 63 TD	10.5 ± 1.7 10.3 ± 1.4 10.5 ± 1.3 (8–12 y)	102 ± 14 103 ± 17 112 ± 11	Free Surfer ROI	Increased GM volume and SA in the left inferior parietal cortex in ASD+ and ASD- Increased GM volume and SA in the left post-central gyrus and the right precentral gyrus in ASD- Increased GM volume and SA in the left precentral gyrus in ASD+

n, number; SD, standard deviation; ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; ASD+, autism spectrum disorder with comorbid ADHD; ASD-, autism spectrum disorder without comorbid ADHD; TD, typically developed; VBM, voxel based morphometry; ROI, region of interest; GM, gray matter; IQ, intelligence quotient.

Hazlett et al. (29) examined young children with ASD (ages 2–5 years) and found increased cortical volumes, but no alterations in cortical thickness implicating that brain enlargement may be associated with increased cortical SA in ASD. Increased cortical thickness in temporal lobes was reported in children (ages 8–12 years) with ASD (30) with greater cortical thinning in ASD over time especially in occipital regions (31). Greater cortical thinning was associated with more severe symptoms in ASD (31). Further investigations pointed toward cortical thinning in adolescents (ages 12–25 years) with ASD (32–34). Studies in adults are divergent with some reporting cortical thinning (35–37) in brain regions involved in social cognition, others cortical thickening within frontal lobe regions (38) or regions from all four lobes (36). A large recent study found no significant difference in overall cortical thickness or surface area between ASD and typically developed (TD) (39).

In children and adults with ADHD, cortical thinning has been described in parietal and frontal regions responsible for executive function and attention (40–42). Another study detected no differences in cortical thickness of ADHD children, but decreased SA and cortical folding (43).

The unclear and puzzling current state requires further studies directly comparing volumetric and cortical thickness parameters between ASD and ADHD. Individuals with isolated autism and individuals, who present comorbid conditions in terms of ADHD, can be distinguished behaviorally as already documented by our research group (44).

Rationale of Our Study

Based on the available evidence, we aimed to study the brain structure in children with ASD with and without comorbid ADHD as well as TD. To address the question of a potential neurobiological overlap between ADHD and ASD, we analyzed the brain scans for shared and disorder-specific abnormalities. We investigated differences in terms of GM as well as cortical thickness and mean curvature.

In doing so, this study represents the first FreeSurfer study comprising ASD and ADHD groups, as well as subjects with co-occurrence of both conditions. Because previous studies showed inconsistent and widely distributed changes, we did not limit the analysis to individual a priori regions of interest (ROIs).

MATERIALS AND METHODS

Participants

The ethics committee of the University Medical Center Freiburg approved the study (approval ID: 279/06). Magnetic resonance imaging (MRI) scans were acquired following written informed consent of the children's parents. Male children with ASD and ADHD were recruited from the Department of Child and Adolescent Psychiatry, Psychotherapy, and Psychosomatics of the University Medical Center Freiburg.

We obtained scans of high quality in 40 male children with a diagnosis of ASD according to ICD-10 and DSM-5 criteria. Twenty-nine ASD patients were included in the final analysis after 7 patients were excluded due to image artifacts, 3 due to IQ < 70 and one patient due to comorbid seizures. All ASD children were high-functioning and with no language delay. Intellectual disability (full scale IQ below 70), comorbid Tourette syndrome or severe neurological diseases were defined as exclusion criteria. With the exception of 2 patients, no ASD participant had comorbid depressive or anxiety symptoms. The diagnostic

process followed international guidelines, including the Autism Diagnostic Observation Schedule [ADOS-G, (45)] and the Autism Diagnostic Interview [ADI-R, (46)]. Psychometric tools included the Child Behavior Checklist [CBCL, (47)], the Social Responsiveness Scale [SRS, (48)], and the diagnostic interview K-SADS-PL (49). According to the K-SADS-PL, 15 autistic children additionally met the diagnostic criteria for ADHD. The ADHD diagnosis was confirmed with the DISYPS-II FBB-ADHD (50), and verified by a multi-professional team of expert clinicians to ensure a comorbid ADHD. The group without (14 patients) and the group with comorbid ADHD (15 patients) were not significantly different for age and IQ.

Additionally, MRI-scans of 50 male patients with ICD-10 and DSM-5 ADHD diagnosis without a comorbid ASD were acquired. Twelve scans were excluded due to poor image quality, 7 due to the low IQ of the subjects (<70) and one due to an arachnoid cyst, so that finally 30 ADHD patients were analyzed. ADHD diagnosis was clinically based on ICD-10 and DSM-5 criteria and additionally confirmed with the DISYPS-II FBB-ADHD (50). ASD symptoms were ruled out applying the SRS score (48), also the CBCL (47) was consulted. With the exception of one ADHD patient, no one suffered from comorbid depression or anxiety disorder as assessed by the K-SADS-PL (49). Methylphenidate medication in children with an ADHD diagnosis was discontinued at least 24 h prior to scanning procedure.

Fourty-eight typically developed male children (TD) were recruited from local schools and sport groups. Control subjects were included after a phone interview with the parents who additionally completed a sociodemographic questionnaire, the CBCL (47) and the SRS (48) for ruling out ASD and ADHD symptoms. Four children were excluded from the TD group, because of the presence of ADHD or autistic symptoms, 7 due to imaging artifacts and one due to an IQ < 70, so that we finally included 36 male TD participants in the study.

Subjects were matched according to IQ assessed with Raven's Standard Progressive Matrices (51), age and sex.

All subjects included in the study accomplished behavioral tasks of executive functions and planning as well. The results are published elsewhere (44).

Image Acquisition
A standard magnetization-prepared rapid gradient echo (MPRAGE) T1-weighted anatomical scan was conducted (relaxation time = 2,200 ms, echo time = 2.15 ms, flip angle = 12°, inversion time = 1,100 ms) on a 3T Siemens TIM Trio Magnetom scanner (Erlangen, Germany). Slice thickness was 1 mm and voxel size $1 \times 1 \times 1$ mm^3.

Brain Segmentation
Cortical reconstruction and segmentation was performed using FreeSurfer version 5.3 (http://surfer.nmr.mgh.harvard.edu/). FreeSurfer is a fully automated suite of tools that enables analysis of key features in the human brain such as segmentation of most macroscopically visible brain structures (52). FreeSurfer allows to compute the volume of subcortical areas and reconstructs the cerebral cortex (53). It also provides information about

mapping of cortical GM thickness (54) and the construction of surface models of the cerebral cortex (55). The technical details of FreeSurfer procedures are described elsewhere (52). Applying FreeSurfer, we removed non-brain tissue and segmented cortical and subcortical GM and WM depending on image intensity. FreeSurfer output was inspected by three blinded trainees and rated on a scale ranging from 1 to 4. A "1" means no visible artifacts, whereas "4" denotes distinct blurred and low-quality images. Manual correction followed recommendations of FreeSurfer developers (https://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/PialEdits_freeview).

MRI scans of poor quality, which showed geometric inaccuracies, were rated "4" (blurred and low quality) or for which the segmentation procedure failed, were excluded.

Region of Interest Parcellation
Individual brains were registered on a spherical atlas for parcellation, taking into account individual cortical folding patterns to match brain geometry between the subjects. FreeSurfer parcelled each brain into 148 GM and 32 subcortical ROIs using the Desikan-Killiany-Atlas (56). Afterwards, ROI labels were transformed back into each subject's individual space to compute the volume of each ROI.

Surface and Cortical Thickness
Cortical surface area was calculated with FreeSurfer based on a 2D representation of cortical surface after estimation of GM/WM boundary and pial surface (54). Cortical thickness was then calculated for each vertex as distance from the GM/WM boundary and the pial surface. FreeSurfer offers better alignment of cortical landmarks than volume-based registration and does not produce an age-associated bias between older and younger children when registering children's brains to a common space (57).

Statistical Analysis
Psychometric Data
Group comparisons of demographic and psychometric data (age, IQ, psychometric scores) were carried out using SPSS software, version 22 (IBM Corp., Armonk, NY, USA). We used analysis of variance (ANOVAs) for the assessment of significance of putative differences.

Analysis of Imaging Data
Further analysis of imaging data was carried out using R statistical computing software (58). We tested for differences in cortical GM volumes respecting all regions of FreeSurfer segmentation according to the Desikan-Killiany-Atlas (56). Additionally, we focused on cortical surface parameters in terms of cortical thickness and mean curvature of ROIs, again defined with the Desikan-Killiany-Atlas (56).

We adjusted volume, mean curvature and thickness data for differences in age and IQ using a linear model applying the groups mean age and IQ.

Type III two-way 2×2 ANOVAs on the adjusted volume, thickness and curvature data were calculated using the independent between-subject factors ASD diagnosis (yes vs. no)

and ADHD diagnosis (yes vs. no; see **Table 2**). Results were corrected for multiple comparisons applying false discovery rate (FDR) correction. FDR corrected $p < 0.05$ were considered significant, and uncorrected $p < 0.05$ were regarded as trends. We didn't restrict our analysis to a priori regions of interest.

Multiple regression models with either SRS total raw score or DISYPS-II FBB-ADHD as independent variables were conducted with the adjusted left Pars orbitalis volume as dependent variables. The regression model included an interaction term with the binary moderator variables ASD and ADHD.

RESULTS

Demographic and Psychometric Data

Table 3 summarizes the demographic and psychometric data of the ASD, ADHD, and TD group. The study included 95 male participants (6–13 years old): 14 with ASD without comorbid ADHD, 15 with ASD and comorbid ADHD, 30 ADHD, and 36 TD controls. Groups did not differ significantly with respect to age and IQ.

Volumetric Results

The two-way ANOVA model showed a significant main effect of the factor ADHD for the left Pars orbitalis volume after FDR correction [$F_{(1, 91)} = 12.63$; $p_{FDR} = 0.039$, $p_{uncorr} < 0.001$]. Children with an ADHD diagnosis exhibited smaller left Pars orbitalis volumes (**Figure 1**). Uncorrected significant effects were regarded as trends (**Table 4**). A main effect of ADHD on trend level could also be observed in the right Pars orbitalis and precuneus cortex, in terms of a volume reduction. An interaction of the factors ADHD and ASD could be detected for the right isthmus cingulate cortex.

Cortical Thickness

Two-way ANOVA models exhibited no significant main effects or interaction surviving FDR correction. The following uncorrected significant effects were regarded as trends (**Table 4**):

A main effect for the diagnosis ASD emerged in the bilateral postcentral gyrus and the left pericalcarine and cuneus cortex, as well as in the right superior parietal cortex, in terms of cortical thinning. ADHD effects were observable in the left Pars orbitalis, again, linked to a cortical thinning. An interaction of diagnosis ADHD and ASD could be observed for the left inferior parietal, parahippocampal, pericalcarine, transverse temporal, and right post-central thickness measures.

TABLE 2 | Factor levels of the 2 × 2 ANOVA model.

	ADHD diagnosis	No ADHD diagnosis
ASD diagnosis	ASD with comorbid ADHD	ASD
No ASD diagnosis	ADHD	TD

TD, typically developed; ASD, autism spectrum disorder; ADHD, attention-deficit/hyperactivity disorder.

Mean Curvature

The two-way ANOVA model showed no significant interaction or main effects for the factors ASD or ADHD on mean curvature after FDR correction. Uncorrected significant results we regard as trends (**Table 4**). The right medial orbitofrontal cortex showed a significant main effect of the factor ASD, as did the left postcentral gyrus and right cuneus cortex. ADHD, in turn, had an effect on mean curvature of the left transverse temporal cortex and right Pars triangularis. Uncorrected significant interactions could be found in the left pericalcarine and right medial orbitofrontal cortex.

SRS-Total Score and DISYPS-II Effect on Main Result

Neither the multiple regression model with the independent variable SRS total score nor DISYPS-II FBB-ADHD revealed any significant effect of these scores or their interaction with the factors ADHD and/or ASD on left Pars orbitalis volume.

DISCUSSION

To our knowledge, this is the first FreeSurfer study that examines children with ADHD, ASD, and comorbid ASD and ADHD in a single study. Our investigation focused on the detection of possible morphometric differences (cortical volume, thickness and mean curvature).

Due to the heterogeneity of findings in earlier studies, we did not limit our analysis to a priori regions of interest.

Volumetric Results

The diagnosis ADHD has a significant effect on the left Pars orbitalis volume with ADHD-diagnosed children showing smaller left Pars orbitalis volumes. These findings suggest that ADHD rather than ASD is related to left Pars orbitalis volume loss. Whether there are weaker "additive" effects on the Pars orbitalis volume of ASD and ADHD cannot be ruled out with a study of the given sample size.

On trend level, we additionally found an ADHD main effect for the right Pars orbitalis and precuneus cortex and an interaction of diagnosis ADHD and ASD for the right isthmus cingulate cortex.

The so-called default-mode network (DMN) has been described as comprising the precuneus/posterior cingulate cortex, the medial prefrontal cortex and the medial, lateral and inferior parietal cortex. It is a network of brain regions associated with task-irrelevant mental processes and mind wandering (59, 60) In line with our results, Castellanos et al. (61) showed ADHD-related decreases in functional connectivity between the precuneus and other DMN components.

The Pars Orbitalis of the Inferior Frontal Gyrus

The Pars orbitalis represents a subdivision of the inferior frontal gyrus which more or less corresponds well to the Brodman Area 47.

TABLE 3 | Demographic and psychometric data.

	ADHD	ASD with comorbid ADHD	ASD	TD	ANOVA
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
N	30	15	14	36	
Age	9.89 ± 2.18	10.32 ± 2.21	10.35 ± 2.47	9.86 ± 2.33	$F(3,91) = 0.274; p = 0.844$
IQ	93.71 ± 13.32	98.24 ± 13.37	93.71 ± 14.17	97.79 ± 13.05	$F(3,91) = 0.789; p = 0.503$
SRS total score	59.23 ± 31.03	92.93 ± 40.60	81.29 ± 22.10	18.83 ± 14.39	$F(3,91) = 36.76; p < 0.001$
DISYPS-II FBB-ADHD	1.44 ± 0.65	1.85 ± 0.61	0.80 ± 0.35	0.20 ± 0.15	$F(3,64) = 25.25; p < 0.001$

N, number; SD, standard deviation; ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; TD, typically developed; IQ, intelligence quotient; SRS, Social Responsiveness Scale; DISYPS, Diagnostik System für Psychische Störungen.
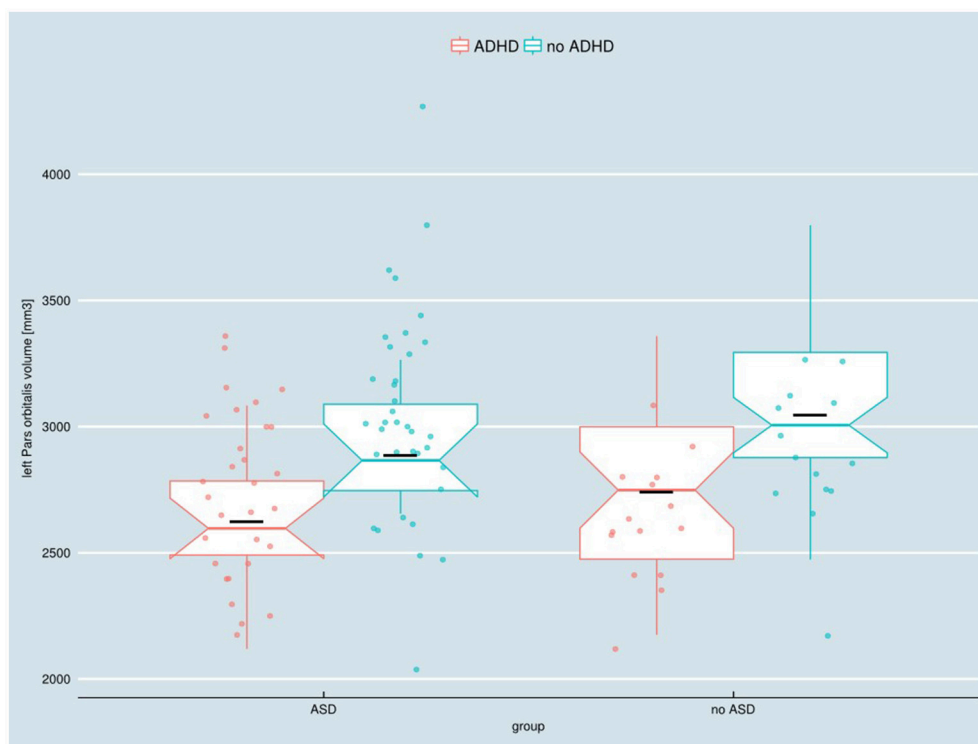
FIGURE 1 | Adjusted left Pars orbitalis volume in children with and without ASD or ADHD, respectively. ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder. Boxes indicate upper/lower quartile as well as the median. The black line indicates the sample mean.

Functionally it has been linked to the recognition of facial expressions of basic emotions (62) and to the modulation of positive emotionality (63). It is also assumed that besides the specific association between the right inferior frontal gyrus and the inhibitory control, the left inferior frontal gyrus is also involved in the successful implementation of inhibitory control over motor responses (64). This could be partly responsible for the impulsive behavior that can be observed in ADHD.

Children with ADHD and autism have a lot of similar features and there is high frequency of ADHD symptoms in autism (65). Also, in autistic patients, difficulties with emotion and facial recognition have been described (66–69). In addition, it was assumed that Brodmann Area

47 is involved in semantic/syntactic processing (70, 71). Previous studies pointed toward abnormalities in the pragmatic understanding and the use of language in ASD (72). Brothers (73) proposed that there is a network of neural regions (the amygdala, the orbito-frontal cortex, the superior temporal sulcus and gyrus) comprising the "social brain." Accordingly, reduced Pars orbitalis volume in ASD with comorbid ADHD might not necessarily be responsible for the aforementioned symptoms in ASD, but it may be a complicating factor.

In a recent publication the Pars orbitalis has been implicated as being part of a critical network for the identification of specific ADHD/ASD subtypes (74).

TABLE 4 | Uncorrected adjusted volume, thickness, and mean curvature results.

Measure	Region	ADHD Mean ± SD	ASD- Mean ± SD	ASD + Mean ± SD	TD Mean ± SD	Two-way ANOVAs (p-values)
Volume	Left pars orbitalis	2738.70 ± 328.53	2884.21 ± 284.83	2621.54 ± 241.09	3043.94 ± 413.61	ASD: $p = 0.148$ **ADHD: $p < 0.001$** ASD × ADHD: $p = 0.784$
	Right pars orbitalis	3387.65 ± 485.42	3676.62 ± 613.57	3285.59 ± 463.18	3656.61 ± 430.33	ASD: $p = 0.896$ **ADHD: $p = 0.027$** ASD × ADHD: $p = 0.572$
	Right isthmus cingulate	3204.79 ± 512.29	3244.11 ± 425.46	2875.96 ± 477.66	3075.76 ± 575.98	ASD: $p = 0.308$ ADHD: $p = 0.320$ **ASD × ADHD: $p = 0.035$**
	Right precuneus	12868.08 ± 1570.15	13284.51 ± 1850.61	12637.10 ± 1968.18	13783.22 ± 1989.24	ASD: $p = 0.392$ **ADHD: $p = 0.047$** ASD × ADHD: $p = 0.745$
Thickness	Left inferior parietal	2.83 ± 0.14	2.85 ± 0.12	2.70 ± 0.21	2.84 ± 0.14	ASD: $p = 0.829$ ADHD: $p = 0.708$ **ASD × ADHD: $p = 0.039$**
	Left post-central	2.24 ± 0.14	2.17 ± 0.12	2.21 ± 0.13	2.31 ± 0.17	**ASD: $p = 0.004$** ADHD: $p = 0.059$ ASD × ADHD: $p = 0.107$
	Right post-central	2.20 ± 0.12	2.11 ± 0.10	2.20 ± 0.14	2.24 ± 0.15	**ASD: $p = 0.004$** ADHD: $p = 0.218$ **ASD × ADHD: $p = 0.030$**
	Right superior parietal	2.44 ± 0.14	2.36 ± 0.12	2.37 ± 0.12	2.48 ± 0.14	**ASD: $p = 0.008$** ADHD: $p = 0.280$ ASD × ADHD: $p = 0.485$
	Left cuneus	2.01 ± 0.18	1.92 ± 0.13	2.00 ± 0.15	2.04 ± 0.16	**ASD: $p = 0.017$** ADHD: $p = 0.346$ ASD × ADHD: $p = 0.104$
	Left parahippocampal	2.89 ± 0.33	2.97 ± 0.29	2.76 ± 0.27	2.84 ± 0.24	ASD: $p = 0.130$ ADHD: $p = 0.491$ **ASD × ADHD: $p = 0.040$**
	Left pars orbitalis	2.89 ± 0.33	2.97 ± 0.29	2.76 ± 0.27	2.84 ± 0.24	ASD: $p = 0.062$ **ADHD: $p = 0.049$** ASD × ADHD: $p = 0.377$
	Left pericalcarine	1.59 ± 0.11	1.54 ± 0.10	1.66 ± 0.19	1.64 ± 0.15	**ASD: $p = 0.032$** ADHD: $p = 0.177$ **ASD × ADHD: $p = 0.010$**
	Left transverse temporal	2.45 ± 0.25	2.39 ± 0.24	2.61 ± 0.22	2.52 ± 0.28	ASD: $p = 0.124$ ADHD: $p = 0.300$ **ASD × ADHD: $p = 0.016$**
Mean curvature	Left transverse temporal	0.141 ± 0.010	0.129 ± 0.010	0.140 ± 0.015	0.133 ± 0.014	ASD: $p = 0.304$ **ADHD: $p = 0.012$** ASD × ADHD: $p = 0.544$
	Left pericalcarine	0.158 ± 0.016	0.160 ± 0.034	0.150 ± 0.013	0.152 ± 0.014	ASD: $p = 0.168$ ADHD: $p = 0.179$ **ASD × ADHD: $p = 0.048$**
	Left post-central	0.143 ± 0.021	0.149 ± 0.021	0.142 ± 0.016	0.137 ± 0.011	**ASD: $p = 0.036$** ADHD: $p = 0.188$ ASD × ADHD: $p = 0.100$
	Right cuneus	0.165 ± 0.008	0.167 ± 0.013	0.166 ± 0.010	0.161 ± 0.010	**ASD: $p = 0.016$** ADHD: $p = 0.650$ **ASD × ADHD: $p = 0.018$**
	Right medial orbitofrontal	0.147 ± 0.010	0.141 ± 0.006	0.150 ± 0.012	0.148 ± 0.010	**ASD: $p = 0.016$** ADHD: $p = 0.650$ **ASD × ADHD: $p = 0.018$**
	Right pars triangularis	0.144 ± 0.010	0.138 ± 0.011	0.142 ± 0.013	0.139 ± 0.009	ASD: 0.873 **ADHD: $p = 0.046$** ASD × ADHD: $p = 0.748$

SD, standard deviation; ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; TD, typically developed. Bold: p < 0.05.

Relationship to Other Publications

The volume reduction in the Pars orbitalis is partly consistent with one previous study which reported a trend toward a lower Pars orbitalis volume in ADHD, but in this investigation, the right Pars orbitalis was affected (75). In the present study, decreased right Pars orbitalis volume could only be observed on an uncorrected level (see **Table 4**). It is assumed that besides the specific association between the right inferior frontal gyrus and the inhibitory control, the left inferior frontal gyrus is also involved in the successful implementation of inhibitory control over motor responses (64). We could not replicate other volumetric results of the few earlier heterogeneous studies that combined ASD and ADHD patients into a single study (3, 10–12, 76). However, it should be mentioned that previous studies are not directly comparable to our study design. Heterogeneous findings are difficult to interpret due to the often arbitrary distinction between both clinical groups without careful consideration of ASD/ADHD comorbidities (74). Furthermore, different methodology in terms of voxel-based morphometry (VBM) was applied in most previous investigations (3, 10, 11) and none of the earlier investigations included ASD– (ASD without comorbid ADHD), ASD+ (ASD with comorbid ADHD), ADHD, and TD participants (3, 10–12).

Additionally, VBM studies examined adolescent samples. Only the study by Mahajan et al. (12) studied children whose average age corresponded to our study (but a smaller age range than our sample). In fact, age and IQ differences across studies are potential factors leading to heterogeneity of results. Even if covariates are used to correct for age effects, the results cannot be transferred to samples from other age groups or age structures.

Dimensional Correlations

Multiple regression models with the independent variable SRS total score or DISYPS-II FBB-ADHD revealed no significant effect of these scores or their interaction with the factors ADHD and/or ASD on left Pars orbitalis volume. It can therefore be assumed that the reduction of the left Pars orbitalis volume is a categorical effect due to ADHD diagnosis and is not due to the severity of different symptoms or traits represented by questionnaires.

Surface Parameters

No significant interaction or main effects for the factors ASD or ADHD on cortical thickness or mean curvature could be detected after FDR correction. The fact that significant group effects only emerged on an uncorrected level might indicate that the effect sizes of possible differences are too small to be detected with the present group size. We decided to interpret the uncorrected significant differences as possible trends.

In doing so, a main effect for ASD diagnosis emerged for the bilateral post-central, left pericalcarine, left cuneus as well as right superior parietal cortical thickness, and an effect for the factor ADHD resulted for the left Pars orbitalis thickness. An interaction of ASD and ADHD diagnosis was detected for left inferior parietal, parahippocampal, pericalcarine, transverse temporal, and right post-central thickness measures.

Our results of cortical thinning in several areas in ASD children (aged 6–13 years) are concordant with studies reporting accelerated thinning in childhood ASD (28). Greater cortical thinning was associated with more severe symptomatology in ASD (31). Another previous study focusing on ASD children (aged 6–12 years) revealed widespread, but mostly left-hemispheric thinning in frontal, temporal, parietal and occipital brain areas related to the theory-of-mind network (77). It should be noted that cortical development in ASD is most likely subject to three different phases: accelerated expansion in early childhood, accelerated thinning in later childhood and adolescence, and decelerated thinning in early adulthood (28). Therefore, when comparing different studies in childhood and adulthood, the exact stage of development must be taken into account.

The detected cortical thinning in the superior parietal cortex in ASD relates well with previous investigations (28, 33). The superior parietal lobule showed decreased activation during learning in ASD and was suggested to play an important role in motor learning and repetitive behaviors (78). Higher SRS- total scores, indicating autistic traits, were associated with thinner cortex in the left superior parietal lobule (79). The postcentral gyrus is important for the representation of haptic and proprioceptive feedback (12). In line with this observation, previous research revealed differences of ASD children in tactile discrimination in comparison to TD (80, 81).

The definite pathomechanisms resulting in cortical thinning are not yet clarified. Regressive (e.g., synaptic pruning) and progressive (e.g., myelination) events are supposed to result in the appearance of GM density reduction or cortical thinning (82), but further research is required.

Methodological Issues and Limitations

The study was conducted with a sample of children with ASD or ADHD being prone to motion artifacts (83). Therefore, many of the MRI brain images were excluded from analysis due to poor image quality, which could have biased the study results. We applied manual inspection and correction as suggested by the recommendations of the developers of FreeSurfer. Nevertheless, we cannot completely rule out any confounding effects induced by head motion. Most previous studies did not quantify the degree of observed motion in groups (1). Therefore, differences in the applied (or not applied) motion correction or exclusion criteria might partly be responsible for the heterogeneity of results across studies. To date, a quantification of head motion as described in diffusion tensor imaging (DTI) studies is not possible in FreeSurfer morphometric studies (84). There is very sparse evidence of utilization of automated quality metrics in FreeSurfer studies (85, 86).

Due to the study's focus on primary forms of ASD the results presented here can not necessarily be generalized to forms of ASD with intellectual impairment or to syndromal-secondary autism (defined as autism with known etiology) (87). Additionally, a larger total sample size would have been desirable to detect more potential subtle differences.

Methylphenidate medication was discontinued at least 24 h prior to scanning procedure. Evidence suggests an impact of long-term neurotropic medication on brain structure with

stimulant medication being associated with normalization of structural abnormalities in ADHD (88). This confounding factor might have influenced our results, yet, given the earlier literature, not in terms of a volume loss as we report in this study (89). Furthermore, for future studies, it would be helpful to choose longitudinal designs to study longitudinal neurodevelopmental trajectories of ASD and ADHD vs. TD.

CONCLUSION

In summary, we detected that ADHD rather than ASD mediates volume loss in the inferior frontal gyrus (Pars orbitalis). The volume reduction in the left Pars orbitalis seems to be primarily a categorical diagnostic effect than to reflect the severity of various traits or symptoms. ASD and ADHD diagnoses tended to have an effect on cortical thickness or mean curvature, which did not survive correction for multiple comparisons. Further studies of more power in larger samples are necessary to investigate the effect of ADHD and ASD on cortical thickness and mean curvature. Additionally, further research is needed to disentangle the precise causal pathways.

AUTHOR CONTRIBUTIONS

MB is principal investigator of the study funded by German Federal Ministry of Education and Research (BMBF; grant number 01GW0710). MB, JU, RR, IM, CK, and CPK designed the study and were responsible for acquisition and analysis of data. SM, KN, and JM performed FreeSurfer analysis of the data. KN, SM, JM, MB, DE, AR, and LTvE were crucially involved in the theoretical discussion and preparation of the manuscript. KN and SM wrote the manuscript. All authors read and approved the final version of the manuscript. They agreed to be accountable for all aspects of the work.

REFERENCES

1. Dougherty CC, Evans DW, Myers SM, Moore GJ, Michael AM. A Comparison of structural brain imaging findings in autism spectrum disorder and attention-deficit hyperactivity disorder. *Neuropsychol Rev*. (2016) 26:25–43. doi: 10.1007/s11065-015-9300-2

2. Gillberg C, Fernell E. Autism plus versus autism pure. *J Autism Dev Disord*. (2014) 44:3274–76. doi: 10.1007/s10803-014-2163-1

3. Brieber S, Neufang S, Bruning N, Kamp-Becker I, Remschmidt H, Herpertz-Dahlmann B, et al. Structural brain abnormalities in adolescents with autism spectrum disorder and patients with attention deficit/hyperactivity disorder. *J Child Psychol Psychiatry* (2007) 48:1251–8. doi: 10.1111/j.1469-7610.2007.01799.x

4. Rommelse NNJ, Geurts HM, Franke B, Buitelaar JK, Hartman CA. A review on cognitive and brain endophenotypes that may be common in autism spectrum disorder and attention-deficit/hyperactivity disorder and facilitate the search for pleiotropic genes. *Neurosci Biobehav Rev*. (2011) 35:1363–96. doi: 10.1016/j.neubiorev.2011.02.015

5. American Psychiatric Association. *Diagnostic and Statistical Manual Of Mental Disorders*. 5th ed. Arlington, TX: American Psychiatric Publishing (2013).

6. Epstein JN, Loren REA. Changes in the definition of ADHD in DSM-5: subtle but important. *Neuropsychiatry* (2013) 3:455–8. doi: 10.2217/npy.13.59

7. Leitner Y. The co-occurrence of autism and attention deficit hyperactivity disorder in children - what do we know? *Front Hum Neurosci*. (2014) 8:268. doi: 10.3389/fnhum.2014.00268

8. Grzadzinski R, Di Martino A, Brady E, Mairena MA, O'Neale M, Petkova E, et al. Examining autistic traits in children with ADHD: does the autism spectrum extend to ADHD? *J Autism Dev Disord*. (2011) 41:1178–91. doi: 10.1007/s10803-010-1135-3

9. Rommelse NNJ, Franke B, Geurts HM, Hartman CA, Buitelaar JK. Shared heritability of attention-deficit/hyperactivity disorder and autism spectrum disorder. *Eur Child Adolesc Psychiatry* (2010) 19:281–295. doi: 10.1007/s00787-010-0092-x

10. O'Dwyer L, Tanner C, van Dongen EV, Greven CU, Bralten J, Zwiers MP, et al. Brain volumetric correlates of autism spectrum disorder symptoms in attention deficit/hyperactivity disorder. *PloS ONE* (2014) 9:e101130. doi: 10.1371/journal.pone.0101130

11. Lim L, Chantiluke K, Cubillo AI, Smith AB, Simmons A, Mehta MA, et al. Disorder-specific gray matter deficits in attention deficit hyperactivity disorder relative to autism spectrum disorder. *Psychol Med*. (2015) 45:965–76. doi: 10.1017/S0033291714001974

12. Mahajan R, Dirlikov B, Crocetti D, Mostofsky SH. Motor circuit anatomy in children with autism spectrum disorder with or without attention deficit hyperactivity disorder. *Autism Res*. (2016) 9:67–81. doi: 10.1002/aur.1497

13. Hazlett HC, Poe M, Gerig G, Smith RG, Provenzale J, Ross A, et al. Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. *Arch Gen Psychiatry* (2005) 62:1366–76. doi: 10.1001/archpsyc.62.12.1366

14. Courchesne E, Carper R, Akshoomoff N. Evidence of brain overgrowth in the first year of life in autism. *J Am Med Assoc*. (2003) 290:337–44. doi: 10.1001/jama.290.3.337

15. Langen M, Bos D, Noordermeer SDS, Nederveen H, van Engeland H, Durston S. Changes in the development of striatum are involved in repetitive behavior in autism. *Biol Psychiatry* (2014) 76:405–11. doi: 10.1016/j.biopsych.2013.08.013

16. Nordahl CW, Lange N, Li DD, Barnett LA, Lee A, Buonocore MH, et al. Brain enlargement is associated with regression in preschool-age boys with autism spectrum disorders. *Proc Natl Acad Sci USA*. (2011) 108:20195–200. doi: 10.1073/pnas.1107560108

17. McFadden K, Minshew NJ. Evidence for dysregulation of axonal growth and guidance in the etiology of ASD. *Front Hum Neurosci*. (2013) 7:671. doi: 10.3389/fnhum.2013.00671

18. Batty MJ, Liddle EB, Pitiot A, Toro R, Groom MJ, Scerif G, et al. Cortical gray matter in attention-deficit/hyperactivity disorder: a structural magnetic resonance imaging study. *J Am Acad Child Adolesc Psychiatry* (2010) 49:229–38. doi: 10.1016/j.jaac.2009.11.008

19. Durston S, Hulshoff Pol HE, Schnack HG, Buitelaar JK, Steenhuis MP, Minderaa RB, et al. Magnetic resonance imaging of boys with attention-deficit/hyperactivity disorder and their unaffected siblings. *J Am Acad Child Adolesc Psychiatry* (2004) 43:332–40. doi: 10.1097/00004583-200403000-00016

20. Hill DE, Yeo RA, Campbell RA, Hart B, Vigil J, Brooks W. Magnetic resonance imaging correlates of attention-deficit/hyperactivity disorder in children. *Neuropsychology* (2003) 17:496–506. doi: 10.1037/0894-4105.17.3.496

21. Batty MJ, Palaniyappan L, Scerif G, Groom MJ, Liddle EB, Liddle PF, et al. Morphological abnormalities in prefrontal surface area and thalamic volume

in attention deficit/hyperactivity disorder. *Psychiatry Res.* (2015) 233:225–32. doi: 10.1016/j.pscychresns.2015.07.004

22. Shaw P, Eckstrand K, Sharp W, Blumenthal J, Lerch JP, Greenstein D, et al. Attention-deficit/hyperactivity disorder is characterized by a delay in cortical maturation. *Proc Natl Acad Sci USA.* (2007) 104:19649–54. doi: 10.1073/pnas.0707741104

23. Nordahl CW, Scholz R, Yang X, Buonocore MH, Simon T, Rogers S, et al. Increased rate of amygdala growth in children aged 2 to 4 years with autism spectrum disorders: a longitudinal study. *Arch Gen Psychiatry* (2012) 69:53–61. doi: 10.1001/archgenpsychiatry.2011.145

24. Barnea-Goraly N, Frazier TW, Piacenza L, Minshew NJ, Keshavan MS, Reiss AL, et al. A preliminary longitudinal volumetric MRI study of amygdala and hippocampal volumes in autism. *Prog Neuropsychopharmacol Biol Psychiatry* (2014) 48:124–128. doi: 10.1016/j.pnpbp.2013.09.010

25. Perlov E, Philipsen A, Tebartz van Elst L, Ebert D, Henning J, Maier S, et al. Hippocampus and amygdala morphology in adults with attention-deficit hyperactivity disorder. *J Psychiatry Neurosci.* (2008) 33:509–15.

26. Plessen KJ, Bansal R, Zhu H, Whiteman R, Amat J, Quackenbush GA, et al. Hippocampus and amygdala morphology in attention-deficit/hyperactivity disorder. *Arch Gen Psychiatry* (2006) 63:795–807. doi: 10.1001/archpsyc.63.7.795

27. Tajima-Pozo K, Yus M, Ruiz-Manrique G, Lewczuk A, Arrazola J, Montañes-Rada F. Amygdala abnormalities in adults with ADHD. *J Atten Disord.* (2016) 22: 671–8. doi: 10.1177/1087054716629213

28. Zielinski BA, Prigge MBD, Nielsen JA, Froehlich AL, Abildskov TJ, Anderson JS, et al. Longitudinal changes in cortical thickness in autism and typical development. *Brain J Neurol.* (2014) 137:1799–1812. doi: 10.1093/brain/awu083

29. Hazlett HC, Poe MD, Gerig G, Styner M, Chappell C, Smith RG, et al. Early brain overgrowth in autism associated with an increase in cortical surface area before age 2 years. *Arch Gen Psychiatry* (2011) 68:467–76. doi: 10.1001/archgenpsychiatry.2011.39

30. Hardan AY, Muddasani S, Vemulapalli M, Keshavan MS, Minshew NJ. An MRI study of increased cortical thickness in autism. *Am J Psychiatry* (2006) 163:1290–92. doi: 10.1176/appi.ajp.163.7.1290

31. Hardan AY, Libove RA, Keshavan MS, Melhem NM, Minshew NJ. A preliminary longitudinal magnetic resonance imaging study of brain volume and cortical thickness in autism. *Biol Psychiatry* (2009) 66:320–326. doi: 10.1016/j.biopsych.2009.04.024

32. Chung MK, Robbins SM, Dalton KM, Davidson RJ, Alexander AL, Evans AC. Cortical thickness analysis in autism with heat kernel smoothing. *NeuroImage* (2005) 25:1256–65. doi: 10.1016/j.neuroimage.2004.12.052

33. Ecker C, Shahidiani A, Feng Y, Daly E, Murphy C, D'Almeida V, et al. The effect of age, diagnosis, and their interaction on vertex-based measures of cortical thickness and surface area in autism spectrum disorder. *J Neural Transm.* (2014) 121:1157–70. doi: 10.1007/s00702-014-1207-1

34. Wallace GL, Eisenberg IW, Robustelli B, Dankner N, Kenworthy L, Giedd JN, et al. Longitudinal cortical development during adolescence and young adulthood in autism spectrum disorder: increased cortical thinning but comparable surface area changes. *J Am Acad Child Adolesc Psychiatry* (2015) 54:464–69. doi: 10.1016/j.jaac.2015.03.007

35. Hadjikhani N, Joseph RM, Snyder J, Tager-Flusberg H. Anatomical differences in the mirror neuron system and social cognition network in autism. *Cereb Cortex* (2006) 16:1276–82. doi: 10.1093/cercor/bhj069

36. Hyde KL, Samson F, Evans AC, Mottron L. Neuroanatomical differences in brain areas implicated in perceptual and other core features of autism revealed by cortical thickness analysis and voxel-based morphometry. *Hum Brain Mapp.* (2010) 31:556–66. doi: 10.1002/hbm.20887

37. Scheel C, Rotarska-Jagiela A, Schilbach L, Lehnhardt FG, Krug B, Vogeley K, et al. Imaging derived cortical thickness reduction in high-functioning autism: key regions and temporal slope. *NeuroImage* (2011) 58:391–400. doi: 10.1016/j.neuroimage.2011.06.040

38. Ecker C, Ginestet C, Feng Y, Johnston P, Lombardo MV, Lai M-C, et al. Brain surface anatomy in adults with autism: the relationship between surface area, cortical thickness, and autistic symptoms. *JAMA Psychiatry* (2013) 70:59–70. doi: 10.1001/jamapsychiatry.2013.265

39. Haar S, Berman S, Behrmann M, Dinstein I. Anatomical Abnormalities in Autism? *Cereb Cortex* (2016) 26:1440–52. doi: 10.1093/cercor/bhu242

40. Hoekzema E, Carmona S, Ramos-Quiroga JA, Richarte Fernández V, Picado M, Bosch R, et al. Laminar thickness alterations in the fronto-parietal cortical mantle of patients with attention-deficit/hyperactivity disorder. *PLoS ONE* (2012) 7:e48286. doi: 10.1371/journal.pone.0048286

41. Makris N, Buka SL, Biederman J, Papadimitriou GM, Hodge SM, Valera EM, et al. Attention and executive systems abnormalities in adults with childhood ADHD: A DT-MRI study of connections. *Cereb Cortex* (2008) 18:1210–20. doi: 10.1093/cercor/bhm156

42. Proal E, Reiss PT, Klein RG, Mannuzza S, Gotimer K, Ramos-Olazagasti MA, et al. Brain gray matter deficits at 33-year follow-up in adults with attention-deficit/hyperactivity disorder established in childhood. *Arch Gen Psychiatry* (2011) 68:1122–34. doi: 10.1001/archgenpsychiatry.2011.117

43. Wolosin SM, Richardson ME, Hennessey JG, Denckla MB, Mostofsky SH. Abnormal cerebral cortex structure in children with ADHD. *Hum Brain Mapp.* (2009) 30:175–184. doi: 10.1002/hbm.20496

44. Unterrainer JM, Rauh R, Rahm B, Hardt J, Kaller CP, Klein C, et al. Development of planning in children with high-functioning autism spectrum disorders and/or attention deficit/hyperactivity disorder. *Autism Res.* (2016). 9:739–51. doi: 10.1002/aur.1574

45. Rühl D, Bölte S, Feineis-Matthews S, Poustka F. Diagnostische beobachtungsskala für autistische störungen. In: von Catherine Lord, Michael Rutter, Pamela C. Dilavore and Susaan Risi, editor. *Deutsche Fassung der Autism Diagnostic Oberservation Schedule (ADOS).* Bern: Huber (2004).

46. Bölte S, Rühl D, Schmötzer G, Poustka F. Diagnostisches Interview für Autismus – Revidiert. In: *Deutsche Fassung des Autism Diagnostic Interview– Revised (ADI-R) von Michael Rutter, Ann Le Couteur und Catherine Lord.* Bern: Huber (2005).

47. Achenbach TM, Rescorla LA. Child Behaviour Checklist. In: *Manual for the ASEBA school-age forms and profiles.* Burlington, VT: University of Vermont: Research Center for Children, Youth and Families (2001).

48. Bölte S, Poustka F. Skala zur Erfassung sozialer Reaktivität – Dimensionale Autismus-Diagnostik. In: von John NC and Gruber CP, editors. *Deutsche Fassung der Social Responsiveness Scale (SRS).* Bern: Huber (2007).

49. Kaufman J, Birmaher B, Brent D, Rao U, Ryan N. *Kiddie-Sas-Present and Lifetime Version (K-SADS-PL) Version 1.0 of October 1996.* Berlin; Bern; Köln; Frankfurt; Hamburg; Jena; Mannheim; Rostock: K-SADS-Arbeitsgruppe (2001).

50. Döpfner M, Görtz-Dorten A, Lehmkuhl G, Breuer D, Goletz H. *Diagnostik-System für psychische Störungen nach ICD-10 und DSM-IV für Kinder und Jugendliche (DISYPS-II, FBB-ADHS).* Bern: Huber (2008).

51. Kratzmeier H, Horn R. *SPM, Raven Matrizen-Test, Standard Progressive Atrices.* Weinheim: Beltz (1979).

52. Fischl B. FreeSurfer. *NeuroImage* (2012) 62:774–81. doi: 10.1016/j.neuroimage.2012.01.021

53. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* (2002) 33:341–55. doi: 10.1016/S0896-6273(02)00569-X

54. Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci USA.* (2000) 97:11050–55. doi: 10.1073/pnas.200033797

55. Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *NeuroImage* (1999) 9:179–94. doi: 10.1006/nimg.1998.0395

56. Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage* (2006) 31:968–80. doi: 10.1016/j.neuroimage.2006.01.021

57. Ghosh SS, Kakunoori S, Augustinack J, Nieto-Castanon A, Kovelman I, Gaab N, et al. Evaluating the validity of volume-based and surface-based brain image registration for developmental cognitive neuroscience studies in children 4-to-11 years of age. *NeuroImage* (2010) 53:85–93. doi: 10.1016/j.neuroimage.2010.05.075

58. R Core Team. *R: A Language And Environment For Statistical Computing.* Vienna: R Foundation for Statistical Computing (2016).

59. Konrad K, Eickhoff SB. Is the ADHD brain wired differently? A review on structural and functional connectivity in attention deficit hyperactivity disorder. *Hum Brain Mapp.* (2010) 31:904–16. doi: 10.1002/hbm. 21058

60. Schilbach L, Eickhoff SB, Rotarska-Jagiela A, Fink GR, Vogeley K. Minds at rest? social cognition as the default mode of cognizing and its putative relationship to the "default system" of the brain. *Conscious Cogn.* (2008) 17:457–67. doi: 10.1016/j.concog.2008.03.013

61. Castellanos FX, Margulies DS, Kelly C, Uddin LQ, Ghaffari M, Kirsch A, et al. Cingulate-precuneus interactions: a new locus of dysfunction in adult attention-deficit/hyperactivity disorder. *Biol Psychiatry* (2008) 63:332–337. doi: 10.1016/j.biopsych.2007.06.025

62. Sprengelmeyer R, Rausch M, Eysel UT, Przuntek H. Neural structures associated with recognition of facial expressions of basic emotions. *Proc Biol Sci.* (1998) 265:1927–31. doi: 10.1098/rspb.1998.0522

63. Volkow ND, Tomasi D, Wang G-J, Fowler JS, Telang F, Goldstein RZ, et al. Positive emotionality is associated with baseline metabolism in orbitofrontal cortex and in regions of the default network. *Mol Psychiatry* (2011) 16:818–25. doi: 10.1038/mp.2011.30

64. Swick D, Ashley V, Turken AU. Left inferior frontal gyrus is critical for response inhibition. *BMC Neurosci.* (2008) 9:102. doi: 10.1186/1471-2202-9-102

65. Dickerson Mayes S, Calhoun SL, Mayes RD, Molitoris S. Autism and ADHD: overalpping and discriminating symptoms. *Res Autism Spectr Disord.* (2012) 6:277–285. doi: 10.1016/j.rasd.2011.05.009

66. Harms MB, Martin A, Wallace GL. Facial emotion recognition in autism spectrum disorders: a review of behavioral and neuroimaging studies. *Neuropsychol Rev.* (2010) 20:290–322. doi: 10.1007/s11065-010-9138-6

67. Kirchner JC, Hatri A, Heekeren HR, Dziobek I. Autistic symptomatology, face processing abilities, and eye fixation patterns. *J Autism Dev Disord.* (2011) 41:158–67. doi: 10.1007/s10803-010-1032-9

68. Lozier LM, Vanmeter JW, Marsh AA. Impairments in facial affect recognition associated with autism spectrum disorders: a meta-analysis. *Dev Psychopathol.* (2014) 26:933–45. doi: 10.1017/S0954579414000479

69. Schaller MU, Rauh R. What difference does it make? implicit, explicit and complex social cognition in autism spectrum disorders. *J Autism Dev Disord.* (2017) 47:961–79. doi: 10.1007/s10803-016-3008-x

70. Levitin DJ, Menon V. Musical structure is processed in "language" areas of the brain: a possible role for Brodmann Area 47 in temporal coherence. *NeuroImage* (2003) 20:2142–52. doi: 10.1016/j.neuroimage.2003.08.016

71. Poldrack RA, Wagner AD, Prull MW, Desmond JE, Glover GH, Gabrieli JD. Functional specialization for semantic and phonological processing in the left inferior prefrontal cortex. *NeuroImage* (1999) 10:15–35. doi: 10.1006/nimg.1999.0441

72. Riedel A, Suh H, Haser V, Hermann I, Ebert D, Riemann D, et al. Freiburg questionnaire of linguistic pragmatics (FQLP): psychometric properties based on a psychiatric sample. *BMC Psychiatry* (2014) 14:374. doi: 10.1186/s12888-014-0374-9

73. Brothers L. The social brain: a project for integrating primate behavior and neurophysology in a new domain. *Cocepts Neurosci.* (1990) 1:27–51.

74. Rommelse N, Buitelaar JK, Hartman CA. Structural brain imaging correlates of ASD and ADHD across the lifespan: a hypothesis-generating review on developmental ASD-ADHD subtypes. *J Neural Transm.* (2017) 124:259–271. doi: 10.1007/s00702-016-1651-1

75. Depue BE, Banich MT, Burgess GC, Bidwell LC, Willcutt EG. Behavioral performance predicts gray matter reductions in the right inferior frontal gyrus in young adults with combined type ADHD. *Psychiatry Res.* (2010) 182:231–37. doi: 10.1016/j.pscychresns.2010.01.012

76. O'Dwyer L, Tanner C, van Dongen EV, Greven CU, Bralten J, Zwiers MP, et al. Decreased left caudate volume is associated with increased severity of autistic-like symptoms in a cohort of ADHD patients and their unaffected siblings. *PloS ONE* (2016) 11:e0165620. doi: 10.1371/journal.pone.0165620

77. Richter J, Henze R, Vomstein K, Stieltjes B, Parzer P, Haffner J, et al. Reduced cortical thickness and its association with social reactivity in children with autism spectrum disorder. *Psychiatry Res.* (2015) 234:15–24. doi: 10.1016/j.pscychresns.2015.06.011

78. Travers BG, Kana RK, Klinger LG, Klein CL, Klinger MR. Motor learning in individuals with autism spectrum disorder: activation in superior parietal lobule related to learning and repetitive behaviors. *Autism Res.* (2015) 8:38–51. doi: 10.1002/aur.1403

79. Wallace GL, Shaw P, Lee NR, Clasen LS, Raznahan A, Lenroot RK, et al. Distinct cortical correlates of autistic versus antisocial traits in a longitudinal sample of typically developing youth. *J Neurosci.* (2012) 32:4856–60. doi: 10.1523/JNEUROSCI.6214-11.2012

80. Blanche EI, Reinoso G, Chang MC, Bodison S. Proprioceptive processing difficulties among children with autism spectrum disorders and developmental disabilities. *Am J Occup Ther.* (2012) 66:621–4. doi: 10.5014/ajot.2012.004234

81. Tomchek SD, Dunn W. Sensory processing in children with and without autism: a comparative study using the short sensory profile. *Am J Occup Ther.* (2007) 61:190–200. doi: 10.5014/ajot.61.2.190

82. Sowell ER, Thompson PM, Leonard CM, Welcome SE, Kan E, Toga AW. Longitudinal mapping of cortical thickness and brain growth in normal children. *J Neurosci.* (2004) 24:8223–31. doi: 10.1523/JNEUROSCI.1798-04.2004

83. Yendiki A, Koldewyn K, Kakunoori S, Kanwisher N, Fischl B. Spurious group differences due to head motion in a diffusion MRI study. *NeuroImage* (2014) 88:79–90. doi: 10.1016/j.neuroimage.2013.11.027

84. Koldewyn K, Yendiki A, Weigelt S, Gweon H, Julian J, Richardson H, et al. Differences in the right inferior longitudinal fasciculus but no general disruption of white matter tracts in children with autism spectrum disorder. *Proc Natl Acad Sci USA.* (2014) 111:1981–6. doi: 10.1073/pnas.1324037111

85. Chalavi S, Simmons A, Dijkstra H, Barker GJ, Reinders AATS. Quantitative and qualitative assessment of structural magnetic resonance imaging data in a two-center study. *BMC Med Imaging* (2012) 12:27. doi: 10.1186/1471-2342-12-27

86. Schilling C, Kühn S, Paus T, Romanowski A, Banaschewski T, Barbot A, et al. Cortical thickness of superior frontal cortex predicts impulsiveness and perceptual reasoning in adolescence. *Mol Psychiatry* (2013) 18:624–30. doi: 10.1038/mp.2012.56

87. Tebartz van Elst L. *Das Asperger-Syndrom im Erwachsenenalter und andere Hochfunktionale Autismus-Spektrum Störungen, 2nd ed.* Berlin: Medizinisch Wissenschaftliche Verlagsgesellschaft (2016).

88. Nakao T, Radua J, Rubia K, Mataix-Cols D. Gray matter volume abnormalities in ADHD: voxel-based meta-analysis exploring the effects of age and stimulant medication. *Am J Psychiatry* (2011) 168:1154–63. doi: 10.1176/appi.ajp.2011.11020281

89. Tebartz van Elst L, Maier S, Klöppel S, Graf E, Killius C, Rump M, et al. The effect of methylphenidate intake on brain structure in adults with ADHD in a placebo-controlled randomized trial. *J Psychiatry Neurosci.* (2016) 41:422–30. doi: 10.1503/jpn.150320

Check for updates

Widespread Genotype-Phenotype Correlations in Intellectual Disability

Emily L. Casanova[1,2]*, Zachary Gerstner[3], Julia L. Sharp[4], Manuel F. Casanova[1,2] and Frank Alex Feltus[3,5,6]

[1] Department of Biomedical Sciences, University of South Carolina School of Medicine at Greenville, Greenville, SC, United States, [2] Department of Pediatrics, Greenville Health System, Greenville, SC, United States, [3] Department of Genetics and Biochemistry, Clemson University, Clemson, SC, United States, [4] Department of Statistics, Colorado State University, Fort Collins, CO, United States, [5] Center for Human Genetics, Clemson University, Clemson, SC, United States, [6] Biomedical Data Science and Informatics Program, Clemson University, Clemson, SC, United States

Background: Linking genotype to phenotype is a major aim of genetics research, yet the underlying biochemical mechanisms of many complex conditions continue to remain elusive. Recent research provides evidence that relevant gene-phenotype associations are discoverable in the study of intellectual disability (ID). Here we expand on that work, identifying distinctive gene interaction modules with unique enrichment patterns reflective of associated clinical features in ID.

Methods: Two hundred twelve forms of monogenic ID were curated according to comorbidities with autism and epilepsy. These groups were further subdivided according to secondary clinical manifestations of complex vs. simple facial dysmorphia and neurodegenerative-like features due to their clinical prominence, modest symptom overlap, and probable etiological divergence. An aggregate gene interaction ID network for these phenotype subgroups was discovered via a public database of known gene interactions: protein-protein, genetic, and mRNA coexpression. Additional annotation resources (Gene Ontology, Human Phenotype Ontology, TRANSFAC/JASPAR, and KEGG/WikiPathways) were utilized to assess functional and phenotypic enrichment patterns within subgroups.

Results: Phenotypic analysis revealed high rates of complex facial dysmorphia in ID with comorbid autism. In contrast, neurodegenerative-like features were overrepresented in ID with epilepsy. Network analysis subsequently showed that gene groups divided according to clinical features of interest resulted in distinctive interaction clusters, with unique functional enrichments according to gene set.

Conclusions: These data suggest that specific comorbid and secondary clinical features in ID are predictive of underlying genotype. In summary, ID form unique clusters, which are comprised of individual conditions with remarkable genotypic and phenotypic overlap.

Keywords: autism spectrum disorder, epilepsy, craniofacial abnormalities, neurodegeneration, infantile proteopathy, genetic phenotype associations

BACKGROUND

Phenomics is a new and emerging area of study, underlying the development of genotype-phenotype mapping and the identification of different disease interaction networks (1). Genotype-phenotype mapping involves the delineation of a relationship between the genetic constitution of an individual and an observable set of characteristics of interest. While the relationship between a phenotype and a given genotype is complex, in the case of rare disorders with high-penetrance mutations determining such a relationship becomes relatively easier, although may still require the aid of computational methods such as those employed in the following study.

Intellectual disability (ID) is a complex and highly heterogeneous group of disorders despite cognitive and behavioral overlap. Genotype-phenotype correlations have been reported within individual ID syndromes and across different mutations within a given gene, yet only recently have there been reports of more extensive and generalizable genotype-phenotype clusters comprising subsets of the condition.

For instance, Casanova et al. (2) produced a manually curated catalog of 650 ID-associated genes that were grouped according to the presence of syndromic ID, non-syndromic ID, and multisystemic disorders. In addition, conditions were further subdivided according to variations in severity and mutation penetrance. The team identified a variety of functional enrichment patterns associated with specific clinical manifestations, such as MAPK, growth factor, and DNA repair signaling in conditions exhibiting short stature and ectodermal anomalies; microcephaly and behavioral features associated with genes enriched for chromatin-related functions, which regulate gene availability and expression patterns; and epilepsy and other neurologic, metabolic, and myopathic abnormalities associated with mitochondrial dysfunction. Some of these findings overlap those presented within the present paper, although we have used alternative methods for phenotype clustering based on our earlier work.

Previously, we reported associations between autism and epilepsy comorbidities in monogenic (single-gene) ID with trends in functional gene enrichment, suggesting these behavioral/neurological phenotypes represent etiological divergence at the molecular level in at least some forms of ID (2). Here we show that additional secondary clinical features are also prominent, such as multiple congenital anomalies (MCA), neurodegeneration, brain atrophy, and motor disorders like upper motor neuron disease (UMND), all of which co-vary to greater or lesser degrees. Because of the prominence of these secondary clinical features, we have elected to extend similar work as Kochinke et al. (3) to perform in depth investigation into functional and modular enrichment in association with these clinical features, in the hopes that in using a more general approach across an array of different disorders we may identify

previously unseen genotype-phenotype associations. It is our aim that this approach may allow us to group IDs into subtypes according to these gene-phenotype relationships, which may afford better understanding of their inherent biologies, as well as provide prognostic powers and potential cross-application of useful treatment paradigms.

In this study, we report multiple unique gene clusters with specific functional enrichment patterns that coincide with distinctive clinical phenotypes, indicating ID genes exhibit broad associations with observable phenotype.

METHODS

Gene-Phenotype Curation

Our gene-ID dataset was curated as described in Casanova et al. (2). To summarize the curation process, a comprehensive list of different forms of ID with known molecular origins was accessed from the Mendelian Inheritance in Man (MIM) database (4). By selecting conditions with ID, we were able to estimate genetic penetrance for the autism and epilepsy phenotypes according to rates of comorbidity. Keywords for initial accession included: "intellectual disability," "mental retardation," "mentally retarded," "global developmental delay," "severe developmental delay," and "profound developmental delay." Any rare conditions not accessed by these call words were not included in the study for the sake of consistency. In addition, conditions were removed if they fulfilled any of the following criteria: (1) the ID was variably expressed and not considered a primary feature; (2) onset of ID was later than 3 years of age; (3) the condition was often lethal in infancy or early childhood; (4) the condition was considered genetically complex (e.g., deletion/duplication syndromes), with the exception of chromosome 2p16.3 deletion syndrome, which contains only the *NRXN1* gene; (5) autism was a defining symptom for diagnosis, as in the case of certain "susceptibility" genes; (6) the condition had <2 reported cases; (7) the condition was a chromosomal instability syndrome, leading to an accumulation of different mutations; and (8) the condition was demarcated by a "?" indicating an unconfirmed or potentially spurious mapping.

The larger group was then subdivided according to comorbidities with autism and epilepsy and their frequencies, which were verified both through MIM and the larger literature (see Additional File 2, **Tables 2–4** tabs). For this study, only conditions with high autism and/or epilepsy rates, or without either comorbidity, were retained.

In addition, all conditions that were not contained within MIM's Clinical Synopses were removed, resulting in a final dataset of 212 different conditions. The autism group with/without epilepsy (referred to here as the "autism group") contained 59 unique conditions; ID with epilepsy but without autism (referred to as the "epilepsy group") was composed of 83 unique conditions; and ID without autism or epilepsy (ID group) was composed of 70 unique conditions. (see Additional File 2, "**Table_1**" tab for full list of IDs according to group and associated genes).

Comorbidity frequencies between ID and autism/epilepsy were obtained from the literature and described in detail in

---

Abbreviations: ID, Intellectual disability; CFD, complex facial dysmorphisms; SFD, simple facial dysmorphisms; NLF, neurodegenerative-like features; MCA, multiple congenital anomalies; UMND, upper motor neuron disease; MMD, multiple movement disorders; PPI, protein-protein interaction.

Casanova et al. (2). (In addition, see Additional File 2, **Tables 2–4** for citation information). A high cut-off for inclusion within both the autism and epilepsy groups was ≥20% for all conditions for the sake of relative homogeneity. Only conditions without any indications of autism or epilepsy comorbidities, including the exclusion of single case examples, were placed within the ID group. This curation process culminated in three groups of conditions with very distinctive clinical and genetic profiles, as will be discussed in the Results section.

All conditions were annotated using the MIM's Clinical Synopses (12/15/2016), which represent common clinical features of a disorder and are organized anatomically. According to Amberger et al. (5), features included within Clinical Synopses:

> … are taken from the literature and incorporated into the synopsis using a semi-controlled vocabulary. Many features include modifiers and additional terminology specific to medical subspecialties that are helpful for delineating overlapping disorders and distinguishing characteristic features. **Among genetically heterogeneous disorders, care is taken to include only those features that are present in patients with mutations in the same causative gene** [our emphasis].

Conditions were annotated according to the presence of congenital anomalies in the following organs/tissues: the facial suite (face, eyes, ears, nose, mouth, dentition, neck); the cranial suite (cranial volume, synostoses, other cranial malformations, e.g., bitemporal narrowing); hands and feet; the limbs; the viscera and genitals (changes to the latter not otherwise due to peripubertal hypogonadism, etc.); hair and skin; and the brain [partial/complete agenesis of the corpus callosum and malformations of cortical development (MCD), the limbic system, the midbrain, and the brainstem, all visible via MRI]. Complex (CFD) and simple facial dysmorphia (SFD) were annotated according to the number of facial regions affected, rather than according to the number of specific dysmorphisms associated with a given condition. Tissue regions include overall facial shape; the nose; the exterior of the mouth; the interior mouth such as tongue, dentition, and jaw shape; the form of the eyes; the midface (cheeks); and the ears. CFD was defined according to three or more malformations in distinct tissue regions, while SFD was defined as 1–2.

Phenotype interactions were analyzed across all congenital anomalies. Following analysis (see Results), CFD was selected as a defining secondary clinical feature for further genetic study, due both to clinical prominence and predictive ability in the presence of MCA syndromes. SFD were also selected as a secondary feature of interest for the sake of contrast, although were generally not predictive of MCA syndromes.

Conditions were also annotated for the presence of: neurodegeneration (confirmed according to literature search); brain atrophy; symptoms indicative of UMND, such as spasticity and hyperreflexia; and the presence of symptoms indicating the co-occurrence of 2 or more distinct movement disorders [UMND, lower motor neuron disease [LMND], disorders of the cerebellum, and disorders of the basal ganglia]. Because brain atrophy and motor disorders were positively associated with neurodegeneration (see Results), all of the above clinical features were collapsed into a single category, "neurodegenerative-like features (NLF)," for the purposes of further genetic study. CFD and NLF phenotypes were further substantiated using the Human Phenotype Ontology (HPO) database, and, when that was insufficient, the general literature in order to ensure reliability of MIM's Clinical Synopsis results for each of the conditions studied (6).

In order to study the association of the above clinical phenotypes with autism, epilepsy, and ID groups, conditions were subdivided according to the overlapping clinical phenotypes presented in **Figure 1**. This resulted in 18 unique gene sets, composed of 216 genes representing 212 different forms of monogenic ID (Additional File 2, "**Table_1**" tab).

Extended Gene Interaction Network

The GeneMANIA gene interaction database [genemania.org; (7)] was queried to discover additional known interactions for all 216 curated seed genes (Additional File 3, **Tables_15-17** tabs). The database provides a report containing several different interaction types including physical, genetic, pathway, predicted, co-localization, co-expression, and shared protein domains. All interactions were obtained from the "networks.data" link, but for the purpose of this study only genes with physical, genetic, or co-expression interactions were included in the finalized network (Additional File 3, **Tables 16-17** tabs).

Visualization and analysis of the network was conducted via Cytoscape (8). The "Network Analysis" Cytoscape app was used to determine topological parameters including node degree distribution fit to the power law, centrality, average connectivity, and clustering co-efficient. The clusterMaker MCL algorithm (granularity 1.2) in the Cytoscape clusterMaker app was used to identify highly connected gene clusters in the extended network (http://www.cgl.ucsf.edu/cytoscape/cluster/clusterMaker.shtml). The clusterMaker algorithm is a plugin that partitions clusters into "meta nodes," allowing interactive exploration of putative associations.

Kochinke et al. (3) reported that nearly half of all ID genes physically interact with one another, with more than a third forming a single large interactive network. Therefore, we tested if phenotype labels, assigned at the gene curation stage, and their extended interactions were non-randomly enriched in MCL gene clusters using the Fisher's Exact Test ($p < 0.001$). Label enrichment was performed on the observed clusters. Fisher's test addresses the potential relationship of these clusters without the need to randomize genes between clusters or create random networks for label enrichment analysis (see **Additional File 1**).

In addition, because there is a portion of genes within the autism gene group that are not currently contained within the syndromic category of the SFARI gene database and may therefore be suspect, we have also assessed nonrandom clustering of syndromic SFARI seed genes to illustrate that similar clustering still occurs with more stringent exclusion criteria. Our approach was identical as in the full network analysis, with the exception
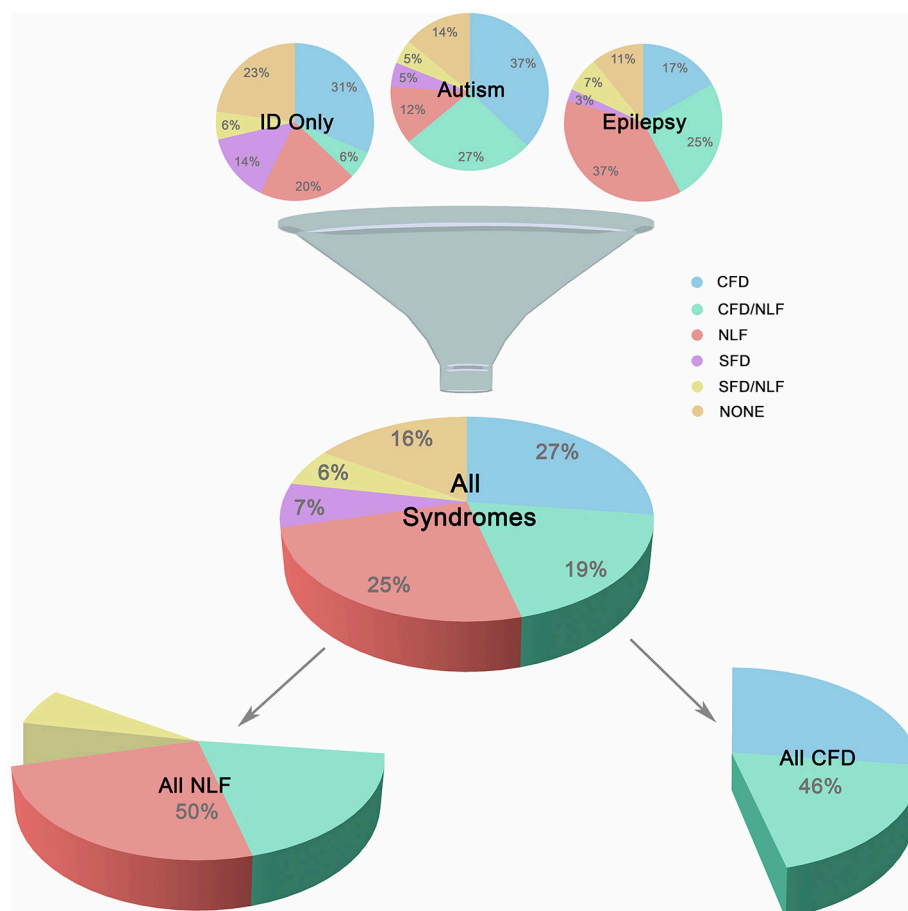
FIGURE 1 | Major Subgroups. Illustration showing breakdown of the three main groups (autism, epilepsy, and ID only) into smaller clinically-related subgroups. Subgroups were defined according to the presence of complex facial dysmorphia (CFD), neurodegenerative-like features (NLF), simple facial dysmorphia (SFD), or a lack of these same features (i.e., "none"). As shown in the lower portion of the image, CFD, and NLF overlapped ~20% of the time, although analyses indicate no clear relationship between the two sets of features, suggesting possible genetic pleiotropy when comorbid.

that only seed genes contained within the syndromic SFARI category were used (9).

Enrichr was used for functional enrichment analysis of each phenotypically-driven subgroup gene list ($N = 570$ total genes) for these annotation categories: Gene Ontology (GO), KEGG/WikiPathways, TRANSFAC/JASPAR Position Weight Matrix (PWM), MGI Mammalian Phenotype (MP), and Human Phenotype Ontology (HPO) (10). The Enrichr platform provides adjusted p-values using the Enrichr list randomization method, which is based on the Fisher's Exact test as well as Z-scores and combined scores for each annotation and is fully explained in Chen et al. (11). As a guide of possible collective gene function for each gene list, we used an adjusted significance p-value threshold of $p < 0.05$.

Statistical Analyses

For phenotype analyses, between- and within-group comparisons were performed using two-sample proportion Chi-square tests with a false discovery rate p-value adjustment (R pairwise.prop.test). Odds ratios with sample size adjustments

(12) were computed to examine associations amongst different congenital anomalies, as well as associations within NLF, the latter without sample size adjustment.

RESULTS

Clinical Features Common in Monogenic Intellectual Disability

Congenital anomalies are prominent features within monogenic forms of ID. In this study, the most common congenital anomaly reported was CFD, occurring in almost half of the conditions studied. Less frequent though still prominent congenital anomalies included (in order of frequency from most to least): microcephaly; organ malformations; brain malformations (visible via MRI); craniosynostoses and other cranial malformations; hand and foot malformations; skin and hair disturbances; SFD; macrocephaly; and limb malformations.

Not only was CFD the most common dysmorphism, it is also strongly associated with other types of dysmorphia

(z = 0.7813–7.1947, p < 0.001–0.014; OR = 2.19–13.461; OR CI = 1.261–6.266, 3.805–28.717), with the exception of specific brain malformations (z = 0.328–2.230, p = 0.055–0.814; OR = 1.159–1.744; 95% CI = 0.479–1.108, 2.654-4.892; see Additional File 3, "Table_11" tab for full results). One primary exception was the strong relationship between complete/partial agenesis of the corpus callosum (ACC) and CFD, suggesting significant etiological links (z = 2.993, p = 0.009; OR = 4.465; 95% CI = 95% CI = 1.762, 15.117). Microcephaly was also only very weakly predictive of MCA (aside from brain and cranium; z = 1.113–2.781, p = 0.014–0.369; OR = 1.393–2.19; 95% CI = 0.734–1.261, 2.498–3.948), and therefore facial dysmorphia were annotated separately from deviations in cranial volume in this study, despite the clinical tradition of grouping all craniofacial malformations together.

Neurodegeneration was also common occurring in ∼20% of ID and was an extremely strong predictive factor for the presence of brain atrophy and various movement disorders, especially UMND (z = 5.110, p < 0.001; OR = 8.61; 95% CI = 3.77, 19.68; see Additional File 3, "Table_13" tab). Another ∼30% of conditions displayed either brain atrophy, UMND, or multiple movement disorders (MMD; or some combination thereof) but are not currently recognized as classical neurodegenerative disorders. However, because of their strong interrelationship suggesting linked etiologies, neurodegeneration, brain atrophy, UMND, and MMD were combined under a single heading, "neurodegenerative-like features" or "NLF," for the purposes of this study (z = 4.69–8.73, p < 0.001; OR = 4.64–56.53; 95% CI = 2.44–22.86, 8.82–139.82). NLF occurred in 50% of the conditions studied, overlapping CFD ∼19% of the time. Despite this large overlap, in the majority of cases these features did not co-occur and, overall, exhibited no statistically significant relationship with one another (p = 0.515; OR = 0.834; 95% CI = 0.483, 1.440). This suggests that while these phenotypes may co-occur in a large minority of these conditions, they are nevertheless unique symptom clusters and may instead reflect genetic pleiotropy (i.e., a single gene influences 2 or more unrelated traits) when comorbid (see **Figure 1**).

Previous results by Casanova et al. (2), utilizing a near-identical dataset, indicate a divergence in functional gene enrichment in ID according to autism and epilepsy comorbidities. Here we report additional clinical phenotype enrichment that varies according to these behavioral/neurological comorbidities. Namely, the autism group was significantly enriched for the presence of CFD (61% vs. 37–40%), suggesting many rare autism syndromes may be dysplastic in nature (χ^2 = 5.42-6.38, p = 0.03) (13–15) (see Additional File 3, "Table_12" tab). Meanwhile, the epilepsy group was similarly enriched for NLF (68% vs. 31–39%), indicating some form of cell stress may be involved in these IDs (χ^2 = 11.18–19.63, p < 0.001) (16, 17). There are additional clinical phenotypes that vary according to group, such as enrichment of neocortical malformations (identified by MRI; z = 4.4566, p < 0.001, OR = 6.4289; 95% CI = 2.836, 14.573) and microcephaly (z = 2.8656, p = 0.011, OR = 2.2778; 95%

CI = 1.297–4.000) in the epilepsy group. (For full results, see Additional File 3, "**Table_14**" tab).

ID Genes Cluster According to Phenotype

Using a list of 216 seed genes divided according to our phenotypes of interest, we have identified an additional 354 interacting genes using the GeneMANIA gene interaction database (genemania.org). This resulted in the formation of 17 unique gene sets composed of a total of 1,195 genes upon which to perform gene module detection according to all protein-protein interaction (PPI), genetic interaction, and mRNA co-expression connections. One of the autism subgroups failed to show any significant intracluster interactions and therefore was not included in the cluster and functional enrichment analyses.

As can be seen in **Figure 2A**, the seed genes plus PPI, genetic interacting, and co-expression loci form 17 sets of relatively non-overlapping gene clusters, constituting tight interaction/coexpression networks. Thirteen of the 17 gene sets form particularly tight clusters and are interconnected via specific hub nodes (**Figures 2B–E**). (For detailed views of the full cluster network, see Additional File 1, **Figure 1**) Overall network degree distribution modestly fits the power law distribution (r = 0.776), indicating the network trends toward scale free behavior (i.e., clustering is non-random and potentially reflects a real gene interaction networks). Other topological parameters of interest include: clustering coefficient = 0.342; centralization = 0.034; and average connectivity = 5.287. SFARI-only syndromic genes likewise formed similar non-random clusters (r = 0.687), indicating the robustness of the autism results overall (**Figure 2F**). Overall, these results indicate that our genes of interest form nonrandom interaction clusters that naturally fall within clusters according to the phenotypes of interest (CFD, NLF, SFD, etc.), suggesting that these phenotypes are strong predictors to which gene cluster, if any, a given gene belongs.

Within the main network, more than half of the gene sets are interconnected via 10 hub nodes (genes onto which the major clusters converge; **Figures 2B–E**). The Rett syndrome-associated gene, *MECP2*, for instance, forms a hub connecting half of the autism-related gene sets, particularly those with secondary clinical features of CFD, combined CFD/NLF, and pure NLF, as well as connecting one of the ID group clusters (**Figure 2B**). In addition, *MECP2* remains an important hub node in the SFARI-only syndromic network, continuing to link CFD, CFD/NLF, and NLF autism subgroups. MECP2's nature as a semi-ubiquitous repressor of long genes, which typifies many neural genes, places it in a key position to regulate development of the central nervous system and thus to potentially interact with many of the genes presented here (18).

Likewise, the Fragile X syndrome-associated gene, *FMR1*, forms a major hub connecting the same clusters as *MECP2* within the main network, although this result is not maintained within the abbreviated SFARI network (**Figures 2B, F**). Interestingly, like MECP2, there is some evidence to suggest that FMRP specifically targets gene products translated from long genes,
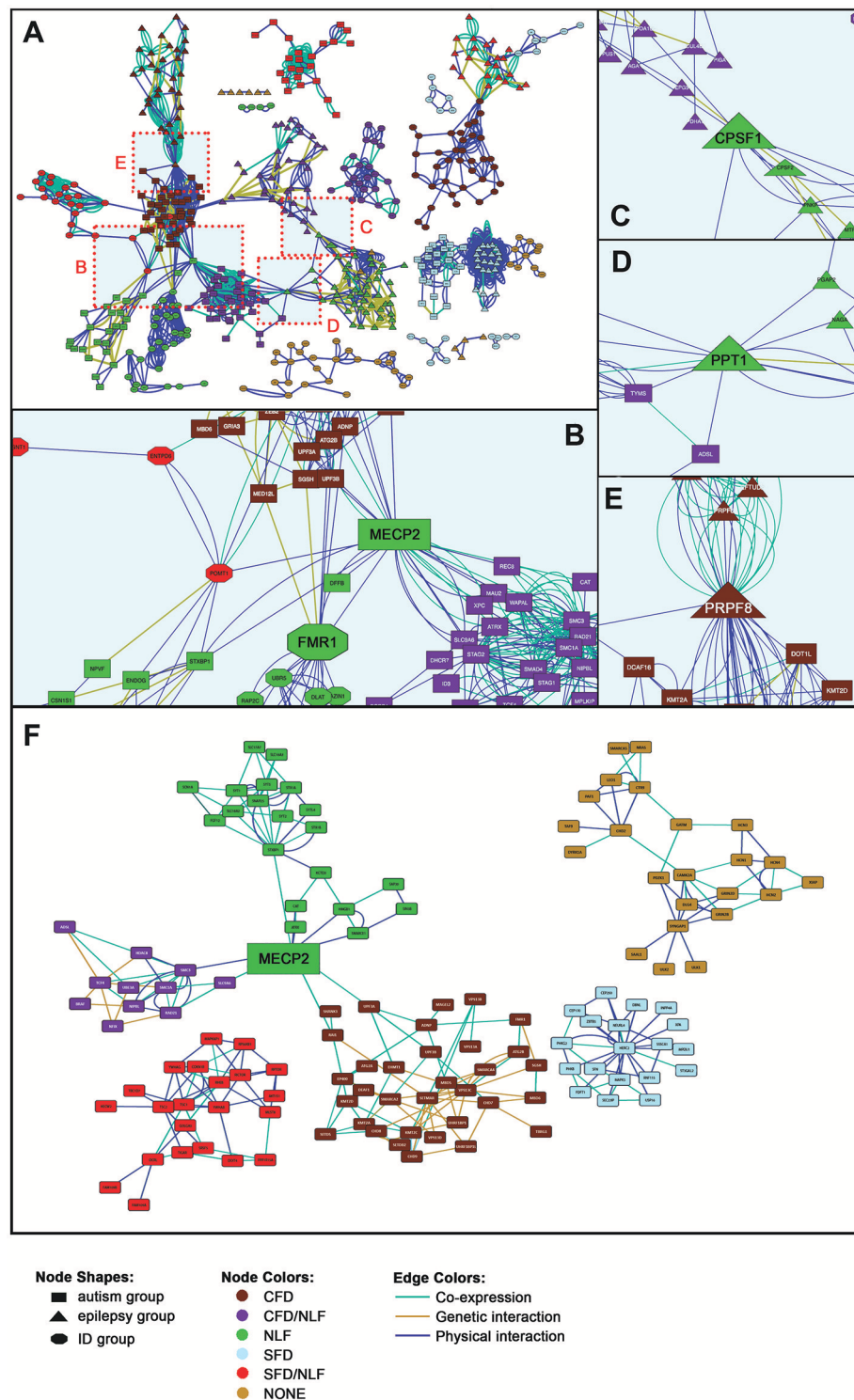
FIGURE 2 | Gene Interaction Network. **(A)** Full gene interaction network. (See **Supplementary Figure 1** for detailed gene network). **(B)** Autism-linked *MECP2* and *FMR1* hubs. **(C)** Epilepsy-linked *CPSF1* hub. **(D)** Epilepsy- and autism-linked *PPT1* hub. **(E)** Epilepsy- and autism-linked *PRPF8* hub. **(F)** Syndromic SFARI gene interaction network. (See **Supplementary Figure 2**, for detailed gene network).

suggesting MECP2 and FMRP may regulate different points along many of the same pathways (19, 20).

Two other major hubs in the main network are involved in mRNA processing: *CPSF1*, which is involved in 3′ processing of mRNA, and *PRPF8*, which acts as a scaffold for spliceosomal complexes and snRNA. As shown in **Figure 2C**, *CPSF1* connects two epilepsy modules with features of NLF; meanwhile, *PRPF8* (**Figure 2E**) connects epilepsy/CFD (EPI/CFD) with autism/CFD (AUT/CFD). As we shall see in the following section, a number of the epilepsy clusters are enriched for mRNA processing. Interestingly, PRPF8 is also essential for sister chromatid cohesion, making it therefore surprising that it forms a hub with AUT/CFD rather than AUT/CFD/NLF, as we shall see in the following section (21).

Finally, the hub, *PPT1*, whose mutation is responsible for the neurodegenerative and lethal condition, Neuronal Ceroid Lipofuscinosis 1, links the EPI/NLF and AUT/CFD/NLF modules (**Figure 2D**). As a glycoprotein involved in catabolism of lipid-modified proteins and a regulator of heat shock proteins, its loss results in excessive generation of reactive oxygen species (ROS) (22, 23). *PPT1*'s role as a hub linking EPI/NLF and AUT/CFD/NLF can potentially be viewed in light of the roles chronic ROS play in the synaptic impairment that ultimately leads to a host of neurodegenerative disorders (24).

Functional Enrichment Trends in Gene Subgroups

The genes sets in some phenotype subgroups showed little obvious trends in functional enrichment, such as EPI/SFD/NLF and ID/SFD. This may be a reflection of etiological diversity in these respective modules and/or the inadequacy of current platforms in estimating disparate functional relationships.

Other groups, however, appeared to show distinctive functional trends, particularly those associated with CFD. For instance, the AUT/CFD gene subgroup is strongly enriched for processes relating to *chromatin modification* ($z = -2.40$, $p < 0.001$), *histone modification* ($z = -2.39$, $p < 0.001$), *methylation* ($z = -2.45$, $p = 0.007$), *transcription factor binding* ($z = -2.16$, $p = 0.026$), and is localized to the nucleus (*nucleolus*; $z = -2.21$, $p = 0.002$; **Figure 3A**). All of these enrichments strongly implicate AUT/CFD genes in the regulation of gene expression and, ultimately, organ and tissue development.

More than a third of AUT/CFD genes are also transcriptional targets for Wilms tumor suppressor 1 (Wt1), a transcription factor that helps regulate cell development and survival ($z = -1.62$, $p = 0.036$). In addition, almost half of AUT/CFD genes are transcriptional targets of Lef1, a positive regulator of the canonical Wnt pathway, which is itself a foundational network involved in organ and tissue morphogenesis ($z = -1.48$, $p = 0.036$) (25).

In contrast, the EPI/CFD gene subgroup, though likewise relegated to the *nucleoplasm* ($z = -2.16$, $p < 0.001$) and involved in *histone modification* ($z = -2.39$, $p < 0.001$), is also enriched for processes involved in *mRNA processing* ($z = -2.37$, $p = 0.003$) and the *spliceosomal complex* ($z = -2.15$, $p < 0.001$).

Similarly, EPI/CFD/NLF was enriched for *RNA polyadenylation* ($z = -2.66$, $p <= 0.003$). Many of these functions concern post-transcriptional stages of gene expression regulation, while enrichments associated with AUT/CFD involve regulation of transcription itself. ID/CFD meanwhile is enriched in *kinase binding* ($z = -2.55$, $p = 0.011$) and *chromatin binding* ($z = -2.45$, $p = 0.031$), while ID/CFD/NLF is enriched for *protein glycosylation* ($z = -2.34$, $p < 0.001$) and is localized to the *Golgi membrane* ($z = -2.29$, $p > 0.001$) and the *lysosome* ($z = -2.31$, $p > 0.001$). All CFD enrichments strongly implicate the role of gene expression regulators in the pathophysiology of complex facial dysmorphia.

When comparing the two autism CFD subgroups to one another, we found that both AUT/CFD and AUT/CFD/NLF are involved in *chromatin binding* ($z = -2.47$, $p < 0.001$). However, AUT/CFD/NLF is also strongly enriched for processes involving the *mitotic cell cycle* ($z = -2.30$, $p < 0.001$) and *sister chromatid cohesion* ($z = -2.67$, $p < 0.001$), which is entirely missing from the AUT/CFD gene subgroup (**Figure 3B**).

In contrast to its CFD counterparts, ID/NLF was enriched in *hydrogen ion membrane transporter activity* ($z = -2.34$, $p = 0.003$) and was involved in the *respiratory chain* ($z = -2.59$, $p < 0.001$) within mitochondria. In addition, it displayed pathway enrichment in relation to *Parkinson's disease* ($z = -1.77$, $p < 0.001$), *Huntington's disease* ($z = -1.85$, $p = 0.002$), and *Alzheimer's disease* ($z = -1.72$, $p = 0.015$). The EPI/NLF gene subgroup, in contrast, was enriched for a variety of terms, such as *myelin sheath* ($z = -2.89$, $p < 0.001$), *mRNA polyadenylation* ($p = 0.007$, $z = -2.71$), *carboxylic acid biosynthetic process* ($z = -2.35$, $p = 0.007$), and *protein folding* ($z = -2.31$, $p = 0.007$), suggesting that despite strong intracluster connectivity, the etiology of the EPI/NLF subgroup is comparatively diverse. Meanwhile, AUT/NLF was modestly enriched for *membrane depolarization* ($z = -2.26$, $p = 0.005$), *regulation of postsynaptic membrane potential* ($z = -2.09$, $p = 0.009$), and *regulation of synaptic plasticity* ($z = -2.15$, $p = 0.027$) (**Figure 3C**). This indicates that disturbances to synaptic proteins in autism could be related to symptoms of NLF, an idea that may be worthy of further exploration in relation to autistic regression given the role of synaptic impairment in the etiologies of many neurodegenerative disorders (26). Interestingly, recent research indicates that autistic individuals with gene disrupting mutations in postsynaptic density genes are more likely to experience autistic regression than individuals with mutations in genes of other functional classes (27) (see Additional File 4, "Table_19" tab for more extensive enrichment results by subgroup).

DISCUSSION

The present study provides evidence of genotype-phenotype correlations throughout multiple ID subsets. In particular, the presence of autism (with or without epilepsy), epilepsy (without autism), CFD, and NLF appear to be general predictors of associated gene function. The AUT/CFD gene subgroup, for instance, is linked with genes localized to the nucleus.
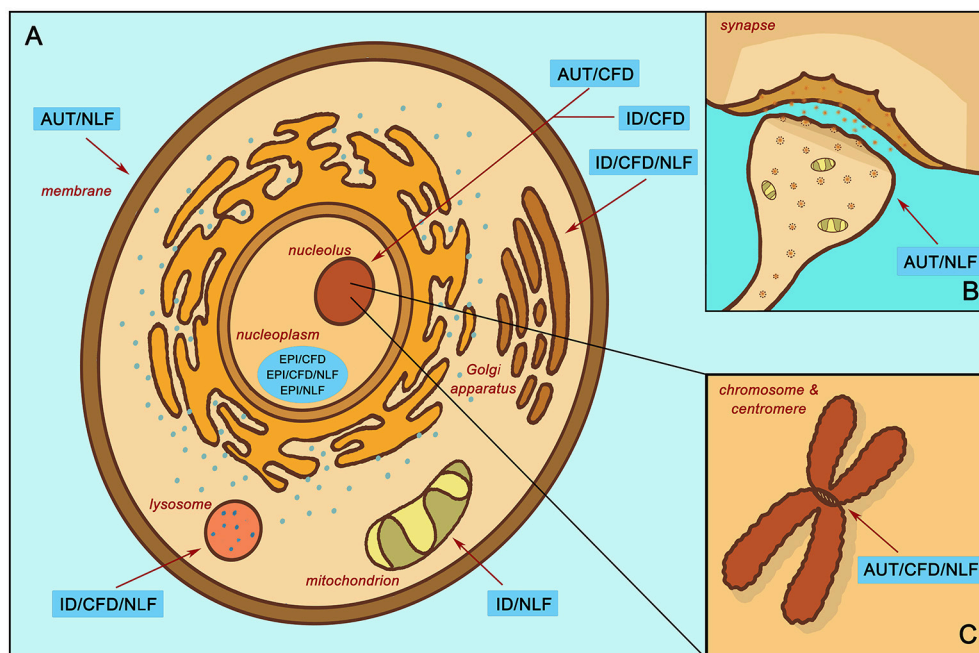
FIGURE 3 | Enrichment localization according to subgroup. **(A)** Localized enrichment according to subgroups within the main body of the cell. **(B)** Subgroup enrichment within the synapse. **(C)** Subgroup enrichment at the chromosome and centromere. Intellectual disability with autism and with/without epilepsy (AUT); intellectual disability with epilepsy and without autism (EPI); intellectual disability without autism or epilepsy (ID); complex facial dysmorphia (CFD); neurodegenerative-like features (NLF).

These genes are involved in chromatin modifications; histone modifications; methylation; transcription factor binding; and are key in regulating embryonic development. In contrast, gene products of the AUT/CFD/NLF subgroup are likewise localized to the nucleus and involved in chromatin binding, but are typically involved in regulation of the cell cycle and sister chromatid segregation. Nuclear localization appears to be a strong risk factor in the developments of both autism and CFD in these subgroups; however, cell cycle involvement may provide an additional risk for NLF as we see in many classical neurodegenerative diseases (28).

Nuclear localization, in general, seems to be a strong predictive factor for the presence of CFD and, more weakly, SFD, although specific functional enrichments vary with the presence of autism, epilepsy, and NLF accordingly. AUT/CFD, EPI/CFD, and ID/CFD all tend to be localized to the nucleus and are at least modestly enriched in processes relating to chromatin binding and modifications. The important roles these gene expression regulators play in organogenesis likely underlie their associations with complex facial dysmorphia and other physical features.

We have also identified a number of major hubs within the clusters analysis, linking otherwise non-overlapping gene sets. Although functional relevance of some of the hubs is currently uncertain, several of the autism hubs are already major foci within the current literature. *MECP2*, the primary gene responsible for Rett syndrome, and *FMR1*, the gene associated with Fragile X syndrome, have both received considerable

attention and *FMR1* in particular has been previously identified as a major pathway of interest in the pathophysiology of autism (29–31). From the clinical perspective, both Rett and Fragile X syndromes share strong associations with autism. Most girls with Rett's present with transient autistic features at a characteristic stage within the prolonged regressive period. Meanwhile, approximately half of individuals with Fragile X present with an enduring autism phenotype (32). Despite their unique clinical phenotypes, our data indicate that both *MECP2* and *FMR1* form foundational pathways underlying autism risk and may overlap in part due to their roles as major regulators of neuronal gene expression and protein translation.

Finally, we have shown that specific secondary clinical phenotypes exhibit strong association with ID according to comorbidities with autism and epilepsy. For instance, the high rates of CFD and MCA in rare autism syndromes are strongly suggestive of a common biology despite genotypic variation. Despite the dearth of obvious brain malformations reported in our autism dataset, the high prevalence of microscopic dysplastic foci in idiopathic autism tends to validate this point (13, 15, 33, 34).

Our results have also shown that close to half of the conditions studied here exhibit features reminiscent of neurodegeneration, although only about a fifth are officially recognized as "neurodegenerative disorders." The occurrence of NLF is particularly prominent in the epilepsy group, although functional enrichment of the ID/NLF subgroup is

more aligned with processes of classic neurodegeneration. However, these data suggest that: (1) postmortem analysis of neurodegeneration may be understudied in some of these conditions, and/or (2) proteopathies with obvious inclusions may comprise only a subset of a broader range of neurodegenerative-like disorders, which have subtler, more complex etiologies with progressions that differ from the typical dementias that occur in later life. In support of this, Sarnat and Flores-Sarnat (35) have recently addressed such concepts within the context of "infantile tauopathies," such as tuberous sclerosis and focal cortical dysplasia 2. At present, recognized infantile proteopathies include only those conditions resultant from MTOR overexpression, a known mechanism of neurodegeneration (36). However, given the range of inclusion bodies associated with adult forms of neurodegeneration and senile dementias, the list of infantile proteopathies is likely to expand in future and may eventually be recognized as a major cause of some developmental and intellectual disabilities (35, 37).

Current Limitations and Future Research

Given the nature of the MIM database, whose purpose is intended to summarize genetic and syndromic disease states, research procedures have varied across individual studies that compose the MIM. The state of the MIM is also potentially incomplete, leading to gaps in our dataset. For these reasons, our results must be extrapolated cautiously, requiring further investigations at the clinical and molecular levels. However, although the MIM data may be incomplete, we feel the current dataset provides an excellent overview of the major gene-phenotype trends that are currently available for data mining. In addition, in order to limit the extent of Type I errors, we have elected to study clinical phenotypes whose medical evaluations are standardized across health fields, ensuring that the clinical data reported here may be relatively reliable (38, 39).

One major exception to this is the field of autism diagnostics, which has changed significantly over the past 25 years. A majority (59%) of seed genes used in this analysis is included within the syndromic category of the SFARI Gene Database, supporting their diagnostic reliability in this study. While we are unable to directly address diagnostic reliability of the remainder of autism genes, we instead assessed robustness of non-random clustering of this subset of syndromic SFARI genes, which like the larger autism gene group exhibited similar clustering. This supports our general findings as well as potential risk status of non-SFARI genes included in this study.

Another limitation of the study is the question of its applicability to a broader range of conditions. The study of severely affected individuals with rare genetic syndromes is a common approach to investigating human illness in order to better understand complex conditions. However, such assumptions are based on symptom similarity rather than biological evidence. As such, our results may not apply to forms of ID, autism, and epilepsy that lack strong genetic roots. However, recent work by Rossi et al. (40) suggest that even those patients with autism but without obvious syndromes often harbor potentially deleterious variants in many of the same genes studied here. Further lines of research will continue to address potential cross-applicability of the data presented here. In the meantime, we believe the subgroups we've described can provide a platform for the further elucidation of common denominator pathways and the regulatory networks underlying these complex conditions, leading to the subtyping of disorders.

CONCLUSIONS

The present study provides strong evidence that ID-associated phenotypes cluster according to related gene function. Specifically, gene modules form according to autism, epilepsy, CFD, and NLF comorbidities. Future research will help to delineate these subgroups in greater detail, as well as determine whether additional genotype-phenotype correlations exist in these and related datasets.

AUTHORS NOTE

Emily L. Casanova, Ph.D., is a postdoctoral fellow in Biomedical Sciences at the University of South Carolina Greenville Medical School with training in developmental and molecular biology and a focus on neurodevelopmental disorders. Zachary Gerstner is a graduate student in Genetics and Biochemistry at Clemson University with training in microbiology, genetics, and computer science. Julia L. Sharp, Ph.D., is an associate professor and director of the Graybill Statistical Laboratory at Colorado State University. She is an applied statistician with expertise in experimental design and mixed models. Manuel F. Casanova, MD, is the SmartState Endowed Chair in Childhood Neurotherapeutics for the University of South Carolina and the Greenville Health System. His clinical and research focus concerns neurodevelopmental disorders with an emphasis on autism. F. Alex Feltus, Ph.D., is an associate professor in the Department of Genetics and Biochemistry at Clemson University with 23 years of broad experience in bioinformatics, systems genetics, and genomics.

AVAILABILITY OF DATA AND MATERIAL

All data generated and analyzed during this study are included in this published article and its Supplementary Information files. Full statistical results are also available, as well as additional results that support the main text, such as Additional File 4, "ID_Comparison" and "No_Cases" tabs.

AUTHOR CONTRIBUTIONS

EC and FF conceived the study. EC curated the phenotypic data and JS performed statistical analyses on that data. ZG and FF performed cluster and enrichment analyses

and associated statistics. MC provided expertise on autism, intellectual disability, and epilepsy and was integral in helping design the study as well as interpret results. All authors contributed substantially to the drafts and have read and approved the final manuscript.

ACKNOWLEDGMENTS

A preprint of this article is available through bioRxiv doi: https://doi.org/10.1101/220608.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2018.00535/full#supplementary-material

REFERENCES

1. Houle D, Govindaraju DR, Omholt S. Phenomics: the next challenge. *Nat Rev Genet*. (2010) 11:855–66. doi: 10.1038/nrg2897
2. Casanova EL, Sharp JL, Chakraborty H, Sumi NS, Casanova MF. Genes with high penetrance for syndromic and non-syndromic autism typically function within the nucleus and regulate gene expression. *Mol Autism*. (2016) 7:18. doi: 10.1186/s13229-016-0082-z
3. Kochinke K, Zweier C, Nijhof B, Fenckova M, Cizek P, Honti F, et al. Systematic phenomics analysis deconvolutes genes mutated in intellectual disability into biologically coherent modules. *Am J Hum Genet*. (2016) 98:149–64. doi: 10.1016/j.ajhg.2015.11.024
4. OMIM. *Online Mendelian Inheritance in Man*. Baltimore, MD: McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (2016).
5. Amberger JS, Bocchini CA, Schiettecatte F, Scott AF, Hamosh A. OMIM. org: Online Mendelian Inheritance in Man (OMIM(R)), an online catalog of human genes and genetic disorders. *Nucleic Acids Res*. (2015) 43:D789–98. doi: 10.1093/nar/gku1205
6. Kohler S, Vasilevsky NA, Engelstad M, Foster E, McMurry J, Ayme S, et al. The human phenotype ontology in 2017. *Nucleic Acids Res*. (2017) 45:D865–76. doi: 10.1093/nar/gkw1039
7. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res*. (2010) 38:W214–20. doi: 10.1093/nar/gkq537
8. Cline MS, Smoot M, Cerami E, Kuchinsky A, Landys N, Workman C, et al. Integration of biological networks and gene expression data using Cytoscape. *Nat Protoc*. (2007) 2:2366–82. doi: 10.1038/nprot.2007.324
9. Abrahams BS, Arking DE, Campbell DB, Mefford HC, Morrow EM, Weiss LA, et al. SFARI Gene 2.0: a community-driven knowledgebase for the autism spectrum disorders (ASDs). *Mol Autism*. (2013) 4:36. doi: 10.1186/2040-2392-4-36
10. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res*. (2016) 44:W90–97. doi: 10.1093/nar/gkw377
11. Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics* (2013) 14:128. doi: 10.1186/1471-2105-14-128
12. Jewell NP. On the bias of commonly used measures of association for 2x2 tables. *Biometrics* (1986) 42:351–8. doi: 10.2307/2531055
13. Casanova MF, El-Baz AS, Kamat SS, Dombroski BA, Khalifa F, Elnakib A, et al. Focal cortical dysplasias in autism spectrum disorders. *Acta Neuropathol Commun*. (2013) 1:67. doi: 10.1186/2051-5960-1-67
14. Jones KL, Adam MP. Evaluation and diagnosis of the dysmorphic infant. *Clin Perinatol*. (2015) 42:243-261, vii-viii. doi: 10.1016/j.clp.2015.02.002
15. Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Marchi E, et al. The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. *Acta Neuropathol*. (2010) 119:755–70. doi: 10.1007/s00401-010-0655-4
16. Lindholm D, Wootz H, Korhonen L. ER stress and neurodegenerative diseases. *Cell Death Differ*. (2006) 13:385–92. doi: 10.1038/sj.cdd.4401778
17. Mancuso C, Scapagini G, Curro D, Giuffrida Stella AM, De Marco C, Butterfield DA, et al. Mitochondrial dysfunction, free radical generation and

cellular stress response in neurodegenerative disorders. *Front Biosci*. (2007) 12:1107–23. doi: 10.2741/2130
18. Kinde B, Wu DY, Greenberg ME, Gabel HW. DNA methylation in the gene body influences MeCP2-mediated gene repression. *Proc Natl Acad Sci USA*. (2016) 113:15114–9. doi: 10.1073/pnas.1618737114
19. Ouwenga RL, Dougherty J. Fmrp targets or not: long, highly brain-expressed genes tend to be implicated in autism and brain disorders. *Mol Autism*. (2015) 6:16. doi: 10.1186/s13229-015-0008-1
20. Zylka MJ, Simon JM, Philpot BD. Gene length matters in neurons. *Neuron* (2015) 86:353–5. doi: 10.1016/j.neuron.2015.03.059
21. Kurtovic-Kozaric A, Przychodzen B, Singh J, Konarska MM, Clemente MJ, Otrock ZK, et al. PRPF8 defects cause missplicing in myeloid malignancies. *Leukemia* (2015) 29:126–36. doi: 10.1038/leu.2014.144
22. Kim SJ, Zhang Z, Saha A, Sarkar C, Zhao Z, Xu Y, et al. Omega-3 and omega-6 fatty acids suppress ER- and oxidative stress in cultured neurons and neuronal progenitor cells from mice lacking PPT1. *Neurosci Lett*. (2010) 479:292–6. doi: 10.1016/j.neulet.2010.05.083
23. Wandinger SK, Suhre MH, Wegele H, Buchner J. The phosphatase Ppt1 is a dedicated regulator of the molecular chaperone Hsp90. *Embo J*. (2006) 25:367–76. doi: 10.1038/sj.emboj.7600930
24. Guo ZH, Mattson MP. Neurotrophic factors protect cortical synaptic terminals against amyloid and oxidative stress-induced impairment of glucose transport, glutamate transport and mitochondrial function. *Cereb Cortex* (2000) 10:50–7. doi: 10.1093/cercor/10.1.50
25. Sokol S. A role for Wnts in morpho-genesis and tissue polarity. *Nat Cell Biol*. (2000) 2:E124–5. doi: 10.1038/35017136
26. Wishart TM, Parson SH, Gillingwater TH. Synaptic vulnerability in neurodegenerative disease. *J Neuropathol Exp Neurol*. (2006) 65:733–9. doi: 10.1097/01.jnen.0000228202.35163.c4
27. Goin-Kochel RP, Trinh S, Barber S, Bernier R. Gene disrupting mutations associated with regression in autism spectrum disorder. *J Autism Dev Disord*. (2017) 47:3600–7. doi: 10.1007/s10803-017-3256-4
28. Lee HG, Casadesus G, Zhu X, Castellani RJ, McShea A, Perry G. Cell cycle re-entry mediated neurodegeneration and its treatment role in the pathogenesis of Alzheimer's disease. *Neurochem Int*. (2009) 54:84–8. doi: 10.1016/j.neuint.2008.10.013
29. Hormozdiari F, Penn O, Borenstein E, Eichler EE. The discovery of integrated gene networks for autism and related disorders. *Genome Res*. (2015) 25:142–54. doi: 10.1101/gr.178855.114
30. Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, et al. De novo gene disruptions in children on the autistic spectrum. *Neuron* (2012) 74:285–99. doi: 10.1016/j.neuron.2012.04.009
31. Parikshak NN, Luo R, Zhang A, Won H, Lowe JK, Chandran V, et al. Integrative functional genomic analyses implicate specific molecular pathways and circuits in autism. *Cell* (2013) 155:1008–21. doi: 10.1016/j.cell.2013.10.031
32. Percy AK. Rett syndrome: exploring the autism link. *Arch Neurol*. (2013) 68:985–9. doi: 10.1001/archneurol.2011.149
33. Bailey A, Luthert P, Dean A, Harding B, Janota I, Montgomery M, et al. A clinicopathological study of autism. *Brain* (1998) 121(Pt 5):889–905. doi: 10.1093/brain/121.5.889
34. Casanova MF, Buxhoeveden DP, Switala AE, Roy E. Minicolumnar pathology in autism. *Neurology* (2002) 58:428–32. doi: 10.1212/WNL.58.3.428

35. Sarnat HB, Flores-Sarnat L. Infantile tauopathies: hemimegalencephaly; tuberous sclerosis complex; focal cortical dysplasia 2; ganglioglioma. *Brain Dev*. (2015) 37:553–62. doi: 10.1016/j.braindev.2014.08.010

36. Pei JJ, Hugon J. mTOR-dependent signalling in Alzheimer's disease. *J Cell Mol Med*. (2008) 12:2525–32. doi: 10.1111/j.1582-4934.2008.00509.x

37. Kovacs GG. *Neuropathology of Neurodegenerative Diseases: A Practicel Guide*. Cambridge: Cambridge University Press (2015).

38. Fahn S, Jankovic J, Hallett M. *Principles and Practice of Movement Disorders: Expert Consult*. Edinburgh: Elsevier (2011).

39. Hall J, Allanson J, Gripp K, Slavotinek A. *Handbook of Physical Measurements*. Oxford: Oxford University Press (2006).

40. Rossi M, El-Khechen D, Black MH, Hagman KF, Tang S, Powis Z. Outcomes of diagnostic exome sequencing in patients with diagnosed or suspected autism spectrum disorder. *Pediatr Neurol*. (2017) S0887–994:30572–0. doi: 10.1016/j.pediatrneurol.2017.01.033

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Increased Left Inferior Temporal Gyrus Was Found in Both Low Function Autism and High Function Autism

Jia Cai[1], Xiao Hu[1], Kuifang Guo[1], Pingyuan Yang[1], Mingjing Situ[1] and Yi Huang[1,2,3*]

[1] Mental Health Center, West China Hospital of Sichuan University, Chengdu, China, [2] Psychiatric Laboratory, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, China, [3] Brain Research Center, West China Hospital of Sichuan University, Chengdu, China

Previous neuroimaging studies of autism spectrum disorder (ASD) have focused on subjects with IQ > 70 or ASD without considering IQ levels. It remains unclear whether differences in brain anatomy in this population are associated with variations in clinical phenotype. In this study, 19 children with low functioning autism (LFA) and 19 children with high functioning autism (HFA) were compared with 27 healthy controls (HC). We found increased gray matter volume (GMV) in the left inferior temporal gyrus in subjects with both HFA and LFA and increased GMV of left middle temporal gyrus BA21 was found only in the LFA group. A significant negative correlation was found between the left inferior temporal gyrus (LITG) and the score of repetitive behavior in the HFA group.

Keywords: low functioning autism, high functioning autism, inferior temporal gyrus, VBM, structural MRI

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that presents with social communication deficits and restricted, repetitive patterns of behavior, interests, and activities (1). ASD is reported to affect 1 in 59 individuals according to the last CDC update of autism's estimated prevalence (2). Among the patients, more than 80% are male and about 70–80% have intelligence disabilities (ID) (3, 4). The symptoms of ASD negatively impact the patients' social outcomes and consequently they become a tremendous burden on both the family and community (5).

According to the 10th revision of the International Statistical Classification of Diseases (ICD-10) by the World Health Organization (WHO) in 2016, there is clinical heterogeneity within this disorder, including cognitive functioning, behaviors, and sensory-motor functioning. Subjects with an IQ < 70 are typically defined in the literature as having low functioning autism (LFA) and those with an IQ ≥ 70 are defined as having high functioning autism (HFA). Those who have normal or even superior intellectual abilities and have no history of developmental language delay are classified as having Asperger syndrome (6, 7). Because of its heterogeneity and frequent comorbidities (epilepsy, intelligence disability, or other mental disorders), it is difficult to elucidate the underlying biological causes of ASD (8, 9). It has been hypothesized that environmental, immunological, and genetic factors all were involved in its etiology (10, 11). Exploring the biological markers for the heterogeneous phenotypes of ASD, using techniques such as magnetic resonance imaging (MRI) of the brain, can help us to better understand the underlying disease mechanisms and improve patient outcomes by targeted interventions.

Several Voxel-based morphometry (VBM) studies (using an MRI technique to investigate focal differences in brain anatomy) have demonstrated abnormalities in the right fusiform gyrus,

right temporal-occipital lobe, left parietal lobe, left middle temporal gyrus, and superior temporal gyrus in patients with ASD, but the conclusions have been inconsistent (12–15). Furthermore, abnormalities were also found in subcortical areas, including increased volume in the caudate nucleus and hippocampus, and decreased volume in the cerebellum and basal ganglia (16–18). Evidence from HFA patients has also shown correlations between clinical symptoms, cognitive functioning, and abnormal regional brain volume. For example, caudate nucleus, parietal lobe, and temporal lobe activity are involved in the repetitive behaviors of ASD patients (19), and the cerebellum is associated with the communication deficits and repetitive behaviors (20). A recent study found that increased gray matter volume (GMV) in the left temporal gyrus was correlated with social interactions and frontal lobe changes were associated with more severe repetitive symptoms in adults with ASD (21). Three studies showed that ASD patients may have an abnormal structure of the "social brain" that may be associated with development of social communication disorder (22–24). The relationship between the autistic symptoms, cognitive functioning and abnormal brain volumes was not clear and most of the studies examining neuroimaging features and manifest behaviors have been conducted in HFA subjects, making it unclear as to whether the results can be generalized to LFA subjects who account for nearly 80% of the ASD population.

Previously, we found four VBM studies which took the individuals' cognitive function into account by separating the whole sample into LFA and HFA. Toal et al. (25) found that adults with ASD had significant reductions in the gray-matter volume of the medial temporal, fusiform, and cerebellar regions that varied with clinical phenotype. Adult patients with autism also demonstrated an increase in gray matter in the frontal and temporal lobes (25). An additional three studies were focused on children with ASD. Riva et al. (18) reported that decreased GMV regions were found in the basal forebrain, nucleus accumbens, and cerebellar hemispheres in the LFA group. Furthermore, the reduction of GMV in the Vermis and CRUS-II was associated with social and interaction deficits in LFA patients (26). Another recent study found increased GMV in the left superior temporal gyrus and left postcentral gyrus in 3- to 7-year-old children with LFA, but no correlation was found between the abnormal GMV and their ASD symptoms (27). In addition, one of two earlier studies that did not use VBM methods reported that cerebral GMV was enlarged in both HFA and LFA compared to controls (28). A larger whole-brain volume was also reported in LFA but not in HFA patients who were 1.9- to 5.2-years old (29). Using semi-automated image analyses, it was reported that autistic individuals have a significantly smaller corpus callosum but not cerebellar area (30). The results of these studies were inconsistent, probably because some researchers recruited only adults or only LFA, while others included both children and adults, making comparisons difficult. The second reason is that some of the research used manual or semi-automated imaging analysis programs which can only be applied to specific brain regions. However, the demonstrated differences in brain anatomy may indicate true biological variability in ASD that accurately reflects its clinical heterogeneity. In any case, to our knowledge

no previous studies have been carried out using VBM-DARTEL to explore the different patterns of brain anatomy in children with ASD with different IQ levels.

In summary, there are relatively few VBM studies of children with ASD distinguishing HFA from LFA. It remains unknown whether there are differences in brain anatomy associated with the variable clinical phenotypes, and if so, how those variations in brain structure are related to the symptoms of ASD. To address these questions, it will be necessary to recruit relatively large samples of subjects, acquire and analyze the data in a similar manner, and compare the brain anatomy of these subjects across the full spectrum of autism, to reveal any correlations between the brain structure and autistic symptoms that may exist.

One of the aims of our study was to compare the GMV among HFA, LFA, and HC by using the method of VBM-DARTEL; the second aim was to explore the relationship between the clinical phenotype of ASD and autistic symptoms. We hypothesized that LFA has more regions of abnormal brain anatomy than HFA and we assumed that there may be some correlations between specific abnormal brain regions and the severity of autistic symptoms.

MATERIALS AND METHODS

Subjects

All subjects were recruited during a 4-year period (2013–2017) from community and clinical sources, including patients from West China Hospital of Sichuan University, special schools, regular schools, and from clinic social skills training groups in Chengdu. Typical developing subjects were recruited from regular schools in Chengdu. After completion of the description of the study purposes to subjects and their parents, written informed consent was obtained. The study was approved by the Ethics Committee of the West China Hospital of Sichuan University. All subjects were ascertained and assessed by a child psychiatrist trained in diagnosis of autism at the Mental Health Center, West China Hospital of Sichuan University. MRI scans, image processing, analysis, and quality control were completed by a professional image technician at the West China Hospital of Sichuan University.

After excluding 16 subjects (including 11 ASD subjects who could not cooperate with MRI scanning even after sedation with chloral hydrate and 5 HC subjects whose MRI images were of low quality) 65 right-handed subjects participated in this study. Among them, there were 19 ASD with HFA (17 males and 2 females, age ranges 5–16 years old, IQ ranges 71–122, excluding 3 Asperger subjects), 19 LFA (15 males and 4 females, age ranges 5–16 years old, IQ ranges 30–67), and 27 HC (26 males and 1 female, age ranges 5–14 years old, IQ ranges 70–130). HFA, LFA, and HC were group matched on gender, age and handedness, and HFA and HC were also matched on IQ.

Diagnosis

First, the child was diagnosed by an experienced child psychiatrist based on the Fourth Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-4). Second, an interview with the child's parents and an assessment of the child was performed by using the Autism Diagnostic Interview-Revised (ADI-R) (31)

and Autism Diagnostic Observation Schedule-Generic (ADOS-G) (32).

The ADI-R is an interview about the individual's current social and early childhood and stereotyped, repetitive behaviors and interests and communication development. The ADOS-G includes activities for young children and activities and an interview for older, verbal children, and it is a semi-structured interactive observation session designed to document signs of autism if they are present. Subjects were tested with one of four different modules depending on their age and verbal ability. Because the caretakers of 2 LFA could not come to our hospital and 14 children could not complete the ADOS-G, we failed to collect the results of ADI-R of 2 LFA children and ADOS-G of 14 children (6 LFA and 8 HFA). All patients were medication-naive and met the criteria of DSM-5. ASD subjects were excluded if they had a history of head injury, seizures, birth asphyxia, and metabolic or genetic disorders such as Fragile-X Syndrome.

HC had no history of developmental, learning, neurological, cognitive, or neuropsychiatric problems and they had no history of psychotropic medication use. To confirm that they were typically developing, all of them had extensive testing, including the ADI-R, IQ, and psychiatric testing.

IQ and Handedness

IQ was measured by different versions of intelligence tests for ASD and HC. The Wechsler Preschool Intelligence Scale (WPPSI) was used for the 5- to 6-year-old subjects. The Wechsler Intelligence Scale for Children (WISC-III) was used for 6- to 16-year-old subjects (33). We used the handedness questionnaire as revised by Li Tianxin in 1983. This questionnaire was formulated by asking about the use of hands in daily life of the subjects (34).

MRI Data Acquisition and Preprocessing

Magnetic resonance images were acquired using a standard quadrature head coil, 8-channel, receive-only on a 3.0 T Verio MRI system (Achieva, Philips, The Netherlands). Foam padding and earplugs were used to diminish the head movement and scanner noise. A number of pulse sequences (T2-weighted, and 2D FLAIR) and image contrasts were collected for clinical review. High-resolution images were obtained with a T1-weighted three-dimensional (3D) spoiled gradient (SPGR) sequence. The parameters were as follows: TR = 8.37 ms; TE = 3.88 ms; flip angle = 7°; in-plane matrix resolution = 256 * 256; field of view = 24 * 24 cm²; voxel size = 1 * 1 * 1 mm³; thickness =1 mm; number of slices = 188.

The 15 ASD and 6 HC who could not cooperate with the scanning were sedated using chloral hydrate with parental consent before the MRI was performed. The dosage of chloral hydrate was based on the child's weight (1 ml/kg). The maximum dosage was usually 20 ml. No complications occurred in the subjects who were sedated.

We used MRI Convert software (http://lcni.uoregon.edu/downloads/mri_convert/mriconvert/view) to transform the DICOM format data collected from magnetic resonance scanning. VBM-DARTEL was conducted with SPM8 (http://www.fil.ion.ucl.ac.uk/spm) software on a Matlab (2012Ra) platform. The pre-processing steps were as follows: (1)

Examining the images for evidence of anatomical abnormalities in each subject; (2) Setting the image origin to the AC-PC line; (3) Segmentation in the DARTEL procedure (A DARTEL was used to create a customized T1 template of our own images rather than a standard T1 template and the segmented gray matter, GM, and white matter, WM, maps were spatially normalized); (4) Affine transform of segmented brain maps into the MNI space; (5) Modulating the segmented images with the Jacobian determinants derived from the spatial normalization; (6) Using standard smoothing by an 8-mm-full width-half maximum Gaussian kernel. This pre-processing yielded the smoothed modulated normalized data (in the MNI space) used for the statistical analysis.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS 22.0 for Windows, IBM Corp., Armonk, NY, USA). Two sample t-tests were used to compare the demographic data between the ASD and HC groups. For the sex data, $\chi 2$ tests were applied. ANOVA was used to compare the demographic data between the LFA, HFA, and HC groups, and two sample t-tests were applied to compare the scores of ADI-R between the LFA and HFA groups. We set the statistical significance level at $F < 0.05$ or $p < 0.05$.

Group differences were evaluated for GMV and white matter volume (WMV) absolute volumes and total cerebral volume (TCV) which were obtained in the brain segmentation step of the VBM-DARTEL pre-processing. TCV was calculated as the sum of the volume of GM, WM and cerebrospinal fluid (CSF).

First, a two sample t-test contained in SPM8 was applied to analyze the differences of regional GMV between the ASD group and the HC group. Intelligence quotient, age, sex, and GMV were treated as covariates. VBM analysis was employed using a $p < 0.001$ significance threshold, False Discovery Rate (FDR) uncorrected with an extent threshold of 50 voxels. An absolute threshold mask of 0.2 was used on the GMV. Each individual cluster that showed significant differences between groups was defined as a region of interest (ROI). The GMVs of individual ROIs were extracted from each subject and compared between groups. Pearson's correlation analysis was used to discuss the relationship between the values of GMV and the score of ADI-R and IQ.

Second, the ASD group was divided into a HFA group and a LFA group and ANOVA analysis was used to analyze the differences of regional GMV between the three groups. Age, sex, IQ, and GMV were treated as covariates. Then, *post hoc* analysis was carried out between these three groups. To assess the functional significance of GMV alterations in patients, the values of GMVs in each ROI were correlated with the score of ADI-R, with IQ, age, sex, and GMV being treated as covariates.

RESULTS

Demographic Characteristics and Clinical Variables

No significant differences were found between the ASD group and the HC group in demographics, including age

($p = 0.111$), gender ($p = 0.224$); IQ in the ASD group was significantly lower than that in the HC group ($p < 0.001$) (**Table 1**).

No significant differences were found among the LFA, HFA, and HC groups in demographics including age ($p = 0.16$) and gender ($p = 0.239$) except that the IQ of the LFA group was significantly lower than the HC group ($p < 0.05$) and the HFA group ($p < 0.05$). To assess the potential effect of IQ on GM group differences, GM volumes were correlated with IQ within each group. Besides, the score of subscales of ADI-R including communication, verbal interaction, non-verbal interaction, and total score of ADI-R in the LFA group were significantly higher than the HF group ($p = 0.044$, $p < 0.05$, $p < 0.05$, $p < 0.05$) (**Table 2**).

Quantitative Structure MRI

No significant differences were found between the ASD and the HC group with regard to whole TCV ($p = 0.132$), GMV ($p = 0.316$), and WMV ($p = 0.185$) (**Table 1**).

Also, no significant differences were found among the three groups (HFA, LFA, and HC) with regard to TCV ($p = 0.283$), GMV ($p = 0.402$), and WMV ($p = 0.401$) (**Table 2**).

VBM-DARTEL Analysis of GMV Differences
Subjects With ASD vs. Controls

Participants with ASD had a large cluster of significant increases in GMV in the left inferior temporal gyrus ($T = 4.3789$, volume $= 189$) and bilateral middle temporal gyrus (Left: $T = 4.1618$, volume $= 283$; Right: $T = 3.8091$, volume $= 67$) as compared to HC. The ASD subjects also had three small areas of decreased GMV including the right precuneus ($T = -4.6267$, volume $= 377$), right cerebellum anterior lobe ($T = -3.9329$, volume $= 84$) and the right angular gyrus ($T = -4.2512$, volume $= 167$) (**Table 3**).

ANOVA Results From Subjects With LFA, HFA, and Controls

The ANOVA showed six increased GMV clusters among the three groups (significant threshold $p < 0.001$, uncorrected) which included the left inferior temporal gyrus ($T = 11.4038$, volume $= 89$); left middle temporal gyrus BA21 ($T = 11.8575$, volume $= 211$); left parahippocampal gyrus BA35 ($T = 9.5346$, volume $= 50$); right inferior parietal lobule ($T = 12.3161$, volume $= 99$); right parahippocampal gyrus ($T = 9.3148$, volume $= 67$); and right precuneus ($T = 10.8188$, volume $= 72$) (**Table 4**).

Subjects With LFA vs. Controls

Compared with the HC group, increased GMV was found in the LFA group in the left inferior temporal gyrus ($T = 4.5422$, volume $= 75$); left middle temporal gyrus BA21 ($T = 4.9095$, volume $= 210$) (**Figure 1**, **Table 4**).

Subjects With HFA vs. Controls

When the HF group was compared with the HC group, increased GMV was found only in left inferior temporal gyrus ($T = 4.9022$, volume $= 87$) (**Figure 1**, **Table 4**).

Subjects With LFA vs. HFA

No significant increases or decreases of GMV were found when the HF group was compared to the LF group (**Figure 1**, **Table 4**).

A two-sample t-test was also carried out between every two of the three groups, and the results are shown in **Supplemental Table 1**.

Correlation Between GMV and Clinical Symptoms in HFA and LFA Patients

A significant negative correlation was found between GMV and the score of repetitive behavior in the HFA group ($p < 0.01$, $r = -0.649$). Yet no significant correlation was found in the LFA or ASD groups (**Supplemental Tables 1–3**, **Figure 2**).

Correlation Between GMV and IQ in the ASD and HC Groups

No significant correlation was found between GM volumes and IQ and clinical ratings within each group (**Supplemental Tables 4–6**).

DISCUSSION

In our study, the unbiased whole brain VBM-DARTEL method was used to assess neuroanatomical differences between children with ASD and HC. We also applied Pearson's correlation analysis to correlate the scores of ADI-R and IQ with abnormalities of brain volume. We found some brain regions that had previously been reported to be abnormal in people with ASD ([17], [25], [35]). We also found preliminary evidence that some of these anatomical differences occurred in both the LFA and HFA groups whereas others vary according to diagnostic categorization based on IQ. More abnormal brain structures were found in the LFA group, which fitted our

TABLE 1 | Demographic characteristics and clinical variables of the ASD and HC groups.

Characteristics	ASD group (N = 38)	HC group (N = 27)	F/t	p
Age (years)	9.56 (3.43)	8.33 (2.3)	1,615	0.111[a]
Gender(Male/Female)	32/6	26/1	/	0.224[b]
IQ(WISC-R)	75.84 (25.1)	98.59 (16.64)	−4.107	0.000[a]
ADI-R(M,SD)	42.79 (18.15)	/	/	/
Communication	16.777 (6.47)	/	/	/
verbal interaction	13.86 (5.64)	/	/	/
non-ver interaction	7.44 (4.79)	/	/	/
Restricted repetitive behavior	3.61 (2.23)	/	/	/
TCV(ml)	1448.36 (148.01)	1395.46 (122.77)	1.524	0.132[a]
GMV(ml)	777.51 (68.21)	761.31 (56.69)	1.01	0.316[a]
WMV(ml)	487.39 (70.98)	465.17 (57.83)	1.34	0.185[a]

[a]The p-values were obtained by two sample t-tests.
[b]The p-values were obtained by chi-square test.

TABLE 2 | Demographic characteristics and clinical variables of the three groups (HFA, LFA, and HC).

Characteristics	LF group (N = 19)	HF group (N = 19)	HC group (N = 27)	F/t	p	Post hoc
Age (years)	9.03 (2.99)	10.08 (3.82)	8.22 (2.3)	1. 89	0.16[a]	/
Gender (Male/Female)	17/2	4/15	26/1	3.321	0.239[b]	/
IQ(WISC-R)	55.89 (10.83)	95.79 (18.37)	98.59 (16.64)	46.9	0.00[a]	LF<HF,HC
ADI-R(M,SD)	54.94 (14.36)	36.42 (10.38)	/	4.468	0.000[c]	/
Communication	19.05 (7.49)	14.73 (4.71)	/	2.095	0.044[c]	/
Verbal interaction	17.41 (5.36)	10.68 (3.69)	/	4.422	0.000[c]	/
Non-ver interaction	10.52 (4.78)	4.68 (2.71)	/	4.573	0.000[c]	/
Restricted repetitive behavior	3.71 (2.84)	3.52 (1.57)	/	0.238	0.814[c]	/
TCV(ml)	1,460.3 (131.05)	1,436.51 (166.0)	1,395.4 (122.77)	1.288	0.283[a]	/
GMV(ml)	786.96 (58.56)	768.06 (77.11)	761.31 (56.69)	0.926	0.402[a]	/
WMV(ml)	490.51 (61.043)	484.28 (81.32)	465.17 (57.83)	0.927	0.401[a]	/

LF, Low Function; HF, High Function; HC, Healthy Control; TCV, total cerebral volume; GMV, gray matter volume; WMV, white matter volume. Statistical significance level: p < 0.05.
[a]*The p-values were obtained by ANOVA.*
[b]*The p-values were obtained by chi-square tests.*
[c]*The p-values were obtained by two-sample t-tests.*

TABLE 3 | Comparison of gray matter volume between the ASD and HC groups.

Volume (mm³)	T	Talairach coordinates (x,y,z)	Anatomical regions
ASD>HC			
189	4.3789	−48, 1.5, −42	L Inferior Temporal Gyrus
67	3.8091	36, 9, −42	R Middle Temporal Gyrus BA38
283	4.1618	−57, 3, −21	L Middle Temporal GyrusBA21
ASD<HC			
84	−3.9329	18, −28.5, −19.5	R Cerebellum Anterior Lobe
377	−4.6267	3, −54, 60	R Precuneus
167	−4.2512	49.5, −64.5, 45	R Angular

All labels are derived from the Anatomical Automatic Atlas (AAL).The threshold was set at p < 0.001 (uncorrected). x,y,z, coordinates of primary peak locations in the MNI space. L, Left; R, Right; BA, Brodmann Area; LF, Low Function; HF, High Function; HC, Healthy Control.

TABLE 4 | Comparison of gray matter volume between the LF, HF, and HC groups.

Volume (mm³)	T	Talairach coordinates (x,y,z)	Anatomical regions
ANOVA			
89	11.4038	−48, 3, −42	L Inferior Temporal Gyrus
211	11.8575	−58.5, 4.5, −21	L Middle Temporal Gyrus BA21
50	9.5346	−22.5, −27, −22.5	L Parahippocampa GyrusBA35
99	12.3161	51, −64.5, 48	R Inferior Parietal Lobule
67	9.3148	19.5, −28.5, −21	R Parahippocampal
72	10.8188	3, −54, 60	R Precuneus
LF>HC			
75	4.5422	−49.5 0 −43.5	L Inferior Temporal Gyrus
210	4.9095	−60 1.5 −12	L Middle Temporal Gyrus BA21
HF>HC			
87	4.9022	−45 −1.5 −37.5	L Inferior Temporal Gyrus

All labels are derived from the Anatomical Automatic Atlas (AAL). The threshold was set at p < 0.001 (uncorrected). x,y,z: coordinates of primary peak locations in the MNI space. L, Left; R, Right; BA, Brodmann Area; LF, Low Function; HF, High Function; HC, Healthy Control.

previous assumptions that LFA has more abnormal brain anatomy than HFA. In addition, significant correlations between increased GMV and autistic symptoms were found in our study.

We demonstrated increased GMV of the left inferior temporal gyrus (ITG) in both the LFA group and the HFA group. This result remains the same while excluding the influence of IQ on brain structure with IQ as covariates. This suggests that the left ITG is implicated in the pathophysiology of ASD across the autistic spectrum at different IQ levels. In agreement with our study, some studies also found an increase of left temporal lobe (including inferior, middle and superior temporal gyrus) in children with ASD (36–38).But another VBM study found those with autism demonstrated a reduction in superior and inferior temporal gyrus (25). This may be explained by the fact that the subjects in their group were all adults with a mean age of 30, whereas the mean age of our subjects was 9.5 years. It has been reported that infants and children with ASD had

a larger brain size than typical of developing children, but that with increased age, the difference in whole brain volume between the two groups was no longer significant (39–41). On the other hand, when the HFA and LFA were combined into one autism group, the heterogeneity of the phenotypes would inevitably be increased. Another study merely found decreased GMV regions including the left ITG, basal forebrain, nucleus accumbens, and cerebellar hemispheres in the LFA group (18). The age of their subjects ranged from 2- to 10-years old, which is younger than the age of our subjects. Moreover, they only included the LFA and HC. As the main brain area to coordinate social communication, emotion and verbal function, the temporal lobe has abnormalities that have been associated with the communication and speech deficits of ASD (42). Early

a:Left inferior temporal gyrus(HF>HC)

b: Left inferior temporal gyrus(LF>HC)

c:Left middle temporal gyrus BA21(LF>HC)
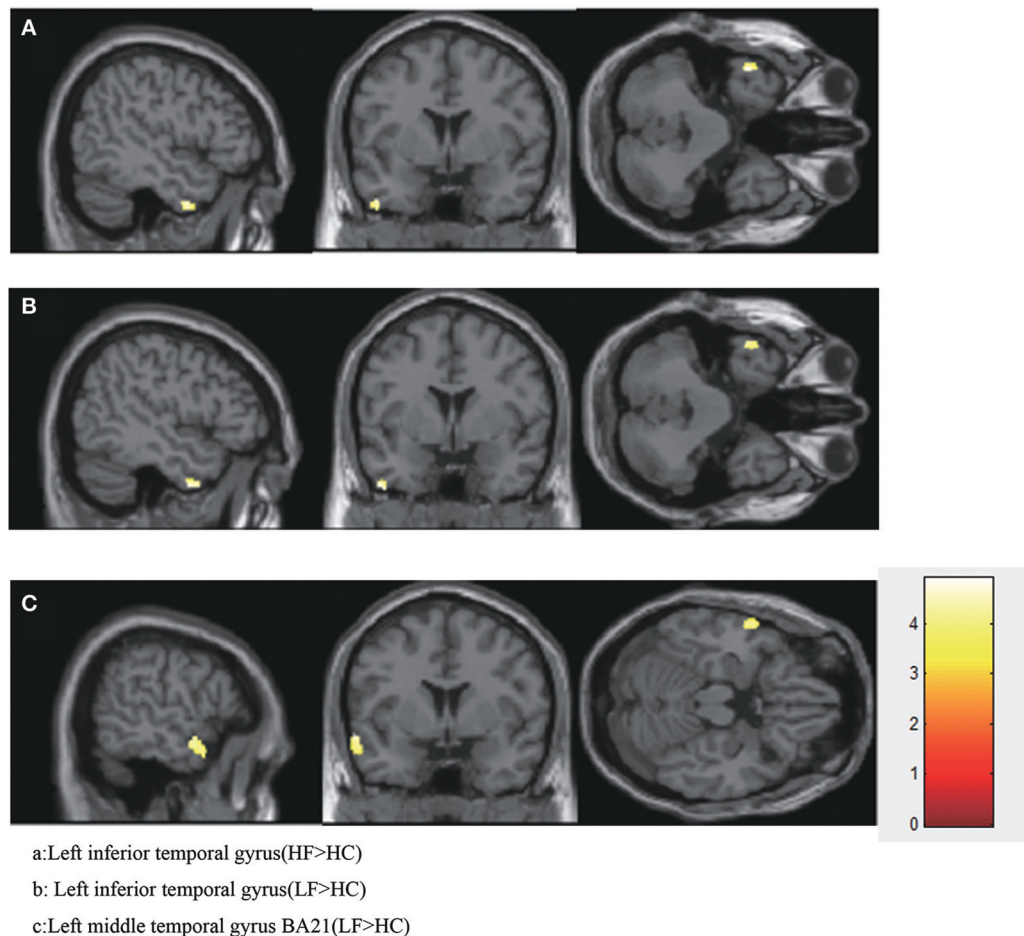
FIGURE 1 | Increased GMV in HFA and LFA group. **(A)** Left inferior temporal gyrus (HF>HC). **(B)** Left inferior temporal gyrus (LF>HC). **(C)** Left middle temporal gyrus BA21 (LF>HC).

studies showed that the inferior temporal surface plays an important role in orthographic decoding, or was the so-called basal temporal language area (43–45). Later, the concept of the "local combination detector" (LCD) was raised by Dehaene et al. (46), who suggested that the function of the inferior temporal gyrus was related to transmitting large amounts of information back and forth, starting by encoding visual features, then single letters, bigrams, quadrigrams, and finally whole words (46, 47). This was later supported by the ERP study (48). These studies suggest that the ITG may play a key role in learning language at an early age. Left ITG is also supposed to be involved in common object perception. A study found fMRI abnormalities in the inferior temporal and fusiform gyri in face discrimination tasks (49) The abnormal structure of left ITG may explain the difficulty in recognizing faces in ASD patients. More importantly, given this importance of the ITG in language acquisition, our results showing increased GMV of the ITG both in HFA and LFA may partly explain the occurrence of delayed language development before age 3 in both the HFA and LFA groups (25, 28).

In our study, increased GMV of the left middle temporal gyrus (MTG) BA21 was only found in the LFA group, and not in the HFA group. This suggests that, neuroanatomically, the LFA sample may represents a more heterogeneous population than HFA. MTG BA21 is a site involved in auditory processing, language and social perception implicated in ASD-associated behaviors (50). It has been reported that abnormal activity occurs in left middle/superior temporal gyrus, especially in region BA21, which may be the key brain region for speech disorders of ASD children (12). Moreover, the middle temporal cortex and superior temporal gyrus are important sites for perceiving and decoding other people's gaze (26). In postmortem BA21 temporal cortex, a study found that compared to controls, ASD patients exhibited altered protein levels of mitochondria respiratory chain protein complexes, decreased Complex I and IV activities, decreased mitochondrial antioxidant enzyme SOD2, and greater oxidative DNA damage (51). Thus it can be seen that MTG is abnormal in both microstructure and macrostructure, indicating that it may be one of the important causes of ASD. In this study, the subscale scores of ADI-R

FIGURE 2 | Correlation between LITG and score of repetitive behavoir in HFA group.
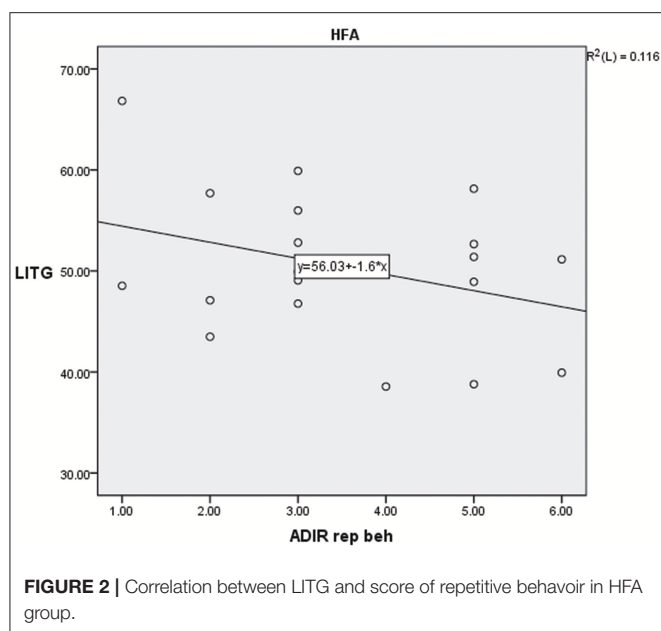
including communication, verbal interaction and non-verbal interaction in the LFA group were significantly higher than in the HFA group. Therefore, it can be postulated that the more impaired the left temporal gyrus, the more obvious the core symptoms of autism, especially with respect to communication and social interaction deficits. However, we failed to find any correlation between left MTG and core autistic symptoms such as communication deficits. This may be explained by the small sample size.

Finally, in the correlation analysis, we reported significant negative correlations between the GMV of the left ITG and the score of repetitive behavior only in the HFA group. The result remains the same with IQ as covariates. A review summarized the brain regions that related to stereotypical repetitive behaviors reported in previous literature were include basal ganglia, caudate, frontal and temporal cortices. Among these brain areas, basal ganglia was the most commonly reported one and Alterations to frontal and temporal cortices are the most varied and difficult to interpret in relation to RRB, as these regions are also implicated in social and/or communication deficits (52). (53) found faster striatal growth was correlated with more severe repetitive behavior (insistence on sameness) at the preschool age. Rojas et al. (19) reported repetitive behavior was positively associated with the left inferior frontal gyrus, caudate nuclei and right amygdala. In their study, the ASD group included both HFA and LFA subjects and the age ranged from 7.8 to 44. This could introduce variability and reduce the effect sizes for some brain structures. In the present study, we only included children who were 5- to 15-years old, and would have fewer developmental covariant influences that could affect the findings. We did not find the same relationship in the LFA group or in the whole ASD group (HFA combined with LFA). This may be explained by three ways. First, LFA and HFA may be two different subtypes of ASD with different

neuroimaging features, so that our results of correlation between GMV of the left ITG and the score of repetitive behavior could only exist in HFA but not in LFA. Second, repetitive behavior reflected by abnormal structures may not particularly evaluated by the ADI-R in LFA patients. Third, the sample size was too small to find any potential relationship in the LFA group.

Strengths and Limitations

The strengths of this study include the enrollment of three groups of young children (LFA, HFA, and HC) and the use of rigorous diagnostic tools, strict and validated imaging methodologies, and exact and original analytic methods. More importantly, so far as we know, this was the first investigation of abnormal brain anatomy in HFA and LFA using VBM, and the first to assess correlations between autistic symptoms, making the results more representative of ASD population. But this study also has limitations. First, all of the subjects were children, so we do not know whether our findings can be generalized to adults. Second, our ASD patients were not followed over time, and so it is difficult to know whether the abnormalities would be altered or extinguished as they grow older. Third, the sample size was relatively small (because we found that it is too difficult for many LFA children to cooperate with MRI scanning). Fourth, we did not include a control group of children with other intellectual disorders for comparison, because the purpose of this research was to find different brain regions in different clinical phenotypes of ASD and HC on various levels of IQ in the same sample. Given the higher proportion of LFA in the overall ASD population, ASD phenotype should include different levels of intelligence to make the results more representative. In attempting to "control" for a domain- non-specific construct such as IQ, variability truly associated with autism could be discarded as "non-specific" (54). In fact, the pathology underlying neurodevelopmental disorders and ASD is itself not well understood (55), so subjects defined on the basis of cognitive function are likely to introduce additional variation related to the causes of the cognitive impairment in ASD research studies (55). Together with the present study, it can be postulated that while understanding biological features of delicate subtypes, research studies on ASD should include subjects with a variety of phenotypes, including different IQ levels, to make the findings more generalizable, so that they can shed light on the biological markers and targeted interventions of ASD in the future.

CONCLUSION

Our findings supported the hypothesis that the neurological findings LFA and HFA reflect abnormal function in the same brain regions, but LFA had involvement of more abnormal regions of the brain than HFA. Increased GMV in the left ITG was found in both HFA and LFA, but increased GMV of the left MTG BA21 was found only in the LFA group. Furthermore, there was a significant negative correlation between GMV of the LITG and the score of repetitive behavior in the HFA group. These

findings seem to provide a theoretical basis for exploration for biological markers and further targeted interventions in ASD. More importantly, our findings indicate that different levels of IQ should be taken into consideration when discussing the biological markers and targeted intervention of ASD in the future research.

AUTHOR CONTRIBUTIONS

JC wrote the manuscript of this work. XH, KG, PY, and MS helped recruit and assess the subjects. YH modified the manuscript.

FUNDING

ACKNOWLEDGMENTS

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2018.00542/full#supplementary-material

REFERENCES

1. Chen R, Jiao Y, Herskovits EH. Structural MRI in autism spectrum disorder. *Pediatr Res.* (2011) 69:63R−8R. doi: 10.1203/PDR.0b013e318212c2b3

2. Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, et al. Prevalence of autism spectrum disorder among children aged 8 years — autism and developmental disabilities monitoring network, 11 sites, United States, 2014. MMWR Surveill Summ. *Centers Dis Control Prevent.* (2018) 67:1–23. doi: 10.15585/mmwr.ss6706a1

3. Kim YS, Leventhal BL, Koh YJ, Fombonne E, Laska E, Lim EC, et al. Prevalence of autism spectrum disorders in a total population sample. *Am J Psychiatry.* (2011) 168:904–12. doi: 10.1176/appi.ajp.2011.10101532

4. Fombonne E. Epidemiology of pervasive developmental disorders. *Pediatr Res.* (2009) 65:591–8. doi: 10.1203/PDR.0b013e31819e7203

5. Picardi, Gigantesco A, Tarolla E, Stoppioni V, Cerbo R, Cremonte M, et al. parental burden and its correlates in families of children with autism spectrum disorder: a multicentre study with two comparison groups. *Clin Pract Epidemiol Ment Health* (2018) 14:143–76. doi: 10.2174/1745017901814010143

6. Szatmari P. The classification of autism, Asperger's syndrome, and pervasive developmental disorder. *Can J Psychiatry* (2000) 45:731–8. doi: 10.1177/070674370004500806

7. Rinehart NJ, Bradshaw JL, Brereton AV, Tonge BJ. A clinical and neurobehavioural review of high-functioning autism and Asperger's disorder. *Aust N Z J Psychiatry* (2002) 36:762–70. doi: 10.1046/j.1440-1614.2002.01097.x

8. Ecker C, Spooren W, Murphy DG. Translational approaches to the biology of Autism: false dawn or a new era? Mol Psychiatry (2013) 18:435–42. doi: 10.1038/mp.2012.102

9. Lai MC, Lombardo MV, Baron-Cohen S. Autism. *Lancet* (2014) 383:896–910. doi: 10.1016/S0140-6736(13)61539-1

10. Matelski L, Van de Water J. Risk factors in autism: thinking outside the brain. *J Autoimmun.* (2016) 67:1–7. doi: 10.1016/j.jaut.2015.11.003

11. Khaiman C, Onnuam K, Photchanakaew S, Chonchaiya W, Suphapeetiporn K. Risk factors for autism spectrum disorder in the Thai population. *Eur J Pediatr.* (2015) 174:1365–72. doi: 10.1007/s00431-015-2544-2

12. Lim L, Chantiluke K, Cubillo AI, Smith AB, Simmons A, Mehta MA, et al. Disorder-specific grey matter deficits in attention deficit hyperactivity disorder relative to autism spectrum disorder. *Psychol Med.* (2015) 45:965–76. doi: 10.1017/S0033291714001974

13. Foster NE, Doyle-Thomas KA, Tryfon A, Ouimet T, Anagnostou E, Evans AC, et al. Structural gray matter differences during childhood development in autism spectrum disorder: a multimetric approach. *Pediatr Neurol.* (2015) 53:350–9. doi: 10.1016/j.pediatrneurol.2015.06.013

14. Riedel, Maier S, Ulbrich M, Biscaldi M, Ebert D, Fangmeier T, et al. No significant brain volume decreases or increases in adults with high-functioning autism spectrum disorder and above average intelligence: a voxel-based morphometric study. *Psychiatry Res Neuroimag.* (2014) 223:67–74. doi: 10.1016/j.pscychresns.2014.05.013

15. Mueller S, Keeser D, Samson AC, Kirsch V, Blautzik J, Grothe M, et al. Convergent findings of altered functional and structural brain connectivity in individuals with high functioning autism: a multimodal MRI study. *PLoS ONE* (2013) 8:e67329. doi: 10.1371/journal.pone.0067329

16. Lai MC, Lombardo MV, Ecker C, Chakrabarti B, Suckling J, Bullmore ET, et al. Neuroanatomy of individual differences in language in adult males with autism. *Cereb Cortex* (2015) 25:3613–28. doi: 10.1093/cercor/bhu211

17. Eilam-Stock T, Wu T, Spagna A, Egan LJ, Fan J. Neuroanatomical alterations in high-functioning adults with autism spectrum disorder. *Front Neurosci.* (2016) 10:237. doi: 10.3389/fnins.2016.00237

18. Riva D, Bulgheroni S, Aquino D, Di Salle F, Savoiardo M, Erbetta A. Basal forebrain involvement in low-functioning autistic children: a voxel-based morphometry study. *AJNR Am J Neuroradiol.* (2011) 32:1430–5. doi: 10.3174/ajnr.A2527

19. Rojas DC, Peterson E, Winterrowd E, Reite ML, Rogers SJ, Tregellas JR. Regional gray matter volumetric changes in autism associated with social and repetitive behavior symptoms. *BMC Psychiatry* (2006) 6:56. doi: 10.1186/1471-244X-6-56

20. D'Mello AM, Crocetti D, Mostofsky SH, Stoodley CJ. Cerebellar gray matter and lobular volumes correlate with core autism symptoms. *Neuroimage Clin.* (2015) 7:631–9. doi: 10.1016/j.nicl.2015.02.007

21. Ecker C, Suckling J, Deoni SC, Lombardo MV, Bullmore ET, Baron-Cohen S, et al. Brain anatomy and its relationship to behavior in adults with autism spectrum disorder: a multicenter magnetic resonance imaging study. *Arch Gen Psychiatry* (2012) 69:195–209. doi: 10.1001/archgenpsychiatry.2011.1251

22. Stanfield AC, McIntosh AM, Spencer MD, Philip R, Gaur S, Lawrie SM. Towards a neuroanatomy of autism: a systematic review and meta-analysis of structural magnetic resonance imaging studies. *Eur Psychiatry* (2008) 23:289–99. doi: 10.1016/j.eurpsy.2007.05.006

23. Nickl-Jockschat T, Habel U, Michel TM, Manning J, Laird AR, Fox PT, et al. Brain structure anomalies in autism spectrum disorder–a meta-analysis of VBM studies using anatomic likelihood estimation. *Hum Brain Mapp.* (2012) 33:1470–89. doi: 10.1002/hbm.21299

24. Duerden EG, Mak-Fan KM, Taylor MJ, Roberts SW. Regional differences in gray and white matter in children and adults with autism spectrum disorders: an activation likelihood estimate (ALE) meta-analysis. *Autism Res.* (2012) 5:49–66. doi: 10.1002/aur.235

25. Toal F, Daly EM, Page L, Deeley Q, Hallahan B, Bloemen O, et al. Clinical and anatomical heterogeneity in autistic spectrum disorder: a structural MRI study. *Psychol Med.* (2010) 40:1171–81. doi: 10.1017/S0033291709991541

26. Riva D, Annunziata S, Contarino V, Erbetta A, Aquino D, Bulgheroni S. Gray Matter reduction in the vermis and CRUS-II is associated with social and interaction deficits in low-functioning children with autistic spectrum disorders: a VBM-DARTEL Study. *Cerebellum* (2013) 12:676–85. doi: 10.1007/s12311-013-0469-8

27. Wang J, Fu K, Chen L, Duan X, Guo X, Chen H, et al. Increased gray matter volume and resting-state functional connectivity in somatosensory cortex and their relationship with autistic symptoms in young boys with autism spectrum disorder. *Front Physiol.* (2017) 8:588. doi: 10.3389/fphys.2017.00588

28. Lotspeich L, Kwon H, Schumann CM, Fryer SL, Goodlin-Jones BL, Buonocore MH, et al. Investigation of neuroanatomical differences between autism and Asperger syndrome. *Arch Gen Psychiatry* (2004) 61:291–8. doi: 10.1001/archpsyc.61.3.291

29. Akshoomoff N, Lord C, Lincoln AJ, Courchesne RY, Carper RA, Townsend J, et al. Outcome classification of preschool children with autism spectrum disorders using MRI brain measures. *J Am Acad Child Adolesc Psychiatry* (2004) 43:349–57. doi: 10.1097/00004583-200403000-00018

30. Boger-Megiddo, Shaw DW, Friedman SD, Sparks BF, Artru AA, Giedd JN, et al. Corpus callosum morphometrics in young children with autism spectrum disorder. *J Autism Dev Disord.* (2006) 36:733–9. doi: 10.1007/s10803-006-0121-2

31. Lord C, Rutter M, Lecouteur A. Autism diagnostic interview-revised - a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord.* (1994) 24:659–85. doi: 10.1007/BF02172145

32. Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, et al. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord.* (2000) 30:205–23. doi: 10.1023/A:1005592401947

33. Gong YaoXian CT. *Chinese-Wechsler Intelligence Scale for Children-the Third Version.* Cai Taisheng: Hunan Map Press (1993).

34. Tianxin L. The distribution of the right and left handedness of Chinese people. *Acta Phycol Sinica* (1983) 3:268–71.

35. Yamasaki S, Yamasue H, Abe O, Suga M, Yamada H, Inoue H, et al. Reduced gray matter volume of pars opercularis is associated with impaired social communication in high-functioning autism spectrum disorders. *Biol Psychiatry* (2010) 68:1141–7. doi: 10.1016/j.biopsych.2010.07.012

36. Hazlett HC, Poe MD, Gerig G, Smith RG, Piven J. Cortical gray and white brain tissue volume in adolescents and adults with autism. *Biol Psychiatry* (2006) 59:1–6. doi: 10.1016/j.biopsych.2005.06.015

37. Knaus TA, Silver AM, Dominick KC, Schuring MD, Shaffer N, Lindgren KA, et al. Age-related changes in the anatomy of language regions in autism spectrum disorder. *Brain Imaging Behav.* (2009) 3:51–63. doi: 10.1007/s11682-008-9048-x

38. Di X, Biswal BB. Similarly expanded bilateral temporal lobe volumes in female and male children with autism spectrum disorder. *Biol Psychiatry Cogn Neurosci Neuroimaging* (2016) 1:178–85. doi: 10.1016/j.bpsc.2015.11.006

39. Radeloff D, Ciaramidaro A, Siniatchkin M, Hainz D, Schlitt S, Weber B, et al. Structural alterations of the social brain: a comparison between schizophrenia and autism. *PLoS ONE* (2014) 9. doi: 10.1371/journal.pone.0106539

40. Wallace GL, Dankner N, Kenworthy L, Giedd JN, Martin A. Age-related temporal and parietal cortical thinning in autism spectrum disorders. *Brain* (2010) 133:3745–54. doi: 10.1093/brain/awq279

41. Courchesne E, Campbell K, Solso S. Brain growth across the life span in autism: age-specific changes in anatomical pathology. *Brain Res.* (2011) 1380:138–45. doi: 10.1016/j.brainres.2010.09.101

42. Courchesne E, Pierce K, Schumann CM, Redcay E, Buckwalter JA, Kennedy DP, et al. Mapping early brain development in autism. *Neuron* (2007) 56:399–413. doi: 10.1016/j.neuron.2007.10.016

43. Burnstine TH, Lesser RP, Hart J Jr, Uematsu S, Zinreich SJ, Krauss GL, et al. Characterization of the basal temporal language area in patients with left temporal lobe epilepsy. *Neurology* (1990) 40:966–70. doi: 10.1212/WNL.40.6.966

44. Schaffler L, Luders HO, Morris HH III, Wyllie E. Anatomic distribution of cortical language sites in the basal temporal language area in patients with left temporal lobe epilepsy. *Epilepsia* (1994) 35:525–8. doi: 10.1111/j.1528-1157.1994.tb02472.x

45. Mani J, Diehl B, Piao Z, Schuele SS, Lapresto E, Liu P, et al. Evidence for a basal temporal visual language center: cortical stimulation producing pure alexia. *Neurology* (2008) 71:1621–7. doi: 10.1212/01.wnl.0000334755.32850.f0

46. Dehaene S, Cohen L, Sigman M, Vinckier F. The neural code for written words: a proposal. *Trends Cogn Sci.* (2005) 9:335–41. doi: 10.1016/j.tics.2005.05.004

47. Vinckier F, Dehaene S, Jobert A, Dubus JP, Sigman M, Cohen L. Hierarchical coding of letter strings in the ventral stream: dissecting the inner organization of the visual word-form system. *Neuron* (2007) 55:143–56. doi: 10.1016/j.neuron.2007.05.031

48. Dien J. The neurocognitive basis of reading single words as seen through early latency ERPs: a model of converging pathways. *Biological Psychol.* (2009) 80:10–22. doi: 10.1016/j.biopsycho.2008.04.013

49. Schultz RT, Gauthier I, Klin A, Fulbright RK, Anderson AW, Volkmar F, et al. Abnormal ventral temporal cortical activity during face discrimination among individuals with autism and Asperger syndrome. *Arch Gen Psychiatry* (2000) 57:331–40. doi: 10.1001/archpsyc.57.4.331

50. Jou RJ, Minshew NJ, Keshavan MS, Vitale MP, Hardan AY. Enlarged right superior temporal gyrus in children and adolescents with autism. *Brain Res.* (2010) 1360:205–12. doi: 10.1016/j.brainres.2010.09.005

51. Tang G, Gutierrez Rios P, Kuo SH, Akman HO, Rosoklija G, Tanji K, et al. Mitochondrial abnormalities in temporal lobe of autistic brain. *Neurobiol Dis.* (2013) 54:349–61. doi: 10.1016/j.nbd.2013.01.006

52. Wilkes BJ, Lewis MH. The neural circuitry of restricted repetitive behavior: magnetic resonance imaging in neurodevelopmental disorders and animal models. *Neurosci Biobehav Rev.* (2018) 92:152–171. doi: 10.1016/j.neubiorev.2018.05.022

53. Langen M, Bos D, Noordermeer SD, Nederveen H, van Engeland H, Durston S, et al. Changes in the development of striatum are involved in repetitive behavior in autism. *Biol Psychiatry* (2014) 76:405–11. doi: 10.1016/j.biopsych.2013.08.013

54. Yeung-Courchesne R, Courchesne E. From impasse to insight in autism research: from behavioral symptoms to biological explanations. *Dev Psychopathol.* (1997) 9:389–419. doi: 10.1017/S0954579497002101

55. Lenroot RK, Yeung PK. Heterogeneity within autism spectrum disorders: what have we learned from neuroimaging studies? *Front Hum Neurosci.* (2013) 7:733. doi: 10.3389/fnhum.2013.00733

Neural Correlates of Duration Discrimination in Young Adults with Autism Spectrum Disorder, Attention-Deficit/Hyperactivity Disorder and Their Comorbid Presentation

Steve D. Lukito [1]*, **Owen G. O'Daly** [2], **David J. Lythgoe** [2], **Susannah Whitwell** [3], **Amanda Debnam** [3], **Clodagh M. Murphy** [3,4,5], **Karen Ashwood** [3,4,5], **Vladimira Stoencheva** [3,4,5], **Emily Simonoff** [1†] and **Katya Rubia** [1†]

[1] Department of Child and Adolescent Psychiatry, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom, [2] Department of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom, [3] The Adult Attention-Deficit Hyperactivity Disorder National Service, Behavioural and Developmental Psychiatry Clinical Academic Group, South London and Maudsley NHS Foundation Trust, London, United Kingdom, [4] Behavioural Genetics Clinic, Adult Autism Service, Behavioural and Developmental Psychiatry Clinical Academic Group, South London and Maudsley NHS Foundation Trust, London, United Kingdom, [5] Department of Forensic and Neurodevelopmental Sciences, Sackler Institute for Translational Neurodevelopmental Sciences, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom

Attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) often co-occur and share neurocognitive deficits. One such shared impairment is in duration discrimination. However, no studies using functional magnetic resonance imaging (fMRI) have investigated whether these duration discrimination deficits are underpinned by the same or different underlying neurofunctional processes. In this study, we used fMRI to compare the neurofunctional correlates of duration discrimination between young adult males with ASD ($n = 23$), ADHD ($n = 25$), the comorbid condition of ASD+ADHD ($n = 24$), and typical development (TD, $n = 26$) using both region of interest (ROI) and whole brain analyses. Both the ROI and the whole-brain analyses showed that the comorbid ASD+ADHD group compared to controls, and for the ROI analysis relative to the other patient groups, had significant under-activation in right inferior frontal cortex (IFG) a key region for duration discrimination that is typically under-activated in boys with ADHD. The findings show that in young adult males with pure ASD, pure ADHD and comorbid ASD+ADHD with no intellectual disability, only the comorbid group demonstrates neurofunctional deficits in a typical duration discrimination region.

Keywords: duration discrimination, neurodevelopment disorder, attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), functional magnetic resonance imaging, comorbidity, time estimation

INTRODUCTION

Autism spectrum disorder (ASD) is characterized by difficulties in reciprocal social communication/interaction and stereotyped and repetitive behaviors, while attention-deficit/hyperactivity disorder (ADHD) is characterized by age-inappropriate symptoms of inattention, hyperactivity and impulsivity (1). Both conditions co-occur despite their distinctive diagnostic criteria even in adulthood [e.g., (2, 3)]. Consequently, studies have compared the cognitive and neural correlates of these conditions, and their comorbid presentation (i.e., ASD+ADHD), with the intention of predicting a model of impairments for the comorbid group in relation to the "pure" disorders (4–6).

Perceptual timing, i.e., "the ability to estimate explicitly attended temporal intervals" (7), could be used for investigating potentially different underlying substrates of the pure disorders and of the comorbid disorder. People with both psychiatric conditions are often impaired in daily functions involving timing and time perception skills, including planning and organizing (8–11). Impaired timing is a major pathway to ADHD (7, 12–14) and symptoms of impulsivity, such as verbal blurting and aversion toward delays, have been found to be correlated with impaired time perception (14–16). Anecdotal accounts from parents and clinicians also suggested timing problems in individuals with ASD (17, 18), which possibly forms the basis for checking behavior or strict adherence to routine (18, 19). Furthermore, time perception difficulties are associated with executive function (EF) deficits [e.g., (20–22)], which have been found to be present in these related conditions (23, 24).

Experimental studies of time perception have consistently reported impaired task performance in ADHD [see (7, 14, 25)]. Consistently, reports show that children and adults with ADHD are less able to detect changes in time intervals within the millisecond range [e.g., (26–28)] and tend to over-estimate supra-second time intervals relative to typically developing controls (12, 29–31). Such difficulties, according to reviews and meta-analyses of fMRI studies in people with ADHD, are related to functional impairments in inferior frontal, inferior parietal, striatal, and cerebellar regions (7, 32, 33). Furthermore, during discrimination of intervals differing by several hundreds of milliseconds specifically, ADHD boys have shown under-activation in right dorsolateral prefrontal (DLPFC), bilateral inferior frontal gyrus (IFG), dorsal anterior cingulate/supplementary motor area (DACC/SMA), striatum, left inferior parietal lobe (IPL) and left cerebellum (14, 34–38).

Experimental findings have also suggested an impairment of time perception in people with ASD (39–44) although there are also some negative results (45–47). Despite evidence for time estimation deficits in ASD, no studies have tested the neural substrates of these deficits or compared patients with ADHD and ASD in time perception.

Several studies, however, have compared the two disorders in other EF domains [e.g., (4, 48, 49)], which have been shown to have some overlap in neural activations with time perception (50). During motor inhibition, ASD-specific over-activation was found in bilateral IFG while ADHD-specific under-activation was observed in ventrolateral prefrontal cortex and basal ganglia (49). Furthermore, shared under-activation of the DLPFC was found in both disorders during sustained attention and working memory tasks (4, 48). Interestingly, a study of temporal discounting (6), which is closely related to timing functions (14, 51, 52), showed weaker brain-behavior association in the ASD+ADHD group in typical areas of temporal discounting such as ventromedial and lateral prefrontal cortex, ventral striatum, and anterior cingulate, indicating increased severity of neural impairments in the comorbid than the pure disorders.

To address the gap in the literature, this study explores the neural correlates of time discrimination in young adult males with ASD, ADHD, and ASD+ADHD relative to age-matched controls. We were particularly interested in the potential impairments in the comorbid group compared to the pure groups to elucidate the mechanisms underpinning the co-occurrence of ASD and ADHD, as this information could be useful clinically for formulating disorder-specific treatments. Based on previous fMRI studies of time perception in ADHD, we hypothesized functional impairments in regions previously implicated with timing deficits in the ADHD group, i.e., in ACC/SMA, IFG, caudate, IPL and cerebellum (14, 34–38), with similar but more pronounced deficits in these timing regions in the comorbid group (6), but potentially different abnormalities in the ASD group, based on more inconsistent findings of time perception deficits in ASD [e.g., (41, 44)].

METHODS AND MATERIALS

Participants

Participants were 107 young adults aged 20–27 years with ASD, ADHD, ASD+ADHD, and TD and full-scale intelligent quotient (FSIQ) $\geq$ 70, estimated using the Wechsler Abbreviated Scale of Intelligence-II (WASI-II) (53). Only males were included to increase homogeneity since ASD and ADHD are highly prevalent among males (54, 55). There were equal proportions of left- and right-handed participants across groups, assessed on the Edinburgh Handedness Inventory (56). Excluded were individuals with epilepsy, personality disorder, current substance abuse/dependence, or lifetime history of bipolar disorder, schizophrenia or head injury. Participants in the clinical groups were invited from adult ASD and ADHD clinics, support organizations, social media and an ASD epidemiological cohort the Special Needs and Autism Project (SNAP) (57). Prescriptions of psychostimulants or selective serotonin reuptake inhibitors (SSRIs) were not exclusion criteria for the clinical groups, but psychostimulants were withdrawn 48 h prior to the study. Participants completed an investigation involving several fMRI tasks and a neurocognitive task battery. For the present study, nine subjects were excluded [seven due to excessive motion (>3 mm), one due to missing behavioral data caused by technical issues and another due to incidental MRI finding]. The final sample comprised 23 ASD, 25 ADHD, 24 ASD+ADHD, and 26 TD subjects (**Table 1**).

In the ASD group, 15 participants had clinical diagnoses confirmed by consultant psychiatrists specialized in ASD (eight with autism; seven with Asperger's syndrome) and eight had research diagnoses of ASD through the SNAP study [three

TABLE 1 | Group differences in socio-demographic variables and clinical measures.

	TD (n = 26)		ASD (n = 23)		ADHD (n = 25)		ASD+ ADHD (n = 24)		Group comparison			Post-hoc
	M	SD	M	SD	M	SD	M	SD	F/t	df	p	
Age	23.4	1.5	23.0	0.7	23.1	1.9	22.9	1.3	0.46	3, 94	0.71	--
FSIQ	117.3	12.0	103.7	18.4	116.0	13.2	106.9	15.9	4.9	3, 94	0.003	ADHD, TD > ASD*
Handedness	66.2	69.3	68.3	63.8	65.2	66.1	51.9	71.9	0.29	3, 94	0.83	--
CAARS ADHD index (*t*-scores)												
Self-rated	41.8	8.5	44.6	11.7	65.2	7.7	59.1	11.8	31.3	3, 94	<0.001	ADHD, ASD+ADHD > ASD***; TD***
Informant-rated	--	--	48.8	7.2	60.4	16.5	64.5	17.4	7.3	2, 69	<0.001	ADHD, ASD+ADHD > ASD*
SDQ17+ Hyperactivity/Inattention (raw scores)												
Self-rated	2.4	1.8	3.4	2.2	7.4	1.5	6.9	2.1	42.8	3, 86	<0.001	ADHD, ASD+ADHD > ASD***; TD***
Informant-rated	--	--	3.1	1.9	7.4	2.0	7.1	1.7	38.1	2, 66	<0.001	ASD+ADHD, ADHD > ASD***
ADHD symptom counts[a]												
Inattention	--	--	--	--	8.2	1.3	7.5	1.1	−1.5	1, 40	0.13	--
Hyperactivity/impulsivity	--	--	--	--	5.1	2.6	4.6	2.8	−1.4	1, 40	0.68	--
Total SRS-2 (*t*-scores)												
Self-rated	48.5	6.1	61.3	8.9	62.7	6.9	66.7	12.2	20.6	3, 93	<0.001	ASD, ADHD, ASD+ADHD > TD***
Informant-rated	--	--	63.8	8.6	56.9	10.5	69.9	11.6	9.4	2, 67	<0.001	ASD+ADHD > ADHD***; ASD > ADHD*
ADOS-2 Module 4[b]												
Communication	--	--	1.8	2.0	--	--	2.1	2.3	−0.44	1, 37	0.66	--
Social interaction	--	--	3.3	2.7	--	--	4.0	3.9	−0.65	1, 37	0.52	--
Communication + social interaction	--	--	5.4	4.1	--	--	6.1	6.0	−0.61	1, 37	0.55	--
Stereotyped behaviors and restricted interest	--	--	0.3	0.9	--	--	1.0	1.3	−1.9	1, 37	0.07	--

TD, Typical development; ASD, Autism Spectrum Disorder; ADHD, Attention Deficit and Hyperactivity Disorder; M, mean; SD, standard deviation; FSIQ, full-scale intelligence quotient; CAARS, Conners Adult ADHD Rating Scale; SRS-2, Social Responsiveness Scale version 2; SDQ17+, Strengths and Difficulties Questionnaires for adults. [a] Current ADHD symptom counts were based on the Diagnostic Interview for Adult ADHD (DIVA 2.0) or the Young Adult Psychiatric Assessment (YAPA), available in 18 participants with ADHD and 16 participants with ASD+ADHD. [b] Current ADOS-2 scores were available in a subset of 18 individuals with ASD and 14 participants with ASD+ADHD. Post-hoc significant threshold: *p < 0.05, ***p < 0.001.

with autism; four with atypical autism, one with pervasive developmental disorder (PDD) unspecified], according to the International Classification of Diseases (ICD-10) criteria (58). Twenty-two ASD diagnoses were supported by gold-standard research instruments, the Autism Diagnostic Observation Schedule (ADOS) (59); three were accompanied by parent interviews on the Autism Diagnostic Interview-Revised (ADI-R) (60). Ten participants met the current cut-off criteria for ASD on at least one of these measures. One participant without ADOS or ADI-R report received childhood ASD diagnosis from a consultant psychiatrist in a specialist neurodevelopmental clinic supported by an assessment on the Diagnostic Interview for Social and Communication Disorders (DISCO) (61) but had no current scores.

All pure ADHD participants met the current DSM5 diagnostic criteria for ADHD, fifteen with combined, nine with predominantly inattentive and one with predominantly hyperactive presentation, diagnosed by consultant psychiatrists in specialist adult ADHD clinics. Twenty-two diagnoses were supported by the Diagnostic Interview for Adult ADHD (DIVA 2.0) (62) and three by the Conners' Adult ADHD Diagnostic Interview for DSM-IV (CAADID) or other type of psychiatric interviews (no current scores) (63). Symptoms

of ADHD are reported on **Table 2**. Five participants were prescribed psychostimulants (four methylphenidate [MPH], one lisdexamphetamine), two SSRIs (sertraline, escitalopram) and one both (MPH, sertraline).

In the ASD+ADHD group, eighteen participants had clinical ASD diagnoses (five with autism; eleven with Asperger's syndrome; two with atypical autism) and six had research diagnoses through SNAP [four with atypical autism, two with PDD unspecified], based on the ICD-10 criteria. Twenty-one diagnoses were supported by the ADOS and/or the ADI-R. Eleven met the current cut-off criteria for ASD on at least one of these measures. Three without ADOS or ADI-R reports received childhood ASD diagnoses from consultant psychiatrists in a specialist neurodevelopmental clinic supported by the DISCO (no current scores). Fifteen had clinical DSM5 ADHD diagnoses, 11 of which were supported by current scores of DIVA 2.0, four clinical diagnoses were supported assessment on the CAADID or other psychiatric interview methods (no current scores). Nine participants had a significant history of ADHD symptoms assessed through SNAP (three of whom had clinical diagnosis of ADHD) and met current ADHD DSM5 criteria on the Young Adult Psychiatric Assessment (YAPA) (64). Sixteen met the criteria for combined and eight for inattentive DSM5 ADHD

TABLE 2 | Behavioral measures of the duration discrimination task across groups.

	TD (n = 26)	ASD (n = 23)	ADHD (n = 25)	ASD+ADHD (n = 24)
% Mean error DD (SD)	21.7 (10.6)	23.3 (15.7)	23.0 (14.2)	28.0 (13.4)
% Mean error TOJ (SD)	15.3 (12.7)	18.7 (6.0)	19.0 (13.3)	17.3 (14.3)
Mean RT DD (SD)	591.3 (115.3)	560.1 (175.3)	618.1 (159.3)	572.3 (135.5)
Mean RT TOJ (SD)	426.4 (91.4)	402.4 (118.5)	437.5 (146.4)	427.4 (106.9)
SDRT DD (SD)	203.9 (72.7)	192.3 (90.0)	220.6 (86.7)	224.9 (101.9)
SDRT TOJ (SD)	141.3 (74.2)	122.4 (63.2)	158.5 (80.2)	183.2 (91.2)

Comparison of measures during the duration discrimination task indicated no difference across groups in performance in accuracy, MRT, and SDRT. The MRT and SDRT are in seconds, whereas accuracy is presented as raw number where the maximum was 30. MRT, Mean response time; SDRT, standard deviation of response time, a measure of response time variability and SD, standard deviation.

subtype. Six were prescribed psychostimulant (five MPH, one dexamphetamine), two SSRIs (sertraline, escitalopram) and one both (MPH, sertraline).

The TD participants were from local communities, had no psychiatric disorders, were medication-free and scored below clinical cut-off for ADHD and ASD traits on the Conners' Adult ADHD Rating Scale (CAARS) (65) and the Social Responsiveness Scale-2 (SRS-2) (66). This study was in accordance of the Declaration of Helsinki and received ethical approval from a local National Health Service Research Ethics Committee (13/LO/0373). Each participant gave written informed consent and was given £50 for their time.

Clinical Measures

ADHD traits were measured using the ADHD index on the CAARS and the hyperactive/impulsive and inattention domain on the Strengths and Difficulties Questionnaires for adults (SDQ17+; provided by Professor Robert Goodman at the IoPPN). Autistic traits were indexed using the total SRS-2 score. All participants completed self-report measures, corroborated by informants (parents/partner/siblings) for those in the clinical groups.

Time Discrimination Task

This block-design time discrimination task (14, 34–37, 67) consisted of ten 30-s blocks, alternating between duration discrimination (DD) and temporal order judgment (TOJ) blocks. During the DD block, the participants indicated which circle stayed for a longer time on the screen. In the TOJ block, the participants indicated the circle that was shown second. Each block began with a 3-s cue. The cue "2" signaled the start of the TOJ block while "L" signaled the DD block (**Figure 1**). Each block consisted of six trials. In each trial, a pair of circles (green and red) appeared sequentially on the left- and right-hand side of the screen and equal number of trials started from the left and the right side first. Each block consisted of two trials comparing a 1,000-ms standard interval against a 1,300-, 1,400-, or 1,500-ms test duration, followed by a 2100-ms response period. Participants responded as soon as the second circle was presented. Discrimination errors were the

primary outcome measures while mean response time (MRT) and standard deviation of response time (SDRT) were secondary measures. Higher values in these variables reflect increased impairments.

fMRI Data Acquisition and Analyses

Neuroimaging data were acquired on a General Electric MR750 3T scanner (Boston, MA, USA) at King's College London. The scanner's body coil was used for RF transmission while an 8-channel head coil was used for signal reception. The echo planar image (EPI) gradient-echo pulse sequence (TR/TE = 2,000/30 ms, flip angle = 75°, FOV = 21 × 21 cm, 64 × 64 matrix, in-plane resolution = 3 mm, slice thickness/gap = 3.0/0.3 mm) was used to acquire 41 consecutive, top-to-bottom, slices of T2*-weighted MR images parallel to the inter-commissural plane covering the entire brain. The 5-min scan produced 153 volumes in time series. A whole-brain high resolution structural T1-weighted scan (Sagittal ADNI Go/2 ACC SPGR) was acquired in the inter-commissural plane with TR/TE = 7.312/3.016 s, 196 slices, FOV = 27 × 27 cm, 256 × 256 matrix and slice thickness of 1.2 mm.

Each participant's echo-planar imaging (EPI) data were slice-time corrected, realigned, co-registered to the individual's structural T1-weighted scan, segmented, normalized to the Montreal Neurological Institute (MNI) EPI template and smoothed with an 8-mm Gaussian kernel. Statistical analyses were completed in two steps on the Statistical Parametric Mapping (SPM8). At the subject-level, BOLD response was predicted using a vector of onsets and durations convolved with the canonical hemodynamic response function. Six nuisance motion regressors (x-, y-, z-translations and rotations) and separate regressors for each spike (>1 mm) controlled the effects of volume-to-volume head motion and abrupt movements. A high-pass filter (128 s) was applied and a first-order autoregressive model corrected the time series correlation. The contrast DD > TOJ indexed neural correlates of time processing.

Within-group activations (reported in Supplement) were analyzed at the second-level analysis with a cluster extent threshold of $p < 0.05$, family-wise error corrected (FWE$_{cor}$) and a voxel threshold of $p < 0.001$. Between-group activations were analyzed using univariate ANCOVA with group as independent factor, covarying total frame-wise head displacement, in spherical region of interests (ROIs, 10-mm radius) associated with perceptual timing in the general population, drawn from a meta-analysis (68), in the bilateral IFG, SMA, left putamen, left pre-central gyrus, right middle temporal gyrus, right DLPFC, right cingulate gyrus, left insula, and left supramarginal gyrus. The influence of IQ alone on the findings was investigated by adding FSIQ as covariate. To examine the influence of current medication alone, medication status (0 = medicated or 1 = non-medicated) was added in the initial model as covariate. The influence of both medication status and IQ on the group difference findings was assessed by adding both factors as covariates. Additionally, a sensitivity analysis was carried out in participants with no psychotropic medication (22 TD, 21 ASD, 18 ADHD, and 17 ASD+ADHD). BOLD activations were extracted

FIGURE 1 | The time discrimination task. This figure illustrates the temporal order judgement and duration discrimination blocks within the task. In this task, pairs of circles (red and green) are presented sequentially. Each temporal order judgment block begins with a screen showing the number "2." In this block, participants are required to identify the circle that appears at later time between the pairs. Each duration discrimination block starts after the letter "L" is displayed instead. The participants are required to identify the circle that appears for longer duration between the pairs.

from the clusters using the MarsBaR toolbox (69) for *post-hoc* pairwise comparisons and further correlational analyses. Finally, to explore neural impairments across groups that are non-specific to ADHD, we conducted a whole-brain ANOVA analysis with Group as predictor and whole-brain t-contrasts between each clinical group against the TD controls (cluster extent threshold of $p < 0.05$ FWE_{cor} and voxel threshold of $p < 0.001$).

Statistical Analyses

Phenotypic, behavioral, and extracted BOLD data were analyzed using IBM SPSS Statistics 22 (IBM Corp., 2013). Demographic data and phenotypic reports were analyzed using univariate ANOVAs. Task performance measures (Errors, MRT, and SDRT) were analyzed using a 4 × 2 mixed-design ANOVA (Group × Condition), prior which error rates were square-rooted to normalize their distribution *post-hoc* pairwise multiple comparisons were corrected with the Tukey-Kramer method. Correlations between BOLD activations and task performance or phenotypic traits were conducted per group.

RESULTS

Participant Characteristics

Groups did not differ in age or handedness, but in FSIQ [$F_{(3, 94)} = 4.9$, $p = 0.003$], which was higher in the ADHD ($p = 0.012$), TD groups ($p = 0.033$) and, at trend level, the ASD+ADHD ($p = 0.089$) relative to the ASD group. Groups differed in self-rated ADHD index [$F_{(3, 94)} = 31.3$, $p < 0.001$], the SDQ score [$F_{(3, 94)} = 42.8$, $p < 0.001$] and the informant-rated scores for these measures [$F_{(3, 94)} = 7.3–38.1$, $ps < 0.001$], with

post-hoc t-tests indicating higher ADHD symptoms in the ADHD and ASD+ADHD groups than the TD ($ps < 0.001$) and the ASD groups ($ps < 0.001$) according to the young adults, which was corroborated by informant-ratings. Self-reported autistic traits were higher in all clinical groups [$F_{(3, 94)} = 20.6$, $p < 0.001$] than controls (all $ps < 0.001$), although informant-rated ASD traits [$F_{(3, 94)} = 9.4$, $p < 0.001$] were significantly higher in the ASD+ADHD ($p < 0.001$) and, at trend-level, in the ASD ($p = 0.064$) relative to the ADHD group.

Performance Results

Errors, MRT and SDRT were greater during DD than TOJ [$Fs_{(1, 94)} \geq 26.1$, $ps < 0.001$] but no main Group effect [$Fs_{(3, 94)} \leq 1.90$, $ps \geq 0.14$] or Group × Condition interaction effect [$Fs_{(3, 94)} \leq 0.67$, $ps \geq 0.67$] were observed.

Neuroimaging Results
Motion

Group difference in the total volume-to-volume head movement in the x-, y-, and z- rotation and translation was significant [$F_{(3, 94)} = 2.65$, $p = 0.05$] and was covaried in the second-level analysis.

Within-Group Brain Activations

The TD group showed activation in right IFC/anterior insula (AI; BA47/13/44/45/46), reaching into the striatum/thalamus; frontally to the dlPFC (BA10/9) and pre-central gyrus (pre-CG; BA6) and medially to the mid-cingulate gyrus/mPFC/SMA (BA32). Activations were also observed in left IFC/AI (BA47/13), reaching into striatum/pallidum; in bilateral

IPL/supramarginal/angular gyri (BA40) and left posterior cerebellum. In ASD, clusters of activations were less extensive than in TD although mostly overlapping, including right IFC/AI (BA44/45/46/13), dlPFC (BA6), mid-cingulate gyrus/SMA (BA32), and premotor and superior frontal gyrus (SFG; BA10). Also included were left IFC/AI (BA45/13), right IPL/supramarginal/angular gyri (BA40), and left cerebellum, and left pre-CG (BA6). Participants with ADHD showed smaller clusters than the TD and ASD groups in right IFC/dlPFC (BA44/45/46/9/8) reaching into pre-CG (BA6), in left posterior cerebellum extending to right cerebellar lobe, in cingulate gyrus reaching to mPFC/SMA areas (BA24/32/6), and in left IFC/pre-CG (BA44/45/6). The clusters in the ASD+ADHD group were the least extensive and found in mPFC/SMA (BA24/32/6), bilateral AI/IFC (BA13/47/45), reaching into caudate/putamen on the right hemisphere (see **Figure 2**).

Between-Group Brain Activations

In the ROIs typically activated during perceptual timing, a group effect was observed in a right IFG cluster [$p = 0.049$, $F = 5.5$, (10, 12, 46), $k_E = 56$ voxels] (see **Figure 3**), with *post-hoc* comparisons showing that the ASD+ADHD group had less activation than the other groups ($ps \leq 0.024$). Covarying for IQ reduced the group effect to a trend level [$p = 0.09$, $F = 4.8$, (10, 12, 46), $k_E = 18$ voxels], preserving the pairwise difference between the ASD+ADHD and the TD or ASD ($ps \leq 0.027$), but not the ADHD group ($p = 0.08$). Group effect findings in right IFG cluster were maintained when medication status alone [$p = 0.019$, $F = 6.5$, (10, 12, 48), $k_E = 235$ voxels], and when both medication status and IQ were covaried [$p = 0.039$, $F = 5.8$, (10, 12, 48), $k_E = 137$ voxels] and the *post-hoc* pairwise analyses consistently showed reduced activation in the ASD+ADHD relative to other groups ($ps \leq 0.040$) in this cluster. Sensitivity analyses excluding participants on medication showed the same group effect [$p = 0.036$, $F = 6.0$, (10, 12, 52), $k_E = 49$ voxels]. However, *post-hoc* t-tests showed reduced activation in the ASD+ADHD group relative to the TD and ASD ($ps \leq 0.008$) but not the ADHD group ($p = 0.51$). No other significant pairwise differences were observed. The right IFG activation cluster correlated negatively with SDRT in the TD [r_{TD} (26) = −0.43; $p = 0.03$]; but not the clinical groups ($|rs| \leq 0.18$; $p \geq 0.38$).

Whole-brain analyses revealed under-activation in right IFG/DLPFC cluster in the ASD+ADHD relative to the TD group [$p = 0.033$, $t = 4.2$, (8, 32, 36), $k_E = 440$], which was preserved when medication status was covaried [$p = 0.002$, $t = 4.5$, (10, 10, 48), $k_E = 864$ voxels] but did not survive after covarying for IQ, after covarying for both medication status and IQ, or in the sensitivity analysis excluding those who were prescribed medication. No other comparisons between the clinical and TD groups yielded significant differences.

DISCUSSION

The study was aimed at elucidating the similarities and differences in the neural correlates of duration discrimination in young adult males with ASD, ADHD, and ASD+ADHD.

The groups had comparable task performance. However, people with ASD+ADHD had under-activation in right IFG relative to the clinical and TD groups in ROIs most consistently activated during time perception (68). In support of this finding, under-activation in right IFG/DLPFC was found only in the ASD+ADHD group relative to TD controls in the exploratory whole-brain analyses. This suggests that in adulthood, only people with ASD+ADHD, but not the pure disorders are impaired in the key region that mediates time discrimination.

The lack of neurofunctional impairment in the ADHD group is not in line with the hypothesized under-activation in right IFG based on reports in adolescents with ADHD during the same task (14, 33–38), which could have several possible explanations. First, previous reports have examined adolescents rather than adults with ADHD. Thus, it is possible that adults with ADHD no longer demonstrate the lateral frontal functional deficits related to time perception observed at younger ages, as has also been shown during response inhibition in adults with ADHD compared to typically developing adults in some previous studies (70–72). Second, compared to previous studies of timing (14, 34–38), the ADHD participants in this study had above-average IQ, which might have moderated time-processing related neural activation deficits, since covarying for IQ reduced the statistical significance of the difference in right IFG activation between the ADHD and the ASD+ADHD group to trend level. Third and most importantly, the lack of neurofunctional abnormalities in the ADHD relative to the TD group could be related to current psychotropic medication prescription in the sample. A sensitivity analysis excluding participants with psychotropic medication (mostly psychostimulants) revealed that the difference between the ASD+ADHD and ADHD groups was no longer significant, suggesting a subtle subthreshold abnormality that may still have been present in the non-medicated ADHD group. This is in line with the typical observation of right IFG under-activation during duration discrimination in medication-naïve ADHD children (14, 34–36), and the findings of an association between single-dose and long-term psychostimulant administration with the upregulation and normalization of right IFG under-activation during timing and other tasks in ADHD children (14, 35, 36, 73–76). This interpretation should, however, be taken with caution as an analysis covarying for medication retained the right IFG under-activation finding in the ASD+ADHD relative to the ASD, ADHD, and TD, which did not differ from one another, suggesting that the exclusion of medicated participants during the sensitivity analyses may have led to reduced power.

The lack of neural impairments in the ASD group was not in line with the initial hypothesis, which, in the absence of fMRI studies of timing in ASD, was formulated based on behavioral findings only [e.g., (39, 42)]. However, negative neurobehavioral findings from some studies (45–47), including a recent study in adults with ASD (77), have suggested heterogeneity in timing impairments in this population. Therefore, although the implication of the present neurofunctional finding is that timing networks in young adults with ASD without intellectual disability are unimpaired, this must be taken mindful of factors that could
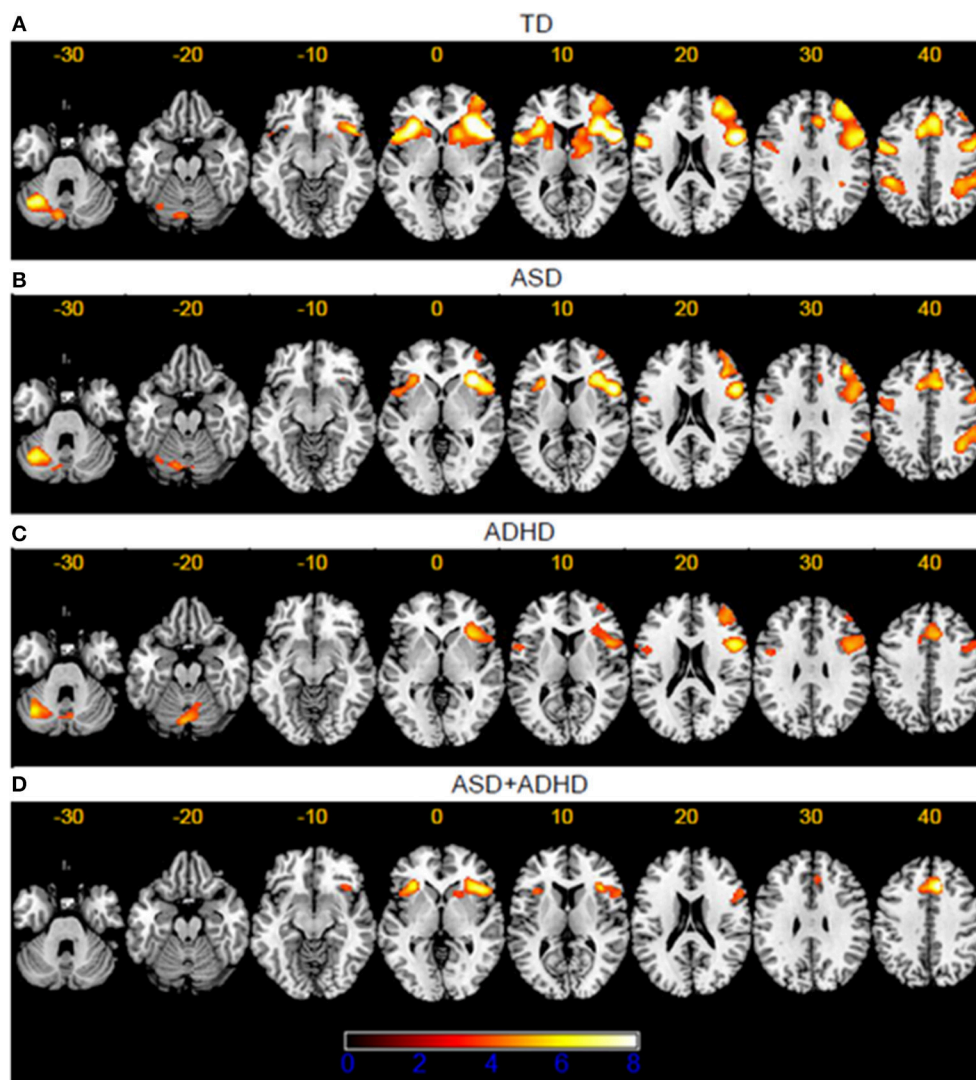
FIGURE 2 | Within-group brain activation clusters contrasting the block duration discrimination vs. temporal order judgement in the **(A)** TD, **(B)** ASD, **(C)** ADHD, and **(D)** ASD+ADHD groups.

have increased the participants' heterogeneity in the study. First, participants in the ASD and ASD+ADHD group in this study had varying presentations (autism, Aspergers, atypical autism, PDD unspecified), which could be associated with heterogeneous neural impairments [see (78, 79)]. Second, a sizeable number of people with ASD in this study are community-sampled and may have less severe impairment than individuals who are clinically referred (80). This is supported by the fact that among those who had current ADOS or ADI-R, only approximately half the participants in the ASD and the ASD+ADHD groups met the clinical cut-off criteria on either measure. Time perception network abnormalities may thus be present in clinically referred children and adults with ASD. Another consideration is the known relationship between time perception and a number of other EF domains such as inhibition (20, 22), working memory and sustained attention (81–83). Meta-analytic findings

suggest that these different cognitive functions are subserved by overlapping regions (50). It is hence possible that poor behavioral performance during time perception tasks found in boys and adult males with ASD in previous studies was mediated by a variety of individually specific deficits in neural networks for other EF domains (4, 6, 48, 49) rather than those subserving time perception *per se.*

The novel finding of right IFG under-activation in the ASD+ADHD group during DD in the ROI and the whole-brain analyses indicate that the addition of ADHD symptoms in adults with ASD lead to increased neurofunctional impairments during DD. This interpretation extends, in the domain of time perception, a previous finding by Chantiluke et al. (6) based on weaker brain-behavior associations in the comorbid group relative to the pure groups in brain regions associated with temporal reward discounting. Since adults with ASD+ADHD

FIGURE 3 | Between-group effects of duration discrimination vs. temporal order judgement. **(A)** Significant right IFG cluster where Group effect was found. **(B)** Average BOLD signal by group is displayed (i) for all data, (ii) after covarying for IQ and (iii) covarying for medication. **(C)** Correlation between BOLD and SDRT by group indicates that reduced BOLD is associated with increased in SDRT. $*p < 0.05$, $**p < 0.01$.

had similar neural impairments as ADHD boys, which are no longer observed in the ADHD adult group, the findings could also indicate more persistent impairments in the comorbid relative to the pure ADHD group. This hypothesis could be further explored by replicating the study involving participants with a wider age range including adolescents and adults.

Analyses of behavioral measures suggest comparable task performance across all participant groups, which was unexpected in people with ADHD where duration discrimination deficits have been reported [e.g., (7, 26–28)]. However, tasks typically lose behavioral sensitivity when adapted for fMRI studies (84, 85), and the recommended sample size for fMRI studies of over 20 (86) may not be sufficiently powered for detecting group differences in behavioral performance on fMRI tasks. In fact, not all findings of neural impairments were accompanied by task performance deficits in ADHD children compared to healthy controls in previous studies of duration discrimination (37, 38). However, a brain-behavior association between increased right IFG activation and reduced response time variability (SDRT) was found in the typically developing group, underlining the importance of this region for task performance in the

healthy population. Increased intra-subject SDRT is an indicator of attentional lapses during cognitive tasks (87, 88) which, during DD, disturbs the perception of the passage of time (89). Thus, increased activation in right IFG in the control group may also reflect better attentional control during time perception.

A strength of the study is the robust characterization of diagnoses of ASD and ADHD in the majority of patients, including the use of ICD-10, DSM5, ADI-R, ADOS, DIVA 2.0, and CAADID, as appropriate (although current scores were not available for everyone). One weakness was the IQ difference across groups which resulted in altered findings of the exploratory whole-brain analysis when IQ was covaried. However, the use of ANCOVA to correct for IQ when it is intrinsically different between groups [e.g., between individuals with neurodevelopmental disorders relative to TD controls (90–92)] and thus when the group memberships are not randomly assigned, is statistically not appropriate since it can lead to artifactual positive or negative results (93–95). Furthermore, covarying for IQ mostly preserved the conclusion from the ROI analyses especially with respect to the under-activation of right IFG in the ASD+ADHD group

relative to other groups, suggesting that the study was not entirely powered for exploratory whole-brain analyses. Another limitation is the inclusion of people currently prescribed medication in the ADHD and ASD+ADHD groups as both SSRIs and stimulants have been shown to affect brain activation in people with these conditions [e.g., (35, 96, 97)]. However, findings remained when we covaried for medication status and sensitivity analyses excluding individuals on medication did not change the primary finding between the ASD+ADHD and TD groups in this study. Additionally, the narrow age range (20–27 years) of the young adult participants limits generalizability of findings to the entire adult ADHD and ASD populations. Finally, fMRI has limitations with respect to temporal resolution. While we were mainly interested in the spatial location of shared or disorder-specific brain abnormalities in relation to the process of temporal judgement in the disorders, adding a temporally better resolved method would have allowed us to also understand differences between disorders in the exact time course of activation deficits. Thus, future studies may consider the combined use of fMRI and physiological (e.g., electroencephalography or magnetoencephalography) approaches with high temporal resolution to complement the fMRI findings during time estimation.

CONCLUSIONS

In summary, only young adult males with comorbid ASD+ADHD showed reduced activation in right IFG during duration discrimination relative to healthy controls and the pure groups who were unimpaired. The findings suggest that right IFG is not neurofunctionally impaired in young adults with ADHD or ASD during time perception, although the finding in

the ADHD group particularly has to be viewed in light of the possible moderating influence of IQ and medication use among the participants in this study.

REFERENCES

1. American Psychiatric Association (APA). *Diagnostic and Statistical Manual of Mental Disorders: DSM-5*. Washington, DC (2013).
2. Joshi G, Wozniak J, Petty C, Martelon MK, Fried R, Bolfek A, et al. Psychiatric comorbidity and functioning in a clinically referred population of adults with autism spectrum disorders: A comparative study. *J Autism Dev Disord.* (2013) 43:1314–25. doi: 10.1007/s10803-012-1679-5
3. Johnston K, Dittner A, Bramham J, Murphy C, Knight A, Russell A. Attention deficit hyperactivity disorder symptoms in adults with autism spectrum disorders. *Autism Res.* (2013) 6:225–36. doi: 10.1002/aur.1283
4. Christakou A, Murphy CM, Chantiluke K, Cubillo AI, Smith AB, Giampietro V, et al. Disorder-specific functional abnormalities during sustained attention in youth with attention deficit hyperactivity disorder (ADHD) and with autism. *Mol Psychiatry* (2013) 18:236–44. doi: 10.1038/mp.2011.185
5. Di Martino A, Zuo XN, Kelly C, Grzadzinski R, Mennes M, Schvarcz A, et al. Shared and distinct intrinsic functional network centrality in autism and attention-deficit/hyperactivity disorder. *Biol Psychiatry* (2013) 74:623–632. doi: 10.1016/j.biopsych.2013.02.011
6. Chantiluke K, Christakou A, Murphy CM, Giampietro V, Daly EM, Ecker C, et al. Disorder-specific functional abnormalities during temporal discounting in youth with attention deficit hyperactivity disorder (ADHD), autism and comorbid ADHD and autism. *Psychiatry Res.* (2014) 223:113–20. doi: 10.1016/j.pscychresns.2014.04.006

7. Noreika V, Falter CM, Rubia K. Timing deficits in attention-deficit/hyperactivity disorder (ADHD): evidence from neurocognitive and neuroimaging studies. *Neuropsychologia* (2013) 51:235–66. doi: 10.1016/j.neuropsychologia.2012.09.036
8. Boyer BE, Geurts HM, Van der Oord S. Planning skills of adolescents with ADHD. *J Atten Disord.* (2014) 22:46–57. doi: 10.1177/1087054714538658
9. Kofler MJ, Sarver DE, Harmon SL, Moltisanti A, Aduen PA, Soto EF, et al. Working memory and organizational skills problems in ADHD. *J Child Psychol Psychiatry* (2018) 59:57–67. doi: 10.1111/jcpp.12773
10. Kenworthy LE, Black DO, Wallace GL, Ahluvalia T, Wagner AE, Sirian LM. Disorganization: the forgotten executive dysfunction in high-functioning autism (HFA) spectrum disorders. *Dev Neuropsychol.* (2005) 28:809–27. doi: 10.1207/s15326942dn2803_4
11. van den Bergh SF, Scheeren AM, Begeer S, Koot HM, Geurts HM. Age related differences of executive functioning problems in everyday life of children and adolescents in the autism spectrum. *J Autism Dev Disord.* (2014) 44:1959–71. doi: 10.1007/s10803-014-2071-4
12. Barkley RA, Murphy KR, Bush T. Time perception and reproduction in young adults with attention deficit hyperactivity disorder. *Neuropsychology* (2001) 15:351–60. doi: 10.1037/0894-4105.15.3.351
13. de Zeeuw P, Weusten J, van Dijk S, van Belle J, Durston S. Deficits in cognitive control, timing and reward sensitivity appear to be dissociable in ADHD. *PLoS ONE* (2012) 7:e51416. doi: 10.1371/journal.pone.0051416
14. Rubia K, Halari R, Christakou A, Taylor E. Impulsiveness as a timing disturbance: neurocognitive abnormalities in attention-deficit

hyperactivity disorder during temporal processes and normalization with methylphenidate. *Philos Trans R Soc Lond B Biol Sci.* (2009) 364:1919–31. doi: 10.1098/rstb.2009.0014

15. Baumann AA, Odum AL. Impulsivity, risk taking, and timing. *Behav Process.* (2012) 90:408–14. doi: 10.1016/j.beproc.2012.04.005

16. Wittmann M, Simmons AN, Flagan T, Lane SD, Wackermann J, Paulus MP. Neural substrates of time perception and impulsivity. *Brain Res.* (2011) 1406:43–58. doi: 10.1016/j.brainres.2011.06.048

17. Hewetson A. *The Stolen Child: Aspects of Autism and Asperger Syndrome.* Westport, CT; London: Bergin & Garvey (2002).

18. Wing L. *The Autistic Spectrum: A Guide for Parents and Professionals.* London: Constable (1996)

19. Peeters T, Gillberg C. *Autism: Medical and Educational Aspects.* London: Whurr (1999).

20. Brown SW, Perreault ST. Relation between temporal perception and inhibitory control in the Go/No-Go task. *Acta Psychol.* (2017) 173:87–93. doi: 10.1016/j.actpsy.2016.12.004

21. Grinblat N, Rosenblum S. Why are they late? Timing abilities and executive control among students with learning disabilities. *Res Dev Disabil.* (2016) 59:105–114. doi: 10.1016/j.ridd.2016.07.012

22. Ogden RS, Salominaite E, Jones LA, Fisk JE, Montgomery C. The role of executive functions in human prospective interval timing. *Acta Psychol.* (2011) 137:352–8. doi: 10.1016/j.actpsy.2011.04.004

23. Craig F, Margari F, Legrottaglie AR, Palumbi R, de Giambattista C, Margari L. A review of executive function deficits in autism spectrum disorder and attention-deficit/hyperactivity disorder. *Neuropsychiatr Dis Treat.* (2016) 12:1191–202. doi: 10.2147/NDT.S104620

24. Rommelse NNJ, Geurts HM, Franke B, Buitelaar JK, Hartman CA. A review on cognitive and brain endophenotypes that may be common in autism spectrum disorder and attention-deficit/hyperactivity disorder and facilitate the search for pleiotropic genes. *Neurosci Biobehav Rev.* (2011) 35:1363–96. doi: 10.1016/j.neubiorev.2011.02.015

25. Falter CM, Noreika V. Time processing in developmental disorders: a comparative view. In: Arstila V, Lloyd D, editors, *Subjective Time.* Cambridge, MA: MIT Press (2014). pp. 557–598.

26. Smith AB, Taylor E, Rogers JW, Newman S, Rubia K. Evidence for a pure time perception deficit in children with ADHD. *J Child Psychol Psychiatry* (2002) 43:529–42. doi: 10.1111/1469-7610.00043

27. Toplak M, Tannock R. Time perception: modality and duration effects in attention-deficit/hyperactivity disorder (ADHD). *J Abnorm Child Psychol.* (2005) 33:639–54. doi: 10.1007/s10802-005-6743-6

28. Valko L, Schneider G, Doehnert M, Müller U, Brandeis D, Steinhausen H-C, et al. Time processing in children and adults with ADHD. *J Neural Transm.* (2010) 117:1213–28. doi: 10.1007/s00702-010-0473-9

29. Barkley RA, Edwards G, Laneri M, Fletcher K, Metevia L. Executive functioning, temporal discounting, and sense of time in adolescents with attention deficit hyperactivity disorder (ADHD) and oppositional defiant disorder (ODD). *J Abnorm Child Psychol.* (2001) 29:541–56. doi: 10.1023/A:1012233310098

30. Bauermeister JJ, Barkley RA, Martinez JV, Cumba E, Ramirez RR, Reina G, et al. Time estimation and performance on reproduction tasks in subtypes of children with attention deficit hyperactivity disorder. *J Clin Child Adolesc Psychol.* (2005) 34:151–62. doi: 10.1207/s15374424jccp3401_14

31. McGee R, Brodeur D, Symons D, Andrade B, Fahie C. Time perception: does it distinguish ADHD and RD children in a clinical sample? *J Abnorm Child Psychol.* (2004) 32:481–490. doi: 10.1023/B:JACP.0000037778.61929

32. Droit-Volet S. Time perception in children: a neurodevelopmental approach. *Neuropsychologia* (2013) 51:220–34. doi: 10.1016/j.neuropsychologia.2012.09.023

33. Hart H, Radua J, Mataix-Cols D, Rubia K. Meta-analysis of fMRI studies of timing in attention-deficit hyperactivity disorder (ADHD). *Neurosci Biobehav Rev.* (2012) 36:2248–56. doi: 10.1016/j.neubiorev.2012.08.003

34. Hart H, Marquand AF, Smith A, Cubillo A, Simmons A, Brammer M, et al. Predictive neurofunctional markers of attention-deficit/hyperactivity disorder based on pattern classification of temporal processing. *J Am Acad Child Adolesc Psychiatry* (2014) 53:569–78.e1. doi: 10.1016/j.jaac.2013.12.024

35. Rubia K, Alegria AA, Cubillo AI, Smith AB, Brammer MJ, Radua J. Effects of stimulants on brain function in attention-deficit/hyperactivity disorder:

36. Smith AB, Cubillo A, Barrett N, Giampietro V, Simmons A, Brammer M, et al. Neurofunctional effects of methylphenidate and atomoxetine in boys with attention-deficit/hyperactivity disorder during time discrimination. *Biol Psychiatry* (2013) 74:615–22. doi: 10.1016/j.biopsych.2013.03.030

37. Smith AB, Taylor E, Brammer M, Halari R, Rubia K. Reduced activation in right lateral prefrontal cortex and anterior cingulate gyrus in medication-naive adolescents with attention deficit hyperactivity disorder during time discrimination. *J Child Psychol Psychiatry* (2008) 49:977–85. doi: 10.1111/j.1469-7610.2008.01870.x

38. Vloet TD, Gilsbach S, Neufang S, Fink GR, Herpertz-Dahlmann B, Konrad K. Neural mechanisms of interference control and time discrimination in attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* (2010) 49:356–67. doi: 10.1097/00004583-201004000-00010

39. Allman MJ, DeLeon IG, Wearden JH. Psychophysical assessment of timing in individuals with autism. *Am J Intellect Dev Disabil.* (2011) 116:165–78. doi: 10.1352/1944-7558-116.2.165

40. Baron-Cohen S, Wheelwright S, Scahill V, Lawson J, Spong A. Are intuitive physics and intuitive psychology independent? A test with children with Asperger syndrome. *J Dev Learn Disord.* (2001) 5:47–78.

41. Falter CM, Noreika V, Wearden JH, Bailey AJ. More consistent, yet less sensitive: interval timing in autism spectrum disorders. *Q J Exp Psychol.* (2012) 65:2093–107. doi: 10.1080/17470218.2012.690770

42. Kargas N, López B, Reddy V, Morris P. The relationship between auditory processing and restricted, repetitive behaviors in adults with autism spectrum disorders. *J Autism Dev Disord.* (2015) 45:658–68. doi: 10.1007/s10803-014-2219-2

43. Maister L, Plaisted-Grant KC. Time perception and its relationship to memory in autism spectrum conditions: time perception in ASC. *Dev Sci.* (2011) 14:1311–22. doi: 10.1111/j.1467-7687.2011.01077.x

44. Lepisto T, Silokallio S, Nieminen-von Wendt T, Alku P, Naatanen R, Kujala T. Auditory perception and attention as reflected by the brain event-related potentials in children with Asperger syndrome. *Clin Neurophysiol.* (2006) 117:2161–71. doi: 10.1016/j.clinph.2006.06.709

45. Jones CR, Happe F, Baird G, Simonoff E, Marsden AJ, Tregay J, et al. Auditory discrimination and auditory sensory behaviours in autism spectrum disorders. *Neuropsychologia* (2009) 47:2850–8. doi: 10.1016/j.neuropsychologia.2009.06.015

46. Kofler MJ, Rapport MD, Bolden J, Sarver DE, Raiker JS. ADHD and working memory: the impact of central executive deficits and exceeding storage/rehearsal capacity on observed inattentive behavior. *J Abnorm Child Psychol.* (2010) 38:149–61. doi: 10.1007/s10802-009-9357-6

47. Wallace GL, Happe F. Time perception in autism spectrum disorders. *Res Autism Spectr Disord.* (2008) 2:447–55. doi: 10.1016/j.rasd.2007.09.005

48. Chantiluke K, Barrett N, Giampietro V, Brammer M, Simmons A, Rubia K. Disorder-dissociated effects of fluoxetine on brain function of working memory in attention deficit hyperactivity disorder and autism spectrum disorder. *Psychol Med.* (2015) 45:1195–205. doi: 10.1017/S0033291714002232

49. Chantiluke K, Barrett N, Giampietro V, Santosh P, Brammer M, Simmons A, et al. Inverse fluoxetine effects on inhibitory brain activation in non-comorbid boys with ADHD and with ASD. *Psychopharmacology* (2015) 232:2071–82. doi: 10.1007/s00213-014-3837-2

50. Radua J, Del Pozo NO, Gómez J, Guillen-Grima F, Ortuño F. Meta-analysis of functional neuroimaging studies indicates that an increase of cognitive difficulty during executive tasks engages brain regions associated with time perception. *Neuropsychologia* (2014) 58:14–22. doi: 10.1016/j.neuropsychologia.2014.03.016

51. Kim BK, Zauberman G. Perception of anticipatory time in temporal discounting. *J Neurosci Psychol Econ.* (2009) 2:91–101. doi: 10.1037/a0017686

52. Jones CR, Jahanshahi M. Dopamine modulates striato-frontal functioning during temporal processing. *Front Integr Neurosci.* (2011) 5:70. doi: 10.3389/fnint.2011.00070

53. Wechsler D. *Wechsler Abbreviated Scale of Intelligence - 2nd Edition (WASI-II).* Bloomington, MN: Pearson (2011).

54. Fayyad J, De Graaf R, Kessler R, Alonso J, Angermeyer M, Demyttenaere K, et al. Cross-national prevalence and correlates of adult

attention-deficit hyperactivity disorder. *Br J Psychiatry* (2007) 190:402–9. doi: 10.1192/bjp.bp.106.034389

55. Fombonne E. Epidemiology of autistic disorder and other pervasive developmental disorders. *J Clin Psychiatry* (2005) 66(Suppl. 10):3–8.

56. Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* (1971) 9:97–113. doi: 10.1016/0028-3932(71)90067-4

57. Baird G, Simonoff E, Pickles A, Chandler S, Loucas T, Meldrum D, et al. Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: the Special Needs and Autism Project (SNAP). *Lancet* (2006) 368:210–5. doi: 10.1016/S0140-6736(06)69041-7

58. World Health Organization (WHO). *ICD-10: International Statistical Classification of Diseases and Related Health Problems Tenth Revision.* Geneva: WHO(2004).

59. Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, et al. The Autism Diagnostic Observation Schedule-Generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord.* (2000) 30:205–23. doi: 10.1023/A:1005592401947

60. Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord.* (1994) 24:659–85.

61. Wing L, Leekam SR, Libby SJ, Gould J, Larcombe M. The Diagnostic Interview for Social and Communication Disorders: background, inter-rater reliability and clinical use. *J Child Psychol Psychiatry* (2002) 43:307–325. doi: 10.1111/1469-7610.00023

62. Kooij JJS. *Adult ADHD: Diagnostic Assessment and Treatment.* Springer: London; New York (2013)

63. Epstein JN, Johnson DE, Conners CK. *Conners' Adult ADHD Diagnostic Interview for DSM-IV.* North Tonawanda, NY: Multi-Health Systems Inc. (2001).

64. Angold A, Cox A, Prendergast M, Rutter M, Simonoff E. *The Young Adult Psychiatric Assessment (YAPA) Version 2.0.3.* Durham, NC: Duke University (2009).

65. Conners CK, Erhardt D, Sparrow E. *Conners' Adult ADHD Rating Scales (CAARS): Technical Manual.* New York, NY; Toronto, ON: Multi-Health Systems Inc. (1999).

66. Constantino JN, Gruber C. *Social Responsiveness Scale^{TM}, Second Edition (SRS^{TM}-2).* Torrance, CA: Western Psychological Services (2012).

67. Smith A, Taylor E, Lidzba K, Rubia K. A right hemispheric frontocerebellar network for time discrimination of several hundreds of milliseconds. *Neuroimage* (2003) 20:344–50. doi: 10.1016/S1053-8119(03)00337-9

68. Wiener M, Turkeltaub P, Coslett HB. The image of time: a voxel-wise meta-analysis. *Neuroimage* (2010) 49:1728–740. doi: 10.1016/j.neuroimage.2009.09.064

69. Brett M, Anton J-L, Valabregue R, Poline JB. Region of interest analysis using an SPM toolbox. Presented at the 8th International Conference on Functional Mapping of the Human Brain, June 2-6, 2002, Sendai, Japan. *Neuroimage* (2002) 16(Supplement 1): 1140–1. doi: 10.1016/s1053-8119(02)90013-3

70. Congdon E, Altshuler LL, Mumford JA, Karlsgodt KH, Sabb FW, Ventura J, et al. Neural activation during response inhibition in adult attention-deficit/hyperactivity disorder: preliminary findings on the effects of medication and symptom severity. *Psychiatry Res Neuroimaging* (2014) 222:17–28. doi: 10.1016/j.pscychresns.2014.02.002

71. Carmona S, Hoekzema E, Antoni Ramos-Quiroga J, Richarte V, Canals C, Bosch R, et al. Response inhibition and reward anticipation in medication-naive adults with attention-deficit/hyperactivity disorder: a within-subject case-control neuroimaging study. *Hum Brain Mapp.* (2012) 33:2350–61. doi: 10.1002/hbm.21368

72. Chen CY, Yen JY, Yen CF, Chen CS, Liu GC, Liang CY, et al. Aberrant brain activation of error processing among adults with attention deficit and hyperactivity disorder. *Kaohsiung J Med Sci.* (2015) 31:179–87. doi: 10.1016/j.kjms.2015.01.001

73. Chou T-L, Chia S, Shang C-Y, Gau SS-F. Differential therapeutic effects of 12-week treatment of atomoxetine and methylphenidate on drug-naive children with attention deficit hyperactivity disorder: a counting Stroop functional MRI study. *Eur Neuropsychopharmacol.* (2015) 25:2300–10. doi: 10.1016/j.euroneuro.2015.08.024

74. Cubillo A, Smith AB, Barrett N, Giampietro V, Brammer MJ, Simmons A, et al. Shared and drug-specific effects of atomoxetine and methylphenidate on inhibitory brain dysfunction in medication-naive ADHD boys. *Cereb Cortex* (2014) 24:174–85. doi: 10.1093/cercor/bhs296

75. Hart H, Radua J, Nakao T, Mataix-Cols D, Rubia K. Meta-analysis of functional magnetic resonance imaging studies of inhibition and attention in attention-deficit/hyperactivity disorder: exploring task-specific, stimulant medication, and age effects. *JAMA Psychiatry* (2013) 70:185–98. doi: 10.1001/jamapsychiatry.2013.277

76. Norman LJ, Carlisi C, Lukito S, Hart H, Mataix-Cols D, Radua J, et al. Structural and functional brain abnormalities in attention-deficit/hyperactivity disorder and obsessive-compulsive disorder: a comparative meta-analysis. *JAMA Psychiatry* (2016) 73:815–25. doi: 10.1001/jamapsychiatry.2016.0700

77. Jones CR, Lambrechts A, Gaigg SB. Using time perception to explore implicit sensitivity to emotional stimuli in autism spectrum disorder. *J Autism Dev Disord.* (2017) 47:2054–66. doi: 10.1007/s10803-017-3120-6

78. McAlonan GM, Cheung C, Cheung V, Wong N, Suckling J, Chua SE. Differential effects on white-matter systems in high-functioning autism and Asperger's syndrome. *Psychol Med.* (2009) 39:1885–93. doi: 10.1017/S0033291709005728

79. McAlonan GM, Suckling J, Wong N, Cheung V, Lienenkaemper N, Cheung C, et al. Distinct patterns of grey matter abnormality in high-functioning autism and Asperger's syndrome. *J Child Psychol Psychiatry* (2008) 49:1287–95. doi: 10.1111/j.1469-7610.2008.01933.x

80. Goodman SH, Lahey BB, Fielding B, Dulcan M, Narrow W, Regier D. Representativeness of clinical samples of youths with mental disorders: a preliminary population-based study. *J Abnorm Psychol.* (1997) 106:3–14.

81. Lee HY, Yang EL. Exploring the effects of working memory on time perception in attention deficit hyperactivity disorder. *Psychol Rep.* (2018). doi: 10.1177/0033294118755674. [Epub ahead of print].

82. Pan Y, Luo QY. Working memory modulates the perception of time. *Psychon Bull Rev.* (2012) 19:46–51. doi: 10.3758/s13423-011-0188-4

83. Polti I, Martin B, van Wassenhove V. The effect of attention and working memory on the estimation of elapsed time. *Sci Rep.* (2018) 8:6690. doi: 10.1038/s41598-018-25119-y

84. Rubia K, Smith AB, Brammer MJ, Toone B, Taylor E. Abnormal brain activation during inhibition and error detection in medication-naive adolescents with ADHD. *Am J Psychiatry* (2005) 162:1067–75. doi: 10.1176/appi.ajp.162.6.1067

85. Smith AB, Taylor E, Brammer M, Toone B, Rubia K. Task-specific hypoactivation in prefrontal and temporoparietal brain regions during motor inhibition and task switching in medication-naive children and adolescents with attention deficit hyperactivity disorder. *Am J Psychiatry* (2006) 163:1044–51. doi: 10.1176/ajp.2006.163.6.1044

86. Thirion B, Pinel P, Mériaux S, Roche A, Dehaene S, Poline JB. Analysis of a large fMRI cohort: statistical and methodological issues for group analyses. *Neuroimage* (2007) 35:105–20. doi: 10.1016/j.neuroimage.2006.11.054

87. Huang-Pollock CL, Karalunas SL, Tam H, Moore AN. Evaluating vigilance deficits in ADHD: a meta-analysis of CPT performance. *J Abnorm Psychol.* (2012) 121:360–71. doi: 10.1037/a0027205

88. Haynes BI, Bauermeister S, Bunce D. Does within-person variability predict errors in healthy adults aged 18-90? *Q J Exp Psychol.* (2017) 70:1722–31. doi: 10.1080/17470218.2016.1204328

89. Zakay D. Relative and absolute duration judgments under prospective and retrospective paradigms. *Percept Psychophys.* (1993) 54:656–64.

90. Charman T, Pickles A, Simonoff E, Chandler S, Loucas T, Baird G. IQ in children with autism spectrum disorders: data from the Special Needs and Autism Project (SNAP). *Psychol Med.* (2011) 41:619–27. doi: 10.1017/s0033291710000991

91. Postorino V, Fatta LM, Sanges V, Giovagnoli G, De Peppo L, Vicari S, et al. Intellectual disability in autism spectrum disorder: investigation of prevalence in an Italian sample of children and adolescents. *Res Dev Disabil.* (2016) 48:193–201. doi: 10.1016/j.ridd.2015.10.020

92. Rommel AS, Rijsdijk F, Greven CU, Asherson P, Kuntsi J. A longitudinal twin study of the direction of effects between ADHD symptoms and IQ. *PLoS ONE* (2015) 10:e0124357. doi: 10.1371/journal.pone.0124357

93. Evans SH, Anastasio EJ. Misuse of analysis of covariance when treatment effect and covariate are confounded. *Psychol Bull.* (1968) 69:225–34. doi: 10.1037/h0025666

94. Dennis M, Francis DJ, Cirino PT, Schachar R, Barnes MA, et al. Why IQ is not a covariate in cognitive studies of neurodevelopmental disorders. *J Int Neuropsychol Soc.* (2009) 15:331. doi: 10.1017/s1355617709090481

95. Miller GM, Chapman JP. (2001) Misunderstanding analysis of covariance. *J Abnorm Psychol.* (2012) 110:40–8. doi: 10.1037/0021-843x.110.1.40

96. Chantiluke K, Barrett N, Giampietro V, Brammer M, Simmons A, Murphy DG, et al. Inverse effect of fluoxetine on medial prefrontal cortex activation during reward reversal in ADHD and autism. *Cereb Cortex* (2015) 25:1757–70. doi: 10.1093/cercor/bht365

97. Murphy DG, Daly E, Schmitz N, Toal F, Murphy K, Curran S, et al. Cortical serotonin 5-HT2A receptor binding and social communication in adults with Asperger's syndrome: an *in vivo* SPECT study. *Am J Psychiatry* (2006) 163:934–6. doi: 10.1176/ajp.2006.163.5.934

Association Between Magnetoencephalographic Interictal Epileptiform Discharge and Cognitive Function in Young Children With Typical Development and With Autism Spectrum Disorders

Tetsu Hirosawa [1,2]*, Mitsuru Kikuchi [1,3], Mina Fukai [4], Shoryoku Hino [4], Tatsuru Kitamura [4], Kyung-Min An [3], Paul Sowman [2], Tetsuya Takahashi [5], Yuko Yoshimura [3], Yoshiaki Miyagishi [1] and Yoshio Minabe [1,3]

[1] Department of Psychiatry and Neurobiology, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan, [2] Department of Cognitive Science, Australian Hearing Hub, Macquarie University, Sydney, NSW, Australia, [3] Research Center for Child Mental Development, Kanazawa University, Kanazawa, Japan, [4] Department of Neuropsychiatry, Ishikawa Prefectural Takamatsu Hospital, Ishikawa, Japan, [5] Health Administration Center, University of Fukui, Fukui, Japan

Electroencephalograms of individuals with autism spectrum disorders (ASD) show higher rates of interictal epileptiform discharges (IEDs), which are known to have an inverse association with cognitive function in typically developed (TD) children. Nevertheless, that phenomenon has not been investigated adequately in children with ASD. From university and affiliated hospitals, 163 TD children (84 male, 79 female, aged 32–89 months) and 107 children (85 male, 22 female, aged 36–98 months) with ASD without clinical seizure were recruited. We assessed their cognitive function using the Kaufman Assessment Battery for Children (K-ABC) and recorded 10 min of MEG. Original waveforms were visually inspected. Then a linear regression model was applied to evaluate the association between the IED frequency and level of their cognitive function. Significantly higher rates of IEDs were found in the ASD group than in the TD group. In the TD group, we found significant negative correlation between mental processing scale scores (MPS) and the IED frequency. However, for the ASD group, we found significant positive correlation between MPS scores and the IED frequency. In terms of the achievement scale, correlation was not significant in either group. Although we found a correlative rather than a causal effect, typically developed children with higher IED frequency might better be followed up carefully. Furthermore, for children with ASD without clinical seizure, clinicians might consider IEDs as less harmful than those observed in TD children.

Keywords: autism spectrum disorder, magnetencephalography, epileptiform discharges, epilepsy, cognitive function

INTRODUCTION

Autism spectrum disorder (ASD) is a lifelong, often severely impairing neurodevelopmental syndrome characterized by impaired social cognition and communication as well as repetitive or obsessive behavior and interests (1). Its increasing prevalence (2–4), a lack of generally accepted pharmacological interventions, profound impact on quality of life, and high costs of education and care for people with ASD all combine to make ASD a public health crisis.

In addition to social impairment, most individuals with ASD have co-occurring intellectual disability (ID) (5). Results of recent studies suggest that ID in ASD might emerge as a consequence of social-communication deficits. According to this model, because social input is crucially important for normal brain development (6), poor sociality in children with ASD stunts their cognitive development by precluding them from social experience (6, 7). Supporting this view, Vianti et al. reported from their recent study that children with greater ASD severity at an initial assessment were more likely to present with poorer cognitive outcomes at later assessment, irrespective of initial cognitive level (8). Furthermore, an intervention program specifically targeting ASD symptoms in young children is known to enhance their cognitive development (9).

The association between ASD and epilepsy has been known since the first described cases (10). The prevalence of seizures in children with ASD is reportedly higher than in the general population [5–46 vs. 2–7% (11–13)]. As many as 32% of patients with epilepsy meet the diagnostic criteria of ASD (14). Moreover, even in the absence of clinical seizures, 6.7–50% of patients with ASD have IEDs shown by their electroencephalograms (EEG) (15, 16), which is much higher than the rate found among typically developing individuals: 1–4% (17, 18). Nevertheless, the clinical significance of IEDs for ASD remains unclear.

IEDs are thought to be representative of excessive neuronal activity (19). Therefore, higher IED frequency implies an excitatory shift in the equilibrium between excitatory (E) glutamatergic and inhibitory (I) gamma-amminobutyric acid (GABA) system. In fact, results of several studies suggest that the GABAergic system is disrupted in cases of ASD. For instance, genetic reports have described consistently that mutations in genes regulating $GABA_A$ receptor expression occur in patients with ASD (20). Furthermore, environmental risk factors for ASD, such as exposure to maternal inflammation in prenatal life, disrupt gene expression across GABA pathways (21–23). Recently, based on such results, E/I imbalance has attracted attention as a candidate of the final "common pathways" in ASD (24).

Relation between IEDs and cognitive function in children remains unclear. In healthy individuals, most studies have found associations between the existence of IEDs and lower cognitive function (25, 26). However, for ASD, few studies have specifically examined this relation. Moreover, their results are conflicting (16, 27–30).

Those conflicting results obtained from ASD individuals might derive from methodological differences. First, the EEG duration varies among the studies. Longer EEG durations might enhance the sensitivity for detection of IEDs. Second, few studies of children with ASD have addressed the IED frequency in previous conventional studies for children with ASD (16, 28–30). Frequency of IEDs is more important than its mere existence if one considers that IEDs might affect cognitive dysfunction. Finally, most of the studies included children and adult ASD participants (14, 25–27). Because animal studies showed that IED effects on cognitive function in children might be different from the effects in adults (31), results found for inhomogeneous populations might not reflect the true relation. A noteworthy exception is a study conducted by Gillian et al. with no such limitations (27). They found no correlation between the IED frequency and cognitive dysfunction in children with ASD.

As described herein, we investigate the association between IEDs and cognitive function in children with ASD and compare it with that in typically developing children of a control group. We hypothesized that higher frequency of IEDs corresponds to lower level of cognitive function in children with ASD as well as TD children. We used magnetoencephalography (MEG) instead of EEG because it is more sensitive to epileptiform activity (32).

MATERIAL AND METHODS

Participants

From Kanazawa University and affiliated hospitals, we recruited 163 typically developed children (TD) (84 male, 79 female, aged 32–89 months) and 107 children (85 male, 22 female, aged 36–98 months) with ASD. The ASD diagnosis was made according to the Diagnostic and Statistical Manual of Mental Disorders (4th edition) (DSM-IV) (1) using the Diagnostic Interview for Social and Communication Disorders (DISCO) (33) or the Autism Diagnostic Observation Schedule-Generic (ADOS-G) (34). The exclusion criteria included known blindness, hearing loss, and ID. Additionally, we excluded participants who had clinical diagnosis of any other neuropsychiatric disorder including epilepsy, and excluded participants who were receiving antiepileptic drugs. Parents agreed to the participation of children. Written informed consent was obtained before participation. The Ethics Committee of Kanazawa University Hospital approved the methods and procedures, all of which were performed in accordance with the Declaration of Helsinki.

Assessment of Cognitive Function

Cognitive function of the participants was assessed using the Japanese version of the K-ABC, (35), which measures the degree of skills in various cognitive domains and which presents them on two global scales: The MPS, which measures fluid intelligence, and the Achievement Scale (ACH), which measures crystallized intelligence. These scores are provided as age-adjusted standardized scores. The scores are normalized to have mean of 100 and standard deviation of 15.

MEG Recordings

Conditions used for MEG recordings were identical to those used in our earlier study (36). MEG data were recorded using a 151-channel Superconducting Quantum Interference Device

(SQUID), whole-head coaxial gradiometer MEG system for children (PQ 1151R; Yokogawa/KIT, Kanazawa, Japan) in a magnetically shielded room (Daido Steel Co., Ltd., Nagoya, Japan) installed at the MEG Center of Ricoh Co., Ltd. (Kanazawa, Japan). The custom child-sized MEG system facilitates the measurement of brain responses in young children, which would otherwise be difficult using conventional adult-sized MEG systems. The child-sized MEG system ensures that the sensors are positioned easily and effectively for the child's brain and ensures that head movements are constrained (37).

The band pass-filtered MEG data (0.16–200 Hz) were collected at a sampling rate of 1,000 Hz. During MEG recording, one staff member escorted each participant into the shielded room, which was decorated with colorful pictures of Japanese (cartoon) characters and those resembling an attractive vehicle adopted from an animation series that is popular with preschool children. During measurements, the staff member stayed in the shielded room comforting and encouraging each participant to maintain a steady body position when necessary. Parent(s)/caretaker(s) were able to observe their child during measurements through a TV monitor. During the MEG recording, the children lay supine on a bed and viewed a video program projected onto a screen (i.e., eye-open condition). The position of the head within the helmet during the MEG recording was determined by measuring the magnetic fields after passing currents through coils attached at three locations on the head surface, which served as fiducial marks for the bilateral mastoid processes and nasion. Before recording, we prepared several video programs that were entertaining for young children. Each participant was shown a video program they had selected. Before recording, each child confirmed that the video program contents had been selected. MEG was recorded for 600 s. The time of MEG recording was between 11 a.m. and 3 p.m. No child showed a clear sign of drowsiness in terms of MEG waveforms.

Assessment of MEG Recordings

One investigator (author T.H.) reviewed the raw MEG signals (i.e., time vs. amplitude waveforms). He had been trained in EEG/MEG and epilepsy for 11 years and had extensive experience in distinguishing epileptiform discharges from other non-epileptic waveforms.

During review, T.H was blinded to the patients' names and their clinical information. To review the MEG record, the band pass filter (0.5–70 Hz) was applied. We divided all sensor pairs into eight groups (frontal/temporal/vertex/occipital region in the left and right side each). It was necessary to review all channels. T.H reviewed every group of channels by switching the montage display. He was allowed to move the records backward and forward at any time to confirm his findings. After counting of epileptiform discharges in 600 s, he reported it as a frequency per 10 s (i.e., the number of epileptiform discharges was divided by 60). Typically, this procedure required 30 min per person.

Epileptic discharges were detected manually by application of the same general principles recommended by the International Federation of Clinical Neurophysiology (38) and were used

in standard EEG interpretation: The sharp transient is clearly different from background activity with an "epileptiform" morphology and a logical spatial distribution (e.g., **Figure 1**). The IED location was defined using an intermediate point of sink and source.

Statistical Analysis

Differences in population descriptors between TD and ASD were tested using Student t-tests for age and cognitive performance (i.e., ACH and MPS in K-ABC), and using Mann–Whitney U-tests for the IED frequency (i.e., number of IEDs per 10 s). Chi-square tests were used for analyses of the IED frequency and sex.

First, we performed a linear regression to predict MPS/ACH scores based on frequency of IEDs, disease condition (TD vs. ASD). Statistical significance was inferred for $P < 0.05$.

Before calculating a linear regression, we applied regression diagnostics to verify how well our data met the regression analysis assumptions. Specifically, we checked the following assumptions: linearity, normality, homogeneity of variance, model specification, influence and collinearity.

We examined the relation between variables using a series of scatterplots and augmented component-plus-residual plots for the variables (i.e., MPS or ACH scores and frequency of IEDs). We found no clear non-linear pattern. Here, there was no reason to check for satisfying the assumption in bivariate categorical variable because the relation is linear by definition. To check for normality in the residuals, we used kernel density plots, histograms, standardized normal probability plots, and quintile-normal plots. Based on results of those tests, we concluded that residuals were normally distributed for all models.

The graphical and the Breusch–Pagan test suggested the possible presence of heteroscedasticity in our model. To address that problem, we decided to use heteroscedasticity-robust standard errors (39).

To check the model specification error, we performed a model specification link test and a regression specification error test. Both tests gave results that were not significant for all models we employed. To check outliers, we used the added-variable plots. All data points were apparently in the range. No outlier was observed. Multicollinearity was tested using the variance inflation factor (VIF). No variable had VIF >10 or 1/VIF <0.10 for any model.

If significant interaction involving the disease condition was found, then a linear regression model was applied within ASD and within TD to ascertain the association between the IED frequency and level of cognitive function (i.e., ACH and MPS in K-ABC) controlling for age and sex. Again, according to the regression diagnostics, we decided to use heteroscedasticity-robust standard errors. Statistical significance was inferred for $P < 0.025$ (Bonferroni correction for multiple comparisons was applied).

In the regression diagnostics, violation was found only for homoscedasticity. Therefore, we decided to use heteroscedasticity-robust standard errors.

Then, because of a possible influence of sex and age on MPS/ACH (31, 40, 41), we entered those in the regression model

FIGURE 1 | Examples of epileptiform discharge. Epileptiform discharge was defined as "sharp transient clearly different from background activity with an "epileptiform" morphology and a logical spatial distribution" (left). MEG topography (right) shows clear pattern of sink (green) and source (red). MEG, magnetoencephalography.
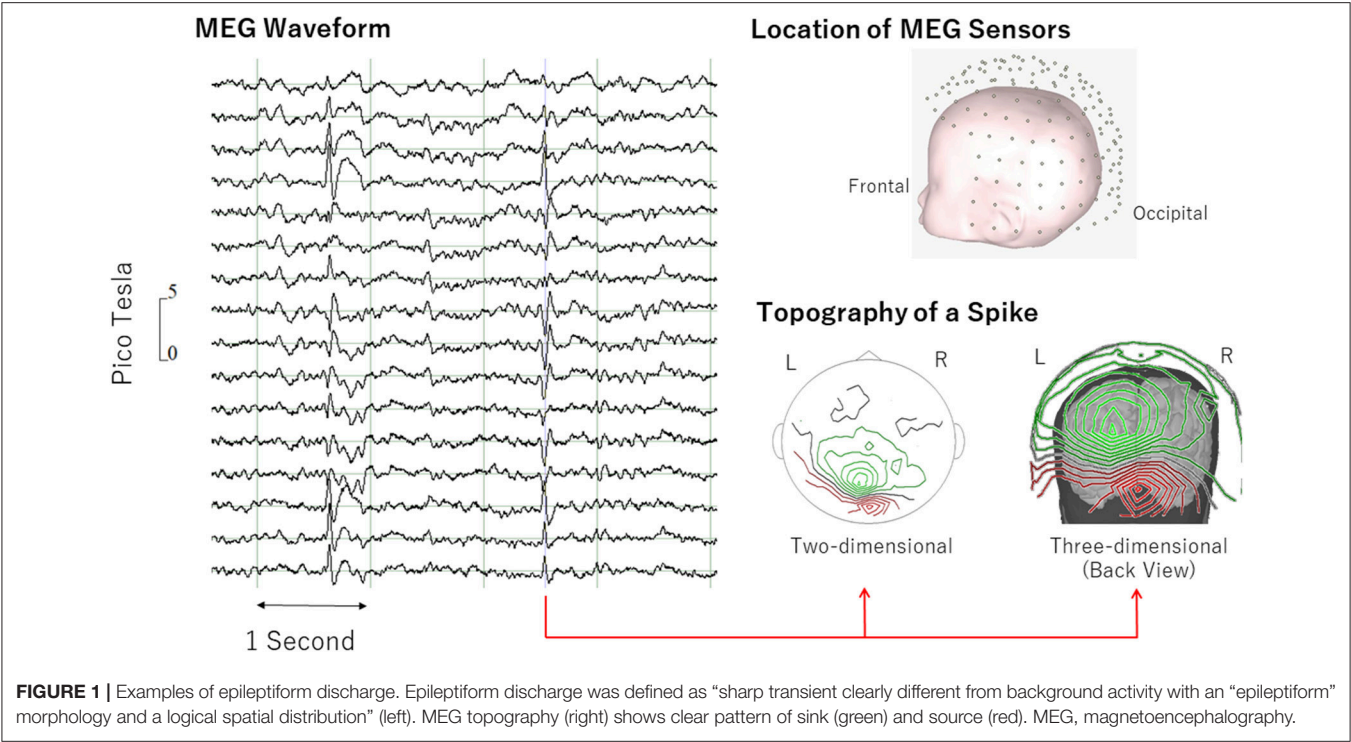
to control for the confounding effects. Against the ACH and MPS scores in K-ABC (i.e., dependent variable), the model included age, sex, IED frequency, child condition (TD vs. ASD), and interaction between the IED frequency and condition. Because the regression diagnostics showed heteroscedasticity, we decided to use heteroscedasticity-robust standard errors. Statistical significance was inferred for $P < 0.05$.

If significant interaction involving the disease condition was found, then we applied a linear regression model within ASD and within TD to ascertain the association between the IED frequency and cognitive function (i.e., ACH and MPS in K-ABC) controlling for age and sex. Again, according to the regression diagnostics, we decided to use heteroscedasticity-robust standard errors. In these models, statistical significance was inferred for $P < 0.025$ (Bonferroni correction for multiple comparisons was applied).

All statistical analyses were performed using software (Stata ver. 15.0; Stata Corp. College Station, TX, USA).

RESULTS

Statistical analyses were conducted after excluding 22 children (7 TD, 15 ASD) who were unable to complete the K-ABC or MEG recording. Actually, 12 of these children (5 TD, 7 ASD) had unreadable MEG recordings because of motion artifacts, noise artifacts or environmental interference. None of the participants received medication. Significant differences were found in age, sex, cognitive performance, prevalence, and the IED frequency between TD and ASD groups. **Tables 1**, **2** and **Figure 2** present results.

TABLE 1 | Characteristics of participants.

	TD	ASD	χ^2	t	z	p	Effect size
n	156	92					
Gender (male/female)*	81/75	72/20	17.0			<0.05	0.26
Months#	56.5 (11.8)	65.5 (12.6)		5.7		<0.05	0.74
Prevalence of IEDs (Negative/positive)*	140/16	73/19	5.2			<0.05	0.14
IED frequency (per 10 s)$	0.06 (0.02)	0.10 (0.04)			2.2	<0.05	0.14
K-ABC scores							
Mental processing scale#	101.7 (12.0)	91.9 (19.8)		−4.8		<0.05	0.63
Achievement scale#	103.1 (14.7)	93.8 (18.6)		−4.3		<0.05	0.83

Numbers are mean (standard deviation) or counts.
Effect sizes were provided by Cramer's V for chi-square test, Cohen's d for Student' t-test and z value divided by square root of sample size for Mann–Whitney U-test.
*Chi-square test.
#Student t-test.
$Mann–Whitney U-test.
ASD, autism spectrum disorder; TD, typically developed controls; K-ABC, Kaufman assessment battery for children.

Association Between the IED Frequency and Level of Cognitive Function

For MPS scores, significant main effects were found for the condition [$t_{(244)} = -4.6$, $p < 0.05$] and the IED frequency [$t_{(244)} = -5.3$, $p < 0.05$]. Significant interaction effects were also found between the IED frequency and disease condition [$t_{(244)} = 4.8$, $p < 0.05$]. The relation between the IED

TABLE 2 | Distribution of IEDs.

	TD		ASD	
	R	L	R	L
Frontal lobe	2	1	4	3
Temporal lobe	0	4	3	4
Parietal lobe	3	2	5	4
Occipital lobe	3	4	1	4
Multiple focus	2		6	

Numbers are counts.
The location of IED was defined using an intermediate point of sink and source.
ASD, autism spectrum disorder; R, right; L, left; TD, typically developed controls.
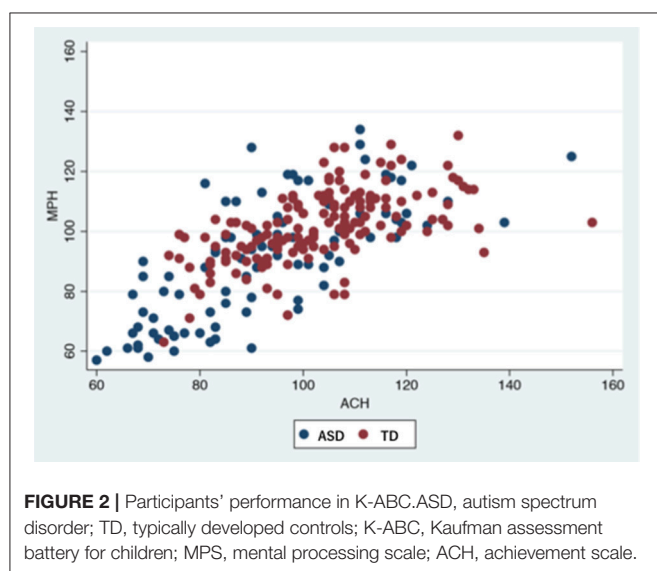
FIGURE 2 | Participants' performance in K-ABC.ASD, autism spectrum disorder; TD, typically developed controls; K-ABC, Kaufman assessment battery for children; MPS, mental processing scale; ACH, achievement scale.

frequency and MPS scores differed depending on the disease condition.

For ACH scores, a significant main effect was found for the disease condition [$t_{(244)} = -4.1$, $p < 0.05$]. No other factors were found to be significant. The results are presented in **Table 3**.

Different Association Between the IED Frequency and Level of Cognitive Function in Each Group

To elucidate the association between the IED frequency and the level of cognitive function, a linear regression model with robust variance estimation was applied within each group.

In the TD group, for MPS scores, a significant main effect of the IED frequency [$t_{(154)} = -5.31$, $p < 0.025$] was found. Higher IED frequency predicted lower MPS scores. For ACH, the main effect was not found to be significant.

In the ASD group, for MPS scores, a significant main effect of the IED frequency on the MPS [$t_{(90)} = 2.47$, $p < 0.025$] was found. Higher IED frequency predicted higher MPS scores. For the ACH, the effect was not found to be significant. The results are presented in **Table 4**.

TABLE 3 | Association between IED frequency and level of cognitive function.

variable	Coeff.	SE	t	95% CI	p
MENTAL PROCESSING SCALE					
Condition (ASD = 1/TD = 0)	−10.8	2.3	−4.6	−15.4 to −6.2	<0.05
IED frequency (/10 s)	−7.7	1.5	−5.3	−10.6 to −4.9	<0.05
Condition × IED frequency	14.0	2.9	4.8	8.2 to 19.7	<0.05
ACHIEVEMENT SCALE					
Condition (ASD = 1/TD = 0)	−9.6	2.3	4.1	−14.2 to −4.9	<0.05
IED frequency (/10 s)	−2.1	3.0	−0.7	−8.1 to 3.8	0.48
Condition × IED frequency	4.0	4.9	0.8	−5.6 to 13.7	0.41

Coef., regression coefficient; SE, robust standard error; CI, confidence interval; ASD, autism spectrum disorder; TD, typically developed controls.

Association Between the IED Frequency and Level of Cognitive Function Controlling for Age and Sex

For MPS scores, significant main effects were found for the condition [$t_{(242)} = -4.77$, $p < 0.05$] and the IED frequency [$t_{(242)} = -4.89$, $p < 0.05$]. Significant interaction effects were also found between the IED frequency and disease condition [$t_{(242)} = 4.45$ $p < 0.05$]. No other factor was found to be significant. The relation between the IED frequency and MPS scores differed depending on the disease condition after controlling for age and sex.

For ACH scores, a significant main effect was found for the disease condition [$t_{(242)} = -3.14$, $p < 0.05$]. ASD children had lower ACH scores compared to TD children. No other factor was found to be significant. The results are presented in **Table 5**.

Different Association Between the IED Frequency and Level of Cognitive Function Controlling for Age and Sex in Each Group

To elucidate the effects of the IED frequency on cognitive function, a linear regression model with robust variance estimation was applied within each group.

In the TD group, for MPS scores, a significant main effect of the IED frequency [$t_{(152)} = -4.77$, $p < 0.025$] was found. No other factor was found to be significant. Higher IED frequency predicted lower MPS scores after controlling for age and sex. For ACH, no factor was found to be significant.

In the ASD group, for MPS scores, a significant main effect of the IED frequency on the MPS [$t_{(88)} = 2.35$, $p < 0.025$) was found. No other factor was found to be significant. Higher IED frequency predicted higher MPS scores after controlling for age and sex. For the ACH, no factor was found to be significant. The results are presented in **Table 6**.

DISCUSSION

This report is the first of a study providing evidence demonstrating that the association between the IED frequency and level of cognitive function in children with ASD differ from those in a control group of TD children. First, using a linear regression model, we demonstrated that the association

TABLE 4 | Association between IED frequency and level of cognitive function in each group.

TD					
Variable	**Coeff.**	**SE**	**t**	**95% CI**	**p**
Mental processing scale					
IED frequency (/10 s)	−7.7	1.4	−5.3	−10.6 to −4.9	<0.025
Achievement scale					
IED frequency (/10 s)	−2.1	3.0	−0.7	−8.0 to 3.8	0.48
ASD					
Variable	**Coeff.**	**SE**	**t**	**95% CI**	**p**
Mental processing scale					
IED frequency (/10 s)	6.2	2.5	2.5	1.2 to 11.2	<0.025
Achievement scale					
IED frequency (/10 s)	1.9	3.9	0.5	−5.8 to 9.6	0.63

Coeff., regression coefficient; SE, robust standard error; CI, confidence interval; ASD, autism spectrum disorder; TD, typically developed controls.

TABLE 5 | Association between IED frequency and level of cognitive function controlling for age and sex.

Variable	Coeff.	SE	t	95% CI	p
MENTAL PROCESSING SCALE					
Condition (ASD = 1/TD = 0)	−12.2	2.6	−4.8	−17.2 to −7.1	<0.05
IED frequency (/10 s)	−8.0	1.6	−4.9	−11.3 to −4.8	<0.05
Condition × IED frequency	14.3	3.2	4.4	8.0 to 20.6	<0.05
Age (months)	0.15	0.8	1.93	−0.0 to 0.3	0.05
Sex (male = 1/female = 0)	0	2	0	−3.9 to 3.8	0.99<
ACHIEVEMENT SCALE					
Condition (ASD = 1/TD = 0)	−8.8	2.8	−3.1	−14.3 to −3.3	<0.05
IED frequency (/10 s)	−1.83	3.1	−0.6	−7.9 to 4.2	0.55
Condition × IED frequency	3.3	4.9	0.7	−6.3 to 13.0	0.49
Age (months)	0	0.1	0	−0.2 to 0.2	0.96
Sex (male = 1/female = 0)	−2.7	2.3	−1.2	−7.3 to 1.8	0.25

Coeff., regression coefficient; SE, robust standard error; CI, confidence interval; ASD, autism spectrum disorder; TD, typically developed controls.

TABLE 6 | Association between IED frequency and level of cognitive function controlling for age and sex in each condition.

TD					
variable	**Coeff.**	**SE**	**t**	**95% CI**	**P**
Mental processing scale					
IED frequency (/10 s)	−7.7	1.6	−4.8	−10.8 to −4.5	<0.025
Age (months)	0.1	0.1	1.2	−0.1 to 0.2	0.25
Sex (male = 1/female = 0)	−2.6	1.9	−1.4	−6.2 to 1.2	0.18
Achievement scale					
IED frequency (/10 s)	−1.8	3.1	−0.6	−7.9 to 4.3	0.57
Age (months)	0	0.1	−0.2	−0.2 to 0.2	0.88
Sex (male = 1/female = 0)	−3.2	2.4	−1.4	−8.0 to 1.4	0.17
ASD					
variable	**Coeff.**	**SE**	**t**	**95% CI**	**p**
Mental processing scale					
IED frequency (/10 s)	7.2	3.0	2.35	1.1 to 13.3	<0.025
Age (months)	0.2	0.2	1.36	−0.1 to 0.5	0.18
Sex (male = 1/female = 0)	6.6	4.8	1.37	−2.9 to 16.1	0.17
Achievement scale					
IED frequency (/10 s)	1.7	4	0.4	−6.2 to 9.6	0.68
Age (months)	0	0.2	0.1	−0.4 to 0.4	0.91
Sex (male = 1/female = 0)	−1.4	5.5	−0.3	−12.2 to 9.5	0.78

Coeff., regression coefficient; SE, robust standard error; CI, confidence interval; ASD, autism spectrum disorder; TD, typically developed controls.

the duration of routine EEG recording (typically about 60 min) and the much shorter duration of our MEG recording (600 s), one might infer that MEG has higher sensitivity for IEDs, perhaps because of inherent differences between MEG and EEG.

We found a higher prevalence of IEDs in children with ASD than in TD children. IEDs are presumed to represent excessive neuronal activity. Therefore, the existence of IEDs can imply a local excitatory shift in the E/I balance. Supporting this view, studies using proton magnetic resonance spectroscopy ([1H]MRS) have shown higher local brain glutamate and lower GABA level in patients with ASD than in healthy controls (45, 46). The higher frequency of IEDs suggest an ongoing regional E/I imbalance in the brains of children with ASD.

The MPS scores represent a person's fluid intelligence, a purely general ability to discriminate and perceive relations between any fundaments (47). In terms of MPS scores, we found a significant interaction effect between the IED frequency and child condition. Results of *post-hoc* analysis showed that higher IED frequency predicted lower MPS scores for TD children. Considering the inverse association between IEDs and level of fluid intelligence for TD children, IEDs can be pathogenetic in this population. Although we found a correlative rather than a causal effect, typically developed children with higher IED frequency must be followed up carefully. In some cases with concurrent cognitive impairment, antiepileptic treatment might be considered (48).

However, for children with ASD, *post-hoc* analysis revealed that higher IED frequency predicted higher MPS scores. Therefore, our results did not support the pathogenicity of IEDs in children with ASD. For children with ASD, clinicians might

between the IED frequency and MPS scores differed depending on the child condition. This association remained significant after controlling for age and sex. Then, *post-hoc* analysis showed that higher IED frequency predicted lower MPS scores for TD children. However, in the ASD group, higher IED frequency predicted higher MPS scores. These associations also remained significant after controlling for age and sex.

In our study, 10.3% of TD children and 20.7% of children with ASD had at least one IED in their MEG recordings. For TD children, the rate was slightly higher than reported previously. However, for children with ASD, reported rates vary: 6.7–61%. (18) This discrepancy might be explained by differences in the type of EEG used in those studies because, for example, prolonged sleep EEG reportedly has higher sensitivity for IEDs than routine EEG has (28). In fact, studies using routine EEGs found similar rates [e.g., 5.7% (42), 18% (43)] to those we observed. Studies using longer durations of EEGs found much higher rates. For example, 61% was reported by (44). Comparing

consider the IEDs as less harmful than those observed in TD children. Rather, a higher frequency of IEDs would correspond to better fluid intelligence. In addition, based on our results, the nature of IEDs might differ in children with ASD from that in TD. Possibly, considering higher prevalence of IED in children with ASD, pathology of ASD can play a role in this phenomenon. For example, E/I imbalance (observed as IEDs) in children with ASD can be an epiphenomenon or compensatory changes to the underlying cause of ASD. In support of this hypothesis, it is noteworthy that Gerhard et al. reported that despite an excitatory shift in E/I balance in the occipital region in ASD participants, the neurons in that region were not severely damaged (45). Furthermore, recent results of results of studies suggest that underlying causes of ASD can have positive effects on their fluid intelligence (49, 50).

The ACH scores represent a person's crystallized intelligence. We found significant main effects of disease condition on ACH scores. Particularly, children with ASD had significantly lower ACH scores than TD children had. Consistent with these results, studies cited above also suggest that the underlying cause of ASD adversely affects their crystallized intelligence (49, 50). Nevertheless, no correlation was found between the IED frequency and the ACH scores of either group. Possible explanations include the lack of statistical power and the existence of potential confounders. For example, because crystallized intelligence relies on acquired knowledge, it might be susceptible to the educational environment. To examine the IED effects on ACH, further study must be undertaken including environmental information such as socioeconomic status, and presence or lack of early childhood education.

Limitations of our study are as follows. First, most of the children with ASD examined in this study were high-functioning. Consequently, the findings of this study might not be applicable to children with "Kanner's autism." However, although we excluded children with known neuropsychiatric disorders other than ASD, the participants still possibly had comorbid disorders. For example, some developmental disorders (e.g., attention deficit/hyperactivity disorder, and learning disability) are difficult to detect at this age. Further studies that consider comorbid disorders using larger sample sizes must be conducted because these comorbidities might affect cognitive performance. Second, the participants were recruited from a small region. Considering environmental risk factors for ASD, our results should be generalized with caution. Third, we assigned only one investigator (T.H.) to review all the MEG recordings. He was instructed to look for transients and to make an "epileptic" or "non-epileptic" decision for every suspicious waveform. Although we had a rigorous definition of epileptic discharge, in truth, sometimes it was difficult to make a decision. It was preferable to employ another rater to cross-check the decisions. Fourth, we were not able to ascertain the precise location of the source of the magnetic field as we could if we used anatomical images such as those gained from MRI. We judged that most of the subjects in our study were unable to endure the examination of conventional magnetic resonance imaging (MRI). Future studies using child-friendly, open-type MRI devices and/or brain models that match the individual brain are necessary to reduce uncertainty in source level estimation. Fifth, we recorded MEG for only 10 min. We would have had higher sensitivity for IEDs if we had recorded MEG for at least 20 min as The American Clinical Neurophysiology Society recommends (51). In addition, presumably because of this short recording time, no child examined for this study showed any clear sign of drowsiness in terms of MEG waveform. Importantly, however, some autistic features are known to occur in the context of sleep IEDs (e.g., the Landau–Kleffner syndrome and electrical status epilepticus in slow wave sleep) (52). To elucidate the relation between IEDs and cognitive function further, future studies must particularly address the difference between the effect of sleep and awake IEDs on cognitive function.

In conclusion, this report described the negative association between the IED frequency and MPS scores in TD children. Although we found a correlative rather than a causal effect, typically developed children with higher IED frequency must be followed up carefully. We also reported a positive association between the IED frequency and MPS scores in children with ASD. For children with ASD without clinical seizure, clinicians might consider the IEDs as less harmful than those observed in TD children.

Our results demonstrate the possibility that E/I imbalance in children with ASD is epiphenomenal or compensatory, rather than causal in ASD. However, drawing such a hard conclusion based purely on our results is inappropriate. In TD children, to clarify a causal relation, studies must be conducted to prove (or disprove) the effectiveness of antiepileptic treatment on various aspects of cognitive function. In ASD children, in view of the developmental aspect of the disease, a longitudinal follow-up study considering the relation between IEDs and cognitive function should be conducted. Change in cognitive function over the course of time in relation to IEDs might provide additional information related to the nature of IEDs in this population. For such clinical trials, given the negative correlation between the IED frequency and cognitive function observed in TD children, not only should the existence of IEDs be assessed; the IED frequency should be assessed as well.

AUTHOR CONTRIBUTIONS

TH and MK designed the study and wrote the protocol. MK and YMin supervised the research. TH wrote the first draft of the manuscript. PS, TT, and TK revised the manuscript and advised colleagues on statistical methods and composition of the manuscript. TH, MF, and SH conducted statistical analyses. YY and K-MA recruited participants. YMiy and YY recorded MEG. All authors contributed to and approved the final manuscript.

FUNDING

REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed Washington, DC (2013).

2. Bertrand J, Mars A, Boyle C, Bove F, Yeargin-Allsopp M, Decoufle P. Prevalence of autism in a United States population: the Brick Township, New Jersey, investigation. *Pediatrics* (2001) 108:1155–61. doi: 10.1542/peds.108.5.1155

3. Christensen DL, Baio J, Van Naarden Braun K, Bilder D, Charles J, Constantino JN, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years – autism and developmental disabilities monitoring network, 11 sites, United States, 2012. *MMWR Surveill Summ.* (2016) 65:1–23. doi: 10.15585/mmwr.ss 6503a1

4. King M, Bearman P. Diagnostic change and the increased prevalence of autism. *Int J Epidemiol.* (2009) 38:1224–34. doi: 10.1093/ije/dyp261

5. Henninger NA, Taylor JL. Outcomes in adults with autism spectrum disorders: a historical perspective. *Autism* (2013) 17:103–16. doi: 10.1177/1362361312441266

6. Kuhl PK. Is speech learn- ing 'gated' by the social brain? *Dev Sci.* (2007) 10:110–20. doi: 10.1111/j.1467-7687.2007.00572.x

7. Dawson G. Early behavioral intervention, brain plastic- ity, and the prevention of autism spectrum disorder. *Dev Psychopathol.* (2008) 20:775–803. doi: 10.1017/S0954579408000370

8. Vivanti G, Barbaro J, Hudry K, Dissanayake C, Margot P. Prior Intellectual development in autism spectrum disorders: new insights from longitudinal studies. *Front Hum Neurosci.* (2013) 7:354. doi: 10.3389/fnhum.2013.00354

9. Dawson G, Rogers S, Munson J, Smith M, Winter J, Greenson J, et al. Randomized, controlled trial of an intervention for toddlers with autism: the early start Denver model. *Pediatrics* (2010) 125:e17–23. doi: 10.1542/peds.2009-0958

10. Kanner L. Autistic disturbances of affective contact. *Acta Paedopsychiatr.* (1968) 35:100–36.

11. Borusiak P, Zilbauer M, Jenke AC. Prevalence of epileptiform discharges in healthy children–new data from a prospective study using digital EEG. *Epilepsia* (2010) 51:1185–8. doi: 10.1111/j.1528-1167.2009.02411.x

12. Bryson SE, Clark BS, Smith IM. First report of a Canadian epidemiological study of autistic syndromes. *J Child Psychol Psychiatry* (1988) 29:433–45. doi: 10.1111/j.1469-7610.1988.tb00735.x

13. Hughes JR, Melyn M. EEG and seizures in autistic children and adolescents: further findings with therapeutic implications. *Clin EEG Neurosci.* (2005) 36:15–20. doi: 10.1177/155005940503600105

14. Clarke DF, Roberts W, Daraksan M, Dupuis A, McCabe J, Wood H, et al. The prevalence of autistic spectrum disorder in children surveyed in a tertiary care epilepsy clinic. *Epilepsia* (2005) 46:1970–7. doi: 10.1111/j.1528-1167.2005.00343.x

15. Kim HL, Donnelly JH, Tournay AE, Book TM, Filipek P. Absence of seizures despite high prevalence of epileptiform EEG abnormalities in children with autism monitored in a tertiary care center. *Epilepsia* (2006) 47:394–8. doi: 10.1111/j.1528-1167.2006.00434.x

16. Rossi PG, Parmeggiani A, Bach V, Santucci M, Visconti P. EEG features and epilepsy in patients with autism. *Brain Dev.* (1995) 17:169–74. doi: 10.1016/0387-7604(95)00019-8

17. Capdevila OS, Dayyat E, Kheirandish-Gozal L, Gozal D. Prevalence of epileptiform activity in healthy children during sleep. *Sleep Med.* (2008) 9:303–9. doi: 10.1016/j.sleep.2007.03.024

18. Ghacibeh GA, Fields C. Interictal epileptiform activity and autism. *Epilepsy Behav.* (2015) 47:158–62. doi: 10.1016/j.yebeh.2015.02.025

19. Avoli M, de Curtis M. GABAergic synchronization in the limbic system and its role in the generation of epileptiform activity. *Prog Neurobiol.* (2011) 95:104–32. doi: 10.1016/j.pneurobio.2011.07.003

20. Ching MS, Shen Y, Tan WH, Jeste SS, Morrow EM, Chen X, et al. Deletions of NRXN1 (neurexin-1) predispose to a wide spectrum of developmental disorders. *Am J Med Genet B Neuropsychiatr Genet.* (2010) 153B:937–47. doi: 10.1002/ajmg.b.31063

21. Fatemi SH, Reutiman TJ, Folsom TD, Huang H, Oishi K, Mori S, et al. Maternal infection engenders abnormal gene regulation and brain atrophy in mouse offspring: implications for genesis of neurodevelopmental disorders. *Schizophr Res.* (2008) 99:56–70. doi: 10.1016/j.schres.2007.11.018

22. Giovanoli S, Weber L, Meyer U. Single and combined effects of prenatal immune activation and peripubertal stress on parvalbumin and reelin expression in the hippocampal formation. *Brain Behav Immun.* (2014) 40:48–54. doi: 10.1016/j.bbi.2014.04.005

23. Meyer U, Feldon J, Dammann O. Schizophrenia and autism: both shared and disorder-specific pathogenesis via perinatal inflammation? *Pediatr Res.* (2011) 69:26R–33R. doi: 10.1203/PDR.0b013e318212c196

24. Ecker C, Spooren W, Murphy DG. Translational approaches to the biology of Autism: false dawn or a new era? *Mol Psychiatry* (2013) 18:435–42. doi: 10.1038/mp.2012.102

25. Ebus S, Arends J, Hendriksen J, van der horst E, de la Parra N, Hendriksen R, et al. Cognitive effects of interictal epileptiform discharges in children. *Eur J Paediatr Neurol.* (2012) 16:697–706. doi: 10.1016/j.ejpn.2012.05.010

26. Galanopoulou AS, Moshe SL. The epileptic hypothesis: developmentally related arguments based on animal models. *Epilepsia* (2009) 50:37–42. doi: 10.1111/j.1528-1167.2009.02217.x

27. Baird G, Robinson RO, Boyd S, Charman T. Sleep electroencephalograms in young children with autism with and without regression. *Dev Med Child Neurol.* (2006) 48:604–8. doi: 10.1017/S0012162206001265

28. Hrdlicka M, Komarek V, Propper L, Kulisek R, Zumrova A, Faladova L, et al. Not EEG abnormalities but epilepsy is associated with autistic regression and mental functioning in childhood autism. *Eur Child Adolesc Psychiatry* (2004) 13:209–13. doi: 10.1007/s00787-004-0353-7

29. Tuchman RF, Rapin I. Regression in pervasive developmental disorders: seizures and epileptiform electroencephalogram correlates. *Pediatrics* (1997) 99:560–6. doi: 10.1542/peds.99.4.560

30. Yasuhara A. Correlation between EEG abnormalities and symptoms of autism spectrum disorder (ASD). *Brain Dev.* (2010) 32:791–8. doi: 10.1016/j.braindev.2010.08.010

31. Baumbach HD, Chow KL. Visuocortical epileptiform discharges in rabbits: differential effects on neuronal development in the lateral geniculate nucleus and superior colliculus. *Brain Res.* (1981) 209:61–76. doi: 10.1016/0006-8993(81)91172-0

32. Lewine JD, Andrews R, Chez M, Patil AA, Devinsky O, Smith M, et al. Magnetoencephalographic patterns of epileptiform activity in children with regressive autism spectrum disorders. *Pediatrics* (1999) 104:405–18. doi: 10.1542/peds.104.3.405

33. Wing L, Leekam SR, Libby SJ, Gould J, Larcombe M. The diagnostic interview for social and communication disorders: background, inter-rater reliability and clinical use. *J Child Psychol Psychiatry* (2002) 43:307–25. doi: 10.1111/1469-7610.00023

34. Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, et al. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J. Autism Dev. Disord.* (2000) 30:205–23. doi: 10.1023/A:1005592401947

35. Alan Kaufman NK. *Kaufman Assessment Battery for Children: Administration and Scoring Manual: American Guidance Service* (Minesota, MN) (1983).

36. Yoshimura Y, Kikuchi M, Shitamichi K, Ueno S, Remijn GB, Haruta Y, et al. Language performance and auditory evoked fields in 2- to 5-year-old children. *Eur J Neurosci.* (2012) 35:644–50. doi: 10.1111/j.1460-9568.2012.07998.x

37. Tesan G, Johnson BW, Reid M, Thornton R, Crain S. Measurement of neuromagnetic brain function in pre-schoolchildren with custom sized MEG. *J. Vis. Exp.* (2010) 36:1693. doi: 10.3791/1693

38. Cobb WA. *IFCN Recommendations for the Practice of Clinical Neurophysiology*. Amsterdam (1983).

39. White H. A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. *Econometrica* (1980) 48:817–30. doi: 10.2307/1912934

40. Ames LB, Gesell Institute of Child Development. *The Gesell Institute's Child From One to Six: Evaluating the Behavior of the Preschool Child*. New York, NY: Harper & Row (1979).

41. Burns CW. Patterns of sex differences in children's information processing with and without independence from g. *J Sch Psychol.* (1988) 26:233–42. doi: 10.1016/0022-4405(88)90003-9

42. Gabis L, Pomeroy J, Andriola MR. Autism and epilepsy: cause, consequence, comorbidity, or coincidence? *Epilepsy Behav.* (2005) 7:652–6. doi: 10.1016/j.yebeh.2005.08.008

43. Hara H. Autism and epilepsy: a retrospective follow-up study. *Brain Dev.* (2007) 29:486–90. doi: 10.1016/j.braindev.2006.12.012

44. Chez MG, Chang M, Krasne V, Coughlan C, Kominsky M, Schwartz A. Frequency of epileptiform EEG abnormalities in a sequential screening of autistic patients with no known clinical epilepsy from 1996 to 2005. *Epilepsy Behav.* (2006) 8:267–71. doi: 10.1016/j.yebeh.2005.11.001

45. Drenthen GS, Barendse EM, Aldenkamp AP, van Veenendaal TM, Puts NA, Edden RA, et al. Altered neurotransmitter metabolism in adolescents with high-functioning autism. *Psychiatry Res.* (2016) 256:44–9. doi: 10.1016/j.pscychresns.2016.09.007

46. Joshi G, Biederman J, Wozniak J, Goldin RL, Crowley D, Furtak S, et al. Magnetic resonance spectroscopy study of the glutamatergic system in adolescent males with high-functioning autistic disorder: a pilot study at 4T. *Eur Arch Psychiatry Clin Neurosci.* (2013) 263:379–84. doi: 10.1007/s00406-012-0369-9

47. Brown RE. Hebb and Cattell: the genesis of the theory of fluid and crystallized intelligence. *Front Hum Neurosci.* (2016) 10:606. doi: 10.3389/fnhum.2016.00606

48. Sanchez Fernandez I, Loddenkemper T, Galanopoulou AS, Moshe SL. Should epileptiform discharges be treated? *Epilepsia* (2015) 56:1492–504. doi: 10.1111/epi.13108

49. Dawson M, Soulieres I, Gernsbacher MA, Mottron L. The level and nature of autistic intelligence. *Psychol Sci.* (2007) 18:657–62. doi: 10.1111/j.1467-9280.2007.01954.x

50. Nader AM, Courchesne V, Dawson M, Soulieres I. Does WISC-IV underestimate the intelligence of autistic children? *J Autism Dev Disord.* (2016) 46:1582–9. doi: 10.1007/s10803-014-2270-z

51. Sinha SR, Sullivan L, Sabau D, San-Juan D, Dombrowski KE, Halford JJ, et al., American clinical neurophysiology society guideline 1: minimum technical requirements for performing clinical electroencephalography. *J Clin Neurophysiol.* (2016) 33:303–7. doi: 10.1097/WNP.0000000000000308

52. Issa NP. Neurobiology of continuous spike-wave in slow-wave sleep and Landau-Kleffner syndromes. *Pediatr Neurol.* (2014) 51:287–96. doi: 10.1016/j.pediatrneurol.2014.04.029

Check for updates

Differential T Cell Levels of Tumor Necrosis Factor Receptor-II in Children With Autism

Paul Ashwood*

Department of Medical Microbiology and Immunology, and The Medical Investigation of Neurodevelopmental Disorders Institute, University of California, Davis, CA, United States

Autism spectrum disorders (ASD) are characterized by impairments in verbal and non-verbal communication, in social interactions, and often accompanied by stereotypical interests and behaviors. A role for immune dysfunction has long been implicated in ASD pathophysiology, behavioral severity, and co-morbidities. The pro-inflammatory cytokine tumor necrosis factor alpha (TNFα) has been associated with ASD in some studies but little is known about its receptors. There are two receptors for TNFα, with TNFRI relaying many of the signals from TNFα, especially those that are rapid, whilst TNFRII relays later more long-term effects of TNFα. Proteolytic cleavage can lead to the soluble versions of these receptors which can neutralize the effects of TNFα. Here, we determined levels of TNFα and its receptors in 36 children with a confirmed diagnosis of ASD and 27 confirmed typically developing (TD) controls, 2–5 years-of-age. Children with ASD had higher levels of TNFRII on T cells compared to controls following cell stimulation. Levels of sTNFRII were decreased in cell supernatants following stimulation in ASD. Overall these data corroborate the role of inflammatory events in ASD and align with previous studies that have shown differential changes in cellular adaptive immunity in children with ASD. Future longitudinal analyzes of cellular immune function and downstream signaling from immune receptors will help further delineate the role of inflammation in ASD.

Keywords: autism, lymphocyte, T cells, TNF-tumor necrosis factor, behavior, cytokine receptors

INTRODUCTION

Autism spectrum disorders (ASD) are neurodevelopmental disorders affecting 1 in 59 children whom are characterized by significant deficits in communication, social interactions and frequently accompanied by stereotyped or restricted behaviors and interests. The etiology of ASD is complex and largely unknown; however, potential genetic candidates linked with ASD include many genes that regulate immune responses, for instance human leukocyte antigen (HLA)-DR, phosphatase and tensin homolog (PTEN), macrophage migration inhibitory factor (MIF), complement C4B, MET tyrosine receptors, interleukin (IL)-4 receptor, and reelin [Reviewed in (1, 2)]. Significant immune dysfunction is also seen in children with ASD, including prominent neuroinflammation in brain specimens, and alterations in adaptive and innate immune responses in the periphery (3–15).

Tumor necrosis factor-α (TNFα) is a pleiotropic and highly regulated pro-inflammatory cytokine secreted by a number of different cells that plays an important role in coordinating early inflammatory processes, cell proliferation and apoptosis (16). Biologically the activities of TNFα are mediated via two different ubiquitously expressed TNFα receptors, TNF receptor type I (TNFRI; also known as CD120a), and type II (TNFRII; CD120b) (17, 18). TNFRI seems to be the main mediator of TNFα rapid signaling and is found on most tissues, whereas TNFRII mediates later more long-term effects of TNFα and are more commonly expressed on immune cells (17, 19). Proteolytic cleavage of these receptors from the cell surface results in soluble forms (sTNFRI and sTNFRII) that can neutralize TNFα and thus modulate its biological activity (20). The measurement of levels of sTNFRs and TNFα together may be more useful and reliable markers of the inflammatory response and TNFα bioactivity than just TNFα alone.

Although many studies have documented increased expression of TNFα in different neuropsychiatric diseases, for example schizophrenia, depressive disorder, and Alzheimer's disease, only a few have evaluated TNFα in serum/plasma or following stimulation of immune cells in ASD, and the results are often conflicting (3, 4, 21–36). Information on the levels of sTNFRs are even more scant in ASD. Finally, no previous study has assessed both levels of TNFRs on the cell surface of immune cells and production of sTNFRs in ASD.

The present study sought to evaluate levels of TNFRs on T cells and sTNFRs in supernatants following immune challenge in ASD patients compared to TD control subjects.

METHODS

Subjects

This study examined 63 participants enrolled through the Childhood Autism Risk from Genetics and Environment (CHARGE) study at U.C. Davis (37). Full details regarding behavioral measures/assessments and recruitment in the CHARGE study protocols have previously been described (37). Children were consecutively assessed. Participants were free of medication and without chronic clinically defined illness or fever at time of blood draw. The participants were 27 typically developing (TD) controls median age 3.9 years [(interquartile range 2.2–6.1), 4 females] and 36 children with ASD [median age 3.6 years (interquartile range 2.5–4.8), 5 females]. Diagnoses of ASD was based on Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria, and defined as autistic disorder. Further evaluation was confirmed using the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS) assessments. Children from the TD groups were screened for autism traits using the Social Communication Questionnaire (SCQ). This study was approved by the UC Davis institutional review board and complied with all requirements regarding human subjects. Parents gave both written and informed consent.

Cell Isolation, Stimulation, and Biochemical Measures

Peripheral blood mononuclear cells (PBMC) were separated from the whole blood by centrifugation over Histopaque-1077 Hybri-Max lymphocyte separation medium (Sigma; St. Louis, MO) before washing twice in Hanks Balanced Salt Solution (HBSS; VWR; Brisbane, CA). PBMC were either cultured in media alone, or stimulated with PHA (10 μg/mL; Sigma), for 24 h at 37°C in 5% CO_2. Following culture, plates were centrifuged before supernatants were harvested and stored at −80°C until cytokine analysis and cells processed for flow cytometry.

The quantification of TNFα and its soluble receptors (sTNFRI and sTNFRII) were assessed by ELISA using standard procedures recommended by the manufacturer (Quantikine, R&D Systems, Minneapolis, Minn., USA). All samples were on unstimulated and stimulated cell culture supernatants, in duplicates. The detection limits for the kits were <0.5 pg/ml. Concentrations obtained below the sensitivity limit of detection (LOD) of the method were calculated as LOD/2 for statistical comparisons. Culture supernatants had not undergone any previous freeze/thaws cycle.

Cells were harvested after culture and were washed three times in FACS buffer (PBS, 1% fetal bovine serum albumin (VWR, USA) and 0.1 % sodium azide (Sigma), before being resuspended and stained in 100 μl FACS buffer containing either the following monoclonal antibodies fluorescein isothiocyanate (FITC)-conjugated mouse anti-human TNFRI (CD120a); phycoerythrin (PE)-conjugated mouse anti-human TNFRII (CD120b); (PE)-Cy5-conjugated mouse anti-human CD3; and allophycocyanin (APC)-conjugated mouse anti-human CD4, CD8 (all antibodies were from BD Biosciences, CA, USA). Appropriate IgG isotype controls (BD bioscience) were used to correct for compensation issues. Cells were incubated at 4°C for 30 min before being spun down and washed with staining buffer. Cells were then analyzed on a LSR II flow cytometer and the data acquired analyzed with FlowJo software (BD Immunocytometry Systems). Lymphocytes were gated using forward scatter and side scatter parameters and $CD3^+$ cells for analysis of cell surface TNFRI and TNFRII expression, with further analysis of CD4 and CD8 expression where each parameter was measured separately on $CD3^+$ populations, $CD3^+CD4^+$ populations, and $CD3^+CD8^+$ populations.

Statistical Analysis

In primary analyses, induced TNFα and soluble receptors and cell surface markers levels (outcome) were compared by group (predictor) and statistical significance was determined using a parametric Student's t-test, following confirmation of normal distribution, with a p-value of <0.05 considered significant. Multiple comparisons were adjusted for by using the Benjamini-Hochberg False Discovery Rate. Using answers to questions regarding loss of language (Q11) and loss of social skills (Q25) of the ADI-R, the autism population was further divided into two groups based on the clinical onset of autistic symptoms; namely, children who regressed in acquired language or social skills after initial typical development, and secondly, children who did not

TABLE 1 | Comparison of the TNFα and soluble receptors (pg/ml) following cell culture in media alone or stimulation with PHA in children with autism ($n = 36$) and typically developing controls ($n = 27$). Data are presented as mean ± standard error of means (SEM).

		TNα	sTNFRI	sTNFRII
Media	ASD	34.38 ± 2.81	57.80 ± 2.13	147.7 ± 13.07
	TD	33.09 ± 3.844	58.23 ± 2.70	182.4 ± 22.80
PHA	ASD	54.50 ± 1.89	63.83 ± 2.48	1,060 ± 77.72
	TD	54.71 ± 1.96	66.17 ± 3.49	1,766 ± 213.61*

*$p = 0.014$ stimulated levels of sTNFRII were decreased in ASD children compared with PHA stimulated levels in typically developing controls.

regress. We also compared ADOS scores with immune outcomes using Pearson correlations. There were too few subjects with clinical co-morbid features such as gastrointestinal symptoms or sleep disorders to determine differences between cytokine production or cell surface receptor expression. All analyses were carried out using SAS version 9.1 (SAS Inc.; Cary, NC) and graphed with Prism 5 Software (GraphPad Software; San Diego, CA).

RESULTS

Cytokine and soluble receptors were measured in harvested supernatants from unstimulated and PHA-stimulated, PBMC cultures. No difference between children with ASD and TD controls were observed in TNFα or its receptors in unstimulated media alone conditions (**Table 1**). Activation with PHA led to an increase in all analytes measured across both groups. Observed levels of sTNFRII after PHA-stimulation were significantly less in ASD group compared to the TD group ($p = 0.014$; **Table 1**). When comparisons were made among children with ASD who had regressed compared to those that had not, unstimulated sTNFRII levels were lower in children with ASD who had not regressed, compared to those that had and to TD controls ($p < 0.04$). However, following PHA-stimulation, both groups were decreased for sTNFRII compared with TD controls and were not significantly different from each other. No other differences were observed in ASD children based on regression. Levels of sTNFRII were significantly negatively correlated with impairments in ADOS social interactions ($r = -0.383$, $p < 0.025$), suggesting lower levels were associated with worse behaviors.

No significant differences in the frequencies of CD3^{+}, CD4^{+}, and CD8^{+} T cells between TD controls and children with ASD, with or without PHA stimulation were observed. The frequency of TNFRI expressing or TNFRII expressing T cell subsets was not different between groups in unstimulated or stimulated conditions (data not shown). Following immune stimulation both the frequency of cells expressing TNFRI (CD120a) and TNFRII (CD120b) on the cell surface were similar in both groups. However, after PHA stimulation, the amount of cell surface TNFRII (CD120b) receptors, as measured by mean fluorescence intensity (MFI), was significantly increased in children with

FIGURE 1 | Comparison of CD120b (TNFRII) on T cells and T cell subsets following PHA stimulation in children with autism (Au: $n = 36$) and frequency and geographically matched typically developing (TD; $n = 27$). Data are expressed as a Mean Fluorescence intensity (MFI) of CD120b on **(A)** CD3^{+} T cells, and **(B)** CD3^{+}CD4^{+} and **(C)** CD3^{+}CD8^{+} T cell subsets. Data is represented as Mean and standard error of mean, exact p-values included.

ASD compared to controls on CD3^{+} T cells as a whole, and both the T helper CD3^{+}CD4^{+}CD120b^{+} subsets and cytolytic CD3^{+}CD8^{+}CD120b^{+} T cells ($p < 0.03$; **Figure 1**). No differences were seen between those children with ASD who had regression and those children with ASD that did not have regression. In the ASD group frequency of CD3^{+}CD120b^{+} were associated with worse social behavior on ADOS ($r = 0.238$, $p < 0.035$).

DISCUSSION

To the best of our knowledge this is the first study to assess levels of TNFRs on immune cells and release of sTNFR into cell culture supernatants following immune challenge in ASD. Our results demonstrated increased levels of TNFRII on T cells and T cell subsets following stimulation, and decreased sTNFRII in supernatants in children with ASD, but no differences in TNFα and cell surface or sTNFRI levels were found.

Moreover, we found associations between TNFRII levels on cells or in the supernatants and more impairments in behavior. It is currently unclear how TNFα can affect neurodevelopmental outcomes and behaviors during childhood in ASD, and the data should be treated with caution. However, of note, numerous studies have shown that impairments in core ASD behaviors and associated co-morbid and aberrant behaviors, are strongly correlated with altered immune profiles (38). Further validation of the link between observed behavioral severity and cytokine and cytokine receptors is warranted.

Multiple studies have demonstrated that TNFα levels are increased in serum of individuals with ASD when compared to controls (21, 23, 25, 26, 34–36); however, studies on plasma have provided conflicting results (4, 28, 35). These discordant results may reflect the matrix used as well as methodological issues, including different assessment instruments, and clinical and demographic characteristics of ASD populations studied. In addition, it is possible that TNFα is produced at affected tissues and degraded shortly after its production. As TNFRs can be induced by TNFα, the cellular expression may relate to TNFα activity. Our data suggest an increase in TNFRII on T cells, with subsequent decreases in supernatants presumably due to decreased shedding. Previous studies by Jyonouchi and colleagues corroborate our findings showing decreased sTNFRII in cell culture supernatants after T cell mitogen stimulation with PHA (29–32); however, cell surface expression of TNFR was not previously determined. Soluble TNFRII is generated by proteolytic cleavage by the metalloproteinase TNFα converting enzyme (TACE, also known as ADAM17) which despite this enzyme being increased in blood of ASD children (39) does not result in increased sTNFRII in our study, perhaps suggesting proteolytic cleavage is not the underlying mechanism and that receptors are retained on cells. Another possibility is that cell signaling after TNFα binding is weak and that proteolysis does not occur due to altered signaling pathways (40). Soluble TNFRII may lead to the inactivation of circulating TNFα by the generation of high affinity complexes. These complexes reduce the binding of TNFα to any cell membrane target receptors thus downregulating the activity and response to TNFα (41). As soluble TNFRs neutralize TNFα, measuring both receptor and cytokine may be more reflective of the net effect of cytokines, in that even though we did not see an increase in TNFα after stimulation, the increased presence of receptors on the cell surface and decreased levels of neutralizing sTNFRII in supernatants may indicate that more TNFα-receptor ligand binding and signaling occurred. Future studies should determine TNFα-TNR responses in immune cells from children with ASD, including other immune cell types not just T cells. Further

studies should also address whether cytokines such as TNFα are involved in pro-apoptotic signaling or pro-life signaling and the net balance of those signals induced by inflammatory responses in ASD.

Differential expression of markers of T cell activation have been shown in children with ASD following immune stimulation including increased CD26, CD38, CD69, HLA-DR, but decreases in CD25 (42–46). Another member of the TNF receptor family, CD137 is a co-stimulatory molecule expressed by activated T cells and enhances T cell proliferation, effector functions, and survival was also increased on T cells in ASD children (43). TNFRII is predominantly found on immune cells and is primarily associated with lymphocyte proliferation (17, 19). The TNFRs differ in their intracellular domains which induce distinct intracellular signals. We recently showed that T cell signaling was altered in children with ASD (40) and may suggest that certain downstream signals such as the mTOR pathway may lead to preferential immune activation. Taken together these results may suggest potential differential activation of T cell subsets in ASD when compared to controls.

In summary, the results presented here are in agreement with prior studies and suggest that inflammatory processes, as evinced by alterations of pro-inflammatory cytokine signaling, could contribute to ASD pathophysiology. It is also possible that other aspects of ASD might provide additional feedback that influences or compounds the altered immune state in ASD. Further, future studies including longitudinal measurements of the same participants might further reveal the putative role for levels of TNFRs as bio signatures of ASD development and progression. More studies will be required to characterize the changes in TNFα and the TNRII and their temporal relationship with ASD development, as well as assessment of cytokine and cognate receptors in the brain.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

ACKNOWLEDGMENTS

REFERENCES

1. Careaga M, Ashwood P. Autism spectrum disorders: from immunity to behavior. *Methods Mol Biol.* (2012) 934:219–40. doi: 10.1007/978-1-62703-071-7_12

2. Mead J, Ashwood P. Evidence supporting an altered immune response in ASD. *Immunol Lett.* (2015) 163:49–55. doi: 10.1016/j.imlet.2014.11.006

3. Ashwood P, Wakefield AJ. Immune activation of peripheral blood and mucosal CD3+ lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms. *J Neuroimmunol.* (2006) 173:126–34. doi: 10.1016/j.jneuroim.2005.12.007

4. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. *Brain Behav Immun.* (2011) 25:40–5. doi: 10.1016/j.bbi.2010.08.003

5. Breece E, Paciotti B, Nordahl CW, Ozonoff S, Van de Water JA, Rogers SJ, et al. Myeloid dendritic cells frequencies are increased in children with autism spectrum disorder and associated with amygdala volume and repetitive behaviors. *Brain Behav Immun.* (2013) 31:69–75. doi: 10.1016/j.bbi.2012.10.006

6. Careaga M, Rogers S, Hansen RL, Amaral DG, Van de Water J, Ashwood P. Immune endophenotypes in children with autism spectrum disorder. *Biol Psychiatry* (2017) 81:434–41. doi: 10.1016/j.biopsych.2015.08.036

7. Enstrom AM, Lit L, Onore CE, Gregg JP, Hansen RL, Pessah IN, et al. Altered gene expression and function of peripheral blood natural killer cells in children with autism. *Brain Behav Immun.* (2009) 23:124–33. doi: 10.1016/j.bbi.2008.08.001

8. Enstrom A, Onore C, Van de Water J, Ashwood P. Differential monocyte responses to TLR ligands in children with autism spectrum disorders. *Brain Behav Immun.* (2010) 24:64–71. doi: 10.1016/j.bbi.2009.08.001

9. Garbett K, Ebert PJ, Mitchell A, Lintas C, Manzi B, Mirnics K, et al. Immune transcriptome alterations in the temporal cortex of subjects with autism. *Neurobiol Dis.* (2008) 30:303–11. doi: 10.1016/j.nbd.2008.01.012

10. Gupta S, Aggarwal S, Rashanravan B, Lee T. Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism. *J Neuroimmunol.* (1998) 85:106–9. doi: 10.1016/S0165-5728(98)00021-6

11. Heuer L, Ashwood P, Schauer J, Goines P, Krakowiak P, Hertz-Picciotto I, et al. Reduced levels of immunoglobulin in children with autism correlates with behavioral symptoms. *Autism Res.* (2008) 1:275–83. doi: 10.1002/aur.42

12. Jyonouchi H, Geng L, Cushing-Ruby A, Quraishi H. Impact of innate immunity in a subset of children with autism spectrum disorders: a case control study. *J Neuroinflamm.* (2008) 5:52–9. doi: 10.1186/1742-2094-5-52

13. Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, Li X-M, et al. Elevated immune response in the brain of autistic patients. *J Neuroimmunol.* (2009) 207:111–6. doi: 10.1016/j.jneuroim.2008.12.002

14. Molloy CA, Morrow AL, Meinzen-Derr J, Schleifer K, Dienger K, Manning-Courtney P, et al. Elevated cytokine levels in children with autism spectrum disorder. *J Neuroimmunol.* (2005) 172:198–205. doi: 10.1016/j.jneuroim.2005.11.007

15. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol.* (2005) 57:67–81. doi: 10.1002/ana.20315

16. McCoy MK, Tansey MG. TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease. *J Neuroinflamm.* (2008) 45:1–13. doi: 10.1186/1742-2094-5-45

17. Hohmann HP, Remy R, Brockhaus M, van Loon AP. Two different cell types have different major receptors for human tumor necrosis factor (TNF alpha). *J Biol Chem.* (1989) 264:14927–34.

18. Brockhaus M, Schoenfeld HJ, Schlaeger EJ, Hunziker W, Lesslauer W, Loetscher H. Identification of two types of tumor necrosis factor receptors on human cell lines by monoclonal antibodies. *Proc Natl Acad Sci USA.* (1990) 87:3127–31. doi: 10.1073/pnas.87.8.3127

19. Grell M, Douni E, Wajant H. The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. *Cell* (1995) 83:793–802. doi: 10.1016/0092-8674(95)90192-2

20. Mohler KM, Torrance DS, Smith CA, Goodwin RG, Stremler KE, Fung VP, et al. Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. *J Immunol.* (1993) 151:1548–61.

21. Al-Ayadhi LY. Pro-inflammatory cytokines in autistic children in central Saudi Arabia. *Neurosciences* (2005) 10:155–8.

22. Ashwood P, Anthony A, Torrente F, Wakefield AJ. Spontaneous mucosal lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms: mucosal immune activation and reduced counter regulatory interleukin-10. *J Clin Immunol.* (2004) 24:664–73. doi: 10.1007/s10875-004-6241-6

23. Chez MG, Dowling T, Patel PB, Khanna P, Kominsky M. Elevation of tumor necrosis factor-alpha in cerebrospinal fluid of autistic children. *Pediatr Neurol.* (2007) 36:361–5. doi: 10.1016/j.pediatrneurol.2007.01.012

24. Croonenberghs J, Bosmans E, Deboutte D, Kenis G, Maes M. Activation of the inflammatory response system in autism. *Neuropsychobiology* (2002) 45:1–6. doi: 10.1159/000048665

25. Ferguson BJ, Marler S, Altstein LL, Lee EB, Mazurek MO, McLaughlin A, et al. Associations between cytokines, endocrine stress response, and gastrointestinal symptoms in autism spectrum disorder. *Brain Behav Immun.* (2016) 58:57–62. doi: 10.1016/j.bbi.2016.05.009

26. Ghaffari MA, Mousavinejad E, Riahi F, Mousavinejad M, Afsharmanesh MR. Increased serum levels of tumor necrosis factor-alpha, resistin, and visfatin in the children with autism spectrum disorders: a case-control study. *Neurol Res Int.* (2016) 2016:9060751. doi: 10.1155/2016/9060751

27. Goines PE, Ashwood P. Cytokine dysregulation in autism spectrum disorders (ASD): possible role of the environment. *Neurotoxicol Teratol.* (2013) 36:67–81. doi: 10.1016/j.ntt.2012.07.006

28. Hu CC, Xu X, Xiong GL, Xu Q, Zhou BR, Li CY, et al. Alterations in plasma cytokine levels in chinese children with autism spectrum disorder. *Autism Res.* (2018) 11:989–999. doi: 10.1002/aur.1940

29. Jyonouchi H, Sun S, Le H. Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. *J Neuroimmunol.* (2001) 120:170–9. doi: 10.1016/S0165-5728(01)00421-0

30. Jyonouchi H, Geng L, Ruby A, Reddy C, Zimmerman-Bier B. Evaluation of an association between gastrointestinal symptoms and cytokine production against common dietary proteins in children with autism spectrum disorders. *J Pediatr.* (2005) 146:605–10. doi: 10.1016/j.jpeds.2005.01.027

31. Jyonouchi H, Geng L, Ruby A, Zimmerman-Bier B. Dysregulated innate immune responses in young children with autism spectrum disorders: their relationship to gastrointestinal symptoms and dietary intervention. *Neuropsychobiology* (2005) 51:77–85. doi: 10.1159/000084164

32. Jyonouchi H, Geng L, Streck DL, Dermody JJ, Toruner GA. MicroRNA expression changes in association with changes in interleukin-1ß/interleukin10 ratios produced by monocytes in autism spectrum disorders: their association with neuropsychiatric symptoms and comorbid conditions (observational study). *J Neuroinflamm.* (2017) 14:229. doi: 10.1186/s12974-017-1003-6

33. Rose D, Ashwood P. Potential cytokine biomarkers in autism spectrum disorders. *Biomark Med.* (2014) 8:1171–81. doi: 10.2217/bmm.14.39

34. Steeb H, Ramsey JM, Guest PC, Stocki P, Cooper JD, Rahmoune H, et al. Serum proteomic analysis identifies sex-specific differences in lipid metabolism and inflammation profiles in adults diagnosed with Asperger syndrome. *Mol Autism.* (2014) 5:4. doi: 10.1186/2040-2392-5-4

35. Suzuki K, Matsuzaki H, Iwata K, Kameno Y, Shimmura C, Kawai S, et al. Plasma cytokine profiles in subjects with high-functioning autism spectrum disorders. *PLoS ONE* (2011) 6:e20470. doi: 10.1371/journal.pone.0020470

36. Xie J, Huang L, Li X, Li H, Zhou Y, Zhu H, et al. Immunological cytokine profiling identifies TNF-α as a key molecule dysregulated in autistic children. *Oncotarget* (2017) 8:82390–8. doi: 10.18632/oncotarget.19326

37. Hertz-Picciotto I, Croen LA, Hansen R, Jones CR, Van de Water J, Pessah IN. The CHARGE study: an epidemiologic investigation of genetic and environmental factors contributing to autism. *Environ Health Perspect.* (2006) 114:1119–25. doi: 10.1289/ehp.8483

38. Onore C, Careaga M, Ashwood P. The role of immune dysfunction in the pathophysiology of autism. *Brain Behav Immun.* (2012) 26:383–92. doi: 10.1016/j.bbi.2011.08.007

39. Corbett BA, Kantor AB, Schulman H, Walker WL, Lit L, Ashwood P, et al. A proteomic study of serum from children with autism showing differential expression of apolipoproteins and complement proteins. *Mol Psychiatry* (2007) 12:292–306. doi: 10.1038/sj.mp.4001943

40. Onore C, Yang H, Van de Water J, Ashwood P. Dynamic Akt/mTOR signaling in children with autism spectrum disorder. *Front Pediatr.* (2017) 5:43. doi: 10.3389/fped.2017. 00043

41. Bemelmans MH, Gouma DJ, Buurman WA. LPS-induced sTNF receptor release *in vivo* in a murine model. investigation of the role of tumor necrosis factor, IL-1, leukemia inhibiting factor, and IFN-gamma. *J Immunol.* (1993) 151:5554–62.

42. Ashwood P, Corbett BA, Kantor A, Schulman H, Van de Water J, Amaral DG. In search of cellular immunophenotypes in the blood of children with autism. *PLoS ONE* (2011) 6:e19299. doi: 10.1371/journal.pone. 0019299

43. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. Altered T cell responses in children with autism. *Brain Behav Immun.* (2011) 25:840–9. doi: 10.1016/j.bbi.2010. 09.002

44. Denney DR, Frei BW, Gaffney GR. Lymphocyte subsets and interleukin-2 receptors in autistic children. *J Autism Dev Disord.* (1996) 26:87–97. doi: 10.1007/BF02276236

45. Plioplys AV, Greaves A, Kazemi K, Silverman E. Lymphocyte function in autism and Rett syndrome. *Neuropsychobiology* (1994) 29:12–6. doi: 10.1159/000119056

46. Mostafa GA, Al Shehab A, Fouad NR. Frequency of CD4+CD25high regulatory T cells in the peripheral blood of Egyptian children with autism. *J Child Neurol.* (2010) 25:328–35. doi: 10.1177/0883073809339393

Anti-*Candida albicans* IgG Antibodies in Children With Autism Spectrum Disorders

Heather K. Hughes and Paul Ashwood*

Department of Medical Microbiology and Immunology, and The Medical Investigation of Neurodevelopmental Disorders (M.I.N.D.) Institute, University of California, Davis, Davis, CA, United States

The gut microbiota are known to have a profound influence on both mucosal and systemic immunity and are important for gastrointestinal (GI) function. In addition, new evidence shows that the microbiota significantly influence neurodevelopment and behavior. Immune dysfunction and GI distress are extremely common in individuals with autism spectrum disorders (ASD). A growing body of evidence suggests that individuals with ASD have significant aberrations in the composition of their gut microbiota, known as dysbiosis. However, these studies have focused on the bacterial components of the microbiota, leaving the fungal microbiota in ASD poorly studied. Increases in fungal species such as *Candida albicans* are associated with inflammatory bowel disorders, and have recently been implicated in several neurological disorders including schizophrenia. We aimed to determine if children with ASD exhibit elevations in antibodies that target *C. albicans*, indicating current or previous overgrowth of this fungal species. We measured anti-*C. albicans* immunoglobulin (IgG) in plasma from 80 children enrolled in the UC Davis MIND Institute CHARGE study. Measurements were acquired using a commercial ELISA kit. Plasma anti-*C. albicans* antibody positivity was found in 36.5% (19/52) of children with ASD. Anti-*C. albicans* antibodies in typically developing controls was (14.3%; 4/28). Overall, ASD children had a higher rate of high-positive values compared to typically developed children with an unadjusted odds ratio of 3.45 (95% confidence interval, 1.0409 to 11.4650; $p = 0.041$, two-tailed). GI dysfunction was found in about half of the ASD children who were positive for anti-*Candida* IgG. This study provides evidence of a new microbial risk factor for ASD.

Keywords: autism, Candida, antibodies, immunity, behavior

INTRODUCTION

Autism spectrum disorders (ASD) are a group of heterogeneous neurodevelopmental disorders defined by deficiencies in social interactions, cognition and communication, and stereotypical and repetitive behaviors (1). Although specific genetic mutations account for 10–20% of causes for ASD, heritability risks are high and could include genes that lead to susceptibility for environmental exposures, or similar epigenetic mechanisms brought about by shared household exposures to environment (2). Environmental risk factors for having a child with ASD include gestational exposure to pollution and pesticides, and maternal infections and inflammation during pregnancy (3–5). ASD is also linked to familial autoimmunity and asthma (6–8) and many individuals with ASD have significant immune dysfunction (9, 10). Gastrointestinal (GI) dysfunction is also common in these individuals, with at least 50% of individuals with ASD experiencing GI issues (11).

Gastrointestinal and immune dysfunction has been linked to aberrant composition of the microbiota, known as dysbiosis (12). Recently, researchers have identified significant bacterial dysbiosis in individuals with ASD (13–23). Bacterial dysbiosis can be caused by a variety of insults including frequent antibiotic use, which can also increase the risk of fungal overgrowth in the GI tract. *Candida albicans* is generally considered a passive commensal yeast of the GI and genitourinary tracts, however, it has polymorphic capabilities and under certain conditions, including altered competition within the gut, it is capable of transitioning to its pathogenic and invasive fungal form (24). The presence of *Candida* species during recolonization after antibiotics also contribute to dysbiosis (25) and are associated with GI disorders such as celiac disease and inflammatory bowel disorders (26–28). These competitive relationships of bacterial versus fungal microbiota are complex and still being investigated, as newer techniques develop to identify them (24).

Overgrowth of *Candida* species has been noted in ASD in a few studies utilizing culture-based techniques (29–31) and more recently in a study using sequencing techniques, which found *Candida* to be present in the stool of ASD children in nearly twice the numbers of typically developed children (21). Elevations of d-arabinitol, a suspected metabolic byproduct of *Candida* species, was found in a study of 21 Italian children with ASD (32). D-arabinitol was also significantly reduced in ASD children after probiotic administration, and this correlated with improved behaviors including ability to concentrate (33). Fungal infections are an emerging area of research interest in ASD, and exposure can also be identified by looking at immunoglobulin (Ig) that target fungal antigens. They may be present in individuals with dysbiosis, as this may lead to breeches in intestinal barrier function and subsequent immune responses to commensal microbiota, including the production of IgG antibodies indicating current or previous overgrowth of this fungal species (34, 35). In schizophrenia, significantly elevated IgG antibodies to fungal microbiota have been seen, especially in males, however, they were also seen in bipolar females associated with lower cognitive scores (36). So far these antibodies have not been studied in individuals with ASD, therefore we aimed to determine if similar antibodies are over-represented in ASD children with and without GI dysfunction compared to their typically developing (TD) counterparts.

METHODS

Study Participants

Eighty participants ranging in age from 3 to 13 years old were enrolled in this study as part of the larger population based cohort Childhood Autism Risk from Genetics and Environment (CHARGE) study (37). ASD diagnoses were confirmed using the Autism Diagnostic Interview-Revised (ADI-R), and the Autism Diagnostic Observation Schedule (ADOS) at the time of enrollment. Social Communication Questionnaire (SCQ) was used to screen for characteristics of ASD in the typically developed children. Criteria for enrollment in the typically developed groups were scores of below 15 on the SCQ and above 70 on the Mullen Scales of Early Learning (MSEL) and

Vineland Adaptive Behavior Score (VABS). There were $n = 52$ ASD subjects (median age 7.42 years (IQR: 5.17–9.42); 8 females) and $n = 28$ TD (median age 6.5 years (IQR: 5.58–8.33); 3 females). All subjects were administered the Aberrant Behavior Checklist (ABC) assessment. Parents also completed a CHARGE GI history (GIH) survey and GI symptom survey, based upon Rome III Diagnostic Questionnaire for the Pediatric Functional GI Disorders (22, 38) to identify symptoms of GI dysfunction including abdominal pain, gas/bloating, diarrhea, constipation, pain on stooling, vomiting, sensitivity to foods, difficulty swallowing, and blood in stool or vomit. Participants were excluded if they had inflammatory bowel disease (IBD) or other GI pathology, recent evidence of a GI infection and/or were taking medication that might alter GI function such as stool softeners which can alter motility or recent antibiotics/antifungals that can induce dysbiosis. In addition, participants with seizure disorder, genetic disorders, or other chronic diseases and/or infections were also excluded.

This study was approved by institutional review boards for the State of California and the University of California, Davis. Both written and informed consent was obtained from a legal guardian for all study participants prior to data collection in accordance with the UC Davis IRB protocol.

Blood Collection and Enzyme-Linked Immunosorbent Assay

Peripheral blood was collected from each subject in acid-citrate dextrose Vacutainers (BD Biosciences; San Jose, Ca). Blood was centrifuged and plasma harvested and stored at $-80°C$ until time of assay. Human anti-*Candida albicans* IgG was measured using enzyme-linked immunosorbent assay with a commercially available kit (Abcam, Cambridge, MA, USA) following the manufacturer's instructions. According to manufacturer's recommendations, 96 well plates were pre-coated with *Candida* capture antigens. Plasma samples were diluted 1:100 with diluent provide, and 100 μL of diluted plasma, positive, negative, and cut-off controls were added to the plate in duplicate, leaving two blank wells per plate, and incubated in the dark for 1 h at 37°C. Wells were aspirated and washed three times with provided washing solution. 100 μL *Candida albicans* anti-IgG HRP Conjugate was added to all wells except for blank wells, and incubated for 30 min in the dark at room temperature, then washed again three times. One hundred microliter 3,3',5,5'-tetramethylbenzidine (TMB) solution was added to the wells and incubated for 15 min, the reaction was stopped with reagent provided by the manufacturer. Absorbance was measured immediately on spectrophotometer at 450 nm with dual wavelength, using 620 nm as reference wavelength.

Statistics

Positivity was identified using background-adjusted absorbance of provided cut-off control. Background-adjusted sample absorbance above the positive control are denoted as high-positive. Comparison of qualitative variables between groups was assessed using Fisher's Exact Probability test with probability (P) of <0.05 considered significant.

RESULTS

Plasma anti-*C. albicans* antibody positivity was found in 36.5% (19/52) of children with ASD. There was a significantly higher percent positivity of plasma anti-*C. albicans* antibodies in children with ASD than in healthy TD controls (14.3%; 4/28), with an unadjusted odds ratio of 3.45 (95% confidence interval, 1.040 to 11.465; $p = 0.041$, two-tailed; **Table 1**). Nine of the 19 positive samples in children with ASD were considered high-positive, versus only one high-positive in the TD population (**Figure 1**). When examining ASD children with positivity for anti-*Candida* IgG, 9 of 19 (47%) had symptoms of GI dysfunction, of the 33 negative for anti-*Candida* IgG 13 had GI symptoms (39%), however no significant differences were observed based on presence or absence of GI symptoms in children with ASD. In comparison, 1 of 4 TD children positive for anti-*Candida* IgG had GI issues (25%) and only 5 of 24 who were negative for anti-*Candida* IgG (21%), however, these data are limited by small sample size of the TD group with GI symptoms.

DISCUSSION

Study of the microbiota-gut-brain axis has exploded over the past few decades, however, fungal microbiota research is still in the early stages. Fungal overgrowth has been implicated

TABLE 1 | The frequency of plasma anti-*Candida albicans* antibodies in children with ASD compared to typically developed children.

Study group	Anti-*C albicans* IgG positive	Anti-*C albicans* IgG negative
ASD	19	33
	36.5%	63.4%
TD	4	24
	14.3%	85.7%
P value	0.04	
OR (95% CI)	3.45	

OR, odds ratio.

FIGURE 1 | *Candida albicans* plasma positivity in ASD vs. typically developing children. Nineteen children with ASD were positive for *C. albicans* IgG antibodies compared to four typically developing children.

in inflammatory bowel disorders (39–41), schizophrenia and bipolar disorder (36), and was recently discovered within the cells of post-mortem brain tissue in Alzheimer's patients (42). Our results indicate that children with ASD have elevations in IgG against this fungal commensal, likely indicating an overgrowth of *Candida albicans* within the GI tract. The source of dysbiosis and *Candida* overgrowth in ASD is currently unknown but may be influenced by the immune dysfunction seen in many children with ASD. Alternatively, this overgrowth may be contributing to immune and GI dysfunction and the behaviors seen in ASD. This raises questions as to whether dysbiosis is present very early in life in ASD and may be involved in the etiology of ASD that need further investigation. IgG antibodies to commensal organisms become more common as individuals age, possibly due to transient intestinal breeches that might occur during enteric infections or dysbiosis from antibiotics, therefore identifying earliest exposure is a key next step to this research. Future research should attempt to identify presence of IgM antibodies which indicate current or very recent infection (35), ideally during infancy and/or early life to see if there is a relationship between infection and ASD symptom emergence.

The presence of *Candida* species is known to alter the assembly of microbiota after antibiotics, contributing to dysbiosis in mice (25). This suggests that if present, a different bacterial composition will occur than when absent. Therefore, if *Candida* is present in very early life it could interfere with initial colonization and the successions of composition during the early transitional stages of microbiota development leading to dysbiosis (43). The early microbiota are typically inherited from maternal sources, especially during vaginal delivery (43). If *Candida* is overgrown in mother during gestation, it could potentially be passed on to offspring as an early colonizer, interfering with normal colonization. Furthermore, the immune response to fungal overgrowth includes elevations of interleukin (IL)-17 (44). This cytokine is implicated in maternal immune activation (MIA) models of ASD, and without it ASD behaviors in MIA offspring were absent (45, 46). Further research utilizing animal models of fungal dysbiosis in early life or gestation could help determine the degree in which fungal microbiota may be contributing to the dysbiosis and behavioral abnormalities in offspring.

To our knowledge, this is the first study to look at anti-*Candida albicans* IgG in children with ASD. Our recent work identified an imbalance in inflammatory versus regulatory cytokines such as transforming growth factor (TGF) beta, with alterations in the microbiota in this same population based cohort (22). Although GI symptoms were not strongly associated with *Candida* IgG positivity, we previously found microbiota changes in individuals with ASD irrespective of GI symptoms (22). This suggests that dysbiosis could occur even in the absence of GI symptoms. This preliminary study was initiated to guide future research on the fungal microbiota and ASD. Further validation of our results could include exploring fungal composition within the gut as well as metabolic byproducts of yeast species such as d-arabinitol and ethanol,

and identifying associations these might have with behaviors in ASD.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of University of California Davis, IRB committee with both written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the IRB committee at UC Davis.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, et al. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. *Mmwr Surveill Summ*. (2018) 67:1–23. doi: 10.15585/mmwr.ss6706a1

2. Abrahams BS, Geschwind DH. Advances in autism genetics: on the threshold of a new neurobiology. *Nat Rev Genet*. (2008) 9:341–55. doi: 10.1038/nrg2346

3. Raz R, Roberts AL, Lyall K, Hart JE, Just AC, Laden F, et al. Autism spectrum disorder and particulate matter air pollution before, during, and after pregnancy: a nested case-control analysis within the Nurses' Health Study II Cohort. *Environ Health Perspect*. (2015) 123:264–70. doi: 10.1289/ehp.1408133

4. Shelton JF, Geraghty EM, Tancredi DJ, Delwiche LD, Schmidt RJ, Ritz B, et al. Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: the CHARGE study. *Environ Health Perspect*. (2014) 122:1103–9. doi: 10.1289/ehp.1307044

5. Atladóttir HÓ, Henriksen TB, Schendel DE, Parner ET. Autism after infection, febrile episodes, and antibiotic use during pregnancy: an exploratory study. *Pediatrics* (2012) 130:e1447-54. doi: 10.1542/peds.2012-1107

6. Chen S-w, Zhong X-s, Jiang L-n, Zheng X-y, Xiong Y-q, Ma S-j, et al. Maternal autoimmune diseases and the risk of autism spectrum disorders in offspring: a systematic review and meta-analysis. *Behav Brain Res*. (2016) 296:61–9. doi: 10.1016/j.bbr.2015.08.035

7. Scott O, Shi D, Andriashek D, Clark B, Goez HR. Clinical clues for autoimmunity and neuroinflammation in patients with autistic regression. *Dev Med Child Neurol*. (2017) 59:947–51. doi: 10.1111/dmcn.13432

8. Wu S, Ding Y, Wu F, Li R, Xie G, Hou J, et al. Family history of autoimmune diseases is associated with an increased risk of autism in children: a systematic review and meta-analysis. *Neurosci Biobehav Rev*. (2015) 55:322–32. doi: 10.1016/j.neubiorev.2015.05.004

9. Onore C, Careaga M, Ashwood P. The role of immune dysfunction in the pathophysiology of autism. *Brain Behav Immun*. (2012) 26:383–92. doi: 10.1016/j.bbi.2011.08.007

10. Mead J, Ashwood P. Evidence supporting an altered immune response in ASD. *Immunol Lett*. (2015) 163:49–55. doi: 10.1016/j.imlet.2014.11.006

11. McElhanon BO, McCracken C, Karpen S, Sharp WG. Gastrointestinal symptoms in autism spectrum disorder: a meta-analysis. *Pediatrics* (2014) 133:872–83. doi: 10.1542/peds.2013-3995

12. Chang C, Lin H. Dysbiosis in gastrointestinal disorders. *Best Pract Res Clin Gastroenterol*. (2016) 30:3–15. doi: 10.1016/j.bpg.2016.02.001

13. Parracho HM, Bingham MO, Gibson GR, McCartney AL. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *J Med Microbiol*. (2005) 54(Pt 10):987–91. doi: 10.1099/jmm.0.46101-0

14. Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, Wolcott RD, et al. Pyrosequencing study of fecal microflora of autistic and control children. *Anaerobe* (2010) 16:444–53. doi: 10.1016/j.anaerobe.2010.06.008

15. Tomova A, Husarova V, Lakatosova S, Bakos J, Vlkova B, Babinska K, et al. Gastrointestinal microbiota in children with autism in Slovakia. *Physiol Behav*. (2015) 138:179–87. doi: 10.1016/j.physbeh.2014.10.033

16. Williams BL, Hornig M, Buie T, Bauman ML, Cho Paik M, Wick I, et al. Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. *PLoS ONE* (2011) 6:e24585. doi: 10.1371/journal.pone.0024585

17. Kang DW, Park JG, Ilhan ZE, Wallstrom G, Labaer J, Adams JB, et al. Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. *PLoS ONE* (2013) 8:e68322. doi: 10.1371/journal.pone.0068322

18. Williams BL, Hornig M, Parekh T, Lipkin WI. Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of Sutterella species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. *MBio* (2012) 3:11. doi: 10.1128/mBio.00261-11

19. Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA. Increased abundance of Sutterella spp, and Ruminococcus torques in feces of children with autism spectrum disorder. *Mol Autism*. (2013) 4:42. doi: 10.1186/2040-2392-4-42

20. Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA. Low relative abundances of the mucolytic bacterium Akkermansia muciniphila and *Bifidobacterium* spp. in feces of children with autism. *Appl Environ Microbiol*. (2011) 77:6718–21. doi: 10.1128/AEM.05212-11

21. Strati F, Cavalieri D, Albanese D, De Felice C, Donati C, Hayek J, et al. New evidences on the altered gut microbiota in autism spectrum disorders. *Microbiome* (2017) 5:24. doi: 10.1186/s40168-017-0242-1

22. Rose DR, Yang H, Serena G, Sturgeon C, Ma B, Careaga M, et al. Differential immune responses and microbiota profiles in children with autism spectrum disorders and co-morbid gastrointestinal symptoms. *Brain Behav Immun*. (2018) 70:354–68. doi: 10.1016/j.bbi.2018.03.025

23. Luna RA, Oezguen N, Balderas M, Venkatachalam A, Runge JK, Versalovic J, et al. Distinct microbiome-neuroimmune signatures correlate with functional abdominal pain in children with autism spectrum disorder. *Cell Mol Gastroenterol Hepatol*. (2017) 3:218–30. doi: 10.1016/j.jcmgh.2016.11.008

24. Mukherjee PK, Sendid B, Hoarau G, Colombel JF, Poulain D, Ghannoum MA. Mycobiota in gastrointestinal diseases. *Nat Rev Gastroenterol Hepatol*. (2015) 12:77–87. doi: 10.1038/nrgastro.2014.188

25. Erb Downward JR, Falkowski NR, Mason KL, Muraglia R, Huffnagle GB. Modulation of post-antibiotic bacterial community reassembly and host response by Candida albicans. *Sci Rep*. (2013) 3:2191. doi: 10.1038/srep02191

26. Li Q, Wang C, Tang C, He Q, Li N, Li J. Dysbiosis of gut fungal microbiota is associated with mucosal inflammation in Crohn's disease. *J Clin Gastroenterol.* (2014) 48:513–23. doi: 10.1097/MCG.0000000000000035

27. Gerard R, Sendid B, Colombel JF, Poulain D, Jouault T. An immunological link between Candida albicans colonization and Crohn's disease. *Critic Rev Microbiol.* (2015) 41:135–9. doi: 10.3109/1040841X.2013.8 10587

28. Harnett J, Myers SP, Rolfe M. Significantly higher faecal counts of the yeasts candida and saccharomyces identified in people with coeliac disease. *Gut Pathogens* (2017) 9:26. doi: 10.1186/s13099-017-0173-1

29. Kantarcioglu AS, Kiraz N, Aydin A. Microbiota-gut-brain axis: yeast species isolated from stool samples of children with suspected or diagnosed autism spectrum disorders and *in vitro* susceptibility against nystatin and fluconazole. *Mycopathologia* (2016) 181:1–7. doi: 10.1007/s11046-015-9949-3

30. Iovene MR, Bombace F, Maresca R, Sapone A, Iardino P, Picardi A, et al. Intestinal dysbiosis and yeast isolation in stool of subjects with autism spectrum disorders. *Mycopathologia* (2017) 182:349–63. doi: 10.1007/s11046-016-0068-6

31. Adams JB, Johansen LJ, Powell LD, Quig D, Rubin RA. Gastrointestinal flora and gastrointestinal status in children with autism–comparisons to typical children and correlation with autism severity. *BMC Gastroenterol.* (2011) 11:22. doi: 10.1186/1471-230X-11-22

32. Noto A, Fanos V, Barberini L, Grapov D, Fattuoni C, Zaffanello M, et al. The urinary metabolomics profile of an Italian autistic children population and their unaffected siblings. *J Matern Fetal Neonatal Med.* (2014) 27(sup2):46–52. doi: 10.3109/14767058.2014.954784

33. Kaluzna-Czaplinska J, Blaszczyk S. The level of arabinitol in autistic children after probiotic therapy. *Nutrition* (2012) 28:124–6. doi: 10.1016/j.nut.2011.08.002

34. Zimmermann K, Haas A, Oxenius A. Systemic antibody responses to gut microbes in health and disease. *Gut Microbes* (2012) 3:42–7. doi: 10.4161/gmic.19344

35. Janeway CJ, Travers P, Walport M. *The Distribution and Functions of Immunoglobulin Isotypes. Immunobiology: The Immune System in Health and Disease. 5th edn.* New York, NY: Garland Science (2001).

36. Severance EG, Gressitt KL, Stallings CR, Katsafanas E, Schweinfurth LA, Savage CL, et al. Candida albicans exposures, sex specificity and cognitive deficits in schizophrenia and bipolar disorder. *NPJ schizophr.* (2016) 2:16018. doi: 10.1038/npjschz.2016.18

37. Hertz-Picciotto I, Croen LA, Hansen R, Jones CR, van de Water J, Pessah IN. The CHARGE study: an epidemiologic investigation of genetic and environmental factors contributing to autism. *Environ Health Perspect.* (2006) 114:1119–25. doi: 10.1289/ehp.8483

38. Walker LS C-DA, Rasquin-Weber A. *Questionnaire on Pediatric Gastrointestinal Symptoms, Rome III Version (QPGS-RIII). Rome III: The Functional Gastrointestinal Disorders.* McLean, VA: Degnon Associates, Inc (2006).

39. Standaert-Vitse A, Sendid B, Joossens M, Francois N, Vandewalle-El Khoury P, Branche J, et al. Candida albicans colonization and ASCA in familial Crohn's disease. *Am J Gastroenterol.* (2009) 104:1745–53. doi: 10.1038/ajg.2009.225

40. Zwolinska-Wcislo M, Brzozowski T, Budak A, Kwiecien S, Sliwowski Z, Drozdowicz D, et al. Effect of Candida colonization on human ulcerative colitis and the healing of inflammatory changes of the colon in the experimental model of colitis ulcerosa. *J Physiol Pharmacol.* (2009) 60:107–18. Available online at: http://www.jpp.krakow.pl/

41. Tamboli CP, Neut C, Desreumaux P, Colombel JF. Dysbiosis in inflammatory bowel disease. *Gut* (2004) 53:1–4. doi: 10.1136/gut.53.1.1

42. Pisa D, Alonso R, Rabano A, Rodal I, Carrasco L. Different brain regions are infected with fungi in Alzheimer's disease. *Sci Rep.* (2015) 5:15015. doi: 10.1038/srep15015

43. RodrÍguez JM, Murphy K, Stanton C, Ross RP, Kober OI, Juge N, et al. The composition of the gut microbiota throughout life, with an emphasis on early life. *Microb Ecol Health Dis.* (2015) 26:26050. doi: 10.3402/mehd.v26. 26050

44. Conti HR, Gaffen SL. IL-17-mediated immunity to the opportunistic fungal pathogen Candida albicans. *J Immunol.* (2015) 195:780–8. doi: 10.4049/jimmunol.1500909

45. Choi GB, Yim YS, Wong H, Kim S, Kim H, Kim SV, et al. The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science* (2016) 351:933–9. doi: 10.1126/science.aa d0314

46. Kim S, Kim H, Yim YS, Ha S, Atarashi K, Tan TG, et al. Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. *Nature* (2017) 549:528–32. doi: 10.1038/nature2 3910

A 16-Year Cohort Analysis of Autism Spectrum Disorder-Associated Morbidity in a Pediatric Population

David Cawthorpe*

Cumming School of Medicine, Departments of Psychiatry and Community Health Sciences, Institute for Child and Maternal Health, The University of Calgary, Calgary, AB, Canada

Introduction: This chapter presents the analysis of physician-diagnosed International Classification of Diseases (ICD version 9) disorders and diseases associated with autism spectrum disorders (ASD) in a 16-year pediatric cohort.

Materials and Methods: The sample ($n = 47,180$; 62% male) consisted of children in the Alberta Health Services Calgary Health Region catchment under the age of 3 years, who received any physician-assigned ICD 9 diagnosis before the age of three between April 1993 and December 31, 1994. There were 111 females and 609 males with ASD diagnosed at any time between 1993 and 2010. The results detail the 16-year odds ratio (OR) associations of ASD diagnosis within the major classes of international classification of diseases (ICD 9) stratified by age and sex in the cohort. Further, for those suffering from ASD and any other disorder or disease, the analysis presents by sex, age, and duration, the proportions of all index physician-assigned ICD diagnoses, arising significantly before and after the index ASD diagnosis.

Results: The rate of treated ASD in the cohort was 1 in 65 and the 16-year population rate of ASD was 62 per 10,000. For males with an ASD over the 16 year period, the ORs were significantly greater than the value one for 15 of the 17 main ICD classes and for 10 of the main ICD classes for females. Different age strata presented a more specific account of the main ICD class OR profiles. More specifically, 28 ICD disorders significantly preceded and 95 ICD disorders significantly followed ASD for females. Thirty-eight ICD disorders significantly preceded and 234 ICD disorders significantly followed ASD for males.

Conclusions: The results largely confirm past studies focusing on more constrained sets of ASD morbidity. The age-stratified ORs gauge the order of risk in time for the cohort. The proportions of specific ICD disorders arising before and after ASD may be useful in respect to informing basic ASD research and ASD clinical management. Limitations are discussed.

Keywords: autism spectrum disorder, temporal hypermorbidity, population cohort, ICD-9, ASD-associated morbidity

INTRODUCTION

Between one in 65 and one in 88 children born in the United States and Canada are diagnosed with ASD (1–4). To date little detailed knowledge has emerged about the different types of morbidity associated with ASD. Based on a literature review spanning the period between 1991 and 2018, it was apparent that most of the ASD morbidity studies have generally focused only on concurrent or prodromal disorders, primarily psychiatric disorders, occasionally physical disorders, and at best only a few disorders within any given study (5–22). The literature review focused on titles containing the terms autism and the stem "morbid." The literature illustrated a basic limitation of the morbidity research design to inform an understanding of the etiology of ASD or its clinical management.

Background

While past studies have identified the ambiguity of comorbidity and multimorbidity definitions (23). Others have sought to remedy the situation, by expanding the conceptualization of comorbidity, multimorbidity, concurrent, and associated disorders. Progress has been made in terms of position papers identifying the scope of the definitions required to begin to understand the associations of disorders, both phenomenological and temporal (24, 25). Jakovljević, in a seminal paper, introduces the terms "anosognosia[1]" in relation to the medical field's approach to the study of multimorbidity, given its range and complexity and the observation that comorbidity and multimorbidity are "under-recognized, under-diagnosed, under-estimated and under-treated" (25). For example, a range of definitions for morbidity including comorbidity and multimorbidity have emerged to better communicate the concepts underpinning the potential relationships of disorders and diseases as these arise in the individual.

In its simplest form, comorbidity exists in from 35 to 88% of all ill people and refers to two or more concurrently co-existing diseases or disorders (25). Researchers have extended the possible definitions of morbidity, comorbidity, and multimorbidity to include terms such as hypercomorbidity and hypocomorbidity that refer to co-occurrence that is greater or lesser than chance, respectively (25). Time necessarily becomes a principle operator in distinguishing sets of definitions related to the directionality of comorbid diseases and their complex nature (26). Also of particular importance is that of each disease's probability in relation to the others' onset (26).

Multimorbidity is defined as the coexistence of multiple chronic diseases or conditions within an individual (27). The definitions of morbidity with inclusion of the often transient nature of temporal morbidity further complicates how morbidity is conceptualized. For example, disorders that are not always present or appear resolved in the individual either before or after the time of ASD onset, may be over-represented in the population of ASD-diagnosed individuals. Definition of morbidity, for the purpose of this paper, required extension to include the concept

of temporality and associated transient diseases or disorders, not necessarily present at the time of index ASD diagnosis, yet present at some past or future time in proportions greater than that expected by chance alone. The present study extends previous work on ASD (28) in describing the full range of unique International Classification of Diseases (ICD) diagnoses associated in time with ASD.

Accompanying updated definitions aiding the conceptualization of morbidity is the advantage of access to large databases, such as the one on which present study and similar studies have been based (29–31). The 16-year database has been the source of numerous publications related to identifying key relationships between biomedical, physical, and mental disorders, in addition to the influence of the temporal occurrence of particular classes of disorders (e.g., mental disorders) in relationship to serious biomedical and somatic disorders (28–33). Identifying a cohort under the age of 3 years within a time frame and following the progress of disorders diagnosed within this group over a 16-year period made possible the examination of the relative emergence of temporal morbidity in relation to the index diagnosis of ASD.

Many disorders are transient (e.g., infections), however, once diagnosed, ASD persists as a comorbid diagnosis in time with all subsequent diagnoses.

Study Objective

The proposition under study is that many temporal physical and biomedical morbidities, transient or persistent, will arise in significant proportions within the ASD-diagnosed population in comparison to those without ASD. When over-represented (hypermorbidity), the pattern of morbidity associated with specific diagnoses in addition to main classes, may provide a better understanding of the etiology or the sequelae of ASD. Etiology might inform basic research *via* the study of specific infections and /or inflammatory processes as well as early diagnosis. Sequelae may facilitate ASD management and care planning when diseases or disorder appear above a given threshold. Of note is that by definition, representation of associated disease might arise in at least one of four main categories in relation to a target (pivot) diagnosis, ASD in this case: significant and non-representative of the sample, non-significant, or significantly before or after an index "pivot" diagnosis (e.g., ASD). Additionally, yet beyond the scope of the present study are the additional categories of transient *vs.* persistent.

MATERIALS AND METHODS

The sample from which the sub-sample for this study[2] derives and the methods employed in this study have been described in detail (28–31, 34). This study extends previous work in describing the specific disorders arising significantly before or after index ASD, the pivot diagnosis.

For this study, a sample was constructed consisting of a regional cohort under 3 years of age between April 1, 1993

---

[1]A deficit of self-awareness, a condition in which a person (in this case system) seems unaware of its existence (e.g., multimorbidity).

[2]This study was approved under Ethics ID: REB_15-1057.

and December 31, 1994 having presented with any physician-diagnosis. All ICD 9 diagnoses assigned to this set of unique individuals over the next approximately 16 years up to November 2010 were merged by unique individual and truncated at maximum age <18 years. Those having an ASD diagnosis were labeled as a group along with all their associated ICD diagnoses, and individuals with unlinked diagnoses making up the comparison group.

TABLE 1 | The all-age odds ratios comparison for the presence and absence of ASD by major classes of ICD 9 diagnoses ranked from highest to lowest by males.

Odds ratio	Males ($n = 29,181$)	Females ($n = 17,898$)
Sense organs	4.94*	2.36*
Respiratory system	3.68*	2.21^{ns}
Symptoms signs ill-defined conditions	3.33*	1.32^{ns}
Nervous system	2.44*	3.22*
Circulatory system	1.7*	2.2*
Congenital anomalies	1.69*	2.58*
Endocrine	1.58*	2.15*
Digestive system	1.56*	1.92*
Perinatal conditions	1.54*	1.31^{ns}
Musculoskeletal system connective tissue	1.4*	1.25^{ns}
Blood-blood-forming organs	1.36*	1.12^{ns}
Skin subcutaneous tissue	1.34*	1.7*
Neoplasms	1.29*	1.66*
Genitourinary system	1.29*	1.71*
Infectious-parasitic diseases	1.28*	1.44^{ns}
Injury poisoning	1.22^{ns}	1.86*
Complications of pregnancy childbirth	0.86^{ns}	1.56^{ns}

* $p < 0.05$, ns, not significant.

The odds ratios (OR) were calculated for this group in relation to the remaining 17 main classes of ICD diseases and disorders (excluding mental disorders). The standard OR formula takes the form of ad/bc, with each of the cells representing a (neither condition), b (one condition), c (the other condition), and d (both conditions) in the 2X2 cross-tabulation. The results were represented in tables and graphs separately for each sex and stratified by age groups. ORs with lower or upper 95% confidence intervals (LCI, UCI) greater or less than the value one were considered to be statistically significant ($p < 0.05$) with z set at value 1.96. Significance and 95% confidence intervals are noted in each table but not in the graphics. In the graphics, lines for each age strata connect the different main ICD classes for ease of comparison within and between age strata.

For those diagnosed with both ASD and any ICD disorder or disease, a count of the occurrence of each ICD diagnosis arising before and after the pivot ASD diagnosis were calculated within each diagnosis and the proportions arising before and after ASD were compared based on upper and lower 95% confidence intervals defined by the standard formula. Additionally, the average duration in days arising before and after ASD, and the average age in years of each disorder were calculated and tabled. Due to the large number of ICD diagnoses (>900), only those with significant proportions arising before and after ASD were tabled. Where the value zero occurred in a cell, the value one was substituted for the purpose of estimating either ORs or proportions and their 95% CIs. In determining the importance of a particular association both the proportion before or after ASD and the proportion before or after of the total sample need to be taken into account.

Note that in the tables the all-age group OR and cell values were the same as the <3 age group OR cell values due to the method used to construct the sample. For example, the only individuals that could be linked in subsequent ages >3 years of age were the individuals within the cohort that remained in the

FIGURE 1 | The odds ratios comparison of the presence and absence of ASD by major class ICD 9 diagnosis ranked by sex from highest to lowest by males.

FIGURE 2 | Main class ICD diagnoses odds ratios by age for females.
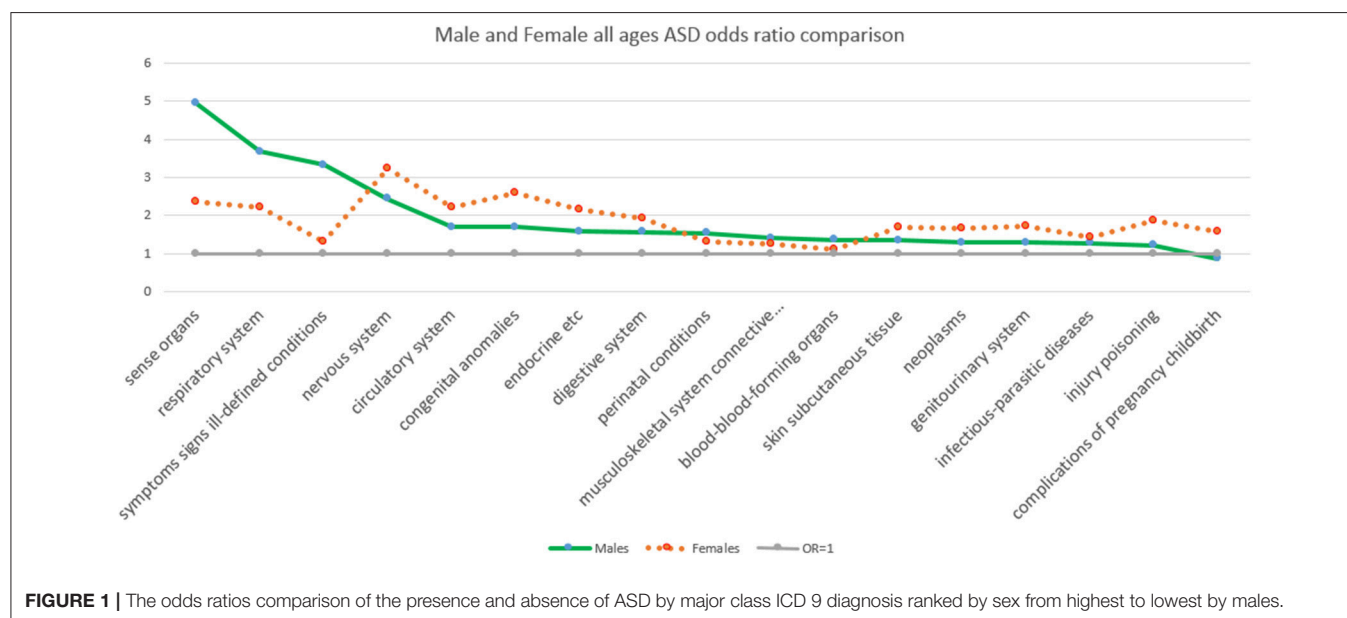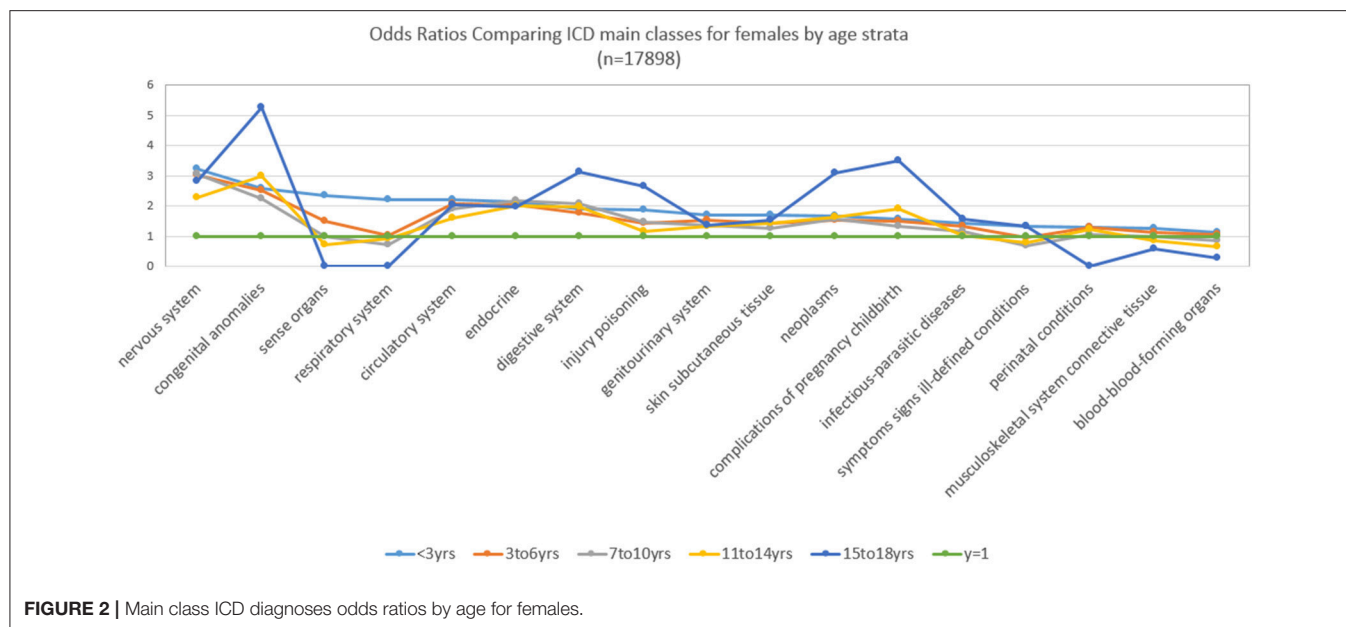
catchment and received a physician-assigned diagnosis at any time over the next 16 years. Variations in the other age groups' ORs and cell values represent actual variations for these main classes of ICD disorders within the age groupings of the cohort over time. While attrition is in this sense may be confounded with absence of disease, the presence of a significant and representative OR association is actual.

Mental disorders were not considered in the present analysis in terms of the calculated ORs, as the different algorithm structure required for analysis was beyond the scope and resources of the present study. Including the temporal morbidity of specific mental disorders other than ASD was possible as output due to the logic structure in the algorithm giving rise to the examination of the specific proportions of ICD diagnoses arising before or after ASD (**Table 4** in Supplementary Material).

RESULTS

There were 17,898 females in the 1993–1994 cohort sample with an average of 61 physician-diagnoses and a range between 1 and 754 diagnoses over the next 16 years for a total of 1,092,752 diagnoses. There were 111 females with ASD diagnosed at some point over the next 16 years. The female mean age of first ASD diagnosis was 7.5 years (std. dev. 3.6) with range from 0 to 16 years.

There were 29,191 males in the sample with an average of 66 physician-diagnoses and a range between 1 and 1,548 diagnoses for a 16-year total of 1,914,955 diagnoses. There were 609 males with ASD diagnosed at some point over the next 16 years. The male mean age of first ASD diagnosis was 7.8 years (std. dev. 3.2) with range from 0 to 17 years.

Of the total 16-year sample (all ages) one in 65 children in the 1993–94 cohort had an ASD diagnosis. The overall all-age OR comparison for the presence or absence of ASD by major classes of ICD diagnoses for males and females (ranked from

highest to lowest by males) are shown in **Table 1**. For males with an ASD over the 16 year period, the ORs were significantly greater than one for all ICD main classes, with the exception of injury poisoning and complications of pregnancy and childbirth. The ORs for females with an ASD over the 16 year period were significantly greater than the value one for ICD main classes: sense organs, nervous system, circulatory system, congenital anomalies, endocrine, digestive system, skin subcutaneous tissue, neoplasms, genitourinary system, and injury/poisoning. The ORs for males with ASD were substantially higher than females for the ICD major classes: sense organs, respiratory system, and symptoms signs ill-defined conditions. The ORs for females with ASD were slightly higher than males for the ICD major classes: nervous system, circulatory system, congenital anomalies, endocrine, digestive system, skin subcutaneous tissue, neoplasms, genitourinary system, and injury/poisoning,

Figure 1 shows for comparison the ASD OR values from **Table 1** by major class ICD 9 diagnosis for males and females (all ages), ranked from highest to lowest by males. For the cohort across all ages, the greatest OR for males given an ASD over 16 year was sensory organs disorders, while for females it was nervous system disorders.

Figure 2 and **Table 2** show for females with an ASD over 16 years, the ORs stratified by age groups, which present a somewhat different picture. For females, the ORs (value > 2) that were significantly and representatively greater at the earliest age for each main ICD class follows: 15–18 years, congenital anomalies; 15–18 years, complications of pregnancy childbirth; < 3 years, nervous system; 15–18 years, neoplasms; 3–6 years, nervous system; <3 years, congenital anomalies; <3 years, sense organs; <3 years, circulatory system; <3 years, endocrine; 7–1 years, 0, digestive system.

Similarly, **Figure 3** and **Table 3** show for males with an ASD over 16 years, the ORs stratified by age groups. For males, the ORs (value >2) that were significantly and representatively greater at

TABLE 2 | Main class ICD diagnoses odds ratios by mean age for females.

Row	Mean age	ICD Main Class	a	b	c	d	Total	OR	LCI	UCI	OR>1	OR<1
1	0–18	Infectious–parasitic diseases	11,022	59	6,765	52	17,898	1.44	0.99	2.09	ns	ns
2	0–18	Neoplasms	15,703	91	2,084	20	17,898	1.66	1.02	2.69	*	ns
3	0–18	Endocrine	15,173	81	2,614	30	17,898	2.15	1.41	3.28	*	ns
4	0–18	Blood-blood-forming organs	16,488	102	1,299	9	17,898	1.12	0.57	2.22	ns	ns
5	0–18	Nervous system	16,382	87	1,405	24	17,898	3.22	2.04	5.07	*	ns
6	0–18	Sense organs	2,112	6	15,675	105	17,898	2.36	1.03	5.37	*	ns
7	0–18	Circulatory system	16,520	95	1,267	16	17,898	2.2	1.29	3.74	*	ns
8	0–18	Respiratory system	1,027	3	16,760	108	17,898	2.21	0.7	6.96	ns	ns
9	0–18	Digestive system	8,718	37	9,069	74	17,898	1.92	1.29	2.86	*	ns
10	0–18	Genitourinary system	10,535	51	7,252	60	17,898	1.71	1.18	2.49	*	ns
11	0–18	Complications of pregnancy childbirth	17,264	106	523	5	17,898	1.56	0.63	3.83	ns	ns
12	0–18	Skin subcutaneous tissue	4,175	17	13,612	94	17,898	1.7	1.01	2.85	*	ns
13	0–18	Musculoskeletal system connective tissue	10,285	58	7,502	53	17,898	1.25	0.86	1.82	ns	ns
14	0–18	Congenital anomalies	15,553	81	2,234	30	17,898	2.58	1.69	3.93	*	ns
15	0–18	Perinatal conditions	12,716	73	5,071	38	17,898	1.31	0.88	1.93	ns	ns
16	0–18	Symptoms signs ill-defined conditions	1,448	7	16,339	104	17,898	1.32	0.61	2.84	ns	ns
17	0–18	Injury poisoning	4,714	18	13,073	93	17,898	1.86	1.12	3.09	*	ns
18	<3	Infectious-parasitic diseases	11,022	59	6,765	52	17,898	1.44	0.99	2.09	ns	ns
19	<3	Neoplasms	15,703	91	2,084	20	17,898	1.66	1.02	2.69	*	ns
20	<3	Endocrine	15,173	81	2,614	30	17,898	2.15	1.41	3.28	*	ns
21	<3	Blood-blood-forming organs	16,488	102	1,299	9	17,898	1.12	0.57	2.22	ns	ns
22	<3	Nervous system	16,382	87	1,405	24	17,898	3.22	2.04	5.07	*	ns
23	<3	Sense organs	2,112	6	15,675	105	17,898	2.36	1.03	5.37	*	ns
24	<3	Circulatory system	16,520	95	1,267	16	17,898	2.2	1.29	3.74	*	ns
25	<3	Respiratory system	1,027	3	16,760	108	17,898	2.21	0.7	6.96	ns	ns
26	<3	Digestive system	8,718	37	9,069	74	17,898	1.92	1.29	2.86	*	ns
27	<3	Genitourinary system	10,535	51	7,252	60	17,898	1.71	1.18	2.49	*	ns
28	<3	Complications of pregnancy childbirth	17,264	106	523	5	17,898	1.56	0.63	3.83	ns	ns
29	<3	Skin subcutaneous tissue	4,175	17	13,612	94	17,898	1.7	1.01	2.85	*	ns
30	<3	Musculoskeletal system connective tissue	10,285	58	7,502	53	17,898	1.25	0.86	1.82	ns	ns
31	<3	Congenital anomalies	15,553	81	2,234	30	17,898	2.58	1.69	3.93	*	ns
32	<3	Perinatal conditions	12,716	73	5,071	38	17,898	1.31	0.88	1.93	ns	ns
33	<3	Symptoms signs ill-defined conditions	1,448	7	16,339	104	17,898	1.32	0.61	2.84	ns	ns
34	<3	Injury poisoning	4,714	18	13,073	93	17,898	1.86	1.12	3.09	*	ns
35	3–6	Infectious-parasitic diseases	9,707	58	6,435	52	16,252	1.35	0.93	1.97	ns	ns
36	3–6	Neoplasms	14,108	90	2,034	20	16,252	1.54	0.95	2.51	ns	ns
37	3–6	Endocrine	13,620	80	2,522	30	16,252	2.03	1.33	3.09	*	ns
38	3–6	Blood-blood-forming organs	14,877	101	1,265	9	16,252	1.05	0.53	2.08	ns	ns
39	3–6	Nervous system	14,784	86	1,358	24	16,252	3.04	1.93	4.79	*	ns
40	3–6	Sense organs	1,284	6	14,858	104	16,252	1.5	0.66	3.42	ns	ns
41	3–6	Circulatory system	14,920	94	1,222	16	16,252	2.08	1.22	3.54	*	ns
42	3–6	Respiratory system	458	3	15,684	107	16,252	1.04	0.33	3.29	ns	ns
43	3–6	Digestive system	7,455	36	8,687	74	16,252	1.76	1.18	2.63	*	ns
44	3–6	Genitourinary system	9,049	50	7,093	60	16,252	1.53	1.05	2.23	*	ns

(Continued)

TABLE 2 | Continued

Row	Mean age	ICD Main Class	a	b	c	d	Total	OR	LCI	UCI	OR>1	OR<1
45	3–6	Complications of pregnancy childbirth	15,650	105	492	5	16,252	1.51	0.61	3.73	ns	ns
46	3–6	Skin subcutaneous tissue	3,166	16	12,976	94	16,252	1.43	0.84	2.44	ns	ns
47	3–6	Musculoskeletal system connective tissue	8,820	57	7,322	53	16,252	1.12	0.77	1.63	ns	ns
48	3–6	Congenital anomalies	14,042	80	2,100	30	16,252	2.51	1.64	3.82	*	ns
49	3–6	Perinatal conditions	11,516	72	4,626	38	16,252	1.31	0.89	1.95	ns	ns
50	3–6	Symptoms signs ill-defined conditions	849	6	15,293	104	16,252	0.96	0.42	2.2	ns	ns
51	3–6	Injury poisoning	3,536	18	12,606	92	16,252	1.43	0.86	2.38	ns	ns
52	7–10	Infectious-parasitic diseases	7,490	50	5,568	44	13,152	1.18	0.79	1.78	ns	ns
53	7–10	Neoplasms	11,234	75	1,824	19	13,152	1.56	0.94	2.59	ns	ns
54	7–10	Endocrine	10,828	65	2,230	29	13,152	2.17	1.4	3.36	*	ns
55	7–10	Blood-blood-forming organs	11,924	87	1,134	7	13,152	0.85	0.39	1.83	ns	ns
56	7–10	Nervous system	11,806	71	1,252	23	13,152	3.05	1.9	4.91	*	ns
57	7–10	Sense organs	697	5	12,361	89	13,152	1	0.41	2.48	ns	ns
58	7–10	Circulatory system	11,956	80	1,102	14	13,152	1.9	1.07	3.36	*	ns
59	7–10	Respiratory system	205	2	12,853	92	13,152	0.73	0.18	3	ns	ns
60	7–10	Digestive system	5,607	25	7,451	69	13,152	2.08	1.31	3.29	*	ns
61	7–10	Genitourinary system	6,700	41	6,358	53	13,152	1.36	0.9	2.05	ns	ns
62	7–10	Complications of pregnancy childbirth	12,633	90	425	4	13,152	1.32	0.48	3.61	ns	ns
63	7–10	Skin subcutaneous tissue	2,022	12	11,036	82	13,152	1.25	0.68	2.3	ns	ns
64	7–10	Musculoskeletal system connective tissue	6,363	46	6,695	48	13,152	0.99	0.66	1.49	ns	ns
65	7–10	Congenital anomalies	11,323	70	1,735	24	13,152	2.24	1.4	3.57	*	ns
66	7–10	Perinatal conditions	9,362	66	3,696	28	13,152	1.07	0.69	1.67	ns	ns
67	7–10	Symptoms signs ill-defined conditions	481	5	12,577	89	13,152	0.68	0.28	1.68	ns	ns
68	7–10	Injury poisoning	2,127	11	10,931	83	13,152	1.47	0.78	2.76	ns	ns
69	11–14	Infectious-parasitic diseases	5,307	42	4,297	35	9,681	1.03	0.66	1.61	ns	ns
70	11–14	Neoplasms	8,098	59	1,506	18	9,681	1.64	0.96	2.79	ns	ns
71	11–14	Endocrine	7,843	53	1,761	24	9,681	2.02	1.24	3.28	*	ns
72	11–14	Blood-blood-forming organs	8,691	72	913	5	9,681	0.66	0.27	1.64	ns	ns
73	11–14	Nervous system	8,544	60	1,060	17	9,681	2.28	1.33	3.93	*	ns
74	11–14	Sense organs	364	4	9,240	73	9,681	0.72	0.26	1.98	ns	ns
75	11–14	Circulatory system	8,710	66	894	11	9,681	1.62	0.85	3.09	ns	ns
76	11–14	Respiratory system	116	1	9,488	76	9,681	0.93	0.13	6.74	ns	ns
77	11–14	Digestive system	3,954	20	5,650	57	9,681	1.99	1.2	3.32	*	ns
78	11–14	Genitourinary system	4,565	31	5,039	46	9,681	1.34	0.85	2.12	ns	ns
79	11–14	Complications of pregnancy childbirth	9,269	72	335	5	9,681	1.92	0.77	4.79	ns	ns
80	11–14	Skin subcutaneous tissue	1,201	7	8,403	70	9,681	1.43	0.66	3.12	ns	ns
81	11–14	Musculoskeletal system connective tissue	4,016	35	5,588	42	9,681	0.86	0.55	1.35	ns	ns
82	11–14	Congenital anomalies	8,349	53	1,255	24	9,681	3.01	1.85	4.9	*	ns
83	11–14	Perinatal conditions	7,011	53	2,593	24	9,681	1.22	0.75	1.99	ns	ns
84	11–14	Symptoms signs ill-defined conditions	294	3	9,310	74	9,681	0.78	0.24	2.49	ns	ns
85	11–14	Injury poisoning	1,153	8	8,451	69	9,681	1.18	0.56	2.45	ns	ns
86	15–18	Infectious-parasitic diseases	2,330	13	1,944	17	4,304	1.57	0.76	3.23	ns	ns
87	15–18	Neoplasms	3,515	18	759	12	4,304	3.09	1.48	6.44	*	ns

(Continued)

TABLE 2 | Continued

Row	Mean age	ICD Main Class	a	b	c	d	Total	OR	LCI	UCI	OR>1	OR<1
88	15–18	Endocrine	3,409	20	865	10	4,304	1.97	0.92	4.23	ns	ns
89	15–18	Blood-blood-forming organs	3,815	29	459	1	4,304	0.29	0.04	2.11	ns	ns
90	15–18	Nervous system	3,712	21	562	9	4,304	2.83	1.29	6.21	*	ns
91	15–18	Circulatory system	3,812	24	462	6	4,304	2.06	0.84	5.07	ns	ns
92	15–18	Digestive system	1,650	5	2,624	25	4,304	3.14	1.2	8.23	*	ns
93	15–18	Genitourinary system	1,728	10	2,546	20	4,304	1.36	0.63	2.91	ns	ns
94	15–18	Complications of pregnancy childbirth	4,095	26	179	4	4,304	3.52	1.22	10.19	*	ns
95	15–18	Skin subcutaneous tissue	426	2	3,848	28	4,304	1.55	0.37	6.53	ns	ns
96	15–18	Musculoskeletal system connective tissue	1,454	14	2,820	16	4,304	0.59	0.29	1.21	ns	ns
97	15–18	Congenital anomalies	3,733	17	541	13	4,304	5.28	2.55	10.92	*	ns
98	15–18	Perinatal conditions	3,358	22	916	8	4,304	1.33	0.59	3	ns	ns
99	15–18	Injury poisoning	356	1	3,918	29	4,304	2.64	0.36	19.4	ns	ns

*$p < 0.05$, ns, not significant.
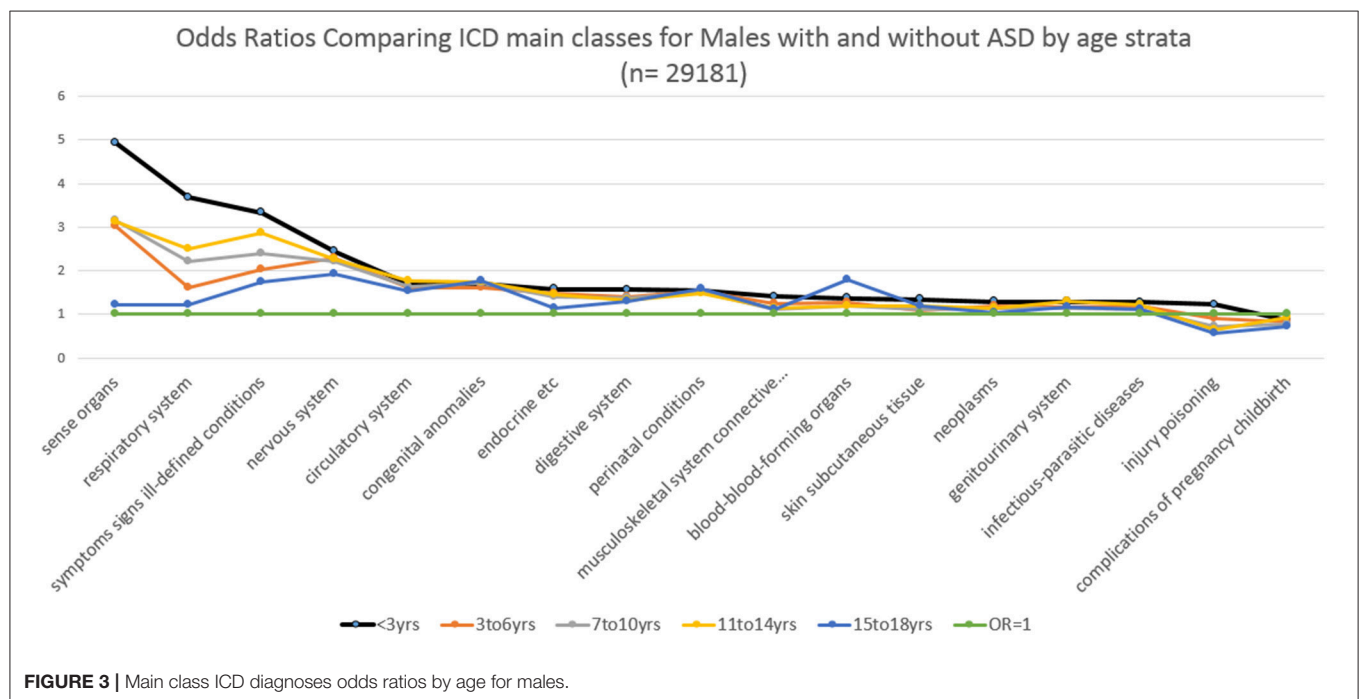
FIGURE 3 | Main class ICD diagnoses odds ratios by age for males.

the earliest age for each main ICD class follow: <3 years, sense organs; <3 years, respiratory system; <3 years, symptoms signs ill-defined conditions; 7–10 years, sense organs; 3–6 years, sense organs; <3 years, nervous system; 7–10 years, symptoms signs ill-defined conditions; 3–6 years, nervous system. Additionally for males, one OR (value < 1) was significantly and representatively less at the earliest age 7–10 years. for injury and poisoning.

Table 4 in Supplementary Material is comprised of 4 sections showing in rows the comparisons of the proportions of distinct physician-assigned ICD diagnoses for males and females arising significantly before and after ASD. The average age in years of individuals within each row is shown together with the average duration in days the ASD arose before or after each ICD

diagnosis. **Table 4** in Supplementary Material is ordered by row number and the ascending numeric sequence of the ICD code for each disorder, first for females in rows 1–28 (28 disorders) where ASD rose significantly before the corresponding ICD disorder, second for females in rows 31–125 (95 disorders) where ASD rose significantly after the corresponding ICD disorder. For males in rows 126–165 (38 disorders), ASD rose significantly before the corresponding ICD disorder and finally, for males in rows 166–401 (234 disorders), where ASD rose significantly after the corresponding ICD disorder.

Examples of disorders with the highest sample proportions diagnosed after and before ASD follow. For females ASD diagnosis preceded the following disorders with the highest

TABLE 3 | Odds ratios by mean age for males.

Row	Mean age	ICD Main Class	a	b	c	d	Total	OR	LCI	UCI	OR>1	OR<1
1	0–18	Infectious-parasitic diseases	19,886	387	8,702	216	29,191	1.28	1.08	1.51	*	ns
2	0–18	Neoplasms	25,411	519	3,177	84	29,191	1.29	1.03	1.63	*	ns
3	0–18	Endocrine	24,425	475	4,163	128	29,191	1.58	1.3	1.93	*	ns
4	0–18	Blood-blood-forming organs	26,219	537	2,369	66	29,191	1.36	1.05	1.76	*	ns
5	0–18	Nervous system	26,526	507	2,062	96	29,191	2.44	1.95	3.04	*	ns
6	0–18	Sense organs	3,005	14	25,583	589	29,191	4.94	2.9	8.41	*	ns
7	0–18	Circulatory system	26,448	530	2,140	73	29,191	1.7	1.33	2.18	*	ns
8	0–18	Respiratory system	1,508	9	27,080	594	29,191	3.68	1.9	7.11	*	ns
9	0–18	Digestive system	13,398	218	15,190	385	29,191	1.56	1.32	1.84	*	ns
10	0–18	Genitourinary system	18,286	349	10,302	254	29,191	1.29	1.1	1.52	*	ns
11	0–18	Complications of pregnancy childbirth	27,874	590	714	13	29,191	0.86	0.49	1.5	ns	ns
12	0–18	Skin subcutaneous tissue	6,874	115	21,714	488	29,191	1.34	1.09	1.65	*	ns
13	0–18	Musculoskeletal system connective tissue	16,670	301	11,918	302	29,191	1.4	1.19	1.65	*	ns
14	0–18	Congenital anomalies	24,002	456	4,586	147	29,191	1.69	1.4	2.04	*	ns
15	0–18	Perinatal conditions	19,411	349	9,177	254	29,191	1.54	1.31	1.81	*	ns
16	0–18	Symptoms signs ill-defined conditions	2,240	15	26,348	588	29,191	3.33	1.99	5.57	*	ns
17	0–18	Injury poisoning	6,051	109	22,537	494	29,191	1.22	0.99	1.5	ns	ns
18	<3	Infectious-parasitic diseases	19,886	387	8,702	216	29,191	1.28	1.08	1.51	*	ns
19	<3	Neoplasms	25,411	519	3,177	84	29,191	1.29	1.03	1.63	*	ns
20	<3	Endocrine	24,425	475	4,163	128	29,191	1.58	1.3	1.93	*	ns
21	<3	Blood-blood-forming organs	26,219	537	2,369	66	29,191	1.36	1.05	1.76	*	ns
22	<3	Nervous system	26,526	507	2,062	96	29,191	2.44	1.95	3.04	*	ns
23	<3	Sense organs	3,005	14	25,583	589	29,191	4.94	2.9	8.41	*	ns
24	<3	Circulatory system	26,448	530	2,140	73	29,191	1.7	1.33	2.18	*	ns
25	<3	Respiratory system	1,508	9	27,080	594	29,191	3.68	1.9	7.11	*	ns
26	<3	Digestive system	13,398	218	15,190	385	29,191	1.56	1.32	1.84	*	ns
27	<3	Genitourinary system	18,286	349	10,302	254	29,191	1.29	1.1	1.52	*	ns
28	<3	Complications of pregnancy childbirth	27,874	590	714	13	29,191	0.86	0.49	1.5	ns	ns
29	<3	Skin subcutaneous tissue	6,874	115	21,714	488	29,191	1.34	1.09	1.65	*	ns
30	<3	Musculoskeletal system connective tissue	16,670	301	11,918	302	29,191	1.4	1.19	1.65	*	ns
31	<3	Congenital anomalies	24,002	456	4,586	147	29,191	1.69	1.4	2.04	*	ns
32	<3	Perinatal conditions	19,411	349	9,177	254	29,191	1.54	1.31	1.81	*	ns
33	<3	Symptoms signs ill-defined conditions	2,240	15	26,348	588	29,191	3.33	1.99	5.57	*	ns
34	<3	Injury poisoning	6,051	109	22,537	494	29,191	1.22	0.99	1.5	ns	ns
35	3–6	Infectious-parasitic diseases	17,775	387	8,283	215	26,660	1.19	1.01	1.41	*	ns
36	3–6	Neoplasms	22,943	518	3,115	84	26,660	1.19	0.95	1.51	ns	ns
37	3–6	Endocrine	22,046	475	4,012	127	26,660	1.47	1.2	1.79	*	ns
38	3–6	Blood-blood-forming organs	23,750	536	2,308	66	26,660	1.27	0.98	1.64	ns	ns
39	3–6	Nervous system	24,065	506	1,993	96	26,660	2.29	1.83	2.86	*	ns
40	3–6	Sense organs	1,756	14	24,302	588	26,660	3.03	1.78	5.17	*	ns
41	3–6	Circulatory system	24,001	529	2,057	73	26,660	1.61	1.26	2.07	*	ns
42	3–6	Respiratory system	625	9	25,433	593	26,660	1.62	0.83	3.14	ns	ns
43	3–6	Digestive system	11,498	217	14,560	385	26,660	1.4	1.18	1.66	*	ns
44	3–6	Genitourinary system	16,078	349	9,980	253	26,660	1.17	0.99	1.38	ns	ns
45	3–6	Complications of pregnancy childbirth	25,377	589	681	13	26,660	0.82	0.47	1.43	ns	ns

(Continued)

TABLE 3 | Continued

Row	Mean age	ICD Main Class	a	b	c	d	Total	OR	LCI	UCI	OR>1	OR<1
46	3–6	Skin subcutaneous tissue	5,284	114	20,774	488	26,660	1.09	0.89	1.34	ns	ns
47	3–6	Musculoskeletal system connective tissue	14,396	300	11,662	302	26,660	1.24	1.06	1.46	*	ns
48	3–6	Congenital anomalies	21,736	455	4,322	147	26,660	1.62	1.35	1.96	*	ns
49	3–6	Perinatal conditions	17,661	349	8,397	253	26,660	1.52	1.29	1.8	*	ns
50	3–6	Symptoms signs ill–defined conditions	1,288	15	24,770	587	26,660	2.03	1.22	3.41	*	ns
51	3–6	Injury poisoning	4,277	108	21,781	494	26,660	0.9	0.73	1.11	ns	ns
52	7–10	Infectious-parasitic diseases	14,263	347	7,244	198	22,052	1.12	0.94	1.34	ns	ns
53	7–10	Neoplasms	18,679	464	2,828	81	22,052	1.15	0.91	1.46	ns	ns
54	7–10	Endocrine	17,908	425	3,599	120	22,052	1.4	1.14	1.73	*	ns
55	7–10	Blood-blood-forming organs	19,470	485	2,037	60	22,052	1.18	0.9	1.55	ns	ns
56	7–10	Nervous system	19,647	451	1,860	94	22,052	2.2	1.75	2.76	*	ns
57	7–10	Sense organs	1,078	9	20,429	536	22,052	3.14	1.62	6.09	*	ns
58	7–10	Circulatory system	19,648	472	1,859	73	22,052	1.63	1.27	2.1	*	ns
59	7–10	Respiratory system	345	4	21,162	541	22,052	2.2	0.82	5.93	ns	ns
60	7–10	Digestive system	8,848	185	12,659	360	22,052	1.36	1.14	1.63	*	ns
61	7–10	Genitourinary system	12,741	305	8,766	240	22,052	1.14	0.96	1.36	ns	ns
62	7–10	Complications of pregnancy childbirth	20,940	534	567	11	22,052	0.76	0.42	1.39	ns	ns
63	7–10	Skin subcutaneous tissue	3,675	86	17,832	459	22,052	1.1	0.87	1.39	ns	ns
64	7–10	Musculoskeletal system connective tissue	10,836	262	10,671	283	22,052	1.1	0.93	1.3	ns	ns
65	7–10	Congenital anomalies	17,900	406	3,607	139	22,052	1.7	1.4	2.07	*	ns
66	7–10	Perinatal conditions	14,580	318	6,927	227	22,052	1.5	1.26	1.79	*	ns
67	7–10	Symptoms signs ill–defined conditions	739	8	20,768	537	22,052	2.39	1.18	4.82	*	ns
68	7–10	Injury poisoning	2,584	87	18,923	458	22,052	0.72	0.57	0.91	ns	*
69	11–14	Infectious-parasitic diseases	9,858	235	5,509	160	15,762	1.22	0.99	1.49	ns	ns
70	11–14	Neoplasms	13,118	330	2,249	65	15,762	1.15	0.88	1.5	ns	ns
71	11–14	Endocrine	12,571	299	2,796	96	15,762	1.44	1.14	1.82	*	ns
72	11–14	Blood-blood-forming organs	13,784	347	1,583	48	15,762	1.2	0.89	1.64	ns	ns
73	11–14	Nervous system	13,834	316	1,533	79	15,762	2.26	1.75	2.9	*	ns
74	11–14	Sense organs	593	5	14,774	390	15,762	3.13	1.29	7.59	*	ns
75	11–14	Circulatory system	13,896	333	1,471	62	15,762	1.76	1.33	2.32	*	ns
76	11–14	Respiratory system	192	2	15,175	393	15,762	2.49	0.62	10.05	ns	ns
77	11–14	Digestive system	6,054	130	9,313	265	15,762	1.33	1.07	1.64	*	ns
78	11–14	Genitourinary system	8,773	200	6,594	195	15,762	1.3	1.06	1.58	*	ns
79	11–14	Complications of pregnancy childbirth	14,952	385	415	10	15,762	0.94	0.5	1.77	ns	ns
80	11–14	Skin subcutaneous tissue	2,221	49	13,146	346	15,762	1.19	0.88	1.61	ns	ns
81	11–14	Musculoskeletal system connective tissue	6,782	161	8,585	234	15,762	1.15	0.94	1.41	ns	ns
82	11–14	Congenital anomalies	12,869	295	2,498	100	15,762	1.75	1.39	2.2	*	ns
83	11–14	Perinatal conditions	10,667	239	4,700	156	15,762	1.48	1.21	1.82	*	ns
84	11–14	Symptoms signs ill-defined conditions	438	4	14,929	391	15,762	2.87	1.07	7.71	*	ns
85	11–14	Injury poisoning	1,245	47	14,122	348	15,762	0.65	0.48	0.89	ns	*
86	15–18	Infectious-parasitic diseases	3,610	93	2,093	60	5,856	1.11	0.8	1.55	ns	ns
87	15–18	Neoplasms	4,654	124	1,049	29	5,856	1.04	0.69	1.56	ns	ns
88	15–18	Endocrine	4,592	120	1,111	33	5,856	1.14	0.77	1.68	ns	ns
89	15–18	Blood-blood-forming organs	5,070	125	633	28	5,856	1.79	1.18	2.73	*	ns

(Continued)

TABLE 3 | Continued

Row	Mean age	ICD Main Class	a	b	c	d	Total	OR	LCI	UCI	OR>1	OR<1
90	15–18	Nervous system	4,990	120	713	33	5,856	1.92	1.3	2.85	*	ns
91	15–18	Sense organs	180	4	5,523	149	5,856	1.21	0.44	3.31	ns	ns
92	15–18	Circulatory system	5,083	129	620	24	5,856	1.53	0.98	2.38	ns	ns
93	15–18	Respiratory system	45	1	5,658	152	5,856	1.21	0.17	8.83	ns	ns
94	15–18	Digestive system	2,116	48	3,587	105	5,856	1.29	0.91	1.82	ns	ns
95	15–18	Genitourinary system	3,127	78	2,576	75	5,856	1.17	0.85	1.61	ns	ns
96	15–18	Complications of pregnancy childbirth	5,547	150	156	3	5,856	0.71	0.22	2.25	ns	ns
97	15–18	Skin subcutaneous tissue	604	14	5,099	139	5,856	1.18	0.67	2.05	ns	ns
98	15–18	Musculoskeletal system connective tissue	1,985	50	3,718	103	5,856	1.1	0.78	1.55	ns	ns
99	15–18	Congenital anomalies	4,747	113	956	40	5,856	1.76	1.22	2.54	*	ns
100	15–18	Perinatal conditions	4,334	102	1,369	51	5,856	1.58	1.12	2.23	*	ns
101	15–18	Symptoms signs ill–defined conditions	129	2	5,574	151	5,856	1.75	0.43	7.13	ns	ns
102	15–18	Injury poisoning	261	12	5,442	141	5,856	0.56	0.31	1.03	ns	ns

*p < 0.05, ns, not significant.

counts ranging from 44 to 7% of the females sample, respectively: psychoses of childhood (299), hyperkinetic syndrome (314), emotional disorder child/adol (313), neurotic disorders (300), conduct disturbance nec (312), depressive disorder nec (311), other viral disease (78), adjustment reaction (309), screen-heart/resp/gu disorder (V81), disorder of menstruation (626), gastritis and duodenitis (535).

For females ASD diagnosis came after the following disorders with the highest counts ranging from 74 to 30% of the females sample, respectively: health supervision child (V20), otitis media, suppur/nos (382), ac up resp inf multiple sites/nos (465), acute nasopharyngitis (460), general symptoms (780), single liveborn (V30), disorders of conjunctiva (372), specific develop delays (315), contact dermatitis (692), ill-defined intest inf (009), nonsuppur otitis media (381), ac bronchitis/bronchiol (466), nutrit/metab/devel symp (783), general medical exam (V70), candidiasis (112), and resp sys/other chest symp (786).

For males ASD diagnosis preceded the following disorders with the highest counts ranging from 47 to 7% of the male sample, respectively: psychoses of childhood (299), hyperkinetic syndrome (314), other viral disease (78), depressive disorder nec (311), other soft tissue disorder (729), screen-heart/resp/gu disorder (V81), and special symptom nec (307).

For males ASD diagnosis came after the following disorders with the highest counts ranging from 79 to 34% of the male sample, respectively: health supervision child (V20), otitis media, suppur/nos (382), ac up resp inf multiple sites/nos (465), acute nasopharyngitis (460), general symptoms (780), ill-defined intest inf (009), disorders of conjunctiva (372), acute bronchitis/bronchiol (466), specific develop delays (315), nonsuppur otitis media (381), acute pharyngitis (462), skin/other integument symp (782), asthma (493), resp sys/other chest symp (786), contact dermatitis (692).

SUMMARY OF RESULTS

The results of the analysis of ASD and the main ICD classes of disease and disorder indicated a significant association that varied with age groups. Given the construction of the sample, the age groupings provided a proxy for the temporal progression of the observed class associations, for which some main class ORs were more pronounced at various ages for one or both sexes. The significant OR associations with main ICD classes were less frequent for females.

The analysis results for specific ICD diseases and disorders provided more precise information about the temporal relationship of each disorder and ASD. For example, concurrently examining the mean age, the number of cases, mean duration in days that the specific diagnosis arose either before or after ASD, together with the significance of their proportions provided additional information related to the temporality of etiology and sequelae of ASD morbidity, especially when the whole sample proportions (e.g., >30%) the before or after ASD counts of each diagnosis were additionally examined for males and females.

DISCUSSION

The estimated prevalence of ASD diagnosis in the cohort sample of treated children was one in 65, and as estimated from the previous study based on those under the age of 19 years (28) was 62 per 10,000 in the entire population, a result similar to other United States and Canadian studies (1, 2) and higher than past studies (1, 35). One difference was that prevalence in the present 1993-94 cohort was prospective in that the value was calculated based on an ASD diagnosis made at any time over the next 16 years. An observation about Canadian data is that there have been few datasets that permit a conclusive estimate of ASD prevalence (36). The average age of ASD diagnosis in the present study was

older than that previously reported for all of Alberta (36). The different study purposes, foci, and designs may have accounted for observed differences in age of diagnosis. The provincial study focused on the prospective Alberta Perinatal Health Program cohort in which the births were actively monitored over the course of the study (36), while the present study focused on the 16-year main ICD class and specific ICD disorder temporal morbidity associated with ASD in a group under 3-years of age between 1993 and 1995.

The emerging field of ASD comorbidity and multimorbidity is evolving and complex. The changing concept of morbidity must necessarily shift health systems and education to configure around multimorbidity from a focus on individual diseases (37). Multimorbidity is the most common chronic condition experienced in significant proportions by older and younger adults: Health system redesign is required to accommodate the emerging body of research (27). Systematic review indicates that the ability to respond clinically to morbidity, comorbidity, and multimorbidity is in its infancy (38).

A number of studies have focused attention on physical disorder comorbidity (39–44) including population studies (45, 46), putative mechanisms underpinning ASD (47–57), and how cross-comorbidity study identifies disease linkage (58), as well as autism-related genes (59) with the potential to identify cause (60).

For basic researchers **Table 4** in Supplementary Material might serve as a reference for comparison to other population studies and inform deeper analysis that might provide more insight into the ASD etiology or sequelae, or both. It is important to take into account when reviewing the proportions of the sample with ASD as the pivot diagnosis, the raw counts also represent a portion of the total female or male sample. For example, in **Table 4** (Supplementary Material), row 31, ASD followed 74% of female cases ($n = 43$ of 51) with ill-defined intestinal infection (009) representing 39% ($n = 43$ of 111) of the female sample with that particular disorder. In **Table 4** (Supplementary Material), row 169, for males ill-defined intestinal infection (009) arose in 85% ($n = 335$ of 393) representing 55% (335 of 609) of the sample. For both males and females, the average age was 7 years and the average duration between the diagnosis ill-defined intestinal infection (009) and ASD was 1,885 and 1,890 days, respectively. The closeness of the age and average duration before ASD for both males and females is interesting, given the preponderance of males that suffer from ASD. The brain-gut axis is an increasing popular focus of research in recent years (60–62). It is possibly mere coincidence, even though many infectious and allergic diseases in the sample had this same profile, that males and females in a population are diagnosed with ASD about the same period on average after suffering from this gastro-intestinal disorder. Similarly, the present study also identifies that sensory and respiratory disorders occur with substantial magnitude before ASD. In respect to development of biome *via* maternal inoculation *via* natural vaginal delivery, study has identified those born under conditions of augmented birth (e.g., cesarean section) are at higher risk of ASD (3). In alignment, the present study indicates that ASD children may have compromised immune systems, possibly associated with the brain-gut axis (61). Comparing the present study findings to other studies focusing on morbidity,

there is similar overlap in the association of inflammatory processes, infection, and the emergence of psychiatric disorders thought to have organic origins, such as schizophrenia (63–65).

How might a clinical temporal, transient, hypercomorbidity profile case stack up, against **Table 4** in Supplementary Material? The present work may contribute in the following ways. In clinical practice repeated visits of any child above a threshold (possibly the before or after counts of disorders in **Table 4** (Supplementary Material)) may signal the need for more detailed investigation, such as thorough assessment of the child's having attained developmental milestones. This seems a sweeping and costly generalization for any practice guide based on a single diagnosis. However, by examining any given child's etiological morbidity profile across multiple diseases and disorders might possibly reveal a more valid constellation of thresholds, the limitations of the present study notwithstanding. In practical terms, the proportions presented in **Table 4** (Supplementary Material) serve as points of reference or signposts. For a clinician treating ASD, interpreting the overall importance of **Table 4** in Supplementary Material is likely most relevant to the clinician's reference case, in other words, the case the clinician is presently treating.

With the exception of specific developmental delays, almost all psychiatric disorders were diagnosed after ASD ranging from 62 to 98% of the sample for females. Similarly for males, psychiatric disorders followed ASD ranging from 56 to 100% of the sample with index psychiatric diagnoses. Before ASD diagnosis was established, pre-ASD psychiatric morbidity was monotonic, that is only specific developmental delay (315) was diagnosed. Any explanation underpinning these results is speculative. For instance, ASD literacy in the mid-1990s may have been low and not favored in psychiatric curricula or graduate continuing medical education. Given the increase in the annual rate of ASD diagnosis between 1993 and 2010 within the catchment area (28), this explanation is plausible. Furthermore, Child and Adolescent Psychiatry only formed in 2010 as a specialization recognized by the Canadian Royal College of Physicians and Surgeons, which could have influenced the dearth of specialized training and sub-specialization in developmental disorders. Hence, it is likely that psychiatric-training-based recognition of ASD was different in the late 1990's and early 2000's (about 7 years being the average age of ASD diagnosis).

LIMITATIONS

The present work has important limitations. One main limitation of the present study is that each major class or individual ICD diagnosis and specific ICD diagnosis proportions before and after ASD was calculated independently of one another. This limitation was addressed in part by stratifying the main classes by age groups and recording the average age and duration before or after ASD, where ASD and the ICD disorder both occurred in the same individual.

Furthermore, it was not possible to examine the linkage of the dataset employed in this study with databases containing prescription data, laboratory results, or to delve further into treatment outcomes *via* case review, or genetic analysis of biological samples. One contemporary database that

links primarily pharmacological prescription data is the United Kingdom's primary care Health Improvement Network (THIN) database (66). A current literature search for the terms "THIN database multimorbidity" produced zero results. A similar search for "THIN database comorbidity" produced 18 results, while "THIN database morbidity" produced 247 results. The majority of these results were focused on a minimal number of associated diseases or disorders and/or prescriptions. Furthermore, evaluation of the THIN database indicated that information loss due to incomplete mapping of medical and drug codes, as well as data structure in the Common Data Model used in THIN limits, its use for all possible epidemiological evaluation studies (66). The database employed in the present study represented the formal physician diagnoses (∼90 Million) for the health service seeking population in the Calgary Health Zone (∼0.75 Million). As noted in previously published limitations respecting this database, over-diagnosis of common disorders, under-diagnosis of rare disorders and misdiagnosis are all likely represented, albeit minimally, in the data (29, 31). Also, the algorithms applied to the data were developed specifically to examine the temporal associations of the diagnosed diseases and disorders. The approaches taken in analysis with this large dataset are relatively novel, as indicated by the dearth of similar studies, such as those emerging from the THIN database. To date the published studies based on the present database have provided largely broad stroke findings, which, while to some extent useful, are signposts that point to the need for further research and development. The present results extend these findings with a description the temporal order of transient hypermorbidity, not only by the proxy of age for the main ICD classes, but also in relation to the full range of specific ICD diagnoses. The provision of age and duration before and after the pivot ASD add value, possibly bringing information into a framework useful to both research and clinical practice.

The results of population-based analysis of multimorbidity are complicated and not necessarily straight forward or intuitive. The results point to some potential possibilities in terms of mechanism, yet there is no possibility that studies focusing only on associative morbidity are conclusive. As with the studies that illustrate a potential confounding of treatment with outcome diagnoses such as ulcerative colitis or cancer (29, 31), confounding exists between epigenetics, individual differences, diathesis, and, importantly, long-term unstudied adverse effects of treatment. While less likely to be the case with diseases, such as ulcerative colitis, the results of this population-based study, where ASD diagnosis alone is the key variable, must be considered as speculative.

CONCLUSIONS

The present work extends the understanding of temporal, transient hyper-multimorbidity associated with ASD. It confirms the ASD association with gastrointestinal problems and immunological disorders, sensory, and neurological disorders, which have been identified in more constrained studies of comorbidity.

The recently published papers from this population dataset have been informative in terms of signaling the

importance of considering mental disorders in relation to physical or biomedical disorders (29–31, 34) and, in the cases of ulcerative colitis and cancer, identifying potential mechanism underlining individual vulnerability (diathesis) or the confounding of the physical or biomedical disease with treatment, or both (29, 31). For example, in both cancers and ulcerative colitis, neuroleptics may disrupt immunological cell-cell communication and represent long-term adverse effects associated with mental disease treatment. This hypothesis is speculative, providing only a direction for detailed basic research.

In conclusion, the present study has the advantage of examining multiform morbidity in a population. The types of analyses including ORs by all ages, stratified by age groups and the proportions before and after any pivot ASD resulted in diagnostic profiles representing novel information potentially informing both basic research, clinical education and practice. The relationship between ASD and the main classes of ICD disorder stratified by age gives a very general indication of the importance of broad diagnostic groupings at different ages and illustrates the need to carefully consider the multiple temporal associations of ASD and specific ICD disorders. The greatest amount of information possibly useful for clinicians is in the proportions of disease before and after ASD, particularly when considered by frequency, age and duration.

NEXT STEPS

It is apparent in considering the study of temporal morbidity in population-based data sets, going forward may require a standardized approach in order for study results to be comparable. Further, noting that the ORs and the specific ICD proportions were independent in the calculation of each main ICD class, hence the resulting ORs (**Tables 1–3**) and proportions (**Table 4** in Supplementary Material) were related only by age and duration before or after ASD. The most important next step in the development of analysis algorithms is to advance an approach to the study of multimorbidity that compares a different matrix format representing the temporal order of specific diagnoses in individuals across all specific ICD diagnoses. The highest levels of observed sequences of disorders that arise and lead to a particular disorder or set of disorders, such as ASD, would represent more a precise analysis than that presently presented. A preliminary algorithm that accomplishes this precision is currently undergoing validation testing.

AUTHOR CONTRIBUTIONS

DC conceptualized the study, organized the data, conducted analyses, and wrote the paper.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2018.00635/full#supplementary-material

REFERENCES

1. Bertrand J, Mars A, Boyle C, Bove F, Yeargin-Allsopp M, Decoufle P. Prevalence of autism in a United States population: the Brick Township, New Jersey, investigation. *Pediatrics* (2001) 108:1155–61. doi: 10.1542/peds.108.5.1155

2. Elsabbagh M, Divan G, Koh YJ, Kim YS, Kauchali S, Marcín C, et al. Global prevalence of autism and other pervasive developmental disorders. *Aut Res.* (2012) 5:160–79. doi: 10.1002/aur.239

3. Gregory SG, Anthopolos R, Osgood CE, Grotegut CA, Miranda ML. Association of autism with induced or augmented childbirth in North Carolina Birth Record (1990-1998) and Education Research (1997-2007) databases. *JAMA Pediatr.* (2013) 167:959–66. doi: 10.1001/jamapediatrics.2013.2904

4. Diallo FB, Fombonne É, Kisely S, Rochette L, Vasiliadis HM, Vanasse A, et al. Prevalence and correlates of autism spectrum disorders in quebec: prevalence et correlats des troubles du spectre de lautisme au Quebec. *Can J Psychiatry* (2018) 63:231–9. doi: 10.1177/0706743717737031

5. Mattila ML, Hurtig T, Haapsamo H, Jussila K, Kuusikko-Gauffin S, Kielinen M, et al. Comorbid psychiatric disorders associated with Asperger syndrome/high-functioning autism: a community- and clinic-based study. *J Aut Dev Dis.* (2010) 40:1080–93. doi: 10.1007/s10803-010-0958-2

6. Zachor D, Yang JW, Itzchak EB, Furniss F, Pegg E, Matson JL, et al. Cross-cultural differences in comorbid symptoms of children with autism spectrum disorders: an international examination between Israel, South Korea, the United Kingdom and the United States of America. *Dev Neurorehabil.* (2011)14:215–20. doi: 10.3109/17518423.2011.568468

7. Kohane IS, McMurry A, Weber G, MacFadden D, Rappaport L, Kunkel L, et al. The co-morbidity burden of children and young adults with autism spectrum disorders. *PLoS ONE* (2012) 7:e33224. doi: 10.1371/journal.pone.0033224

8. Strunz S, Dziobek I, Roepke S, [Comorbid psychiatric disorders and differential diagnosis of patients with autism spectrum disorder without intellectual disability]. *Psychother Psychosom med Psychol.* (2014) 64:206–13. doi: 10.1055/s-0033-1358708

9. Larson FV, Lai MC, Wagner AP, MRC AIMS Consortium, Baron-Cohen S, Holland AJ. Testing the "Extreme Female Brain" theory of psychosis in adults with autism spectrum disorder with or without co-morbid psychosis. *PLoS ONE* (2015) 10:e0128102. doi: 10.1371/journal.pone.0128102

10. Peterson BS, Goh S, Dong Z, Dager SR, Corrigan NM, Shaw DW. Brain lactate as a potential biomarker for comorbid anxiety disorder in autism spectrum disorder-reply. *JAMA Psychiatry* (2015) 190–1. doi: 10.1001/jamapsychiatry.2014.2425

11. Chiang, H.-L, Gau, S. S.-F. Comorbid psychiatric conditions as mediators to predict later social adjustment in youths with autism spectrum disorder. *J Child psychol Psychiatry* 57:103–11. doi: 10.1111/jcpp.12450

12. Pan, P.-Y, Yeh, C.-B. The comorbidity of disruptive mood dysregulation disorder in autism spectrum disorder. *Psychiatry Res.* (2016) 108–9. doi: 10.1016/j.psychres.2016.05.001

13. Frye RE, Rossignol DA. Identification and treatment of pathophysiological comorbidities of autism spectrum disorder to achieve optimal outcomes. Clinical medicine insights. *Pediatrics* (2016) 10:43–56. doi: 10.4137/CMPed.S38337

14. Koski S, Gabriels RL, Beresford C. Interventions for paediatric surgery patients with comorbid autism spectrum disorder: a systematic literature review. *Arch Dis Childhood* (2016) 101:1090–4. doi: 10.1136/archdischild-2016-310814

15. Brookman-Frazee L, Stadnick N, Chlebowski C, Baker-Ericzén M, Ganger W. Characterizing psychiatric comorbity in children with autism spectrum disorder receiving publicly funded mental health services. *Autism* (2017) 22:938–52. doi: 10.1177/1362361317712650

16. Sokolova E, Oerlemans AM, Rommelse NN, Groot P, Hartman CA, Glennon JC, et al. A causal and mediation analysis of the comorbidity between attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD). *J Aut Dev Disor.* (2017) 47:1595–604. doi: 10.1007/s10803-017-3083-7

17. Stadnick N, Chlebowski C, Baker-Ericzén M, Dyson M, Garland A, Brookman-Frazee L. Psychiatric comorbidity in autism spectrum disorder: correspondence between mental health clinician report and structured parent interview. *Autism* (2017) 21:841–51. doi: 10.1177/13623613166 54083

18. Espluga-Frigola N, Cardoner N, Pàmias-Massana M, Palao-Vidal DJ. Comorbidity of autism spectrum disorder and bipolar disorder. *Actas espanolas de psiquiatria.* (2017) 79–88.

19. Flor J, Bellando J, Lopez M, Shui A. Developmental functioning and medical Co-morbidity profile of children with complex and essential autism. *Aut Res.* (2017) 10:1344–52. doi: 10.1002/aur.1779

20. Haruvi-Lamdan N, Horesh D, Golan O. PTSD and autism spectrum disorder: co-morbidity, gaps in research, and potential shared mechanisms. *Psychol Trauma* 10:290–9. doi: 10.1037/tra0000298

21. Bozzi Y, Provenzano G, Casarosa S. Neurobiological bases of autism-epilepsy comorbidity: a focus on excitation/inhibition imbalance. *Eur J Neurosci.* (2018) 47:534–48. doi: 10.1111/ejn.13595

22. Kantzer AK, Fernell E, Westerlund J, Hagberg B, Gillberg C, Miniscalco C. Young children who screen positive for autism: Stability, change and "comorbidity" over two years. *Res Dev Disabil.* 72:297–307. doi: 10.1016/j.ridd.2016.10.004

23. van den Akker M, Buntinx F, Knottnerus JA. Comorbidity or multimorbidity, *Eur J General Pract.* (1996) 2:65–70. doi: 10.3109/13814789609162146

24. Angold A, Costello EJ, Erkanli A. Comorbidity. *J Child Psychol Psychiatry* (1999) 40:57–87.

25. Jakovljevic M, Ostojic L. Comorbidity and multimorbidity in medicine today: challenges and opportunities for bringing separated branches of medicine closer to each other. *Psychiatria Danubina* (2013) 25(Suppl. 1):18–28.

26. Bonavita V, De Simone R. Towards a definition of comorbidity in the light of clinical complexity. *Neurol Sci.* (2008) 29(Suppl. 1):S99–102. doi: 10.1007/s10072-008-0898-1

27. Tinetti ME, Fried TR, Boyd CM. Designing health care for the most common chronic condition – Multimorbidity. *JAMA* (2012) 307:2493–4. doi: 10.1001/jama.2012.5265

28. Cawthorpe D. Comprehensive description of comorbidity for autism spectrum disorder in a general population. *Permanente J* (2017) 21:16-088. doi: 10.7812/TPP/16-088

29. Cawthorpe D, Davidson M. Temporal comorbidity of mental disorder and ulcerative colitis. *Permanente J.* (2015) 19:52–57. doi: 10.7812/TPP/14-120

30. Chartier G, Cawthorpe D. From "Big 4" to "Big 5": a review and epidemiological study on the relationship between psychiatric disorders and World Health Organization preventable diseases. *Curr Opin Psychiatry* (2016) 29:316–21. doi: 10.1097/YCO.0000000000000270

31. Cawthorpe D, Kerba M, Narendran A, Ghuttora H, Chartier G, Sartorius N. Temporal order of cancers and mental disorders in an adult population. *BJPsych open* (2018b) 4:95–105. doi: 10.1192/bjo.2018.5

32. Cawthorpe D, Wilkes TC, Guyn L, Li B, Lu M. Association of mental health with health care use and cost: a population study. *Can J Psychiatry* (2011) 56:490–4. doi: 10.1177/070674371105 600807

33. Wilkes TC, Guyn L, Li B, Lu M, Cawthorpe D. Association of child and adolescent psychiatric disorders with somatic or biomedical diagnoses: do population-based utilization study results support the adverse childhood experiences study? *Permanente J* (2012) 16:23–6.

34. Cawthorpe D. A novel population-based health index for mental disorder. *Permanente J.* (2013) 17:50–4. doi: 10.7812/TPP/12-081

35. Yeargin-Allsopp M, Rice C, Karapurkar T, Doernberg N, Boyle C, Murphy C. Prevalence of autism in a US metropolitan area. *JAMA* (2003) 289:49–55.

36. Burstyn I, Sithole F, Zwaigenbaum L. Autism spectrum disorders, maternal characteristics and obstetric complications among singletons born in Alberta, Canada. *Chronic Dis Can.* 30:125–34. doi: 10.1186/1471-2288-11-2

37. Barnett K, Mercer SW, Norbury M, Watt G, Wyke S, Guthrie B. Epidemiology of multimorbidity and implications for health care, research, and medical education: a cross-sectional study. *Lancet* (2012) 380:37–43. doi: 10.1016/S0140-6736(12)60240-2

38. Smith SM, Wallace E, O'Dowd T, Fortin M. Interventions for improving outcomes in patients with multimorbidity in primary care and community settings. *Cochrane Database Syst Rev.* (2012) 4:CD006560. doi: 10.1002/14651858.CD006560.pub2

39. Bauman ML. Medical comorbidities in autism: challenges to diagnosis and treatment. *Neurotherapeutics* (2010) 7:320–7. doi: 10.1016/j.nurt.2010.06.001

40. Ahmedani BK, Hock RM. Health care access and treatment for children with co-morbid autism and psychiatric conditions. *Soc Psychiatry Psychiatric Epidemiol.* (2012) 47:1807–14. doi: 10.1007/s00127-012-0482-0

41. Inga Jácome MC, Morales Chacòn LM, Vera Cuesta H, Maragoto Rizo C, Whilby Santiesteban M, and Ramos Hernandez L, et al. Peripheral inflammatory markers contributing to comorbidities in autism. *Behav Sci.* (2016) 6:29. doi: 10.3390/bs6040029

42. Somekh J, Peleg M, Eran A, Koren I, Feiglin A, Demishtein A, et al. A model-driven methodology for exploring complex disease comorbidities applied to autism spectrum disorder and inflammatory bowel disease. *J Biomed Inform.* (2016) 63:366–78. doi: 10.1016/j.jbi.2016.08.008

43. Muskens JB, Velders FP, Staal WG. Medical comorbidities in children and adolescents with autism spectrum disorders and attention deficit hyperactivity disorders: a systematic review. *Eur Child Adolesc Psychiatry* (2017) 26:1093–103. doi: 10.1007/s00787-017-1020-0

44. Vohra R, Madhavan S, Sambamoorthi U. Comorbidity prevalence, healthcare utilization, and expenditures of Medicaid enrolled adults with autism spectrum disorders. *Autism*(2017) 21:995–1009. doi: 10.1177/1362361316665222

45. Garg S, Lehtonen A, Huson SM, Emsley R, Trump D, Evans DG, et al. Autism and other psychiatric comorbidity in neurofibromatosis type 1: evidence from a population-based study. *Dev Med Child Neurol.* (2013) 55:139–145. doi: 10.1111/dmcn.12043

46. Doshi-Velez F, Ge Y, Kohane I. Comorbidity clusters in autism spectrum disorders: an electronic health record time-series analysis. *Pediatrics* (2014) 133:e54–63. doi: 10.1542/peds.2013-0819

47. Hogart A, Wu D, LaSalle JM, Schanen NC. The comorbidity of autism with the genomic disorders of chromosome 15q11.2-q13. *Neurobiol Dis* (2010) 38:181–91. doi: 10.1016/j.nbd.2008.08.011

48. Noterdaeme MA, Hutzelmeyer-Nickels A. [Comorbidity in autism spectrum disorders - II. Genetic syndromes and neurological problems]. *Zeitschrift fur Kinder- und Jugendpsychiatrie und Psychotherapie* (2010) 38:267–72. doi: 10.1024/1422-4917/a000046

49. Bejerot S, Humble MB, Gardner A. Endocrine disruptors, the increase of autism spectrum disorder and its comorbidity with gender identity disorder–a hypothetical association. *Int J Androl.* 34:e350. doi: 10.1111/j.1365-2605.2011.01149.x

50. Ragunath P, Chitra R, Mohammad S, Abhinand P. A systems biological study on the comorbidity of autism spectrum disorders and bipolar disorder. *Bioinformation* (2011) 7:102–6.

51. Abbeduto L, McDuffie A, Thurman AJ. The fragile X syndrome-autism comorbidity: what do we really know? *Front Genet.* (2014) 5:355. doi: 10.3389/fgene.2014.00355

52. Hollocks MJ, Howlin P, Papadopoulos AS, Khondoker M, Simonoff E. Differences in HPA-axis and heart rate responsiveness to psychosocial stress in children with autism spectrum disorders with and without co-morbid anxiety. *Psychoneuroendocrinology* (2014) 46:32–45. doi: 10.1016/j.psyneuen.2014.04.004

53. Polyak A, Kubina RM, Girirajan S. Comorbidity of intellectual disability confounds ascertainment of autism: implications for genetic diagnosis. *Am J Med Genet B Neuropsychiatr Genet.* (2015) 168:600–608. doi: 10.1002/ajmg.b.32338

54. David MM, Enard D, Ozturk A, Daniels J, Jung JY, Diaz-Beltran L, et al. Comorbid analysis of genes associated with autism spectrum disorders reveals differential evolutionary constraints. *PLoS ONE* (2016) 11:e0157937. doi: 10.1371/journal.pone.0157937

55. Markkanen E, Meyer U, Dianov GL. DNA damage and repair in schizophrenia and autism: implications for cancer comorbidity and beyond. *Int J Mol Sci.* (2016) 17:E856. doi: 10.3390/ijms17060856

56. Jyonouchi H, Geng L, Streck DL, Dermody JJ, Toruner GA. MicroRNA expression changes in association with changes in interleukin-1ss/interleukin10 ratios produced by monocytes in autism spectrum disorders: their association with neuropsychiatric symptoms and comorbid conditions (observational study). *J Neuroinflamm.* (2017) 14:229. doi: 10.1186/s12974-017-1003-6

57. Larson FV, Arrand JR, Tantam D, Jones PB, Holland AJ. Copy number variants in people with autism spectrum disorders and co-morbid psychosis. *Eur J Med Genet.* (2018) 61:230–4. doi: 10.1016/j.ejmg.2017.12.005

58. Rose DR, Yang H, Serena G, Sturgeon C, Ma B, Careaga M, et al. Differential immune responses and microbiota profiles in children with autism spectrum disorders and co-morbid gastrointestinal symptoms. *Brain Behav Immun* (2018) 70:354–68. doi: 10.1016/j.bbi.2018.03.025

59. Diaz-Beltran L, Esteban FJ, Varma M, Ortuzk A, David M, Wall DP. Cross-disorder comparative analysis of comorbid conditions reveals novel autism candidate genes. *BMC Genom.* (2017) 18:315. doi: 10.1186/s12864-017-3667-9

60. Mayer EA, Padua D, Tillisch K. Altered brain-gut axis in autism: comorbidity or causative mechanisms? *BioEssays* (2014) 36:933–9. doi: 10.1002/bies.201400075

61. Vela G, Stark P, Socha M, Sauer AK, Grabrucker AM. Zinc in gut-brain interaction in autism and neurological disorders. *Neural Plasticity* (2015) 2015:972791. doi: 10.1155/2015/972791

62. Mangiola F, Ianiro G, Franceschi F, Fagiuoli S, Gasbarrini G, Gasbarrini A. Gut microbiota in autism and mood disorders. *World J Gastroenterol.* (2016) 22:361–8. doi: 10.3748/wjg.v22.i1.361

63. Stevens JR, Langloss JM, Albrecht P, Yolken R, Wang YN. A search for cytomegalovirus and herpes viral antigen in brains of schizophrenic patients. *Arch Gener Psychiatry* (1984) 41:795–801.

64. Leucht S, Burkard T, Henderson J, Maj M, Sartorius N. Physical illness and schizophrenia: a review of the literature. *Acta psychiatrica Scand.* (2007) 116:317–33. doi: 10.1111/j.1600-0447.2007.01095.x

65. Prusty BK, Gulve N, Govind S, Krueger GRF, Feichtinger J, Larcombe L, et al. Active HHV-6 infection of cerebellar purkinje cells in mood disorders, *Front Microbiol.* (2018) 9:1955. doi: 10.3389/fmicb.2018.01955

66. Zhou X, Murugesan S, Bhullar H, Liu Q, Cai B, Wentworth C, et al. An evaluation of the THIN database in the OMOP common data model for active drug safety surveillance. *Drug Safety* (2013) 36:119–34. doi: 10.1007/s40264-012-0009-3

frontiers
in Psychiatry

Autism, Joint Hypermobility-Related Disorders and Pain

Carolina Baeza-Velasco [1,2]*, David Cohen [3,4], Claude Hamonet [5], Elodie Vlamynck [6], Lautaro Diaz [3], Cora Cravero [3], Emilie Cappe [1] and Vincent Guinchat [3]

[1] Laboratory of Psychopathology and Health Processes, University Paris Descartes-Sorbonne Paris Cité, Boulogne-Billancourt, France, [2] INSERM U1061, Neuropsychiatry: Epidemiological and Clinical Research, Department of Emergency Psychiatry and Acute Care, CHU Montpellier, Montpellier, France, [3] Department of Child and Adolescent Psychiatry, Reference Center for Rare Psychiatric Diseases, APHP, Groupe Hospitalier Pitié-Salpêtrière, Université Sorbonne, Paris, France, [4] Institut des Systèmes Intelligents et Robotiques, CNRS UMR 7222, Université Sorbonne, Paris, France, [5] University Paris-Est Créteil, Créteil, France, [6] UMR-S 1075 INSERM/UNICAEN, Caen, France

Autism Spectrum Disorder (ASD) and Joint Hypermobility-Related Disorders are blanket terms for two etiologically and clinically heterogeneous groups of pathologies that usually appears in childhood. These conditions are seen by different medical fields, such as psychiatry in the case of ASD, and musculoskeletal disciplines and genetics in the case of hypermobility-related disorders. Thus, a link between them is rarely established in clinical setting, despite a scarce but growing body of research suggesting that both conditions co-occur more often than expected by chance. Hypermobility is a frequent sign of hereditary disorders of connective tissue (e.g., Ehlers-Danlos syndromes, Marfan syndrome), in which the main characteristic is the multisystem fragility that prone to proprioceptive and motor coordination dysfunction and hence to trauma and chronic pain. Considering the high probability that pain remains disregarded and untreated in people with ASD due to communication and methodological difficulties, increasing awareness about the interconnection between ASD and hypermobility-related disorders is relevant, since it may help identify those ASD patients susceptible to chronic pain.

Keywords: autism, joint hypermobility, Ehlers-Danlos syndrome, pain, genetic disorders, comorbidity

INTRODUCTION

Autism Spectrum Disorders (ASD) is a blanket term for an etiologically and clinically heterogeneous group of neurodevelopmental disorders commencing in early childhood. The core characteristics of ASD are impairments in communication, social interaction, and restricted repetitive and stereotyped behaviors (1). The prevalence of ASD is estimated to be around 1% (2). Most of the cases are "idiopathic" (i.e., unknown cause), and approximately 10% of cases are considered as "secondary autism" since these coincide with a genetic syndrome with identified etiology (3).

The burden of these long-lasting and disabling conditions is enhanced by an important degree of comorbidity, which is higher than observed in general pediatric population (4). In autistic adults, it has been reported that only 16% present good physical health (5). Unfortunately, somatic comorbidities in ASD have not been well-addressed in research settings (6). There are, however, more and more genetic syndromes that are identified as associated with autism (7).

Concerning painful conditions specifically, these are highly prevalent in the general population but remain under-diagnosed and under-researched in ASD (8). This is due to communication and

methodological difficulties, but also to the late awareness among the medical community of the ability of autistic people to feel and express pain (8, 9). Indeed, this was questioned for decades. Nevertheless, nowadays it is well-accepted that individuals with autism do experience and express pain but in an atypical way (e.g., altered sensory thresholds, hypo- and hyper-responsiveness including behavioral problems) (2, 9, 10).

Although the magnitude of the co-occurrence between chronic pain and neurodevelopmental disorders such as ASD remains unknown (11), some data suggest that chronic pain is frequent among the ASD population. Bursch et al. (12) reported that more than 20% of pediatric patients in a pain clinic in US presented with ASD traits. In addition, potential pathological sources of pain such as neurologic disorders (seizures and epilepsy) and gastrointestinal problems are known to be frequent in ASD (13, 14). In addition, people with ASD are particularly exposed to pain due to aberrant behaviors such as self-injuries, aggressions, and agitation (13). Conversely, these disruptive behaviors as well as acute behavioral crisis can be manifestations of an underlying pain-associated pathology (15).

Thus, as Clarke [(2), p. 1] stated, "failure to recognize ASD as a common cause of pain can lead to late diagnosis, inappropriate treatment, distress, and further disability." In this sense, it is necessary to disseminate knowledge concerning somatic pain conditions associated with ASD. This will help overcome the challenge of recognizing pain-related suffering which could worsen ASD symptoms and the general state of those affected.

Joint hypermobility (JH) refers to an exaggerated increase in the range of a given joint's mobility. This somatic trait is more frequent in infancy, decreases with age, and is more common in women than in men (16). When hypermobility is polyarticular (five joints or more), it is thought to be a congenital and hereditary trait caused by an alteration of collagen synthesis (17). Its prevalence has been estimated between 10–30% in males and 20–40% in females (16).

To have JH implies increased flexibility but also a propensity for trauma and pain since the tissues are more fragile. According to Grahame [(18) p. 485] "Even a single hypermobile joint may suffer any or all of the consequences of laxity, including a tendency to dislocate, develop traumatic synovitis or premature osteoarthritis, or it may just hurt for no visibly obvious reason." Thus, far from being trivial, the presence of JH should draw attention and lead to a deeper exploration in order to track associated problems such as ligament and tendon problems, joint dislocation/subluxation, chronic arthralgia/myalgia, fatigue, abnormal stature, autonomic, cardiovascular, ocular, neuromuscular, visceral, auditory, and dental pathologies, etc. (19). These should be considered suggestive of an underlying pathology such as a Heritable Disorders of Connective Tissue (HDCTs). Indeed, in this group of disorders, JH is a prominent feature along with fragility of tissues, abnormal skin texture, dysfunctional vessels, and internal organs (20). The HDCTs includes classically Marfan syndrome, Ehlers-Danlos syndromes (EDS), Osteogeneses Imperfecta, and a large list of other genetic disorders, some of them very rare (21). The affected genes encode various connective tissue matrix proteins (collagen, elastin, tenascin, and fibrillin). As

consequence, the biochemical structure of fibrous proteins is compromised, altering their physical qualities and resulting in hyperlaxity and mechanical defect (21). In this regard, pain may be present in any HDCTs, but is more prevalent in EDS (19).

With the current specialization and fragmentation of care, patients with ASD and hypermobility-related disorders (HRDs) are seen by different medical fields, such as psychiatry in the case of ASD, and musculoskeletal disciplines and genetics in the case of HRDs. Therefore, a link between these conditions is rarely established in clinical setting despite a scarce but growing body of research suggesting that both conditions co-occur more often than expected by chance (22–24).

This work proposes an overview of the link between ASD and HRDs. We expect to raise awareness among health professionals on the interconnection between these clinical entities, in order to better identify those patients with ASD who may be susceptible to chronic pain.

AUTISM, JOINT HYPERMOBILITY (JH) AND HYPERMOBILITY-RELATED DISORDERS (HRDS)

Current clinical descriptions of young children with autism include hypotonia, joint laxity, clumsiness, apraxia, and toe walking as common findings (25). Interestingly, similar features have been also described in people with HRDs (26–28).

To the best of our knowledge, the first systematic study exploring the association between JH (non-syndromic) and autism according to DSM-IV criteria (1) is that of Shetreat-Klein et al. (29). These authors assessed the range of joint mobility at the elbow, wrist, metacarpo-phalangeal joint, and ankle in children with ASD aged 4 years old in average, and in matched healthy children ($n = 38$ in each group). Results showed that the joints of children with autism were significantly more supple than their typically developing peers. In the same vein, the study of Eccles et al. (30) explored JH and autonomic dysfunction in a group of adult patients with neurodevelopmental disorders ($n = 205$), including patients with autism although the exact number of these subjects was not reported. Results showed that the rate of JH and autonomic symptoms were significantly higher among people with neurodevelopmental disorders than in the control group. More recently, Glans et al. (31) explored the potential association between JH and autistic traits in the general population. One thousand thirty-nine Swedish adults responded to the Five-point questionnaire for JH (32), and others instruments assessing neurodevelopmental traits including the abridged version of the 50-item Autism Spectrum Quotient (33). No link was observed between JH and autistic traits in this study, which lead the authors to suggest that this association is limited to clinical populations only.

Most of the data linking ASD and JH, center around genetic syndromes featuring JH and/or HDCTs. For instance, Fragile X syndrome which is caused by an alteration of the FMR1 gene, is the second cause of intellectual disability among males, and the most frequent genetic comorbidity of ASD (30–50%). In this syndrome, an underlying connective tissue anomaly is presumed

since signs such as JH (50%), soft skin, scoliosis, flat feet, and pectus excavatum among others are common in those affected (34).

The Chromosome 2q37 Deletion Syndrome, which is characterized by three major clinical features (developmental delay, intellectual disability, skeletal malformations, and facial dysmorphism) also include in their phenotypical description JH, hypotonia, and dislocations. It has been reported that around 17–50% of patients with this syndrome, also have ASD (35).

Concerning HDCTs, the work by Blair et al. (36) which examined comorbidity among Mendelian and complex diseases by mining the medical records of over 110 million patients, observed significant clinical comorbidities between Marfan syndrome (i.e., HDCTs characterized by marfanoid habitus, aortic aneurysm, and ectopia lentis) and neuropsychiatric conditions such as ASD. These results had not been reported before. Recently, Balasubramanian et al. (37) reported a higher incidence of ASD in people with Osteogeneses Imperfecta (i.e., HDCTs characterized by brittle bones and blue sclerae). Ten out 102 patients in their cohort have ASD while in the general population ASD is estimated of 1 in 100.

Among HDCTs, Ehlers-Danlos syndromes exhibit the greatest clinical overlap with ASD in literature.

EHLERS-DANLOS SYNDROMES (EDS) AND AUTISM

EDS is not a single disease, but a group of clinically and genetically heterogeneous conditions characterized by JH, skin hyperextensibility and tissue fragility (38). Since collagen is widely distributed through the body, the manifestations of EDS are multi-systemic and often pain-associated.

The Revised 2017 International Classification for EDS (38) describe 13 subtypes (**Figure 1**) which range from mild (although debilitating) to life threatening. The prevalence of all EDS is estimated at 1/5,000 (20). The hypermobile subtype (hEDS) is the most common and accounts for 80–90% of EDS cases (39). As in ASD, the genetic background of hEDS has not yet been elucidated, therefore this is the only EDS subtype for which the diagnosis remains clinical. EDS, and especially hEDS are often under-recognized (40). The diagnostic delay was estimated at 14 years for half of EDS patients, and 28 years for a quarter of patients constituting the longest diagnostic delay among 16 rare diseases (41). Recent changes in EDS nosology now indicated that hEDS lies at the end of a spectrum (the so-called Hypermobility Spectrum Disorders; **Figure 1**) which includes intermediate phenotypes presenting JH plus other symptoms but not fulfilling criteria for an EDS (17). Thus, the Joint Hypermobility Syndrome described by rheumatologists (42) is now part of the hypermobility spectrum.

It is worth mentioning that there remains disagreement amongst experts regarding the new EDS nosology is not an easy issue (43, 44). As in ASD, there is ongoing debate concerning the description of hEDS in particular, as to whether it is a genetic disease or a clinical syndrome is still relevant. In this regard,

these new criteria will be revised during 2018 by the International Consortium on EDS.

The first attempt to characterize EDS patients from a psychosocial perspective was the study by Lumley et al. (45). In this study, 44 adults and 7 children with EDS of various subtypes were tested and interviewed. The results in children (aged 7–12-years-old) showed some features compatibles with ASD such as impaired social competence (57 % of the sample), internalizing problems (43%), and aberrant behaviors (29%) although no specific assessment for autism was applied in this study. Moreover, to date eight case reports have been published concerning the potential association between ASD and EDS (22, 46, 47). First, Fehlow and Tennstedt (48) in a German publication presented the case of a 15-year-old boy with an autistic syndrome and EDS type I (current classical type) having JH, skin extensibility, moderate bleeding tendencies and deformity of the thorax. Sieg (49) for his part, described a 13-year-old boy presenting ASD symptoms such as impaired social skills, unusual interests, language delay, mannerisms, lack of awareness of feelings of others, and physical particularities such as JH, Gorlin's sign (i.e., the ability to touch the tip of the nose with the tongue), hyperextensible skin with velvety texture, and a trend to dislocations among others. A diagnosis of EDS type II (current classical type) was confirmed by geneticists. Later, Tantam et al. (50) highlighted the co-occurrence of Asperger's syndrome (now part of ASD) and HDCTs through the presentation of three cases (two girls and one man) with lifelong hyperlaxity and muscular incoordination among others. The authors discarded EDS as a diagnosis due to the absence of skin elasticity in these patients, and concluded a Marfan-like disorder of connective tissue. However, the clinical descriptions are compatible with hEDS (47) in which skin hyperextensibility may be absent. In addition, another case combining high-functioning autistic disorder and EDS was reported by Takei et al. (51). In which a 17-years-old boy presented with highly flexible fingers and toes, JH and skin hyperelasticity. His mother was also diagnosed with EDS, but the subtype in both cases was not mentioned. Similarly, we reported a case of a 12-years-old boy diagnosed with ASD who was referred to rehabilitation medicine due to articular and muscular pain, gait problems and chronic fatigue. The physical exploration revealed JH, history of recurrent sprains and blocks, thin skin with abnormal scarring, easy bruising, cutaneous hyperesthesia, hypotonia, dysautonomia symptoms (excessive sweating, poor thermoregulation, unexplained fever episodes, dry eyes and mouth, dizziness), gastrointestinal problems, severe headaches, and proprioceptive dysfunction (clumsiness, frequent trips and falls, difficulties in gripping and holding objects). The patient's father and brother had similar signs (although milder) and the patient's mother had been diagnosed with fibromyalgia. Finally, a diagnosis of hEDS was obtained for this patient (22). The last case report is that by Cravero et al. (52), who described a 21-years-old man with Cornelia de Lange, Ehlers-Danlos syndrome (classic type), and severe autistic syndrome. Concerning EDS features, he presented JH, pale and hyperextensible skin, abnormal healing with widened anthropic scars, hemorrhagic syndrome, and a family history of EDS.

FIGURE 1 | Categories involving joint hypermobility. Joint hypermobility (JH) is frequent in the general population and is not a problem per se. When JH is accompanied by symptoms, it could underlie a hypermobility-related disorder such as a Heritable Disorder of the Connective Tissue (HDCT). HDCT classically include Marfan syndrome, Osteogenesis imperfecta and Ehlers-Danlos syndromes (EDS). The 2017 EDS classification describes 13 subtypes. It also describes the so-called Hypermobility Spectrum Disorders (HSD), which is a group of clinical conditions with symptomatic JH but not fulfilling criteria of any EDS subtype. HSD is understood as a continuum on which JH ranges from asymptomatic JH through to hypermobile EDS (hEDS) as part of the EDS.

Recently, Lipsker et al. (11) described the case of a 6-years-old girl with severe chronic pain since very early age (headaches, joint and muscle pain), and comorbid ASD and attention deficit/hyperactivity disorder (ADHD). The authors also describe JH and fatigue in this patient, as well as antecedents of chronic pain in her relatives. After consulting several clinics and trying different treatments, improvements in pain and functioning were obtained with methylphenidate medication and parental behavioral training. Although the possibility of a HDCTs was not evoked, considering the combination of JH, chronic pain and neurodevelopmental disorders, a hypermobility-related disorder such as EDS should be hypothesized (23), as was noted by Fernell and Ronge (53) in a letter to the editor about this case report.

The anecdotal evidence provided by the aforementioned clinical descriptions, which correspond mainly to patients with secondary rather than idiopathic ASD, is supported by the study of Cederlöf et al. (54) in a large cohort of EDS and hypermobility syndrome patients ($N = 1,771$). In this work, EDS and hypermobility syndrome subjects were compared to matched controls in relation to antecedents of psychiatric disorders. Results showed that ASD was overrepresented in EDS patients (2.9% vs. 0.4% in controls; RR 7.4, 95% CI 5.2–10.7). Similarly, ASD was diagnosed in 1.6% of patients with hypermobility syndrome compared to 1.4% in controls (1.6%

vs. 1.2%; RR 1.4, 95% CI 1.1–1.6). In addition, more cases of ASD were found in hypermobility syndrome siblings compared to control sibling (ASD in 0.6% vs. 0.5%, respectively; RR 1.3, 95% CI 1.1–1.7).

EXPLAINING THE LINK BETWEEN AUTISM AND HRDS

The etiological mechanisms underlying the comorbidity between ASD and HRDs are poorly understood. According to Tamtam et al. (50), a disorder of the connective tissue may result in central nervous system abnormalities. This was probably the case of an EDS patient with epilepsy reported by Cupo et al. (55), in which postmortem explorations showed structural brain abnormalities that may be related to the connective tissue disorder. Moreover, Eccles et al. (56), reported structural brain differences between subjects with and without JH in areas involved in emotion processing, attention, cognitive control of pain, and negative emotions (bilateral amygdala, anterior cingulate, parietal lobe), as well as a negative correlation between JH and superior temporal volume, which is an area related to processing social and emotional signals. Differences in amygdala and superior temporal cortex anatomy have been also observed in autism (57).

Brain heterotopias (i.e., neuronal migrational abnormalities) have been reported in ASD (58) and in EDS (55, 59, 60) providing another clue in understanding the overlap between both conditions. In addition, studies in human and animal models indicate immunological dysfunction in ASD (61), while recent research highlights the co-ocurrence of mast cell dysregulation in EDS (62, 63) suggesting problems in the immune system. Moreover, endocrine dysregulation has been postulated as a potential risk factor of ASD [e.g., maternal diabetes, polycystic ovary syndrome, etc.; (64)]. Endocrine involvement has also been identified in EDS. Hugo-Rodin et al. (65) reported a high prevalence of gynecological symptoms in women with hEDS, of which a subgroup was sensitive to hormonal fluctuations with an increase in symptoms severity during puberty, prior to menstruation, during the postpartum period, and on oral contraception. Authors suggested that hormones may play a modulatory effect in hEDS. Recently, the study of Casanova et al. (24) observed that women with ASD and JH reported significantly more immune- and endocrine-mediated conditions than those without JH. These results shed new light on the potential comorbidity between ASD and HRDs.

Tamtam et al. (50) also evoked the hypothesis of an indirect link between ASD and HRDs. This raises the question whether connective tissue abnormalities alter motor development and proprioception preventing the optimal acquisition of non-verbal communication skills, which may lead to autistic traits such as impairments in social interactions. The **Figure 2** illustrate this idea showing possible connections between HRDs (specially hEDS and hypermobility spectrum disorders), and neurodevelopmental outcomes including autistics traits (66).

Finally, ASD and HRDs may be pleiotropic manifestations of a common genetic milieu that deserve to be better scrutinized.

AUTISM, HRDS AND PAIN

As has been suggested, a link between ASD and HRDs (specially EDS) theoretically implies a susceptibility to a wide range of pain (mainly musculoskeletal but also headaches and visceral pain). In this sense, there is evidence of higher rates of pain symptoms in EDS patients when there is comorbidity with a psychiatric disorder (69). In addition, mast cell activation syndrome, which has been associated to several painful conditions [e.g., migraine, atopic dermatitis, pelvis and bladder pain, inflammatory bowel pain, fibromyalgia, vulvodynia, self-injurious behaviors associated pain, etc. (70)] is frequent in ASD (71) and EDS (62).

Despite these suggestive data, confirmation by systematic studies about the presence of pain in ASD with HRDs is needed. In this sense, Casanova et al. (24) contributed some of the first data. Through a survey via internet, this group explored adult women on the autism spectrum with and without JH ($n = 85$ vs. $n = 20$, respectively) and estimated the prevalence of immune and endocrine mediated conditions. It was observed that the hypermobile ASD group presented significantly higher
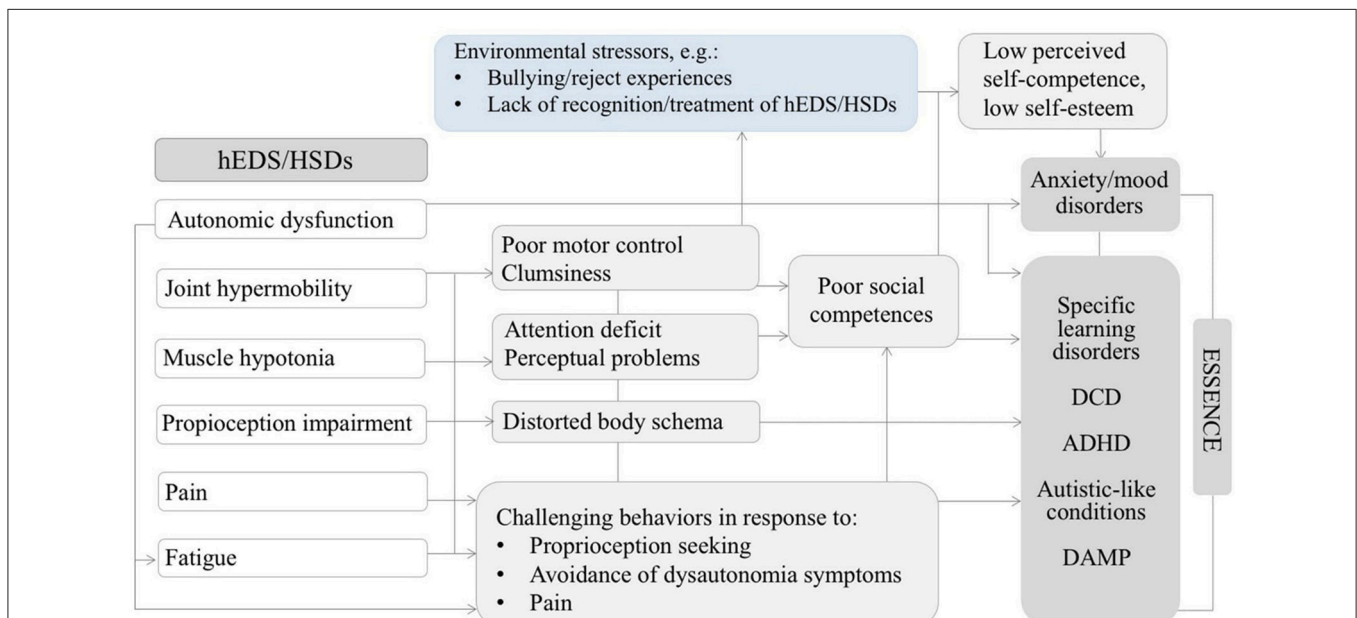
FIGURE 2 | Diagram illustrating possible relationships between some features of hEDS/HSDs might contributing to neurodevelopmental disorders and psychopathology in the developmental age. Adapted by permission from Springer Nature: ADHD Attention Deficit and Hyperactivity Disorders. Attention-deficit/hyperactivity disorder, joint hypermobility-related disorders and pain: expanding body-mind connections to the developmental age, Baeza-Velasco et al. (66) Copyright 2018. hEDS/HSDs: hypermobile Ehlers-Danlos syndrome/hypermobility spectrum disorders. ADHD: attention deficit/hyperactivity disorder. DCD: developmental coordination disorder. DAMP: deficits in attention, motor control and perceptual abilities (67). ESSENCE: early symptomatic syndromes eliciting neurodevelopmental clinical examinations (68).

rates of autoimmune disorders (45% vs. 13%; $p = 0.02$), but also pain-associated endocrine symptomatology such as dysmenorrhea and endometriosis (85% vs. 28%; $p < 0.001$ and 30% vs. 5%; $p = 0.01$ respectively) compared to the non-hypermobile ASD group. In addition, all participants in the hypermobile ASD group suffered arthralgia, and 75% from other types of chronic pain (including fibromyalgia) compared to 29 and 31%, respectively, in the non-hypermobile ASD group ($p < 0.001$). These results concern only females and a subpopulation capable of answering online self-questionnaires, for which authors assume an IQ > 70. In this regard, studies in males and individuals with autism and intellectual disability are needed in order to extend explorations to a more representative ASD population.

CONCLUSION

ASD and HRDs, specially hEDS, are conditions with a strong genetic component, a polymorphic clinical presentation, appearing both in infancy, and sharing several phenotypical features (35). Although existing data does not allow to ascertain increase prevalence of ASD in HRDs, as well as shared underlying patho-mechanisms between both conditions, there is increasing evidence suggesting that these co-occur more often than expected by chance. This requires be confirmed by further investigation which should consider the recent nosological changes both in EDS and the hypermobility spectrum disorders [see (17, 38)], and in ASD (72).

Disseminate knowledge about this potential connection can be highly useful in clinical context since it allows the clinician the awareness of potential pain-related symptoms in a population in which it is extremely challenging to screen for and manage pain. Beyond the methodological barriers to explore pain in people with ASD, and as (69) stated, mental-health related stigma can prevent more depth investigations into an underlying cause of systemic complaints, or to the exacerbation of behavioral problems and/or comorbid psychopathology in the case of ASD patients, delaying the recognition of HRDs. Conversely, patients primarily treated for painful conditions related to hypermobility, should be screened for neurodevelopmental abnormalities. A broad image of each patient, including somatic and psychological aspects, will help to ensuring proper care.

Moreover, once the association between autism, HRDs and pain have been established, it is necessary to consider antalgic strategies targeted to this specific subgroup of patients. Such therapeutic strategies are unexplored so far, hence clinicians are underprepared to manage complex clinical pictures. Thus, future avenues for research that deserves more attention includes the confirmation of comorbidity between ASD and HRDs, the elucidation of its etiology and clinical significance, and the most appropriate management approach for these cases.

AUTHOR CONTRIBUTIONS

ACKNOWLEDGMENTS

REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. Washington, DC: American Psychiatric Association (1994).
2. Clarke C. Autism spectrum disorder and amplified pain. *Case Rep Psychiatry* (2015) 2015:930874. doi: 10.1155/2015/930874
3. Glennon JM, Karmiloff-Smith A, Thomas MSC. Syndromic autism: progressing beyond current levels of description. *Rev J Autism Dev Disord.* (2017) 4:321–7. doi: 10.1007/s40489-017-0116-2
4. Doshi-Velez F, Ge Y, Kohane I. Comorbidity cluster in Autism Spectrum Disorders: an electronic health record time-series analysis. *Pediatrics* (2014) 133:e54–63. doi: 10.1542/peds.2013-0819
5. O'Rourke L. *Psychiatric and Physical Comorbidity in Adults with Autism Spectrum Disorder* [MD. Thesis]. Dublin: Royal College of Surgeons in Ireland (2016).
6. Matson ML, Matson JL, Beighley JS. Comorbidity of physical and motor problems in children with autism. *Res Dev Disabil* (2011) 32:2304–8. doi: 10.1016/j.ridd.2011.07.036
7. Zafeiriou DI, Ververi A, Dafulis V, Kalyva E, Vargiami E. Autism Spectrum Disorders: the quest for genetic syndromes. *Am J Med Genet Part B* (2013) 162B:327–66. doi: 10.1002/ajmg.b.32152
8. Allely CS. Pain sensitivity and observer perception of pain in individuals with autistic spectrum disorders. *ScientificWorldJournal* (2013) 2013: 916178. doi: 10.1155/2013/916178
9. Dubois A, Rattaz C, Pry R, Baghdadli A. Autism and Pain—A literature review. *Pain Res Manage.* (2010) 15:245–53. doi: 10.1155/2010/749275
10. Tordjman S, Anderson GM, Charrier A, Oriol C, Kermarrec S, Canitano R, et al. Relationships between self-injurious behaviors, pain reactivity, and β-Endorphin in children and adolescentes with autism. *J Clin Psychiatry* (2018) 79:16m10889. doi: 10.4088/JCP.16m10889
11. Lipsker CW, von Heijne M, Bölte S, Wicksell RK. a case report and literature review of autism and attention deficit hyperactivity disorder in paediatric chronic pain. *Acta Paediatr.* (2018) 107:753–8. doi: 10.1111/apa.14220
12. Bursch B, Ingman K, Vitti L, Hyman P, Zeltzer LK. Chronic pain in individuals with previously undiagnosed Autistic Spectrum Disorders. *J Pain* (2004) 5:290–5. doi: 10.1016/j.jpain.2004.04.004
13. Dubois A, Michelon C, Rattaz C, Zabalia M, Baghdadli A. Daily living pain assessment in children with autism: exploratory study. *Res Dev Disabil.* (2017) 62:238–46. doi: 10.1016/j.ridd.2017.01.003
14. Muskens JB, Velders FP, Staal WG. Medical comorbidities in children and adolescents with autism spectrum disorders and attention deficit hyperactivity disorders: a systematic review. *Eur Child Adolesc Psychiatry* (2017) 26:1093–103. doi: 10.1007/s00787-017-1020-0
15. Guinchat V, Cravero C, Diaz L, Périsse D, Xavier J, Amiet C, et al. Acute behavioral crises in psychiatric inpatients with autism spectrum disorders (ASD): recognition of concomitant medical or non-ASD psychiatric conditions predicts enhanced improvement. *Res Dev Disabil.* (2015) 38:242–55. doi: 10.1016/j.ridd.2014.12.020
16. Hakim A, Grahame R. Joint hypermobility. *Best Pract Res Clin Rheumatol.* (2003) 17:989–1004. doi: 10.1016/j.berh.2003.08.001

17. Castori M, Tinkle B, Levy H, Rahame R, Malfait F, Hakim A. A framework for the classification of joint hypermobility and related conditions. *Am J Med Genet.* (2017) 175C:148–57. doi: 10.1002/ajmg.c.31539

18. Grahame R. Time to take hypermobility seriously (in adults and children). *Rheumatology* (2001) 40:485–7. doi: 10.1093/rheumatology/40.5.485

19. Hakim A, Malfait F, De Paepe A, Sahota A. The heritable disorders of connective tissue: epidemiology, nosology and clinical features. In: Hakim A, Keer R, Grahame R, editors. *Hypermobility, Fibromyalgia and Chronic Pain.* London: Churchill Livingstone Elsevier (2010) 3–17. doi: 10.1016/B978-0-7020-3005-5.00001-X

20. Callewaert B, Malfait F, Loeys B, De Paepe A. Ehlers-Danlos syndromes and Marfan syndrome. *Best Prac Res Clin Rheumatol.* (2008) 22:165–89. doi: 10.1016/j.berh.2007.12.005

21. Grahame, R. Hypermobility and the heritable disorders of the connective tissue. In: Keer R, Grahame R. editors. *Hypermobility Syndrome. Recognition and Management for Physiotherapist.* London: Butterworth Heinemann. (2003). doi: 10.1016/B978-0-7506-5390-9. 50006-X

22. Baeza-Velasco C, Pailhez G, Bulbena A, Baghdadli A. Joint hypermobility and the heritable disorders of connective tissue: clinical and empirical evidence of links with psychiatry. *Gen Hospital Psychiatry* (2015) 37:24–30. doi: 10.1016/j.genhosppsych.2014.10.002

23. Baeza-Velasco C, Grahame R, Bravo JF. A connective tissue disorder may underlie ESSENCE problems in childhood. *Res Dev Disabil.* (2017) 60:232–42. doi: 10.1016/j.ridd.2016.10.011

24. Casanova EL, Sharp JL, Edelson SM, Kelly DP, Casanova, MF. A cohort study comparing women with Autism Spectrum Disorder with and without generalized joint hypermobility. *Behav Sci.* (2018) 8:E35. doi: 10.3390/bs8030035

25. Rapin I. Autism. *N Engl J Med.* (1997) 337:97–104. doi: 10.1056/NEJM199707103370206

26. Colombi M, Dordoni C, Chiarelli N, Ritelli M. Differential diagnosis and diagnostic flow chart of joint hypermobility syndrome/ehlers–danlos syndrome hypermobility type compared to other heritable connective tissue disorders. *Am J Med Genet C* (2015) 169C:6–22. doi: 10.1002/ajmg.c. 31429

27. Castori M. Ehlers-Danlos Syndrome, hypermobility type: an underdiagnosed hereditary connective tissue disorder with mucocutaneous, articular, and systemic manifestations. *Dermatology* (2012) 2012:751768. doi: 10.5402/2012/751768

28. Ghibellini G, Brancati F, Castori M. Neurodevelopmental attributes of joint hypermobility syndrome/Ehlers–Danlos syndrome, hypermobility type: update and perspectives. *Am J Med Genet C* (2015) 169C:107–16. doi: 10.1002/ajmg.c.31424

29. Shetreat-Klein M, Shinnar S, Rapin I. Abnormalities of joint mobility and gait in children with autism spectrum disorders. *Brain Dev.* (2014) 36:91–6. doi: 10.1016/j.braindev.2012.02.005

30. Eccles JA, Lodice V, Dowell NG, Owens A, Hughes L, Skipper S, et al. Joint hypermobility and autonomic hyperactivity: relevance to neurodevelopmental disorders. *J Neurol Neurosurg Psychiatry* (2014) 85:e3. doi: 10.1136/jnnp-2014-308883.9

31. Glans M, Bejerot S, Humble MB. Generalised joint hypermobility and neurodevelopmental traits in a non-clinical adult population. *BJPsy Open* (2017) 3:236–42. doi: 10.1192/bjpo.bp.116.004325

32. Hakim A, Grahame R. A simple questionnaire to detect hypermobility: an adjunct to the assessment of patients with diffuse musculoskeletal pain. *Int J Clin Pract.* (2003b) 57:163–6.

33. Allison C, Auyeung B, Baron-Cohen S. Toward brief "red flags" for autism screening: the short Autism Spectrum Quotient and the short Quantitative Checklist in 1,000 cases and 3,000 controls. *J Am Acad Child Adolesc Psychiatry* (2012) 51:202–12.e7. doi: 10.1016/j.jaac.2011.11.003

34. Ciaccio C, Fontana L, Milani D, Tabano S, Miozzo M, Esposito S. Fragile X syndrome: a review of clinical and molecular diagnoses. *Ital J Pediatr.* (2017) 43:39. doi: 10.1186/s13052-017-0355-y

35. Baeza-Velasco C, Hamonet C, Baghdadli A, Brissot R. Autism Spectrum Disorders and Ehlers-Danlos Syndrome hypermobility-type: similarities in clinical presentation. *Cuadernos Med Psicosomática y Psiquiatría Enlace* (2016) 118:49–58.

36. Blair DR, Lyttle CS, Mortensen JM, Bearden CF, Jensen AB, Khiabanian H, et al. A non-degenerate code of deleterious variants in mendelian loci contributes to complex disease risk. *Cell* (2013) 155:70–80. doi: 10.1016/j.cell.2013.08.030

37. Balasubramanian M, Jones R, Milne E, Marshall CH, Arundel P, Smith K, et al. Autism and heritable bone fragility: a true association? *Bone Rep.* (2018) 8:156–62. doi: 10.1016/j.bonr.2018.04.002

38. Malfait F, Francomano C, Byers P, Belmont J, Berglund B, Black J, et al The 2017 international classification on the Ehlers–Danlos syndromes. *Am J Med Genet.* (2017) 175C:148–57 doi: 10.1002/ajmg.c.31552

39. Tinkle B, Castori M, Berglund B, Cohen H, Grahame R, Kazkaz H, et al. Hypermobile Ehlers–Danlos Syndrome (a.k.a. Ehlers–Danlos syndrome type III and Ehlers–Danlos syndrome hypermobility type): clinical description and natural history. *Am J Med Genet.* (2017) 175C:48–69. doi: 10.1002/ajmg.c.31538

40. Hamonet C, Ducret L, Layadi K, Baeza-Velasco C. Ehlers-Danlos-Tschernogobow syndromes: Historical and current aspects. *Cuadernos Neuropsicol/Panam J Neuropsychol.* (2016) 10:17–31. doi: 10.1111/j.1365-2141.2008.06994.x

41. Kole A, Faurisson F. Rare diseases social epidemiology: analysis of inequalities. *Adv Exp Med Biol.* (2010) 686:22–50. doi: 10.1007/978-90-481-9485-8_14

42. Grahame R, Bird HA, Child A. The revised (Brighton 1998). criteria for the diagnosis of benign joint hypermobility syndrome (BJHS) *J Rheumatol.* (2000) 27:1777–9.

43. Grahame, R. The new EDS classification. In: *Conference at the 1st International School of Ehlers-Danlos Syndroms and Hyperaxity.* October 27th, University of Murcia, Murcia, Spain. (2017).

44. Hamonet C, Brissot R, Gompel A, Baeza-Velasco C, Guinchat V, Brock I, et al. Ehlers-Danlos syndrome (EDS). – Contribution to clinical diagnosis – A prospective study of 853 patients. *EC Neurol.* (2018) 10.6:428–39.

45. Lumley MA, Jordan M, Rubenstein R, Tsipouras P, Evans MI. Psychosocial functioning in the Ehlers–Danlos syndrome. *Am J Med Genet.* (1994) 53:149–52. doi: 10.1002/ajmg.1320530206

46. Bulbena-Cabré A, Baeza-Velasco C, Pailhez G, Martín-Lopez LM, Mallorquí-Bagué N, Bulbena A. Psychopathology of joint hypermobility. *Cuadernos Neuropsicol/Panam J Neuropsychol.* (2016) 10:61–70.

47. Sinibaldi L, Ursini G, Castori M. Psychopathological manifestations of joint hypermobility and joint hypermobility syndrome/Ehlers-Danlos syndrome, hypermobility type: the link between connective tissue and psychological distress revised. *Am J Med Genet.* (2015) 169C:97–106. doi: 10.1002/ajmg.c.31430

48. Fehlow P, Tennstedt A. Concomitant neuropsychiatric symptoms in a case of Ehlers–Danlos syndrome. *Psychiatr Neurol Med Psychol (Leipz).* (1985) 37:215–20.

49. Sieg KG. Autism and Ehlers Danlos syndrome. *J Am Acad Child Adolesc Psychiatry* (1992) 31:173. doi: 10.1097/00004583-199201000-00034

50. Tantam D, Evered C, Hersov L. Asperger's syndrome and ligamentous laxity. *J Am Acad Child Adolesc Psychiatry* (1990) 29:892–6. doi: 10.1097/00004583-199011000-00008

51. Takei A, Mera K, Sato Y, Haraoka Y. High-functioning autistic disorder with Ehlers–Danlos syndrome. *Psychiatry Clin Neurosci.* (2011) 65:605–6. doi: 10.1111/j.1440-1819.2011.02262.x

52. Cravero C, Guinchat V, Barete S, Consoli, A. Cornelia de Lange and Ehlers-Danlos: comorbidity of two rare syndromes. *BMJ Case Rep.* (2016) 2016:bcr2015210925. doi: 10.1136/bcr-2015-210925

53. Fernell E, Ronge E. A possible connection between childhood pain, joint hypermobility and neurodevelopmental disorders. *Acta Paediatr.* (2018) 36:91–6. doi: 10.1111/apa.14359

54. Cederlöf M, Larsson H, Lichtenstein P, Almqvist C, Serlachius E, Ludvigsson JF. Nationwide population-based cohort study of psychiatric disorders in individuals with Ehlers-Danlos syndrome or hypermobility syndrome and their siblings. *BMC Psychiatry* (2016) 16:207. doi: 10.1186/s12888-016-0922-6

55. Cupo LN, Pyeretz R, Olson J, McPhee SJ, Hutchins M, McKusick VA. Ehlers-Danlos syndrome with abnormal collagen fibrils, sinus of Valsava aneurysm, myocardial infarction, panacinar emphysema, and cerebral heterotopias. *Am J Med.* (1981) 71:1051–8. doi: 10.1016/0002-9343(81)90341-7

56. Eccles JA, Beacher FD, Gray MA, Jones CL, Minati L, Harrison NA, et al. Brain structure and joint hypermobility: relevance to the expression of psychiatric symptoms. *Br J Psychiatry* (2012) 200:508–9. doi: 10.1192/bjp.bp.111.092460

57. Boddaert N, Chabane N, Gervais H, Good CD, Bourgeois M, Plumet MH, et al. Superior temporal sulcus anatomical abnormalities in childhood autism: a voxel-based morphometry MRI study. *NeuroImage* (2004) 23:364–9. doi: 10.1016/j.neuroimage.2004.06.016

58. Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Marchi E, et al. The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. *Acta Neuropathol.* (2010) 119:755–70. doi: 10.1007/s00401-010-0655-4

59. Gomez-Garre P, Seijo M, Guiterrez-Delicado E, Castro del Rio M, de la Torre C, Morales-Corraliza J, et al. Ehlers-Danlos syndrome and periventricular nodular heterotopia in a Spanish family with a single FNLA mutation. *J Med Genet.* (2006) 43:232–7. doi: 10.1136/jmg.2004.029173

60. Sheen VL, Walsh CA. Periventricular heterotopia: new insights into Ehlers-Danlos syndrome. *Clin Med Res.* (2005) 3:229–33. doi: 10.3121/cmr.3.4.229

61. Marchezan J, Winkler Dos Santos EGA, Deckmann I, Riesgo RDS. Inmunological dysfunction in autism spectrum disorder: a potential target for therapy. *Neuroimmunomodulation* (2018) 5:1–20. doi: 10.1159/000492225

62. Seneviratne SL, Maitland A, Afrin L. Mast cell disorders in Ehers-Danlos syndrome. *Am J Med Genet Part C* (2017) 175C:226–36. doi: 10.1002/ajmg.c.31555

63. Chau AS. Allergic and Immunologic Dysregulation in Ehlers-Danlos Syndrome: a case series. *J Allergy Clin Immunol.* (2018) 141:AB125. doi: 10.1016/j.jaci.2017.12.398

64. Hisle-Gorman E, Susi A, Stokes T. Prenatal, perinatal, and neonatal risk factors of autism spectrum disorder. *Pediatr Res* (2018) 84:190–8. doi: 10.1038/pr.2018.23

65. Hugo-Rodin J, Lebègue G, Becourt S, Hamonet C, Gompel A. Gynecologic symptoms and the influence on reproductive life in 386 women with hypermobility type Ehlers-Danlos syndrome: a cohort study. *Orphanet J Rare Dis.* (2016) 11:124. doi: 10.1186/s13023-016-0511-2

66. Baeza-Velasco C, Sinibaldi L, Castori M. Attention deficit/hyperactivity disorder, joint hypermobility related disorders and pain: expanding body-mind connections to the developmental age. *ADHD Attent Def Hyp Disord.* (2018) 10:163–75. doi: 10.1007/s12402-018-0252-2

67. Gillberg C, Rasmussen P, Carlström G, Svenson B, Waldenström E. Perceptual, motor and attentional deficits in six-years-old children. Epidemiological aspects. *J Child Psychol Psychiatry* (1982) 23:131–44. doi: 10.1111/j.1469-7610.1982.tb00058.x

68. Gillberg C. The ESSENCE in child psychiatry: early symptomatic syndromes eliciting neurodevelopmental clinical examinations. *Res Dev Disabil.* (2010) 31:1543–51. doi: 10.1016/j.ridd.2010.06.002

69. Hershenfeld SA, Wasim S, McNiven V, Parikh M, Majewski P, Faghfoury H, et al. Psychiatric disorders in Ehlers-Danlos syndrome are frequent, diverse and strongly associated with pain. *Rheumatol Int.* (2016) 36:341–8. doi: 10.1007/s00296-015-3375-1

70. Chatterjea D, Martinov T. Mast cells, versatile gatekeepers of pain. *Mol Immunol.* (2015) 63:38–4. doi: 10.1016/j.molimm.2014.03.001

71. Theoharides TC, Angelidou A, Alysandratos KD, Zhang B, Asadi S, Francis K, et al. Mast cell activation in autism. *Biochim Biophys Acta* (2012) 1822:34–41. doi: 10.1016/j.bbadis.2010.12.017

72. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders 5th ed.* Arlington: American Psychiatric Publishing. (2013). doi: 10.1176/appi.books.9780890425596

The Presence of Comorbid ADHD and Anxiety Symptoms in Autism Spectrum Disorder: Clinical Presentation and Predictors

Einat Avni[1]*, Esther Ben-Itzchak[2] and Ditza A. Zachor[1,3]

[1] *The Autism Center, Department of Pediatrics, Assaf Harofeh Medical Center, Tel Aviv, Israel,* [2] *Bruckner Center for Research in Autism Spectrum Disorder, Communication Disorder Department, Ariel University, Ariel, Israel,* [3] *Department of Pediatrics, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*

High rates of attention deficit/hyperactivity disorder (ADHD) and anxiety symptoms have been documented in autism spectrum disorder (ASD), and have been associated with social and adaptive impairments. The study examined the frequency of clinically elevated ADHD and anxiety symptoms in an ASD group in comparison to a non-clinical group, compared the clinical presentation in the ASD group with and without ADHD and anxiety, assessed which child and familial variables add to the severity of Inattention, Hyperactivity/Impulsivity (HI), and anxiety symptoms, and evaluated whether having clinically elevated ADHD and/or anxiety symptoms adds to the prediction of adaptive functioning in ASD. The study included 260 participants diagnosed with ASD (mean age: 7.5 ± 1.1), using standardized tests. The rate of clinically elevated ADHD and anxiety symptoms in ASD was 62.7 and 44.6%, respectively, and symptom severity was significantly greater than the non-clinical sample. The entire population was divided into four subgroups: ASD alone, ASD+ADHD, ASD+anxiety, ASD+ADHD+anxiety, based on the parental behavioral questionnaire. The ASD alone group showed less severe autism symptoms in comparison to the other groups. Having ASD+ADHD symptoms was associated with greater impairments in socialization adaptive skills. Only the group with ASD+ADHD+anxiety was associated with poorer daily living adaptive skills. Regression analyses for prediction of ADHD and anxiety symptoms revealed that being a female and having lower adaptive skills scores predicted higher Inattention severity; being older, having better cognition, and more severe Restrictive Repetitive Behavior symptoms predicted more severe HI symptoms; being older and having more severe social impairments predicted higher anxiety scores. A regression analysis for the prediction of adaptive skills revealed that in addition to cognition and autism severity, the severity of Inattention symptoms added to the prediction of overall adaptive skills. In light of these findings, clinicians should diagnose these comorbidities in ASD early on, and provide effective interventions to reduce their negative impact on functioning, thereby improving outcome.

Keywords: autism (ASD), ADHD (attention deficit and hyperactivity disorder), anxiety, autism severity, adaptive skills, cognitive abilities

INTRODUCTION

Autism spectrum disorder (ASD) is a neurobehavioral disorder defined by social- communication deficits and restricted and repetitive behaviors that are typically detectable in early childhood and continue into adulthood (1). ASD-specific behaviors have been found to negatively impact various aspects of the lives of individuals with this disorder (2). The highly heterogeneous nature of ASD is often reflected in the child's characteristics, including clinical variability in the severity of autism symptoms, cognitive ability, and language skills (3). In addition, substantial individual differences are apparent with regard to the occurrence of co-morbidities such as attention-deficit/hyperactivity disorder (ADHD) and anxiety (4–9).

ADHD is a common neurodevelopmental disorder with a prevalence estimated at 7.2% in the general population (10). ADHD is characterized by a persistent pattern of inattention and/or hyperactivity and impulsivity that is pervasive across settings and leads to various degrees of functional impairment (11, 12). Children and adolescents with ASD have shown high rates of ADHD symptoms (16–85%) (5–7, 9) and overlap between ASD and ADHD symptoms has been described as well (13). However, the recognition that the diagnoses of ADHD and ASD can occur together has been formalized only in the DSM-5 (1). Previous studies have examined the co-morbidity of ADHD in ASD and described more severe autism symptoms (14), higher rates of cognitive impairment (10), more deficits in adaptive skills (2, 15) and lower quality of life (10) in individuals with ASD and ADHD in comparison to ASD alone. These studies were all cross-sectional and groups were not based on having an ADHD diagnosis but rather on ADHD symptom severity. These studies used different ADHD rating scales and behavioral checklists, completed by different informants (parents, teachers or both) (2, 10, 14, 15). Using standardized tests, Sprenger et al. reported a greater severity of autistic symptoms in children with ASD and ADHD symptoms, especially in the social interaction domain, than in those with ASD alone (14). Sikora et al. (2) found greater impairment in adaptive functioning among children with ASD and clinically significant ADHD symptoms in comparison to children with ASD and fewer ADHD symptoms. In contrast, Ashwood et al. (15) found significant associations between reduced adaptive functioning and autism symptoms, but not with ADHD symptoms.

The prevalence of anxiety disorders in the general population is 6.5% (16), ranging from 3 to 24% in various studies (17). Anxiety is among the most common mental health problems in ASD (4, 8). Similarly to ADHD, higher frequencies of anxiety difficulties (11–84%) have been reported in children with ASD in comparison to the general population (18) and up to 40% are diagnosed with at least one DSM anxiety disorder at some point in their lives (19, 20). Anxiety disorders may include features of excessive fear and related behavioral disturbances (1). Some studies have noted that ASD is a vulnerability for stress and anxiety (20). In contrast, according to some cross-sectional research, anxiety may be an underlying cause of several symptoms of ASD. For example, anxiety affects the stereotypical and rigid behaviors (21) and social functioning difficulties (22)

that children with an ASD often face. Furthermore, anxiety in children with ASD has a negative impact on adaptive functioning, daily living skills (DLS), and relationships with peers, teachers, and family (23, 24). For example, Factor et al. (25) found more social difficulties in youth with ASD and anxiety symptoms in comparison to youth with only ASD. Most of the studies that examined ASD and anxiety were cross-sectional ones, and anxiety symptoms were assessed using different anxiety scales.

Rates of anxiety in the presence of ADHD in the general population range from 13 to 50%, and these comorbidities together are associated with greater risk of long term impairments than children with either condition (26). However, only a few studies have examined the frequency or the clinical impact of anxiety in children with both ASD and ADHD. Craig et al. (27) found that individuals with ASD and ADHD had higher frequency of anxiety, as compared to individuals with only ASD. Since having ASD and ADHD is already known to negatively affect functioning (2, 10, 14, 15), it is assumed that having anxiety in addition to ADHD will have additive negative impact on children with ASD. Other studies have suggested that some of the anxiety symptoms overlap with ASD symptoms (21, 22).

In light of this, it is possible that the clinical presentation in ASD would not be worsened by having anxiety symptoms. Therefore, is it important to further investigate the contribution of having anxiety symptoms in ASD with comorbid ADHD on various aspects of the clinical presentation.

The study has several aims. First, to evaluate the frequency of ADHD and anxiety symptoms in the participants diagnosed with ASD and to compare them to non-clinical standardized samples. Second, to compare the clinical presentation in the ASD participants with and without ADHD and anxiety, in terms of autism symptom severity, adaptive skills, and cognitive ability. Third, to assess the relationships between the severity of the ADHD and anxiety symptoms and the autism severity and adaptive functioning. The fourth aim is to assess which variables (including age, sex, parental educational attainment, cognition, autism severity, and adaptive skills) add to the severity of Inattention, Hyperactivity/Impulsivity, and anxiety symptoms individually. The fifth aim is to assess whether having ADHD and/or anxiety symptoms adds to the prediction of adaptive skills functioning in the ASD participants, beyond the role of the child's other characteristics.

We have several hypotheses:

1. Clinically elevated ADHD and anxiety symptoms frequently occur in children with ASD. Rates of ADHD and anxiety in the ASD group will be significantly higher compared to the non-clinical standardized samples.
2. The participants with ASD and clinically elevated ADHD and/or anxiety symptoms, and particularly those with ASD+ADHD+anxiety, will present with more severe autism symptomatology, lower cognition, and lower adaptive skills, in comparison to those without these comorbid symptoms.
3. Higher cognition, more severe autism symptoms and lower adaptive skills will be associated with and predict more severe ADHD and anxiety symptoms.

4. More severe ADHD and anxiety symptoms in ASD will be associated with and predict lower adaptive functioning

This study is the first to examine a large group diagnosed with ASD and two major comorbidities and assesses their relationship to the clinical presentation.

METHODS

Measures

Autism Diagnostic Interview-Revised (ADI–R)

A semi-structured interview administered to parents, designed to make a diagnosis of autism according to DSM-IV criteria. Diagnosis of ASD was made on meeting the cutoff points of the ADI-R algorithm scores in the three domains: social interaction, communication, and restricted repetitive behavior (RRB) (28). Autism severity was assessed based on the level of the scores; higher scores in each ADI subdomain reflected more severe autism symptoms.

Autism Diagnosis Observation Schedule (ADOS)

A semi-structured, interactive schedule conducted by skilled professionals designed to assess social and communicative functioning in individuals who may have ASD (29). The scores of each of the ADOS subdomains, social affect (SA) and RRB, were used for the calculation of each subdomain severity score using the SA and RRB-calibrated severity scale (CSS) (30). Higher scores in the ADOS subdomains reflect more severe autism symptoms.

Wechsler Preschool and Primary Scale of Intelligence—Third Edition (WPPSI-IIIHEB)

An intelligence test for children aged 2:6–7:3, conducted by skilled psychologists. It consists of 4 subscales: Verbal IQ, Performance IQ, Processing Speed and Full Scale IQ. All the indices including Full Scale IQ were represented as standard scores: mean = 100; SD = 15 (31).

Wechsler Intelligence Scale for Children IV (WISC-IV)

A measure of intellectual ability and cognitive processing for children aged 6:0–16:11, conducted by skilled psychologists. It has ten core and five supplemental subtests. The subtests can be clustered into composite quotients for four indices: Verbal Comprehension, Perceptual Reasoning, Working Memory and Processing Speed. All the indices including full scale IQ were represented as standard scores: mean = 100; SD = 15 (32).

Vineland Adaptive Behavior Scales (VABS)

A standardized caregiver interview designed to assess adaptive behaviors in children between the ages of birth and 18 years. The VABS yields a composite score as well as scores in four domains: Communication, Daily Living Skills, Socialization, and Motor Skills (until the age of 6 years), each of which yields a standard score (33). Higher scores in the subdomains reflect better functioning.

Conners' Rating Scales–Revised: Long Form (CRS-R:L)

Parent and teacher questionnaires normed for children and adolescents 3–17 years old that assess ADHD symptomatology (i.e., inattention, hyperactivity, impulsivity), anxiety, and other co-morbid behaviors. Estimates of symptom severity are obtained using T-scores (mean = 50, SD = 10); higher T-scores reflect greater psychopathology (34). For ADHD symptoms, we used three scales of the parents' questionnaire: ADHD Index, DSM-IV inattentive and the DSM-IV hyperactive. For anxiety symptoms, we used the anxious-shy scale (8 items; i.e., easily frightened, afraid of people, afraid of new situations, afraid to be alone, overly attached to caregivers, and so forth). Only scores ≥60 in the corresponding scale were considered significant.

Procedure and Participants

The study was conducted at a national tertiary center for autism that is involved in diagnosis, treatment, and research in ASD. Data from clinical records were collected on 1,143 children who were assessed for a possible ASD diagnosis between January 2010 and December 2015. The children underwent a comprehensive evaluation including a neurological assessment by a pediatric developmental neurologist, and behavioral and cognitive evaluations conducted by a skilled interdisciplinary team. Information about previous diagnoses was obtained from previous assessments performed in other centers. Assessment of ASD was obtained using standardized tests, the Autism Diagnosis Interview-Revised (ADI-R) (28) and the Autism Diagnosis Observation Schedule (ADOS) (29), and meeting criteria for ASD based on DSM-IV (35) or DSM-5 criteria (1), depending on the year of diagnosis. The ADOS and ADI-R were administered by child neurologists and Masters' level psychologists, all of whom established at least 80% concurrence with research reliable psychologists on three consecutive administrations. Inclusion criteria were having an ASD diagnosis and being in the age range of 5–12. Out of the 1,143 children who were evaluated at the center, only 264 matched our study's criteria for inclusion. Participants with diagnoses of specific genetic syndromes and sensory impairments (n = 4) were excluded from the study. The final cohort included 260 participants (228 males, 32 females), with a mean age of 7 years and 5 months (SD = 1 year and 1 month). All the participants were Caucasians, and Hebrew was the spoken language.

The results for the ADI-R were available for all 260 participants and for the ADOS for 255 participants. Cognitive and developmental abilities (IQ/DQ) were assessed using the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) (31) or the Wechsler Intelligence Scale for Children IV (WISC-IV) (32), according to the child's age and language level, and results were available for 232 participants. The assessment of adaptive skills was obtained using the Vineland Adaptive Behavior Scales (VABS) (33) and was available for all 260 participants. All the children underwent a thorough neurological assessment, which included a semi-structured interview for comorbidities, such as ADHD, anxiety, oppositional defiant disorder, and mood disturbances. The severity of ADHD

symptomatology (i.e., inattention, hyperactivity, and impulsivity) and anxiety were assessed using the Conners' Rating Scales–Revised: Long form (CRS-R:L) (34). T-score ≥60 supported significant symptoms in the correlating scale.

In the current research, although parents' and teachers' CRS scores are presented and compared, only the parents' questionnaire was used to define the groups, in order to avoid the impact of ADHD medications given during the school day, which may affect the severity of ADHD symptoms as reported by the teachers. The study participants were divided into four groups based on the ADHD index and anxiety scales of the Conners Parents' Rating Scales–Revised: Long (CPRS-R:L) questionnaire (results were available for all 260 participants): the first group, with an ADHD index <60, and anxiety <60, was designated as ASD alone ($N = 68$); the second group, with an ADHD index ≥60, and anxiety <60, was designated as ASD with ADHD (ASD+ADHD; $N = 76$); the third group, with an ADHD index <60 and Anxiety ≥60, was designated as ASD with anxiety (ASD+anxiety; $N = 29$); the fourth group, with an ADHD index ≥60 and anxiety ≥60, was designated as ASD with anxiety and ADHD (ASD+ADHD+anxiety; $N = 87$).

Data Analysis

To compare the CPRS subdomain mean scores of the ASD study group with data on a non-clinical group taken from the CRS manual, a one sample T-test analysis was used.

To examine the effect of the coder who completed the CRS (parent/teacher), a one-way MANOVA with a repeated measure of coder was performed for the Inattention, HI, and Anxiety subdomain scores.

Comparisons of demographic data between the four examined groups (ASD alone, ASD+ADHD, ASD+anxiety, ASD+ADHD+anxiety) included sex using non-parametric chi-square analysis, age using one-way ANOVAs, and parental ages and educational attainment using one-way MANOVAs. Comparisons between the four examined groups of the dependent variables—including IQ/DQ scores, ADI-R subdomain (social interaction, communication and RRB) scores, ADOS subdomain (SA- and RRB-CSS) scores, and VABS composite and subdomain (Communication, DLS, Socialization) scores—were performed using one-way ANOVAs and MANOVAs. When a MANOVA yielded a significant group effect, individual ANOVAs were performed for each of the examined variables. In the analyses that yielded a significant group effect, post-hoc Scheffe tests were performed.

Next we examined the correlations between CPRS subdomain (IA, HI and anxiety) scores with IQ scores and VABS, ADI-R, and ADOS subdomain scores using Pearson correlation analyses.

Finally, to identify the variables that contributed to the explained variance in the CPRS subdomains, three five-step hierarchical linear regression analyses were performed with CPRS Inattention, HI, and Anxiety scores as the dependent variables.

To identify the variables that contributed to the explained variance in the VABS composite scores, another five-step hierarchical linear regression analysis was performed.

Compliance With Ethical Standards

All procedures performed in this study were approved by the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The Helsinki committee of the medical center waived the need for informed consent, as the study was retrospective and all the data were derived from the clinical charts.

RESULTS

Prevalence of ADHD and Anxiety Symptoms

The frequency of clinically elevated ADHD symptoms as reported by the parents (CPRS ADHD index ≥60) in the entire ASD group was 62.7%. "Inattention" symptoms (CPRS DSM-IV inattentive ≥60) were reported in 67.3% of the participants, and "hyperactivity/impulsivity" symptoms (CPRS DSM-IV hyperactive ≥60) in 56.5% of the participants.

The frequency of clinically elevated anxiety symptoms as reported by the parents (CPRS anxious-shy ≥60) in the entire ASD group was 44.6%.

At the time of evaluation at the Autism Center, 74.5% ($n = 194$) of the study participants were not previously diagnosed with ASD. Among those participants, 52.9% had at least one previous medical, neurodevelopmental and/or behavioral diagnosis. The most prevalent diagnosis was ADHD ($n = 61$; 31.4%) followed by developmental delay ($n = 48$, 29.3%) and language disorder ($n = 19$, 9.8%). Among the group with a previous ASD diagnosis (25.5%, $n = 66$), 10 participants (15%) received an ADHD diagnosis in addition to the principal diagnosis of ASD. Comparing the CPRS subdomain mean scores of the ASD group to the mean scores of the non-clinical group (ages 3–12) taken from the CRS manual revealed significantly higher scores in the Inattention [$t_{(258)} = 26.33$, $p < 0.01$], HI [$t_{(258)} = 23.53$, $p < 0.01$] and Anxiety [$t_{(258)} = 15.40$, $p < 0.01$] subdomains for the current study's ASD group.

Parents' and Teachers' Reports of ADHD and Anxiety Symptoms

As presented in **Table 1**, parent and teacher CRS ratings were compared, and yielded a significant coder effect [$F_{(3, 247)} = 38.53$, $p < 0.001$, $\eta^2 = 0.32$]. For the clinically elevated ADHD symptoms (Inattention and Hyperactivity/Impulsivity), the teachers' CRS scores were lower than the parental CRS. In contrast, for clinically elevated anxiety symptoms, teachers' CRS scores were higher than the parents'. For the clinically elevated CRS Hyperactivity/Impulsivity and the CRS anxiety scores, the effect size was medium. However, for the clinically elevated CRS Inattention scores the effect size was small.

Clinical Presentation of ASD With and Without Clinically Elevated ADHD and Anxiety Symptoms

We compared the four defined groups based on the CPRS ADHD index scores and the anxious-shy scores. The male:female ratio

did not differ significantly among the groups in a non-parametric chi-square analysis [$\chi^2_{(3)} = 3.18$, $p > 0.05$]. As presented in **Table 2**, parental ages at pregnancy and educational attainment in the four examined groups were not significantly different using one-way MANOVAs. Only the ANOVA for the child's age yielded a significant main effect, however *post-hoc* Scheffe analysis did not reveal any significant difference between each pair of the groups.

Cognitive Ability

Regarding cognitive ability, using a one-way ANOVA, no group main effect was found (**Table 3**).

Adaptive Skills

Regarding adaptive behavior skills, using a one-way MANOVA, a group effect was found [$F_{(9, 765)} = 3.68$, $p < 0.001$, $\eta^2 = 0.04$]. When examining each subdomain separately, the daily living skills and the socialization subdomains yielded a significant group main effect with medium effect size (**Table 3**). For the DLS subdomain, the ASD alone group had significantly higher

scores than the ASD+ADHD+anxiety group ($p < 0.001$); for the socialization subdomain, the ASD alone group had significantly higher scores than the ASD+ADHD group ($p < 0.01$) and the ASD+ADHD+anxiety group ($p < 0.05$) (*post-hoc* Scheffe).

Autism Severity

We then compared autism severity in the four examined groups, using the ADI-R scores, which were based on parental reports, and the ADOS scores, which were based on professional assessments. Comparing ADI-R subdomain scores between the four examined groups using a one-way MANOVA revealed a significant group effect [$F_{(9, 768)} = 4.99$, $p < 0.001$, $\eta^2 = 0.05$]. Separately examining each ADI-R subdomain using one way ANOVAs yielded a significant group effect in all ADI-R subdomains (**Table 4**). For the social interaction subdomain, the ANOVA yielded a large effect size. The ASD alone group had lower scores than the other three groups ($0.001 < p < 0.05$) and the ASD+ADHD group had significantly lower scores than the ASD+ADHD+anxiety group ($p < 0.05$). For the communication and RRB subdomains, the ANOVAs yielded medium effect sizes. For the communication subdomain, only the ASD alone group had significantly lower scores than the ASD+ADHD+anxiety group ($p < 0.001$). For the RRB subdomain, the ASD alone group had significantly lower scores than the other three examined groups ($0.001 > p < 0.01$) (*post-hoc* Scheffe tests).

Comparing the ADOS-CSS between the defined groups, the one-way MANOVA yielded a significant group main effect [$F_{(6, 502)} = 2.16$, $p < 0.05$, $\eta^2 = 0.02$], however, the separated ANOVAs for each subdomain did not yield a significant main effect.

TABLE 1 | Mean standard scores of parent and teacher ratings on CRS subdomains.

	Parents	**Teacher**	$F_{(1, 249)}$	η^2
CRS IA	65.08 (11.28)	61.26 (11.06)	24.73***	0.09
CRS HI	62.59 (11.94)	58.18 (11.26)	31.69***	0.11
CRS Anxiety	61.40 (13.75)	68.25 (13.37)	44.14***	0.15

*CRS, Conners' rating scales; IA, inattention; HI, hyperactivity/impulsivity. ***p < 0.001.*

TABLE 2 | Mean scores and standard deviation of demographic variables in the ASD alone, ASD+ADHD, ASD+anxiety, and ASD+ADHD+anxiety groups.

	ASD **N = 68**	**ASD+ADHD** **N = 76**	**ASD+Anxiety** **N = 29**	**ASD+ADHD+Anxiety** **N = 87**	**F**	μ^2
Age	6:10 (1:11)	7:4 (1:11)	7:10 (2:1)	7:7 (1:11)	2.83*	0.03
Maternal age	30:4 (4:6)	29:10 (4.00)	30:0 (4:6)	29:8 (4:11)	0.26	0.00
Paternal age	33:8 (6:1)	32:4 (5:0)	32:6 (5:2)	32:4 (5:5)	1.04	0.01
Maternal education	15:6 (2:7)	15:8 (2:4)	14:8 (2:4)	15:4 (3:1)	0.88	0.00
Paternal education	14:8 (2:7)	15:1 (2:6)	14:10 (4:0)	15:1 (3:6)	2.2	0.00

*ASD, autism spectrum disorder; ADHD, attention-deficit/hyperactivity disorder. *p < 0.05.*

TABLE 3 | Mean scores and standard deviation of IQ and VABS domains in the ASD alone, ASD+ADHD, ASD+anxiety, and ASD+ADHD+anxiety groups.

	ASD **N = 68**	**ASD+ADHD** **N = 76**	**ASD+Anxiety** **N = 29**	**ASD+ADHD+Anxiety** **N = 86**	**F**	μ^2
Cognition	90.22 (17.14)	90.41 (22.72)	83.93 (17.77)	84.58 (19.92)	1.71	0.02
VABS communication	86.90 (13.11)	83.10 (16.0)	86.10 (14.12)	81.33	2.20^	0.02
VABS DLS	83.23 (9.59)	79.56 (13.02)	78.24 (8.89)	76.03 (12.05)	5.10**	0.06
VABS socialization	80.73 (11.67)	73.90 (11.85)	75.00 (14.08)	70.83 (10.78)	9.25***	0.10
VABS composite scores	81.59 (10.07)	76.99 (12.03)	78.34 (10.01)	74.57 (10.63)	5.32***	0.06

*VABS, adaptive behavior scales; ASD, autism spectrum disorder, ADHD, attention-deficit/hyperactivity disorder; DLS, daily living skills. ***p < 0.001, **p < 0.01, ^p < 0.1.*

Correlations Between ADHD and Anxiety Symptom Severity and the Clinical Presentation

We examined the correlations of all three CPRS subdomains–Inattention (IA) Hyperactivity/Impulsivity (HI) and Anxiety, with VABS, ADI-R, and ADOS CSS subdomain scores (**Table 5**). We used continuous scores for these analyses. CPRS subdomains correlated negatively and significantly with all VABS subdomains and composite scores (except for CPRS HI and VABS communication subdomain) and positively with all ADI-R subdomains. Most correlations were in the medium range. The correlations of CPRS IA and ADI-R RRB, CPRS HI and VABS DLS and CPRS anxiety and VABS communication were all in the small range.

Factors Predicting the Severity of ADHD and Anxiety Symptoms

To identify variables that contributed to the variance in the CPRS scores, we applied three five-step hierarchical linear regression analyses using the CPRS Inattention, Hyperactivity/Impulsivity, and Anxiety scores as the dependent variables. We used continuous scores for these analyses. The independent variables of age and sex were entered in the first step, maternal education in the second step, cognitive scores in the third step, the ADI-SI, ADI Communication, and ADI RRB scores in the fourth step, and the VABS composite score in the fifth step.

Inattention

As presented in **Table 6**, the first regression analysis with the CPRS Inattention scores as the dependent variable explained 12.3% of the variance. In the final step, when all predictors were entered together, only sex and VABS composite scores were significant independent predictors over and above all the other variables entered.

Hyperactivity/Impulsivity

The second regression analysis with CPRS IH scores as the dependent variable (**Table 7**) explained 18% of the variance. In the final step, when all predictors were entered together, only age and ADI-R-RBB scores were significant independent predictors over and above all the other variables entered.

Anxiety

The third regression analysis with CPRS anxiety scores as the dependent variable (**Table 8**) explained 18.0% of the variance. In the final step, when all predictors were entered together, only age and ADI-R social interaction scores were significant independent predictors over and above all the other variables entered.

Predictors of Adaptive Functioning

An additional five-step hierarchical linear regression analysis using the VABS composite scores as the dependent variable was applied to identify variables that contributed to the variance in the child's adaptive behavior. Independent variables included age and sex in the first step, maternal education in the second step, cognitive scores in the third step, ADI-R SI, Communication and RRB subdomain scores in the fourth step, and CPRS Inattentive, HI and Anxiety scores in the fifth step. As presented in **Table 9**, the total model explained 44.0% of the variance. In the final step, when all predictors were entered together, age, IQ scores, ADI–R SI and Communication scores, and CPRS inattention scores were

TABLE 4 | Mean scores and standard deviation of ADOS and ADI-R subdomains in the ASD alone, ASD+ADHD, ASD+anxiety, and ASD+ADHD+anxiety groups.

	ASD	ASD+ADHD	ASD+ Anxiety	ASD+ ADHD+Anxiety	F ASD±ADHD	μ^2
ADOS–SA-CSS	8.14 (1.68)	7.40 (2.13)	8.00 (2.27)	8.11 (1.76)	2.38^	0.03
ADOS-RRB-CSS	8.33 (2.29)	8.40 (1.92)	7.55 (2.57)	8.26 (1.82)	1.26	0.01
ADI-R SI	9.59 (5.26)	12.33 (5.70)	13.34 (6.00)	15.10 (5.72)	12.43***	0.13
ADI-R communication	8.96 (4.00)	10.85 (4.33)	10.44 (4.66)	12.19 (4.81)	6.8***	0.07
ADI-R RRB	4.23 (2.56)	5.78 (2.67)	6.21 (2.68)	6.52 (2.84)	9.70***	0.10

ADOS, autism diagnosis observation schedule; ADI-R, autism diagnostic interview-revised; ASD, autism spectrum disorder; ADHD, attention-deficit/hyperactivity disorder; SA, social affect; RRB, restricted repetitive behavior; CSS, calibrated severity scale; SI, social interaction. ***p < 0.001, ^p < 0.1.

TABLE 5 | Correlations of CPRS subdomains scores with VABS, ADI–R, and ADOS subdomain scores.

	VABS communication	VABS DLS	VABS socialization	VABS composite	ADI-R SI	ADI-R communication	ADI-R RRB	ADOSSA CSS	ADOS RRB CSS
CPRS IA	−0.24***	−0.28***	−0.27***	−0.30***	0.23**	0.26***	0.15**	−0.05	0.05
CPRS HI	−0.10	−0.17**	−0.30***	−0.20***	0.27***	0.27***	0.38***	−0.01	0.03
CPRS anxiety	−0.13*	−0.23***	−0.30***	−0.22*	0.34***	0.28***	0.28***	−0.08	−0.09

CPRS, Conners' parents' rating scales; VABS, adaptive behavior scales; ADI-R, autism diagnostic interview–revised; ADOS, autism diagnosis observation schedule; IA, inattention; HI, hyperactivity/impulsivity; DLS, daily living skills; SI, social interaction; RRB, restricted repetitive behavior; SA, social affect; CSS, calibrated severity scale. ***p < 0.001, **p < 0.01, *p < 0.5.

TABLE 6 | Linear regression model for the CPRS inattentive scores.

Variable	B	SE.B	β	R^2	ΔR^2
Step 1				0.04**	0.04**
Sex	6.47	2.24	0.19**		
Age	−0.01	0.03	−0.02		
Step 2				0.04*	0.00
Sex	6.47	2.24	0.19**		
Age	−0.01	0.03	−0.02		
Maternal education	0.12	0.28	0.03		
Step 3				0.04*	0.00
Sex	6.23	2.26	0.18**		
Age	−0.01	0.03	−0.02		
Maternal education	0.16	0.27	0.04		
Cognition	−0.03	0.04	−0.06		
Step 4				0.09***	0.05**
Sex	6.76	2.23	0.20***		
Age	−0.02	0.03	−0.05		
Maternal education	0.10	0.28	0.02		
Cognition	−0.01	0.04	−0.02		
ADI-R–SI	0.18	0.18	0.09		
ADI-R–communication	0.40	0.22	0.16*		
ADI-R–RRB	0.04	0.31	0.01		
Step 5				0.12***	0.03**
Sex	6.62	2.20	0.20***		
Age	−0.03	0.03	−0.07		
Maternal education	0.14	0.28	0.03		
Cognition	0.04	0.04	0.06		
ADI-R–SI	0.06	0.18	0.03		
ADI-R–communication	0.30	0.22	0.12		
ADI-R–RRB	0.04	0.30	0.01		
VABS composite scores	−0.23	0.09	−0.22**		

CPRS, conners' parents' rating scales; ADI-R, autism diagnostic interview–revised; SI, social interaction; RRB, restricted repetitive behavior; VABS, adaptive behavior scales. ***p < 0.001, **p < 0.01, *p < 0.5.

TABLE 7 | Linear regression model for the CPRS HI scores.

Variable	B	SE.B	β	R^2	ΔR^2
Step 1				0.03*	0.03*
Sex	1.05	2.36	0.03		
Age	0.08	0.03	0.17**		
Step 2				0.03*	0.00
Sex	1.07	2.36	0.03		
Age	0.08	0.03	0.17**		
Maternal education	0.16	0.30	0.03		
Step 3				0.04*	0.01
Sex	1.37	2.38	0.04		
Age	0.08	0.03	0.17**		
Maternal education	0.11	0.30	0.02		
Cognition	0.04	0.04	0.07		
Step 4				0.18***	0.14***
Sex	3.04	2.23	0.09		
Age	0.07	0.03	0.15**		
Maternal education	−0.05	0.28	−0.01		
Cognition	0.08	0.04	0.14**		
ADI-R–SI	0.08	0.18	0.04		
ADI-R–communication	0.25	0.22	0.09		
ADI-R–RRB	1.33	0.31	0.32***		
Step 5				0.18	0.00
Sex	3.00	2.23	0.08		
Age	0.07	0.03	0.15**		
Maternal education	−0.04	0.28	−0.01		
Cognition	0.10	0.04	0.17**		
ADI-R–SI	0.04	0.18	0.02		
ADI-R–communication	0.21	0.22	0.08		
ADI-R–RRB	1.32	0.31	0.32***		
VABS composite score	−0.08	0.09	−0.07		

CPRS, conners' parents' rating scales; HI, hyperactivity/impulsivity; ADI-R, autism diagnostic interview–revised; SI, social interaction; RRB, restricted repetitive behavior; VABS, adaptive behavior scales. ***p < 0.001, **p < 0.01, *p < 0.5.

significant independent predictors over and above all the other variables entered.

DISCUSSION

Prevalence of Clinically Elevated ADHD and Anxiety Symptoms

The current study found an increased frequency of clinically elevated ADHD symptoms (62.7%) as reported by the parents in a large, well-characterized group diagnosed with ASD. Both clinically elevated 'inattention' symptoms and 'hyperactivity/impulsivity' symptoms were documented at high rates in the ASD group (67 and 57% respectively). These findings were emphasized when comparing the current study's ASD group with the CRS manual's non-clinical group. This was in accordance with our hypothesis. The current study supported previous studies that reported higher ADHD symptom frequencies (16–85%) in ASD (5–7, 9, 25).

In addition, increased frequency of clinically elevated anxiety symptoms (44%) was found in the entire ASD group of the current study. These findings were emphasized when comparing the current study's ASD group with the CRS manual's non-clinical group. The clinically elevated anxiety symptoms are in accordance with previous reports (24, 27) and with our initial hypothesis. Furthermore, the ASD participants with clinically elevated ADHD symptoms in the current study had almost twice the frequency of clinically elevated anxiety symptoms (53%) than the participants with ASD without ADHD symptoms (29.8%). Craig et al. (27) also reported similar findings in a subgroup of ASD and ADHD.

Parents' and Teachers' Reports

Differences in informant reports of clinically elevated ADHD and anxiety symptoms were also of interest. Parents reported more severe ADHD symptoms, while teachers reported more severe anxiety symptoms. This can be explained as almost one third of the study participants received medication for ADHD, which

TABLE 8 | Linear regression model for the CPRS anxiety scores.

Variable	B	SE.B	β	R^2	ΔR^2
Step 1				0.04**	0.04
Sex	−3.44	2.75	−0.08		
Age	0.10	0.04	0.18**		
Step 2				0.05**	0.01
Sex	−3.47	2.75	−0.08		
Age	0.11	0.04	0.19**		
Maternal education	−0.42	0.35	−0.08		
Step 3				0.07**	0.02**
Sex	−4.22	2.74	−0.10		
Age	0.10	0.04	0.18**		
Maternal education	−0.30	0.35	−0.06		
Cognition	−0.10	0.05	−0.15**		
Step 4				0.18***	0.11***
Sex	−3.10	2.62	−0.07		
Age	0.08	0.04	0.13*		
Maternal education	−0.44	0.33	−0.08		
Cognition	−0.06	0.04	−0.08		
ADI-R–SI	0.50	0.21	0.20**		
ADI-R–communication	0.32	0.25	0.10		
ADI-R–RRB	0.46	0.36	0.09		
Step 5				0.18***	0.00
Sex	−3.12	2.62	−0.07		
Age	0.07	0.04	0.13		
Maternal education	−0.44	0.33	−0.08		
Cognition	−0.05	0.05	−0.08		
ADI-R–SI	0.48	0.22	0.20**		
ADI-R–communication	0.31	0.26	0.10		
ADI-R–RRB	0.46	0.36	0.09		
VABS composite scores	−0.02	0.11	−0.02		

CPRS, conners' parents' rating scales; ADI-R, autism diagnostic interview–revised; SI, social interaction; RRB, restricted repetitive behavior; VABS, adaptive behavior scales.
***p < 0.001, **p < 0.01, *p < 0.5.

TABLE 9 | Linear regression model for the VABS composite scores.

Variable	B	SE.B	β	R^2	ΔR^2
Step 1				0.04**	0.04**
Sex	−1.54	2.09	−0.05		
Age	−0.08	0.03	−0.18**		
Step 2				0.04**	0.00
Sex	−1.52	2.09	−0.05		
Age	−0.08	0.03	−0.18**		
Maternal education	0.32	0.26	0.08		
Step 3				0.24***	0.20***
Sex	0.18	1.88	0.01		
Age	−0.07	0.03	−0.17**		
Maternal education	0.06	0.24	0.01		
Cognition	0.24	0.03	0.45***		
Step 4				0.42***	0.18***
Sex	−0.60	1.67	−0.02		
Age	−0.04	0.02	−0.10*		
Maternal education	0.16	0.21	0.04		
Cognition	0.19	0.03	0.37***		
ADI-R -SI	−0.54	0.13	−0.29***		
ADI-R-communication	−0.43	0.16	−0.18**		
ADI-R-RRB	−0.02	0.23	−0.01		
Step 5				0.44***	0.02*
Sex	0.34	1.70	0.01		
Age	−0.05	0.02	−0.11*		
Maternal education	0.18	0.21	0.04		
Cognition	0.19	0.03	0.36***		
ADI-R–SI	−0.52	0.13	−0.28***		
ADI-R–communication	−0.38	0.16	−0.16**		
ADI-R–RRB	−0.06	0.24	−0.01		
CPRS Inattention	−0.18	0.06	−0.16**		
CPRS HI	0.03	0.06	0.03		
CPRS Anxiety	0.01	0.04	0.01		

VABS, adaptive behavior scales; ADI-R, autism diagnostic interview–revised; SI, social interaction; RRB, restricted repetitive behavior; CPRS, conners' parents' rating scales; HI, hyperactivity/impulsivity. ***p < 0.001, **p < 0.01, *p < 0.5.

decreased symptom severity in the structured school setting. In contrast, the school environment provides more stressful social situations, which may lead to more noticeable anxiety symptoms. Therefore, we chose to use only the parental reports for the assessment of comorbidities (see Limitations).

Llanes et al. (36) compared parents' and teachers' reports on ADHD and anxiety symptoms in preschool and school-aged children, and found a low concordance between these informers, with teachers reporting fewer problems overall.

Clinical Presentation
Autism Severity

When looking at ASD with and without specific comorbidities (ADHD and/or anxiety), the subgroup of ASD without comorbid symptoms showed less severe autism symptoms in comparison to the subgroup of ASD with ADHD and/or anxiety. These differences were noted only by parental reports on the ADI-R (socialization, communication, and RRB) with a large effect size, but were not found in the professionals' observations

(ADOS). One possible explanation is that children with ASD and comorbidities (ADHD and/or anxiety) exhibit aberrant behaviors at home more than children with ASD without these comorbidities. They may be less attentive, need more support and have more difficulties in change of routines, and consequently are harder to raise. Therefore, they are potentially perceived by their parents as having more severe "autism" than children with only ASD. The professional assessment using the ADOS focuses on specific behaviors in a stimulating and interesting setting and is less affected by ADHD or anxiety-related behavior problems. In addition, the ADOS is a relatively short "snapshot" in real time of a child's observed behaviors in a quiet clinical room 1:1 with a professional. The ADI-R, on the other hand, is based on information given by the child's parents, and includes the child's overall behavior in the "real world," providing a broader picture than the time-specific ADOS. These findings are in accordance with previous research

(14, 37) which suggested that the difference between parental reports and professional assessments in regard to autism severity when clinically elevated ADHD symptoms are present, might be explained by a possible bias of the professional's observation, who may rate ASD symptoms as less severe because some of these symptoms are attributed to the co-morbid ADHD symptoms. These findings were in partial accordance with our hypothesis, as having comorbidities was associated with more severe autism symptoms only as reported by the parents, and having more than one comorbidity was not associated with greater autism severity.

Adaptive Skills

Regarding adaptive skills, having ASD with clinically elevated ADHD symptoms was associated with greater impairments in socialization adaptive skills. Only the subgroup with ASD and the two comorbidities was associated with poorer daily living adaptive skills. These findings partially supported our hypothesis, as not all the adaptive skills domains were associated with the comorbidities. However, having two comorbidities was associated with more adaptive functioning domains, albeit with a medium effect size. The current research is in line with previous findings that individuals with ASD and ADHD have a tendency toward greater impairments in adaptive functioning in all the VABS subdomains as compared to those with only ASD (2, 37, 38).

Cognitive Ability

Although ASD with clinically elevated symptoms of ADHD and/or anxiety was associated with lower adaptive skills functioning, surprisingly and not in accordance with our hypothesis, it was not associated with lower cognitive ability. Other studies found more impairments in verbal and non-verbal abilities, spatial working memory, response inhibition and executive functions in ASD with ADHD (37, 38). A lack of association between clinically elevated ADHD and/or anxiety symptoms and cognitive ability may be explained by the notion that cognition is an innate ability, which theoretically is not affected by comorbidities. The diagnosis center's team has a great deal of experience working with children with ASD, and it may be that they were able to help the children reach their best potential in terms of cognitive ability.

Regarding our third aim, in accordance with our hypothesis, the severity of clinically elevated ADHD and anxiety symptoms were associated with more severe autism symptoms and poorer overall functioning.

Predictors of ADHD and Anxiety Symptom Severity

Our Forth aim was to assess which variables added to the severity of Inattention, Hyperactivity/Impulsivity, and anxiety symptoms individually. For Inattention symptom severity, the regression model explained 12.3% of the variance. Being a female and having lower adaptive skills scores were greater predictors of higher severity of Inattention symptoms, beyond the contribution of age, parental educational attainment, cognition, and autism severity. It is possible that lower functioning is associated with the severity of inattention as perceived by parents. The finding regarding

females is important and should be further examined in future studies on inattention in a subgroup of females with ASD. For Hyperactivity/impulsivity symptom severity, the regression model explained 18.5% of the variance. Being older, having greater cognitive abilities and more severe RRB symptoms were greater predictors of higher severity of HI symptoms, beyond sex, parental educational attainment, adaptive skills, and autism severity in the social interaction and communication domains. It may be that ADHD is easier to diagnose in older children with greater cognitive abilities. In addition, RRB symptoms may overlap with HI symptoms (i.e., motor stereotypies, repetitive behaviors), and therefore children with more RRB symptoms will be perceived as having more HI symptoms. For anxiety symptom severity, the regression model explained 21% of the variance. Being older and having more severe social impairments were greater predictors of higher anxiety scores, beyond sex, parental educational attainment, adaptive skills, and autism severity in the communication and RRB domains. This may reflect that anxiety symptoms are more easily recognized in older children. Social impairments and anxiety symptoms often overlap or augment each other. Overall our hypothesis regarding the association between the comorbidities and cognitive level, autism severity and adaptive functioning was partially supported, as each comorbidity was associated with different factors.

Predictors of Adaptive Functioning

Adaptive functioning refers to behaviors critical in everyday life in terms of communication, socialization, daily living, and motor skills. Adaptive skills are one of the most important measures in evaluating functioning in ASD, as they reflect the generalization of acquired skills in everyday life. Therefore, we thought it important to identify the variables that may predict overall adaptive skills by using regression models as outlined in our Fifth aim. For overall level of adaptive skills, the regression model explained 44% of the variance. Younger age, better cognition, less severe social and communication impairments, and less severe inattention symptoms, all as reported by parents were greater predictors of better overall adaptive skills, beyond sex, maternal education, autism severity in the RRB domain, and the severity of HI and anxiety symptoms. These findings suggest that in ASD, in addition to the role of cognitive ability and autism severity, the severity of inattention symptoms adversely relates to the overall adaptive functioning. Interestingly, only the severity of Inattention and not that of Hyperactivity/impulsivity or anxiety contributed to lower adaptive functioning, over and above other ASD-related characteristics. These findings partially support our hypothesis, as only inattention symptoms but not HI and anxiety symptoms predicted the level of adaptive skills.

Strengths and Limitations

The current study has several strengths. One is the large sample size of a well-characterized ASD sample. The sample contained both males and females. There were no significant differences between the four examined groups in any demographic parameters. The assessment of ASD was conducted using gold standard tests (ADI-R; ADOS)

and ADHD and anxiety symptomatology were obtained using standardized questionnaires (CRS). The use of these comprehensive assessments promotes a broader understanding of the clinical presentation in the subgroup diagnosed with ASD and ADHD and/or anxiety.

This study has several limitations. There is a lack of a control group of children with neither ASD nor comorbidities; in the future, it will be important to design such a study. In addition, there were missing data points for some participants in the cognitive test scores (28) and the ADOS severity scores (5). Severity of symptoms of ADHD and anxiety was based on standardized questionnaires, which allowed for the quantification of a continuous variable, and not on the clinical diagnosis of these disorders. We compared parents' and teachers' CRS scores, but used only the parental CRS to examine the relationship between the severity of ADHD and anxiety symptoms and the levels of autism severity, cognitive ability and adaptive skills. Since more than a quarter of our study participants were previously diagnosed with ADHD, and many were treated with medications during the school day, the parents' reports were used to avoid the potential effects of medication on the reported severity of the ADHD symptoms. Previous research has used only parental CRS as well (25). Indeed, teachers' reports were less severe than the parents' in terms of ADHD symptoms. However, the teachers reported more severe anxiety symptoms as compared to the parents, and this was not reflected in the analyses that were based on parental reports. Finally, the anxiety subscale of the CSR was used to assess anxiety, instead of a specific anxiety questionnaire.

Study's Significance

The novelty of the current study is that it is the first to examine the relationship between having ADHD and/or anxiety in ASD and the clinical presentation in autism severity, adaptive skills and cognitive ability. In addition, the current study investigated for the first time which variables are associated with symptoms of inattention, hyperactivity/impulsivity and anxiety in ASD. Finally, the study addressed which variables may comprehensively affect adaptive skills in ASD by considering the contribution of ADHD and anxiety co-morbidities in addition to more traditional variables, such as the child's age, sex, cognitive ability, and autism severity. This led to the unique finding that only a specific ADHD symptom—inattention—contributes to lower adaptive functioning in ASD.

This study has theoretical significance. The very high frequency of ADHD symptoms in over two thirds of the ASD group suggests that some of the symptoms of both disorders may overlap. ASD and ADHD are described with similar brain abnormalities in specific regions, including the medial frontal and prefrontal cortex, which play an important role in executive functions (39), and reduced activation in the striato-thalamic region, prefrontal, and parietal cortex (40). Our finding of the significant co-occurrence of ADHD symptoms in ASD, along with the CNS abnormalities in both disorders, implies a common neurobiological origin. Significant anxiety symptoms may aggravate certain ASD symptoms, such as

social withdrawal, avoidance of exposure to different stimuli, and increased ritual/stereotypic behaviors, which may affect overall functioning. Therefore, it is possible that there may be overlap between symptoms of ASD and anxiety. Structural and functional abnormalities of the amygdala were found in ASD (41), suggesting more extensive impairment and more biological insults, which may manifest in a more severe clinical presentation.

Implications and Future Research

The study has several clinical implications. In light of the finding that ADHD and anxiety symptoms are related to poorer adaptive behaviors and to more severe autism symptoms as perceived by the parents, it is highly important during the ASD diagnostic process to assess ADHD and anxiety symptomatology. Since having ADHD and/or anxiety symptoms with ASD is associated with parents' negative perceptions of their child's functioning, parents should be provided with support and guidance.

In addition, since many children in this study were diagnosed first with ADHD and only later with ASD, it may be that the professionals were influenced by the dominant ADHD symptoms and they associated the social communication and RRB symptoms with the ADHD diagnosis. A very important takeaway for developmental behavioral pediatricians, pediatric neurologists and child psychiatrists is to consider a possible diagnosis of ASD when a young child exhibits significant ADHD symptomatology and when there are social difficulties and evidence for sensory or repetitive restrictive behaviors too.

In light of the study's findings, it will be important to diagnose these comorbidities early on in children with ASD, and to explore effective medical and behavioral interventions to reduce the impact of these comorbidities, thereby hopefully leading to improved functioning.

Future studies should further explore the clinical presentation in females. In addition, it would be interesting to explore the impact of medication and specific intervention programs to lower anxiety on the clinical presentation of children with ASD and ADHD and/or anxiety symptoms. It is also important to investigate genetic and imaging differences between children with ASD alone, ASD and ADHD, and ASD, ADHD and anxiety.

AUTHOR CONTRIBUTIONS

ACKNOWLEDGMENTS

REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders (DSM-5®)*. Arlington, TX: American Psychiatric Publishing (2013).

2. Sikora DM, Vora P, Coury DL, Rosenberg D. Attention-deficit/hyperactivity disorder symptoms, adaptive functioning, and quality of life in children with autism spectrum disorder. *Pediatrics* (2012) 130(Suppl.):S91–7. doi: 10.1542/peds.2012-0900G

3. Johnson CP, Myers SM, Disabilities and the Council on Children with disabilities. Identification and evaluation of children with autism spectrum disorders. *Pediatrics* (2007) 120:1183–215. doi: 10.1542/peds.2007-2361

4. Ghaziuddin M. Asperger syndrome: associated psychiatric and medical conditions. *Focus Autism Other Dev Disabl.* (2012) 17:138–44. doi: 10.1177/10883576020170030301

5. Gjevik E, Eldevik S, Fjæran-Granum T, Sponheim E. Kiddie-SADS reveals high rates of DSM-IV disorders in children and adolescents with autism spectrum disorders. *J Autism Dev Disord.* (2011) 41:761–9. doi: 10.1007/s10803-010-1095-7

6. Hanson E, Cerban BM, Slater CM, Caccamo LM, Bacic J, Chan E. Brief report: prevalence of attention deficit/hyperactivity disorder among individuals with an autism spectrum disorder. *J Autism Dev Disord.* (2013) 43:1459–64. doi: 10.1007/s10803-012-1677-7

7. Murray MJ. Attention-deficit/hyperactivity disorder in the context of autism spectrum disorders. *Curr Psychiatry Rep.* (2010) 12:382–88. doi: 10.1007/s11920-010-0145-3

8. Wood JJ, Gadow KD. Exploring the nature and function of anxiety in youth with autism spectrum disorders. *Clin Psychol.* (2010) 17:281–92. doi: 10.1111/j.1468-2850.2010.01220.x

9. Yoshida Y, Uchiyama T. The clinical necessity for assessing attention deficit/hyperactivity disorder (AD/HD) symptoms in children with high-functioning pervasive developmental disorder (PDD). *Eur Child Adolesc Psychiatry* (2004) 13:307–14. doi: 10.1007/s00787-004-0391-1

10. Thomas S, Sciberras E, Lycett K, Papadopoulos N, Rinehart N. Physical functioning, emotional, and behavioral problems in children with ADHD and comorbid ASD: a cross-sectional study. *J Atten Disord.* (2015) 22:1002–7. doi: 10.1177/1087054715587096

11. Biederman J, Faraone SV. Attention-deficit hyperactivity disorder. *Lancet* (2005) 366:237–48. doi: 10.1016/S0140-6736(05)66915-2

12. Klassen AF, Miller A, Fine S. Health-related quality of life in children and adolescents who have a diagnosis of attention-deficit/hyperactivity disorder. *Pediatrics* (2004) 114:e541–7. doi: 10.1542/peds.2004-0844

13. Sokolova E, Oerlemans AM. A causal and mediation analysis of the comorbidity between attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD). *J Aut Dev Disord.* (2017) 47:1595–604. doi: 10.1007/s10803-017-3083-7

14. Sprenger L, Bühler E, Poustka L, Bach C, Heinzel-Gutenbrunner M, Kamp-Becker I, et al. Impact of ADHD symptoms on autism spectrum disorder symptom severity. *Res Dev Disabil.* (2013) 34:3545–52. doi: 10.1016/j.ridd.2013.07.028

15. Ashwood KL, Tye C, Azadi B, Cartwright S, Asherson P, Bolton P. Brief report: Adaptive functioning in children with ASD, ADHD and ASD + ADHD. *J Autism Dev Disord.* (2015) 45:2235–42. doi: 10.1007/s10803-014-2352-y

16. Polanczyk GV, Salum GA, Sugaya LS, Caye A, Rohde LA. Annual research review: a meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. *J Child Psychol Psychiatry* (2015) 56:345–65. doi: 10.1111/jcpp.12381

17. Cartwright-Hatton S, McNicol K, Doubleday E. Anxiety in a neglected population: prevalence of anxiety disorders in pre-adolescent children. *Clin Psychol Rev.* (2006) 26:817–33. doi: 10.1016/j.cpr.2005.12.002

18. White SW, Oswald D, Ollendick T, Scahill L. Anxiety in children and adolescents with autism spectrum disorders. *Clin Psychol Rev.* (2009) 29:216–29. doi: 10.1016/j.cpr.2009.01.003

19. Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G. Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. *J Am Acad Child Adolesc Psychiatry* (2008) 47:921–9. doi: 10.1097/CHI.0b013e318179964f

20. Van Steensel FJA, Bögels SM, Perrin S. Anxiety disorders in children and adolescents with autistic spectrum disorders: a meta-analysis. *Clin Child Fam Psych Rev.* (2011) 14:302–17. doi: 10.1007/s10567-011-0097-0

21. Rodgers J, Glod M, Connolly B, McConachie H. The relationship between anxiety and repetitive behaviours in autism spectrum disorder. *J Aut Dev Disord.* (2012) 42:2404–9. doi: 10.1007/s10803-012-1531-y

22. Chang Y, Quan J, Wood JJ. Effects of anxiety disorder severity on social functioning in children with autism spectrum disorder. *J Dev Phys Disabil.* (2012) 24:235–45. doi: 10.1007/s10882-012-9268-2

23. Hallett V, Lecavalier L, Sukhodolsky DG, Cipriano N, Aman MG, McCracken JT, et al. Exploring the manifestations of anxiety in children with autism spectrum disorders. *J Aut Dev Disord.* (2013) 43:2341–52. doi: 10.1007/s10803-013-1775-1

24. Kim JA, Szatmari P, Bryson SE, Streiner DL, Wilson FJ. The prevalence of anxiety and mood problems among children with autism and Asperger syndrome. *Autism* (2000) 4:117–32. doi: 10.1177/1362361300004002002

25. Factor RS, Ryan SM, Farley JP, Ollendick TH, Scarpa A. Does the presence of anxiety and ADHD symptoms add to social impairment in children with autism spectrum disorder? *J Aut Dev Disord.* (2017) 47:1122–34. doi: 10.1007/s10803-016-3025-9

26. Manasis K, Tannock R, Young A, Francis-John S. Cognition in anxious children with attention deficit hyperactivity disorder: a comparison with clinical and normal children. *Behav Brain Funct.* (2007) 3:4. doi: 10.1186/1744-9081-3-4

27. Craig F, Lamanna AL, Margari F, Matera M, Simone M, Margari L. Overlap between autism spectrum disorders and attention deficit hyperactivity disorder: searching for distinctive/common clinical features. *Autism Res.* (2015) 8:328–37. doi: 10.1002/aur.1449

28. Le Couteur A, Rutter M, Lord C, Rios P, Robertson S, Holdgrafer M, et al. Autism diagnostic interview: a standardized investigator-based instrument. *J Autism Dev Disord.* (1989) 19:363–87. doi: 10.1007/BF02212936

29. Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, et al. The ADOS-G (Autism Diagnostic Observation Schedule-Generic): a standard measure of social communication deficits associated with autism spectrum disorders. *J Aut Dev Disord.* (2000) 30:205–23. doi: 10.1023/A:1005592401947

30. Hus V, Gotham K, Lord C. Standardizing ADOS domain scores: Separating severity of social affect and restricted and repetitive behaviors. *J Autism Dev Disord.* (2014) 44:2400–12. doi: 10.1007/s10803-012-1719-1

31. Wechsler D. *Wechsler Preschool and Primary Scale of Intelligence-Revised*. New York, NY: The Psychological Corporation (1989).

32. Wechsler D. *Wechsler Intelligence Scale for Children*. 4th ed. San Antonio, TX: The Psychological Corporation (2003).

33. Sparrow SS, Cicchetti DV, Balla DA. *Vineland Adaptive Behavior Scales: (Vineland II), Survey*. Livonia, MI: Pearson Assessments (2005).

34. Conners CK. *Conners' Rating Scales–Revised*. North Tonawanda, NY: MHS (1997).

35. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)*. Washington, DC (1994).

36. Llanes E, Blacher J, Stavropoulos K, Eisenhower A. Parent and teacher reports of comorbid anxiety and ADHD symptoms in children with ASD. *J Aut Dev Disord.* (2018) 1–12. doi: 10.1007/s10803-018-3701-z. [Epub ahead of print].

37. Yerys BE, Wallace GL, Sokoloff JL, Shook DA, James JD, Kenworthy L. Attention deficit/hyperactivity disorder symptoms moderate cognition and behavior in children with autism spectrum disorders. *Autism Res.* (2009) 2:322–33. doi: 10.1002/aur.103

38. Rao PA, Landa RJ. Association between severity of behavioral phenotype and comorbid attention deficit hyperactivity disorder symptoms in children with autism spectrum disorders. *Autism* (2014) 18:272–80. doi: 10.1177/1362361312470494

39. Brieber S, Neufang S, Bruning N, Kamp-Becker I, Remschmidt H, Herpertz-Dahlmann B, et al. Structural brain abnormalities in adolescents with autism spectrum disorder and patients with attention

deficit/hyperactivity disorder. *J Child Psychol Psychiatry* (2007) 48:1251–8. doi: 10.1111/j.1469-7610.2007.01799.x

40. Christakou A, Murphy CM, Chantiluke K, Cubillo AI, Smith AB, Giampietro V, et al. Disorder-specific functional abnormalities during sustained attention in youth with attention deficit hyperactivity disorder (ADHD) and with autism. *Mol Psychiatry* (2012) 18:1–9. doi: 10.1038/mp. 2011.185

41. Herrington JD, Maddox BB, Kerns CM, Rump K, Worley JA, Bush JC, et al. Amygdala volume differences in autism spectrum disorder are related to anxiety. *J Autism Dev Disord.* (2017) 47:3682–91. doi: 10.1007/s10803-017-3206-1

Characterizing the Interplay Between Autism Spectrum Disorder and Comorbid Medical Conditions: An Integrative Review

Charlotte Tye[1]*, Abigail K. Runicles[1], Andrew J. O. Whitehouse[2,3] and Gail A. Alvares[2,3]

[1] Child & Adolescent Psychiatry, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingdom, [2] Telethon Kids Institute, University of Western Australia, Perth, WA, Australia, [3] Cooperative Research Centre for Living with Autism (Autism CRC), Brisbane, QLD, Australia

Co-occurring medical disorders and associated physiological abnormalities in individuals with autism spectrum disorder (ASD) may provide insight into causal pathways or underlying biological mechanisms. Here, we review medical conditions that have been repeatedly highlighted as sharing the strongest associations with ASD—epilepsy, sleep, as well as gastrointestinal and immune functioning. We describe within each condition their prevalence, associations with behavior, and evidence for successful treatment. We additionally discuss research aiming to uncover potential aetiological mechanisms. We then consider the potential interaction between each group of conditions and ASD and, based on the available evidence, propose a model that integrates these medical comorbidities in relation to potential shared aetiological mechanisms. Future research should aim to systematically examine the interactions between these physiological systems, rather than considering these in isolation, using robust and sensitive biomarkers across an individual's development. A consideration of the overlap between medical conditions and ASD may aid in defining biological subtypes within ASD and in the development of specific targeted interventions.

Keywords: autism spectrum disorder, comorbidity, epilepsy, gastrointestinal disorders, immune function, sleep

INTRODUCTION

Autism spectrum disorders (ASD) are a group of complex and heterogeneous developmental conditions characterized by reduced social interaction and communication, as well as restricted range of interests and/or stereotypic behaviors (1, 2). Many of the core cognitive and behavioral symptoms associated with ASD are thought to arise from dysfunction of the central nervous system (3). However, accumulating and converging evidence across several areas of medicine have strongly emphasized comorbid medical conditions and associated peripheral/central physiological abnormalities in children with ASD as providing potential clues to additional etiological factors. Epidemiological evidence indicates that medical disorders are more prevalent in children with ASDs compared to children in the general population (4), with further evidence for specific clusters of medical conditions in ASD (5). Whilst multisystem dysfunction across a range of organs and physiological systems has been proposed in other brain-based conditions

(including neurodegenerative disorders, schizophrenia, bipolar disorder and depression), the elevated incidence of medical conditions and associated markers of dysfunction appear to be particularly perturbed in ASD (6). These medical conditions, and accompanying disabilities, can have significant impacts on broader development outcomes, social functioning, and education/employment outcomes. Despite the high prevalence of medical comorbidities in this population, many of these conditions are not routinely screened for in ASD evaluations (7, 8). This may reflect variability in the symptoms expressed by individuals with varying language capacity as well as the ability of health professionals to recognize behavioral symptoms that may be better explained by other medical conditions. In addition to better medical treatment, the increasing understanding of systemic abnormalities in ASD may provide clues to etiological factors as well as identifying biological pathways for more targeted and effective treatments.

Prevalence estimates of medical comorbidities vary greatly depending on the population studied. One of the largest studies of hospital records of children and young adults with ASD in the US found that prevalence estimates of a range of co-morbid disorders, particularly gastrointestinal and seizure disorders, greatly exceeded that compared to the general hospital population (4). Other studies have highlighted greater prevalence of abnormal neurological findings and clinical neuropathology in children with ASD (9). Strikingly, reports indicate elevated mortality ratios of up to 2.4 in ASD (10–12), which is thought to be due to largely due to complications arising from comorbid medical conditions (13). Put another way, premature mortality in individuals with ASDs is over twice the rate of that experienced by the general population (12, 13). Although prevalence estimates for some conditions are higher in studies with smaller samples, the overall evidence suggests that at least 10% of individuals with ASD present with comorbid medical symptoms that require formal medical evaluation (4). This has significant implications for subsequent development, prognosis and treatment plans for individuals and families as well as the additional pressures comorbidities place on health care and disability systems to provide adequate and necessary supports across the lifespan.

There have been several reviews and empirical studies focusing on specific medical, [e.g., (14–17)], behavioral [e.g., (18–20)], and genetic [e.g., (21, 22)] comorbidities of ASD. Here, we review medical disorders that have been repeatedly highlighted as sharing the strongest associations with ASD—epilepsy, sleep problems, gastrointestinal disorders and immune dysfunction—with the purpose of integrating mechanistic theories of their overlap. For each domain, we describe the prevalence and associations with behavior and cognition in ASD, before moving on to focus on work aiming to uncover shared aetiological mechanisms. A proposed mechanistic model integrating ASD and these medical comorbidities is then presented. We argue that a consideration of overlap between medical problems and ASD will provide insight into shared mechanisms and implications for treatment, based on proposed models of comorbidity (**Figure 1**).

META-SYNTHESIS OF THEORIES UNDERLYING COMMON MEDICAL COMORBIDITIES OF ASD

Epilepsy
Prevalence

Epilepsy, defined as two unprovoked seizures of any type, can be extended out to include multiple disorders with various etiologies, pathophysiology and outcomes (23). Prevalence of epilepsy in the general population is between 1 and 2%, whilst general estimates suggest a prevalence of ∼25–30% in individuals with ASD by adolescence (24, 25). In particular, two peak periods of epilepsy onset have been described in ASD—one in early childhood and a second in adolescence (26, 27), although prospective longitudinal studies have failed to replicate this bimodal distribution (28). Rates of ASD are higher in certain genetic disorders; for example, 47.4% of individuals with Dravet syndrome meet criteria for ASD, with the main seizure type being focal seizures manifesting in clusters (29). Whilst rates of ASD in tuberous sclerosis complex (TSC) are thought to approach 60%, individuals with TSC often experience different types of seizures (30, 31). Even in the absence of diagnosed epilepsy, there is considerable debate concerning the significance of abnormal electroencephalography (EEG) findings observed in ASD not associated with clinical seizure activity (24). A significant proportion of individuals with ASD display significant EEG paroxysmal abnormalities during sleep without the presence of clinical seizures, with reports as high as 60% (32–34). Retrospective studies indicate similar rates of cognitive impairment and cerebral lesions in ASD patients with abnormal EEGs with and without epilepsy (27). Due to this debate, clinical EEGs are not generally recommended as routine practice for children with ASD unless seizure activity is suspected.

All seizure types appear to be associated with ASD but vary in prevalence depending on the population studied (see **Table 1**). In a Swedish study, the most prevalent seizures in ASD were complex partial, atypical absence, myoclonic, and tonic-clonic seizures (35); by comparison, an American study reported that generalized tonic-clonic and atypical absence seizures were the most common in ASD (36). Some more recent studies argue that complex partial seizures are most prevalent in ASD (27, 34, 37). Clinically, this latter seizure type is particularly significant as some symptoms of complex partial seizures may be difficult to differentiate from common associated behaviors in ASD, such as not responding to calling name or repetitive movements. Of significance for clinical intervention, it has also been reported that treatment-resistant epilepsy is also of particularly high prevalence in ASD (38). One of the most severe forms of comorbid epilepsy in ASD is epileptic encephalopathy, a process whereby the epileptic activity contributes to severe cognitive and behavioral impairments above and beyond the underlying pathology alone (39, 40). It is characterized by intractable seizures as well as frequent ictal or interictal epileptiform activity (39), which may be idiopathic or syndromic. Infants with epileptic
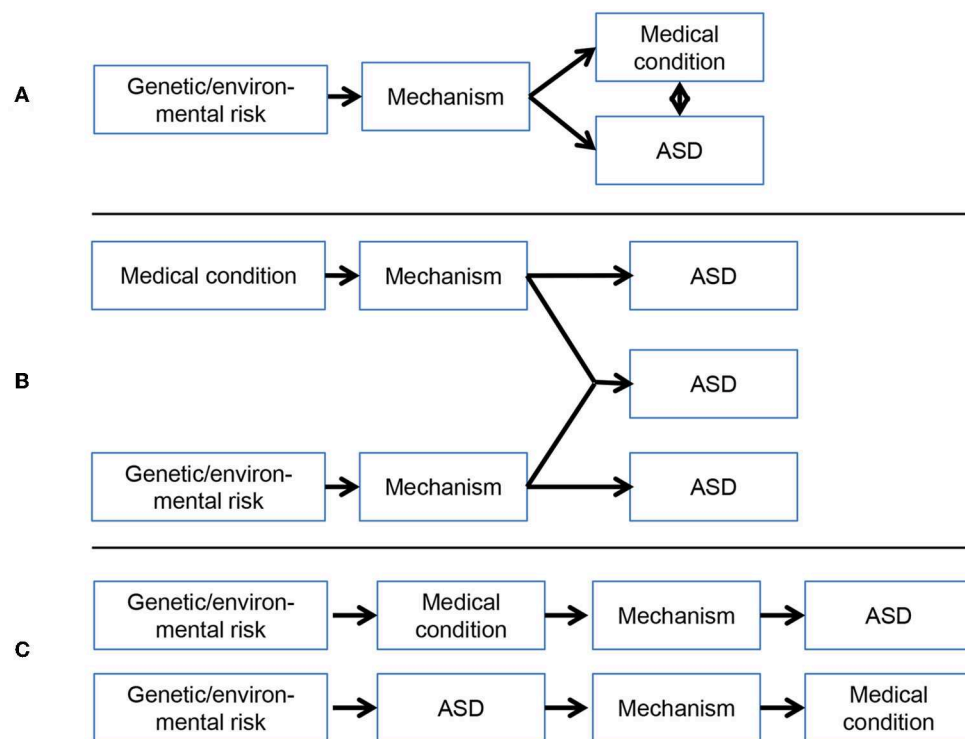
FIGURE 1 | Possible models of the association between medical conditions and ASD: **(A)** Overlap between medical conditions and ASD arises from a *common* mechanism; **(B)** Overlap between medical conditions and ASD arises from the *independent* pathways or *cumulative* impact of impairments in two or more developmental pathways (possibly subgroups of individuals); **(C)** Overlap between medical conditions and ASD arises from the *effect of medical abnormalities* on underling mechanisms, or vice versa. These models are not mutually exclusive and more than one pathway may be involved.

TABLE 1 | Overview of studies examining specific seizure types in individuals with ASD.

Study	Sample size	Prevalence	Subtypes
Steffenburg et al. (35)	$N = 90$, 24 ASD, 53 with at least 1 psychiatric diagnosis.	59% at least 1 psychiatric diagnosis, 27% ASD, 11% had ASD-like condition.	Complex partial, atypical absence, myoclonic, tonic clonic.
Tuchman et al. (36)	$N = 314$ ASD, $N = 237$ dysphasic non-ASD.	14% of ASD and 8% of dysphasic had epilepsy.	Generalized tonic-clonic and atypical absence seizures.
Matsuo et al. (37)	$N = 519$ with epilepsy, $N = 79$ ASD.	15.2% of sample with epilepsy had ASD.	Most frequent type—complex partial seizures.
Yasuhara (34)	$N = 1,014$ ASD.	37% had epilepsy.	Most frequent type—complex partial seizures.
Parmeggiani et al. (27)	$N = 345$ ASD.	24.9% epilepsy, 45.5% EEG paroxysmal abnormalities.	Most frequent type—complex partial seizures.

encephalopathy are at a higher risk for an ASD diagnosis and lasting cognitive impairments (41). In particular, 19.9% of children with infantile spasms will have ASD (29).

Of concern, is the increased 2-fold higher mortality rate in individuals with comorbid ASD and epilepsy compared to the general population, which is higher still in females with these comorbidities (11, 13, 42). These studies describe sudden unexpected death due to epilepsy at a higher frequency than expected, indicating that these individuals are likely to require increased medical monitoring to prevent avoidable death over their lifespan.

Associations With Behavior and Cognition in ASD

Research has suggested that there is a relationship between specific symptoms and epilepsy in individuals with ASD. Turk et al. (43) found that a co-morbid diagnosis of ASD and epilepsy led to greater difficulties in in social interactions with peers and unusual eye contact. Those with epilepsy also received an ASD diagnosis later than those who were not diagnosed with epilepsy. Conversely, other studies have found that increased frequency of epilepsy has been associated with an earlier age of ASD diagnosis, higher rates of repetitive object use, and greater unusual sensory interests (44). Viscidi et al. (45) completed a

large-scale study showing that children with both epilepsy and ASD displayed significantly more maladaptive behaviors linked to ASD. This included a decrease in scores on social cognition, communication and motivation when compared to ASD patients without epilepsy. Participants with both epilepsy and ASD also display higher levels of self-injurious, compulsive and sameness behaviors. Finally, a meta-analysis of social cognition in epilepsy and autism and found that rates of impaired facial recognition and theory of mind were higher in patients with epilepsy and ASD compared to controls (46). These studies suggest that there is a link between epilepsy and ASD symptoms and cognitive correlates. However, the close relationship with IQ suggests a dynamic interplay between ASD symptoms, and subsequent diagnostic age, IQ, and epilepsy.

Evidence from meta-analyses confirms an overall greater risk for epilepsy in children with ASD and intellectual disability (21.5%) compared to those without comorbid intellectual disability (8%) (47), as well as an increased risk in children with ASD and intellectual disability after the age of 12 years (48). This implies that the neurobiological mechanisms underpinning common associations between epilepsy and ASD may be derived from these subgroups within ASD. Other researchers have also pointed to the relationship between behavioral regression in ASD and greater incidence of epilepsy (49–51). Berg and Pliophys (52) suggest that the role of intellectual disability is so vital that it could explain most, if not all of the relationship between epilepsy and ASD. In a case study of TSC, the child's development was typical until 21 months of age at which seizure onset occurred; by 24 months of age the child met criteria for both ASD and intellectual disability (53). Taken together, this suggests that there is a dynamic interplay between seizures, intellectual disability and ASD with the onset of seizures playing a significant role in developmental regression and ASD diagnostic symptoms.

A diagnosis of comorbid epilepsy and ASD is a negative prognostic indicator, associated with greater risk of poorer outcomes (54). Children with both conditions report lower quality of life, score lower on social maturity scales, and exhibit higher use of psychotropic medications (26, 55, 56), in addition to poorer social outcomes (43). A large prospective follow-up study found significantly higher levels of cognitive impairments in adults followed from childhood, with seizure frequency reported to have the most significant impact on individual functioning (28). Even in children with ASD but without diagnosed epilepsy, EEG abnormalities and seizures have been associated with greater reports of aberrant behavior [(57); see **Table 2**].

Treatment Effects

As epilepsy may contribute to elevated mortality rates in ASD, appropriate detection and treatment of elevated seizure activity in individuals with ASD is a clear and urgent treatment need [(48); see **Table 3**]. Treatment of children with both epilepsy and ASD is based on guidelines aimed at treating childhood epilepsy with anti-epileptic drugs (AEDs), chosen based on seizure type and response to medication. Research into treatments that best control seizures in children with ASD is critical, as some children with ASD appear to be more sensitive to the side effects of AEDs (24). When epileptiform activity is

TABLE 2 | Overview of studies examining behavior and cognitive differences in individuals with ASD and epilepsy.

Study	Sample size	Features in ASD + epilepsy compared to ASD
Turk et al. (43)	N = 60 ASD + epilepsy, N = 60 ASD.	Higher incidence of motor difficulties, developmental delays & challenging behaviors.
Cuccaro et al. (44)	N = 577 ASD, N = 64 ASD + epilepsy.	Higher rates of repetitive object use and unusual sensory interests.
Viscidi et al. (45)	N = 2,645 ASD, N = 139 ASD + epilepsy.	Higher rates of irritability (20% higher) and hyperactivity (24% higher).

present, therapeutic interventions to reduce seizure activity may also improve language outcomes and ASD symptoms [reviewed in (62)]. Valproic acid, lamotrigine and Levetiacetam are the mostly highly rated AEDs in terms of effectiveness and tolerability in ASD, with valproic acid also found to improve core ASD symptoms (58). While alternative treatments, such as diets (e.g., ketogenic, modified Atkin's or gluten and casein free), have shown some efficacy for treating epilepsy, their suitability and safety for individuals with ASD require further study. Recent treatment advances for syndromic ASD may have particular relevance in improving either or both seizures and symptoms associated with ASD. For example, mTOR inhibitor treatment (Everolimus), associated with reduction in cell proliferation, angiogenesis and glucose uptake, has shown efficacy for some of the physical manifestations of TSC (63). In a case series, treatment with Everolimus was associated with a reduction in the severity and frequency of seizures, as well as improvements in repetitive behavior and social contact (59), although a recent study showed limited effect of everolimus on neuropsychiatric features in children (60). Furthermore, vigabatrin is recommended for children with infantile spasms and TSC, linked to improved later outcomes, including a decrease in adverse cognitive and behavioral outcomes such as ASD (61). Such treatment effects may help to explain the underlying pathophysiology and interaction between genetic mutations, epilepsy and ASD, for example, through normalization of altered brain activity and excitatory/inhibitory balance (see section Aetiological Mechanisms).

Aetiological Mechanisms

Seizure disorders can be due to many causes, including acute or chronic focal or diffuse brain pathology, genetic mutations, metabolic causes, or idiopathic reasons; as such, attempts to identify common causal pathways between ASD and epilepsy have proven to be a complex endeavor. While a number of reports have suggested common neuropathology in children with ASD and epilepsy (64), the vast majority of evidence suggests that epilepsy is not causal to the development of ASD, although the notable exception may be in the case of infants with an epileptic encephalopathy (see section Prevalence). It has been hypothesized that comorbidity of these conditions

TABLE 3 | An overview of different therapeutic approaches to treating epileptic symptoms in ASD.

Studies	Therapeutic approach	Benefits of therapeutic approach	Caveats of therapeutic approach
Frye et al. (58)	Anti-epileptic drugs (AEDs; e.g., Valproic acid, lamotrigine, levetiracetam and ethosuximide)	Improvement in seizures and limited impact on other clinical factors	Rate of side effects higher for AEDs compared to non-AED treatments
Frye et al. (58)	Ketogenic diet	Improvement in seizures, favorable effects on sleep, communication, behavior, attention and mood Low incidence of adverse effects	Can result in severe acidosis
Kilincaslan et al. (59) Krueger et al. (60)	mTOR inhibitor (Everolimus)	Reduction in severity and frequency of seizures, improvements in repetitive behavior and social contact (59)	Limited effect on neuropsychiatric features (60)
Bombardieri et al. (61)	Vigabatrin	Decrease in adverse cognitive and behavioral outcomes	Only indicated in patient with TSC and infantile spasms

reflects shared neurodevelopmental pathways or common etiopathologies (65), such as abnormalities in synaptic plasticity and excitatory/inhibitory imbalance early in development and/or shared multiple genome variants (25, 40).

Many candidate genes associated with ASD and epilepsy are involved in synaptic formation and maintenance (e.g., NRXN1, CNTNAP2) and GABAergic neurotransmission [e.g., ARX, Mecp2; for review see (66)]. The possibility of common genes is especially pertinent for those with intellectual disability. Recurrent structural abnormalities are shown at 15q13.3, 16p13.11, and 16p13.3 in patients with epilepsy, which overlap with genomic hotspots reported in ASD and ID (47, 67, 68). Likewise, copy number variations (CNVs) identified in patients with epileptic encephalopathies (Landau-Kleffner syndrome and continuous spike-wave during slow-wave sleep syndrome) corresponded to genomic regions or genes associated with ASD or related behaviors, particularly those encoding cell adhesion proteins (69).

The association between genetic disorders, epilepsy and ASD could provide a further indication of the aetiological mechanisms underlying ASD and epilepsy (40). Genetic disorders associated with epilepsy, such as TSC, Dravet syndrome and Angelman syndrome, have been linked to higher rates of ASD (30, 70–72). For example, TSC is caused by a mutation of TSC1 or TSC2 that disrupt the mTOR pathway and is characterized by multisystem growths of tumor-like lesions called hamartomas, which can affect a wide-range of bodily systems. Cortical tubers and peritubular cortex act as epileptogenic foci which increase the risk of epilepsy up to 90% and are linked to intellectual impairment and behavioral disturbances including ASD (70, 73). Likewise, Dravet syndrome (severe myoclonic epilepsy in infancy) is often caused by mutations in the SCN1A gene, which regulates movement of sodium ions, helping to propagate electrical signals along neurons (74). Dravet syndrome is associated with prolonged refractory seizures within the first year of life and later cognitive impairment, motor deficits and behavioral disorders such as ASD (71). Duplications on chromosome 15q11.3-q13.1 (Dup15q syndrome) lead to overexpression of several genes, including those involved in GABA transmission and are associated with high risk for early onset epilepsy, ASD and intellectual disability (75). The association between

ASD and epilepsy within genetic syndromes may help to identify a shared etiology, whereby disruption downstream of the genetic mutation affects both epileptogenesis and behavior [for review see (76)]. The genes implicated in several of these syndromes are associated with synaptic function. The diverse genetic etiologies identified may therefore converge by altering excitatory/inhibitory balance in the cerebral cortex, due to defects in GABAergic fibers of GABA-receptor function (77).

Despite many common associations found in both disorders, there is a limited amount of evidence investigating shared environmental and/or neuroinflammation pathways leading to comorbid epilepsy and ASD, for example advanced maternal/paternal age (78) or increased activation of astroglia/microglia in children with ASD (79) or epilepsy (80).

Summary
The well-documented overlap between ASD and epilepsy points toward shared aetiological mechanisms. Current findings do not suggest a causative role for epilepsy and abnormal epileptiform activity in the development of ASD; rather, presence of comorbid conditions predicts poorer prognostic outcome in individuals, and as such, indicates need for greater monitoring and interventions for associated factors. Research to date has not yet been able to fully determine the anatomical or molecular causes of why these conditions converge, as it is difficult to distinguish the effects of the underlying pathology from the neurological effects of the seizures themselves (25).

Sleep Problems
Prevalence
Sleep problems occur in a significantly higher proportion of children with ASD compared to typically developing children and children with other developmental delays (81). Prevalence estimates range between 50 and 80% as compared with 9–50% of typically developing children (81–84). These sleep disturbances not only affect daytime functioning but impact on the quality of life of the whole family (85).

A range of sleep disorders may be present in children with ASD, including insomnia (including difficulties with sleep initiation, duration, consolidation, or sleep quality, bedtime

resistance, night awakenings, or inability to sleep independently), sleep disordered breathing (that is, disorders related to airway obstruction, including obstructive sleep apnoea), parasomnias (including nightmares, wake screaming, complex movements, and dreams), and sleep related movement disorders (for example, rhythmical movement disorder and restless legs syndrome). Using parental reports, Goldman and colleagues found that younger children with ASD experienced more sleep anxiety, bedtime resistance, night wakefulness, and parasomnias; adolescents, however, tended to have more difficulty falling asleep, getting enough sleep, and experiencing daytime sleepiness (86). Actigraphy data confirms this, with children with ASD experiencing greater latency to fall asleep, longer periods of awakenings, and more night-time activity compared to typically developing children (87). Data from overnight polysomnography also demonstrates shorter sleep times and lower rapid eye movement (REM) sleep in children with ASD (88). Overall, insomnia (difficulty falling asleep and staying asleep) appears to be the most reliably observed phenomena to characterize sleep disturbances across the spectrum [(89–91); see **Table 4**].

Associations With Behavior and Cognition

Sleep has a vital role to play in child development as it serves multiple functions, such as energy conservation, brain growth, cognition and memory consolidation (15). The relationship between behavior in ASD and sleep dysfunction are likely to be bi-directional; whilst challenging daytime behaviors and associated comorbid conditions (e.g., attention deficit hyperactivity disorder (ADHD), anxiety and depression) contribute to sleep difficulties (see also section Treatment Effects), inefficient sleep can exacerbate or promote ASD behaviors. For example, sleep disorders are predictive of symptom severity in children with ASD (92, 93), including the level of social interaction difficulties (94). Different sleep problems appear to be differentially related to behavioral difficulties. For example, decreased sleep duration (and associated daytime sleepiness) has been associated with increased severity of core ASD symptoms such as repetitive behaviors and social communication difficulties, as well as pronunciation of more specific features such as failure to develop peer relationships and adherence to non-functional routines or rituals (95). Sleep onset delay is associated with stereotyped behaviors and social interaction deficits, but not communication deficits (96), while parasomnias has been linked to symptom severity, communication problems and an increased in stereotyped behaviors (96). Alterations in specific sleep stages may also be associated with different ASD phenotypes; prolonged REM latency has been associated with regression (97). Other maladaptive behaviors, such as self-injury, tantrums and aggression, are associated with shorter sleep durations in ASD (15), and children with ASD with poorer sleep quality also have higher rates of internalizing and externalizing behavioral disorders and lower levels of adaptive functioning (98, 99).

Sleep disorders may be caused by core ASD symptoms; for example, children may have reduced sensitivity to environmental cues that help signal the sleep/wake circadian systems, perseverate on activities or thoughts that may interfere with sleep onset or promote nocturnal awakenings, have limited communication to understand parental expectations for bedtime, or have more challenging behaviors such as hyperactivity or environmental hypersensitivities that may preclude ability to settle down to sleep (100). However, comorbidities such as anxiety or depression may also contribute; in typically developing children, sleep disorders are often related to anxiety and depression (15, 101). Children with ASD are especially vulnerable to co-occurring psychopathologies with prevalence rates ranging from 25 to 70% within the ASD population (18). For example, insomnia can be a consequence of elevated levels of anxiety in individuals with ASD (102), and higher percentage of time spent in REM sleep is associated with greater internalizing behavior in ASD (103). Interestingly, melatonin treatment may alleviate symptoms of both insomnia and anxiety in ASD (104), supporting a shared etiology.

Rates of co-occurring ASD and ADHD are also high, with the prevalence of ADHD symptoms in individuals with ASD ranging from 40 to 70% (105). The key symptoms of ADHD, inattention and hyperactivity, can have an impact on both an individual's ASD symptoms and thus the development of a sleep disorder (85). It has been hypothesized that symptoms of sleeplessness do not always manifest as sleepiness but as overactivity, which at extreme levels can be classified as hyperactivity (85). Interestingly research has shown that melatonin treatment can also have a positive effect on reducing both sleep disorder and hyperactivity (104), suggesting that insufficient sleep may result in behavioral symptoms often seen in patients with co-morbid ASD and ADHD.

Treatment Effects

Potential treatments for sleep disturbances range from behavioral to pharmacological, with the goal to not only improve sleep quality and daytime functioning but also reduce caregiver stress (106). Although a number of behavioral and medical sleep interventions are available, only melatonin appears to have been systematically investigated for its efficacy in ASD (107, 108). The summary evidence for melatonin supplementation for sleep problems in ASD suggests significantly improved sleep duration and sleep onset latency compared to placebo, and significantly improved daytime behaviors, with minimal side effects (107, 108). Because sleep deprivation can contribute to emotional reactivity and interpretation of nonverbal social cues (109, 110), sleep disturbances and daytime sleepiness may also contribute to efficacy of daytime behavioral interventions or impact on educational outcomes.

Conversely, it has also been argued that medications often prescribed to treat symptoms such as anxiety in individuals with ASD may negatively influence sleep. For example, it has been suggested that anti-psychotics and serotonin reuptake inhibitors (SSRIs) may disrupt the sleep cycle and thus have a role to play in sleep disorder (111). Some medications also increase risk of metabolic side effects like obesity (112); rates of overweight and obesity are increased in children with ASD, particularly those with co-occurring sleep disorders (113). The mechanisms and directional impact of sleep problems and treatment in ASD therefore remain complex.

TABLE 4 | An overview of sleep difficulties in individuals with ASD.

Source	Sample size	Methodology	Prevalence	Sleep disturbance
Goldman et al. (86)	$N = 1,859$ ASD	Parent-report questionnaire	67.3% categorized as good sleepers, 31.5% as bad sleepers	Younger children had sleep anxiety bedtime resistance, night waking and parasomnias. Adolescents had problems with falling asleep, getting enough consistently and daytime sleepiness
Souders et al. (87)	$N = 59$ ASD, $N = 40$ Controls	Actigraphy	66% in ASD group had moderate sleep disturbances compared to 45% mild sleep disturbance in controls	Behavioral insomnia sleep-onset type
Buckley et al. (88)	$N = 60$ ASD, $N = 15$ Controls	Overnight polysomnographic recording	–	Shorter total sleep time and smaller REM sleep percentage
Baker et al. (90)	$N = 34$ ASD, $N = 27$ Controls	Sleep questionnaire, sleep diary, and actigraphy	Three times more likely to report sleep problems compared to controls	Decreased sleep efficiency and fatigue

Aetiological Mechanisms

Development of a circadian rhythm has a vital role to play in the development of a competent sleep cycle, controlling different biological rhythms within a 24-h period and modified by both internal and external factors. It is thought that this rhythm regulates both biological and behavioral functions and how an individual can anticipate and adapt to environmental changes (114). When the circadian cycle is dysregulated by underlying alterations in neurophysiological and neurochemistry, an individual can be vulnerable to sleep disorders and physiological disturbances (15, 114). Neurochemistry factors involved in normal sleep include neurotransmitters such as GABA (115), serotonin, and melatonin (116).

Genes known to be associated with the human circadian clock have an important role to play in controlling sleep phase and duration (117) and have a widespread physiological effect on mood, cognition and reward-related behaviors. Mutations in clock genes have been implicated in ASD, thus resulting in the dysregulation of the circadian cycle (114, 118). For example, mutations in the Per1 and NPAS2 clock genes are associated with ASD and related to the morningness-eveningness phenotype (119, 120), and missense changes in six clock genes have been identified in individuals with ASD and sleep problems (118). In addition, the SCN controls the sleep-wake cycle by stimulating the pineal gland to produce melatonin through expression of melatonin-related genes (TPH2, DDC, AANAT, ASMT). Altered melatonin production appears to be feature of children with ASD, including below average physiological levels of melatonin or its metabolites and abnormal coupling of melatonin with the circadian rhythm (107). For example, studies have shown that variations in AMST are associated with decreased melatonin production in ASD (116). Exposure to external environmental factors, such as morning light and external clocks may also contribute to disruptions in sleep cycles. Some individuals with ASD may exhibit variations in light sensitivity, thus leading to a possible misalignment between the circadian phase and light/dark cycle (121). Bidirectional associations between genetic and environmental factors, as well as co-occurring behaviors, are likely to affect underlying biological networks involved in sleep (**Figure 2**).

Summary

Current findings suggest that sleep disorders in children with ASD may be associated with altered circadian rhythms, which may reflect mutations in clock genes and genes involved in melatonin production. Furthermore, sleep disorders in ASD have been associated with behavioral and psychiatric co-morbidities, such as anxiety or ADHD. The directional impact of these underlying mechanisms remains complex when additionally considering the impact of pharmacological interventions, however the broader evidence based suggests co-occurrence of sleep problems with behavioral symptoms, rather than an aetiological mechanism.

Gastrointestinal Dysfunction
Prevalence

Estimates for the prevalence of any gastrointestinal (GI) problem in children with ASD vary between 9 and 70% (7, 14, 122, 123), but may even range to as high as 91% (124). These problems can range from mild gastro-esophageal reflux to more severe symptoms, such as chronic constipation, abdominal pain, and persistent diarrhea (125, 126). The most common of these appears to be chronic constipation, with a median prevalence of 22% (14). Without treatment, these problems can lead to encopresis, delayed continence, pain, and maladaptive daytime behaviors (127–130). Although a subset of children with ASD have GI pathology related directly to ASD, many children also have functional GI disorders relating to selective eating, medications that have effects on GI motility, and differences in sensory processing. For example, Prosperi et al. (131) found that 27% of children with ASD have problems with food selectivity and that this was frequently associated with GI problems. Despite differences in prevalence between studies, the collective evidence suggests that there is an unusually high prevalence of GI symptoms in children with ASD, implying a possible underlying pathophysiology contributing to both conditions. GI functioning in ASD also encompasses nutrition, including food allergies, metabolic abnormalities, pre-existing nutritional deficiencies, nutrition-related medication side effects, as well as behavioral factors including problematic eating [(132); see **Table 5**].

FIGURE 2 | Interdependent bidirectional associations between ASD and sleep problems. GI, gastrointestinal; RRBIs, restricted and repetitive behaviours and interest.
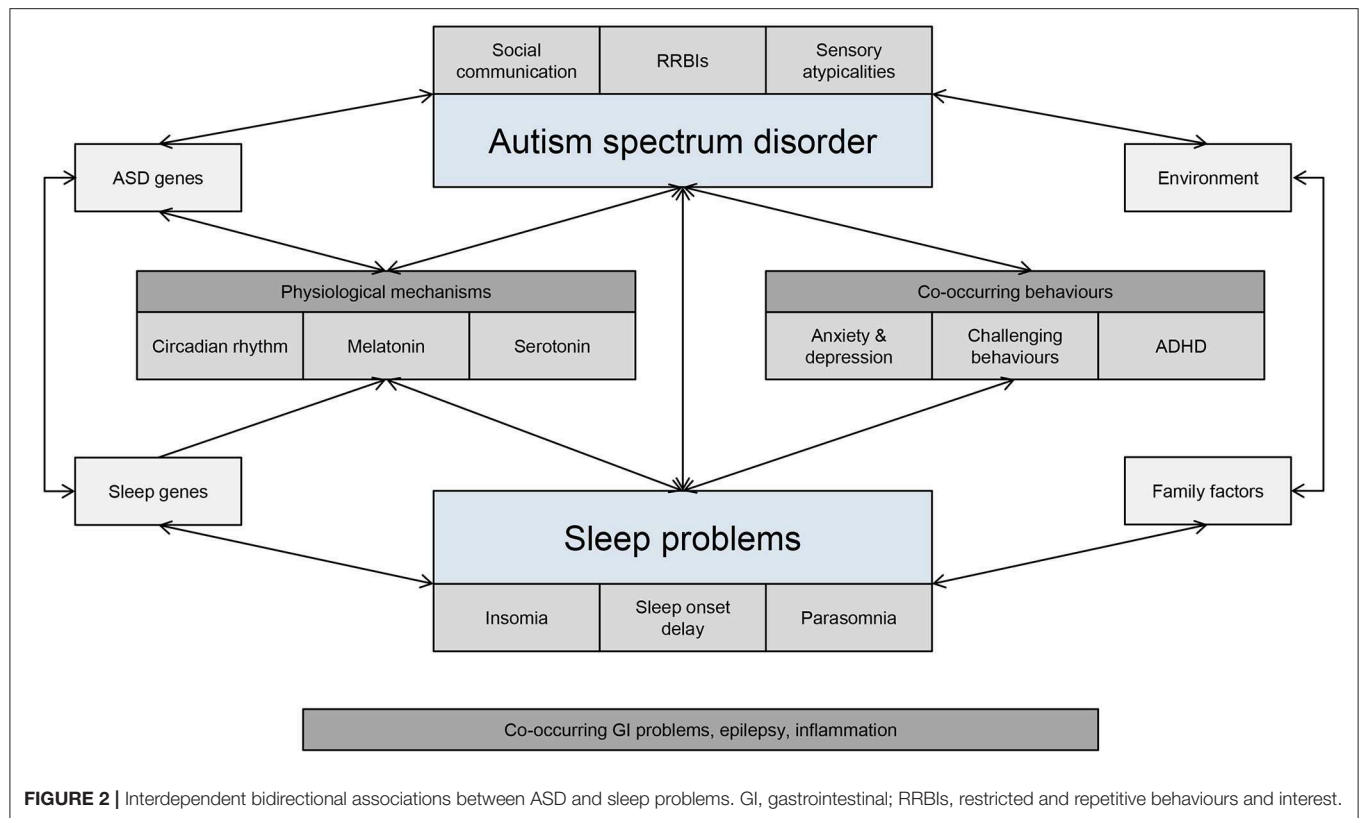
TABLE 5 | An overview of gastrointestinal (GI) symptoms reported in individuals with ASD.

Source	Sample size	Prevalence	GI symptoms
McElhanon et al. (125)	N = 15 studies	Higher rates of diarrhea (OR, 3.63), constipation (OR, 3.96), abdominal pain (OR, 2.45).	Diarrhea, constipation, abdomen pain
Mazurek et al. (128)	N = 2,973 ASD	24% had at least one type of chronic GI problem.	Constipation, abdominal pain, bloating, diarrhea and/or nausea lasting 3 months or more. Sensory over-responsivity and anxiety were highly associated with GI symptoms.
Mazefsky et al. (129)	N = 95 ASD	61% reported at least one GI symptom.	Abdominal pain, not hungry, bloating. Participants with GI problems also had significantly higher levels of affective problems.
Chandler et al. (130)	N = 132 ASD, N = 81 other developmental conditions, N = 82 Controls	46.5% ASD had at least one individual GI symptom, relative to 29.2% other developmental conditions and 21.8% in Controls.	Vomiting, diarrhea, abdominal pain, constipation. No association between GI symptoms and ASD severity.
Prosperi et al. (131)	N = 163 ASD	25.8% had at least one severe GI symptom.	Constipated (22.1%), Painful bowel movement (7.4%).

Associations With Behavior and Cognition in ASD

It has been speculated that underlying and undiagnosed GI problems may contribute to some of the behavioral difficulties observed in some children with ASD. Chaidez et al. (133) suggested that there is a strong and significant relationship between GI symptoms and increased instances of irritability, social withdrawal and hyperactivity. Furthermore, non-verbal children with ASD often come to clinical attention due to self-injurious behavior and aggression. It is thought that this behavior could be a result of being unable to communicate their pain and/or discomfort effectively. However, there has been limited evidence to suggest a significant association between presence or frequency of GI symptoms and ASD symptom severity over and above those without significant GI complaints (127, 130,

134, 135), although there is some evidence for associations with language impairment (136). Whilst some reports suggest that increased ASD severity is associated with significantly more GI problems (133, 137), a number of studies have failed to support this association (129). In addition, the interplay between GI problems and anxiety symptoms has been supported in both typical and ASD populations. A large-scale study indicated that anxiety, sensory over-responsivity and GI problems are interrelated in children with ASD (128).

Treatment Effects

A wide range of diets have been purported to exert some efficacy in alleviating GI symptoms in children with ASD. However, a systematic review found little evidence for the beneficial effect of nutritional supplements or gluten/casein-free diets on ASD symptoms (138). Further to this, the long-term effects of these therapies are not well understood and may have unintentional physical health consequences. For example unconventional food preference may result in reductions in bone cortical thickness in boys with autism, a reduction that is greater for those on casein-free diets (139). However, suboptimal bone development in ASD has also been linked to combinations of a lack of exercise, GI problems, as well as clinically compromised vitamin D and calcium intake due to restrictive diets.

Microbiota transfer therapy has recently been suggested as a potential therapy in ASD to target GI symptoms based on the premise of differences in microbiome composition (132). However, whilst the subject has been widely investigated, findings have been inconclusive and contradictory (see **Table 6**). For example, in a review of the literature, researchers concluded that inconsistent findings in the field are complicated by use of different methodologies, high incidence of antibiotic use, special diets and/or have repetitive dietary behaviors (144). For example, Kang et al. (142) suggested that GI symptoms in ASD were characterized by less diverse gut microbial composition with findings indicating lower levels of Prevotella, Coproccus, and unclassified Veillonellaceae. It has been argued that a higher diversity of gut bacteria protects the human intestine from stresses such as pathogenic gut microbes and lower diversity in ASD may explain increased risk of GI disturbances. Conversely, Finegold et al. (143) found that there was a significantly higher diversity of bacteria found in feces of participants with ASD. Parracho et al. (124) conducted a study that found that there was a higher incidence of the Clostridium histolyticum group of bacteria. Clostridium histolyticum are known to be toxin-producers and it has been hypothesized that this may contribute toward gut dysfunction with metabolic products having a systemic effect. Based on this evidence, clinical trials have now started investigating whether alteration of microbiota profiles may impact on GI symptoms and ASD behaviors. In a recent open-label trial, microbiota transfer therapy incurred an 80% reduction in GI symptoms, including a significant improvement in symptoms such as constipation and abdominal pain that persisted after 8 weeks, in addition to ASD symptoms (145). These results provide very preliminary evidence that, by targeting the gut ecosystem, potentially both ASD and GI symptoms can be impacted, supporting a potential shared mechanism.

Aetiological Mechanisms

The underlying mechanism of GI dysfunction and how this relates to the pathophysiology of ASD is still not well understood. One theory in particular has gained particular momentum, that abnormal neurodevelopment in ASD may be caused by increased GI permeability, so-called "leaky gut," that facilitates the absorption of toxic by-products of incompletely digested proteins [see (146) for review]. The production of metabolites by certain microbiota produces neuroactive compounds, including 5-HT, dopamine and GABA. These can cross the "leaky gut" resulting in the entry of toxins and bacteria into the bloodstream, to influence brain function and the hypothalamic-pituitary-adrenal (HPA) axis [see (147) for review]. This theory has been so influential, that despite limited evidence for efficacy in improving core ASD symptoms, casein and gluten-free diets, as well as dietary supplementations, are increasingly popular alternative treatments pursued by families (148).

Serotonin is critical for gut function, with 80–95% of 5-HT receptors localized to the gut and alterations to 5-HT signaling related to many GI disorders (for example, Crohn's disease, ulcerative colitis, irritable bowel syndrome, and chronic constipation). It has been speculated that altered gut 5-HT signaling may be an important contributor to the presence of GI disorders in children with ASD (149, 150); a cascade of events resulting from gut inflammation may lead to reduced levels of brain 5-HT, thereby resulting in mood and cognitive disturbances associated with ASD (149), although this may also be associated with an overall reduced brain availability of 5-HT (151). A well-established connection links the gut to the brain in a bidirectional pathway (see also **Figure 3**) (152)–autonomic projections from the brain regulate digestive reflexes, signals traveling from the gut signal satiety to the brain, whilst neural signals of stress and anxiety influence gut function and sensitivity. Dysregulation of the HPA axis has been implicated in children with GI problems and emerging evidence suggests a role in ASD (128, 153). This may provide a strong rationale for use of selective serotonin reuptake inhibitors (SSRIs) in the treatment of ASD symptoms by increasing availability of 5-HT, although research to date has established limited efficacy (154). However, if reduced 5-HT results from intestinal inflammation, then possibly targeting 5-HT metabolism by restoring availability of tryptophan for 5-HT synthesis may represent a viable alternate therapeutic mechanism (149).

Increasing evidence suggests GI complications can arise as a result of genetic and environmental risk factors for ASD. For example, variants in the c-Met gene encoding for MET receptor tyrosine kinase are associated with ASD in individuals with co-occurring GI dysfunction (155). The role of MET hypofunction is supported by decreased protein expression in post-mortem brains from autistic individuals compared to typical controls (156). In addition, alterations in the serotonin reuptake transporter (SERT) are implicated in ASD (157)

TABLE 6 | An overview of studies investigating microbiota composition in ASD.

References	Sample size	Methodology	Significantly increased in ASD	Significantly decreased in ASD
Williams et al. (140)	$N = 15$ ASD, $N = 7$ Controls; all with GI symptoms	Pyrosequencing and PCR, biopsies from ileal and cecal region	Firmicutes and proteobacteria; Sutterella	Bacteroidetes
Gondalia et al. (141)	$N = 23$ ASD without GI dysfunction, $N = 28$ ASD with GI dysfunction, $N = 53$ siblings	Pyrosequencing of fecal material	No significant difference	No significant difference
Kang et al. (142)	$N = 20$ ASD, $N = 20$ Controls	Pyrosequencing—fecal DNA samples	–	Prevotella, Coproccus and unclassified Veillonellaceae
Parracho et al. (124)	$N = 58$ ASD, $N = 10$ Controls, $N = 12$ Siblings	Fluorescence *in situ* hybridization—fecal material	Clostridium histolyticum group (I & II)	–
Finegold et al. (143)	$N = 33$ ASD, $N = 8$ Controls, $N = 7$ Siblings	Pyrosequencing—fecal microflora	Bacteroidetes, Desulfovibrio, Bacteroides vulgatus, Actinobacterium and Proteobacterium phyla	Firmicutes

and are likely to disrupt GI serotonin metabolism (158). Expression of the most common SERT variant (Ala56) in mice is associated with ASD-like behaviors [repetitive behaviors, reduced vocalizations, and social contact; (159)], as well as fewer gut neurons, a badly maintained gut lining and slow gut activity (160). Treatment with a 5-HT agonist prevented these GI manifestations (160).

Dysfunctional immune responses (see section Immune Dysfunction), in particular mucosal immune cells, may also have adverse effects on GI functioning in ASD. Endoscopic investigations suggest diffuse inflammation in the intestinal tract of children with ASD (7). There have also been reports of increased gastrointestinal complaints associated with autoimmune responses or a family history of autoimmunity in children in with ASD (161–163), although smaller studies have failed to replicate this increased prevalence (164). For example, increased autoantibodies directed toward central nervous system proteins have been observed in children with ASD and their mothers (165–168). One speculation is that these autoantibodies may signal presence of heightened inflammatory processes or an autoimmune component that could decrease the integrity of the mucosal barrier, or even reflect a downstream effect of previous mucosal infection (123). Notably, these maternal autoantibodies are strongly associated with the functional c-Met C allele associated with susceptibility to ASD and comorbid GI dysfunction (169), forming a promising convergent pathway (see section Immune Dysfunction).

Summary

Individuals with ASD are at higher risk of experiencing GI disturbances, with GI problems further linked to increased ASD symptoms. Abnormal neurodevelopment, dysfunctional immune responses and altered serotonergic transmission have been suggested potential mechanisms underlying the overlap between ASD and GI dysfunction.

Immune Dysfunction
Prevalence

The immune system comprises a group of defense mechanisms triggered to protect against disease or illness causing pathogens. Antigens, proteins found on the surface of pathogens, are recognized by a healthy immune system and trigger the production of antibodies that identify and neutralize or remove the antigen. The immune system comprises both innate and adaptive systems. The innate system develops early in fetal development, using genetically encoded receptors and nonspecific mechanisms for defense. In this way, the innate system plays more of a "housekeeping" role. The adaptive system is responsible for threat response, developing a memory of the response for future threats, and does not fully mature until early childhood. This system is more dynamic, responding to potential pathogens and toxins upon exposure. Both systems work together to achieve homeostasis, and importantly, disruption in either can affect neuronal development and functioning (170). Optimal immune functioning is characterized by the homeostatic maintenance of the innate and acquired immune responses, balancing pro- and anti-inflammatory signaling in response to potential pathogenic threats. Aberrant immune functioning can manifest in many ways, such as upregulation of inflammation or immune deficiency that comprises innate host defense mechanisms. Conditions associated with dysfunction of the immune system can include allergies, asthma, and autoimmune disorders.

Prevalence of immune conditions in individuals with ASD varies considerably between studies. Increased rates of food allergies, allergic rhinitis, and atopic dermatitis (i.e., eczema) (133, 171, 172), as well as autoimmune disorders such as psoriasis (4, 173), have been reported in children with ASD in case-control analyses of electronic medical records. In general, asthma is reported at increased frequencies in children with ASD (171, 174), however decreased rates have also been reported (173). Additionally, atopic conditions (asthma, atopic dermatitis, allergic rhinitis, or allergic conjunctivitis) in early childhood is

FIGURE 3 | Bidirectional associations between alterations in central nervous system circuitry (epilepsy, sleep), immune system and gastrointestinal function and behaviors characteristic of ASD interacting with genetic and environmental risk.
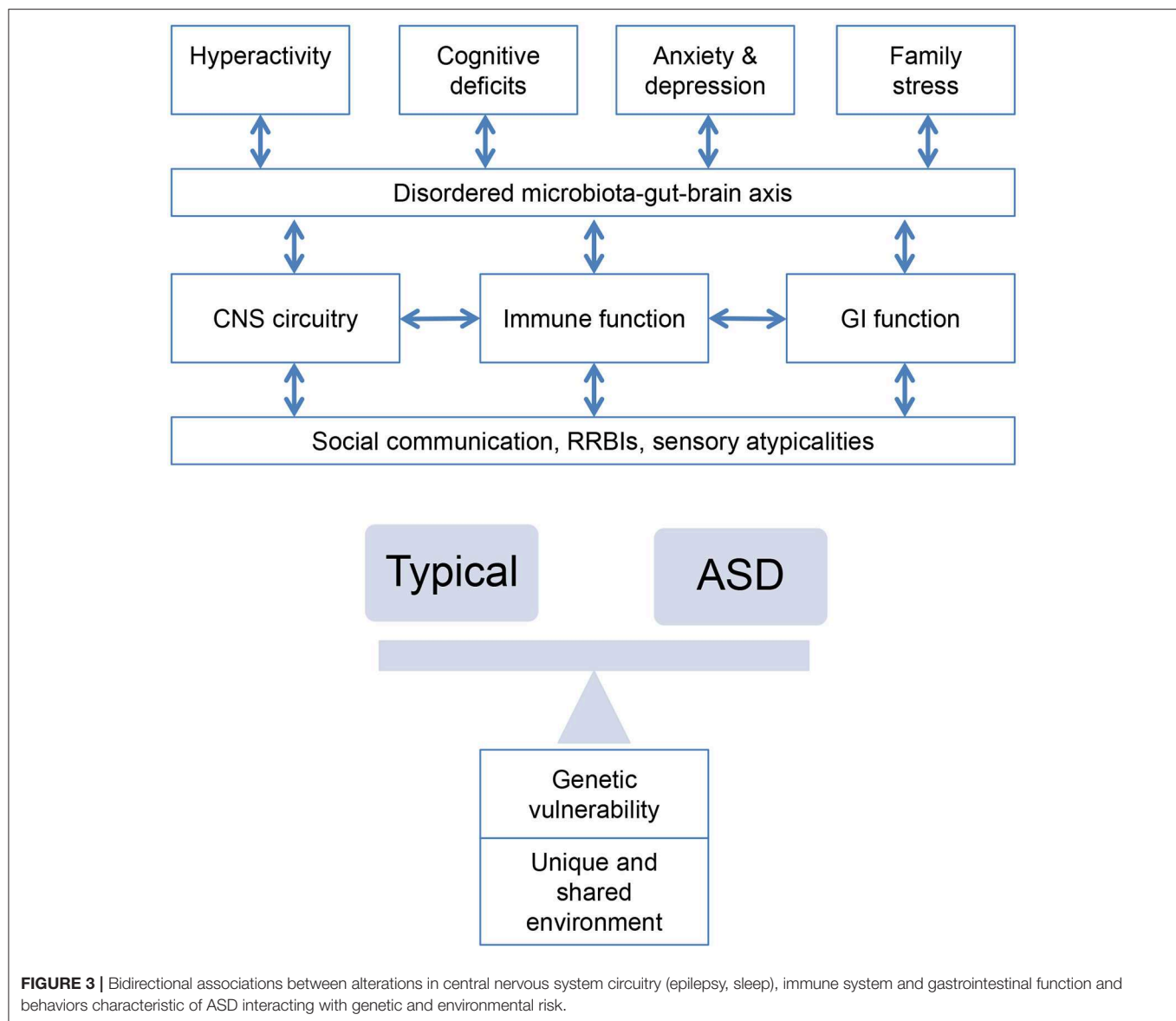
associated with increased rates of later diagnosis of ASD and ADHD, with increased number of atopic conditions associated with stronger likelihood of a later ASD diagnosis (175). Taken together, these studies of medical records or retrospective parental reports support an increased prevalence of specific immune-related conditions, which may constitute an immune-mediated subtype of ASD; see **Table 7**.

Associations With Behavior and Cognition in ASD

An extension of this research is the question of whether changes in peripheral cytokine expression are associated with differences in individual behaviors within individuals with ASD. If so, this would then suggest that interventions that target the immune system might have some benefit in improving symptoms. Chemokine levels have been associated with impaired communication, increased parent-reported behavior problems,

decreased cognitive and adaptive functions, and worse adaptive behavior (176). Increased levels of proinflammatory cytokines (section Aetiological Mechanisms) have been associated with the severity of ASD in a sample of children with ASD in Egypt (177), whilst decreased levels of an immunosuppressant cytokine, transcription growth factor beta 1, significantly predicts worse behavioral symptoms and lower adaptive levels (178). Al-Ayadhi and Mostafa (179) also found that children with more severe ASD symptoms exhibited greater levels of a proinflammatory cytokine compared to children with more milder symptom presentations. More recently, we observed decreased levels of a range of cytokines associated with symptom severity in children with ASD, with differences in cytokine expressions between male and female children (180). There is also some evidence that different presentations of ASD symptoms may be associated with family histories of immune dysfunction; Molloy and colleagues

TABLE 7 | An overview of immune-mediated conditions reported at increased prevalence in individuals with ASD.

Study	Sample size	Conditions	Prevalence
Miyazaki et al. (172)	Meta-analysis; $N = 10$ studies	Atopic dermatitis, asthma, atopic rhinitis, food allergies	Increased rates of asthma (OR 1.66) and atopic rhinitis (OR 1.66) in ASD; no increase in prevalence of food allergies and trend for increase in atopic dermatitis
Chen et al. (171)	$N = 1,598$ ASD, $N = 6,392$ Controls	Type 1 diabetes	Trend for increase in Type 1 diabetes (0.3 vs. 0.1%); OR 4.00
Chen et al. (171)	$N = 1,598$ ASD, $N = 6,392$ Controls	Crohn's disease	Trend for increase in Crohn's disease (1.4 vs. 1.0%); OR 1.46
Chen et al. (171)	$N = 1,598$ ASD, $N = 6,392$ Controls	Urticaria	Increased rates of urticaria (8.4 vs. 6.3%); OR 1.38

reported that familial autoimmune thyroid disease was more common in children who regressed compared to those children with an early onset form of ASD (181). Combined, these results suggest a spectrum of altered inflammatory responses associated with differences in symptom profiles within individuals with ASD.

Treatment Effects

There is some preliminary evidence for cytokine changes in response to medical treatments in ASD. Choi et al. showed that levels of some cytokines significantly reduced after risperidone treatment in children with ASD, and interleukin 5 was specifically increased in a small sample of treatment responders (182). However, others have not observed changes in cytokine serum levels after risperidone treatment compared to placebo in a much larger sample of children (183). A further study demonstrated that anti-inflammatory agents combined with risperidone had a superior effect in treating symptoms in children, although inflammatory responses were not assessed (184). This latter study implies that modulating the inflammatory response in children with ASD may facilitate the efficacy of treatments such as risperidone. This idea has been supported by some historical and case study accounts of modest reductions in the severity of ASD symptoms when treating gastrointestinal inflammation with corticosteroids or antibiotics (185).

Immune-modulatory agents have also been trialed to specifically target ASD symptoms (186). Significant improvements have been described in open-label investigations of corticosteroids (187) and lenalidomide, an immune-modulatory agent (188), as well as double-blind investigations of celecoxib, an anti-inflammatory drug (189). However, varying clinical efficacy and limited randomized double-blind investigations, combined with serious side effects noted in corticosteroids (189), indicates that this area requires further investigation.

Aetiological Mechanisms

The mechanisms by which dysfunctional immune systems may contribute etiologically to ASD is an area of active investigation (190). Here, we briefly outline the main streams of research [see (191) for extensive recent review on aetiological mechanisms of immune dysfunction in ASD]. Several reports of associations

between ASD and family history of autoimmune or immune-mediated disorders have emerged (161, 192, 193). Several high confidence genes identified for ASD converge on pathways important in synapse formation, neuronal migration, and immune function (194, 195). Of relevance here, restriction of HLA genes has been reported as conferring a greater risk for the development of ASD, involved in immune function and also associated with risk for autoimmune conditions (196–199). Located within the large genome regions known as the major histocompatibility complex (MHC) on chromosome 6, several HLA haplotypes appear to be more frequent in children with ASD (200, 201). Some failures to replicate this have suggested that possible genetic differences may lie more generally within this MHC region rather than specifically confined to the HLA genes only (202, 203). For example, the gene coding for the complement protein C4 located in the MHC region is important for innate immunity. Deficiencies in the C4B allele, as well as several other complement proteins, may be differentially produced in some individuals with ASD (204–208). In addition, the MET receptor tyrosine kinase has been associated with ASD (209), which is implicated in both neurodevelopment and immune function.

In addition to examining diagnosed immune conditions, significant research has focused on measurements of cytokine signaling profiles as indicators of broader changes in inflammatory processes in individuals with ASD (17). Cytokines are proteins produced and expressed by neurons that regulate immune responses, including hematopoiesis, inflammation, and immune cell proliferation and differentiation (210). Some cytokines act to make disease worse (proinflammatory) whereas others reduce inflammation and promote healing by suppressing the activity of proinflammatory cytokines (anti-inflammatory). Cytokines also play a role in normal neurodevelopment, including the processes of neuronal migration and synaptic plasticity (211). These processes are tightly regulated and a dysregulation in the balance of signals mediated by cytokines can have a variety of detrimental effects that contribute to changes in neurodevelopment and behavior.

A recent meta-analysis of cytokine levels derived from plasma and serum in unmedicated individuals with ASD (mostly children) found an overall abnormal cytokine profile, characterized by elevations in proinflammatory cytokines and reduced levels of anti-inflammatory cytokines (17). This suggests

that some individuals with ASD may exhibit a heightened inflammatory state and altered cytokine profile, observed in peripheral tissues, suggestive of broader immune system dysregulation in ASD. While elevated levels of inflammatory cytokines observed in the central nervous system of individuals with ASD may reflect inflammatory processes that modulate neuronal function and change behavior (79, 212), altered cytokines in peripheral tissues indicate more widespread inflammatory involvement (213–216). This meta-analysis also observed significant heterogeneity between studies, likely reflecting important methodological differences between studies and the prevalent use of siblings as control subjects for analysis, who are at higher risk of exhibiting broader ASD symptoms themselves (217) or having similar immune system profiles (218).

One well-researched area has explored early maternal infection and inflammation during pregnancy and later risk for an ASD diagnosis (219–225). Pregnancy requires a complex and dynamic response from the maternal immune system to protect the mother from pathogens or infections but to also support the fetal tissue that contains many "non-self" antigens from the father to promote fetal health and development. As this is a complex regulatory system to maintain such balance, this period represents an extremely vulnerable period for both mother and the developing fetus. Disturbances in immune regulation during this period has been well-established to provide a substantial risk factor for alterations in neurodevelopment (226). Incidence of maternal viral and bacterial infections has been proposed as a risk factor for the development of ASD (220, 227–229). Perturbation of the maternal immune system may modify either the placenta or the fetal brain to then later neurodevelopment (230, 231), of which the cytokine IL-6 appears to play a major role (231, 232). Involvement of the maternal immune system during pregnancy does not just appear to influence neurodevelopment, but may also modify ongoing immune dysfunction in offspring (233–236) and later symptom presentation (237). The maternal immune environment has also been proposed as a key factor influencing the increased risk of ASD diagnoses in children born very preterm (226).

Summary

Disruptions in both innate and adaptive immunity have clear consequences for neurodevelopment, with the cumulative evidence suggestive of a disrupted immune profile in for some individuals with ASD as well as links to the early maternal pregnancy environment in shaping later immune profiles and neurodevelopment. There evidence to suggest a direct association between perturbed immune profiles and impact on subsequent behavioral and symptomatic profiles, with limited studies for potential changes in immune profiles in response to treatment or changes in immune functioning through anti-inflammatory markers to facilitate reductions in core ASD symptoms. Taken together, this evidence suggests that an immune-mediated subtype of ASD may be amenable to specific, targeted and/or personalized treatments based on individual immune profiles.

DISCUSSION

Mechanisms of Associations Between Medical Comorbidities and ASD

This review highlights that the interactions between observed comorbid medical conditions and physiological abnormalities in children with ASD are complex. Disorders of GI function and seizures appear to be parallel comorbid conditions, with possible common aetiological mechanisms resulting from yet unknown neurological causes. Sleep disorders, however, are likely to be a consequence of ASD symptoms or may be, in turn, associated with GI symptoms or other comorbidities that can cause sleep disturbances. Whilst the evidence discussed suggests a causative role for metabolic and immunological pathways for some individuals later diagnosed with ASD, evidence for these remains preliminary and based on group level findings, rather than evidence from prospective and longitudinal observations. What is clear, however, is that these systems and pathways do not work in isolation from each other; rather, complex interactions imply dysregulation in any one system may cause a cascade of events cumulating in a cluster of symptoms associated with ASD (**Figure 3**). Because these systems are very complex, and no perturbations in any one appear to be common across individuals with ASD, it is likely that the heterogeneity in ASD reflects the potential myriad of different disturbances along any one of these pathways. Further complicating this model is the high likelihood that any given individual with ASD may also present with multiple medical comorbidities or elevated abnormalities, which also interact with co-occurring behavioral comorbidities, such as hyperactivity and anxiety, and developmental delay (238). Thus, attempts to subgroup individuals based on medical conditions alone may be complicated by the prospects of individuals belonging to multiple subgroups. Importantly, identification of shared or distinct biochemical or neurocognitive mechanisms will be key in elucidating causal pathways.

Integrating Mechanistic Models of ASD: Implications for Etiology and Treatment

As discussed in each section, complicating interactions between these systems suggest a greater likelihood for individuals with alterations in one system to have alterations across multiple systems. Still, the overlap between medical and behavioral features associated with ASD may point toward convergent platforms for a final common pathway to ASD across varied causes, with implications for targeted treatment. For example, children who have sleep problems and ASD are two times more likely to have GI issues and seizures (6).

Review of the proposed aetiological mechanisms underlying the comorbidity between these medical comorbidities and ASD allows some degree of integration. Processes with shared involvement in ASD and multiple medical disorders include gene transcriptional regulation; cellular growth and proliferation; and synapse development, stability and function. Importantly, the potential role of the microbiota-gut-brain axis in multiple elements of medical comorbidity has been implicated (**Figure 3**), whereby short-chain fatty acids can cross the blood-brain

barrier and enter the brain (section Gastrointestinal Dysfunction, **Table 6**), gut microbiota modulate the immune response by stimulating secretion of cytokines and microbiota can deliver signals to the brain via the vagus nerve [see (239) for review]. Beneficial therapeutic effects may be afforded by focusing on the microbiota-gut-brain axis, although more systematic study of its role in ASD is required.

Associations between sleep and other disorders are also apparent; while classically functioning to regulate circadian and seasonal rhythms, melatonin additionally affects cardiovascular and immune systems, regulates body fat mass, insulin secretion, and metabolism of glucose and lipids, as a close derivative of serotonin (5-HT). Due to these additional functions, emerging research has highlighted a role for melatonin administration in humans to attenuate metabolic symptoms induced by antipsychotic use (240) and to potentially improve GI functioning via effects on intestinal permeability (241). Given additional physiological functions of melatonin, effective supplementation may have secondary effects in improving GI functioning in ASD; however there has not been any systematic investigation into this as yet.

Similarly, comorbid medical conditions and associated medication use may result in sleep disturbance; for example, physical symptoms such as abdominal pain in some GI disorders, or medications prescribed for seizures can cause difficulty sleeping. Children with epilepsy also exhibit significant alterations in sleep latency, sleep efficiency, and number of awakenings (242), and epilepsy may be characterized by seizures during sleep (e.g., Landau-Kleffner). It is therefore of critical importance to determine whether sleep problems are better explained by associated medical conditions which should be treated rather than attempting to address sleep difficulties alone (100).

Finally, alterations in immune system profiles, dysregulated gastrointestinal symptoms and disordered sleep patterns clearly impact upon behavioral profiles, including elevated anxiety, increased social and communication difficulties, reduced adaptive functioning, and increased maladaptive behaviors. Recent meta-analytic evidence confirms an association between sleep disturbances and increased inflammation, suggesting that improvements in one system could potentially positively impact upon other regulatory systems (243). Generating evidence for such hypotheses require large and detailed biological datasets from children with ASD across their developmental course [e.g., (244–246).

A key aim for future research will be to examine the interactions between these systems using established biomarkers along their pathways within ASD, rather than examining each in isolation (**Box 1**). Possible networks of disturbances may then be mapped out to determine potential subgroups of individuals with ASD classified by patterns of abnormal biological profiles. A second question of interest will be how modulation of these biological substrates through targeted pharmacological interventions may affect core ASD symptoms (247). Initial promising findings in small sampled trials suggest some efficacy for such approaches. However, if subgroups of individuals with specific biological profiles exist along the spectrum, then

---

Box 1 | Future directions and common themes.

- Using expertise from a diverse range of disciplines—genetics, neuroscience, biochemistry and developmental psychology—to disentangle biological mechanisms underpinning medical comorbidity.
- Improved understanding of the link between comorbid medical conditions and co-occurring mental health problems and psychopathology in ASD.
- Understanding the longitudinal course of the full range of medical comorbidities in ASD.
- Treatment and intervention studies to systematically assess whether treating medical issues has additional positive effects on other medical issues (e.g., sleep) or on behavioral domains.

---

interventions must be appropriately targeted for individuals with certain profiles, rather than a universal approach. Lastly, the true efficacy of such targeted interventions will lie in the use of robust and sensitive biomarkers to determine treatment response, rather than a sole reliance on parent-reported and observational outcomes.

An important consideration is the time at which symptoms or markers are assessed. As a developmental condition, the symptoms of ASD change over the course of the child's lifespan, and there is increasing acknowledgment for the importance in understanding an individual's trajectory over time to grasp the full complexity of the heterogeneous symptoms presentations within ASD (248). Current physiological states are dependent on history of previously received interventions and past characteristics, such as regression, that may later affect developmental trajectories. In addition, there are likely to be sensitive periods within development where particular comorbidities may be more apparent or exhibit higher risk; for example, sensitive periods for prevalence of epilepsy-related disorders in childhood (249). Prospective longitudinal studies are required to systematically measure medical disorders and biomarkers within cohorts of individuals diagnosed or with clinical risk indicators of ASD. This may then provide sufficient power to identify subgroups of children with different clusters of symptoms that converge on similar pathways. Likewise, a developmental perspective will provide insight on the nature of the association with health conditions as a resulting or co-occurring model.

Clinical Implications

Assessment of co-occurring medical problems is of critical importance in the initial diagnostic procedure for individuals with ASD as well as in ongoing treatment management. As part of a multidisciplinary approach, systematic and evidence-based screening should be recommended at the time of diagnostic evaluations as well as during ongoing health monitoring. A key conclusion from this review is the importance of monitoring and maintaining general health and wellbeing in individuals with ASD. Although many of the medical conditions or abnormalities may not be present in many individuals with ASD, the overall increased risk of all-cause mortality

in this population indicates a need for increased vigilance. For example, comorbid epilepsy, particularly accompanied by intellectual disability, is associated with an increased mortality risk; thus, there is a significant imperative for clinicians and parents/careers to provide additional care and monitoring around overall health and wellbeing for these individuals to potentially attenuate this risk. Cumulative evidence highlights increased prevalence of obesity and poor diet in individuals with ASD, and decreased use of general health services, such as for oral health, due to barriers to accessing health care that supports individuals with special needs. There is also a critical need for healthcare services to provide supportive and accessible environments for individuals with ASD.

The burden of such medical conditions in increasing mortality is likely compounded by impaired communication and increased sensory sensitivities that present a significant barrier to the delivery of health care services (both preventative and targeted treatment). A growing area of research has therefore focused on the necessity for effective and targeted health care services that are designed for ASD, as well as identifying potential barriers to access and use (250). Appropriately delivered health care services that can manage difficulties in communication and sensory sensitivities, particularly those that are pre-emptive, are likely to have benefits beyond just medical treatment but may drastically improve the quality of life for both the individual and their caregiver(s) and potentially decrease long-term financial burden. Despite higher health care utilization in this population, the decreased ability for the health care system to effectively manage behavioral symptoms, impaired communication, and specific sensory issues is a significant barrier for most preventative health care, especially for those on the severe end of the spectrum.

CONCLUSION

The presence of comorbid medical conditions in ASD highlights the vast heterogeneity within the disorder. Using a model-based approach to understand the interacting systems potentially involved in the etiology and symptoms of ASD may lead to hypothesis generation and potential avenues for clinical trials. We require a better understanding of how variability in these systems results in similar or different functional profiles in ASD, and more integrative studies that consider the interaction between systems and the environment to produce behaviors characteristic of ASD. Such an approach can then lend hope to more specific biomedical treatments aimed at targeted these biomedical abnormalities and improving core and associated symptoms in ASD, ultimately to improve long-term outcomes.

AUTHOR CONTRIBUTIONS

CT and GA led in the conception and design of the review. CT, AR, and GA drafted the paper. AW provided substantial revision.

ACKNOWLEDGMENTS

REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 5th edn. Arlington, VA: American Psychiatric Publishing (2013). doi: 10.1176/appi.books.9780890425596

2. World Health Organization. *The ICD-10 Classification of Mental and Behavioural Disorders: Clinical Descriptions and Diagnostic Guidelines.* Geneva: World Health Organization (1993).

3. Bourgeron T. From the genetic architecture to synaptic plasticity in autism spectrum disorder. *Nat Rev Neurosci.* (2015) 16:551–63. doi: 10.1038/nrn3992

4. Kohane IS, McMurry A, Weber G, MacFadden D, Rappaport L, Kunkel L, et al. The co-morbidity burden of children and young adults with autism spectrum disorders. *PLoS ONE* (2012) 7:e33224. doi: 10.1371/journal.pone.0033224

5. Doshi-Velez F, Ge Y, Kohane I. Comorbidity clusters in autism spectrum disorders: an electronic health record time-series analysis. *Pediatrics* (2014) 133:e54–63. doi: 10.1542/peds.2013-0819

6. Aldinger KA, Lane CJ, Veenstra-VanderWeele J, Levitt P. Patterns of risk for multiple co-occurring medical conditions replicate across distinct cohorts of children with autism spectrum disorder. *Autism Res.* (2015) 8:771–81. doi: 10.1002/aur.1492

7. Buie T, Campbell DB, Fuchs GJ, Furuta GT, Levy J, VandeWater J, et al. Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. *Pediatrics* (2010) 125(Suppl. 1):S1–18. doi: 10.1542/peds.2009-1878C

8. Malow BA, Byars K, Johnson K, Weiss S, Bernal P, Goldman SE, et al. A practice pathway for the identification, evaluation, and management of insomnia in children and adolescents with autism spectrum disorders. *Pediatrics* (2012) 130(Suppl. 2):S106–24. doi: 10.1542/peds.2012-0900I

9. Isaksen J, Bryn V, Diseth TH, Heiberg A, Schjølberg S, Skjeldal OH. Children with autism spectrum disorders–the importance of medical investigations. *Eur J Paediatr Neurol.* (2013) 17:68–76. doi: 10.1016/j.ejpn.2012.08.004

10. Shavelle RM, Strauss DJ, Pickett J. Causes of death in autism. *J Autism Dev Disord.* (2001) 31:569–76. doi: 10.1023/A:1013247011483

11. Gillberg C, Billstedt E, Sundh V, Gillberg IC. Mortality in autism: a prospective longitudinal community-based study. *J Autism Dev Disord.* (2010) 40:352–7. doi: 10.1007/s10803-009-0883-4

12. Mouridsen SE, Brønnum-Hansen H, Rich B, Isager T. Mortality and causes of death in autism spectrum disorders: an update. *Autism* (2008) 12:403–14. doi: 10.1177/1362361308091653

13. Hirvikoski T, Mittendorfer-Rutz E, Boman M, Larsson H, Lichtenstein P, Bölte S. Premature mortality in autism spectrum disorder. *Br J Psychiatry* (2016) 208:232–8. doi: 10.1192/bjp.bp.114.160192

14. Holingue C, Newill C, Lee L, Pasricha PJ, Daniele Fallin M. Gastrointestinal symptoms in autism spectrum disorder: a review of the literature on ascertainment and prevalence. *Autism Res.* (2018) 11:24–36. doi: 10.1002/aur.1854

15. Cohen S, Conduit R, Lockley SW, Rajaratnam SMW, Cornish KM. The relationship between sleep and behavior in autism spectrum disorder (ASD): a review. *J Neurodev Disord.* (2014) 6:44. doi: 10.1186/1866-1955-6-44

16. Jeste SS, Tuchman R. Autism spectrum disorder and epilepsy: two sides of the same coin? *J Child Neurol.* (2015) 30:1963–71. doi: 10.1177/0883073815601501

17. Masi A, Quintana DS, Glozier N, Lloyd AR, Hickie IB, Guastella AJ. Cytokine aberrations in autism spectrum disorder: a systematic review and meta-analysis. *Mol Psychiatry* (2014) 20:440–6. doi: 10.1038/mp.2014.59

18. Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G. Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. *J Am Acad Child Adolesc Psychiatry* (2008) 47:921–9. doi: 10.1097/CHI.0b013e318179964f

19. Leyfer OT, Folstein SE, Bacalman S, Davis NO, Dinh E, Morgan J, et al. Comorbid psychiatric disorders in children with autism: interview development and rates of disorders. *J Autism Dev Disord.* (2006) 36:849–61. doi: 10.1007/s10803-006-0123-0

20. Rommelse NNJ, Geurts HM, Franke B, Buitelaar JK, Hartman CA. A review on cognitive and brain endophenotypes that may be common in autism spectrum disorder and attention-deficit/hyperactivity disorder and facilitate the search for pleiotropic genes. *Neurosci Biobehav Rev.* (2011) 35:1363–96. doi: 10.1016/j.neubiorev.2011.02.015

21. Richards C, Jones C, Groves L, Moss J, Oliver C. Prevalence of autism spectrum disorder phenomenology in genetic disorders: a systematic review and meta-analysis. *Lancet Psychiatry* (2015) 2:909–16. doi: 10.1016/S2215-0366(15)00376-4

22. Tye C, Varcin KJ, Bolton P, Jeste SS. Early developmental pathways to autism spectrum disorder in tuberous sclerosis complex. *Advances in Autism* (2016) 2:84–93. doi: 10.1108/AIA-01-2016-0004

23. Guerrini R. Epilepsy in children. *Lancet* (2006) 367:499–524. doi: 10.1016/S0140-6736(06)68182-8

24. Spence SJ, Schneider MT. The role of epilepsy and epileptiform EEGs in autism spectrum disorders. *Pediatr Res.* (2009) 65:599–606. doi: 10.1203/PDR.0b013e31819e7168

25. Tuchman R, Cuccaro M. Epilepsy and autism: neurodevelopmental perspective. *Curr Neurol Neurosci Rep.* (2011) 11:428–34. doi: 10.1007/s11910-011-0195-x

26. Hara H. Autism and epilepsy: a retrospective follow-up study. *Brain Dev.* (2007) 29:486–90. doi: 10.1016/j.braindev.2006.12.012

27. Parmeggiani A, Barcia G, Posar A, Raimondi E, Santucci M, Scaduto MC. Epilepsy and EEG paroxysmal abnormalities in autism spectrum disorders. *Brain Dev.* (2010) 32:783–9. doi: 10.1016/j.braindev.2010.07.003

28. Danielsson S, Gillberg IC, Billstedt E, Gillberg C, Olsson I. Epilepsy in young adults with autism: a prospective population-based follow-up study of 120 individuals diagnosed in childhood. *Epilepsia* (2005) 46:918–23. doi: 10.1111/j.1528-1167.2005.57504.x

29. Strasser L, Downes M, Kung J, Cross JH, De Haan M. Prevalence and risk factors for autism spectrum disorder in epilepsy: a systematic review and meta-analysis. *Dev Med Child Neurol.* (2018) 60:19–29. doi: 10.1111/dmcn.13598

30. Jeste SS, Sahin M, Bolton P, Ploubidis GB, Humphrey A. Characterization of autism in young children with tuberous sclerosis complex. *J Child Neurol.* (2008) 23:520–5. doi: 10.1177/0883073807309788

31. Wong V. Study of the relationship between tuberous sclerosis complex and autistic disorder. *J Child Neurol.* (2006) 21:199–204. doi: 10.2310/7010.2006.00046

32. Chez MG, Chang M, Krasne V, Coughlan C, Kominsky M, Schwartz A. Frequency of epileptiform EEG abnormalities in a sequential screening of autistic patients with no known clinical epilepsy from 1996 to 2005. *Epilepsy Behav.* (2006) 8:267–71. doi: 10.1016/j.yebeh.2005.11.001

33. Kim HL, Donnelly JH, Tournay AE, Book TM, Filipek P. Absence of seizures despite high prevalence of epileptiform EEG abnormalities in children with autism monitored in a tertiary care center. *Epilepsia* (2006) 47:394–8. doi: 10.1111/j.1528-1167.2006.00434.x

34. Yasuhara A. Correlation between EEG abnormalities and symptoms of autism spectrum disorder (ASD). *Brain Dev.* (2010) 32:791–8. doi: 10.1016/j.braindev.2010.08.010

35. Steffenburg S, Gillberg C, Steffenburg U. Psychiatric disorders in children and adolescents with mental retardation and active epilepsy. *Arch Neurol.* (1996) 53:904–12. doi: 10.1001/archneur.1996.00550090114017

36. Tuchman RF, Rapin I, Shinnar S. Autistic and dysphasic children. II: Epilepsy. *Pediatrics* (1991) 88:1219–25.

37. Matsuo M, Maeda T, Sasaki K, Ishii K, Hamasaki Y. Frequent association of autism spectrum disorder in patients with childhood onset epilepsy. *Brain Dev.* (2010) 32:759–63. doi: 10.1016/j.braindev.2010.05.005

38. Sansa G, Carlson C, Doyle W, Weiner HL, Bluvstein J, Barr W, et al. Medically refractory epilepsy in autism. *Epilepsia* (2011) 52:1071–5. doi: 10.1111/j.1528-1167.2011.03069.x

39. Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE commission on classification and terminology, 2005–2009. *Epilepsia* (2010) 51:676–85. doi: 10.1111/j.1528-1167.2010.02522.x

40. Srivastava S, Sahin M. Autism spectrum disorder and epileptic encephalopathy: common causes, many questions. *J Neurodev Disord.* (2017) 9:23. doi: 10.1186/s11689-017-9202-0

41. Tuchman R, Moshé SL, Rapin I. Convulsing toward the pathophysiology of autism. *Brain Dev.* (2009) 31:95–103. doi: 10.1016/j.braindev.2008.09.009

42. Mouridsen SE, Rich B, Isager T. A longitudinal study of epilepsy and other central nervous system diseases in individuals with and without a history of infantile autism. *Brain Dev.* (2011) 33:361–6. doi: 10.1016/j.braindev.2010.07.002

43. Turk J, Bax M, Williams C, Amin P, Eriksson M, Gillberg C. Autism spectrum disorder in children with and without epilepsy: impact on social functioning and communication. *Acta Paediatr.* (2009) 98:675–81. doi: 10.1111/j.1651-2227.2008.01184.x

44. Cuccaro ML, Tuchman RF, Hamilton KL, Wright HH, Abramson RK, Haines JL, et al. Exploring the relationship between autism spectrum disorder and epilepsy using latent class cluster analysis. *J Autism Dev Disord.* (2012) 42:1630–41. doi: 10.1007/s10803-011-1402-y

45. Viscidi EW, Johnson AL, Spence SJ, Buka SL, Morrow EM, Triche EW. The association between epilepsy and autism symptoms and maladaptive behaviors in children with autism spectrum disorder. *Autism* (2014) 18:996–1006. doi: 10.1177/1362361313508027

46. Richard AE, Scheffer IE, Wilson SJ. Features of the broader autism phenotype in people with epilepsy support shared mechanisms between epilepsy and autism spectrum disorder. *Neurosci Biobehav Rev.* (2017) 75:203–33. doi: 10.1016/j.neubiorev.2016.12.036

47. Amiet C, Gourfinkel-An I, Bouzamondo A, Tordjman S, Baulac M, Lechat P, et al. Epilepsy in autism is associated with intellectual disability and gender: evidence from a meta-analysis. *Biol Psychiatry* (2008) 64:577–82. doi: 10.1016/j.biopsych.2008.04.030

48. Woolfenden SUE, Sarkozy V, Ridley G, Coory M, Williams K. A systematic review of two outcomes in autism spectrum disorder–epilepsy and mortality. *Dev Med Child Neurol.* (2012) 54:306–12. doi: 10.1111/j.1469-8749.2012.04223.x

49. Hrdlicka M. EEG abnormalities, epilepsy and regression in autism: a review. *Neuro Endocrinol Lett.* (2008) 29:405–9.

50. Giannotti F, Cortesi F, Cerquiglini A, Miraglia D, Vagnoni C, Sebastiani T, et al. An investigation of sleep characteristics, EEG abnormalities and epilepsy in developmentally regressed and non-regressed children with autism. *J Autism Dev Disord.* (2008) 38:1888–97. doi: 10.1007/s10803-008-0584-4

51. Oslejskova H, Dusek L, Makovska Z, Pejcochova J, Autrata R, Slapak I. Complicated relationship between autism with regression and epilepsy. *Neuro Endocrinol Lett.* (2008) 29:558–70.

52. Berg AT, Plioplys S. Epilepsy and autism: is there a special relationship? *Epilepsy Behav.* (2012) 23:193–8. doi: 10.1016/j.yebeh.2012.01.015

53. Humphrey A, Neville BGR, Clarke A, Bolton PF. Autistic regression associated with seizure onset in an infant with tuberous sclerosis. *Dev Med Child Neurol.* (2006) 48:609–11. doi: 10.1017/S0012162206001277

54. Tuchman R, Alessandri M, Cuccaro M. Autism spectrum disorders and epilepsy: moving towards a comprehensive approach to treatment. *Brain Dev.* (2010) 32:719–30. doi: 10.1016/j.braindev.2010.05.007

55. Shinnar S, Pellock JM. Update on the epidemiology and prognosis of pediatric epilepsy. *J Child Neurol.* (2002) 17(Suppl. 1):S4–17. doi: 10.1177/08830738020170010201

56. Bolton PF, Carcani-Rathwell I, Hutton J, Goode S, Howlin P, Rutter M. Epilepsy in autism: features and correlates. *Br J Psychiatry* (2011) 198:289–94. doi: 10.1192/bjp.bp.109.076877

57. Hartley-McAndrew M, Weinstock A. Autism spectrum disorder: correlation between aberrant behaviors, EEG abnormalities and seizures. *Neurol Int.* (2010) 2:e10. doi: 10.4081/ni.2010.e10

58. Frye RE, Sreenivasula S, Adams JB. Traditional and non-traditional treatments for autism spectrum disorder with seizures: an on-line survey. *BMC Pediatr.* (2011) 11:37. doi: 10.1186/1471-2431-11-37

59. Kilincaslan A, Kok BE, Tekturk P, Yalcinkaya C, Ozkara C, Yapici Z. Beneficial effects of everolimus on autism and attention-deficit/hyperactivity disorder symptoms in a group of patients with tuberous sclerosis complex. *J Child Adolesc Psychopharmacol.* (2017) 27:383–8. doi: 10.1089/cap.2016.0100

60. Krueger DA, Sadhwani A, Byars AW, de Vries PJ, Franz DN, Whittemore VH, et al. Everolimus for treatment of tuberous sclerosis complex-associated neuropsychiatric disorders. *Ann Clin Transl Neurol.* (2017) 4:877–87. doi: 10.1002/acn3.494

61. Bombardieri R, Pinci M, Moavero R, Cerminara C, Curatolo P. Early control of seizures improves long-term outcome in children with tuberous sclerosis complex. *Eur J Paediatr Neurol.* (2010) 14:146–9. doi: 10.1016/j.ejpn.2009.03.003

62. Di Martino A, Tuchman RF. Antiepileptic drugs: affective use in autism spectrum disorder. *Pediatr Neurol.* (2001) 25:199–207. doi: 10.1016/S0887-8994(01)00276-4

63. Krueger DA, Care MM, Holland K, Agricola K, Tudor C, Mangeshkar P, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med.* (2010) 363:1801–11. doi: 10.1056/NEJMoa1001671

64. Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Marchi E, et al. The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. *Acta Neuropathol.* (2010) 119:755–70. doi: 10.1007/s00401-010-0655-4

65. Gilby KL, O'Brien TJ. Epilepsy, autism, and neurodevelopment: kindling a shared vulnerability? *Epilepsy Behav.* (2013) 26:370–4. doi: 10.1016/j.yebeh.2012.11.002

66. Lo-Castro A, Curatolo P. Epilepsy associated with autism and attention deficit hyperactivity disorder: is there a genetic link? *Brain Dev.* (2014) 36:185–93. doi: 10.1016/j.braindev.2013.04.013

67. Tuchman R. Autism and social cognition in epilepsy: implications for comprehensive epilepsy care. *Curr Opin Neurol.* (2013) 26:214–8. doi: 10.1097/WCO.0b013e32835ee64f

68. Mefford HC, Batshaw ML, Hoffman EP. Genomics, intellectual disability, and autism. *N Engl J Med.* (2012) 366:733–43. doi: 10.1056/NEJMra1114194

69. Lesca G, Rudolf G, Labalme A, Hirsch E, Arzimanoglou A, Genton P, et al. Epileptic encephalopathies of the Landau-Kleffner and continuous spike and waves during slow-wave sleep types: genomic dissection makes the link with autism. *Epilepsia* (2012) 53:1526–38. doi: 10.1111/j.1528-1167.2012.03559.x

70. Bolton PF, Park RJ, Higgins JNP, Griffiths PD, Pickles A. Neuro-epileptic determinants of autism spectrum disorders in tuberous sclerosis complex. *Brain* (2002) 125:1247–55. doi: 10.1093/brain/awf124

71. Wolff M, Cassé-Perrot C, Dravet C. Severe myoclonic epilepsy of infants (Dravet syndrome): natural history and neuropsychological findings. *Epilepsia* (2006) 47:45–8. doi: 10.1111/j.1528-1167.2006.00688.x

72. Trillingsgaard A, Østergaard JR. Autism in Angelman syndrome: an exploration of comorbidity. *Autism* (2004) 8:163–74. doi: 10.1177/1362361304042720

73. Tye C, Thomas LE, Sampson JR, Lewis J, O'Callaghan F, Yates JRW, et al. Secular changes in severity of intellectual disability in tuberous sclerosis complex: a reflection of improved identification and treatment of epileptic spasms? *Epilepsia Open.* (2018) 3:276–80. doi: 10.1002/epi4.12111

74. Dravet C. The core Dravet syndrome phenotype. *Epilepsia* (2011) 52:3–9. doi: 10.1111/j.1528-1167.2011.02994.x

75. Al Ageeli E, Drunat S, Delanoë C, Perrin L, Baumann C, Capri Y, et al. Duplication of the 15q11-q13 region: clinical and genetic study of 30 new cases. *Eur J Med Genet.* (2014) 57:5–14. doi: 10.1016/j.ejmg.2013.10.008

76. Lee BH, Smith T, Paciorkowski AR. Autism spectrum disorder and epilepsy: disorders with a shared biology. *Epilepsy Behav.* (2015) 47:191–201. doi: 10.1016/j.yebeh.2015.03.017

77. Jacob J. Cortical interneuron dysfunction in epilepsy associated with autism spectrum disorders. *Epilepsia* (2016) 57:182–93. doi: 10.1111/epi.13272

78. Vestergaard M, Mork A, Madsen KM, Olsen J. Paternal age and epilepsy in the offspring. *Eur J Epidemiol.* (2005) 20:1003–5. doi: 10.1007/s10654-005-4250-2

79. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol Off J Am Neurol Assoc Child Neurol Soc.* (2005) 57:67–81. doi: 10.1002/ana.20315

80. Vezzani A, Aronica E, Mazarati A, Pittman QJ. Epilepsy and brain inflammation. *Exp Neurol.* (2013) 244:11–21. doi: 10.1016/j.expneurol.2011.09.033

81. Krakowiak P, Goodlin-Jones B, Hertz-Picciotto I, Croen LA, Hansen RL. Sleep problems in children with autism spectrum disorders, developmental delays, and typical development: a population-based study. *J Sleep Res.* (2008) 17:197–206. doi: 10.1111/j.1365-2869.2008.00650.x

82. Liu X, Hubbard JA, Fabes RA, Adam JB. Sleep disturbances and correlates of children with autism spectrum disorders. *Child Psychiatry Hum Dev.* (2006) 37:179–91. doi: 10.1007/s10578-006-0028-3

83. Polimeni MA, Richdale AL, Francis AJ. A survey of sleep problems in autism, Asperger's disorder and typically developing children. *J Intellect Disabil Res.* (2005) 49(Pt 4):260–8. doi: 10.1111/j.1365-2788.2005.00642.x

84. Wiggs L, Stores G. Sleep patterns and sleep disorders in children with autistic spectrum disorders: insights using parent report and actigraphy. *Dev Med Child Neurol.* (2004) 46:372–80. doi: 10.1017/S0012162204000611

85. Richdale AL, Schreck KA. Sleep problems in autism spectrum disorders: prevalence, nature, & possible biopsychosocial aetiologies. *Sleep Med Rev.* (2009) 13:403–11. doi: 10.1016/j.smrv.2009.02.003

86. Goldman SE, Richdale AL, Clemons T, Malow BA. Parental sleep concerns in autism spectrum disorders: variations from childhood to adolescence. *J Autism Dev Disord.* (2012) 42:531–8. doi: 10.1007/s10803-011-1270-5

87. Souders MC, Mason TB, Valladares O, Bucan M, Levy SE, Mandell DS, et al. Sleep behaviors and sleep quality in children with autism spectrum disorders. *Sleep* (2009) 32:1566–78. doi: 10.1093/sleep/32.12.1566

88. Buckley AW, Rodriguez AJ, Jennison K, Buckley J, Thurm A, Sato S, et al. Rapid eye movement sleep percentage in children with autism compared with children with developmental delay and typical development. *Arch Pediatr Adolesc Med.* (2010) 164:1032–7. doi: 10.1001/archpediatrics.2010.202

89. Lipton J, Becker RE, Kothare S V. Insomnia of childhood. *Curr Opin Pediatr.* (2008) 20:641–9. doi: 10.1097/MOP.0b013e32831897cb

90. Baker E, Richdale A, Short M, Gradisar M. An investigation of sleep patterns in adolescents with high-functioning autism spectrum disorder compared with typically developing adolescents. *Dev Neurorehabil.* (2013) 16:155–65. doi: 10.3109/17518423.2013.765518

91. Richdale AL, Baker E, Short M, Gradisar M. The role of insomnia, pre-sleep arousal and psychopathology symptoms in daytime impairment in adolescents with high-functioning autism spectrum disorder. *Sleep Med.* (2014) 15:1082–8. doi: 10.1016/j.sleep.2014.05.005

92. Hoffman CD, Sweeney DP, Gilliam JE, Apodaca DD, Lopez-Wagner MC, Castillo MM. Sleep problems and symptomology in children with autism. *Focus Autism Other Dev Disabl.* (2005) 20:194–200. doi: 10.1177/10883576050200040101

93. Schreck KA, Mulick JA, Smith AF. Sleep problems as possible predictors of intensified symptoms of autism. *Res Dev Disabil.* (2004) 25:57–66. doi: 10.1016/j.ridd.2003.04.007

94. Malow BA, Marzec ML, McGrew SG, Wang L, Henderson LM, Stone WL. Characterizing sleep in children with autism spectrum disorders: a multidimensional approach. *Sleep* (2006) 29:1563–71. doi: 10.1093/sleep/29.12.1563

95. Veatch OJ, Sutcliffe JS, Warren ZE, Keenan BT, Potter MH, Malow BA. Shorter sleep duration is associated with social impairment and comorbidities in ASD. *Autism Res.* (2017) 10:1221–38. doi: 10.1002/aur.1765

96. Tudor ME, Hoffman CD, Sweeney DP. Children with autism: sleep problems and symptom severity. *Focus Autism Other Dev Disabl.* (2012) 27:254–62. doi: 10.1177/1088357612457989

97. Giannotti F, Cortesi F, Cerquiglini A, Vagnoni C, Valente D. Sleep in children with and without autistic regression. *J Sleep Res.* (2011) 20:338–47. doi: 10.1111/j.1365-2869.2010.00882.x

98. Cohen S, Fulcher BD, Rajaratnam SMW, Conduit R, Sullivan JP, St Hilaire MA, et al. Behaviorally-determined sleep phenotypes are robustly associated with adaptive functioning in individuals with low functioning autism. *Sci Rep.* (2017) 7:14228. doi: 10.1038/s41598-017-14611-6

99. Cohen S, Fulcher BD, Rajaratnam SMW, Conduit R, Sullivan JP, St Hilaire MA, et al. Sleep patterns predictive of daytime challenging behavior in individuals with low-functioning autism. *Autism Res.* (2018) 11:391–403. doi: 10.1002/aur.1899

100. Reynolds AM, Malow BA. Sleep and autism spectrum disorders. *Pediatr Clin North Am.* (2011) 58:685–98. doi: 10.1016/j.pcl.2011.03.009

101. Wallace ML, McMakin DL, Tan PZ, Rosen D, Forbes EE, Ladouceur CD, et al. The role of day-to-day emotions, sleep, and social interactions in pediatric anxiety treatment. *Behav Res Ther.* (2017) 90:87–95. doi: 10.1016/j.brat.2016.12.012

102. Tani P, Lindberg N, Nieminen-von Wendt T, von Wendt L, Virkkala J, Appelberg B, et al. Sleep in young adults with Asperger syndrome. *Neuropsychobiology* (2004) 50:147–52. doi: 10.1159/000079106

103. Bruni O, Ferri R, Vittori E, Novelli L, Vignati M, Porfirio MC, et al. Sleep architecture and NREM alterations in children and adolescents with Asperger syndrome. *Sleep* (2007) 30:1577–85. doi: 10.1093/sleep/30.11.1577

104. Paavonen EJ, Nieminen-von Wendt T, Vanhala R, Aronen ET, von Wendt L. Effectiveness of melatonin in the treatment of sleep disturbances in children with Asperger disorder. *J Child Adolesc Psychopharmacol.* (2003) 13:83–95. doi: 10.1089/104454603321666225

105. Antshel KM, Zhang-James Y, Wagner KE, Ledesma A, Faraone S V. An update on the comorbidity of ADHD and ASD: a focus on clinical management. *Expert Rev Neurother.* (2016) 16:279–93. doi: 10.1586/14737175.2016.1146591

106. Goldman SE, Surdyka K, Cuevas R, Adkins K, Wang L, Malow BA. Defining the sleep phenotype in children with autism. *Dev Neuropsychol.* (2009) 34:560–73. doi: 10.1080/87565640903133509

107. Rossignol DA, Frye RE. Melatonin in autism spectrum disorders: a systematic review and meta-analysis. *Dev Med Child Neurol.* (2011) 53:783–92. doi: 10.1111/j.1469-8749.2011.03980.x

108. Gringras P, Findling RL, Nir T, Zisapel N. Short and long term prolonged release melatonin treatment for sleep disorders in children with autism spectrum disorders: results of a phase III randomized clinical trial. *Sleep Med.* (2017) 40:e119. doi: 10.1016/j.sleep.2017.11.347

109. Gujar N, McDonald SA, Nishida M, Walker MP. A role for REM sleep in recalibrating the sensitivity of the human brain to specific emotions. *Cereb Cortex* (2011) 21:115–23. doi: 10.1093/cercor/bhq064

110. van der Helm E, Gujar N, Walker MP. Sleep deprivation impairs the accurate recognition of human emotions. *Sleep* (2010) 33:335–42. doi: 10.1093/sleep/33.3.335

111. Hollway JA, Aman MG. Pharmacological treatment of sleep disturbance in developmental disabilities: a review of the literature. *Res Dev Disabil.* (2011) 32:939–62. doi: 10.1016/j.ridd.2010.12.035

112. Maayan L, Correll CU. Weight gain and metabolic risks associated with antipsychotic medications in children and adolescents. *J Child Adolesc Psychopharmacol.* (2011) 21:517–35. doi: 10.1089/cap.2011.0015

113. Broder-Fingert S, Brazauskas K, Lindgren K, Iannuzzi D, Van Cleave J. Prevalence of overweight and obesity in a large clinical sample of children with autism. *Acad Pediatr.* (2014) 14:408–14. doi: 10.1016/j.acap.2014.04.004

114. Charrier A, Olliac B, Roubertoux P, Tordjman S. Clock genes and altered sleep–wake rhythms: their role in the development of psychiatric disorders. *Int J Mol Sci.* (2017) 18:938. doi: 10.3390/ijms18050938

115. Pizzarelli R, Cherubini E. Alterations of GABAergic signaling in autism spectrum disorders. *Neural Plast.* (2011) 2011:297153. doi: 10.1155/2011/297153

116. Melke J, Botros HG, Chaste P, Betancur C, Nygren G, Anckarsäter H, et al. Abnormal melatonin synthesis in autism spectrum disorders. *Mol Psychiatry* (2007) 13:90–8. doi: 10.1038/sj.mp.4002016

117. Bourgeron T. The possible interplay of synaptic and clock genes in autism spectrum disorders. *Cold Spring Harb Symp Quant Biol.* (2007) 72:645–54. doi: 10.1101/sqb.2007.72.020

118. Yang Z, Matsumoto A, Nakayama K, Jimbo EF, Kojima K, Nagata K, et al. Circadian-relevant genes are highly polymorphic in autism spectrum disorder patients. *Brain Dev.* (2016) 38:91–9. doi: 10.1016/j.braindev.2015.04.006

119. Ebisawa T. Circadian rhythms in the CNS and peripheral clock disorders: human sleep disorders and clock genes. *J Pharmacol Sci.* (2007) 103:150–4. doi: 10.1254/jphs.FMJ06003X5

120. Nicholas B, Rudrasingham V, Nash S, Kirov G, Owen MJ, Wimpory DC. Association of Per1 and Npas2 with autistic disorder: support for the clock genes/social timing hypothesis. *Mol Psychiatry* (2007) 12:581. doi: 10.1038/sj.mp.4001953

121. Glickman G. Circadian rhythms and sleep in children with autism. *Neurosci Biobehav Rev.* (2010) 34:755–68. doi: 10.1016/j.neubiorev.2009.11.017

122. Campbell DJ, Chang J, Chawarska K. Early generalized overgrowth in autism spectrum disorder: prevalence rates, gender effects, and clinical outcomes. *J Am Acad Child Adolesc Psychiatry* (2014) 53:1063–73. doi: 10.1016/j.jaac.2014.07.008

123. Coury DL, Ashwood P, Fasano A, Fuchs G, Geraghty M, Kaul A, et al. Gastrointestinal conditions in children with autism spectrum disorder: developing a research agenda. *Pediatrics* (2012) 130(Suppl. 2):S160–8. doi: 10.1542/peds.2012-0900N

124. Parracho HMRT, Bingham MO, Gibson GR, McCartney AL. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *J Med Microbiol.* (2005) 54:987–91. doi: 10.1099/jmm.0.46101-0

125. McElhanon BO, McCracken C, Karpen S, Sharp WG. Gastrointestinal symptoms in autism spectrum disorder: a meta-analysis. *Pediatrics* (2014) 133:872–83. doi: 10.1542/peds.2013-3995

126. Buie T, Fuchs GJ, Furuta GT, Kooros K, Levy J, Lewis JD, et al. Recommendations for evaluation and treatment of common gastrointestinal problems in children with ASDs. *Pediatrics* (2010) 125(Suppl. 1):S19–29. doi: 10.1542/peds.2009-1878D

127. Nikolov RN, Bearss KE, Lettinga J, Erickson C, Rodowski M, Aman MG, et al. Gastrointestinal symptoms in a sample of children with pervasive developmental disorders. *J Autism Dev Disord.* (2009) 39:405–13. doi: 10.1007/s10803-008-0637-8

128. Mazurek MO, Vasa RA, Kalb LG, Kanne SM, Rosenberg D, Keefer A, et al. Anxiety, sensory over-responsivity, and gastrointestinal problems in children with autism spectrum disorders. *J Abnorm Child Psychol.* (2013) 41:165–76. doi: 10.1007/s10802-012-9668-x

129. Mazefsky CA, Schreiber DR, Olino TM, Minshew NJ. The association between emotional and behavioral problems and gastrointestinal symptoms among children with high-functioning autism. *Autism* (2014) 18:493–501. doi: 10.1177/1362361313485164

130. Chandler S, Carcani-Rathwell I, Charman T, Pickles A, Loucas T, Meldrum D, et al. Parent-reported gastro-intestinal symptoms in children with autism spectrum disorders. *J Autism Dev Disord.* (2013) 43:2737–47. doi: 10.1007/s10803-013-1768-0

131. Prosperi M, Santocchi E, Balboni G, Narzisi A, Bozza M, Fulceri F, et al. Behavioral phenotype of ASD preschoolers with gastrointestinal symptoms or food selectivity. *J Autism Dev Disord.* (2017) 47:3574–88. doi: 10.1007/s10803-017-3271-5

132. Geraghty ME, Depasquale GM, Lane AE. Nutritional intake and therapies in autism: a spectrum of what we know: part 1. *ICAN Infant Child Adolesc Nutr.* (2010) 2:62–9. doi: 10.1177/1941406409358437

133. Chaidez V, Hansen RL, Hertz-Picciotto I. Gastrointestinal problems in children with autism, developmental delays or typical development. *J Autism Dev Disord.* (2014) 44:1117–27. doi: 10.1007/s10803-013-1973-x

134. Maenner MJ, Arneson CL, Levy SE, Kirby RS, Nicholas JS, Durkin MS. Brief report: association between behavioral features and gastrointestinal problems among children with autism spectrum disorder. *J Autism Dev Disord.* (2012) 42:1520–5. doi: 10.1007/s10803-011-1379-6

135. Molloy CA, Manning-Courtney P. Prevalence of chronic gastrointestinal symptoms in children with autism and autistic spectrum disorders. *Autism* (2003) 7:165–71. doi: 10.1177/1362361303007002004

136. Gorrindo P, Williams KC, Lee EB, Walker LS, McGrew SG, Levitt P. Gastrointestinal dysfunction in autism: parental report, clinical evaluation, and associated factors. *Autism Res.* (2012) 5:101–8. doi: 10.1002/aur.237

137. Wang LW, Tancredi DJ, Thomas DW. The prevalence of gastrointestinal problems in children across the United States with autism spectrum disorders from families with multiple affected members. *J Dev Behav Pediatr.* (2011) 32:351–60. doi: 10.1097/DBP.0b013e31821bd06a

138. Sathe N, Andrews JC, McPheeters ML, Warren ZE. Nutritional and dietary interventions for autism spectrum disorder: a systematic review. *Pediatrics* (2017) 139:e20170346. doi: 10.1542/peds.2017-0346

139. Hediger ML, England LJ, Molloy CA, Yu KF, Manning-Courtney P, Mills JL. Reduced bone cortical thickness in boys with autism or autism spectrum disorder. *J Autism Dev Disord.* (2008) 38:848–56. doi: 10.1007/s10803-007-0453-6

140. Williams BL, Hornig M, Buie T, Bauman ML, Cho Paik M, Wick I, et al. Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. *PLoS ONE* (2011) 6:e24585. doi: 10.1371/journal.pone.0024585

141. Gondalia S V, Palombo EA, Knowles SR, Cox SB, Meyer D, Austin DW. Molecular characterisation of gastrointestinal microbiota of children with autism (with and without gastrointestinal dysfunction) and their neurotypical siblings. *Autism Res.* (2012) 5:419–27. doi: 10.1002/aur.1253

142. Kang D-W, Park JG, Ilhan ZE, Wallstrom G, LaBaer J, Adams JB, et al. Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. *PLoS ONE* (2013) 8:e68322. doi: 10.1371/journal.pone.0068322

143. Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, Wolcott RD, et al. Pyrosequencing study of fecal microflora of autistic and control children. *Anaerobe* (2010) 16:444–53. doi: 10.1016/j.anaerobe.2010.06.008

144. Louis P. Does the human gut microbiota contribute to the etiology of autism spectrum disorders? *Dig Dis Sci.* (2012) 57:1987–9. doi: 10.1007/s10620-012-2286-1

145. Kang D-W, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, et al. Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome* (2017) 5:10. doi: 10.1186/s40168-016-0225-7

146. Ding HT, Taur Y, Walkup JT. Gut microbiota and autism: key concepts and findings. *J Autism Dev Disord.* (2017) 47:480–9. doi: 10.1007/s10803-016-2960-9

147. Li Q, Han Y, Dy ABC, Hagerman RJ. The gut microbiota and autism spectrum disorders. *Front Cell Neurosci.* (2017) 11:120. doi: 10.3389/fncel.2017.00120

148. Mulloy A, Lang R, O'Reilly M, Sigafoos J, Lancioni G, Rispoli M. Gluten-free and casein-free diets in the treatment of autism spectrum disorders: a systematic review. *Res Autism Spectr Disord.* (2010) 4:328–39. doi: 10.1016/j.rasd.2009.10.008

149. de Theije CGM, Wu J, da Silva SL, Kamphuis PJ, Garssen J, Korte SM, et al. Pathways underlying the gut-to-brain connection in autism spectrum disorders as future targets for disease management. *Eur J Pharmacol.* (2011) 668(Suppl. 1):S70–80. doi: 10.1016/j.ejphar.2011.07.013

150. Israelyan N, Margolis KG. Serotonin as a link between the gut-brain-microbiome axis in autism spectrum disorders. *Pharmacol Res.* (2018) 132:1–6. doi: 10.1016/j.phrs.2018.03.020

151. Chugani DC, Muzik O, Behen M, Rothermel R, Janisse JJ, Lee J, et al. Developmental changes in brain serotonin synthesis capacity in autistic and nonautistic children. *Ann Neurol.* (1999) 45:287–95. doi: 10.1002/1531-8249(199903)45:3<287::AID-ANA3>3.0.CO;2-9

152. Mayer EA. Gut feelings: the emerging biology of gut-brain communication. *Nat Rev Neurosci.* (2011) 12:453–66. doi: 10.1038/nrn3071

153. De Palma G, Collins SM, Bercik P, Verdu EF. The microbiota–gut–brain axis in gastrointestinal disorders: stressed bugs, stressed brain or both? *J Physiol.* (2014) 592:2989–97. doi: 10.1113/jphysiol.2014.273995

154. Williams K, Wheeler DM, Silove N, Hazell P. Selective serotonin reuptake inhibitors (SSRIs) for autism spectrum disorders (ASD). *Cochrane Database Syst Rev.* (2013) 6:CD004677. doi: 10.1002/14651858.CD004677.pub3

155. Campbell DB, Buie TM, Winter H, Bauman M, Sutcliffe JS, Perrin JM, et al. Distinct genetic risk based on association of MET in families with co-occurring autism and gastrointestinal conditions. *Pediatrics* (2009) 123:1018–24. doi: 10.1542/peds.2008-0819

156. Campbell DB, D'Oronzio R, Garbett K, Ebert PJ, Mirnics K, Levitt P, et al. Disruption of cerebral cortex MET signaling in autism spectrum disorder.

Ann Neurol Off J Am Neurol Assoc Child Neurol Soc. (2007) 62:243–50. doi: 10.1002/ana.21180

157. Prasad HC, Steiner JA, Sutcliffe JS, Blakely RD. Enhanced activity of human serotonin transporter variants associated with autism. *Philos Trans R Soc B Biol Sci.* (2009) 364:163–73. doi: 10.1098/rstb.2008.0143

158. Berger M, Gray JA, Roth BL. The expanded biology of serotonin. *Annu Rev Med.* (2009) 60:355–66. doi: 10.1146/annurev.med.60.042307.110802

159. Veenstra-VanderWeele J, Muller CL, Iwamoto H, Sauer JE, Owens WA, Shah CR, et al. Autism gene variant causes hyperserotonemia, serotonin receptor hypersensitivity, social impairment and repetitive behavior. *Proc Natl Acad Sci USA.* (2012) 109:5469–74. doi: 10.1073/pnas.1112345109

160. Margolis KG, Li Z, Stevanovic K, Saurman V, Israelyan N, Anderson GM, et al. Serotonin transporter variant drives preventable gastrointestinal abnormalities in development and function. *J Clin Invest.* (2016) 126:2221–35. doi: 10.1172/JCI84877

161. Atladóttir HÓ, Pedersen MG, Thorsen P, Mortensen PB, Deleuran B, Eaton WW, et al. Association of family history of autoimmune diseases and autism spectrum disorders. *Pediatrics* (2009) 124:687–94. doi: 10.1542/peds.2008-2445

162. Comi AM, Zimmerman AW, Frye VH, Law PA, Peeden JN. Familial clustering of autoimmune disorders and evaluation of medical risk factors in autism. *J Child Neurol.* (1999) 14:388–94. doi: 10.1177/088307389901400608

163. Enstrom AM, Van de Water JA, Ashwood P. Autoimmunity in autism. *Curr Opin Investig Drugs* (2009) 10:463–73.

164. Valicenti-McDermott M, McVicar K, Rapin I, Wershil BK, Cohen H, Shinnar S. Frequency of gastrointestinal symptoms in children with autistic spectrum disorders and association with family history of autoimmune disease. *J Dev Behav Pediatr.* (2006) 27(Suppl. 2):S128–36. doi: 10.1097/00004703-200604002-00011

165. Wills S, Cabanlit M, Bennett J, Ashwood P, Amaral DG, Van de Water J. Detection of autoantibodies to neural cells of the cerebellum in the plasma of subjects with autism spectrum disorders. *Brain Behav Immun.* (2009) 23:64–74. doi: 10.1016/j.bbi.2008.07.007

166. Croen LA, Braunschweig D, Haapanen L, Yoshida CK, Fireman B, Grether JK, et al. Maternal mid-pregnancy autoantibodies to fetal brain protein: the early markers for autism study. *Biol Psychiatry* (2008) 64:583–8. doi: 10.1016/j.biopsych.2008.05.006

167. Singer HS, Morris CM, Gause CD, Gillin PK, Crawford S, Zimmerman AW. Antibodies against fetal brain in sera of mothers with autistic children. *J Neuroimmunol.* (2008) 194:165–72. doi: 10.1016/j.jneuroim.2007.11.004

168. Zimmerman AW, Connors SL, Matteson KJ, Lee LC, Singer HS, Castaneda JA, et al. Maternal antibrain antibodies in autism. *Brain Behav Immun.* (2007) 21:351–7. doi: 10.1016/j.bbi.2006.08.005

169. Heuer L, Braunschweig D, Ashwood P, Van de Water J, Campbell DB. Association of a MET genetic variant with autism-associated maternal autoantibodies to fetal brain proteins and cytokine expression. *Transl Psychiatry* (2011) 1:e48. doi: 10.1038/tp.2011.48

170. Moynihan JA, Santiago FM. Brain behavior and immunity: twenty years of T cells. *Brain Behav Immun.* (2007) 21:872–80. doi: 10.1016/j.bbi.2007.06.010

171. Chen M-H, Su T-P, Chen Y-S, Hsu J-W, Huang K-L, Chang W-H, et al. Comorbidity of allergic and autoimmune diseases in patients with autism spectrum disorder: a nationwide population-based study. *Res Autism Spectr Disord.* (2013) 7:205–12. doi: 10.1016/j.rasd.2012.08.008

172. Miyazaki C, Koyama M, Ota E, Swa T, Amiya RM, Mlunde LB, et al. Allergies in children with autism spectrum disorder: a systematic review and meta-analysis. Rev *J Autism Dev Disord.* (2015) 2:374–401. doi: 10.1007/s40489-015-0059-4

173. Zerbo O, Leong A, Barcellos L, Bernal P, Fireman B, Croen LA. Immune mediated conditions in autism spectrum disorders. *Brain Behav Immun.* (2015) 46:232–6. doi: 10.1016/j.bbi.2015.02.001

174. Gurney JG, McPheeters ML, Davis MM. Parental report of health conditions and health care use among children with and without autism: National Survey of Children's Health. *Arch Pediatr Adolesc Med.* (2006) 160:825–30. doi: 10.1001/archpedi.160.8.825

175. Chen M-H, Su T-P, Chen Y-S, Hsu J-W, Huang K-L, Chang W-H, et al. Is atopy in early childhood a risk factor for ADHD and ASD? A longitudinal study. *J Psychosom Res.* (2014) 77:316–21. doi: 10.1016/j.jpsychores.2014.06.006

176. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders. *J Neuroimmunol.* (2011) 232:196–9. doi: 10.1016/j.jneuroim.2010.10.025

177. Hashim H, Abdelrahman H, Mohammed D, Karam R. Association between plasma levels of transforming growth factor-β1, IL-23 and IL-17 and the severity of autism in Egyptian children. *Res Autism Spectr Disord.* (2013) 7:199–204. doi: 10.1016/j.rasd.2012.08.007

178. Ashwood P, Enstrom A, Krakowiak P, Hertz-Picciotto I, Hansen RL, Croen LA, et al. Decreased transforming growth factor beta1 in autism: a potential link between immune dysregulation and impairment in clinical behavioral outcomes. *J Neuroimmunol.* (2008) 204:149–53. doi: 10.1016/j.jneuroim.2008.07.006

179. Al-Ayadhi LY, Mostafa GA. Elevated serum levels of interleukin-17A in children with autism. *J Neuroinflammation* (2012) 9:158. doi: 10.1186/1742-2094-9-158

180. Masi A, Breen EJ, Alvares GA, Glozier N, Hickie IB, Hunt A, et al. Cytokine levels and associations with symptom severity in male and female children with autism spectrum disorder. *Mol Autism.* (2017) 8:63. doi: 10.1186/s13229-017-0176-2

181. Molloy CA, Morrow AL, Meinzen-Derr J, Dawson G, Bernier R, Dunn M, et al. Familial autoimmune thyroid disease as a risk factor for regression in children with autism spectrum disorder: a CPEA study. *J Autism Dev Disord.* (2006) 36:317–24. doi: 10.1007/s10803-005-0071-0

182. Choi JE, Widjaja F, Careaga M, Bent S, Ashwood P, Hendren RL. Change in plasma cytokine levels during risperidone treatment in children with autism. *J Child Adolesc Psychopharmacol.* (2014) 24:586–9. doi: 10.1089/cap.2013.0108

183. Tobiasova Z, van der Lingen KHB, Scahill L, Leckman JF, Zhang Y, Chae W, et al. Risperidone-related improvement of irritability in children with autism is not associated with changes in serum of epidermal growth factor and interleukin-13. *J Child Adolesc Psychopharmacol.* (2011) 21:555–64. doi: 10.1089/cap.2010.0134

184. Asadabadi M, Mohammadi MR, Ghanizadeh A, Modabbernia A, Ashrafi M, Hassanzadeh E, et al. Celecoxib as adjunctive treatment to risperidone in children with autistic disorder: a randomized, double-blind, placebo-controlled trial. *Psychopharmacol.* (2013) 225:51–9. doi: 10.1007/s00213-012-2796-8

185. Chez MG, Guido-Estrada N. Immune therapy in autism: historical experience and future directions with immunomodulatory therapy. *Neurotherapeutics* (2010) 7:293–301. doi: 10.1016/j.nurt.2010.05.008

186. McDougle CJ, Landino SM, Vahabzadeh A, O'Rourke J, Zurcher NR, Finger BC, et al. Toward an immune-mediated subtype of autism spectrum disorder. *Brain Res.* (2014) 1617:72–92. doi: 10.1016/j.brainres.2014.09.048

187. Chez MG, Loeffel M, Buchanan CP, Field-Chez M. Pulse high-dose steroids as combination therapy with valproic acid in epileptic aphasia patients with pervasive developmental delay or autism. *Ann Neurol.* (1998) 44:539.

188. Chez M, Low R, Parise C, Donnel T. Safety and observations in a pilot study of lenalidomide for treatment in autism. *Autism Res Treat.* (2012) 2012:291601. doi: 10.1155/2012/291601

189. Deshmukh CT. Minimizing side effects of systemic corticosteroids in children. *Indian J Dermatol Venereol Leprol.* (2007) 73:218–21. doi: 10.4103/0378-6323.33633

190. Onore C, Careaga M, Ashwood P. The role of immune dysfunction in the pathophysiology of autism. *Brain Behav Immun.* (2012) 26:383–92. doi: 10.1016/j.bbi.2011.08.007

191. Hughes HK, Mills Ko E, Rose D, Ashwood P. Immune dysfunction and autoimmunity as pathological mechanisms in autism spectrum disorders. *Front Cell Neurosci.* (2018) 12:405. doi: 10.3389/fncel.2018.00405

192. Chen S-W, Zhong X-S, Jiang L-N, Zheng X-Y, Xiong Y-Q, Ma S-J, et al. Maternal autoimmune diseases and the risk of autism spectrum disorders in offspring: a systematic review and meta-analysis. *Behav Brain Res.* (2016) 296:61–9. doi: 10.1016/j.bbr.2015.08.035

193. Wu S, Ding Y, Wu F, Li R, Xie G, Hou J, et al. Family history of autoimmune diseases is associated with an increased risk of autism in children: a systematic review and meta-analysis. *Neurosci Biobehav Rev.* (2015) 55:322–32. doi: 10.1016/j.neubiorev.2015.05.004

194. Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R, et al. Functional impact of global rare copy number variation in autism spectrum disorders. *Nature* (2010) 466:368–72. doi: 10.1038/nature09146

195. De Rubeis S, He X, Goldberg AP, Poultney CS, Samocha K, Cicek AE, et al. Synaptic, transcriptional and chromatin genes disrupted in autism. *Nature* (2014) 515:209–15. doi: 10.1038/nature13772

196. Daniels WW, Warren RP, Odell JD, Maciulis A, Burger RA, Warren WL, et al. Increased frequency of the extended or ancestral haplotype B44-SC30-DR4 in autism. *Neuropsychobiology* (1995) 32:120–3. doi: 10.1159/000119223

197. Lee LC, Zachary AA, Leffell MS, Newschaffer CJ, Matteson KJ, Tyler JD, et al. HLA-DR4 in families with autism. *Pediatr Neurol.* (2006) 35:303–7. doi: 10.1016/j.pediatrneurol.2006.06.006

198. Stubbs EG, Magenis RE. HLA and autism. *J Autism Dev Disord.* (1980) 10:15–9. doi: 10.1007/BF02408429

199. Torres AR, Maciulis A, Stubbs EG, Cutler A, Odell D. The transmission disequilibrium test suggests that HLA-DR4 and DR13 are linked to autism spectrum disorder. *Hum Immunol.* (2002) 63:311–6. doi: 10.1016/S0198-8859(02)00374-9

200. Ashwood P, Van de Water J. A review of autism and the immune response. *Clin Dev Immunol.* (2004) 11:165–74. doi: 10.1080/10446670410001722096

201. Torres AR, Sweeten TL, Cutler A, Bedke BJ, Fillmore M, Stubbs EG, et al. The association and linkage of the HLA-A2 class I allele with autism. *Human Immunol.* (2006) 67:346–51. doi: 10.1016/j.humimm.2006.01.001

202. Guerini FR, Bolognesi E, Manca S, Sotgiu S, Zanzottera M, Agliardi C, et al. Family-based transmission analysis of HLA genetic markers in Sardinian children with autistic spectrum disorders. *Hum Immunol.* (2009) 70:184–90. doi: 10.1016/j.humimm.2008.12.009

203. Guerini FR, Bolognesi E, Chiappedi M, De Silvestri A, Ghezzo A, Zanette M, et al. HLA polymorphisms in Italian children with autism spectrum disorders: results of a family based linkage study. *J Neuroimmunol.* (2011) 230:135–42. doi: 10.1016/j.jneuroim.2010.10.019

204. Corbett BA, Kantor AB, Schulman H, Walker WL, Lit L, Ashwood P, et al. A proteomic study of serum from children with autism showing differential expression of apolipoproteins and complement proteins. *Mol Psychiatry* (2007) 12:292–306. doi: 10.1038/sj.mp.4001943

205. Mostafa GA, Shehab AA. The link of C4B null allele to autism and to a family history of autoimmunity in Egyptian autistic children. *J Neuroimmunol.* (2010) 223:115–9. doi: 10.1016/j.jneuroim.2010.03.025

206. Odell D, Maciulis A, Cutler A, Warren L, McMahon WM, Coon H, et al. Confirmation of the association of the C4B null allele in autism. *Hum Immunol.* (2005) 66:140–5. doi: 10.1016/j.humimm.2004.11.002

207. Warren RP, Yonk J, Burger RW, Odell D, Warren WL. DR-positive T cells in autism: association with decreased plasma levels of the complement C4B protein. *Neuropsychobiology* (1995) 31:53–7. doi: 10.1159/000119172

208. Warren RP, Burger RA, Odell D, Torres AR, Warren WL. Decreased plasma concentrations of the C4B complement protein in autism. *Arch Pediatr Adolesc Med.* (1994) 148:180–3. doi: 10.1001/archpedi.1994.02170020066011

209. Campbell DB, Li C, Sutcliffe JS, Persico AM, Levitt P. Genetic evidence implicating multiple genes in the MET receptor tyrosine kinase pathway in autism spectrum disorder. *Autism Res.* (2008) 1:159–68. doi: 10.1002/aur.27

210. Dinarello CA. Proinflammatory cytokines. *Chest J.* (2000) 118:503–8. doi: 10.1378/chest.118.2.503

211. Bauer S, Kerr BJ, Patterson PH. The neuropoietic cytokine family in development, plasticity, disease and injury. *Nat Rev Neurosci.* (2007) 8:221–32. doi: 10.1038/nrn2054

212. Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, Li X-M, et al. Elevated immune response in the brain of autistic patients. *J Neuroimmunol.* (2009) 207:111–6. doi: 10.1016/j.jneuroim.2008.12.002

213. Ashwood P, Anthony A, Torrente F, Wakefield AJ. Spontaneous mucosal lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms: mucosal immune activation and reduced counter regulatory interleukin-10. *J Clin Immunol.* (2004) 24:664–73. doi: 10.1007/s10875-004-6241-6

214. Jyonouchi H, Sun S, Le H. Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. *J Neuroimmunol.* (2001) 120:170–9. doi: 10.1016/S0165-5728(01)00421-0

215. Singh VK. Plasma increase of interleukin-12 and interferon-gamma. Pathological significance in autism. *J Neuroimmunol*. (1996) 66:143–5. doi: 10.1016/0165-5728(96)00014-8

216. Singh VK, Warren RP, Odell JD, Cole P. Changes of soluble interleukin-2, interleukin-2 receptor, T8 antigen, and interleukin-1 in the serum of autistic children. *Clin Immunol Immunopathol*. (1991) 61:448–55. doi: 10.1016/S0090-1229(05)80015-7

217. Constantino JN, Todd RD. Autistic traits in the general population: a twin study. *Arch Gen Psychiatry* (2003) 60:524–30. doi: 10.1001/archpsyc.60.5.524

218. Saresella M, Marventano I, Guerini FR, Mancuso R, Ceresa L, Zanzottera M, et al. An autistic endophenotype results in complex immune dysfunction in healthy siblings of autistic children. *Biol Psychiatry* (2009) 66:978–84. doi: 10.1016/j.biopsych.2009.06.020

219. Atladóttir HÓ, Henriksen TB, Schendel DE, Parner ET. Autism after infection, febrile episodes, and antibiotic use during pregnancy: an exploratory study. *Pediatrics* (2012) 130:e1447–54. doi: 10.1542/peds.2012-1107

220. Atladóttir HÓ, Thorsen P, Østergaard L, Schendel DE, Lemcke S, Abdallah M, et al. Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. *J Autism Dev Disord*. (2010) 40:1423–30. doi: 10.1007/s10803-010-1006-y

221. Brown AS, Sourander A, Hinkka-Yli-Salomäki S, McKeague IW, Sundvall J, Surcel HM. Elevated maternal C-reactive protein and autism in a national birth cohort. *Mol Psychiatry* (2013) 19:259–64. doi: 10.1038/mp.2012.197

222. Chess S. Autism in children with congenital rubella. *J Autism Child Schizophr*. (1971) 1:33–47. doi: 10.1007/BF01537741

223. Deykin EY, MacMahon B. Viral exposure and autism. *Am J Epidemiol*. (1979) 109:628–38. doi: 10.1093/oxfordjournals.aje.a112726

224. Zerbo O, Iosif A-M, Walker C, Ozonoff S, Hansen RL, Hertz-Picciotto I. Is maternal influenza or fever during pregnancy associated with autism or developmental delays? Results from the CHARGE (CHildhood Autism Risks from Genetics and Environment) study. *J Autism Dev Disord*. (2013) 43:25–33. doi: 10.1007/s10803-012-1540-x

225. Zerbo O, Qian Y, Yoshida C, Grether JK, Van de Water J, Croen LA. Maternal infection during pregnancy and autism spectrum disorders. *J Autism Dev Disord*. (2013) 45:4015–25. doi: 10.1007/s10803-013-2016-3

226. Meldrum SJ, Strunk T, Currie A, Prescott SL, Simmer K, Whitehouse AJO. Autism spectrum disorder in children born preterm—role of exposure to perinatal inflammation. *Front Neurosci*. (2013) 7:123. doi: 10.3389/fnins.2013.00123

227. Chess S. Follow-up report on autism in congenital rubella. *J Autism Child Schizophr*. (1977) 7:69–81. doi: 10.1007/BF01531116

228. Libbey JE, Sweeten TL, McMahon WM, Fujinami RS. Autistic disorder and viral infections. *J Neurovirol*. (2005) 11:1–10. doi: 10.1080/13550280590900553

229. Sweeten TL, Posey DJ, McDougle CJ. Brief report: autistic disorder in three children with cytomegalovirus infection. *J Autism Dev Disord*. (2004) 34:583–6. doi: 10.1007/s10803-004-2552-y

230. Meyer U, Nyffeler M, Yee BK, Knuesel I, Feldon J. Adult brain and behavioral pathological markers of prenatal immune challenge during early/middle and late fetal development in mice. *Brain Behav Immun*. (2008) 22:469–86. doi: 10.1016/j.bbi.2007.09.012

231. Hsiao EY, Patterson PH. Activation of the maternal immune system induces endocrine changes in the placenta via IL-6. *Brain Behav Immun*. (2011) 25:604–15. doi: 10.1016/j.bbi.2010.12.017

232. Smith SE, Li J, Garbett K, Mirnics K, Patterson PH. Maternal immune activation alters fetal brain development through interleukin-6. *J Neurosci*. (2007) 27:10695–702. doi: 10.1523/JNEUROSCI.2178-07.2007

233. Lasala N, Zhou H. Effects of maternal exposure to LPS on the inflammatory response in the offspring. *J Neuroimmunol*. (2007) 189:95–101. doi: 10.1016/j.jneuroim.2007.07.010

234. Mandal M, Marzouk AC, Donnelly R, Ponzio NM. Preferential development of Th17 cells in offspring of immunostimulated pregnant mice. *J Reprod Immunol*. (2010) 87:97–100. doi: 10.1016/j.jri.2010.06.156

235. Mandal M, Marzouk AC, Donnelly R, Ponzio NM. Maternal immune stimulation during pregnancy affects adaptive immunity in offspring to promote development of TH17 cells. *Brain Behav Immun*. (2011) 25:863–71. doi: 10.1016/j.bbi.2010.09.011

236. Surriga O, Ortega A, Jadeja V, Bellafronte A, Lasala N, Zhou H. Altered hepatic inflammatory response in the offspring following prenatal LPS exposure. *Immunol Lett*. (2009) 123:88–95. doi: 10.1016/j.imlet.2009.02.009

237. Patel S, Masi A, Dale RC, Whitehouse AJO, Pokorski I, Alvares GA, et al. Social impairments in autism spectrum disorder are related to maternal immune history profile. *Mol Psychiatry* (2018) 23:1794–7. doi: 10.1038/mp.2017.201

238. Neumeyer AM, Anixt J, Chan J, Perrin JM, Murray D, Coury DL, et al. Identifying associations among co-occurring medical conditions in children with autism spectrum disorders. *Acad Pediatr*. (2018). doi: 10.1016/j.acap.2018.06.014. [Epub ahead of print].

239. Li Q, Zhou JM. The microbiota–gut–brain axis and its potential therapeutic role in autism spectrum disorder. *Neuroscience* (2016) 324:131–9. doi: 10.1016/j.neuroscience.2016.03.013

240. Romo-Nava F, Alvarez-Icaza González D, Fresán-Orellana A, Saracco Alvarez R, Becerra-Palars C, Moreno J, et al. Melatonin attenuates antipsychotic metabolic effects: an eight-week randomized, double-blind, parallel-group, placebo-controlled clinical trial. *Bipolar Disord*. (2014) 16:410–21. doi: 10.1111/bdi.12196

241. Sommansson A, Saudi WSW, Nylander O, Sjöblom M. Melatonin inhibits alcohol-induced increases in duodenal mucosal permeability in rats *in vivo*. *Am J Physiol Liver Physiol*. (2013) 305:G95–105. doi: 10.1152/ajpgi.00074.2013

242. Eriksson SH. Epilepsy and sleep. *Curr Opin Neurol*. (2011) 24:171–6. doi: 10.1097/WCO.0b013e3283445355

243. Irwin MR, Olmstead R, Carroll JE. Sleep disturbance, sleep duration, and inflammation: a systematic review and meta-analysis of cohort studies and experimental sleep deprivation. *Biol Psychiatry* (2016) 80:40–52. doi: 10.1016/j.biopsych.2015.05.014

244. Alvares GA, Dawson PA, Dissanayake C, Eapen V, Gratten J, Grove R, et al. Study protocol for the Australian autism biobank: an international resource to advance autism discovery research. *BMC Pediatr*. (2018) 18:284. doi: 10.1186/s12887-018-1255-z

245. Reilly J, Gallagher L, Chen JL, Leader G, Shen S. Bio-collections in autism research. *Mol Autism*. (2017) 8:34. doi: 10.1186/s13229-017-0154-8

246. Fischbach GD, Lord C. The Simons Simplex Collection: a resource for identification of autism genetic risk factors. *Neuron* (2010) 68:192–5. doi: 10.1016/j.neuron.2010.10.006

247. Loth E, Charman T, Mason L, Tillmann J, Jones EJH, Wooldridge C, et al. The EU-AIMS Longitudinal European Autism Project (LEAP): design and methodologies to identify and validate stratification biomarkers for autism spectrum disorders. *Mol Autism*. (2017) 8:24. doi: 10.1186/s13229-017-0146-8

248. Frye RE, Rossignol DA. Treatments for biomedical abnormalities associated with autism spectrum disorder. *Front Pediatr*. (2014) 2:66. doi: 10.3389/fped.2014.00066

249. Thomas MSC, Annaz D, Ansari D, Scerif G, Jarrold C, Karmiloff-Smith A. Using developmental trajectories to understand developmental disorders. *J Speech Lang Hear Res*. (2009) 52:336–58. doi: 10.1044/1092-4388(2009/07-0144)

250. Zablotsky B, Kalb LG, Freedman B, Vasa R, Stuart EA. Health care experiences and perceived financial impact among families of children with an autism spectrum disorder. *Psychiatr Serv*. (2014) 65:395–8. doi: 10.1176/appi.ps.201200552

Corrigendum: Characterizing the Interplay Between Autism Spectrum Disorder and Comorbid Medical Conditions: An Integrative Review

Charlotte Tye[1]*, Abigail Runicles[1], Andrew J.O. Whitehouse[2,3] and Gail A. Alvares[2,3]

[1] Child & Adolescent Psychiatry, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingdom, [2] Telethon Kids Institute, The University of Western Australia, Perth, WA, Australia, [3] Cooperative Research Centre for Living with Autism (Autism CRC), Brisbane, QLD, Australia

Keywords: autism spectrum disorder, comorbidity, epilepsy, gastrointestinal disorders, immune function, sleep

A Corrigendum on

Characterizing the Interplay Between Autism Spectrum Disorder and Comorbid Medical Conditions: An Integrative Review
Tye C, Runicles AK, Whitehouse AJO and Alvares GA (2019). Front. Psychiatry 9:751. doi: 10.3389/fpsyt.2018.00751

There was an omission in the **Funding** statement. The grant number for "Medical Research Council" is "G9817803".

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

Psychophysiological Arousal and Auditory Sensitivity in a Cross-Clinical Sample of Autistic and Non-autistic Anxious Adults

David Nicholas Top Jr.[1], Steven G. Luke[1,2], Kevin G. Stephenson[3] and Mikle South[1,2*]

[1] Department of Psychology, Brigham Young University, Provo, UT, United States, [2] Neuroscience Center, Brigham Young University, Provo, UT, United States, [3] Nationwide Children's Hospital, Columbus, OH, United States

Many autistic people report overwhelming sensory experiences and also elevated levels of anxiety. Understanding how these experiences are linked to each other can contribute to improved support and intervention for reducing sensory overload and anxiety. This study included 95 young adult participants including autistic adults, non-autistic adults reporting to a psychotherapy clinic with high levels of anxiety, and neurotypical adults with no psychiatric concerns. We measured pupil size using including a baseline task with no auditory stimulus followed by two blocks of simple auditory habituation. In a subset of 80 participants we also measured self-report levels of sensory processing, anxious apprehension, and intolerance of uncertainty. The autism group showed atypical sensory processing on all four measured domains of the Adolescent and Adult Sensory Profile including sensory sensitivity, sensory seeking, sensory avoidance, and low registration subscales. Dimensional analyses across all participants showed significant positive correlations between sensory sensitivity, sensory seeking, and sensory avoidance domains with scores from the Intolerance of Uncertainty Scale-Short Form and Penn State Worry Questionnaire. The autism group showed significantly larger pupil size than other groups at baseline, before any auditory stimulation. There were no group differences in the rate of auditory habituation, nonetheless the overall, absolute larger pupil size remained in the autism group throughout the experiment. We suggest that this and other findings could indicate chronic hyperarousal in many autistic people. Treatment for anxiety in autism should be informed by knowledge of unique aspects of anxiety in autism and consider the role of sensory experience and everyday psychophysiological arousal.

Keywords: autism spectrum disorder, sensory processing, anxiety, intolerance of uncertainty, anxious arousal, pupillometry, habituation

INTRODUCTION

Many autistic people report unusually intense sensory processing, including hypersensitivity to multiple sensory modalities and high levels of distress even to low-threshold sensory stimuli (1, 2). Atypical sensory processing has been reported in between 45 and 95% of autistic samples (3–5) and is included in the most recent definitions of autism (6, 7). Many autistic people also experience

elevated levels of anxiety (8–10). These anxiety symptoms can cause significant additional distress, and have been linked to increased levels of problematic behavior (11, 12), difficulty with decision making (13), and considerable stress on family systems (14). While some autistic individuals manifest anxiety in ways typical of other anxious people, there are also unexpected expressions of anxiety in autism that may go overlooked, such as different underlying drives toward compulsive behavior or social avoidance than typically seen in anxiety (9).

There is growing evidence for a strong link between atypical sensory processing and elevated anxiety in autism (15–20). Effective sensory processing is a critical evolutionary component for managing stress and danger [see (21–23)] and there are demonstrated links between sensory sensitivity and affective disorders, including anxiety, outside of autism (24–27). However, side-by-side comparisons of young autistic and neurotypical children suggest that the relationship between sensory processing and anxiety may be much more prominent in autism than neurotypical development (28). Green et al. (29) found that sensory over-responsivity emerges earlier than anxiety in autism and that sensory sensitivity predicts later anxiety symptoms in autism.

To date, most studies of the relationship between sensory processing, anxiety and autism traits have focused on child samples. One of the few studies of adults featured mothers with autistic children (30). Among these mothers, 98% of the sample had scores at least a standard deviation above the mean on at least one sensory domain. In a study focused on emotion processing (i.e., alexithymia) in autistic adults, Milosavljevic et al. (31) reported self-report data from autistic adults using the Adolescent Adult Sensory Profile [AASP; (32)] that were somewhat above published norms for the AASP. However, the authors did not administer the AASP to comparison groups and did not report analyses of association between sensory processing and anxiety. Thus, the first primary aim of our study was to directly compare sensory processing behaviors, alongside measures of anxious apprehension and autism traits, in a cross-clinical sample of autistic, anxious, and neurotypical adults.

Intolerance of uncertainty (IU), a transdiagnostic psychological construct that refers to decreased thresholds for ambiguity and enhanced discomfort with ambiguity (33), has emerged as a critical mediator between sensory processing and anxiety in autism and other anxiety disorders (18–20, 28, 34–36). Although IU is typically a factor associated with generalized anxiety disorder, IU has shown to negatively affect depression as well as other anxiety disorders (37, 38). Because many autistic individuals prefer things to be predictable and dislike change, it has been argued that characteristics of IU share some common features with the insistence on sameness seen in autism (34). Multiple studies have now established the link between IU, anxiety, and ASD symptomology (28, 34, 35, 39). A study by Boulter et al. (34) reported a "causal meditational model" in which IU almost completely mediated the relationship between the diagnostic group and anxiety scores. Another study, using an autism only sample, found a link between sensory over-responsiveness, IU, and anxiety in which IU mediated the relationship between sensory processing and anxiety (20).

Neil et al. (28), replicating the (20) study with a larger sample that includes typically developing individuals, found that IU had a direct effect on sensory sensitivity and anxiety. Given the evidence of IU in modulating the anxiety symptoms in autism, we further evaluated associations between sensory processing, anxiety, and IU in this study.

Another characteristic of studies in this area is a reliance on questionnaires including parent-report surveys (16, 17, 20, 34, 40) or self-report surveys (19, 31, 41). There have been a few notable studies involving psychophysiological measures. Corbett et al. (15) reported that cortisol response to stress was higher for autistic children than neurotypical controls, during an ecologically-relevant peer interaction. In that study greater sensory dysfunction was associated with increased stress, and diagnosis was a significant moderator of the relationship between sensory function and stress response. An emerging idea from our lab and the work of others is that everyday psychophysiological arousal may be elevated in autism (35, 42, 43). We do not know of any studies that examine possible links between ambulatory arousal and sensory and/or emotional sensitivity. It is likely that the sensory and performance demands of laboratory settings would exacerbate such links.

With this limitation in mind, some studies have found that autism samples have a larger tonic pupil size–indicative of elevated physiological arousal–than neurotypical comparison groups (44, 45) though others have found no difference (46) or the opposite trend (47). Takahashi et al. (42) found an elevated startle response in autistic children to the mild stimuli as well as a longer peak-startle latency, while a different, threat-modulated startle study found elevated startle response during baseline but not during habituation conditions (35). Our second aim was thus to evaluate evidence for elevated arousal in autism. To do this we designed an explicit extended baseline period to measure tonic pupil size without any other task demands, as well as tracked their pupil size throughout the duration of the task.

The study of habituation may be useful for understanding the link between sensory processing, anxiety and autism especially with regard to amygdala and insula function in the brain (48–50). In experimental work with both mice and humans, Herry et al. (51) have reported that unpredictability in sequences of sound pulses, which disrupts habituation, is associated with anxiety-like behavior, and is further associated with enhanced/sustained amygdala activity in both animal and human models. The authors suggest that uncertainty at initial encoding (including the amygdala) decreases the flexibility of downstream emotional response. Atypical habituation in autism could therefore underlie inflexible and anxious behavior.

Two fMRI studies of cognitively-typical autistic youth (52, 53) have shown that, during a challenge of mildly aversive sensory stimuli, the autism sample showed more activation than controls in primary sensory areas, amygdala, and orbitofrontal cortex. This activation was correlated with parent-reported anxiety and also with sensory over-responsiveness beyond the association with anxiety. Brain activity in the ASD samples was especially heightened when multiple sensory modalities (auditory and tactile) appeared simultaneously. The authors highlighted difficulties with habituation as a possible underlying feature

of sensory overresponsiveness. Takahashi et al. (42) did not find differences in habituation between autistic and neurotypical children during a acoustic startle response paradigm, but a number of other studies have shown reduced or atypical habituation (or increased sensitization, which is the opposite of habituation) in autistic children for various stimulus modalities (54–56). Given the limited literature on sensory experience in adults, our third aim was to characterize unimodal sensory habituation in autistic adults, during a simple auditory habituation task while measuring pupil dilation at baseline and then during two sets of trials which increased in stimulus aversiveness. The sample included autistic adults with typical cognitive performance (AUT group) alongside two IQ-matched adult comparison groups: a sample of highly anxious, (ANX group) and a sample of neurotypical adults who reported no psychiatric concerns (NT group). The inclusion of a highly-anxious group allowed for more direct comparison of the relative contributions of sensory traits and physiological arousal vis-à-vis anxiety in autism.

Aim 1: Evaluate sensory processing behaviors, and their link to measures of anxious apprehension and autism traits, in autistic adults vis-à-vis clinical and non-clinical comparison groups. We predicted three-tier outcomes where the autism group would score highest (AUT>ANX>NT) on sensory experience, intolerance of uncertainty, autism trait measures, while the ANX group highest on a measure of anxious apprehension (ANX>AUT>NT). Following our previous study that used a dimensional approach to examine trait-based associations (39), we planned to pool all participants for correlation analyses. We predicted strong associations between sensory experience, anxious apprehension, and intolerance of uncertainty. We also conducted follow-up analyses of correlations within each group separately.

Aim 2: Compare baseline (non-task) physiological arousal and general physiological arousal (whole experiment) across the autism and comparison groups. We explicitly measured baseline arousal before the start of the habituation protocol used in this study. We predicted increased pupil size at baseline in the AUT group compared to neurotypical controls. Given previous mixed literature we did not have a firm prediction on whether the AUT group might be equal to or exceed baseline arousal compared to the ANX group. We also predicted a three-tier difference in general arousal throughout the duration of the experiment (ASD<ANX<CON), meaning that the ASD group's general arousal would decrease over time less than the other groups.

Aim 3: Evaluate sensory habituation in an auditory stimulation task using pupillometry to index psychophysiological arousal. For this aim we also predicted a three-tier habituation response (AUT<ANX<NT), meaning that pupil dilation would take longest to decrease over each set of trials in the AUT group.

MATERIALS AND METHODS
Participants
Pupillometry data were collected from 95 young adults including 31 AUT group (24 males), 28 ANX group (11 males) and 36 NT group (22 males) participants. A subset of this sample completed

the Adolescent Adult Sensory Profile and other behavioral measures (AUT n = 24, ANX n = 20, NT n = 36).

The majority of participants in the AUT group were recruited from a pre-existing database of persons who had participated in previous studies and consented to be contacted for future. Other AUT participants were recruited from the community via recruitment fliers as approved by the Brigham Young University Institutional Review Board. Members in the AUT group had a confirmed diagnosis of autism spectrum disorder informed by the Autism Diagnostic Observation Schedule, Second Edition [ADOS-2; (57)] administered by a research reliable clinician who was also an author of this study.

The ANX group was recruited from individuals with no reported history of autism, who were presenting for psychotherapy at a counseling center of a large private university and had not yet begun, or only just begun psychotherapy. Invitations were sent to individuals who scored above established cutoffs on at least one of the two anxiety subscales (*Generalized Anxiety* and *Social Anxiety*) of the Counseling Center Assessment of Psychological Symptoms [CCAPS; (58)], and who also scored below the 80th percentile for non-anxiety subscales. Formal psychiatric diagnoses are not generally given in the counseling center and thus were not available. The NT group was recruited via the psychology department research participation system and reported no history of autism spectrum diagnosis or any elevated psychiatric concern or history of diagnosis.

As shown in **Table 1**, the AUT group was significantly older than the ANX and NT groups. There were no significant differences in cognitive performance as measured by the Wechsler Abbreviated Scales of Intelligence – Second Edition (WASI-II). All participants who agreed to participate in this study were able to complete the auditory habituation protocol.

Behavioral Measures
Autism Spectrum Quotient
The Autism Spectrum Quotient [ASQ; (59)] is a 50-item questionnaire that asks participants to indicate the extent to which they can identify with statements describing behaviors and attitudes that reflect core autistic traits. The ASQ has been used as a dimensional measure of autism traits in clinical populations and in the general public, and has been demonstrated to be sensitive to a range of intensity of autism symptoms (60).

Penn State Worry Questionnaire
The Penn State Worry Questionnaire (PSWQ) is a 16-item questionnaire that measures the severity of anxious apprehension or worry, in both clinical and nonclinical populations (61) The PSWQ has been shown to have good discriminant validity and convergent validity; to be unrelated to measures of depression (e.g., the Beck Depression Inventory) and to be sensitive to cognitive oriented treatment (61, 62).

Intolerance of Uncertainty Scale-12
The Intolerance of Uncertainty Scale-12 (IUS-12) (63) is a 12-item measure that includes questions about the unknown regarding one's prospective anxiety (e.g., "Unforeseen events upset me greatly") and inhibitory anxiety (e.g., "Uncertainty

TABLE 1 | Demographic characteristics and behavioral questionnaire responses.

	Mean ± SD			F (df)	p	Direction
	AUT	ANX	NT			
Age	24.47 ± 6.14	21.90 ± 2.80	20.94 ± 1.72	6.62 (2.92)	0.002	AUT>ANX=NT
FSIQ	112.36 ± 10.63	112.16 ± 12.13	111.95 ± 8.21	2.32 (2.92)	0.993	AUT=ANX=NT
ASQ	27.77 ± 8.96	23.33 ± 7.18	15.61 ± 5.42	23.18 (2.92)	0.000	ASD=ANX>NT
PSWQ	50.92 ± 15.01	63.11 ± 8.75	46.69 ± 11.98	14.62 (2.86)	0.000	ANX>AUT=NT
IUS-12	28.00 ± 7.13	40.89 ± 9.56	38.96 ± 10.08	19.91 (2.85)	0.000	AUT=ANX>NT
AASP						
Sensitivity	44.91 ± 10.11	39.25 ± 9.90	33.22 ± 6.75	22.36 (2.77)	0.000	AUT=ANX>NT
Avoiding	48.75 ± 9.46	39.85 ± 9.49	37.19 ± 5.56	14.22 (2.77)	0.000	AUT>ANX=NT
Low Reg.	39.83 ± 7.14	32.20 ± 7.14	31.91 ± 5.85	11.82 (2.77)	0.000	AUT>ANX=NT
Seeking	52.69 ± 6.54	44.95 ± 8.80	38.67 ± 8.36	24.40 (2.77)	0.000	AUT>ANX>NT

AUT, autistic adults; ANX, highly anxious adults; NT, neurotypical adults; FSIQ, full scale IQ from the Wechsler Abbreviated Scales of Intelligence–Second Edition; AASP, adolescent adult sensory profile; Sensory Sensitivity, Sensation Avoiding, Low Registration and Sensory Seeking subscales.

keeps me from living a full life"). The IUS-12 total score was used in the current study.

Adolescent/Adult Sensory Profile

The Adolescent/Adult Sensory Profile [AASP; (32)] is a 60-item questionnaire measuring four sensory processing categories based on Dunn's (64) model of sensory processing: *low registration* (i.e., easily misses sensory information), *sensation seeking* (seeks out sensory stimulation), *sensory sensitivity* (heightened awareness of sensory stimuli), and *sensation avoiding* (withdraws from overwhelming sensory input). The four subscales of the AASP reflect typical sensory processing where extreme scores (higher or lower) reflect differences from typical development.

Eye-Tracking Apparatus and Measurement

The experiment was conducted in a $6' \times 15'$ room with a single window that was facing southeast. The window's blind were closed for all participants. The rooms lights consisted of four fluorescents ceiling lights that were on while the participants completed the study. Pupils were recorded via an SR Research Eyelink 1000 Plus tower mount eye tracker (spatial resolution of $0.01°$) sampling at 1000 Hz. Subjects were seated 60 cm away from a 24″ LCD screen with their back toward the window to reduce effects of luminance from the outside environment. Head movements were minimized with a chin and headrest. Although viewing was binocular, recordings were taken from the right eye only. Prior to recording, the eye tracker was calibrated using a nine-point calibration routine. The experiment was controlled with SR Research Experiment Builder software.

Auditory Habituation Protocol

After the eye-tracking equipment was calibrated to the participant, each participant was shown the instructions on the computer screen while the experimenter also read the instructions out loud. The instructions were as follows, "During this experiment, you will be staring at the fixation cross in the center of the screen. While staring at the cross you will be hearing noises in the headphones. Please keep your eyes focused on the cross throughout the experiment. Failure to look at the fixation cross will pause the experiment. Do you have any questions?" After answering any participant questions the experimenter started the protocol. The auditory habituation protocol consisted of three blocks with 10 trials per block for a total of 30 trials. The first block included only "Silence" trials consisting of a silent tone generated using Audacity software. The second block included only Sound1 trials, consisting of a 2000 Hz sinewave tone, also generated using Audacity and presented at 60 db. The last block included only Sound2 trials, consisting of a 2000 Hz sawtooth tone (which is scratchier and slightly more aversive than the sinewave tone), also generated using Audacity and presented at 80 db. Each trial began with 500 ms silence followed by the corresponding sound (Silence, Sound1, or Sound2) with a jittered duration from 1800 to 2200 ms (mean = 2000 ms). This was followed by jittered inter-trial-interval ranging from 18000 to 22000 ms (mean = 20000 s). Each participant received each block in the same order (Silence, Sound1, Sound2). During each block, the fixation-cross remained on screen continuously, and there were no visual changes to the screen to indicate that one trial had ended and another had begun. The eye-tracker was programmed so that if the eyes left a pre-defined invisible area around the fixation cross, the experiment would pause until the eyes returned to the fixation cross. Participants were instructed to stare at a black fixation cross of 200×200 pixels cross that was located in the center of a white screen.

Ethical Considerations

This study was submitted to and approved by the Brigham Young University Institutional Review Board (BYU IRB). All clients were recruited in accordance to BYU IRB guidelines. In accordance with the Declaration of Helsinki, all participants signed the IRB-approved consent form that has been verbally including information that participants could withdraw from the study at any time. All data for this studied was de-identified during the data preparation phase. Participants were compensated $15 upon the completion of this study.

TABLE 2A | Association of sensory experience and intolerance of uncertainty.

AASP scale	Combined		AUT		ANX		NT	
	r	p	r	p	r	p	r	p
Sensory sensitivity	0.565	<0.001	0.514	0.020	0.376	0.102	0.337	0.045
Sensory avoidant	0.570	<0.001	0.532	0.016	0.329	0.157	0.636	<0.001
Low registration	0.380	0.001	0.417	0.067	0.398	0.083	0.105	0.544
Sensory seeking	−0.478	<0.001	0.147	0.536	−0.308	0.187	−0.387	0.020

TABLE 2B | Association of sensory experience and anxious apprehension.

AASP scale	Combined		AUT		ANX		NT	
	r	p	r	p	r	p	r	p
Sensory sensitivity	0.400	<0.001	0.237	0.300	0.318	0.172	0.451	<0.001
Sensory avoidant	0.271	0.017	−0.030	0.898	0.349	0.131	0.444	0.007
Low registration	0.256	0.025	0.601	0.004	0.238	0.311	0.083	0.628
Sensory seeking	−0.160	0.154	0.061	0.794	0.066	0.782	0.011	0.948

AUT n = 20, ANX n = 21, NT n = 36. AASP, Adolescent Adult Sensory Profile; PSWQ, Penn State Worry Questionnaire; IUS-12, Intolerance of Uncertainty Scale-12.

Data Cleaning and Preparation

All data preparation was completed using R statistical software (65).

Because the data were originally in arbitrary area units, we converted the data to mm diameter by running the experiment with a 10 mm artificial pupil and using the resulting data to compute pupil diameter of the actual participants. Data were cleaned by manually removing samples that occurred during blinks and saccades. The data was then smoothed using a loess filter with a span of 0.25. Pupil size at time 0 (the moment before sound onset) was used as a baseline, and pupil size change was computed by subtracting this baseline value from each sample. Finally, before analysis, outlier samples greater than or less than 2.5 standard deviations from the participant's mean were removed (less than 4% of the total data were removed; the amount removed did not differ by group), and the pupil data were grouped into 250 ms bins via averaging (66).

RESULTS

Aim 1: Sensory Processing in Autistic Adults

We first examined between-group differences on behavioral measures, as summarized in **Table 1**. The ASQ, IUS-12 total score, and AASP *sensory sensitivity* subscale had non-normal distributions and we followed standard ANOVA analyses with Kruskal-Wallis tests (with Dunn's test of multiple comparisons of rank sums using the "dunntest" package of STATA 14. The ANOVA and Kruskal-Wallis tests provided identical results in all cases.

As expected, scores for the AUT group were significantly different than the NT group on all subscales of the AASP sensory questionnaire, including higher scores on the atypical sensory experience scales and lower scores on the typical *sensory seeking*

scale. ANX group scores were equivalent to the AUT group for the *sensory seeking* subscale, equivalent to the NT group for the *low registration* and *sensory avoidance* scales, and between the AUT and NT group for *sensory seeking*. The ANX group had the highest scores on the PSWQ (anxious apprehension), while the AUT and ANX groups were equivalent for the IUS-12 total (intolerance of uncertainty). In line with our previous findings regarding autism trait measures in highly anxious adults (67), the AUT and ANX groups were statistically equal for the ASQ total score.

Associations With Anxiety and Sensory Processing

As shown in **Tables 2A**, **2B**, dimensional analyses of all participants combined across groups ($n = 77$) found strong significant correlations between the AASP subscales and the IUS-12 and PSWQ total scores. This is in line with our previous paper that looked at dimensional associations with autism and neurotypical groups in the same analysis (39). Breaking down the correlations by group showed a few different patterns between groups although lower statistical power due to the sample separation affects interpretation. There were no significant correlations between the pupillometry measures and any of the behavioral measures.

Aim 2: Baseline and General Physiological Arousal

We calculated the average pupil size across each of the 10 trials of the baseline Silence condition to calculate the tonic pupil size for each group as our baseline measure. **Table 3** reports group differences in this measure and the following pupillometry measures. Analysis revealed significant differences between the groups [$F(2, 92) = 3.32$, $p = 0.044$]. *Post-hoc* analysis showed the AUT group had a significantly greater tonic pupil size than both the NT group and ANX groups. **Figure 1** depicts this group

	Mean ± SD			F (df)	p	Direction
	AUT	**ANX**	**NT**			
Baseline Pupil Size	3.92 ± 0.54	3.59 ± 0.28	3.63 ± 0.42	3.32 (2.92)	0.044	AUT=ANX>NT
Sound1 Response	0.311 ± 0.33	0.272 ± 0.32	0.298 ± 0.34	0.67 (2.92)	0.514	AUT=ANX=NT
Sound2 Response	0.715 ± 0.45	0.667 ± 0.37	0.905 ± 0.57	14.62 (2.86)	0.334	AUT=ANX=NT

AUT, autistic adults; ANX, highly anxious adults; NT, neurotypical adults; Baseline Pupil Size, tonic pupil size averaged across all trials of the baseline "no-sound" condition. Sound1 and 2 initial responses are the difference of the peak pupil size in the 2000 ms following the first onset of that sound minus the pupil size at Time 0 immediately before the onset of that sound.
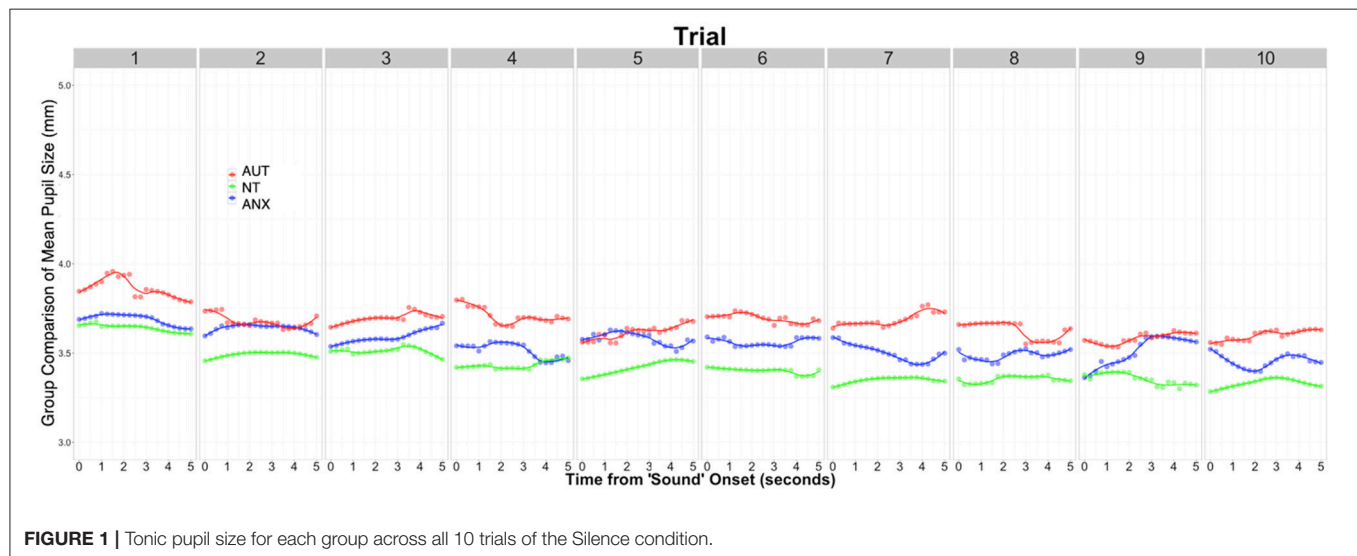
FIGURE 1 | Tonic pupil size for each group across all 10 trials of the Silence condition.

difference in tonic pupil size on a trial-by-trial basis. Standard ANOVA analysis showed identical results. In detail, **Figure 1** shows that pupil size was largest for the AUT group at the beginning of the block, with the ANX group in-between the AUT and NT groups. This pattern remained constant throughout each 5-second block of "silence" trials.

We used Hierarchal Linear Modeling (HLM) to calculate pre-experiment pupil size and the effects of that starting point on general physiological arousal throughout the whole experiment. HLM is especially useful for these analyses because the models account for inter-individual variability with the aim to separate "true effects" from "random effects" created by individual variability (68). The AUT group's pupil size at Time 0, immediately before the Silent block began—equivalent to the intercept of the model–was significantly larger than the NT group ($t = -2.20$, $p = 0.031$), but not significantly different from the ANX group ($t=-1.83$, $p=0.071$), indicating higher arousal of the AUT group compared to the NT group at the beginning of the experiment. Pupil size for the ANX group was in-between that of the AUT and NT groups, and secondary analysis using the ANX group as the reference showed no difference from the other two groups. Throughout the duration of the experiment, the AUT group's pupil size did not change significantly compared to the null slope of zero ($t = 0.74$, $p = 0.428$). The NT group slope was not significantly different than the AUT group ($t = 1.86$, $p = 0.063$), indicating that their mean pupil size also did

not change during the course of the task. However, the ANX group showed decreased pupil size across the duration of the experiment compared to the AUT group ($t = -2.69$, $p = 0.007$) and the NT group ($t = -2.01$, $p = 0.045$). Putting these analyses together, the AUT group started with a larger pupil size than the NT group but was not significantly different than the ANX group, but the ANX group decreased over the course of the experiment while the AUT group did not, so that the difference between the two groups increased significantly over the course of the experiment.

Aim 3: Auditory Response and Habituation

As is common with psychophysiology measurements, most pupillometry data were positively skewed and we analyzed data using Kruskal-Wallis tests with Dunn's tests for *post-hoc* comparisons. Follow-up ANOVA analyses reported identical results in every case. We divided our analyses regarding habituation into three steps. First was to compare the initial response to hearing each sound, as a measure of arousal when orienting to novel stimuli. Second was to track the rate of decline in pupil size from the offset of the sound stimulus to the beginning of the next trial. Third was to track the slope of response magnitude from trial-to-trial as a measure of habituation to each sound over the duration of the stimulus block.

Initial Response to Sound Stimuli

We examined initial pupil response to each of the two sounds by looking at the peak pupil change within the first 2000 ms following sound onset, during the first trial for that sound. There was no between-groups difference for either sound: Sound1 [$F(2, 92) = 0.67$, $p = 0.51$], Sound2 [$F(2, 92) = 2.29$, $p = 0.11$]. Thus, there were no overall group differences in the initial response to each tone.

Recovery After Sound Stimulus

We next analyzed potential group differences in recovery following the sound stimuli. Our first analysis showed that, across the combined Sound1 and Sound2 trials, there was no significant between-groups difference in pupil size at the time of sound offset. We then calculated the slope of pupil size for the duration from sound offset to the beginning of the next trial, using the number of seconds from sound offset as the time variable. Visual inspection of the data and unconditional growth curve models suggested that a quadratic transformation of the time (in seconds) showed the best model fit (See **Supplemental Table 1**). All three groups showed significant decrease in pupil size from the offset of the sound to the start of the next trial (**Supplemental Table 2**). The AUT group showed slower recovery than the NT group ($t = -5.51$, $p < 0.001$) and faster recovery than the ANX group ($t = 68.65$, $p < 0.001$).

Auditory Habituation to the Sound Stimuli

Our critical question of habituation was analyzed by calculating change in per-trial pupil response across all of the sound trials. We began by calculating the difference between the baseline for each trial (i.e., mean pupil size during the 500 ms silence) and the peak pupillary response during presentation of the sound stimulus (2000 ms). We utilized HLM to model change in this response over time. Visual inspection of the data and unconditional growth models indicated that a natural log transformation of trial ["ln(trial)"] variable provided the best fit of the data for both Sounds blocks (see **Supplemental Tables 3, 4**). The final model for the peak change in pupil size across the Sounds blocks included fixed effects of group and trial, the group-by-trial interaction, and the random effects of trial.

Figures 2, **3** depict the habituation trends. Results for Sound 1 block showed a significant effect for trial but non-significant effects for group or the group-by-trial interaction. Thus, the three groups habituated to Sound1 at similar rates. There was likewise a strong habituation response for the Sound2 trials, but non-significant group main effects or group-by-trial interaction effects (see **Supplemental Tables 5, 6**).

DISCUSSION

There were two separate types of measures in this study: the first is tonic pupil size, that is, pupil size in the absence of any explicit sensory stimulation, which may index everyday physiological arousal that co-exists with feelings of anxiety. The second is the change in pupil size in response to sensory (i.e., auditory) stimulation which may relate t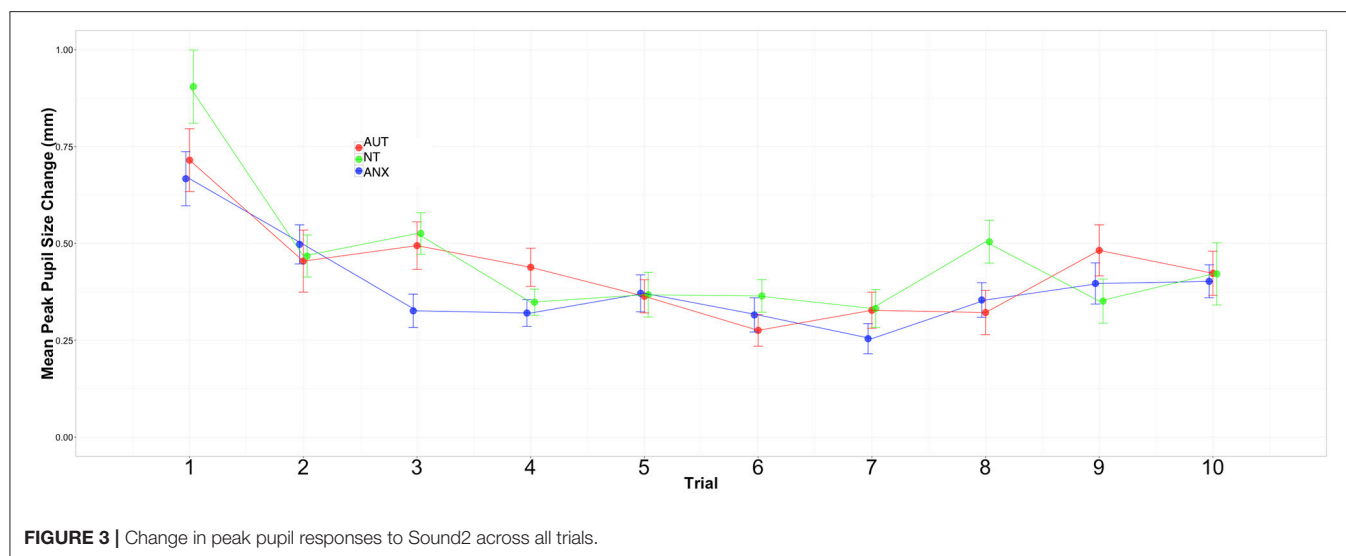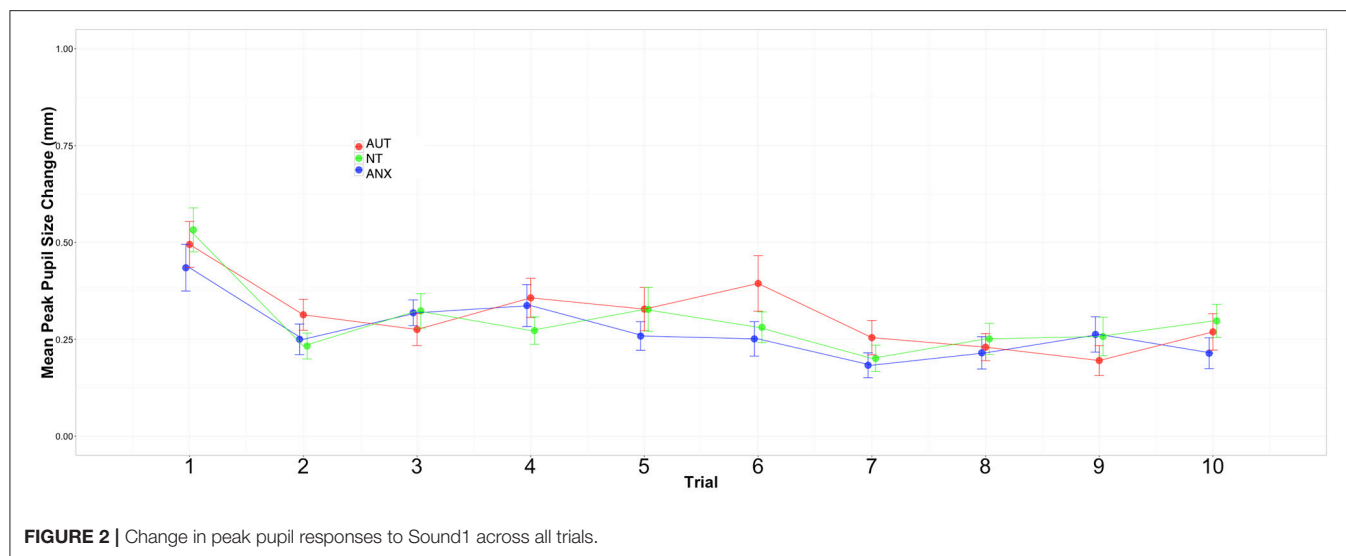o basic sensory processes. There is growing evidence to suggest that sensory sensitivity and anxiety are especially related to each other in autism (18). To our knowledge, this is the first comparison of a cross-clinical sample of autistic adults, non-autistic anxious adults and neurotypical non-anxious adults, looking directly at these measures of baseline arousal and subsequent reactivity and habituation to sensory stimuli. We will discuss the findings of this study according its three aims.

Aim 1: Sensory Processing in Autistic Adults

The first aim of this study was to evaluate sensory processing behaviors, and their link to measures of anxious apprehension and autism traits, in autistic adults vis-à-vis clinical and non-clinical comparison groups. The Adolescent and Adult Sensory Profile (AASP) measures sensory experience extensively across four domains, and the AUT group scored higher than the NT sample in every domain, with the ANX group falling in-between. These self-report data match earlier findings from child samples that relied on parent-report surveys, and confirm that challenging sensory experiences in autism persist into adulthood. While the anxiety group did report differences from the NT sample in the domains of sensory sensitivity and sensory seeking behavior, these differences are much more pronounced in the AUT group. These findings suggest a link between anxiety and sensory experiences in which atypical sensory processing may contribute to heightened anxiety, serving as evidence that a potential mechanism for the increased in anxiety in autism is atypical sensory processing. Although the sample size in this study did not permit more sophisticated statistical modeling, our dimensional data further suggest that intolerance of uncertainty and anxiety (at least for a measure of worry/anxious apprehension) may be related to sensory processing. Further research with larger sample sizes is needed to test models of the underlying mechanisms for anxiety in autism similar to studies such as those done with alexithymia (31, 39).

Aim 2: Baseline Arousal and General Physiological Arousal in Autistic Adults

A notable finding from our pupillometry measures is increased pupil dilation in the ASD group at baseline (before any auditory stimulation). In the absence of a known physical reason for between-group differences in pupil size, it is possible that this difference reflects ongoing elevated physiological arousal in the autism sample. As reviewed in Aim 3 below, the measure of auditory habituation showed that the autism group habituated to the stimuli at similar rates as the comparison groups. However, larger absolute pupil size persisted in the autism group throughout the course of the experiment. That is, even as pupil size decreased with habituation to the sound stimulus for all groups, pupil size in the AUT group never came down to match the other groups. This elevation may reflect frequently increased activation of the sympathetic nervous system in autism that does not diminish over time. Such chronic hyperarousal could function as a mechanism and/or consequence of anxiety in autism. While not a universal finding in the autism literature,

FIGURE 2 | Change in peak pupil responses to Sound1 across all trials.

FIGURE 3 | Change in peak pupil responses to Sound2 across all trials.

there are an increasing number of suggestions that everyday physiological arousal is atypical in many autistic people and could include chronic physiological arousal (35, 42, 69).

This idea was also suggested by the first ever fMRI study of fear conditioning in autism (43), where we found much stronger amygdala activation to threat vs. safe cues in a neurotypical adult sample, but significantly reduced differentiation between threat and safe in the autistic sample. We wondered whether elevated baseline arousal—either in everyday life and/or as a function of the intense sensory environment of the MRI setting—provided a sort of ceiling effect for amygdala activation so that additional, task-based activation was less likely. We have recently undertaken a study of physiolgical arousal over the course of a psychotherapy session which may shed light on this. Studies with ambulatory or other, more ecologically valid approaches would certianly be useful for elucidating these possibilities.

Aim 3: Auditory Response and Habituation

Habituation is defined as an exponential decrement of a response to an initially novel stimulus that is presented repeatedly over time (55, 70). Both animal and human research provide strong support for links between less successful habituation with less flexible adaptation that may underlie anxiety. One intriguing model of autism (71, 72) suggests that challenges integrating prior and current environmental input—for example difficulties with sensory habituation—could drive a unique sensory-perceptual experience that can make the autistic world seem "too real" and overwhelming. However, our study found no group differences in the initial response to sound stimuli (i.e., pupil size change when hearing the first sound) or in habituation to those stimuli over time. Thus, our data do not support a link between atypical sensory habituation and anxiety. However, this may be because ours was a very simple task that required no activity or active learning. It may be that increased task demands

could over-tax sensory integration systems, as suggested by fMRI studies from Green et al. (52, 53) who sowed atypical sensory response only for simultaneous stimulation of multiple sensory systems, and not for single sensory modalities presented separately. Possible questions for future research include: Could it be that chronically elevated physiological arousal, and/or an elevated response to lab-based stressors, could modulate findings in habituation studies (43)? Is habituation decreased for unimodal sensory stimulation (i.e., only auditory or only tactile) or do difficulties appear only in the integration of multiple stimuli at once (as is common in real life) (52)? Does uncertainty associated with sensory processing challenges directly contribute to the intolerance of uncertainty that seems so prominent in autism (18, 20)?

Limitations

There are several limitations for this study. Firstly, the compositions of our groups were different on multiple levels. For instance, the ASD group was significantly older than the ANX and NT groups. However, HLM analyses indicated that age was not a significant predictor of pupil responses. There were more females in the ANX group than in the ASD or NT groups. Some research has shown that females have larger pupil responses than males to neutral stimuli (73). The ANX group was not formally diagnosed with anxiety disorders, and we did not assume a formal diagnosis in our conceptual or experimental findings. They were a group of individuals (a) who were actively seeking treatment for emotional distress; (b) who scored high on common intake measures of anxiety used widely in college counseling centers, and not so high on depression; (c) scored high on study measures of anxious apprehension/worry (the PSWQ) as well as on the intolerance of uncertainty measure (IUS-12). But subsequent studies with carefully characterized clinical groups including anxiety are necessary before making any stronger conclusions about the overlap of anxiety and autism. As noted above, the limited sample size precluded mediation modeling and other useful approaches. The lack of correlation between our self-report questionnaires and observed psychophysiological responses is predicted by recent arguments from LeDoux et al. that psychophysiological defense mechanisms are separate from the subjective, conscious experience of fear (74, 75) although this framework is quite controversial. Linking psychophysiology with questionnaire data has been traditionally problematic in autism (76) and more research about how different systems might feed into each other is an important and ripe area for research.

This study also has some strength. We believe that the involvement of additional clinical samples such as anxiety is an essential approach for research moving forward, as is now happening in many research groups. Pupillometry is a simple and non-invasive physiological measure that precluded participant attrition. The auditory habituation task was the simplest possible protocol to test our hypotheses, examining basic sensory processes that are less reliant on higher-level cognitive processes.

Clinical Implications

While many autistic adults figure out how to compensate for differences in social styles and motivation, and could find success in relationships, employment and other settings, success is often impeded by overwhelming feelings of anxiety. Many autistic adults continue to be bothered by sensory stimulation that is disruptive in its own right and may further exacerbate anxiety. One autistic adult in our study reported that he feels "at war with the world" because of frequently overwhelming sensory stimulation. This can lead to frequent feelings of confusion and uncertainty that mediate the link between sensory experience and anxiety, and could contribute to everyday feelings of challenge and heightened physiological arousal.

Attention to sensory experience is not a standard element of cognitive behavioral therapy (CBT) or other treatment modalities. In light of increased awareness of how sensory experience and anxiety uniquely interact in autism, an explicit focus on sensory processing challenges will likely be beneficial for many children and adults in home, school, work, and therapeutic settings (18, 77–79). Consultation with the autistic student/employee/client and those who know them well can be essential for understanding the nature of sensory and anxiety experiences and learning how to utilize that information to build supports and/or interventions to alleviate sensory challenges (80, 81). Such approaches could include additional environmental supports or changes (including those used in occupational therapy), as well the autistic person learning how to manage sensory challenges more effectively.

As understanding grows of cognitive, emotional, and sensory contributions to anxiety in autism, it is imperative to assimilate targeted treatment approaches—certainly behavioral and possibly pharmacological approaches—into autism interventions (9, 36, 39, 79, 82, 83). Anxiety in autism *is different* than anxiety without autism, and intervention approaches need to adapt accordingly. At the same time, it is essential to further explore heterogeneity in autism. Sensory experience, and anxiety experience (and alexithymia and intolerance of uncertainty and many other constructs) are not universal within autism. Several recent studies have highlighted the importance of examining varying levels of anxiety within large autism samples (48, 84).

Consulting with the autistic person on what challenges are most detrimental to their success is essential. Understanding that typical approaches to anxiety have considerable efficacy in autism is helpful [e.g., (85–89)]. But it is equally necessary to realize that there are important, unique aspects of anxiety in autism including (a) differences in central and autonomic nervous system function (15, 48, 84); the validity of typical anxiety symptom questionnaires (90–92); and helpful modifications for treatment (80, 81, 93). Thus, behavioral and pharmacological treatments for anxiety in autism should think outside the box, including explicit and dedicated attention to the impact of atypical sensory experience in so many autistic children and adults.

AUTHOR CONTRIBUTIONS

DT, SL, and MS conceptualized and designed the project. DT and KS were involved in data collection with equipment provided by SL. DT, KS, and SL completed data processing and all

REFERENCES

1. Tomchek SD, Dunn W. Sensory processing in children with and without autism: a comparative study using the short sensory profile. *Am J Occup Ther Off Publ Am Occup Ther Assoc.* (2007) 61:190–200. doi: 10.5014/ajot.61.2.190

2. Tomchek SD, Huebner RA, Dunn W. Patterns of sensory processing in children with an autism spectrum disorder. *Res Autism Spectr Disord.* (2014) 8:1214–24. doi: 10.1016/j.rasd.2014.06.006

3. Baranek GT, Boyd BA, Poe MD, David FJ, Watson LR, MacLean Jr WE. Hyperresponsive sensory patterns in young children with autism, developmental delay, and typical development. *Am J Ment Retard.* (2007) 112:233–45. doi: 10.1352/0895-8017(2007)112[233:HSPIYC]2.0.CO;2

4. Green D, Chandler S, Charman T, Simonoff E, Baird G. Brief report: DSM-5 sensory behaviours in children with and without an autism spectrum disorder. *J Autism Dev Disord.* (2016) 46:3597–606. doi: 10.1007/s10803-016-2881-7

5. Leekam SR, Nieto C, Libby SJ, Wing L, Gould J. Describing the sensory abnormalities of children and adults with autism. *J Autism Dev Disord.* (2006) 37:894–910. doi: 10.1007/s10803-006-0218-7

6. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition. DSM-V.* American Psychiatric Association Publishing (2013).

7. Christensen DL, Baio J, Braun KVN, Bilder D, Charles J, Constantino JN, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years — autism and developmental disabilities monitoring network, 11 Sites, United States, 2012. *MMWR Surveill Summ.* (2016) 65:1–23. doi: 10.15585/mmwr.ss6503a1

8. Buck TR, Viskochil J, Farley M, Coon H, McMahon WM, Morgan J, et al. Psychiatric comorbidity and medication use in adults with autism spectrum disorder. *J Autism Dev Disord.* (2014) 44:3063–71. doi: 10.1007/s10803-014-2170-2

9. Kerns CM, Kendall PC, Berry L, Souders MC, Franklin ME, Schultz RT, et al. Traditional and atypical presentations of anxiety in youth with autism spectrum disorder. *J Autism Dev Disord.* (2014) 44:2851–61. doi: 10.1007/s10803-014-2141-7

10. van Steensel FJA, Bögels SM, de Bruin EI. Psychiatric comorbidity in children with autism spectrum disorders: a comparison with children with ADHD. *J Child Fam Stud.* (2013) 22:368–76. doi: 10.1007/s10826-012-9587-z

11. Gotham K, Bishop SL, Hus V, Huerta M, Lund S, Buja A, et al. Exploring the relationship between anxiety and insistence on sameness in autism spectrum disorders. *Autism Res Off J Int Soc Autism Res.* (2013) 6:33–41. doi: 10.1002/aur.1263

12. Rodgers J, Glod M, Connolly B, McConachie H. The relationship between anxiety and repetitive behaviours in autism spectrum disorder. *J Autism Dev Disord.* (2012) 42:2404–9. doi: 10.1007/s10803-012-1531-y

13. Luke L, Clare ICH, Ring H, Redley M, Watson P. Decision-making difficulties experienced by adults with autism spectrum conditions. *Autism Int J Res Pract.* (2012) 16:612–21. doi: 10.1177/1362361311415876

14. Conner CM, Maddox BB, White SW. Parents' state and trait anxiety: relationships with anxiety severity and treatment response in adolescents with autism spectrum disorders. *J Autism Dev Disord.* (2013) 43:1811–8. doi: 10.1007/s10803-012-1728-0

15. Corbett BA, Muscatello RA, Blain SD. Impact of sensory sensitivity on physiological stress response and novel peer interaction in children with

and without autism spectrum disorder. *Front Neurosci.* (2016) 10:278. doi: 10.3389/fnins.2016.00278

16. Green SA, Ben-Sasson A. Anxiety disorders and sensory over-responsivity in children with autism spectrum disorders: is there a causal relationship? *J Autism Dev Disord.* (2010) 40:1495–504. doi: 10.1007/s10803-010-1007-x

17. Lidstone J, Uljarević M, Sullivan J, Rodgers J, McConachie H, Freeston M, et al. Relations among restricted and repetitive behaviors, anxiety and sensory features in children with autism spectrum disorders. *Res Autism Spectr Disord.* (2014) 8:82–92. doi: 10.1016/j.rasd.2013.10.001

18. South M, Rodgers J. Sensory, emotional and cognitive contributions to anxiety in autism spectrum disorders. *Front Hum Neurosci.* (2017) 11:20. doi: 10.3389/fnhum.2017.00020

19. Uljarević M, Carrington S, Leekam S. Brief report: effects of sensory sensitivity and intolerance of uncertainty on anxiety in mothers of children with autism spectrum disorder. *J Autism Dev Disord.* (2015) 46:315–9. doi: 10.1007/s10803-015-2557-8

20. Wigham S, Rodgers J, South M, McConachie H, Freeston M. The interplay between sensory processing abnormalities, intolerance of uncertainty, anxiety and restricted and repetitive behaviours in autism spectrum disorder. *J Autism Dev Disord.* (2015) 45:943–52. doi: 10.1007/s10803-014-2248-x

21. Gasquoine PG. Contributions of the insula to cognition and emotion. *Neuropsychol Rev.* (2014) 24:77–87. doi: 10.1007/s11065-014-9246-9

22. Lübke KT, Pause BM. Always follow your nose: the functional significance of social chemosignals in human reproduction and survival. *Horm Behav.* (2015) 68:134–44. doi: 10.1016/j.yhbeh.2014.10.001

23. Soumiya H, Godai A, Araiso H, Mori S, Furukawa S, Fukumitsu H. Neonatal whisker trimming impairs fear/anxiety-related emotional systems of the amygdala and social behaviors in adult mice. *PLoS ONE* (2016) 11:e0158583. doi: 10.1371/journal.pone.0158583

24. Acevedo BP, Jagiellowicz J, Aron E, Marhenke R, Aron A, Acevedo B. Sensory processing sensitivity and childhood quality's effects on neural responses to emotional stimuli. *Clin Neuropsychiatry* (2017) 14:359–73.

25. Ahadi B, Basharpoor S. Relationship between sensory processing sensitivity, personality dimensions and mental health. *J Appl Sci.* (2010) 10:570–4. doi: 10.3923/jas.2010.570.574

26. Aron EN, Aron A. Sensory-processing sensitivity and its relation to introversion and emotionality. *J Pers Soc Psychol.* (1997) 73:345–68.

27. Wallis K, Sutton D, Bassett S. Sensory modulation for people with anxiety in a community mental health setting. *Occup Ther Ment Health* (2018) 34:122–37. doi: 10.1080/0164212X.2017.1363681

28. Neil L, Olsson NC, Pellicano E. The relationship between intolerance of uncertainty, sensory sensitivities, and anxiety in autistic and typically developing children. *J Autism Dev Disord.* (2016) 46:1962–73. doi: 10.1007/s10803-016-2721-9

29. Green SA, Ben-Sasson A, Soto TW, Carter AS. Anxiety and sensory over-responsivity in toddlers with autism spectrum disorders: Bidirectional effects across time. *J Autism Dev Disord.* (2012) 42:1112–9. doi: 10.1007/s10803-011-1361-3

30. Uljarević M, Prior MR, Leekam SR. First evidence of sensory atypicality in mothers of children with Autism Spectrum Disorder (ASD). *Mol Autism* (2014) 5:26. doi: 10.1186/2040-2392-5-26

31. Milosavljevic B, Carter Leno V, Simonoff E, Baird G, Pickles A, Jones CRG, et al. Alexithymia in adolescents with autism spectrum disorder: its relationship to internalising difficulties, sensory modulation

and social cognition. *J Autism Dev Disord.* (2016) 46:1354–67. doi: 10.1007/s10803-015-2670-8

32. Brown C, Tollefson N, Dunn W, Cromwell R, Filion D. The adult sensory profile: measuring patterns of sensory processing. *Am J Occup Ther.* (2001) 55:75–82. doi: 10.5014/ajot.55.1.75

33. Dugas MJ, Gagnon F, Ladouceur R, Freeston MH. Generalized anxiety disorder: a preliminary test of a conceptual model. *Behav Res Ther.* (1998) 36:215–26. doi: 10.1016/S0005-7967(97)00070-3

34. Boulter C, Freeston M, South M, Rodgers J. Intolerance of uncertainty as a framework for understanding anxiety in children and adolescents with autism spectrum disorders. *J Autism Dev Disord.* (2014) 44:1391–402. doi: 10.1007/s10803-013-2001-x

35. Chamberlain PD, Rodgers J, Crowley MJ, White SE, Freeston MH, South M. A potentiated startle study of uncertainty and contextual anxiety in adolescents diagnosed with autism spectrum disorder. *Mol Autism* (2013) 4:31. doi: 10.1186/2040-2392-4-31

36. Keefer A, Kreiser NL, Singh V, Blakeley-Smith A, Duncan A, Johnson C, et al. Intolerance of uncertainty predicts anxiety outcomes following CBT in youth with ASD. *J Autism Dev Disord.* (2016) 47:3949–58 doi: 10.1007/s10803-016-2852-z

37. Einstein DA. Extension of the transdiagnostic model to focus on intolerance of uncertainty: a review of the literature and implications for treatment. *Clin Psychol Sci Pract.* (2014) 21:280–300. doi: 10.1111/cpsp.12077

38. McEvoy PM, Mahoney AEJ. To be sure, to be sure: intolerance of uncertainty mediates symptoms of various anxiety disorders and depression. *Behav Ther.* (2012) 43:533–45. doi: 10.1016/j.beth.2011.02.007

39. Maisel ME, Stephenson KG, South M, Rodgers J, Freeston MH, Gaigg SB. Modeling the cognitive mechanisms linking autism symptoms and anxiety in adults. *J Abnorm Psychol.* (2016) 125:692–703. doi: 10.1037/abn0000168

40. Ben-Sasson A, Hen L, Fluss R, Cermak SA, Engel-Yeger B, Gal E. A meta-analysis of sensory modulation symptoms in individuals with autism spectrum disorders. *J Autism Dev Disord.* (2008) 39:1–11. doi: 10.1007/s10803-008-0593-3

41. Liss M, Mailloux J, Erchull MJ. The relationships between sensory processing sensitivity, alexithymia, autism, depression, and anxiety. *Person Individ Differ.* (2008) 45:255–9. doi: 10.1016/j.paid.2008.04.009

42. Takahashi H, Komatsu S, Nakahachi T, Ogino K, Kamio Y. Relationship of the acoustic startle response and its modulation to emotional and behavioral problems in typical development children and those with autism spectrum disorders. *J Autism Dev Disord.* (2016) 46:534–43. doi: 10.1007/s10803-015-2593-4

43. Top Jr DN, Stephenson KG, Doxey CR, Crowley MJ, Kirwan CB, South M. Atypical amygdala response to fear conditioning in autism spectrum disorder. *Biol Psychiatry Cogn Neurosci Neuroimaging* (2016) 1:308–15. doi: 10.1016/j.bpsc.2016.01.008

44. Anderson CJ, Colombo J. Larger tonic pupil size in young children with autism spectrum disorder. *Dev Psychobiol.* (2009) 51:207–11. doi: 10.1002/dev.20352

45. Anderson CJ, Colombo J, Unruh KE. Pupil and salivary indicators of autonomic dysfunction in autism spectrum disorder. *Dev Psychobiol.* (2013) 55:465–82. doi: 10.1002/dev.21051

46. Nuske HJ, Vivanti G, Dissanayake C. No evidence of emotional dysregulation or aversion to mutual gaze in preschoolers with autism spectrum disorder: an eye-tracking pupillometry study. *J Autism Dev Disord.* (2015) 45:3433–45 doi: 10.1007/s10803-015-2479-5

47. Martineau J, Hernandez N, Hiebel L, Roché L, Metzger A, Bonnet-Brilhault F. Can pupil size and pupil responses during visual scanning contribute to the diagnosis of autism spectrum disorder in children? *J Psychiatr Res.* (2011) 45:1077–82. doi: 10.1016/j.jpsychires.2011.01.008

48. Herrington JD, Maddox BB, Kerns CM, Rump K, Worley JA, Bush JC, et al. Amygdala volume differences in autism spectrum disorder are related to anxiety. *J Autism Dev Disord.* (2017) 47:3682–91. doi: 10.1007/s10803-017-3206-1

49. Uddin LQ. Salience processing and insular cortical function and dysfunction. *Nat Rev Neurosci.* (2015) 16:55–61. doi: 10.1038/nrn3857

50. Uddin LQ, Menon V. The anterior insula in autism: under-connected and under-examined. *Neurosci Biobehav Rev.* (2009) 33:1198–203. doi: 10.1016/j.neubiorev.2009.06.002

51. Herry C, Bach DR, Esposito F, Salle FD, Perrig WJ, Scheffler K, et al. Processing of temporal unpredictability in human and animal amygdala. *J Neurosci.* (2007) 27:5958–66. doi: 10.1523/JNEUROSCI.5218-06.2007

52. Green SA, Hernandez L, Tottenham N, Krasileva K, Bookheimer SY, Dapretto M. Neurobiology of sensory overresponsivity in youth with autism spectrum disorders. *JAMA Psychiatry* (2015) 72:778–86. doi: 10.1001/jamapsychiatry.2015.0737

53. Green SA, Rudie JD, Colich NL, Wood JJ, Shirinyan D, Hernandez L, et al. Overreactive brain responses to sensory stimuli in youth with autism spectrum disorders. *J Am Acad Child Adolesc Psychiatry* (2013) 52:1158–72. doi: 10.1016/j.jaac.2013.08.004

54. Järvinen A, Ng R, Crivelli D, Neumann D, Arnold AJ, Woo-VonHoogenstyn N, et al. Social functioning and autonomic nervous system sensitivity across vocal and musical emotion in williams syndrome and autism spectrum disorder. *Dev Psychobiol.* (2015) 58:17–26. doi: 10.1002/dev.21335

55. Madsen GF, Bilenberg N, Cantio C, Oranje B. Increased prepulse inhibition and sensitization of the startle reflex in autistic children. *Autism Res.* (2014) 7:94–103. doi: 10.1002/aur.1337

56. Puts NAJ, Wodka EL, Tommerdahl M, Mostofsky SH, Edden RAE. Impaired tactile processing in children with autism spectrum disorder. *J Neurophysiol.* (2014) 111:1803–11. doi: 10.1152/jn.00890.2013

57. Lord C, Risi S, Lambrecht L, Cook EH, Leventhal BL, DiLavore PC, et al. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord.* (2000) 30:205–23. doi: 10.1023/A:1005592401947

58. Locke BD, Buzolitz JS, Lei P-W, Boswell JF, McAleavey AA, Sevig TD, et al. Development of the counseling center assessment of psychological symptoms-62 (CCAPS-62). *J Couns Psychol.* (2011) 58:97–109. doi: 10.1037/a0021282

59. Baron-Cohen S, Wheelwright S, Skinner R, Martin J, Clubley E. The autism-spectrum quotient (AQ): evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians. *J Autism Dev Disord.* (2001) 31:5–17. doi: 10.1023/A:1005653411471

60. Bishop DVM, Maybery M, Maley A, Wong D, Hill W, Hallmayer J. Using self-report to identify the broad phenotype in parents of children with autistic spectrum disorders: a study using the Autism-Spectrum Quotient. *J Child Psychol Psychiatry* (2004) 45:1431–6. doi: 10.1111/j.1469-7610.2004.00849.x

61. Meyer TJ, Miller ML, Metzger RL, Borkovec TD. Development and validation of the Penn State Worry Questionnaire. *Behav Res Ther.* (1990) 28:487–95.

62. Dear BF, Titov N, Sunderland M, McMillan D, Anderson T, Lorian C, et al. Psychometric comparison of the generalized anxiety disorder scale-7 and the penn state worry questionnaire for measuring response during treatment of generalised anxiety disorder. *Cogn Behav Ther.* (2011) 40:216–27. doi: 10.1080/16506073.2011.582138

63. Carleton RN, Norton MAPJ, Asmundson GJG. Fearing the unknown: A short version of the Intolerance of Uncertainty Scale. *J Anxiety Disord.* (2007) 21:105–17. doi: 10.1016/j.janxdis.2006.03.014

64. Dunn W. The impact of sensory processing abilities on the daily lives of young children and their families: a conceptual model. *Infants Young Child* (1997) 9:23–35.

65. R Core Team. *R: A Language and Environment for Statistical Computing.* Vienna: R Foundation for Statistical Computing (2013). Available online at: http://www.R-project.org/

66. Sirois S, Brisson J. Pupillometry. *Wiley Interdiscip Rev Cogn Sci.* (2014) 5:679–92. doi: 10.1002/wcs.1323

67. South M, Carr AW, Stephenson KG, Maisel ME, Cox JC. Symptom overlap on the srs-2 adult self-report between adults with asd and adults with high anxiety. *Autism Res Off J Int Soc Autism Res.* (2017) 10:1215–20. doi: 10.1002/aur.1764

68. Singer JD, Willett JB, Willett CWEPJB, Willett JB. *Applied Longitudinal Data Analysis: Modeling Change and Event Occurrence.* Oxford, UK: Oxford University Press (2003).

69. Chiu TA, Anagnostou E, Brian J, Chau T, Kushki A. Specificity of autonomic arousal to anxiety in children with autism spectrum disorder. *Autism Res.* (2016) 9:491–501. doi: 10.1002/aur.1528

70. Sinclair D, Oranje B, Razak KA, Siegel SJ, Schmid S. Sensory processing in autism spectrum disorders and Fragile X syndrome-from the clinic to animal models. *Neurosci Biobehav Rev.* (2016) 76:235–53. doi: 10.1016/j.neubiorev.2016.05.029

71. Pellicano E, Burr D. When the world becomes "too real": a Bayesian explanation of autistic perception. *Trends Cogn Sci.* (2012) 16:504–10. doi: 10.1016/j.tics.2012.08.009

72. Lawson RP, Rees G, Friston KJ. An aberrant precision account of autism. *Front Hum Neurosci.* (2014) 8:302. doi: 10.3389/fnhum.2014.00302

73. Partala T, Surakka V. Pupil size variation as an indication of affective processing. *Int J Hum Comput Stud.* (2003) 59:185–98. doi: 10.1016/S1071-5819(03)00017-X

74. LeDoux JE, Brown R. A higher-order theory of emotional consciousness. *Proc Natl Acad Sci USA.* (2017) 114:E2016–25. doi: 10.1073/pnas.1619316114

75. LeDoux JE, Pine DS. Using neuroscience to help understand fear and anxiety: a two-system framework. *Am J Psychiatry* (2016) 173:1083–93. doi: 10.1176/appi.ajp.2016.16030353

76. Geurts HM, Corbett B, Solomon M. The paradox of cognitive flexibility in autism. *Trends Cogn Sci.* (2009) 13:74–82. doi: 10.1016/j.tics.2008.11.006

77. Howe FEJ, Stagg SD. How sensory experiences affect adolescents with an autistic spectrum condition within the classroom. *J Autism Dev Disord.* (2016) 46:1656–68. doi: 10.1007/s10803-015-2693-1

78. Ozsivadjian A, Knott F. Anxiety problems in young people with autism spectrum disorder: a case series. *Clin Child Psychol Psychiatry* (2011) 16:203–14. doi: 10.1177/1359104511404749

79. van Steensel FJA, Heeman EJ. Anxiety levels in children with autism spectrum disorder: a meta-analysis. *J Child Fam Stud.* (2017) 26:1753–67. doi: 10.1007/s10826-017-0687-7

80. Rodgers J, Hodgson A, Shields K, Wright C, Honey E, Freeston M. Towards a treatment for intolerance of uncertainty in young people with autism spectrum disorder: development of the coping with uncertainty in everyday situations (CUES©) programme. *J Autism Dev Disord.* (2016) 47:3959–66. doi: 10.1007/s10803-016-2924-0

81. Rodgers J, Ofield A. Understanding, recognising and treating co-occurring anxiety in autism. *Curr Dev Disord Rep.* (2018) 5:58–64. doi: 10.1007/s40474-018-0132-7

82. Vasa RA, Keefer A, Reaven J, South M, White SW. Priorities for advancing research on youth with autism spectrum disorder and co-occurring anxiety. *J Autism Dev Disord.* (2017) 48:925–34. doi: 10.1007/s10803-017-3320-0

83. White SW, Mazefsky CA, Dichter GS, Chiu PH, Richey JA, Ollendick TH. Social-cognitive, physiological, and neural mechanisms underlying emotion regulation impairments: understanding anxiety in autism spectrum disorder. *Int J Dev Neurosci Off J Int Soc Dev Neurosci.* (2014) 39:22–36. doi: 10.1016/j.ijdevneu.2014.05.012

84. Hollocks MJ, Howlin P, Papadopoulos AS, Khondoker M, Simonoff E. Differences in HPA-axis and heart rate responsiveness to psychosocial stress in children with autism spectrum disorders with and without co-morbid anxiety. *Psychoneuroendocrinology* (2014) 46:32–45. doi: 10.1016/j.psyneuen.2014.04.004

85. Reaven J, Blakeley-Smith A, Leuthe E, Moody E, Hepburn S. Facing your fears in adolescence: cognitive-behavioral therapy for high-functioning autism

86. Storch EA, Arnold EB, Lewin AB, Nadeau JM, Jones AM, De Nadai AS, et al. The effect of cognitive-behavioral therapy versus treatment as usual for anxiety in children with autism spectrum disorders: a randomized, controlled trial. *J Am Acad Child Adolesc Psychiatry* (2013) 52:132–142.e2. doi: 10.1016/j.jaac.2012.11.007

87. van Steensel FJA, Bögels SM. CBT for anxiety disorders in children with and without autism spectrum disorders. *J Consult Clin Psychol.* (2015) 83:512–23. doi: 10.1037/a0039108

88. Wood JJ, Drahota A, Sze K, Har K, Chiu A, Langer DA. Cognitive behavioral therapy for anxiety in children with autism spectrum disorders: a randomized, controlled trial. *J Child Psychol Psychiatry* (2009) 50:224–234. doi: 10.1111/j.1469-7610.2008.01948.x

89. Wood JJ, Ehrenreich-May J, Alessandri M, Fujii C, Renno P, Laugeson E, et al. Cognitive behavioral therapy for early adolescents with autism spectrum disorders and clinical anxiety: a randomized, controlled trial. *Behav Ther.* (2015) 46:7–19. doi: 10.1016/j.beth.2014.01.002

90. Glod M, Creswell C, Waite P, Jamieson R, McConachie H, Don South M, et al. Comparisons of the factor structure and measurement invariance of the spence children's anxiety scale-parent version in children with autism spectrum disorder and typically developing anxious children. *J Autism Dev Disord.* (2017) 47:3834–46. doi: 10.1007/s10803-017-3118-0

91. Magiati I, Lerh JW, Hollocks MJ, Uljarevic M, Rodgers J, McConachie H, et al. The measurement properties of the spence children's anxiety scale-parent version in a large international pooled sample of young people with autism spectrum disorder. *Autism Res.* (2017) 10:1629–52. doi: 10.1002/aur.1809

92. Rodgers J, Wigham S, McConachie H, Freeston M, Honey E, Parr JR. Development of the anxiety scale for children with autism spectrum disorder (asc-asd). *Autism Res.* (2016) 9:1205–15. doi: 10.1002/aur.1603

93. Kerns CM, Roux AM, Connell JE, Shattuck PT. Adapting cognitive behavioral techniques to address anxiety and depression in cognitively able emerging adults on the autism spectrum. *Cogn Behav Pract.* (2016) 23:329–40. doi: 10.1016/j.cbpra.2016.06.002

Prevalence and Correlates of Psychiatric Symptoms in Minimally Verbal Children and Adolescents With ASD

Daniela Plesa Skwerer[1]*, Robert M. Joseph[1,2], Brady Eggleston[1], Steven R. Meyer[1] and Helen Tager-Flusberg[1]

[1] Department of Psychological and Brain Sciences, Boston University, Boston, MA, United States, [2] Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA, United States

Despite many studies documenting the prevalence of various co-occurring psychiatric symptoms in children and adults with ASD, less is known about how these symptoms relate to subtypes defined by particular phenotypic features within the ASD population. We examined the severity and prevalence of comorbid symptoms of psychopathology, emotion dysregulation, and maladaptive behaviors, as well as adaptive functioning, in a group of 65 minimally verbal children ($n = 33$) and adolescents ($n = 32$) with ASD. On the *Child and Adolescent Symptom Inventory* (CASI-5), for all the symptom classifications except oppositional defiant disorder and conduct disorder, more participants in our sample showed elevated or clinically concerning severity scores relative to the general population. On the *Emotion Dysregulation Inventory (EDI),* the mean scores for Reactivity and Dysphoria factors in our sample were lower than in the autism calibration sample, which included a large number of inpatient youth with ASD. Overall, few differences were found between the children and adolescents within this severely impaired group of ASD individuals based on clinical cutoff scores on the CASI-5 and EDI factor scores. Psychiatric comorbidities and emotion dysregulation measures were not correlated with autism symptom severity or with measures of adaptive functioning, and were largely unrelated to IQ in our sample. The number of clinically significant psychiatric symptoms on the CASI-5 emerged as the main predictor of maladaptive behaviors. Findings suggest a wide range of co-occurring psychopathology and high degree of maladaptive behavior among minimally verbal children and adolescents with ASD, which are not directly attributable to autism symptom severity, intellectual disability or limitations in adaptive functioning.

Keywords: psychopathology, minimally verbal autism spectrum disorder, maladaptive behavior, children, adolescents

INTRODUCTION

Interest in the presence of comorbid conditions in individuals with autism spectrum disorder (ASD) has increased considerably in recent years, particularly in research aimed at characterizing the substantial phenotypic heterogeneity found in ASD and its possible underlying etiology (1–4). While many studies have documented the prevalence of various co-occurring psychiatric symptoms

in children and adults with ASD, little is known about how these symptoms relate to specific phenotypic characteristics, such as expressive language ability, which varies significantly among individuals with ASD (5). When researchers have focused on particular subgroups of individuals with ASD, they were typically distinguished by the presence or absence of intellectual disability (ID), without explicit consideration of the ability to use spoken language functionally.

The majority of these studies have indicated a higher prevalence and number of different psychiatric conditions in youth with ASD compared to age-matched control groups without ASD (2, 6, 7), however the reported prevalence of these conditions has varied across studies. In a literature review of existing research on comorbid conditions in individuals with ASD, Mannion and Leader (2) attributed inconsistent findings to the use of different instruments, and differences in study participants' intellectual and communication abilities, among other factors (8–10). For instance, rates of anxiety in individuals with ASD have been estimated between 11 and 84% (11, 12), and rates of ADHD co-occurring with ASD have varied between 28 and 87% (13–15). The inconsistencies in findings across studies could be related to the diagnostic instruments commonly used to characterize psychiatric and behavior dysregulation symptoms in children and adults, which have not been specifically designed to screen for comorbid psychopathology in people with ASD [e.g., the Aberrant Behavior Checklist, ABC; (16, 17); Child Behavior Checklist, CBCL; (18); CASI-5; (19)].

Several studies have employed instruments specifically designed for use with adults with ASD [e.g., Autism Spectrum Disorders—Comorbidity for Adults, ASD-CA; (20)] or children with ASD [e.g., Baby and Infant Screen for Children with Autism Traits; BISCUIT; (21); Autism Comorbidity Interview-Present and Lifetime; ACI-PL; (22); Autism Spectrum Disorders-Comorbidity for Children, ASD-CC; (23, 24)]. Using the Autism Comorbidity Interview [ACI-PL; (22)], which was developed based on an adaptation of the Kiddie-Schedule for Affective Disorders and Schizophrenia [K-SADS; (25)], Leyfer et al. (22) found that the most prevalent psychiatric comorbidity in a sample of verbal 5-to-17-year-olds was specific phobia, present in 44% of the sample, followed by obsessive-compulsive disorder (OCD) present in 37% of the sample. In contrast, Simonoff et al. (14) reported that only 8% of their sample had a diagnosis of OCD, while the most prevalent psychiatric comorbidity was social anxiety (29%), followed by ADHD (28%) in their ASD sample between the ages of 11 and 14 years, most of whom had IQ scores over 70. The utility of these measures for assessing psychiatric comorbidities in non- or minimally verbal (MV) individuals with ASD, many of whom also have intellectual disability, is not clear, given that these studies enrolled primarily individuals with spoken language.

Thus, despite interest in the comorbid psychiatric conditions in people with ASD, there is a paucity of studies that focus on the segment of the population most severely affected, the ∼30% of individuals with ASD who remain non- or minimally verbal beyond school-age (26, 27). In an effort to fill this gap, a research collaborative between several academic and medical institutions was established in 2014, with the goal of conducting common comprehensive assessments on children and adolescents with ASD admitted to inpatient psychiatry care units [the Autism Inpatient Collection; AIC; (28)]. A preliminary paper reporting on 147 participants showed that expressive language impairment (being non-, or minimally verbal) affected 52% of the sample, 43% had intellectual disability, and 27% engaged in self-injurious behavior. Elevated behavioral disturbance was common in this cohort as reflected by high average scores on the Irritability and Hyperactivity subscales of the Aberrant Behavior Checklist [ABC; (16)], which are indicative of clinically concerning externalizing symptoms (4).

A second paper based in part on the same sample (29) directly compared 165 minimally verbal individuals with ASD with a cohort of 268 verbal youth with ASD, drawn from several referral sources, mostly outpatient clinics, on the *Child and Adolescent Symptom Inventory* [CASI-4 and—5; (19, 30)] parent-report rating scales. The main findings were that, regardless of verbal ability status, inpatient participants showed greater severity and were more likely to exceed clinical cutoffs than those in outpatient settings across almost all CASI-4 and—5 psychiatric classifications. However, in comparisons between the minimally verbal and verbal groups, the verbal group had higher symptom severity, and a higher percentage of participants exceeded clinical cut-offs for depression, general anxiety disorder and oppositional defiant disorder. These results were consistent with other reports in the literature that have suggested that better functional communication was associated with higher levels of anxiety in individuals with ASD (31–34), although these other studies did not specifically enroll or investigate *minimally verbal* individuals with ASD as a group. In contrast to the profile of psychiatric comorbidities reported for verbal individuals with ASD, the MV participants assessed by Lerner et al. (29) were more likely to meet the clinical cutoff for ADHD-Hyperactivity/Impulsivity type relative to the verbal participants (50% compared to 37%), when controlling for non-verbal IQ, age, and ADOS calibrated symptom severity scores.

In sum, the majority of previous studies did not enroll non- or minimally verbal youths with ASD and only more recent studies have focused specifically on this neglected "severe end of the spectrum" (26, 28). Expanding on this research, here we examined the type, frequency, and severity of psychiatric symptoms using the CASI-5 as our primary outcome measure, and the *Emotion Dysregulation Inventory* [*EDI*; (35)], a new measure designed to assess emotion control difficulties independently of IQ and verbal ability in individuals with ASD, in a group of MV children and adolescents with ASD who have never received inpatient psychiatric care. Given that emotion dysregulation has been proposed as one potential explanatory mechanism for the high rates of psychiatric comorbidities found among individuals with ASD (36), we hypothesized that the EDI reactivity and dysphoria scales would be correlated with several of the CASI-5 symptom classifications indicative of externalizing and internalizing disorders, respectively. Because minimal capacity for spoken language is often associated with intellectual disability (ID) in ASD, we examined whether CASI-5 psychiatric comorbidities and EDI reactivity and dysphoria were associated with maladaptive behaviors in our MV-ASD sample, independently of age and ID.

The overarching aim of our study was to characterize the profile of comorbid symptoms of psychopathology and emotion dysregulation in an exclusively *outpatient* sample of children and adolescents who remained minimally verbal by school age (5 years and older). In particular, based on CASI-5 scores, we examined the severity and frequency of psychiatric symptoms in 5-to-18-year-old children and adolescents with ASD, relative to population norms. In addition, based on EDI scores, we investigated the frequency of parent-reported symptoms of emotional reactivity and dysphoria.

The specific goals of this study were:

1) To investigate whether individual differences, such as age and gender, influence the presentation of psychiatric comorbidities and emotion dysregulation among MV individuals with ASD. In particular, we examined whether children (5; 0 to 11; 11 years) differed from adolescents (12; 1 to 18; 6 years) in the type, prevalence and severity of symptoms reported by caregivers on the CASI-5 and EDI;

2) To examine the relationships between ratings of psychiatric symptoms and of emotion dysregulation and other characteristics of the sample, such as cognitive ability (IQ), adaptive functioning, maladaptive behavior, and autism symptom severity.

3) To examine whether the overall burden of psychiatric comorbidities and emotion dysregulation predict maladaptive behaviors, over and above variability in IQ and age, in MV individuals with ASD.

METHODS

Participants

Sixty-five participants diagnosed with ASD who had limited verbal abilities (i.e., few to no words used spontaneously) were included in the study. Participants had enrolled in a larger phenotyping study of minimally verbal (MV) individuals with ASD conducted at a University-affiliated research center. They were recruited from a variety of resources in the community including schools, clinics, and social media and came from predominantly English-speaking homes, had normal or corrected-to-normal vision and hearing, and did not have significant neurological impairment. Based on a medical history survey answered by caregivers, none of the participants had ever been hospitalized in a psychiatric care unit prior to participation in our research. Informed consent was obtained from the parents, and the Boston University Institutional Review Board approved study procedures.

ASD diagnoses of the children and adolescents enrolled in the study were confirmed using the Autism Diagnostic Interview—Revised [ADI-R; (37)] conducted with primary caregivers and the ADOS. Participants aged 5 through 11 years ($n = 33$) were assessed with Module 1 of the Autism Diagnostic Observation Schedule-2 [ADOS-2; (38)]. Participants aged 12 through 18 years ($n = 32$) were assessed with Module 1 of the Adapted ADOS [A-ADOS; (39)], which uses play materials more appropriate and engaging for adolescents. The ADOS Module 1 was specifically designed to assess ASD symptomatology in children with few to

no words and is therefore appropriate for defining minimally verbal ASD (40). Social-affective and restrictive and repetitive behavior symptom severity were calculated with the ADOS calibrated symptom severity scores, which are comparable across ADOS modules (41). For both the ADI-R and the ADOS assessments, higher diagnostic algorithm and calibrated symptom severity scores indicate more severe ASD symptoms. Participant characteristics are reported in **Tables 1A**, **1B** by age group.

Measures and Procedure

All participants were administered a battery of cognitive diagnostic assessments and parents completed several questionnaires and interviews about their child's developmental history and current behavioral profile, either in their homes, or when their child was being tested.

Measures of Cognitive and Adaptive Functioning

Non-verbal IQ (NVIQ) was assessed with the Leiter International Performance Scale -Third Edition [Leiter-3; (42)], a test commonly used with minimally- and low-verbal individuals with ASD (43) because it does not require verbal instructions or verbal responding. Parents completed the Vineland Adaptive Behavior Scales, Second Edition [VABS-II; (44)], a measure administered in a semi-structured interview format. In addition to assessing the level of an individual's personal and social skills required for everyday living, the VABS-II also yields a maladaptive

TABLE 1A | Participant characteristics, by age group.

	Child	Adolescent	p[a]
	$N = 33$	$N = 32$	
	Mean (SD)	Mean (SD)	
Age	7.59 (1.99)	14.79 (1.9)	0.001
ADI-R SCORES			
Social interaction	25.94 (2.72)	26.04 (3.8)	ns
Nonverbal communication	11.9 (2.22)	12.59 (1.53)	ns
Repetitive behaviors	5.35 (1.49)	5.96 (2.47)	ns
ADOS SYMPTOM SEVERITY SCORES			
Social affect	7.12 (1.19)	7.47 (1.74)	ns
Restricted and repetitive behaviors	8.91 (1.18)	7.94 (1.70)	0.009
Total (overall CSS)	7. 70 (1.18)	7.59 (1.74)	ns
Leiter-3 nonverbal IQ	70.53 (14.69)	48.97(12.97)	0.001
VINELAND ADAPTIVE BEHAVIOR SCALES (VABS–II)[b]			
Communication domain	54.91 (12.26)	43.39 (9.74)	0.001
Socialization domain	55.66 (8.27)	43.85 (6.37)	0.001
Daily living skills	62.0 (10.38)	50.48 (11.02)	0.001
Adaptive behavior composite	57.13 (8.88)	44.54 (9.18)	0.001
Maladaptive behavior index[c]	19.25 (1.54)	19.14 (1.18)	ns
Internalizing behaviors[c]	19.38 (2.03)	19.64 (1.50)	ns
Externalizing behaviors[c]	17.28 (1.81)	17.79 (1.55)	ns

[a] *Independent-samples t-test.*
[b] *Data on VABS-II was not available for 4 adolescent participants.*
[c] *v-Scale scores.*

TABLE 1B | Demographic characteristics of the participants.

	Child	Adolescent	P[a]
	$N = 33$	$N = 32$	
	Mean (SD)	Mean (SD)	
Gender (Male/Female)	27/6	22/10	ns
Race/Ethnicity			ns
White	60.6%	68.8%	
Hispanic	6.0%	3.1%	
Native Hawaiian/Pacific islander	0	3.1%	
More than one race / unknown	9.1%	3.1%	
Maternal education			ns
Less than high school	0	0	
High school/GED	6.0%	6.2%	
Some college	27.3%	19.4%	
Bachelor's degree	18.2%	35. 5%	
Graduate degree	45.4%	32.3%	
Other (e.g., trade vocational school)	3%	6.5%	
Household income			ns
< $50,000.00	12.12%	6.25%	
$50,000 to $100,000	12.12%	6.25%	
>$100,000.00	48.5%	53.13%	
No response	27.3%	34.37%	

[a] χ^2 test.

behaviors index (including separate scores for externalizing and internalizing maladaptive behaviors), based on caregiver ratings of problematic or challenging behaviors that interfere with a person's optimal daily functioning.

Parent-Report Measures of Comorbid Psychopathology and Emotion Dysregulation

Child and Adolescent Symptom Inventory (CASI-5)

We used the parent-report version of the CASI-5 to examine the frequency and severity of comorbid psychiatric symptoms in our sample. The parent version of the CASI-5 includes 173 items, which rate behaviors as occurring *never, sometimes, often and very often*. The items assess symptoms of DSM-5 psychiatric disorders, including Attention Deficit Hyperactivity Disorder (inattentive, hyperactive/impulsive, & combined types), anxiety disorders (generalized anxiety, social anxiety/'social phobia', and separation anxiety), conduct disorder and oppositional-defiant disorder, mood disorders (major depressive episode, dysthymia, and manic episode) and eating disorders (anorexia, bulimia). In addition, a limited number of symptoms characteristic of the following disorders are also included: posttraumatic stress disorder, obsessive-compulsive disorder, schizophrenia and schizoid personality, specific phobia, panic disorder, selective mutism, trichotillomania, motor tics, vocal tics, and substance use. For the majority of items, symptoms rated as occurring *often* and *very often* are considered clinically significant, and those rated as *never* or *sometimes* are not.

The CASI-5 yields several types of scores that correspond to two approaches to assessing psychiatric symptomatology. One approach is based on a dimensional scoring method that uses normative data to generate T-scores (with a mean of 50 and *SD* = 10) for each symptom classification based on the participant's gender and age. For the purposes of this study, we considered severity T-scores > 65 (i.e., > 1.5 SD above the mean and at the 93rd percentile or higher relative to the norm sample) as clinically and functionally significant. The second approach to scoring is categorical and involves determining whether an individual meets criteria for a particular DSM-5 screening diagnosis based on the number of clinically concerning symptoms shown (e.g., items rated *often and very often*); this number—labeled S*ymptom Count Score*—is compared to a *Symptom Count Cutoff* /criterion score for each disorder classification. It should be noted that symptom count cutoff scores do not indicate a psychiatric diagnosis, and their relevance is restricted to screening purposes for clinically concerning symptoms related to a specific disorder. According to recent reviews [e.g., (45)], the CASI-5 subscale scores generally show a high degree of correspondence with psychiatric diagnoses (predictive validity) and correlate well with other commonly used dimensional scales (concurrent validity), demonstrating satisfactory psychometric properties for a diversity of youth, including those with ASD (30).

Emotion Dysregulation Inventory (EDI)

Emotion dysregulation was assessed with the 66-item version of the EDI, a caregiver-report questionnaire designed to capture emotional distress and a wide range of problems with emotion regulation in youth with ASD ages 6 years and above. The items describe observable indicators of poor emotion regulation, which are rated by caregivers on a 0 to 4 scale from "*not at all—never happens*" to "*very severe—almost always happening and causes a serious problem.*" The EDI is comprised of two scales: a *Reactivity scale*, which captures intense, rapidly escalating, sustained, and poorly regulated negative emotional reactions, and a *Dysphoria scale*, characterized by minimal positive affect and motivation, and the presence of nervousness and sadness. The EDI reactivity and dysphoria scales yield raw scores that were converted into T-scores based on tables provided to us by the instrument's author. It should be noted that the calibration sample for this instrument consisted of a large combined sample of 1,751 community and psychiatric inpatients with ASD. Therefore, the results we present are relative to the autism norms and scoring that were validated for this population (36).

Statistical Analysis

Descriptive statistics were calculated using means and standard deviations for continuous variables and frequencies and proportions for categorical variables. First, children and adolescent groups were compared on demographic variables, including scores on standardized tests of cognitive and adaptive functioning and autism symptom severity, using independent sample *t*-tests or χ^2 tests, as appropriate. Variables on which significant group differences were found (e.g., NVIQ) were controlled for (entered as covariates) in analyses of the CASI-5 and EDI factors variables, conducted to compare psychiatric

comorbidities and emotion dysregulation ratings across the age groups.

We conducted several types of analyses of the CASI-5 parent ratings to characterize the profile of psychiatric comorbidities in our sample. First, we examined the prevalence of different types of comorbidities based on the frequencies of scores exceeding the clinical cut-off for each CASI-5 classification (categorical scoring approach). Then we explored the distribution of clinically significant CASI-5 symptom severity scores (i.e., T-score > 65) in our sample relative to the distribution expected in the general population, using χ^2 to test for significant differences. We further examined age and gender group differences in the severity of comorbid symptoms (dimensional scoring) using a multivariate analysis of variance approach with the CASI-5 T-scores as the dependent variables, while co-varying NVIQ. More specifically, the continuous CASI-5 *symptom severity* variables (T-scores) for the psychiatric classifications relevant for both age groups (nine classifications or subscales) were entered into a multivariate analysis of covariance (MANCOVA) to determine if symptom severity-scores across classifications differed as a function of age-group and gender, after controlling for NVIQ. To further examine group differences, the multivariate test was followed by univariate ANOVAs and *post-hoc* tests, as needed, using Bonferroni corrected significance levels. A similar MANCOVA was conducted with the two EDI factors as dependent variables, age-group and gender as between-subjects factors and NVIQ as a covariate, to examine possible group differences in aspects of emotion dysregulation, after controlling for cognitive ability. Relationships between psychiatric comorbidities, emotion dysregulation factors and level of cognitive functioning (NVIQ), were examined using Pearson correlations. We further investigated relationships between selected psychiatric comorbidities, emotion dysregulation factors, autism symptom severity and ratings of maladaptive behavior on the VABS-II while controlling for NVIQ, using partial correlations and the Holm-Bonferroni method to correct for multiple testing. Finally, to investigate if psychiatric comorbidities or emotion dysregulation factors contributed significantly to ratings of maladaptive behavior on the VABS-II, a stepwise multiple regression analysis was conducted, entering age and NVIQ on the first step, followed by the number of clinically significant comorbidities endorsed by parents on the CASI-5 and the two EDI emotion dysregulation factors as an independent variables, with VABS-II Maladaptive behavior index scores as the dependent variable.

RESULTS

Tables 1A,B present demographic characteristics of the participants, by age group. No differences were found between males and females on any characteristic listed. The child and adolescent groups did not differ in gender, race/ethnicity, parent education, and household income distributions (based on χ^2 tests, all $p > 0.25$), or on ADI-R or ADOS overall calibrated symptom severity scores. Independent-samples t-tests showed that younger participants obtained, on average, higher standard

scores on several measures of cognitive and adaptive functioning than the adolescent group (see **Table 1A**). Non-verbal IQ and VABS-II Adaptive Behavior Composite scores were highly correlated ($r = 0.782$, $p < 0.0001$), even when adjusting for age differences ($r_p = 0.649$, $p < 0.0001$). Therefore, all analyses of group differences on the variables of interest from the CASI-5 and the EDI survey were conducted co-varying NVIQ.

Sample Characterization With the CASI-5

Table 2 presents the prevalence of different psychiatric comorbidities in our sample for children and adolescents, based on the CASI-5 categorical scoring, which takes into account whether an individual meets criteria for a particular DSM-5 diagnosis based on the number of clinically concerning symptoms shown (i.e., exceeds a *Symptom Count Cutoff* criterion for a particular CASI-5 classification). All participants met cutoff criteria for at least one CASI-5 classification, and the number of categorical classifications parents endorsed ranged from 1 to 15, with a mode and a median of 6 classifications. **Figure 1** shows

TABLE 2 | Prevalence of participants meeting clinical cut-off scores on CASI-5 symptom classifications.

| N	Children	Adolescents	Entire sample
	33	32	65
	% of sample	% of sample	% of sample
EXTERNALIZING DISORDERS			
ADHD			
Inattentive type	48.5	31.25	40
Hyperactive/impulsive type	21.21	25	23.1
Combined type	18.18	18.75	18.5
Oppositional defiant disorder	3.03	3.13	3.1
Conduct disorder	3.03	6.25	4.6
INTERNALIZING DISORDERS			
Generalized anxiety disorder	3.03	3.13	3.1
Major depressive disorder	0	3.13	1.5
Dysthymic disorder	3.03	3.13	3.1
Social phobia/social anxiety	12.12	6.2	9.2
Separation anxiety disorder	3.03	3.03	3.1
OTHER DISORDERS			
Specific phobia	45.5	40.6	43.1
Panic disorder	0	0	0
Obsessions	6.06	3.13	4.6
Compulsions	36.4	31.25	33.8
Posttraumatic stress	15.2	12.5	13.8
Motor tics	42.4	50	46.2
Vocal tics	54.6	78.13	66.2
Somatic symptoms	3.03	0	1.5
Enuresis	60.6	6.06	52.3
Anorexia nervosa	3.03	3.13	3.1
Bulimia nervosa	6.06	25	15.4
Schizoid personality disorder	12.1	28.1	20
Schizophrenia	0	0	0
Bipolar disorder/Manic episode	3.03	3.13	3.1
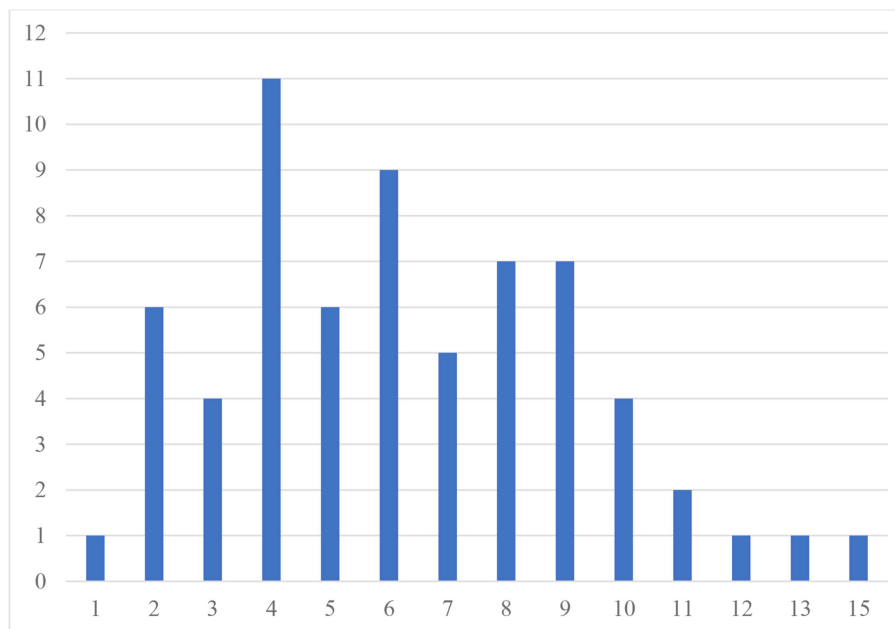
FIGURE 1 | Frequency of participants by number of CASI−5 symptom classifications with clinically significant ratings. Number of psychiatric comorbidities exceeding clinical cutoff scores.

the distribution of participants by the number of symptom classifications on which they met clinical cutoff criteria on the CASI-5.

The most frequent symptom classification endorsed by parents in our sample was *vocal tics*, with 66.2% of the sample meeting clinical cutoff. Forty-six percent of the sample met the clinical cutoff for *motor tics*, followed by *specific phobia*, reported to be present in 43% of the sample. A large proportion of the sample met the clinical cutoff for ADHD Inattentive type (40%), whereas 23% met clinical cutoff for the ADHD Hyperactive-impulsive type, followed by ADHD Combined type (18.5%). Compulsions were reported in about a third of the sample. However, except for vocal tics, no single psychiatric symptom was found significantly elevated in more than 50% of the sample, reflecting the wide variety and complex combinations of clinically significant psychiatric symptoms among our MV participants. The one exception was enuresis, which was reported for 60.6% of children but only for 6% of adolescents, suggesting that this comorbid condition is likely to resolve over time.

We also characterized the profile of psychiatric comorbidities in this population based on analyses of symptom severity, afforded by the dimensional scoring approach for the CASI-5 classifications. Symptom severity scores on the CASI-5 indicate whether an individual shows symptom levels of clinical concern, even if not having the number of symptoms required to screen positive for a DSM-5 psychiatric diagnosis. Therefore, we examined the distribution of T-scores in our sample, taking as a threshold for clinical significance T-scores higher than 1.5 standard deviation from the mean (i.e., T-scores of 65 and above). **Table 3** shows the percentage of participants who obtained T-scores above and below 65. When comparing this distribution of

scores to the normal distribution, we found that for all symptom categories, except oppositional defiant disorder and conduct disorder, more participants showed clinically concerning severity scores than expected based on general population norms.

Symptom severity ratings of psychiatric comorbidities (CASI-5 T-scores) for the 9 symptom classifications common to children and adolescents (see **Table 4**) were entered into a MANCOVA with age-group and gender as between-subjects factors and NVIQ as covariate. This analysis yielded a significant multivariate effect of age group, $F_{(9,51)} = 3.291$, $p = 0.003$, Wilks' $\Lambda = 0.633$, partial $\eta^2 = 0.367$, with no other significant effects or interactions. Follow-up univariate ANOVAs indicated that children and adolescents differed significantly in the severity of their symptoms for ADHD Inattentive type, $F_{(1,59)} = 7.10$, $p = 0.01$, partial $\eta^2 = 0.11$, with the children showing more impairment than the adolescents (mean = 67.9, $SD = 14.9$ vs. mean = 63.7, $SD = 12.3$, respectively) and on Major depressive episode, $F_{(1,59)} = 6.32$, $p = 0.015$, partial $\eta^2 = 0.10$, with the adolescents (mean = 61.97, $SD = 13.6$) showing more impairment than the children (mean = 52.06, $SD = 9.9$) in this category of psychiatric comorbidity.

Sample Characterization on the EDI

Table 5 presents the T-scores for the children and adolescents in our sample on the EDI reactivity and dysphoria scales. A MANCOVA with EDI reactivity and dysphoria T-scores as dependent variables, age-group and gender as between-subjects factors, and NVIQ as a covariate did not yield any main effects or interactions indicating that, in our sample, severity of emotion dysregulation was largely independent of age-group, $[F_{2,49} = 1.54$, $p = 0.224$, Wilks' $\Lambda = 0.941$, partial $\eta^2 = 0.06]$, gender

TABLE 3 | Proportion of participants (%) by Distribution of clinically significant T-scores on the CASI-5.

T-score	<65	>65	χ^2	p
Normal distribution %	93%	7%		
EXTERNALIZING DISORDERS				
ADHD				
Inattentive type	55.4	44.6	141.3,	0.0001
Hyperactive/impulsive type	52.3	47.7	165.3,	0.0001
Combined type	44.6	55.4	233.7,	0.0001
Oppositional defiant disorder	96.9	3.1	1.54,	ns
Conduct disorder	96.9	3.1	1.54,	ns
INTERNALIZING DISORDERS				
Generalized anxiety disorder	72.3	27.7	42.75,	0.0001
Depressive disorders				
Major depressive disorder	80	20	16.87,	0.0001
Dysthymic disorder	83.1	16.9	9.83,	0.002
Social phobia[a]	72.1	27.9	28.9,	0.0001
Separation anxiety disorder	84.6	15.4	7.02,	0.008
OTHER DISORDERS[b]				
Schizophrenia	58.6	41.4	52.6,	0.0001
Schizoid personality disorder	38.7	61.3	140.4,	0.0001
Bipolar disorder/Manic episode	81.3	18.8	6.79,	0.009

[a] Classification T-score applies only to children (5–12 years).
[b] Classification T-scores apply only to adolescents (12–18 years).

$[F_{2,49} = 0.953, p = 0.392,$ Wilks' $\Lambda = 0.963,$ partial $\eta^2 = 0.037]$, or NVIQ $[F_{2,49} = 0.761, p = 0.473,$ Wilks' $\Lambda = 0.796,$ partial $\eta^2 = 0.03]$.

Overall, relative to the normative autism calibration sample, the participants in this study did not show significantly elevated symptoms of reactivity or dysphoria on the EDI, according to parent report for current behaviors. The range of T-scores was 30 to 60 for reactivity and 36 to 58 for dysphoria in our sample, suggesting that few caregivers rated behaviors indicative of emotion dysregulation as *very severe problems,* although across participants the full scale (0 to 4) was used for most items. Some items included in the dysphoria scale, however, never received a rating of *severe* or *very severe* (e.g., "seems sad or unhappy," "appears uneasy through the day").

Relationships Among Parent-Report Measures of Psychiatric Symptoms, Emotion Dysregulation, IQ, Adaptive Functioning, Maladaptive Behaviors, and Autism Severity

First, we examined correlations between cognitive functioning (NVIQ) and severity scores for the CASI-5 classifications and the EDI factors. NVIQ was significantly negatively correlated with CASI-5 T- scores only for symptoms of ADHD (for ADHD Hyperactivity-impulsive type, $r_{(64)} = -0.381, p = 0.002,$ and $r_{(64)} = -0.274, p = 0.024$ for ADHD Combined type). No other psychiatric comorbidities rated on the CASI-5 correlated significantly with NVIQ. On the EDI, neither the reactivity nor the dysphoria factors were correlated with NVIQ. However,

TABLE 4 | Mean (and Standard Deviations) of CASI-5 severity scores (T-scores) for symptom classifications.

	Child	Adolescent	p*
	Mean (SD)	Mean (SD)	
DISRUPTIVE BEHAVIOR DISORDERS			
ADHD			
Inattentive type	67.85 (14.86)	63.75 (12.31)	0.01
Hyperactive/impulsive type	62.12 (10.72)	69.19 (11.17)	ns
Combined type	67.0 (13.58)	67.8 (12.82)	ns
Oppositional defiant disorder	45.24 (5.96)	47.09 (11.21)	ns
Conduct disorder	46.82 (2.8)	48.84 (7.38)	ns
ANXIETY DISORDERS			
Generalized anxiety disorder	57.24 (9.06)	58.13 (10.9)	ns
Separation anxiety disorder	50.18 (8.69)	57.59 (16.67)	ns
MOOD DISORDERS			
Major depressive episode	52.06 (9.89)	61.97 (13.63)	0.015
Dysthymic disorder	53.23 (10.05)	61.47 (15.67)	ns

*Pairwise comparisons among estimated marginal means, controlling for NVIQ.

TABLE 5 | Means (and Standard Deviations) of T-scores on EDI factors by age-group.

	Child	Adolescent	p*
	Mean (SD)	Mean (SD)	
EMOTION DYSREGULATION (EDI)			
Reactivity[a]	41.91 (4.94)	43.65 (6.51)	ns
Dysphoria[a]	42.13 (4.96)	43.93 (6.30)	ns

[a] T-scores. *Pairwise comparisons between estimated marginal means, controlling for NVIQ.

because the two age groups differed significantly in NVIQ scores, we conducted all other correlational analyses controlling for NVIQ. Adaptive functioning scores (VABS-II Adaptive Behavior Composite scores) were not correlated with any CASI-5 severity T-scores or with the EDI factors in our sample.

Next, we examined autism symptom severity scores as related to measures of psychiatric comorbidities and emotion dysregulation severity, controlling for NVIQ. We found no significant correlations between T- scores on the CASI-5 or the EDI factors and ADOS calibrated severity scores. To investigate whether psychiatric comorbidities and emotion dysregulation contributed to behavioral dysregulation as assessed by ratings of maladaptive behaviors (i.e., internalizing and externalizing behaviors) on the VABS-II, we examined correlations between CASI-5 and EDI factors T-scores, and VABS-II indices of internalizing and of externalizing behaviors, controlling for NVIQ. We selected from the CASI-5 the symptom classifications for which T-scores are provided for both children and adolescents, and for which our sample showed elevated severity (T scores > 65, cf. **Table 3**). **Table 6** presents partial correlations among our primary measures from the CASI-5 and the EDI, and indices of maladaptive behavior from the VABS-II, controlling for

NVIQ. As expected based on the theoretical model of emotion dysregulation underlying the EDI, the reactivity factor index on the EDI was significantly correlated with ratings of externalizing behaviors on the VABS-II after controlling for cognitive ability, $r_{p(53)} = 0.383$, $p = 0.006$. The dysphoria factor, however, was not significantly correlated with internalizing behaviors scores on the VABS-II. While EDI reactivity and dysphoria T-scores were both correlated with the number of clinically significant CASI-5 symptom classifications (a measure of "psychiatric burden"), only the EDI dysphoria factor was significantly correlated with the generalized anxiety CASI-5 classification, $r_p(54) = 0.398$, $p = 0.002$, after adjusting for multiple testing (**Table 6**).

Finally, we were interested in exploring to what extent the burden of psychiatric comorbidities and emotion dysregulation predicted caregivers' ratings of maladaptive behaviors in their children, as reported on the VABS-II maladaptive behavior index. To this end, we conducted a stepwise multiple regression analysis entering chronological age and NVIQ on the first step, followed by entering the two EDI factors (dysphoria and reactivity) and the total number of clinically significant symptom classifications from CASI-5 on the second step. Because neither the ASD symptom severity calibrated score, nor the overall adaptive functioning measure (VABS-II Adaptive Behavior Composite) were correlated with any of the psychiatric comorbidity or emotion dysregulation T- scores in our sample, we did not enter these variables in the analysis. Results revealed that, when entered in stepwise fashion, only the number of comorbid symptom classifications on the CASI-5 was retained as predictor, explaining 9.5% of variance in the VABS-II maladaptive index scores [adjusted $R^2 = 0.095$; $F_{1,50} = 5.22$. $p = 0.027$], whereas the EDI factors did not make a significant contribution to the model (EDI reactivity $\beta = 0.125$, t = 0.829, ns, and EDI dysphoria $\beta = -0.092$, t = -0.610, ns).

DISCUSSION

In this first study of a relatively large and never hospitalized outpatient sample of minimally verbal children and adolescents with ASD, our main goal was to investigate co-occurring psychiatric symptoms using several different measures to provide a comprehensive phenotypic characterization of MV individuals as related to psychiatric and emotion dysregulation symptomatology. We found high rates of psychiatric symptomatology on the CASI-5, but relatively low rates of emotional dysregulation, especially dysphoria on the EDI. We also found that the number of different psychiatric symptom classifications endorsed on the CASI-5 was a key predictor of maladaptive behavior. The overall picture to emerge from this study is that minimally verbal children and adolescents present with extremely heterogeneous profiles of co-morbid psychopathology that are not easily predicted by autism symptom severity, intellectual disability, or limitations in communication.

Our main analyses focused on the CASI-5, a well-validated parent-report psychiatric screening measure. Perhaps the most striking finding was that virtually every participant met cut-off

criteria for at least one co-morbid condition, and the average number of different co-morbidities across the sample was six. The most common conditions included tics and phobias and among psychiatric categories, ADHD was the most common. In contrast, we found low rates of oppositional defiant disorder and conduct disorder, perhaps reflecting the limited opportunities for exhibiting signs of these disorders in the population we studied, or caregivers' construal of their children's behavior. In general, our findings are consistent with other reports in the literature. In particular, the pattern of psychopathology we found matches what Lerner and his colleagues reported (29) in their sample of minimally verbal youth with ASD, although the rates that we found were lower. This is not surprising since our sample had never been hospitalized whereas Lerner et al.'s minimally verbal participants were drawn largely from a current inpatient sample. Finally, we note that on the CASI-5, as well as our other measures of co-morbid psychopathology, we found very few differences between children and adolescents. Rates of different disorders as well as the number of different conditions were similar across the sample. We also did not find differences between males and females, although the small number of females who provided data in this study precludes drawing valid conclusions about gender-related similarities or differences in the profile of psychiatric comorbidities of MV individuals with ASD.

Our second key measure for assessing psychopathology in this study was the newly developed instrument, the EDI. This measure, which taps two different emotion dysregulation factors, reactivity and dysphoria, was specifically designed for and normed on an ASD sample (35, 36). In contrast to the significant psychopathology reported on the CASI-5, we found relatively low rates of clinically significant dysregulation compared to the instrument norms, with rates for dysphoria especially low. One reason for our lower rates might again be related to the difference in our sample since Mazefsky and colleagues normed the EDI on a large sample of both inpatient and outpatient children and adolescents with ASD and included both verbal and minimally verbal individuals. Since rates of certain co-morbid psychopathological conditions, particularly anxiety, depression and ODD are significantly more prevalent among more verbal individuals, and the EDI is correlated with CASI-5 psychopathology, it is likely that our EDI findings reflect the lower end of the ASD distribution of scores on this instrument. A second explanation for the lower EDI scores in our sample may be that parents either have trouble discerning the internal emotional states of their minimally verbal children, or that they interpret their child's behavior and affect more in terms of their primary diagnosis of ASD coupled with their severely limited communicative abilities. Thus, if a child cannot say, for example, they are unhappy or do not want to go to school, parents fail to interpret behaviors that are consistent with these emotional states in this way and therefore do not endorse those items on the EDI.

We investigated the relationship between comorbid psychopathology and other behavioral characteristics, including non-verbal IQ, adaptive functioning, and autism symptom severity. Among minimally verbal children and adolescents, autism severity scores were not related to any of our measures of

TABLE 6 | Partial correlations between severity scores for selected psychiatric symptom classifications (CASI-5), emotion dysregulation (EDI) factors, and maladaptive behavior (VABS-II), controlling for cognitive ability (NVIQ).

	VABS-II internalizing behavior	VABS-II externalizing behavior	EDI reactivity	EDI dysphoria	Number of comorbid symptoms
ADHD inattentive type	0.202	0.269	0.060	0.267	0.440*
ADHD hyperactive /impulsive type	0.113	0.474*	0.214	0.181	0.425*
ADHD combined type	0.186	0.385*	0.129	0.274	0.472*
Generalized anxiety disorder	0.284	0.342	0.298	0.398*	0.577**
Major depressive disorder	−0.228	−0.056	0.209	0.302	0.342*
Number of comorbid symptoms	0.245	0.244	0.407*	441*	
EDI Dysphoria	0.100	0.076	0.554**		
EDI Reactivity	0.079	0.383*			

Significance level after Holm-Bonferroni correction: *p < 0.001; **p < 0.0001.

psychopathology and even IQ and adaptive behavior scores were either not related or only to a modest degree (with r-values all below 0.4). The lack of correlations between CASI-5 scores and a general measure of adaptive functioning (VABS-II composite scores) may seem surprising, in light of findings from other research that documented associations between co-occurring psychopathology and adaptive behavior in individuals with ASD (32, 46, 47). However, these findings were based primarily on samples of individuals with ASD without intellectual disabilities (46, 48–51) or on mixed samples including both individuals with IQ in the average range and those with ID, but whether the mixed samples included MV-ASD participants remained unspecified [see (52) for a review]. In the few studies that focused on individuals with ASD and ID (53, 54) researchers examined primarily associations between psychiatric comorbidities and "problem behaviors" that directly impact adaptive functioning. In our MV-ASD sample we also found expected associations between psychiatric burden tapped by CASI-5 scores, the Dysregulation factor of the EDI and maladaptive behaviors on the VABS-II.

Consistent with the underlying model for the EDI, we found correlations between the CASI-5 and the EDI. Both EDI factors correlated significantly with most of the CASI-5 symptom classifications severity scores and with the overall number of different CASI-5 symptom classifications endorsed by each respondent. Nevertheless, in our regression analysis exploring which variables were the strongest concurrent predictors of overall maladaptive behavior, only the number of different CASI-5 symptom classifications accounted for 9.5% of the variance; Although this is not a large portion of the variance, it was significant in the context of model tested, whereas no other predictor variables, including any of the EDI factors, was significant. In some respect then, the number of different symptom classifications may function as a cumulative risk index for maladaptive behavior. Future research should explore further whether models of this cumulative risk of co-morbid psychopathology for minimally verbal individuals with ASD would be more sensitive if certain comorbid conditions were weighted higher than others were, but this would require a significantly larger sample of individuals than we had available.

It would also be important to begin exploring whether there are other factors that may protect some children and adolescents from higher levels of maladaptive behavior, despite carrying a significant burden of co-morbid psychopathology.

There are some notable limitations to this study. Most importantly, we relied exclusively on parent report questionnaires, which are generally less accurate than either in-depth caregiver interviews or direct observation and evaluation of psychiatric conditions. Since minimally verbal individuals are, by definition, not able to report on their own feelings and behavior, even a direct evaluation would depend largely on parent report coupled with observations in a clinical setting. Still, our findings may have been enhanced had they been coupled with such observations carried out by an expert clinician. We focused here specifically on an outpatient group of minimally verbal children and adolescents, thus complementing the work reported by Lerner and his colleagues (26; see also 33). Because of the nature of the larger lab-based research study in which the participants were enrolled, we excluded those with the most severe behavior problems including aggression, self-injury or non-compliance, and therefore our findings must be viewed in the context of whom our participants represent. Nevertheless, this study is an important step forward in work that characterizes the minimally verbal end of the autism spectrum who have so often been excluded from earlier studies (cf. 24).

In sum, our study highlights the wide-ranging profiles of comorbid psychopathology and degree of maladaptive behavior among minimally verbal children and adolescents with ASD. The findings do not suggest that the presence of these comorbidities represent a subtype within the ASD population; on the contrary, comorbid psychopathology is the norm rather than the exception. This suggests that beginning at an early age every minimally verbal person with ASD should have ongoing clinical diagnostic and treatment services that focus on these comorbid conditions, which may well change over time within each child. It is likely that the burden of care for minimally verbal people is related as much to their comorbid conditions, including both the absence of functional language and psychopathology. As we work toward including this end of

the spectrum into our fuller understanding of ASD, we need to embrace the highly complex and unique behavioral profiles of each individual.

AUTHOR CONTRIBUTIONS

HT-F, DPS, and RJ conceived of the study and wrote the manuscript. DPS, BE, and SM collected, coded and analyzed the data. RJ contributed to data analysis. BE and SM reviewed and approved the manuscript.

REFERENCES

1. Levy SE, Giarelli E, Lee LC, Schieve LA, Kirby RS, Cunniff C, et al. Autism spectrum disorder and co-occurring developmental, psychiatric, and medical conditions among children in multiple populations of the United States. *J Dev Behav Pediatr.* (2010) 31:267–75. doi: 10.1097/DBP.0b013e3181d5d03b

2. Mannion A, Leader G. Comorbidity in autism spectrum disorder: a literature review. *Res Autism Spectr Disord.* (2013) 7:1595–616. doi: 10.1016/j.rasd.2013.09.006

3. Matson JL, Golding RL. Comorbidity in autism: trends, topics and future directions. *Res Autism Spectr Disord.* (2013) 7:1228–33. doi: 10.1016/j.rasd.2013.07.003

4. Siegel M, Smith KA, Mazefsky C, Gabriels RL, Erickson C, Kaplan D, et al. The autism inpatient collection: methods and preliminary sample description. *Mol Autism* (2015) 6:61. doi: 10.1186/s13229-015-0054-8

5. Kim SH, Paul R, Tager-Flusberg H, Lord C. Language and communication in autism. In: Volkmar FR, Paul R, Rogers SJ, Pelphrey KA, editors. *Handbook of Autism and Pervasive Developmental Disorders Volume 1: Diagnosis, Development, and Brain Mechanisms.* 4th ed. New York, NY: Wiley (2014). p. 230–62.

6. Matson JL, Williams LW. The making of a field: the development of comorbid psychopathology research for persons with intellectual disabilities and autism. *Res Dev Disabil.* (2014) 35:234–8. doi: 10.1016/j.ridd.2013.09.043

7. Mazefsky CA, Borue X, Day TN, Minshew NJ. Emotion regulation patterns in adolescents with high-functioning autism spectrum disorder: comparison to typically developing adolescents and association with psychiatric symptoms. *Autism Res.* (2014) 7:344–54. doi: 10.1002/aur.1366

8. Brereton AV, Tonge BJ, Einfeld SL. Psychopathology in children and adolescents with autism compared to young people with intellectual disability. *J Autism Dev Disord.* (2006) 36:863–70. doi: 10.1007/s10803-006-0125-y

9. McCarthy J, Hemmings C, Kravariti E, Dworzynski K, Holt G, Bouras N, et al. Challenging behavior and co-morbid psychopathology in adults with intellectual disability and autism spectrum disorders. *Res Dev Disabil.* (2010) 31:362–6. doi: 10.1016/j.ridd.2009.10.009

10. Tureck K, Matson JL, Cervantes P, Konst M. An examination of the relationship between autism spectrum disorder, intellectual functioning, and comorbid symptoms in children. *Res Dev Disabil.* (2014) 35:1766–72. doi: 10.1016/j.ridd.2014.02.013

11. White SW, Oswald D, Ollendick T, Scahill L. Anxiety in children and adolescents with autism spectrum disorders. *Clin Psychol Rev.* (2009) 29:216–29. doi: 10.1016/j.cpr.2009.01.003

12. de Bruin EI, Ferdinand RF, Meester S, de Nijs PF, Verheij F. High rates of psychiatric co-morbidity in PDD-NOS. *J Autism Dev Disord.* (2007) 37:877–86. doi: 10.1007/s10803-006-0215-x

13. Ames CS, White SJ. Brief report: are ADHD traits dissociable from the autistic profile? Links between Cognition and Behaviour. *J Autism Dev Disord.* (2011) 41:357–63. doi: 10.1007/s10803-010-1049-0

14. Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Barid G. Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. *J Am Acad Child Adolesc Psychiatry* (2008) 47:921–9. doi: 10.1097/CHI.0b013e318179964f

15. Salazar F, Baird G, Chandler S, Tseng E, O'Sullivan T, Howlin P, et al. Co-occurring psychiatric disorders in preschool and elementary school-aged children with autism spectrum disorder. *J Autism Dev Disord.* (2015) 45:2283–94. doi: 10.1007/s10803-015-2361-5

16. Aman M, Singh N, Stewart A, Field CJ. The aberrant behavior checklist: a behavior rating scales for the assessment of treatment effects. *Am J Ment Defic.* (1985) 89:485–91.

17. Aman MG. Aberrant behavior checklist: current identity and future developments. *Clin Exp Pharmacol.* 2:e114. doi: 10.4172/2161-1459.1000e114

18. Achenbach TM, Rescorla LA. *Manual for the ASEBA School-Age Forms and Profiles.* Burlington, VT: University of Vermont, Research Center for Children, Youth, and Families (2001).

19. Gadow KD, Sprafkin J. *Child and Adolescent Symptom Inventory-5.* Stony Brook, NY: Checkmate Plus (2013).

20. Matson JL, Terlonge C, González ML. *Autism Spectrum Disorders – Comorbidity – Adult Version.* Baton Rouge, LA: Disability Consultants, LLC (2006).

21. Matson JL, Boisjoli J, Wilkins J. *The Baby and Infant Screen for Children With Autism Traits (BISCUIT).* Baton Rouge, LA: Disability Consultants, LLC (2007). p. 376–86.

22. Leyfer OT, Folstein SE, Bacalman S, Davis NO, Dinh E, Morgan J, et al. Comorbid psychiatric disorders in children with autism: interview development and rates of disorders. *J Autism Dev Disord.* (2006) 36:849–61. doi: 10.1007/s10803-006-0123-0

23. Matson JL, Gonzalez ML. *Autism Spectrum Disorders – Comorbidity – Child Version.* Baton Rouge, LA: Disability Consultants LLC (2007).

24. Matson JL, LoVullo SV, Rivet TT, Boisjoli JA. Validity of the autism spectrum disorder – comorbid for children (ASD-CC). *Res Autism Spectr Disord.* (2009) 3:345–57. doi: 10.1016/j.rasd.2008.08.002

25. Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (K-SADSPL): initial reliability and validity data. *J Am Acad Child Adolesc Psychiatry* (1997) 36:980–8. doi: 10.1097/00004583-199707000-00021

26. Tager-Flusberg H, Kasari C. Minimally verbal school-aged children with autism spectrum disorder: the neglected end of the spectrum. *Autism Res.* (2013) 6:468–78. doi: 10.1002/aur.1329

27. Tager-Flusberg H, Plesa Skwerer D, Joseph RM, Brukilacchio B, Decker J, Eggleston B, et al. Conducting research with minimally verbal participants with autism spectrum disorder. *Autism* (2016) 21:852–61. doi: 10.1177/1362361316654605

28. Siegel M. The severe end of the spectrum: insights and opportunities from the autism inpatient collection (AIC). *J Autism Dev Disord.* (2018) 48:3641–6. doi: 10.1007/s10803-018-3731-6

29. Lerner MD, Mazefsky CA, Weber RJ, Transue E, Siegel M, Gadow KD. Verbal ability and psychiatric symptoms in clinically referred inpatient and outpatient youth with ASD. *J Autism Dev Disord.* (2018) 48:3689–701. doi: 10.1007/s10803-017-3344-5

30. Gadow KD, Sprafkin J. *Child Symptom Inventory-4 Screening and Norms Manual.* Stony Brook, NY: Checkmate Plus (2002).

31. Davis TE, Moree BN, Dempsey T, Reuther ET, Fodstad JC, Hess JA, et al. The relationship between autism spectrum disorders and anxiety: the moderating effect of communication. *Res Autism Spectr Disord.* (2011) 5:324–9. doi: 10.1016/j.rasd.2010.04.015

32. Kerns CM, Kendall PC, Zickgraf H, Franklin ME, Miller J, Herrington J. Not to be overshadowed or overlooked: functional impairments associated with

comorbid anxiety disorders in youth with ASD. *Behav Ther.* (2015) 46:29–39. doi: 10.1016/j.beth.2014.03.005

33. Sukhodolsky DG, Scahill L, Gadow KD, Arnold LE, Aman MG, McDougle CJ, et al. Parent-rated anxiety symptoms in children with pervasive developmental disorders: frequency and association with core autism symptoms and cognitive functioning. *J Abnorm Child Psychol.* (2008) 36:117–28. doi: 10.1007/s10802-007-9165-9

34. Witwer AN, Lecavalier L. Validity of comorbid psychiatric disorders in youngsters with autism spectrum disorders. *J Dev Phys Disabil.* (2010) 22:367–80. doi: 10.1007/s10882-010-9194-0

35. Mazefsky CA, Day TN, Siegel M, White SW, Yu L, Pilkonis PA. Development of the emotion dysregulation inventory: a PROMIS® ing method for creating sensitive and unbiased questionnaires for autism spectrum disorder. *J Autism Dev Disord.* (2016) 48:1–11. doi: 10.1007/s10803-016-2907-1

36. Mazefsky CA, Yu L, White SW, Siegel M, Pilkonis PA. The emotion dysregulation inventory: psychometric properties and item response theory calibration in an autism spectrum disorder sample. *Autism Res.* (2018) 11:928–41. doi: 10.1002/aur.1947

37. Rutter M, Le Couteur A, Lord C. *Autism Diagnostic Interview – Revised.* Los Angeles, CA: Western Psychological Services (2003).

38. Lord C, Rutter M, DiLavore PC, Risi S, Gotham K, Bishop S. *Autism Diagnostic Observation Schedule–Second Edition (ADOS-2).* Los Angeles, CA: Western Psychological Services (2012).

39. Hus V, Maye M, Harvey L, Guthrie W, Liang J, Lord C. The adapted ADOS: preliminary findings using a modified version of the ADOS for adults who are nonverbal or have limited language. In: *Poster presented at the International Meeting for Autism Research* (San Diego, CA) (2011).

40. Bal VH, Katz T, Bishop SL, Krasileva K. Understanding definitions of minimally verbal across instruments: evidence for subgroups within minimally verbal children and adolescents with autism spectrum disorder. *J Child Psychol Psychiatry* (2016) 57:1424–33. doi: 10.1111/jcpp.12609

41. Hus V, Gotham K, Lord C. Standardizing ADOS categories scores: separating severity of social affect and restricted and repetitive behaviors. *J Autism Dev Disord.* (2014) 44:2400–12. doi: 10.1007/s10803-012-1719-1

42. Roid GH, Miller LJ, Pomplun M, Koch C. *Leiter International Performance Scale, (Leiter 3).* Torrance, CA: Western Psychological Services (2013).

43. Kasari C, Brady N, Lord C, Tager-Flusberg H. Assessing the minimally verbal school-aged child with autism spectrum disorder. *Autism Res.* (2013) 6:479–93. doi: 10.1002/aur.1334

44. Sparrow S, Cicchetti D, Balla D. *Vineland Adaptive Behavior Scales, Second Edition (VABS-II).* Circle Pines, MN: Pearson Assessments (2005).

45. Gadow KD. *The Symptom Inventories: An Annotated Bibliography.* Stony Brook, NY: Checkmate Plus (2015).

46. Kraper CK, Kenworthy L, Popal H, Martin A, Wallace GL. The gap between adaptive behavior and intelligence in autism persists into young adulthood and is linked to psychiatric co-morbidities. *J Autism Dev Disord.* (2017) 47:3007–17. doi: 10.1007/s10803-017-3213-2

47. Tager-Flusberg H, Dominick KC. Co-morbid disorders. In: Hollander E, Kolevzon A, Coyle J, editors. *Textbook of Autism Spectrum Disorders.* Washington, DC: American Psychiatric Publishing Company (2011). p. 209–18.

48. Farley MA, McMahon WM, Fombonne E, Jenson WR, Miller J, Gardner M, et al. Twenty-year outcome for individuals with autism and average or near-average cognitive abilities. *Autism Res.* (2009) 2:109–18. doi: 10.1002/aur.69

49. Bal VH, Kim SH, Cheong D, Lord C. Daily living skills in individuals with autism spectrum disorder from 2 to 21 years of age. *Autism* (2015) 19:774–84. doi: 10.1177/1362361315575840

50. Mattila ML, Hurtig T, Haapsamo H, Jussila K, Kuusikko-Gauffin S, Kielinen M, et al. Comorbid psychiatric disorders associated with Asperger syndrome/high-functioning autism: a community-and clinic-based study. *J. Autism Dev. Disord.* (2010) 40:1080–93. doi: 10.1007/s10803-010-0958-2

51. Matthews NL, Smith CJ, Pollard E, Ober-Reynolds S, Kirwan J, Malligo A. Adaptive functioning in autism spectrum disorder during the transition to adulthood. *J Autism Dev Disord.* (2015) 45:2349–60. doi: 10.1007/s10803-015-2400-2

52. Matson JL, Cervantes PE. Commonly studied comorbid psychopathologies among persons with autism spectrum disorder. *Res Dev Disabil.* (2014) 35:952–62. doi: 10.1016/j.ridd.2014.02.012

53. Melville C, Cooper S-A, Morrison J, Smiley E, Allan L, Jackson A, et al. The Prevalence and Incidence of Mental Ill-Health in Adults with Autism and Intellectual Disabilities. *J Autis Develop Disor.* (2008) 38:1676–88. doi: 10.1007/s10803-008-0549-7.

54. Forster S, Gray KM, Taffe J, Einfeld SL, Tonge BJ. Behavioural and emotional problems in people with severe and profound intellectual disability. *J Intellect Disabil Res.* (2011) 55:190–8. doi: 10.1111/j.1365-2788.2010.01373.x

Check for updates

Prevalence of Overweight and Obesity Among Children and Adolescents With Autism Spectrum Disorder and Associated Risk Factors

Norazlin Kamal Nor*, Azilawati Hanim Ghozali and Juriza Ismail

Department of Paediatrics, Faculty of Medicine, UKM Medical Center, The National University of Malaysia, Kuala Lumpur, Malaysia

Introduction: Prevalence of obesity in Autism Spectrum Disorder (ASD) has been reported to be higher than in the general population. Determining prevalence may help increase awareness of obesity in ASD and potentially lead to initiatives to reduce obesity. In order to understand obesity in ASD children, common risk factors were assessed including physical activity, feeding problems and sleep disturbances.

Methods: This is a cross-sectional study performed at the Child Development Center at Universiti Kebangsaan Malaysia Medical Center on 151 ASD children aged 2–18 years. Anthropometric and demographic information were obtained and parents completed three questionnaires; Children Sleep Habits Questionnaire (CSHQ), Physical Activity for Older Children Questionnaire (PAQ-C) and Brief Autism Mealtime Behavior Questionnaire (BAMBI).

Results: For ASD children in our sample, the prevalence of overweight (BMI ≥85th to <95th percentiles) was 11.3% and the prevalence of obesity (BMI ≥95th percentile) was 21.9%. The overweight/obese ASD children's median age was higher at 8.5 years (IQR 5.81–10.13) compared to the normal/underweight group of 6.33 years (IQR 4.75–7.7) with a *p*-value of 0.001. The two groups also differed significantly for maternal BMI and paternal age. The median maternal BMI in the overweight/obese group was 26.05 (IQR 23.35–32.25), statistically significantly higher (*p* = 0.003) than in the non-overweight/obese group, 24.7 (IQR 21–27.9). The median paternal age of 40 years (IQR 37–44) was statistically significantly higher (*p* = 0.039) in the overweight/obese group, compared to the median paternal age in the non-overweight/obese group of 38 (IQR 35–42). The male overweight/obese children had median PAQ-C score of 2.44 (IQR 2.00–3.00) vs. 2.89 (IQR 2.35–3.53) in the counterpart group with a *p*-value of 0.01. Using the multiple linear regression stepwise method, three predictors associated with BMI percentiles reached a statistical level of significance; PAQ-C score in males (*p* < 0.001), the BAMBI domains of Food Refusal (*p* = 0.001) and Limited Variety of Food (*p* = 0.001).

Conclusions: The prevalence of obesity and overweight is high among Malaysian ASD children and adolescents. Older child age, high maternal BMI, older paternal age, low physical activity, low likelihood of food refusal and high likelihood of food selectivity were found to be risk factors for high BMI in these children.

Keywords: autism, overweight, obesity, sleep habits, physical activity, feeding problems

INTRODUCTION

The prevalence of childhood obesity is increasing rapidly in Malaysia in tandem with increasing global prevalence. In the U.S., 13.9% of children and adolescents aged 2–19 were obese in 1999 and the trend showed a significant increase to 17.1% between 2003 and 2004. The prevalence remains high, having been reported in a recent study to be approximately 17.2% (1). In 2004 in Malaysia, a study reported the prevalence of overweight and obesity among school children and adolescents to be 7.3% (2). Recent studies found that the prevalence of overweight and obesity among children in Malaysia have increased significantly to 18.2–19.9 and 15.2%, respectively (3, 4). Obesity has become a significant global public health problem with unhealthy excess weight posing health risks for chronic diseases such as Type 2 diabetes mellitus, hypertension, dyslipidaemia, orthopedic problems, and sleep disordered breathing (5–9). The study of childhood obesity in a country like Malaysia, in which children below 18 years make up approximately 30% of the population, may provide a good representation of the direction of obesity prevalence of children worldwide.

Interestingly, several studies have revealed that the prevalence of obesity is significantly greater among children with autism spectrum disorder (ASD), compared to the general population. Based on previous literature on ASD, the prevalence of obesity among ASD children ranged from 17 to 32%, and the prevalence of overweight ranged between 13 and 33% (10–18). A recent study in the U.S based on the 2016 National Survey of Children's Health reported that the prevalence of overweight and obesity are higher in the ASD group in comparison to typically developing children, with 19 and 23% of children with ASD reported to be overweight and obese, respectively, compared to 14 and 15% of typically developing children (18).

ASD is a neurodevelopmental disorder characterized by impairments in communication, behavior, and social functioning beginning in childhood. There are no local epidemiological studies on ASD prevalence in Malaysia. However, a feasibility study on the use of Modified Checklist for Autism in Toddlers (M-CHAT) among children aged 18–36 months by the Ministry of Health Malaysia found the prevalence of ASD to be approximately 1.6 in 1,000, significantly lower than those reported by recent worldwide ASD prevalence studies, and likely an underestimation (19). Autism prevalence has been showing an increasing trend over the past few decades.

There are several risk factors that might contribute to obesity among ASD children. A significant proportion of ASD children have been reported to have sleeping and feeding problems, and difficulty in engaging in physical activity (20–22). Children with

ASD have been found to be less physically active and fit compared to typically developing children (20). Studies by McCoy et al. (23) and Healy et al. (24) compared physical activity and obesity among adolescents with and without ASD and found that the ASD group are more likely to be obese and less likely to engage in regular physical activity or sports (23, 24).

There is a clear association between short sleep duration and the risk of childhood obesity in the general population (25, 26). Children with ASD have poorer sleep quantity and quality compared to typically developing children and these issues do not appear to improve with age (21). They commonly have a shorter duration of sleep and problems with sleep tend to endure. Humphreys et al. (27) investigated longitudinal sleep patterns in children with ASD and found that sleep duration in children with ASD reduced from 30 months of age onwards and persisted until adolescence. Night-time sleep duration was shortened by later bedtimes and earlier waking times (27).

Children with ASD are frequently associated with picky eating and aversions to specific textures, colors, smells, temperatures, and brand names of foods. The degree of food selectivity appears to be an important indicator of acceptance or rejection of food and might be related to obesity. Children with ASD exhibit greater likelihood of food selectivity, eat a narrower range of foods, and have been frequently reported to have behavioral concerns during mealtime such as screaming or crying and food refusal (22, 28–30). A possible risk factor for obesity is the use of food as a primary reinforcer to calm these children down, which could also lead to obesity (31). Another possible mechanism leading to food selectivity or refusal in ASD children is "gut-brain" axis disruption leading to gastrointestinal disturbances, which may potentially be amenable to dietary manipulation or supplementary management (32).

Obesity is especially challenging to manage in children with ASD and therefore it is important to identify significant modifiable risk factors for potential remedial measures. The standard management for obesity in children and adolescents in Malaysia, and in general worldwide, is the reduction of energy intake by dietary modifications, increasing physical activity, and reducing physical inactivity. These are carried out using behavior modification techniques for modifying eating habits and improving activity patterns, and the involvement of the family in weight management (Malaysian Obesity Clinical Practice Guideline). Obtaining adequate night time sleep has also been proven to be associated with 40% lower prevalence of obesity (33).

Previous studies have focused on the prevalence rate of overweight and obesity among the ASD population. There is, however, a paucity of research on physical activity and its impact

on body mass index among ASD children, with no previous study yet reported in Malaysia. To the best of our knowledge, there is little research to identify other possible risk factors apart from physical activity associated with overweight and obesity in children with ASD, specifically the impact of sleep habits and mealtime behavior. The aims of this study are to assess the prevalence of overweight and obesity among Malaysian ASD children and adolescents its associated risk factors, and to determine the relationship between the level of physical activity, sleep habits and mealtime behavior with the BMI status of Malaysian ASD children.

METHODOLOGY

This study was carried out at an outpatient setting on 151 children and adolescents aged 2–18 years who were diagnosed with ASD based on DSM IV or DSM 5 criteria and were on follow up at the Child Development Center (CDC) UKMMC. Patients' records were reviewed to obtain the diagnosis, co-morbidities, age, and sex. Stadiometer and weighing scale were used to obtain height and weight. Measured values for weight and height were used to calculate the body mass index (BMI) and these were charted on growth charts to determine percentiles by gender and age. Centers for Disease Control and Prevention criteria for BMI were used to define overweight and obesity (≥85th to <95th percentiles and ≥95th percentiles, respectively). Parental height, weight, and BMI were also measured. Questionnaires were given to parents who had signed the consent form and the questionnaires were filled up while they were waiting for their appointment. Questionnaires used were Children Sleep Habit Questionnaire (CHSQ), Physical Activity for Older Children Questionnaire (PAQ-C), and Brief Autism Mealtime Behavior Inventory (BAMBI). Exclusion criteria were children who were on Ritalin and antipsychotics.

This is a cross sectional study. The sample size was calculated based on the formula n' = NZ^2 P(1–P)/d^2(N−1) + Z^2 P(1–P) whereby N is the population size, Z is the statistic for a level of confidence, P is the expected proportion, and d is precision (34). The estimated number of ASD patients under CDC follow up is 300 children, Z value is 1.96 for the level of confidence interval 95%, the expected prevalence of overweight plus obesity based on previous studies is 0.5 and the precision is 5%. The calculated sample size was 165.

Ethical approval was obtained from the Research Ethics Committee UKM (Research code: FF-2018-247). This ethical approval allowed for us to collect data from human subjects in the form of questionnaires and anthropometric data, with confidentiality maintained for all study subjects. Each participant (parent) was provided with an explanation of the study, both in written and verbal forms, and requested to sign a consent form prior to collection of data.

QUESTIONNAIRES
Children Sleep Habit Questionnaire (CSHQ)
CSHQ is a retrospective 34-item questionnaire, developed by Owens et al. (35) that has been used to examine sleep behavior

in young children aged 4–12 years (35). It is not intended to diagnose specific sleep problems but instead was designed to screen for the most common sleep problems. It is based on parental report and contains 8 subscales: bedtime resistance, sleep onset delay, sleep duration, sleep anxiety, night waking, parasomnias, sleep disordered breathing, and daytime sleepiness. The answers are based on a Likert scale. Items are rated on a 3-point scale; "usually" if the sleep behavior occurred 5–7 times per week, "sometimes" for 2–4 times per week and "rarely" for 0–1 time per week. The response "usually" is scored as 3, "sometimes" scored as 2, and "rarely" scored as 1. A total score of over 41 indicates a pediatric sleep disturbance. The questionnaire had been translated into the Malay version by Firouzi et al. (36) and was pretested on a sample of 17 children. The Cronbach's alpha of the questionnaire was 0.895, which indicates high reliability (36).

Physical Activity for Older Children Questionnaire (PAQ-C)
The PAQ-C is a self-administered, 7-day recall questionnaire developed by Kowalski et al. (37) to assess general levels of physical activity of children aged ∼8–14 years old (37). It has 10 items, 9 of which are scored on a 5-point scale. The final PAQ-C activity summary score is the mean value from 1 to 5 for each of 9 items. A score of 1 indicates low physical activity whereas a score of 5 indicates high physical activity. Reliability and validity data have been proven by Kowalski et al. (37). This questionnaire has been translated into the Malay language and validation performed by Mohd Zaki et al. on 73 children aged 10–17 years old, which showed that the Malay version has good internal consistency with Cronbach alpha 0.75–0.77 (38). Based on a study by Voss et al. (39) on 7,226 children aged 10–15 years old to determine the cut-off point for PAQ-C score, it was found that PAQ-C scores of more than 2.9 for boys and more than 2.7 for girls indicated "sufficiently active" group vs. "low active" group (39).

Brief Autism Mealtime Behavior Inventory (BAMBI)
BAMBI was developed by Lukens (40) to measure mealtime behavioral problems seen in autistic children aged between 3 and 11 years old. It is a parental report and consists of 18 items that are in three categories of eating behavior: limited variety of food, food refusal, and features of autism. Items were rated on a 5-point Likert scale, where "1" referred to never/rarely, "2" for seldom, "3" for occasionally, "4" for often and "5" for almost every meal. It has been validated by Lukens (40). DeMand et al. (41) explored the psychometric properties of BAMBI in 273 children with

---

TABLE 1 | Body Mass Index (BMI) status of 151 children with ASD.

BMI status by percentiles	N	%
Underweight (BMI <5th percentile)	11	7.3
Normal (BMI ≥5th to <85th percentiles)	90	59.6
Overweight (BMI ≥ 85th to <95th percentiles)	17	11.3
Obese (BMI ≥ 95th percentiles)	33	21.9

autism and derived a cut-off for BAMBI total score. The cut off total score of more than 34 indicates problematic feeders (41). Permission from Dr. Colleen Taylor Lukens to use and translate BAMBI has been obtained for this study. BAMBI has been translated into the Malay language using forward and backward translation methods. The original BAMBI was translated into the Malay language by a bilingual person. The Malay version of BAMBI was then back-translated into English by another translator who had not seen the original English version. The two different translators were fluent in both the Malay language and English. The two versions of translation were found to be comparable after being checked by two pediatricians. A pilot study was conducted using the Malay version of the BAMBI on 30 subjects for validation. The Cronbach's alpha was found to be 0.83, which indicates good reliability.

The median age was 6.75 years old. One hundred and thirty two were male (87.4%) and 19 were female (12.6%). Ethnic distribution were 99 Malays (65.6%), 41 Chinese (27.2%), 8 Indians (5.3%), and 3 of "other" ethnicity (2%). Nine children had a co-morbidity of attention deficit hyperactive disorder (ADHD) (6%), 11 had global developmental delay (GDD) (7.3%), 4 had intellectual disability (ID) (2.6%), and 8 of the children had other co-morbidities including dysmorphism, epilepsy, *beta*-thalassemia, atopic eczema and speech language impairment (SLI) (5.3%). One child was on a gluten-restriction diet.

Statistical Analysis

Data analysis was carried out using Statistical Package for Social Sciences (SPSS) Version 23.0. To compare the median difference or percentage difference of the variables between obese,

TABLE 2 | Comparison of characteristics of overweight and obese with normal and underweight groups.

Characteristics	Normal + Underweight BMI <85th percentiles (n = 101)	Overweight + Obese BMI ≥ 85th percentiles (n = 50)	p-value
	Median (IQR) or n (%)	Median (IQR) or n (%)	
Age (years)	6.33 (4.75–7.71)	8.54 (5.81–10.13)	**0.001***
SEX			
Female	12 (11.9%)	7 (14%)	0.712
Male	89 (88.1%)	43 (86%)	
Birth weight (kg)	3.01 (2.73–3.32)	2.99 (2.70–3.20)	0.459
RACE			
Malay	69 (68.3%)	30 (60%)	0.252
Chinese	26 (25.7%)	15 (30%)	
Indian	4 (4%)	4 (8%)	
Others	2 (2%)	1 (2%)	
Paternal BMI	26.00 (23.6–29.00)	27.65 (24.43–31.25)	0.096
Maternal BMI	24.7 (21–27.9)	26.05 (23.35–32.25)	**0.003***
Paternal age (years)	38 (35–42)	40 (37–44)	**0.039***
Maternal age (years)	36 (34–39)	37 (34.75–40.25)	0.108
Monthly income (RM)	6,000 (4,000–10,000)	5,000 (3,000–10,000)	0.184
Number of siblings	2 (2–3)	2 (2–3)	0.393
CO-MORBIDITIES			
None	77 (76.2%)	42 (84%)	0.247
ADHD	7 (6.9%)	2 (4%)	
GDD	10 (9.9%)	1 (2%)	
ID	3 (3%)	1 (2%)	
Others	4 (4%)	4 (8%)	
DIETARY RESTRICTION			
None	97 (96%)	50 (100%)	0.514
Restriction	4 (4%)	0 (0%)	
PATERNAL EDUCATION			
Tertiary	73.5 (72.3%)	31 (62%)	0.644
Secondary	28 (27.7%)	19 (38%)	
MATERNAL EDUCATION			
Tertiary	75 (74.3)	31 (62%)	0.369
Secondary	26 (25.7%)	19 (38%)	

*Significant median difference at p < 0.05 using Mann-Whitney U test/Significant number difference at p < 0.05 using Chi square test. ADHD, attention deficit hyperactive disorder; GDD, global developmental delay; ID, intelectual disability. The bold values are statistically significant at p < 0.05.

overweight, normal weight, and underweight groups, the Mann-Whitney U test was used for continuous data and Chi squared test for categorical data. The variables included age, ethnicity, co-morbidities, number of siblings, socioeconomic status, parental BMI, parental age, and parental education. The Spearman Correlation was applied to determine the correlation between the variables. Multiple Linear Regression analysis utilizing stepwise method was performed for the association between sleep habits, physical activity level and mealtime behavior and odds of being overweight and obese.

The data was analyzed between two groups of ASD children based on BMI, comparing those with BMI $\geq$85th percentile (overweight + obese, $n = 50$) to those with BMI <85th percentile (underweight + normal, $n = 101$), to determine associations between being overweight/obese and its risk factors. Normality skewness and kurtosis test was performed, and it showed that the data was skewed. Therefore, non-parametric tests were utilized.

RESULTS

Table 1 demonstrates the breakdown of ASD children by BMI category. The prevalence of overweight individuals in ASD children in this study was 11.3%, with 17 of the 151 children having BMI $\geq$85th to <95th percentile. The prevalence of obesity was 21.9% with 33 of the 151 children having BMI $\geq$95th percentile. The total prevalence of overweight and obesity in ASD children in the study was therefore 33.2%, with 50 of the children having BMI $\geq$85th percentile.

Table 2 shows the characteristics of the study sample, comparing those categorized as overweight and obese to those who were not. There was a significant difference in median age between the two groups, whereby the overweight/obese ASD children's median age was 8.5 years (IQR 5.81–10.13), in

comparison to non-overweight/obese group which was 6.33 years (IQR 4.75–7.7) with a p-value of 0.001. **Figure 1** demonstrates the association between BMI percentiles and mean age, whereby increasing age is associated with higher BMI. The two groups also differed significantly in terms of maternal BMI and paternal age, whereby in the overweight/obese group, the maternal median BMI was statistically significantly higher than the non-overweight/obese group. The maternal median BMI in overweight/obese was 26.05 (IQR 23.35–32.25) and maternal BMI in non-overweight/obese was 24.7 (IQR 21–27.9).

Table 3 describes the frequency of sleep disturbance based on Total Sleep Disturbance Index, physical activity level and problematic feeders among the ASD children. All ASD children in this study (except two) have pediatric sleep disturbance. Parents of seven ASD children did not answer the PAQ-C questionnaire relating to physical activity as the questions were not applicable to their children, who at 3 years of age were too young. From a total of 144 children, 41.7% were sufficiently active whereas 53.6% had levels of activity that were low. From the

TABLE 3 | Frequency of sleep disturbance, physical activity level and problematic feeder among 151 children with ASD.

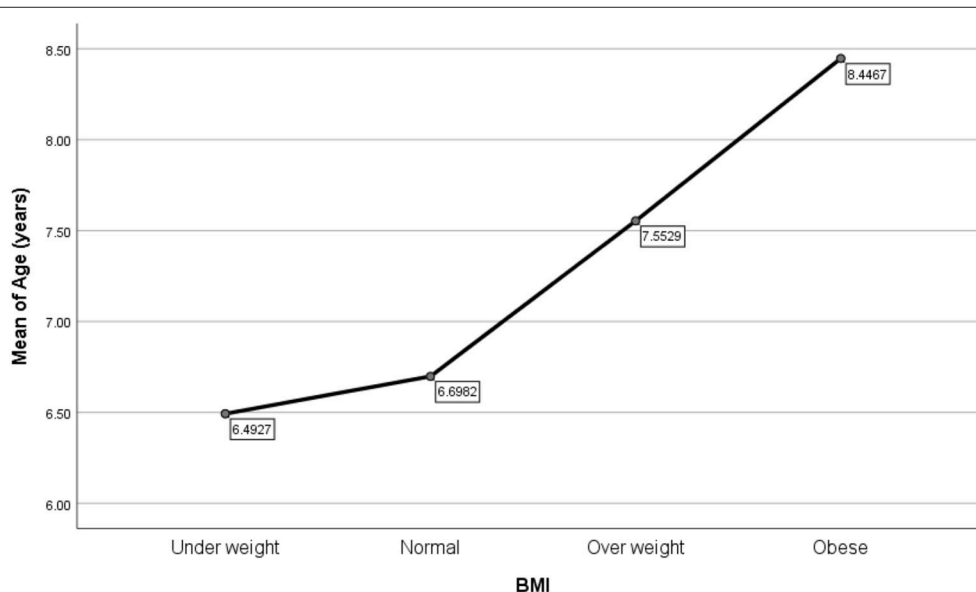| Risk factors for Overweight/Obesity (Questionnaires)		N	%
Pediatric sleep disturbance (CSHQ)	Yes	149	98.7
	No	2	1.3
Physical activity level (PAQ-C)	Sufficiently active	63	41.7
	Low active	81	53.6
	NA	7	4.6
Problematic feeder (BAMBI)	Yes	138	91.4
	No	13	8.6

FIGURE 1 | Weight status in relation to mean age (years).

feeding questionnaire, a majority (91.4%) of the children were problematic feeders.

In **Table 4**, obese and overweight ASD children were compared to ASD children who were underweight and had normal weight in relation to the studied risk factors. One hundred and forty nine out of 151 ASD children were identified as having disturbance with sleep based on Total Sleep Disturbance Index of the Children Sleep Habit Questionnaire (CSHQ). The median duration of sleep for the whole study sample was 9 h. Median sleep hours for overweight/obese group was 9 (IQR 8–10; $n = 80$) and for underweight/normal group the median sleep hours was 8.5 (7.63–9.88; $n = 40$). No significant difference was noted in Total Sleep Disturbance Index among the two groups. Stratifying by gender did not reveal a significant difference for Total Sleep Disturbance Index ($p = 0.458$). Thirty-one parents did not answer the total duration of sleep in hours.

Based on gender stratification, there was a significant difference in the Physical Activity Questionnaire for Older Children (PAQ-C) score between the two groups amongst male ASD children. The male overweight/obese children had a median PAQ-C score of 2.44 (IQR 2.00–3.00) vs. 2.89 (IQR 2.35–3.53) in the counterpart group with a p-value of 0.01. The lower score indicates less physical activity. For the questionnaire on physical activity, seven of the children were too young for the questionnaire, resulting in assessment of 144 out of 151 children for this part of the analysis.

Of the 151 children in the study, 138 were problematic feeders based on BAMBI scoring. There was no significant difference between the two groups in the BAMBI score: however, one of the domains of BAMBI, the Food Refusal component, showed a significant difference between the two groups. The overweight/obese group had lower scores in the Food Refusal domain as compared to non-overweight/obese. The median score in overweight/obese group was 7 (IQR 5–9.25) and the median score in non-overweight/obese group was 9 (IQR 7–11). Gender stratification did not reveal a significant difference for total BAMBI score ($p = 0.986$).

Using multiple linear regression stepwise method, three predictors associated with BMI percentiles reached a statistical level of significance. These were the PAQ-C score in males ($p < 0.001$, beta coefficients -0.37) and the BAMBI domains of Food Refusal ($p = 0.001$, beta coefficients -0.71) and Limited Variety of Food ($p = 0.001$, beta coefficients 0.39). The beta coefficients for PAQ-C score in males and Food Refusal score showed a negative beta coefficients value, which indicates that they contribute to higher weight in ASD children. Whereas, Limited Variety of Food score has a positive beta coefficients value, which indicates that it is protective against higher weight status. **Table 5** illustrates the strength of association between PAQ-C score for male ASD, and the two BAMBI domains mentioned previously. Based on the analysis, 74% of the change in BMI percentiles was significantly affected by three factors:

TABLE 4 | Comparison between normal and underweight ASD children with overweight and obese ASD children based on CSHQ, PAQ-C and BAMBI scoring.

	Normal & Underweight ($n = 101$)	Overweight & Obese ($n = 50$)	p-value
	Median (IQR 25–75)	Median (IQR 25–75)	
i. CSHQ: TOTAL INDEX, TOTAL DURATION OF SLEEP AND EIGHT DOMAINS OF SLEEP HABIT			
Total sleep disturbance index	54 (50.5–58)	55 (51–58)	0.909
Total duration of sleep (hours)	9 (8–10) ($n = 80$)	8.5 (7.63–9.88) ($n = 40$)	0.197
A. Bedtime resistance	12.5 (11–14)	12 (10–14)	0.414
B. Sleep onset delay	3 (2–3)	3 (2–3)	0.601
C. Sleep duration	3 (3–3)	3 (3–3)	1.000
D. Sleep anxiety	7 (5–8)	6 (5–8)	0.904
E. Night waking	4 (3–5)	3 (3–4)	0.081
F. Parasomnias	9 (7–10)	8 (7–10)	0.552
G. Sleep disordered breathing	3 (3–4)	3 (3–4.25)	0.124
H. Daytime sleepiness	14 (13–15.75)	14 (12.75–15)	0.608
ii. PAQ-C			
PAQC score			
Male	2.89 (2.35–3.53) ($n = 84$)	2.44 (2.00–3.00) ($n = 42$)	**0.010***
Female	2.67 (2.14–3.27) ($n = 12$)	3.17 (2.81–3.75) ($n = 6$)	0.205
iii. BAMBI AND DOMAINS			
BAMBI total score	44 (40–48)	45 (42–50.5)	0.257
A. Limited variety of food	25 (22–27)	26 (23–28)	0.052
B. Food refusal	9 (7–11)	7 (5–9.25)	**0.005***
C. Features of autism	11 (10–13)	12 (9.75–13.25)	0.196

*Significant median difference at p < 0.05 using Mann-Whitney U test. CSHQ, children sleep habit questionnaire; PAQ-C, physical activity questionnaire for older children; BAMBI-brief autism mealtime behavior inventory. The bold values are statistically significant at p < 0.05.

TABLE 5 | Predictors of overweight/ obesity among children and adolescents with ASD.

Risk factors	B	95% CI	p-value
PAQ-C score for male ASD	−0.37	−2.95, −1.14	<0.001*
Total Food refusal score	−0.71	−1.01, −0.41	0.001*
Total Limited Variety of Food score	0.39	0.22, 0.57	0.001*

$R^2 = 0.74$ (adjusted $R^2 = 0.69$), *significant p-value < 0.05.

Physical Activity Questionnaire for Older Children (PAQ-C) score, total scores for BAMBI domains of Food Refusal and Limited Variety of Food.

DISCUSSION

The prevalence of overweight and obesity in ASD children in our study were 21.9 and 11.3%, respectively. Our results for prevalence of obesity in ASD children are consistent with previous studies of obesity prevalence in ASD, although our study showed a lower prevalence of overweight individuals (10–18). The prevalence of overweight individuals in ASD children in our study was similar to that reported by Memari et al. (12). A possible hypothesis for this lower prevalence in overweight ASD children is that in our population, those who had difficulty maintaining an ideal weight were more severe in phenotype and thus more likely to present to a tertiary center such as ours. However, we did not assess for severity in this study and were not able to investigate this further. Similar to other published studies, our findings showed a higher prevalence of obesity in ASD children compared to what has been reported in children from the general population (11, 14). A recent study of Malaysian children from the general population found an obesity prevalence of 15% (4). Our study is timely as obesity is becoming a greater problem in children in general, especially in ASD children.

We found that the BMI status of ASD children change as they age. The younger age group tend to be underweight and the older age group tend to be obese. Hill et al. (17) found that the prevalence of overweight and obesity were significantly higher in the age groups of 2–5 years and 12–17 years (17). Our findings showed similar results in the older age group, although we did not find comparable results for the younger children. However, the majority of the children in our study were above 4 years of age and this may account for the dissimilar findings. Must et al. (42) also demonstrated that the prevalence of obesity in ASD children increased from the age of 10–17 years, consistent with the trend observed in our study. They also found that typically developing children who were obese became less obese with increasing age, whereas children with ASD did not (42). A study by MacDonald et al. (43) found that there is a decline in physical activity as children with ASD age, especially for moderate to vigorous physical activities (43). One possible explanation for this is that children with ASD may have challenges in engaging in physical activity due to motor and social skills, and communication difficulties, that result in more time spent sedentarily (44). A higher BMI in older ASD children may be the result of less

physical activity as children with ASD age, which could be due to increasing difficulty for parents to manage older children with ASD.

We found that overweight and obesity among ASD children were associated with higher maternal BMI. In our study, the median maternal BMI in overweight/obese group was 26, which is classified as overweight. Some probable causes may be an unhealthy family diet and lack of family-centered exercise, both of which could result in mother and child concurrently having a higher weight. Paternal BMI in overweight/obese ASD children also showed an increasing trend, but did not reach statistical significance. A postulation for why maternal BMI was found to be associated with obesity in ASD children is that children are more likely to have a similar diet and activity level as their mothers, since in our setting mothers are the most probable primary caretaker. Overweight and obesity is also found to be associated with older paternal age. It is possible that older fathers may be less likely to engage children in physical activity, and thus these children spend more time sedentarily, potentially leading to increased risk of obesity. However, we did not specifically explore this in our study.

The majority (98.7%) of children in our study had sleep disturbance when scored on the Total Sleep Disturbance Index. Previous studies based on parental reports found that children with ASD are more likely to have disturbed sleep as compared to typically developing children, including short sleep duration, frequent night waking and long sleep onset latency (27, 45). ASD children with sensory over-responsivity may be particularly predisposed to sleep disturbance and hyperarousal (46). The median duration of sleep for all patients in this study was 9 h. Based on a meta-analysis conducted by Chen et al. (25), the recommended sleep duration for children age 5–10 years old is at least 10 h (25). This shows that the majority of ASD children in this study were not getting the recommended duration of sleep for their age. However, there was no significant difference in sleep habits and sleep duration between overweight/obese ASD children and their non-overweight/non-obese counterpart. Sleep disturbance is common in ASD, but our study did not show that it was a risk factor for obesity.

Analysis for level of physical activity was stratified based on gender. Stratification was done because the cut-off point for males and females were different. Girls require a lower score to be defined as sufficiently active compared to boys. Low physical activity level was found in male ASD children who were overweight and obese. This result is consistent with a previous study by McCoy et al. (23) which reported that adolescents with ASD were more likely to be overweight and obese and less likely to engage in regular physical activity (23). Reduction in activity has been recognized as a risk factor for obesity in children (47). This suggests that ASD children who have low levels of physical activity are at risk of overweight and obesity, and the mainstay of intervention for obesity in these children is greater participation in physical activity. In addition, healthcare practitioners should advocate prevention of obesity in ASD children by routinely enquiring about physical activity levels during clinical assessment and advising more physical activity, in order to reduce the risk of obesity. No significant difference was observed in physical

activity level among girls with ASD, which may be due to the very small number of female patients.

The majority (91.4%) of subjects have problems with feeding. Previous studies have reported that ASD is associated with feeding problems (22, 28–30). Reasons for feeding difficulties may be related to core autistic features such as insistence on sameness and rigidity as well as sensory issues. Gastrointestinal disturbances have also been frequently reported among the ASD population and this could potentially affect food intake and food choice (32). There was no significant difference in the BAMBI score between the two groups. However, food refusal was observed more frequently in the non-overweight/non-obese group. This may reduce the likelihood of them being obese or overweight. There was less food refusal observed in those with high BMI. In our study we also found that higher BMI in ASD children was associated with food selectivity. Children with high BMI are thus more likely to choose only specific foods, but not refuse food in general. Some ASD children have been noted to have a diet with a narrow repertoire of food types, for example, a preference for crunchy foods, which is likely related to sensory-seeking behavior. Children with ASD who have high food selectivity may choose foods that are high in calories, leading to increased BMI. The results of our study are consistent with previous studies in which it has been demonstrated that ASD children are more likely to have feeding problems. However, further research is required to assess the possible mechanisms by which feeding problems may contribute to the development of obesity.

LIMITATIONS

This study was only conducted on children with ASD. There was no data from typically developing children from the general population. As such, the prevalence rate in our study was compared to existing prevalence of obesity in children from the general population, based on studies conducted earlier.

The selection of subjects was not randomized. The 151 children in this study were taken as convenient sampling in the CDC during the period of data collection. Ideally, a larger sample size of 320, considering the effect size, is required to accurately represent the Malaysian ASD population in studies where subjects are not randomized. However, due to time constraints, the sample size obtained for this study was less than the calculated value to correct for precision. This may potentially impact the precision of the results. The value of precision that we obtained in our study using the sample size, was smaller, $d = 0.02$, which means that the statistical cut-off level should be less than $p < 0.02$. Having said that, almost all of our significant results had a p-value of less than 0.02. Therefore, the results of our analysis were still valid for most of our findings. The questionnaire used

for physical activity was designed for children aged 8–14 years, but in this study, we utilized it to include younger children from 3 years and above, which is not ideal. PAQ-C questionnaire has been previously used and validated in Malaysia.

This study combined normal and underweight children which may affect analysis, however because the underweight group is small, we feel it is unlikely to affect the overall result.

CSHQ is a screening tool for sleep disturbance in children from the general population. As children with ASD have been reported to have a greater likelihood of sleep difficulties, it is not surprising that the vast majority of our children were found to have sleep disturbance. However, no specific questionnaire has been developed to ascertain sleep disturbance in ASD children.

CONCLUSION

From this study, the prevalence of overweight and obesity is high among Malaysian ASD children and adolescents, at 21.9 and 11.3%, respectively. Older age group, high maternal BMI, older paternal age, low physical activity, low likelihood of food refusal and higher likelihood of food selectivity, were all found to be risk factors for high BMI in these children. Clinicians who manage children with ASD need to have a greater awareness of the increased risk of overweight and obesity in ASD so that they can provide meaningful preventive measures regarding obesity.

DATA AVAILABILITY

Datasets are available on request: The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

NK and JI contributed to the concept and design of the study. AG and NK organized the database and performed the statistical analysis. NK, AG, and JI drafted the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

ACKNOWLEDGMENTS

REFERENCES

1. Ogden CL, Carroll MD, Fryar CD, Flegal KM. Prevalence of obesity among adults and youth: United States, 2011–2014. *NCHS Data Brief* (2015) 219:1–8.
2. Moy FM, Gan CY, Zaleha MK. Body mass status of school children and adolescents in Kuala Lumpur, Malaysia. *Asia Pac J Clin Nutr.* (2004) 13:324–9.
3. Naidu BM, Mahmud SZ, Ambak R, Sallehuddin SM, Mutalip HA, Saari R, et al. Overweight among primary school-age children in Malaysia. *Asia Pac J Clin Nutr.* (2013):22:408–15. doi: 10.6133/apjcn.2013.22.3.18
4. Lee PY, Cheah WL, Chang CT, Siti Raudzah G. Childhood obesity, self-esteem and health-related quality of life among urban primary schools children in Kuching, Sarawak, Malaysia. *Mal J Nutr.* (2012) 18:207–19.

5. Goran MI, Ball GDC, Cruz ML. Obesity and risk of type 2 diabetes and cardiovascular disease in children and adolescents. *J Clin Endocrinol Metab.* (2003) 88:1417–27. doi: 10.1210/jc.2002-021442

6. Friedemann C, Heneghan C, Mahtani K, Thompson M, Perera R, Ward AM. Cardiovascular disease risk in healthy children and its association with body mass index: systematic review and meta-analysis. *BMJ* (2012) 345:e4759. doi: 10.1136/bmj.e4759

7. Freedman DS, Mei Z, Srinivasan SR, Berenson GS, Dietz WH. Cardiovascular risk factors and excess adiposity among overweight children and adolescents: the Bogalusa Heart Study. *J Pediatr.* (2007) 150:12–7.e2. doi: 10.1016/j.jpeds.2006.08.042

8. Taylor ED, Theim KR, Mirch MC. Orthopedic complications of overweight in children and adolescents. *Pediatrics* (2006) 117:2167–74. doi: 10.1542/peds.2005-1832

9. Bixler EO, Vgontzas AN, Lin HM. Sleep disordered breathing in children in a general population sample: prevalence and risk factors. *Sleep* (2009) 32:731–6. doi: 10.1093/sleep/32.6.731

10. Chen AY, Kim SE, Houtrow AJ, Newacheck PW. Prevalence of obesity among children with chronic conditions. *Obesity* (2009) 18:210–3. doi: 10.1038/oby.2009.185

11. Curtin C, Anderson SE, Must A, Bandini L. The prevalence of obesity in children with autism: a secondary data analysis using nationally representative data from the National Survey of Children's Health. *BMC Pediatrics* (2010) 10:11. doi: 10.1186/1471-2431-10-11

12. Memari AH, Kordi R, Ziaee V, Mirfazeli FS, Setoodeh MS. Weight status in Iranian children with autism spectrum disorders: investigation of underweight, overweight and obesity. *Res Autism Spectrum Disord.* (2012) 6:234–9. doi: 10.1016/j.rasd.2011.05.004

13. Egan AM, Dreyer ML, Odar CC, Beckwith M, Garrison CB. Obesity in young children with autism spectrum disorders: prevalence and associated factors. *Childhood Obes.* (2013) 9:125–31. doi: 10.1089/chi.2012.0028

14. Bicer AH, Alsaffar AA. Body mass index, dietary intake and feeding problems of Turkish children with autism spectrum disorder (ASD). *Res Dev Disabil.* (2013) 34:3978–87. doi: 10.1016/j.ridd.2013.08.024

15. Broder-Fingert S, Brazauskas K, Lindgren K, Iannuzzi D, Van Cleave J. Prevalence of overweight and obesity in a large clinical sample of children with autism. *Acad Pediatr.* (2014) 14:408–14. doi: 10.1016/j.acap.2014.04.004

16. Zuckerman KE, Hill AP, Guion K, Voltolina L, Fombonne E. Overweight and obesity: prevalence and correlates in a large clinical sample of children with autism spectrum disorder. *J Autism Dev Disord.* (2014) 44:1708–19. doi: 10.1007/s10803-014-2050-9

17. Hill AP, Zuckerman KE, Fombonne E. Obesity and autism. *Pediatrics* (2015) 136:1051–61. doi: 10.1542/peds.2015-1437

18. Healy S, Aigner CJ, Haegele JA. Prevalence of overweight and obesity among US youth with autism spectrum disorder. *Autism* (2018). doi: 10.1177/1362361318791817. [Epub ahead of print].

19. Family Health Division. *Prosiding Mesyuarat Membincangkan Hasil Kajian Saringan dan Pengendalian Masalah Autisme.* Kuala Lumpur: Malaysian Health Technology Assessment Section (MaHTAS), Ministry of Health Malaysia, Federal Government of Malaysia (2006).

20. Tyler K, MacDonald M, Menear K. Physical activity and physical fitness of school-aged children and youth with autism spectrum disorders. *Autism Res Treat.* (2014) 2014:312163. doi: 10.1155/2014/312163

21. Hodge D, Carollo TM, Lewin M, Hoffman CD, Sweeney DP. Sleep patterns in children with and without autism spectrum disorders: developmental comparisons. *Res Dev Disabil.* (2014) 35:1631–8. doi: 10.1016/j.ridd.2014.03.037

22. Schreck KA, Williams K, Smith AF. A comparison of eating behaviors between children with and without autism. *J Autism Dev Disord.* (2004) 34:433–8. doi: 10.1023/B:JADD.0000037419.78531.86

23. McCoy SM, Jakicic JM, Gibbs BB. Comparison of obesity, physical activity, and sedentary behaviors between adolescents with autism spectrum disorders and without. *J Autism Dev Disord.* (2016) 46:2317–26. doi: 10.1007/s10803-016-2762-0

24. Healy S, Haegele JA, Grenier M, Garcia JM. Physical activity, screen-time behavior, and obesity among 13-year olds in Ireland with and without autism spectrum disorder. *J Autism Dev Disord.* (2016) 47:49–57. doi: 10.1007/s10803-016-2920-4

25. Chen X, Beydoun MA, Wang Y. Is sleep duration associated with childhood obesity? A systematic review and meta-analysis. *Obesity* (2008) 16:265–74. doi: 10.1038/oby.2007.63

26. Caputi P, Iverson D. Lack of sleep could increase obesity in children and too much television could be partly to blame. *Acta Paediatr.* (2014) 103:e27–31. doi: 10.1111/apa.12447

27. Humphreys JS, Gringras P, Blair PS, Scott N, Henderson J, Fleming PJ, et al. Sleep patterns in children with autistic spectrum disorders: a prospective cohort study. *Arch Dis Child* (2013) 99:114–8. doi: 10.1136/archdischild-2013-304083

28. Bandini LG, Anderson SE, Curtin C, Cermak S, Evans EW, Scampini R, et al. Food selectivity in children with autism spectrum disorders and typically developing children. *J Pediatr.* (2010) 157:259–64. doi: 10.1016/j.jpeds.2010.02.013

29. Evans EW, Must A, Anderson SE, Curtin C, Scampini R, Maslin M, et al. Dietary patterns and body mass index in children with autism and typically developing children. *Res Autism Spectr Disord.* (2012) 6:399–405. doi: 10.1016/j.rasd.2011.06.014

30. Attlee A, Kassem H, Hashim M, Obaid RS. Physical status and feeding behavior of children with autism. *Indian J Pediatr.* (2015) 82:682–7. doi: 10.1007/s12098-015-1696-4

31. Kral TVE, Souders MC, Tompkins VH, Remiker AM, Eriksen WT, Pinto-Martin JA. Child eating behaviors and caregiver feeding practices in children with autism spectrum disorders. *Public Health Nurs.* 32:488–97. doi: 10.1111/phn.12146

32. Santocchi E, Guiducci L, Fulceri F, Billeci L, Buzzigoli E, Apicella F, et al. Gut to brain interaction in autism spectrum disorders: a randomized controlled trial on the role of probiotics on clinical, biochemical and neurophysiological parameters. *BMC Psychiatry* (2016) 16:183. doi: 10.1186/s12888-016-0887-5

33. Anderson SE, Whitaker RC. Household routines and obesity in US preschool-aged children. *Pediatrics* (2010) 125:420–8. doi: 10.1542/peds.2009-0417

34. Naing L, Winn T, Rusli BN. Practical issues in calculating the sample size for prevalence studies. *Arch Orofacial Sci.* (2006) 1:9–14.

35. Owens JA, Spirito A, Mc Guinn M. The Children's Sleep Habits Questionnaire (CSHQ): psychometric properties of a survey instrument for school-aged children. *Sleep* (2000) 23:1043–51. doi: 10.1093/sleep/23.8.1d

36. Firouzi S, Poh BK, Ismail MN, Sadeghilar A. Sleep habits, food intake, and physical activity levels in normal and overweight and obese Malaysian children. *Obes Res Clin Prac.* (2014) 8:e70–8. doi: 10.1016/j.orcp.2012.12.001

37. Kowalski KC, Crocker PRE, Faulkner RA. Validation of the physical activity questionnaire for older children. *Pediatr Exer Sci.* (1997) 9:174–86. doi: 10.1123/pes.9.2.174

38. Mohd Zaki NA, Sahril N, Omar MA, Ahmad MH, Baharudin A, Mohd Nor NS. Reliability and validity of the physical activity questionnaire for older children (PAQ-C) in Malay language. *Int J Public Health Res.* (2016) 6:670–6.

39. Voss C, Ogunleye AA, Sandercock GR. Physical activity questionnaire for children and adolescents: English norms and cut-off points. *Pediatr Int.* (2013) 55:498–507. doi: 10.1111/ped.12092

40. Lukens CT, Linscheid TR. Development and validation of an inventory to assess mealtime behavior problems in children with autism. *J Autism Dev Disord.* (2008) 38:342–52. doi: 10.1007/s10803-007-0401-5

41. DeMand A, Johnson C, Foldes E. Psychometric properties of the brief autism mealtime behaviors inventory. *J Autism Dev Disord.* (2015) 45:2667–73. doi: 10.1007/s10803-015-2435-4

42. Must A, Eliasziw M, Phillips SM, Curtin C, Kral TVE, Segal M, et al. The effect of age on the prevalence of obesity among US youth with autism spectrum disorder. *Childh Obes.* (2016) 13:25–35. doi: 10.1089/chi.2016.0079

43. MacDonald M, Esposito P, Ulrich D. The physical activity patterns of children with autism. *BMC Res Notes* (2011) 4:422. doi: 10.1186/1756-0500-4-422

44. Finlay J, Bejerot S, Hanley M. Motor deficits in children with autism spectrum disorder: a cross-syndrome study. *Autism Res.* (2014) 7:664–76. doi: 10.1002/aur.1408

45. Wiggs L, Stores G. Sleep patterns and sleep disorders in children with autistic spectrum disorders: insights using parent report and actigraphy. *Dev Med Child Neurol.* (2004) 46:372–80. doi: 10.1017/S0012162204000611

46. Mazurek MO, Petroski GF. Sleep problems in children with autism spectrum disorder: examining the contributions of sensory over-responsivity and anxiety. *Sleep Med.* (2015) 16:270–9. doi: 10.1016/j.sleep.2014.11.006

47. Steinbeck KS. The importance of physical activity in the prevention of overweight and obesity in childhood: a review and an opinion. *Obes Rev.* (2001) 2:117–30. doi: 10.1046/j.1467-789x.2001.00033.x

Autistic Symptoms in Schizophrenia Spectrum Disorders: A Systematic Review and Meta-Analysis

Franco De Crescenzo [1,2,3†], Valentina Postorino [4,5†], Martina Siracusano [6,7], Assia Riccioni [8], Marco Armando [9], Paolo Curatolo [8] and Luigi Mazzone [8*]

[1] Department of Psychiatry, University of Oxford, Oxford, United Kingdom, [2] Pediatric University Hospital-Department, Bambino Gesù Children's Hospital, Rome, Italy, [3] Department of Epidemiology, Lazio Regional Health Service, Rome, Italy, [4] Department of Pediatrics, School of Medicine, University of Colorado Anschutz Medical Campus, JFK, Aurora, CO, United States, [5] Brain and Body Integration - Mental Health Clinic, Denver, CO, United States, [6] Department of Biomedicine and Prevention, University of Rome Tor Vergata, Rome, Italy, [7] Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy, [8] Child Neurology and Psychiatry Unit, System Medicine Department, University of Rome Tor Vergata, Rome, Italy, [9] Developmental Imaging and Psychopathology Lab, Department of Psychiatry, School of Medicine, University of Geneva, Geneva, Switzerland

Background: Recent studies have examined the association between autism spectrum disorder and schizophrenia spectrum disorders, describing a number of cognitive features common to both conditions (e.g., weak central coherence, difficulties in set-shifting, impairment in theory of mind). Several studies have reported high levels of autistic symptoms in population with schizophrenia spectrum disorders. Our study systematically reviews and quantitatively synthetizes the current evidence on the presence of autistic symptoms in individuals with schizophrenia spectrum disorders.

Methods: A comprehensive literature search of the PubMed/MEDLINE, Cochrane Library, CINHAL, and Embase databases was performed from the date of their inceptions until March 2018. The primary outcome measure was the Autism Spectrum Quotient (AQ). As secondary outcome measures, we analyzed the AQ subscales. Data were extracted and analyzed by using a conservative model and expressed by standardized mean difference (SMD).

Results: Thirteen studies comprising a total of 1,958 individuals were included in the analysis. Results showed that individuals with schizophrenia spectrum disorders have higher levels of autistic symptoms compared to healthy controls [SMD: 1.39, 95% confidence interval (CI): 1.11 to 1.68] and lower levels of autistic symptoms compared to individuals with autism (SMD: −1.27, 95% CI: −1.77 to −0.76).

Conclusions: Current findings support that individuals with schizophrenia spectrum disorders have higher autistic symptoms than healthy controls. Therefore, further studies are needed in order to shed light on the association between these two conditions.

Keywords: schizophrenia, autism, development, comorbidity, meta-analysis

INTRODUCTION

According to the current diagnostic classification systems, Schizophrenia Spectrum Disorders (SSDs) include schizophrenia, schizophreniform disorder, and other psychotic disorders (1). These disorders are defined by abnormalities in one or more of the following five domains: delusions, hallucinations, disorganized thinking (speech), grossly disorganized or abnormal motor behavior (including catatonia), and negative symptoms. The onset of schizophrenia spectrum disorders is usually between late teens and mid-30s. Onset prior to adolescence is rare (2).

By contrast, Autism Spectrum Disorder (ASD) is an early onset lifelong condition characterized by persistent deficits in social communication, as well as restricted and repetitive patterns of behavior (3). Until a century ago, autism was considered as an early expression of schizophrenia. Later on, different studies have demonstrated that these conditions are separate (3–5). Despite their differences, SSDs and ASD appear to show several similar symptoms and research into the possible link between these disorders has grown considerably with studies showing shared genetic risk factors as well as potential links in specific clinical characteristics of these two disorders [for a systematic review see Kincaid et al. (6)]. Recent studies have shown a genetic overlap between ASD and SSDs (7). For example, studies assessing copy number variation (CNV) in ASD and SSDs have repeatedly observed heterozygous deletions eliminating exons of the neurexin-1α gene (but not the neurexin-1β gene) in patients with ASD and SSDs (8–10). Furthermore, several studies have reported high number of shared CNV deletions and duplications, including 1q21, 15q11.2, 15q13.3, 16p11.2, 22q12, and Neurexin 1 loci, in ASD and SSDs (8, 11).

Moreover, an overlap between early autistic symptoms and psychotic experiences during adolescence was reported in longitudinal studies reporting that 20–50% of individuals with childhood-onset schizophrenia met criteria for premorbid ASD (12–16). In addition, social difficulties and language impairment are common to both conditions. Specifically, deficits in reciprocal social interactions are considered part of the core clinical symptoms of ASD. In fact, individuals with ASD exhibit deficits in eye contact, non-verbal communication (e.g., descriptive, conventional, and emphatic gestures), and difficulties to develop age-appropriate relationships (17). Similarly, social withdrawal is documented in individuals with SSDs. Indeed, impairment in social functioning common to both conditions may be due to underlying mechanisms (e.g., deficits in theory of mind) that are common to both conditions (18–24). Studies investigating social functioning deficits in these conditions have shown contrasting findings. For example, Couture et al. (23) completed a battery of social cognitive measures in 44 individuals with schizophrenia, 36 individuals with high functioning autism, and 41 non-clinical controls and reported that individuals with schizophrenia and individual with high functioning autism were both impaired on a variety of social cognitive tasks. By contrast, Sasson et al. (24) comparing the visual scanning patterns and emotion judgments of individuals with autism, individuals with schizophrenia, and controls, suggested that both individuals with autism and individuals with schizophrenia fixate faces less than controls. However, their results also found that only individuals with autism fail to orient to faces more rapidly based on the presence of facial information (24).

In clinical practice, distinguishing between these conditions has proved to be challenging given the symptom overlap between ASD and SSDs. For example, social communication deficits and restricted and repetitive behaviors typical of ASD can be misinterpreted as possible signs of a SSD (25). Some perceptions reported by individuals with ASD are misinterpreted as hallucinations (26). Deficits in emotion recognition leading to misinterpretations of the actions of others is a core symptom of ASD and is also common in SSDs (26). Moreover, difficulties with emotional reciprocity or speech delay in ASD can be misinterpreted as blunted affect or alogia (poverty of speech) in SSDs (26). Furthermore, catatonic features are present in both disorders (26, 27). Previous systematic reviews suggested elevated rates of co-occurrence of ASD and SSDs (28, 29). For example, Kincaid et al. (6) in a recent systematic review reported a prevalence rate of ASD that ranged from <1 to 52% across outpatient and inpatient populations with a diagnosis of schizophrenia or other psychotic disorders. However, considering ASD as dimensional disorder, separate from the issue of ASD diagnosis is the matter of autistic traits. Autistic traits refer to symptoms that are typical of ASD at the time of the assessment; however, these symptoms are generally not reported during childhood, which is essential for an ASD diagnosis (6).

Studying autistic symptoms in individuals with SSDs can give further insight to understand the overlap and distinction between these conditions, which can have important diagnostic and treatment implications (22). For example, previous studies reported that individuals with SSDs with autistic features had a longer duration of illness compared to individuals with SSDs without autistic symptoms (30, 31). Moreover, these studies also suggest that a longer duration of illness is associated with poorer long-term outcomes and a higher symptom severity in this clinical population (32). Therefore, an early screening of autistic symptoms in individuals with SSDs might be able to inform both psychological and pharmacological treatments, and possibly modify the clinical outcome in this clinical population (6). Kincaid et al. (6) systematic review reported prevalence rates of autistic symptoms across outpatient and inpatient populations with a diagnosis of schizophrenia or other psychotic disorders ranging from 9.6 to 61%. However, to our knowledge, no previous meta-analysis was conducted in order to quantify the presence of autistic symptoms in SSD populations. Therefore, the aim of this systematic review and meta-analysis was to systematically review and quantitatively synthetize the current evidence on the presence of autistic symptoms in individuals with SSDs compared to healthy controls and individuals with autism.

METHODS

Literature Search

The electronic databases of PubMed, Medline, CINAHL, ISI web of knowledge were searched up from the date of their

FIGURE 1 | PRISMA flow diagram.
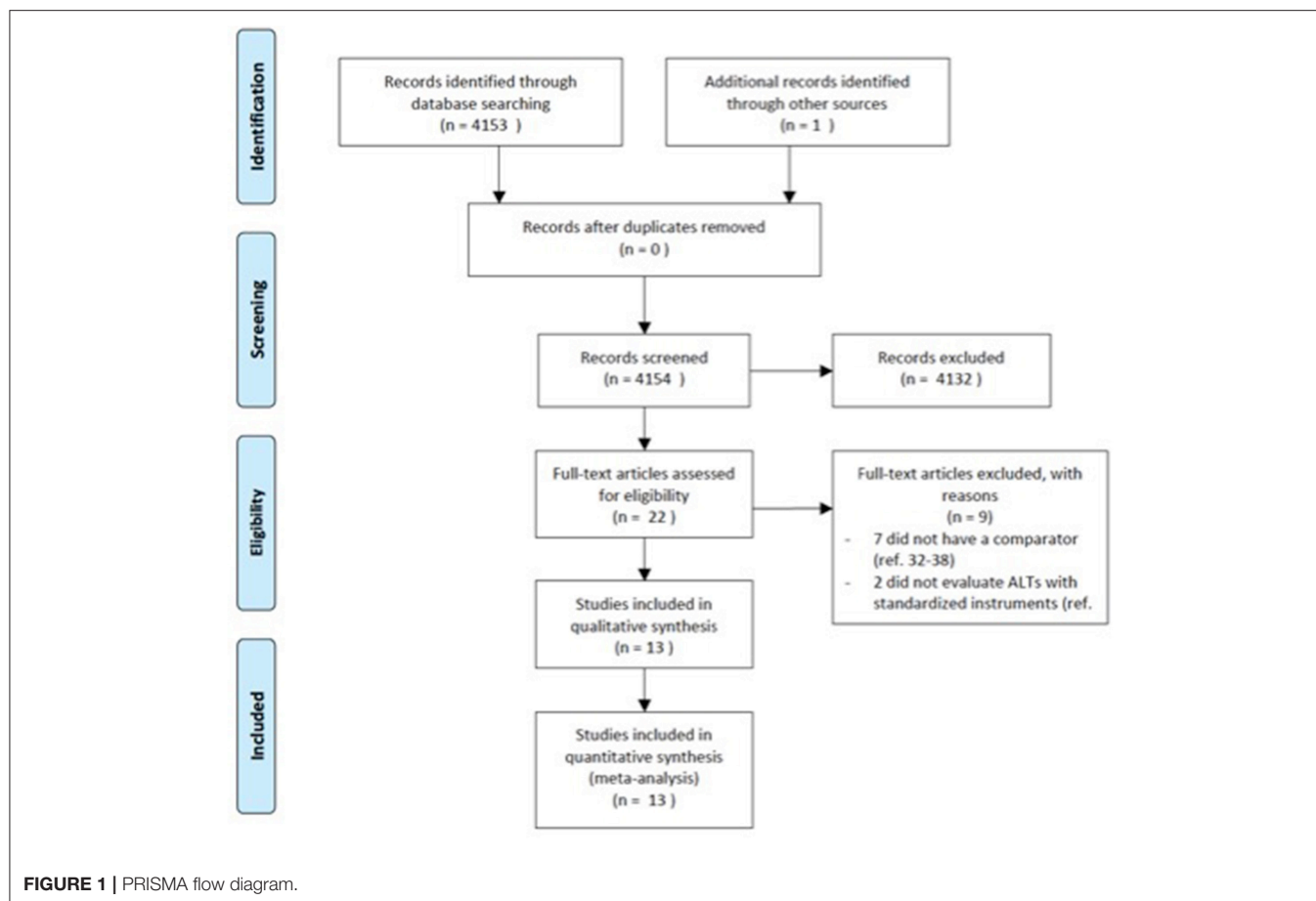
inceptions until March 2018. We used a search algorithm based on a combination of the terms: autist*, "autistic disorder," "autism," "child development disorders, pervasive," "Asperger syndrome," and "schizophrenia," "schizophrenia spectrum and other psychotic disorders," "schizophrenia, paranoid," "schizophrenia, disorganized," "schizophrenia, childhood," "schizotypal personality disorder." Reference lists of eligible papers were also screened for relevant studies. No language limit was used. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline (33) (**Figure 1**).

Study Selection

All studies using recognized assessment scales to measure autistic symptoms in individuals with SSDs, compared to healthy controls or autism, were eligible for inclusion. We included study-defined diagnosis of first-episode psychosis or SSDs [i.e., schizophrenia, psychotic disorder not otherwise specified (NOS), schizoaffective disorder, schizophreniform disorder, delusional disorder]. The exclusion criteria were: (a) articles not within the field of interest of this review; (b) review articles, editorials, or letters, comments, conference proceedings; (c) case reports or case series; (d) studies dated before 1990 if the system used for the diagnosis of schizophrenia did not use operationalized

criteria, but only disease names with no diagnostic criteria (i.e., ICD-9); (e) We excluded mood disorders with psychotic features (e.g., major depression with psychotic symptoms, bipolar disorder with psychotic symptoms). Two researchers (MS, VP) independently identified potential titles from all databases and screened the abstracts for relevance. Full-texts were then retrieved and read to determine eligibility. Disagreements were resolved by consensus.

Data Extraction

For each included study, the same two reviewers independently documented information about the publication (i.e., author's names, journal, year of publication, setting), patients' and comparison's characteristics (i.e., gender, age, diagnostic criteria, outcomes). We assessed the quality and potential sources of bias for each study by using the Newcastle Ottawa scale (NOS) (34) (**Table 1**).

Outcome Measures

The Autism Questionnaire (AQ), expressed as continuous variable was used as the primary outcome measure. AQ subscales were used as secondary outcome measures. The AQ is a 50-item questionnaire consisting of five different areas: social skill, attention switching, attention to detail, communication, and imagination (47). Whenever the AQ was not measured or

TABLE 1 | Risk of bias table for assessing the quality of cohort studies by using the Newcastle-Ottawa Scale.

Study	Representativenes of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cohorts on the basis of the gender	Comparability of cohorts on the basis of the age	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow up of cohorts
Konstantareas et al. (35)	*	*	*		*	*	*	*	*
Naito et al. (36)	*	*	*		*			*	*
Sasamoto et al. (37)	*	*	*		*	*		*	*
Solomon et al. (18)	*	*	*		*	*			*
Guo et al. (38)	*	*	*						*
Jalbrzikowski et al. (39)	*	*	*		*	*			*
Lugnegard et al. (40)	*	*	*		*			*	*
de Bildt et al. (41)	*	*	*		*	*	*	*	*
Matsuo et al. (42)	*	*	*		*			*	*
Martinez et al. (43)	*	*	*		*	*		*	*
Zhang et al. (44)	*	*	*		*	*		*	*
Ota et al. (45)	*		*					*	*
Upthegrove et al. (46)			*		*				*

*item present. i.e., low risk of bias for for the variable considered.

reported, the following scales measuring symptoms of autism were used: the Social Responsiveness Scale (SRS) (48), the Autism Diagnostic Observation Schedule-Second Edition (ADOS-2) (49, 50), the Autism Spectrum Screening Questionnaire (ASSQ) (51), and the Childhood Autism Rating Scale (CARS) (52, 53).

The SRS is a 65-item rating scale focusing on social awareness and avoidance (54, 55).

The ADOS-2 is a semi-structured, standardized assessment of communication, social interaction, play, and repetitive behaviors. The ADOS-2 includes five modules, The choice of the module is based on the level of expressive language and chronological age of the individual being evaluated (i.e., Module Toddler: from 12 to 30 months of age who do not consistently use phrase speech; Module 1: children 31 months and older who do not consistently use phrase speech; Module 2: children of any age who use phrase speech, but are not verbally fluent; Module 3: verbally fluent children or adolescents; Module 4: verbally fluent older adolescents and adults) (49, 50).

The ASSQ is a teacher's or parent's rating scale that investigates four main fields: social difficulties, tic/motor/obsessive-compulsive disorders, and autistic style (51).

The CARS is a clinician-rated observation scale evaluating socialization, communication, emotional responses, and sensory sensitivities. This scale has shown good reliability, a high degree of correlation with the Diagnostic and Statistical Manual of Mental Disorders (DSM)- Fourth Edition-Text Revised criteria, and a good agreement with diagnoses made using the ADOS-2 and the Autism Diagnostic Interview-Revised (ADI-R) (56).

Data Analysis

We synthesized study included in the current review consistently with meta-analytic recommendations (57). Data analysis were performed using STATA (version 13.1). data analysis involved the following steps: (a) calculating standardized mean difference (SMD) for each comparison with confidence intervals (CI) (95%); (b) determining an overall average SMD; (c) estimating heterogeneity. We considered SMD "small" if <0.40, "moderate" from 0.40 to 0.70, and "large" if >0.7 (58). The combined use of slightly different scales suggested application of the random effects model, which is more conservative than the fixed-effects model. Qualitative data were presented descriptively. The I^2 statistic was used to assess the heterogeneity of effect sizes (57). The I^2 statistic rages from 0 to 100 and measures the percent of variation across effect sizes due to heterogeneity compared to chance. A high I^2 index indicates greater heterogeneity and greater variation in effect size across studies. We used I^2 thresholds of 25, 50, and 75% to differentiate low, moderate and high heterogeneity. We also undertook subgroup analyses for studies on adults, =studies on children, studies on adolescents, studies including only patients diagnosed with schizophrenia, studies including also individuals diagnosed with SSDs, and studies including also individuals with an unspecified psychotic episode.

RESULTS

Selected Studies

The literature search generated 4,153 articles. 4,131 articles were excluded due to the fact that they did not meet the inclusion criteria. Twenty-two articles were screened for eligibility by full-text review. Of these, seven did not have a comparator group (32, 59–64) and two did not evaluate autistic symptoms with standardized instruments (65, 66). A total of 13 studies comprising 1,958 individuals were included in the current meta-analysis.

Study Characteristics

The characteristics of the included studies are presented in **Table 2**. The study population mean age was 25.4 years. Three studies included only children and adolescents (18, 38, 39). Four studies were undertaken in Japan (36, 37, 42, 45), four in Europe (35, 40, 41, 46), three in the U.S. (18, 39, 43), and two in China (38, 44). Most of the studies included a high percentage of males, with the exception of three studies (40, 42, 45). Most of the studies included patients with a diagnosis of schizophrenia. Nine studies included a healthy control group, one study included first degree relatives as control group (41), and eight studies included a group with a diagnosis of autism. Seven studies assessed the presence of autistic symptoms in other clinical groups (i.e., psychopathy, attention-deficit/hyperactivity disorder, ultra-high risk (UHR) to develop psychosis, first episode psychosis, obsessive compulsive disorder, major depressive disorder, bipolar disorder) (18, 38, 39, 41, 42, 44, 46). To support the diagnosis, one study used the DSM-Third Edition-Text Revised criteria (35), one study used the DSM-Fourth Edition criteria (38), four studies used the DSM-Fourth Edition-Text Revised criteria (18, 36, 39, 44), and one study used the DSM-Fifth Edition criteria in one study (45). The quality assessment is described in **Table 1**. Of note, we used self-reported scales which may introduce a bias for the assessment of the outcome.

Meta-Analysis

The results of the pairwise meta-analysis for individuals with SSDs vs. healthy controls and individuals with autism compared for the presence of autistic symptoms in our study are presented in **Table 3** (See Forest Plot, **Figure 2**).

Individuals with SSDs have significantly higher autistic symptoms than healthy controls (SMD: 1.39; 95% CI: 1.11 to 1.68) and lower autistic symptoms than individuals with autism (SMD: −1.27; 95% CI: −1.77 to −0.76). Individuals with SSDs have significantly higher autistic symptoms than healthy controls on the AQ total (SMD: 1.68; 95% CI: 0.84 to 2.51), the AQ Social subscale (SMD: 1.24; 95% CI: 0.94 to 1.54), the AQ Attention Switching subscale (SMD: 0.75; 95% CI: 0.03 to 1.47), the AQ Communication subscale (SMD: 1.38; 95% CI: 0.99 to 1.77), and the AQ Imagination subscale (SMD: 0.96; 95% CI: 0.67 to 1.25). However, no significant difference was found between individuals with SSDs and healthy controls on the AQ Attention to Details subscale (SMD: 0.27; 95% CI: −0.01 to 0.54).

TABLE 2 | Included studies.

Studies	Year	Setting	Participants (n)	Diagnostic criteria	Mean Age (SD)	Gender (% males)	Outcomes	Outcome Measures Total Score Mean (SD)
Konstantareas et al. (35)	2001	Continuing care program of a psychiatric research institute	Autism (14) Schizophrenia (14)	DSM-III-; Leiter; SANS; SAPS; SCID.	25.3 (4.4) 25.3 (4.4)	100 100	CARS	35.36 (4.4) 19.36 (2.1)
Naito et al. (36)	2010	Kobe university hospital Kansai-Seishonen Sanatorium	Autism (51) Schizophrenia (46)	DSM-IV-TR; S-scale; WAIS-R; WAIS-III.	28.8 (9.4) 34.1 (9.6)	78 50	AQ Adult Japanese Version	32.6 (6.8) 21.8 (7.4)
Sasamoto et al. (37)	2011	Department of Neuropsychiatry, Kyoto University Hospital	Schizophrenia (20) Healthy controls (25)	JART; PANSS; SCID-I/P (version 2.0); WAIS–R (vocabulary and block design).	34.5 (8.8) 34.5 (9.4)	70 64	AQ Adult Japanese Version	25.35 (6.6) 14.48 (6.9)
Solomon et al. (18)	2011	UC Davis EDAPT clinic	Clinical High risk for psychosis (met criteria for the attenuated positive symptom risk state) (15) First episode schizophrenia (16) Autism (20) Healthy controls (20)	ADOS-G mod 3, 4; CCC-2; DSM-IV-TR; SCID-I/P; SCQ; SIPS; WASI.	14.1 (2.2) 17.0 (1.8) 15.1 (2.2) 14.8 (2.1)	66 75 80 65	SRS	(13.9) 72.5 (20.7) 79.55 (11.8) 42.8 (9.3)
Guo et al. (38)	2011	Institute of mental health Peking University	Autism (94) ADHD (45) Childhood onset schizophrenia (26) Healthy controls (120)	DSM-IV	6.7 (3.9) 8.8 (2.2) 13.8 (3) 6.0 (1.3)	Not Reported	CH-ASSQ Mandarine Chinese Version	25.3 (9.2) 10.4 (7.1) 12.2 (10.6) 25.3 (9.2)
Jalbrzikowski et al. (39)	2013	CAPPS or the adolescent brain and behavior reaserch center	Youths at clinical high risk (58) Adolescents with a psychotic disorder (20) Healthy controls (36)	DSM-IV-TR; K-SADS; SCID-I/P; SIPS; WASI.	15.5 (1.9) 15.7 (1.6) 15.0 (1.5)	64 45 50	GFS; GFR; aSRS	67.2 (15) 70.7 (12.2) 47.8 (11.4)
Lugnegard et al. (40)	2014	Department of Psychiatry Central Hospital Karlstad Sweden	Asperger Syndrome (51) Schizophrenic psychosis (schizophrenia, schizoaffective disorder, schizophreniform disorder) (36) Healthy controls (49)	DISCO-11; SCID-I; WAIS-III (vocabulary)	27.1 (4.1) 29.1 (4.2) 28.6 (9.2)	47 63 38	AQ Adult Version	26.7 (8.9) 22.7 (6.2) 13.4 (6.4)
de Bildt et al. (41)	2015	University Medical Center Groningen	ASD-high functioning (38) Schizophrenia (18) Psychopathy (16) Controls (first degree relatives) (21)	DSM-IV-TR	31.8 (11.2) 37.0 (10.7) 39.0 (10.6) 34.2 (9.1)	100 100 100 100	ADOS-2 module 4 revised algorithm (Hus and Lord 2014)	10.37 (5.7) 7.28 (4.1) 3.31 (2) 2.67 (2.3)

(Continued)

TABLE 2 | Continued

Studies	Year	Setting	Participants (n)	Diagnostic criteria	Mean Age (SD)	Gender (% males)	Outcomes	Outcome Measures Total Score Mean (SD)
Matsuo et al. (42)	2015	National Center of Neurology and Psychiatry Hospital, Tokyo	Schizophrenia (44) Major depressive disorder (125) Bipolar disorder (56) Healthy controls (65)	DSM-IV-TR; HDRS-17; Japanese version of Mini International Neuropsychiatric Interview; PANSS; WAIS-III; YMRS.	36.9 (7.5) 41.5 (9.2) 40.4 (7.8) 42.2 (8.2)	46 56 46 28	SRS for Adults	59.6 (25) 48.7 (25) 55.4 (25.8) 32.5 (19.1)
Martinez et al. (43)	2016	Service Hospitalo-Universitaire in Sainte-Anne Hospital, Paris	Schizophrenia (36) Autism (19) Healthy controls (20)	ADI-R; BPRS-24; DIGS; DSM-IV-TR; MASC-FR; PANSS; TLC; WAIS III.	23.4 (3.5) 22.7 (4.1) 23.4 (3.6)	83 78 85	AQ	20.9 (7.5) 30.2 (8.3) 14.1 (10.1)
Zhang et al. (44)	2016	Mental Health Center of Anhui Province	Autism (32) Schizophrenia (37) Obsessive-compulsive disorder (38) Healthy controls (38)	DSM IV-TR; Raven test.	19.4 (3.8) 20.9 (3.6) 21.2 (3.1) 21.3 (3.3)	81 81 81 78	AQ Mandarin Chinese Version	133.4 (10.1) 120.5 (6.8) 118.3 (8.3) 103.5 (8.5)
Ota et al. (45)	2017	National Center of Neurology and Psychiatry Hospital, Tokyo	Schizophrenia (37) Healthy controls (62)	DSM-5; Japanese Mini-International Neuropsychiatric Interview; PANSS.	36.2 (9.5) 40.6 (13.)	45 27	SRS for Adults	60.6 (24) 32.2 (16)
Upthegrove et al. (46)	2017	Birmingham Early Intervention Services	First episode psychosis (99) Healthy controls (381)	AQ; BHS; CAPEp; CESD-R; PANSS; SBQ-R.	25.6 (5.0) 20.6 (3.0)	67 21	AQ	20.76 (8) 15.32 (6)

ADOS-G, Autism Diagnostic Observation Schedule, Generic; ADOS-2, Autism Diagnostic Observation Schedule, Second Edition; ADI-R, Autism Diagnostic Interview – Revised; AQ, Autism-Spectrum Quotient; BHS, Beck Hopelessness Scale; BPRS, Brief Psychiatric Rating Scale; CAPEp, Community Assessment of Psychotic Experiences; CARS, Childhood Autism rating Scale; CCC-2, Children's Communication Checklist-2; CESD-R, The Center for Epidemiologic Studies Depression Scale-Revised; CH-ASSQ, Autism Spectrum Screening Questionnaire; DIGS, Diagnosis interview for Genetic Studies ; DISCO 11, The diagnostic interview for Social and Communication Disorders version 11; DSM III-R, Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revision; DSM-IV-TR, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; JART, Japanese version of the National Adult Reading Test; K-SADS, Kiddie Schedule for Affective Disorders and Schizophrenia; HDRS-17, Hamilton Depression Rating Scale; LEITER, Leiter International Performance Scale; MASC-FR, Movie for the Assessment of Social Cognition; PANSS, Positive and Negative Syndrome Scale; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; SBQ-R, The Suicide Behaviours Questionnaire-Revised; S-scale, Additional questions about psychotic symptoms; SCID-I/P, Structured Clinical Interview for DSM-IV-TR; SCQ, Social Communication Questionnaire; SIPS, Structured Interview for Prodromal Symptoms; SRS, Social Responsiveness Scale; TLC, Thought Language and Communication Scale; WAIS, Wechsler Adult Intelligence Scale; WASI, Wechsler Abbreviated Scale of Intelligence; YMRS, YoungMania Rating Scale.

Individuals with SSDs have significantly lower autistic symptoms than individuals with autism on the AQ total (SMD: −1.17; 95% CI: −1.69 to−0.65) and on all the AQ subscales (see **Table 3**).

We undertook subgroup analyses on studies on children, on studies on adolescents, and on studies reporting a diagnosis of SSD, and we found that results did not substantially change (see **Table 3**).

DISCUSSION

The aim of this meta-analysis was to provide a synthesis of existing literature on the presence of autistic symptoms in individuals with SSDs. The meta-analysis results show that individuals with SSDs have significantly higher autistic symptoms than healthy controls and lower autistic symptoms than individuals with autism. The results of this meta-analysis

TABLE 3 | Data analysis results.

Comparison	Outcome	Studies	SMD	95% CI	z-score	p-value	I²
Schizophrenia vs. healthy controls							
	all scales	11	1.39	1.11 to 1.68	9.50	0.000	72.9%
	AQ total	5	1.68	0.84 to 2.51	3.93	0.000	93.0%
	AQ social	3	1.24	0.94 to 1.54	8.08	0.000	0.0%
	AQ switching	3	0.75	0.03 to 1.47	2.04	0.042	83.4%
	AQ attention to details	3	0.27	−0.01 to 0.54	1.90	0.057	0.0%
	AQ communication	3	1.38	0.99 to 1.77	7.01	0.000	35.2%
	AQ immagination	3	0.96	0.67 to 1.25	6.45	0.000	0.0%
Schizophrenia vs. autism							
	all scales	8	−1.27	−1.77 to−0.76	4.95	0.000	84.0%
	AQ total	4	−1.17	−1.69 to−0.65	4.43	0.000	76.9%
	AQ social	3	−0.66	−1.09 to−0.23	3.05	0.002	64.2%
	AQ switching	3	−0.87	−1.13 to−0.61	6.59	0.000	0.0%
	AQ attention to details	3	−0.59	−0.84 to−0.34	4.59	0.000	0.0%
	AQ communication	3	−0.70	−1.38 to−0.02	2.02	0.043	85.4%
	AQ immagination	3	−0.56	−1.07 to−0.06	2.02	0.028	74.5%
SUBGROUP ANALYSES							
Schizophrenia vs. healthy controls							
	Age > 18 y, all scales	8	1.35	1.02 to 1.68	8.05	0.000	74.3%
	Age < 18 y, all scales	3	1.56	0.81 to 2.31	4.09	0.000	76.9%
	Only schizophrenia, all scales	8	1.27	0.96 to 1.59	7.84	0.000	73.4%
	Schizophrenia spectrum, all scales	3	1.70	1.35 to 2.05	9.48	0.000	0.0%
Schizophrenia vs. autism							
	Age > 18 y, all scales	5	−1.06	−1.52 to −0.60	4.54	0.000	75.1%
	Age < 18 y, all scales	3	−1.96	−3.54 to −0.38	2.44	0.015	92.5%
	Only schizophrenia, all scales	1	−2.80	−3.36 to −2.24	9.75	0.000	0.0%
	Schizophrenia spectrum, all scales	2	−0.48	−0.85 to −0.12	2.61	0.009	0.0%

support a shared symptomatology between these conditions. For example, language deficits often found in individuals with autism are also frequently found in prodromal symptoms of SSDs and were highlighted by the difference on the AQ Communication subscale between individuals with SSDs and healthy controls (SMD: 1.38) (67). Moreover, tangential thought, formal language, and focus on favorite subjects are often present in individuals with autism, and these language symptoms are similar to formal thought disorder which is characterized by disorganized speech (22). Furthermore, individuals with autism may present with language deficits characterized by a lack of verbal initiation and poverty of content similar to individuals with schizophrenia and negative symptoms (22). Likewise, social deficits seem to be present in both conditions (18, 22). For example, social isolation and difficulties to maintain age-appropriate peer relationships are observed in individuals with autism as well as in individuals with schizophrenia. Therefore, it is not surprising that the AQ Communication subscale, which measure the communication skills, and the AQ Socialization subscale, which measure the socialization ability, reported that highest scores in this group. On the other hand, it has to be noted that the level of autistic symptoms changes among the AQ subscales. Indeed, the difference in the AQ sub-scale scores between individuals with SSDs and healthy controls showed

higher levels of autistic symptoms in individuals with SSDs on the AQ Communication subscale (SMD: 1.38), on the AQ Social subscale (SMD: 1.24), the AQ Imagination subscale (SMD: 0.96), and the AQ Attention Switching subscale (SMD: 0.75). Notably, no significant difference was found between individuals with SSDs and healthy controls on the AQ Attention to Details subscale (SMD: 0.27), thus suggesting that in this clinical population the difficulties on some autistic symptoms are not as severe. In general, in clinical practice it is difficult to discern between positive symptoms of SSDs and autism symptoms. For example, sensory issues present in individuals with autism may be misdiagnosed as hallucinations in schizophrenia with significant treatment implications. Similarly, it can be also difficult to discern between negative symptoms of schizophrenia and autism symptoms. For example, the lack of emotional reciprocity that is present in individuals with autism resemble the blunt affect or alogia (i.e., poverty of speech) in schizophrenia. Therefore, it is possible that individuals with SSDs develop autistic-like symptoms as a result of their negative symptoms. Our results are consistent with previous research in this area reporting that autistic symptoms seem to be prevalent in SSDs, ranging from 9.6 to 61% (6).

However, the findings of this meta-analysis should be interpreted with some caution. Limitations include the paucity of
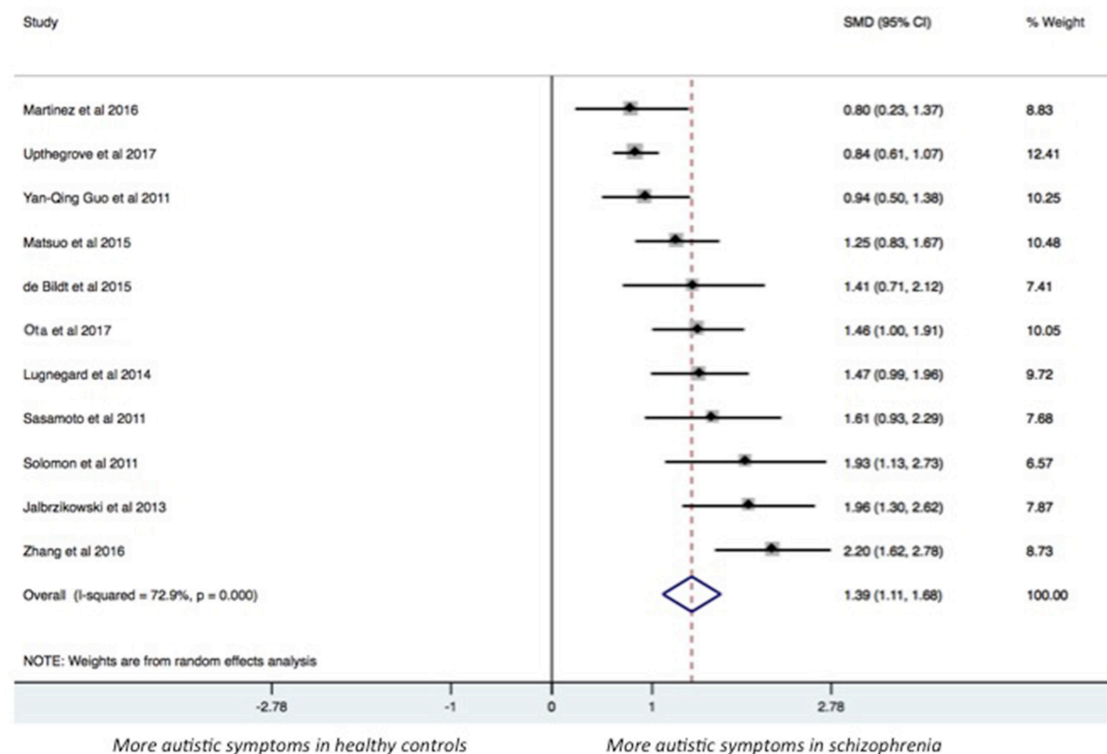
FIGURE 2 | Forest plot.

studies analyzed, different diagnostic criteria and measures, and differences in the age of the samples.

In our meta-analysis, we included only 13 studies. The pool of 13 studies was small and did not allow meta-regression analyses to examine how participants characteristics (e.g., gender, age, illness duration) can predict autistic symptoms. However, the total number of participants included in the current meta-analysis is large ($n = 1,958$) and the results are strong, with high SMDs, and precise 95%CIs.

Furthermore, we found studies with different diagnostic criteria, which is a reason for increased heterogeneity. However, it has to be noted that all the diagnostic criteria used by studies included on the current meta-analysis were well-validated and all studies used either a DSM based diagnosis, or the Structured Interview for Prodromal Symptoms (SIPS), or the Positive and Negative Syndrome Scale (PANSS) for the diagnosis of SSDs. Indeed, the AQ is a self-report scale, which may introduce reporting bias. Moreover, this measure was not always available, and we needed to use other instruments measuring autistic symptoms. Therefore, given the different scales used in the current meta-analysis, we analyzed data by SMDs as opposed to mean differences.

Three studies included only children and adolescents (18, 38, 39), which may differ from adults in terms of diagnosis stability and autistic symptoms. Therefore, we decided to undertake subgroup analyses taking into account the age. ASD by definition

is a disorder present from early childhood. It is important to consider the individual's developmental history in order to distinguish between the presence of an ASD and autistic symptoms. However, it is still unclear whether difficulties such as those present in individuals with autism are present before the onset of schizophrenia or whether they are dependent by the schizophrenic state itself (e.g., resulting from thought dysfunction), thus not truly autistic.

Further studies investigating the etiology of autism symptoms in children and adolescents with SSDs are needed in order to shed light on this issue. Furthermore, longitudinal studies investigating autistic symptoms in high-ultra risk state populations can clarify whether these symptoms are stable features of this clinical population or are dependent by the schizophrenic state of illness. Indeed, it has to be noted that in the present meta-analysis only one study (41) used a diagnostic tool for autism (i.e., ADOS-2). All other studies used instruments that were designed as screening measures for autism. Therefore, in order to distinguish between trait and state, future studies would benefit by the use of diagnostic measures (e.g., ADOS-2) along with developmental history in order to confirm whether any deficit associated with autism was present before the onset of SSDs. Indeed, it is well-known that individual with autism are at an increased risk to develop other mental-health conditions and a ASD diagnosis is particularly difficult in adults, especially when knowledge of early developmental history is missing.

Therefore, longitudinal studies investigating autistic symptoms in individuals with SSDs in different psychopathological phases could clarify whether these characteristics persist after recovery from SSDs.

CONCLUSION

In conclusion, we found that individuals with SSDs have higher autistic symptoms than healthy controls and lower autistic symptoms than individuals with autism. To our knowledge, this is the first systematic review and meta-analysis trying to quantitatively pool all evidences on the topic. Our study has some limitations, including the use of self-report scales, which may introduce reporting bias. Therefore, further studies investigating the etiology of autism symptoms are needed to shed light on the association between these conditions.

AUTHOR CONTRIBUTIONS

MS and VP overviewed and examined the literature. FD performed the statistical analysis. FD, VP, AR, and MS wrote the manuscript. LM, MA, and PC designed the study, contributed to theoretical interpretation read and final proof reading. Each author read and approved the final version of the manuscript.

REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders-5*. Washington, DC: American Psychiatric Association (2013).
2. Miettunen J, Immonen J, McGrath J, Isohanni M, Jääskeläinen E. The age of onset of schizophrenia spectrum disorders. *Schizophr Bull.* (2018) 44(Suppl.1):S270. doi: 10.1093/schbul/sby017.659
3. Bender L. Childhood schizophrenia; clinical study on one hundred schizophrenic children. *Am J Orthopsychiatry* (1947) 17:40–56.
4. Kolvin I. Studies in the childhood psychoses: diagnostic criteria and classification. *Br J Psychiatry* (1971) 118:381–4.
5. Rutter M. Childhood schizophrenia reconsidered. *J Autism Dev Disord.* (1972) 2:315–37.
6. Kincaid DL, Doris M, Shannon C, Mulholland C. What is the prevalence of autism spectrum disorder and ASD traits in psychosis? A systematic review. *Psychiatry Res.* (2017) 250:99–105. doi: 10.1016/j.psychres.2017.01.017
7. Gandal MJ, Haney JR, Parikshak NN, Leppa V, Ramaswami G, Hartl C et al. Shared molecular neuropathology across major psychiatric disorders parallels polygenic overlap. *Science* (2018) 359:693–7. doi: 10.1126/science.aad6469
8. Carroll LS, Owen MJ. Genetic overlap between autism, schizophrenia and bipolar disorder. *Genome Med.* (2009) 1:102. doi: 10.1186/gm102
9. Burbach JP, van der Zwaag B. Contact in the genetics of autism and schizophrenia. *Trends Neurosci.* (2009) 32:69–72. doi: 10.1016/j.tins.2008.11.002
10. Reichelt AC, Rodgers RJ, Clapcote SJ The role of neurexins in schizophrenia and autistic spectrum disorder. *Neuropharmacology* (2012) 62:1519–26. doi: 10.1016/j.neuropharm.2011.01.024
11. St Clair D. Copy number variation and schizophrenia. *Schizophr Bull.* (2009) 35:9–12. doi: 10.1093/schbul/sbn147
12. Bevan Jones R, Thapar A, Lewis G, Zammit S. The association between early autistic traits and psychotic experiences in adolescence. *Schizophr Res.* (2012) 135:164–9. doi: 10.1016/j.schres.2011.11.037
13. Sullivan S, Rai D, Golding J, Zammit S, Steer C. The association betweenautism spectrum disorder and psychotic experiences in the Avon longitudinalstudy of parents and children (ALSPAC) birth cohort. *J Am Acad Child Adolesc Psychiatry* (2013) 52: 806–14.e2. doi: 10.1016/j.jaac.2013.05.010
14. Volkmar FR, Cohen DJ. Comorbid association of autism and schizophrenia. *Am J Psychiatry* (1991) 148:1705–7.
15. Leyfer O, Folstein S, Bacalman S, Davis N, Dinh E, Morgan J, et al. Comorbid psychiatric disorders in children with autism: Interview development and rates of disorders. *J Autism Dev Disord.* (2006) 36:849–61. doi: 10.1007/s10803-006-0123-0
16. Rapoport J, Chavez A, Greenstein D, Addington A, Gogtay N. Autism spectrum disorders and childhood-onset schizophrenia: clinical and biological contributions to a relation revisited. *J Am Acad Child Adolesc. Psychiatry* (2009) 48:10–8. doi: 10.1097/CHI.0b013e31818b1c63
17. Losh M, Adolphs R, Poe MD, Couture S, Penn D, Baranek GT et al. Neuropsychological profile of autism and the broad autism phenotype. *Arch Gen Psychiatry* (2009) 66:518–26. doi: 10.1001/archgenpsychiatry.2009.34

18. Solomon M, Olsen E, Niendam T, Ragland JD, Yoon J, Minzenberg M, et al. From lumping to splitting and back again: atypical social and language development in individuals with clinical-high-risk for psychosis, first episode schizophrenia, and autism spectrum disorders. *Schizophr Res.* (2011) 131(1–3): 146–151. doi: 10.1016/j.schres.2011.03.005
19. Larson FV, Wagner AP, Jones PB, Tantam D, Lai MC, Baron-Cohen S, et al. Psychosis in autism: a comparison of the features of both conditions in a dually-affected cohort. *Br J Psychiatry* (2017) 210:269–75. doi: 10.1192/bjp.bp.116.187682
20. Bora E, Yucel M, Pantelis C. Theory of mind impairment in schizophrenia: meta-analysis. *Schizophr Res.* (2009) 109:1–9. doi: 10.1016/j.schres.2008.12.020
21. Sprong M, Becker H, Schothorst P, Swaab, H, Ziermans, T, Dingemans P, et al. Pathways to psychosis: a comparison of the pervasive developmental disorder subtype multiple complex developmental disorder and the "at risk mental state". *Schizophr Res.* (2008) 99:38–47. doi: 10.1016/j.schres.2007.10.031
22. Chisholm K, Lin A, Abu-Akela A, Wood SJ. The association between autism and schizophrenia spectrum disorders: a review of eight alternate models of co-occurrence. *Neurosci Biobehav Rev.* (2015) 55:173–83. doi: 10.1016/j.neubiorev.2015.04.012
23. Couture SM, Penn DL, Losh M, Adolphs R, Hurley R, Piven J. Comparison of social cognitive functioning in schizophrenia and high functioning autism: more convergence than divergence. *Psychol Med.* (2010) 40:569–79. doi: 10.1017/S003329170999078X
24. Sasson N, Tsuchiya N, Hurley R, Couture SM, Penn DL, Adolphs R, et al. Orienting to social stimuli differentiates social cognitive impairment in autism and schizophrenia. *Neuropsychologia* (2007) 45:2580–8. doi: 10.1016/j.neuropsychologia.2007.03.009
25. Cochran DM, Dvir Y, Frazier JA. "Autism-plus" spectrum disorders: intersection with psychosis and the schizophrenia spectrum. *Child Adolesc Psychiatr Clin N Am.* (2013) 22:609–27. doi: 10.1016/j.chc.2013.04.005
26. Chisholm K, Lin A, Armando M. Schizophrenia Spectrum Disorder and ASD. In: Mazzone L, Vitiello B, editors. *The Psychiatric Comorbidities in Autism Spectrum Disorder.* Springer (2016). p. 51–66. doi: 10.1007/978-3-319-29695-1
27. Mazzone L, Postorino V, Valeri G, Vicari S. Catatonia in patients with autism: prevalence and management. *CNS Drugs* (2014) 28:205–15. doi: 10.1007/s40263-014-0143-9
28. Padgett FE, Miltsiou E, Tiffin PA. The co-occurrence of nonaffective psychosis and the pervasive developmental disorders: a systematic review. *J Intellect Dev Disabil.* (2010) 35:187–98. doi: 10.3109/13668250.2010.494596
29. Skokauskas N, Gallagher L. Psychosis, affective disorders and anxiety in autistic spectrum disorder: prevalence and nosological considerations. *Psychopathology* (2010) 43:8–16. doi: 10.1159/000255958
30. Rabinowitz J, Levine SZ, Häfner H. A population based elaboration of the role of age of onset on the course of schizophrenia. *Schizophr Res.* (2006) 88:96–101. doi: 10.1016/j.schres.2006.07.007
31. Carbon M, Correll CU. Clinical predictors of therapeutic response to antipsychotics in schizophrenia. *Dialogues Clin Neurosci.* (2014) 16:505–24. Availabe online at: https://academicworks.medicine.hofstra.edu/articles/978

32. Barlati S, Deste G, Gregorelli M, Vita A. Autistic traits in a sample of adult patients with schizophrenia: prevalence and correlates. *Psychol Med.* (2018) 20:1–9. doi: 10.1017/S0033291718000600

33. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol.* (2009) 62:1006–12. doi: 10.1016/j.jclinepi.2009.06.005

34. Wells, GA, Shea, B, O'Connell, D, et al. *The Newcastle Ottawa scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analysis.* (2011). Available online at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

35. Konstantareas MM, Hewitt T. Autistic disorder and schizophrenia: diagnostic overlaps. *J Autism Dev Disord.* (2001) 31:19–28. doi: 10.1023/A:1005605528309

36. Naito K, Matsui Y, Maeda K, Tanaka K. Evaluation of the validity of the Autism Spectrum Quotient (AQ) in differentiating high-functioning autistic spectrum disorder from schizophrenia. *Kobe J Med Sci.* (2010) 56:E116–24.

37. Sasamoto A, Miyata J, Hirao K, Fujiwara H, Kawada R, Fujimoto S, et al. Social impairment in schizophrenia revealed by Autism-Spectrum Quotient correlated with gray matter reduction. *Soc Neurosci.* (2011) 6:548–58. doi: 10.1080/17470919.2011.575693

38. Guo YQ, Tang Y, Rice C, Lee LC, Wang YF, Cubells JF. Validation of the Autism Spectrum Screening Questionnaire, Mandarin Chinese Version (CH-ASSQ) in Beijing, China. *Autism* (2011) 15:713–27. doi: 10.1177/1362361310396383

39. Jalbrzikowski M, Krasileva KE, Marvin S, Zinberg J, Andaya A, Bachman P, et al. Reciprocal social behavior in youths with psychotic illness and those at clinical high risk. *Dev Psychopathol.* (2013) 25(4 Pt 1):1187–97. doi: 10.1017/S095457941300045X

40. Lugnegård T, Hallerbäck MU, Gillberg C. Asperger syndrome and schizophrenia: overlap of self-reported autistic traits using the Autism-spectrum Quotient (AQ). *Nord J Psychiatry* (2015) 69:268–74. doi: 10.3109/08039488.2014.972452

41. De Bildt A, Sytema S, Meffert H, Bastiaansen JACJ. The Autism Diagnostic Observation Schedule, Module 4: Application of the Revised Algorithms in an Independent, Well-Defined, Dutch Sample (*n* = 93). *J Autism Dev Disord.* (2016) 46:21–30. doi: 10.1007/s10803-015-2532

42. Matsuo J, Kamio Y, Takahashi H, Ota M, Teraishi T, Hori H, et al Autistic-like traits in adult patients with mood disorders and schizophrenia. *PLoS ONE* (2015) 10:e0122711. doi: 10.1371/journal.pone.0122711

43. Martinez G, Alexandre C, Mam-Lam-Fook C, Bendjemaa N, Gaillard R, Garel P et al. Phenotypic continuum between autism and schizophrenia: Evidence from the Movie for the Assessment of Social Cognition (MASC). *Schizophr Res.* (2017) 185:161–166. doi: 10.1016/j.schres.2017.01.012

44. Zhang L, Sun Y, Chen F, Wu D, Tang J, Han X. Psychometric properties of the Autism-Spectrum Quotient in both clinical and non-clinical samples: Chinese version for mainland China. *BMC Psychiatry* (2016) 16:213. doi: 10.1186/s12888-016-0915

45. Ota M, Matsuo J, Sato N, Teraishi T, Hori H, Hattori K, et al. Correlation of reduced social communicational and interactional skills with regional grey matter volumes in schizophrenia patients. *Acta Neuropsychiatr* (2017) 29:374–81. doi: 10.1017/neu.2017.9

46. Upthegrove R, Abu-Akel A, Chisholm K, Lin A, Zahid S, Pelton M, et al. Autism and psychosis: clinical implications for depression and suicide. *Schizophr Res.* (2018) 195:80–85. doi: 10.1016/j.schres.2017.08.028

47. Baron-Cohen S, Wheelwright S, Skinner R, Martin J, Clubley E. The autism-spectrum quotient (AQ): evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians. *J Autism Dev Disord.* (2001) 31:5–17. doi: 10.1023/A:1005653411471

48. Constantino J, Gruber C. *Social Responsiveness Scale.* Los Angeles, CA: Western Psychological Services (2005).

49. Lord C, Luyster RJ, Gotham K, Guthrie W. *Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) Manual (Part II): Toddler Module.* Torrance, CA: Western Psychological Services (2012).

50. Lord C, Rutter M, DiLavore P, Risi S, Gotham K, Bishop SL. *Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) Manual (Part I): Modules 1-4.* Torrance, CA: Western Psychological Services (2012).

51. Ehlers S, Gillberg C, Wing L. A screening questionnaire for Asperger syndrome and other high-functioning autism spectrum disorders in school age children. *J Autism Dev Disord.* (1999) 29:129–41.

52. Garfin DG, McCallon D, Cox R. Validity and reliability of the Childhood Autism Rating Scale with autistic adolescents. *J Autism Dev Disord.* (1988) 18:367–78.

53. Perry A, Condillac RA, Freeman N, Dunn-Geier J, Belair J. Multi-site study of the Childhood Autism Rating Scale (CARS) in five clinical groups of young children. *J Autism Dev Disord.* (2005) 35:625–34. doi: 10.1007/s10803-005-0006-9

54. Bölte S, Poustka F, Constantino JN. Assessing autistic traits: cross-cultural validation of the social responsiveness scale (SRS). *Autism Res.* (2008) 1:354–63. doi: 10.1002/aur.49

55. Constantino JN. *The Social Responsiveness Scale.* Los Angeles, CA: Western Psychological Services (2002).

56. Ventola PE, Kleinman J, Pandey J, Barton M, Allen S, Green J, et al. Agreement among four diagnostic instruments for autism spectrum disorders in toddlers. *J Autism Dev Disord.* (2006) 36:839–47. doi: 10.1007/s10803-006-0128-8

57. Higgins JP, Thompson SG, Deeks JJ, Altman DG Measuring inconsistency in meta-analyses. *BMJ* (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557

58. Higgins JP, Green S. *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0.* The Cochrane collaboration (2011). Available online at: www.cochrane-handbook.org

59. Chang HL, Juang YY, Wang WT, Huang CI, Chen CY, Hwang YS. Screening for autism spectrum disorder in adult psychiatric outpatients in a clinic in Taiwan. *Gen Hosp Psychiatry* (2003) 25:284–8. doi: 10.1016/S0163-8343(03)00053-7

60. Fraser R, Cotton S, Gentle E, Angus B, Allott K, Thompson A. Non-expert clinicians' detection of autistic traits among attenders of a youth mental health service. *Early Interv Psychiatry* (2012) 6:83–6. doi: 10.1111/j.1751-7893.2011.00288.x

61. Mandell DS, Lawer LJ, Branch K, Brodkin ES, Healey K, Witalec R, et al. Prevalence and correlates of autism in a state psychiatric hospital. *Autism* (2012) 16:557–67. doi: 10.1177/1362361311412058

62. Hallerback MU, Lugnegard T, Gillberg C. Is autism spectrum disorder common in schizophrenia? *Psychiatry Res.* (2012) 198:12–7. doi: 10.1016/j.psychres.2012.01.016

63. Waris P, Lindberg N, Kettunen K, Tani P. The relationship between Asperger's syndrome and schizophrenia in adolescence. *Eur Child Adolesc Psychiatry* (2013) 22:217–23. doi: 10.1007/s00787-012-0338-x

64. Davidson C, Greenwood N, Stansfield A, Wright S. Prevalence of Asperger syndrome among patients of an early intervention in psychosis team. *Early Interv Psychiatry* (2014) 8:138–46. doi: 10.1111/eip.12039

65. Kästner A, Begemann M, Michel TM, Everts S, Stepniak B, Bach C, et al. Autism beyond diagnostic categories: characterization of autistic phenotypes in schizophrenia. *BMC Psychiatry* (2015) 13:115. doi: 10.1186/s12888-015-0494-x

66. Morrison KE, Pinkham AE, Penn DL, Kelsven S, Ludwig K, Sasson NJ. Distinct profiles of social skill in adults with autism spectrum disorder and schizophrenia. *Autism Res.* (2017) 10:878–87. doi: 10.1002/aur.1734

67. Owen MJ, O'Donovan MC, Thapar A, Craddock N. Neurodevelopmental hypothesis of schizophrenia. *Br J Psychiatry* (2011) 198:173–5. doi: 10.1192/bjp.bp.110.0843

Parental Perspectives on Psychiatric Comorbidity in Preschoolers With Autism Spectrum Disorders Receiving Publicly Funded Mental Health Services

Filippo Muratori [1,2], Marco Turi [3], Margherita Prosperi [1,2], Antonio Narzisi [1], Giovanni Valeri [4], Silvia Guerrera [4], Elisa Santocchi [1], Fabio Apicella [1], Caterina Lattarulo [3], Sara Calderoni [1,2]* and Stefano Vicari [4]

[1] Department of Developmental Neuroscience, IRCCS Fondazione Stella Maris, Pisa, Italy, [2] Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy, [3] Fondazione Stella Maris Mediterraneo, Potenza, Italy, [4] Child and Adolescence Neuropsychiatry Unit, Department of Neuroscience, Children Hospital Bambino Gesù, Rome, Italy

An increased prevalence of psychiatric comorbidity (PC) in individuals with Autism Spectrum Disorders (ASD) is consistently reported. While several studies have examined PC in school-aged children, adolescents and adults with ASD, investigations on PC in preschoolers are less common. In this study, we explore the prevalence and the type of PC in a sample of 989 preschoolers with ASD through the DSM-Oriented Scales (DOS) of the Child Behavior Checklist (CBCL 1½-5) and their possible links with the core features of ASD and cognitive functioning. Results indicated that 37.8% of the sample had at least one PC in addition to ASD; these subjects displayed significantly higher Total score ($p = 0.02$) and Social Affect score ($p = 0.003$) on the ADOS-based calibrated severity scores (CSS), as well as lower ($p \leq 0.0001$) performance IQ (pIQ) compared to ASD individuals without PC. As far as the specific DOS, Affective Problems (AP) were detected in 23.4% of the whole sample, ADHD Problems (ADHD) in 17.3%, Anxiety Problems (AXP) in 16.7%, and Oppositional Problems (OP) in 7.9%. These different comorbidities were isolated in 195 subjects (Mono-comorbid group: 19.7% of the whole sample), while 179 subjects (18.1% of the whole sample) had two or more types of PC (Multi-comorbid group). One-way ANOVA revealed that subjects with multi-comorbidity have statistically significant lower pIQ and higher Total score and Social Affect score on CSS-ADOS. Specific differences for each type of comorbidity and gender differences were also discussed. Taken together, results indicate a considerable presence of PC in preschoolers with ASD that should be accurately considered during the assessment and diagnosis process in order to plan a tailored intervention based not only on core symptoms of ASD, but also on comorbid psychiatric condition since preschool age.

Keywords: child behavior checklist 1.5–5, affective problems, anxiety problems, young children, oppositional problems, ADHD problems, multicomorbidity, sleep problems

INTRODUCTION

Autism Spectrum Disorders (ASD) are neurodevelopmental disorders characterized by persistent social communication difficulties as well as restricted interests, repetitive activities and sensory abnormalities (1). Substantial heterogeneity exists in ASD in terms of genetic susceptibility (2), neural underpinnings (3), clinical presentation (4), medical and psychiatric comorbidities (5), response to treatment (6), and developmental trajectories (7). In particular, studies consistently reported an increased prevalence of psychiatric comorbidities (PC) in individuals with ASD compared with typically developing (TD) controls (8–10). The type and the prevalence rate of PC in ASD considerably vary across studies, according to the demographic and clinical features of patients (e.g., sex, age, core symptom severity, intellective functioning) as well as the assessment modalities (11). PC of children with ASD predicts poorer prognosis (12), and are associated with psychological distress in their parents—see the recent systematic review and meta-analysis (13). It is worth mentioning that the presentation of PC in the ASD population could be different than PC in the general population. Therefore, there is a considerable risk for mis– or under–diagnosis of PC (and consequently under–treatment) if symptoms are presumed to be part of ASD (i.e., diagnostic overshadowing) (14). Vice versa, there is also the possibility that the co–occurrence of psychiatric disorders may mask or obscure the core symptoms of ASD and thus contribute to difficulties of accurate and timely diagnosis of ASD (15).

Moreover, developmental characteristics such as age, intellectual functioning, and socio–communicative abilities may interfere on the presentation and expression of PC in individuals with ASD. In particular, difficulties in communication are intrinsically part of the ASD features and could impact– especially if there is an associated intellectual disability– on the ability of patients to express their own emotional and behavioral problems, and this is particularly true in the preschool years.

While several studies have examined PC in school–aged children (16), adolescents (17), and adults (18–20) with ASD, investigations on the presence of PC in preschoolers with ASD are less common. Importantly, several studies demonstrated a high presence of multiple PC in ASD children and adolescents. For example, Simonoff et al. (21) used structured assessments in a sample of 255 ASD children aged 10–14 years detecting that 41% had two or more co–occurring disorders and more than a third had three or more disorders in addition to ASD. Specifically, Social Anxiety Disorder (29%), Attention–Deficit/Hyperactivity Disorder (28%), and Oppositional Defiant Disorder (28%) were the most common PC; while the prevalence of Major Depressive Disorder (0.9%), Dysthymic Disorder (0.5%), and Conduct Disorder (3%) appeared minimal. Also, Leyfer et al. (9) implemented a modified version of the Kiddie Schedule for Affective Disorders (K–SADS) in a sample of participants aged 5–17 years and found that the majority of their sample had at least two PC in addition to ASD. However, the authors suggested a likely underestimation of the diagnosis of multiple comorbidity resulting from the methodology adopted. A high multiple PC was

subsequently confirmed in other ASD samples of children and adolescents with ASD (22–27).

The difficulty to find reliable and accurate diagnostic tools to detect PC in preschoolers with ASD could contribute to the relatively sparse studies in this area (27). Among instruments used to measure comorbid psychopathology in young children with ASD, the Child Behavior Checklist (CBCL) was considered robust in their measurement properties –see Hanratty et al. (28) for a recent systematic review on this topic. In fact, the CBCL's syndrome scales demonstrated good instrument quality and validity (29, 30), and the CBCL's DSM–Oriented Scales (DOS) showed similitudes in psychometric properties with regard to consistency, reliability and cross–informant agreement (31, 32). In addition, previous studies have shown that DOS are valid for discriminating related DSM–diagnoses in participants both in the CBCL 6–18 (33–35) and in the CBCL 1.5–5 (36). The use of checklist measures allowed the clinicians to highlight a strong *continuum* between preschool behavioral and emotional problems and psychopathology in later childhood (37–40) and even adulthood (41). In addition, it has been shown similitudes in psychopathology between preschoolers and older children and adolescents with a high concordance between parental report at early age and the following direct evaluations of the same participants at an older age (42). The use of the same clinical CBCL thresholds in both school children and preschoolers is under discussion, since the applying of lower threshold scores in preschoolers has proved useful (43). In fact, a tendency of parents to underestimate affective and atypical reactions in preschoolers as compared to older children emerges, in particular for depressed symptoms (44) and disruptive behaviors (45).

Some previous studies used the CBCL 1½−5 to investigate the PC of preschoolers with ASD. Hartley et al. (46) evaluated 169 young children with ASD and found that about one third (34.3%) of the sample had a Total Problems score in the clinically significant range, while the most frequent clinically significant scores in syndrome scales were Withdrawn (70.4%), Attention (38.5%) and Aggression (22.5%), with a high degree of comorbidity. Hartley and Sikora (47) examined coexisting emotional and behavior problems in a sample of 157 boys and 42 girls with ASD aged 1.5–3.9 years. Results indicated that female toddlers exhibited more sleep and affective problems than matched males. Tseng et al. (48) identified more severe internalizing problems and higher scores in Withdrawn, Social Problems, Thought Problems, and Attention Problems scale in ASD toddlers than in typically developing children; moreover, 73.1% of the patient sample—composed of 67 ASD preschoolers—had at least one CBCL syndrome scale score in the clinically significant range, while 47.7% had two or more. Giovagnoli et al. (49) reported significantly higher rates of behavioral and emotional problems in children with ASD as compared to their TD peers: specifically, in all the three broadband scales (total, internalizing, and externalizing problems), and in all syndrome scales, with exception of Somatic Complaints and Sleep Problems. Vaillancourt et al. (50) conducted a longitudinal investigation across four time points of children with ASD aged 3 to 6 years and detected that internalizing and externalizing behaviors co–occurred at high

rates across time, and, on average, declined slightly over time. However, high/stable course of internalizing or externalizing problems were found in a considerable part of the sample (23.2 and 13.5%, respectively).

While the abovementioned investigations used the broadband and the syndrome scales of the CBCL 1½−5, in the present paper we preferred the DOS to investigate PC in a more precise way. The same method was applied in a recent study on the prevalence of Anxiety Problems and Attention Deficit Hyperactivity Disorder Problems in a sample of preschool and early elementary aged children with ASD (51). Compared to this study we widened the number of PC investigated, describing the presence and the type of four PC (Affective Problems, Anxiety Problems, Attention Deficit Hyperactivity Disorder Problems and Oppositional Problems) through the DOS of the CBCL 1½−5–Parent Report Form in a much larger sample of ASD preschoolers. Possible correlations with demographic and clinical variables (gender, intellective functioning, core ASD features) were also evaluated.

METHODS

Participants

The sample (**Table 1**) included 989 preschoolers with ASD between 16 and 75 months of age (mean age: 44.0 months; *SD*: 13.8 months) recruited by three different Italian care centers for children: specifically, 498 children from IRCCS Fondazione Stella Maris in Pisa, 323 children from Bambin Gesù Children's Hospital in Rome, and 168 children from Stella Maris Mediterraneo Foundation in Matera. These children were selected among individuals who received a diagnosis of ASD according to DSM−5 criteria (52), or of autistic disorder, Asperger disorder, and pervasive developmental disorder– not otherwise specified according to DSM–IV criteria (53), performed by a multidisciplinary team including a senior child psychiatrist and an experienced clinically trained research child psychologist.

Clinical diagnosis was confirmed by ADOS, the gold–standard standardized interviewer–rated measure for child observation and assessment of skills in communication, social interaction, quality of play and imagination. In this study, ADOS–G (54) and ADOS−2 (55) were applied. According to two already published algorithms (56, 57), the Calibrated Severity Score (CSS) was obtained for each participant based on ADOS Total score and sub–scores Social Affect (SA) and Restricted Repetitive Behaviors (RRB). CSS range is 1–10 and it allows comparing different versions and modules of ADOS. Moreover, the CSS provides a measure of autism symptoms that is independent of age and language ability and thus is better suited than the ADOS scores for assessing the severity of ASD (58). The scores of ADOS– G were previously converted in ADOS−2 scores (SA, RRB and Total score) on the basis of the new algorithm proposed by Gotham et al. algorithm (59). The total and the CSS domains were calculated for Toddler Module of ADOS−2 on the basis of Esler et al. (60) to facilitate a direct comparison to other modules of ADOS−2.

TABLE 1 | Demographic, clinical characteristics, CBCL broad–band, and DSM–IV Oriented scales scores in the total sample (*n* = 989).

	Total sample (*n* = 989)
Gender (Male/Female)	820 (83%):169 (17%)
	Mean (SD); Range
Age (months)	44.01 (13.76); 16–75.15
Performance IQ	79.21 (23.30); 30–138
ADOS Calibrated Severity Score—Social Affect	6.11 (1.96); 1–10
ADOS Calibrated Severity Score—RRB	6.95 (0.06); 1–10
ADOS Calibrated Severity Score—Total score	6.26 (0.06); 3–10

CBCL	**T–Score Mean (SD); Range**	**Number (%) of subjects with CBCL score in the borderline or clinical range**
CBCL—Total problems	58.40 (10.87); 50–94	363 (36.7)
CBCL—Internalizing problems	60.13 (10.27); 50–93	479 (48.4)
CBCL—Externalizing problems	54.90 (9.62); 50–97	220 (22.2)
CBCL—Pervasive developmental problems	68.64 (9.71); 50–98	682 (69)

CBCL DSM–IV oriented scales	**T–Score Mean (SD); Range**	**Number (%) of subjects with CBCL score in the borderline or clinical range**
CBCL—Affective problems	58.82 (8.78); 50–95	231 (23.4)
CBCL—Anxiety problems	56.74 (8.13); 50–100	165 (16.7)
CBCL—Attention Deficit/Hyperactivity problems	57.80 (6.97); 50–76	171 (17.3)
CBCL—Oppositional problems	54.52 (6.09); 50–80	78 (7.9)

Number of subjects with CBCL score in the borderline or clinical range are reported.

As far as cognitive evaluation, a number of standardized tests were used to assess intellectual abilities due to differences in the verbal skills and functioning level of children. These included: the Leiter International Performance Scale–Revised (LIPS– R) (61), the Griffiths Mental Developmental Scales–Extended–Revised (GMDS–ER) (62), and the Italian version of Wechsler Preschool and Primary Scale of Intelligence (WPPSI) (63). When the tool provides a mental age (MA), IQ was estimated dividing MA by the child's chronological age (CA): MA/CA × 100. For this study, we have considered the non–verbal IQ scores (performance IQ). Sixty-two patients were not evaluable with standardized intelligence tests.

Males and females were represented in a different percentage in the total sample (83% vs. 17%; 820 males and 169 females) with a ratio between ASD males and ASD females similar to that reported in the literature (4.9:1). All cases of syndromic autism or with a known cause for ASD were excluded. No participant used psychotropic drugs in the last two months before the evaluation.

The current study was carried out according to the standards for good ethical practice and in accordance with the guidelines of the Declaration of Helsinki. Written informed consent from a parent/guardian of each participant was obtained when filling out the questionnaire.

Measures
CBCL 1½−5
The Italian version of the Child Behavior Checklist (CBCL 1½−5) (64, 65) is one of the most widely used checklists consisting of 100 statements about the child's behaviors. The parents are asked to rate the frequency of each behavior on a three−point Likert scale (0, not true; 1, somewhat or sometimes true; 2, very true or often true). The CBCL provides seven syndrome scales scores (i.e., Emotionally Reactive, Anxious/Depressed, Somatic Complaints, Withdrawn, Aggressive Behavior, Attention Problems and Sleep Problems) and three summary scales scores (i.e., Internalizing, Externalizing and Total Problems). A T−score between 60 and 63 for summary scales, and a T−score between 65 and 69 for syndrome scales is considered in the borderline or clinical range. For this study we have in particular considered the DSM−Oriented scales (DOS): Affective Problems (AP), Anxiety Problems (AXP), Attention Deficit Hyperactivity Disorder Problems (ADHD), and Oppositional Defiant Problems (OP); for these four scales a T−score between 65 and 69 (borderline range) or above 70 is indicative for a clinically significant score. The items composing the syndromic scales and the DOS of the CBCL are unique confronting them to each other and also independent from the Pervasive Developmental Problems (PDP) scale.

In this study, we adopted the borderline clinical elevation cut−off score (T score $\geq$ 60 for summary scales and T score $\geq$ 65 for DOS), according to previous studies on screening (66–68) and comorbidity (16, 51) in young children with ASD.

Procedure
All participants received a clinical diagnosis of ASD, were assessed with ADOS and were evaluated with psychometric tests when it was possible. Parents completed the CBCL at the beginning of the diagnostic process based on the behavior of their child in the last 6 months. For this study, the CBCL completed by mothers were preferred; when this was not possible, the CBCL was completed by fathers or by another close caregiver. Firstly, we examined the whole sample comparing different groups identified on the basis of single or multiple PC. Then, we looked for gender differences in PC. We also examined PC dividing the whole group on the base of higher or lower autistic behaviors measured by the PDP DOS on the CBCL.

TABLE 2 | ANCOVA (controlling for gender and age) on ADOS and Performance IQ between ASD with or without Psychiatric Comorbidity.

	ASD−only (N = 615)	ASD+Psychiatric comorbidity (N = 374)	ANCOVA		
			F−values	p−value	Effect size
ADOS CSS—Social Affect	**5.98 (1.95)**	**6.33 (1.95)**	**9.12 (1,985)**	**0.003**	$\eta^2 = 0.01$
ADOS CSS—RRB	6.93 (2.09)	6.98 (2.06)	0.73 (1,985)	0.40	–
ADOS CSS—Total score	**6.17 (1.95)**	**6.40 (1.95)**	**5.05 (1,985)**	**0.02**	$\eta^2 = 0.003$
Performance IQ	**81.20 (22.91)**	**75.93 (23.16)**	**13.15 (1,939)**	**<0.0001**	$\eta^2 = 0.014$

Significant comparison are highlighted in bold.

Data Analysis
All the continuous variables were examined for normality using skewness tests and Kolgomorov–Smirnov testing. The descriptive analyses, chi–square analysis and t–test were used for categorical and continuous independent variables, respectively. One–way analysis of variance (ANOVA) with Scheffe post–hoc test for multiple comparisons was performed to evaluate differences in age and CBCL scales among all groups. Analysis of covariance (ANCOVA) with Bonferroni post–hoc test for multiple comparisons was used to assess differences among the groups on CBCL scales, controlling for gender and age. In order to evaluate effect size, we measured: Cohen's d (d) for independent sample t–test, eta squared (η^2 that represent the variance accounted for) for analysis of Variance, and Phi (ϕ) for non–parametric statistics (Chi–square). In order to evaluate the effect of PC on the severity of autism, we compared the group composed by ASD children without PC with different groups characterized by the presence of PC (Mono– or Multi–Comorbidity). To understand in which way each single type of PC (Affective Problems, Anxiety Problems, ADHD, Oppositive Problems) could specifically be associated with ASD level or IQ we compared the ADOS scores and the IQ scores among the ASD–only group and groups with a specific PC (mono–comorbid or multi–comorbid).

RESULTS
37.8% (374 participants) of our sample had a score over the borderline cutoffs on one or more of the DOS of the CBCL. It means that these participants had at least one PC in addition to ASD. In order to evaluate the relationship of PC with the severity of autism and cognitive level, we compared this PC group with the group of ASD children without PC (615 participants; 62.2% of the whole group). One–way ANCOVA (controlling for age and gender) revealed significantly higher mean scores on CSS–AS ($p = 0.003$) and on CSS–Total ($p = 0.02$) score in the PC group vs. the ASD–only group. Significantly higher performance IQ mean scores were present in the ASD–only group ($p \leq 0.0001$) compared to the PC group (**Table 2**).

Preschoolers presented a full range of different types of DOS (**Table 1**): Affective Problems were over the borderline cutoff in 23.4% of the whole sample; ADHD Problems in 17.3%; Anxiety Problems in 16.7%; Oppositional Problems in 7.9%. They were isolated (Mono–comorbid group) in 195 participants (52.1% of the 374 PC group; 19.7% of whole sample); 105 participants (28.1% of the 374 PC group; 10.6% of whole sample) had two types of PC; 56 participants (15% of the 374 PC group; 5.7% of whole sample) had three types of PC; 18 participants (4.8% of the 374 PC group; 1.8% of whole sample) were over the cutoff on all four DOS (**Figure 1**). One–way ANOVA revealed that participants with one ($n = 195$) or more PC ($n = 179$) have no statistically significant differences as far as IQ and ADOS scores are regarded. However, **Figure 1** shows that participants who have four PC have also a significantly lower pIQ score ($p = 0.002$, $\eta^2 = 0.02$). Post–hoc analyses using the Scheffe post–hoc criterion for significance indicated that the average IQ is significantly higher in the no PC group ($M = 81.20$, $SD = 22.9$) than in the group with at least one PC ($M = 75.25$, $SD = 22.5$, $p = 0.02$).

Affective Problems

Two hundred and thirty-one participants had scores over the borderline cutoff on the Affective Problems DOS (**Table 3**). We found that this AP group (which has positive clinically significant scores in AP not taking in account mono– or multi–comorbidity) compared to participants without any borderline/clinical score on DOS (ASD–only group), had Higher CSS–AS ($p = 0.001$, $d = 0.25$) and CSS–Total Score ($p = 0.01$, $d = 0.11$), and lower pIQ ($p = 0.001$, $d = 0.27$). The AP group is composed of 81 participants with an isolated PC (mono–comorbid group) and of 150 multi–comorbid participants. ANCOVA with a Bonferroni post hoc test revealed significantly higher scores on CSS–SA ($p = 0.004$, $\eta^2 = 0.017$) and CSS–Total ($p = 0.01$, $\eta^2 = 0.01$) and significant lower score on pIQ ($p = 0.002$, $\eta^2 = 0.02$) when the ASD–only group is compared to the mono–comorbid group; these differences were not found for multi–comorbid group. No significant difference was found for CSS–RRB scores within the groups identified.

Anxiety Problems

One hundred sixty-five participants had scores over the borderline cutoff on the Anxiety Problems DOS; this sample was composed of 43 children with mono–comorbidity (positive only on AXP scale) and of 122 multi–comorbid children (positive to AXP scale and other DOS). ANCOVA revealed no significant difference on CSS–SA scores, CSS–RRB scores, CSS–Total scores and pIQ between AXP and ASD–only or for mono– and multi–comorbid AXP children.

ADHD Problems Cluster

One hundred seventy-one children had scores over the borderline cutoff on the ADHD problems of the DOS (**Table 3**). Differences between this group (mono–comorbid and multi–comorbid) and the ASD–only group showed that ADHD group was associated with Higher CSS–SA ($p = 0.04$, $d = 0.09$) and lower IQ ($p = 0.006$, $d = 0.25$) compared with the ASD–only children. The ADHD sample was composed of 62

participants with an isolated PC (positive only on ADHD scale) and 109 multi–comorbid participants (positive to ADHD scale and other DOS). ANCOVA failed to reveal significant differences on all CSS scores among groups, but multi–comorbid ADHD group showed lower statistically significant scores on pIQ ($p = 0.003$, $\eta^2 = 0.012$) compared to the ASD–only group.
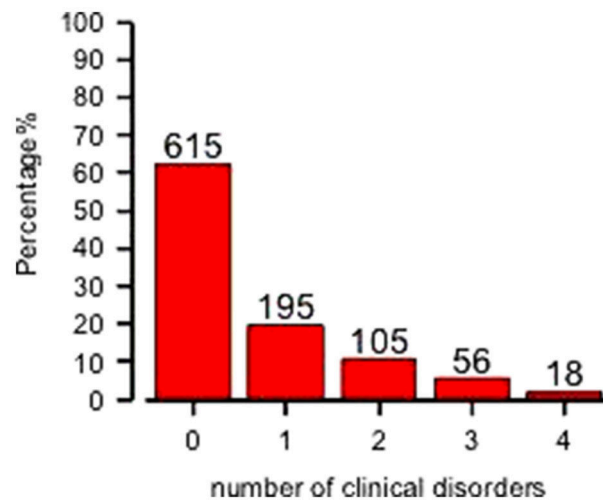
Oppositional Problems

Eighty children showed scores over the borderline cutoff on the Oppositional Problems DOS (**Table 3**). This group, composed of mono–comorbid and multi–comorbid participants, showed lower pIQ ($p < 0.0001$, $d = 0.46$) compared to the group of children without any comorbidity (ASD–only group). This OP sample is composed of 9 children with an isolated PC (mono–comorbid group) and 71 multi–comorbid children (positive to OP scale and to other DOS). ANCOVA with a Bonferroni post hoc test revealed a significantly higher score on CSS–SA ($p = 0.02$, $\eta^2 = 0.006$) and lower score on pIQ ($p = 0.002$, $\eta^2 = 0.03$) when mono–comorbid group is compared to the ASD–only group. In addition, by comparing CSS–RRB scores between groups we found a significant interaction between group and age [$F_{(2, 689)} = 4.03$ $p = 0.02$, $\eta^2 = 0.01$].

Moreover, we compared groups based on the type of psychiatric mono–comorbidity and ANCOVA was not able to detect differences on the clinical variables considered (**Table 4**). In order to evaluate the effect of having different clusters of multi–comorbidity we compared groups composed of any type of different clustering (i.e., AP and AXP, AP, and ADHD, ADHD and AXP, AP, and AXP and OP, etc.); also in this case different ANCOVA did not reveal any significant differences among groups on the considered clinical variable.

Finally, we have considered the distribution of PC taking into account the severity of autism and gender differences. In order to investigate the effect of the severity of autism, we have divided the whole group on the basis of having a PDP score over (PDP+; 682 participants) or under (PDP–; $n = 307$) the borderline cut–off on this scale. The PDP+ group show significantly higher mean scores on all DOS and higher number of participants with PC compared to the group of ASD preschoolers with the score under the borderline cut–off (**Table 5**).

Regarding gender differences, we found a statistically significant difference on RRB–CSS where males show a higher score when compared to females with ASD. No significant differences were found for age, pIQ, CSS Total score and CSS–SA. Males and females did not show any difference on mean DOS score over the borderline cut-off (**Table 6**).

Finally, in order to study possible differences between younger (≤ 36 months) and older (> 36 months) subjects on ASD symptom severity and PC, the sample was dichotomized on the basis of age in 333 children younger or equal than 36 months (33% of sample) and 656 older than 36 months (67% of sample). The comparison between the two groups revealed higher scores for the younger groups on CSS–SA [$t_{(987)} = 3.58$, $p < 0.0001$, $d = 0.23$], CSS–RRB [$t_{(987)} = 2.75$, $p = 0.006$,

	Comorbidities					ANOVA		
	0	**1**	**2**	**3**	**4**	**F**-values	**p**-value	**Effect**
	(N = 615)	**(N = 195)**	**(N = 105)**	**(N = 56)**	**(N = 18)**			**size**
ADOS CSS—Social Affect	5.98 (1.95)	6.34 (1.96)	6.20 (1.94)	6.57 (1.75)	6.17 (2.47)	2.16 (4,984)	0.07	–
ADOS CSS—RRB	6.93 (2.07)	6.89 (2.08)	7.08 (1.92)	6.84 (2.07)	7.83 (2.43)	1.00 (4,984)	0.40	–
ADOS CSS—Total score	6.17 (1.95)	6.35 (1.91)	6.38 (1.97)	6.55 (1.87)	6.56 (2.54)	0.97 (4,984)	0.42	–
Performance IQ	**81.20 (22.9)**	**75.25 (22.5)**	**79.75 (24.82)**	**73.49 (25.17)**	**68.83 (21.50)**	**4.14 (4,938)**	**0.002**	$\eta_2 = 0.02$

FIGURE 1 | Distribution of mono– and multi–comorbidity across the whole sample and clinical comparisons. One–way ANOVA on ADOS scores and Performance IQ (means and SD) for all comorbidities groups are reported. Significant comparison are highlighted in bold.

$d = 0.17$], and CSS–Total scores [$t_{(987)} = 4.22$ $p < 0.0001$, $d = 0.27$], but no difference as far as prevalence of PC is regarded (**Table 7**).

DISCUSSION

This study aims to explore psychiatric comorbidity in a wide sample of ASD preschoolers, searching for the impact of gender, symptom severity and intelligence on PC. For this purpose, we have used the DSM Oriented Scales (DOM) of the CBCL 1½−5 that have proven validity to identify PC in ASD preschoolers (51). Nevertheless, their use is sparse in literature and, to our knowledge, this is the first time that they are applied in a very large sample of preschoolers with ASD.

Results revealed that 37.8% of the participants had at least one PC in addition to ASD. This finding is not surprising since significant genetic overlap between the diverse group of neurodevelopmental disorders—for instance ASD and ADHD—(69) and between different psychiatric diseases—e.g., ASD and depression—has been identified (70). The relative low rate of PC in our sample can be interpreted from a developmental perspective. Specifically, it is plausible that in the toddler–age the majority of the child's emotional and behavioral problems could be explained by a diagnosis of ASD, whereas, with increasing age

and consequently social, adaptive and cognitive demands, new internalizing and externalizing disorders emerge. Accordingly, 71% of children (21), 74% of adolescents (23), and 73% of adults with ASD (8) have been described as affected by at least one other psychiatric diagnosis.

Our results indicated that children with ASD combined with one or more PC had higher severity of autism symptoms and lower IQs than ASD children without PC, in contrast to previous findings where no relationship with these clinical features was found (21, 71), even taking in account the effect of gender and age, according to this we found a small to medium ($\eta^2 = 0.014$ to 0.02) effect size in our analysis. Of particular importance is the detection of PC in individuals with ASD plus intellectual disability in order to avoid the diagnostic overshadowing (14) that is the attribution of all symptoms to intellectual impairment instead of to specific PC.

Moreover, 18.1% of our whole sample had two or more PC associated with ASD (multi–comorbid group). This percentage is lower than that reported by Simonoff et al. (21); in this study 52% of participants had multiple PC and 38% had three or more PC, while in our sample 10.6% participants had two types of PC, 5.7% participants had three types of PC, and only 1.8% satisfy all the four PC we considered. Furthermore, the cumulative percentage of our children with at least two PC in addition

TABLE 3 | Clinical characteristics of ASD subjects grouped on the base of specific PC.

					a	b	c			
AP	**AP+** (N = 231)	**ASD–only** (N = 615)	**t**	**p (ES)**	**Mono– Comorbidity** (N = 81)	**Multi– Comorbidity** (N = 150)	**ASD–only** (N = 615)	**F**	**p–value**	**Effect Size**
CSS–SA	6.48 (1.97)	5.98 (1.95)	**3.25**	**0.001** (d = 0.25)	6.68 (1.88)	6.37 (2.02)	5.98 (1.95)	**6.66** (2,841)	0.001 a>c	η2 = 0.017
CSS–RRB	7.17 (1.97)	6.93 (2.09)	1.54	0.12 (–)	7.17 (1.79)	7.17 (2.06)	6.93 (2.09)	1.57 (2,841)	0.19 ns	–
CSS–Total score	6.56 (1.95)	6.17 (1.95)	**2.51**	**0.01** (d = 0.11)	6.65 (1.68)	6.51 (2.08)	6.17 (1.95)	**4.19** (2,841)	**0.01** a>c	η2 = 0.01
Performance IQ	74.87 (23.48)	81.20 (22.91)	**−3.46**	**0.001** (d = 0.27)	72.96 (20.26)	75.93 (25.05)	81.20 (22.91)	**7.09** (2,801)	**0.001** c>a c>b	η2 = 0.02
ADHD	**ADHD+** (N = 171)	**ASD–only** (N = 615)	**t**	**p**	**Mono– Comorbidity** (N = 62)	**Multi– Comorbidity** (N = 109)	**ASD–only** (N = 615)			
CSS–SA	6.15 (1.87)	5.98 (1.95)	**2.05**	**0.04** (d = 0.09)	6.21 (1.90)	6.39 (1.86)	5.98 (1.95)	2.74 (2,781)	0.06 ns	–
CSS–RRB	6.91 (2.19)	6.93 (2.09)	−0.78	0.43 (–)	6.42 (2.30)	6.99 (2.11)	6.93 (2.09)	1.86 (2,781)	0.13 ns	–
CSS–Total score	6.61 (1.95)	6.17 (1.95)	1.15	0.24 (–)	6.15 (2.10)	6.49 (1.86)	6.17 (1.95)	2.05 (2,781)	0.10 ns	–
Performance IQ	75.05 (25.24)	81.20 (22.91)	**−2.77**	**0.006** (d = 0.25)	76.88 (24.67)	74.57 (25.63)	81.20 (22.91)	**4.58** (2,743)	**0.003** c>b	η2 = 0.012
OP	**OP+** (N = 80)	**ASD–only** (N = 615)	**t**	**p**	**Mono– Comorbidity** (N = 9)	**Multi– Comorbidity** (N = 71)	**ASD–only** (N = 615)			
CSS–SA	6.42 (1.99)	5.98 (1.95)	1.86	0.06 (–)	6.33 (2.73)	6.43 (1.89)	5.98 (1.95)	**3.13** (2,690)	**0.02** b>c	η2=0.006
CSS–RRB	7.13 (2.07)	6.93 (2.09)	0.80	0.42 (–)	7.78 (1.71)	7.04 (2.11)	6.93 (2.09)	0.74 (2,690)	0.52 ns	–
CSS–Total score	6.56 (2.13)	6.17 (1.95)	1.66	0.09 (–)	6.89 (2.80)	6.52 (2.05)	6.17 (1.95)	1.98 (2,690)	0.11 ns	–
Performance IQ	70.55 (22.48)	81.20 (22.91)	**−3.79**	**0.0001** (d = 0.46)	68.33 (22.82)	70.85 (22.60)	81.20 (22.91)	**6.17** (2,660)	**<0.001** c>a	η2 = 0.03

Each specific PC is compared to ASD–only subjects without PC. AP, Affective Problems; ADHD, Attention Deficit/Hyperactivity problems; OP, Oppositional Problems. Significant comparison after Bonferroni correction are highlighted in bold.

to autism was significantly lower (18.2 vs. 50% approximately) than that reported in another study (9). The lower average age of our sample as well as the exclusion of other types of psychiatric disorders not detected by the CBCL (e.g., obsessive compulsive disorder, specific phobias) could partly justify this finding. However, some behaviors of ASD children could be misinterpret by clinicians or parents; difficulties in disentangling symptoms of PC from ASD symptoms (i.e., withdrawn) could create an under–estimation—but also an over–estimation—of PC in ASD subjects depending on setting or on the training of the professionals (72).

As far as the specific PC, we confirm the considerable additional presence of affective problems, ADHD, and anxiety problems among our participants, as previously highlighted by other studies (73, 74).

The overall rate of affective problems that we found (23.4%) is similar to the one reported by Leyfer et al. (9) in a sample of 9–years–old ASD subjects, using a modified version of the K–SADS. Conversely, Salazar et al. (27) detected that only 14.6% of their preschool and elementary–school aged children met criteria for major depressive disorder, using the Preschool Age Psychiatric Assessment (PAPA) interview (39). A significant lower rate of major depression and dysthymic disorder (1.4%) was observed by Simonoff et al. (21) in a sample of ASD children and adolescents aged 10–14 years, using the Child and Adolescent Psychiatric Assessment–parent version (CAPA) (75). The fact that in our sample the affective symptoms have been evaluated through the CBCL may have had an impact on results. In fact, it is worthy of note that four out ten items that make up the Affective Problems (AP) scale of the CBCL 1 ½−5 (i.e., 24: doesn't eat

TABLE 4 | Clinical differences among group of ASD subjects with specific psychiatric mono–comorbidity.

	DSM–IV oriented scales				ANCOVA		
	AP+ *(N = 81)*	AXP+ *(N = 43)*	ADHD+ *(N = 62)*	OP+ *(N = 9)*	*F*–values	*p*–value	Effect size
CSS–SA	6.58 (1.83)	5.91 (2.01)	6.11 (1.81)	6.33 (2.73)	1.49 (3,189)	0.21	–
CSS–RRB	7.18 (1.74)	6.84 (2.24)	6.30 (2.33)	7.78 (1.71)	1.86 (3,189)	0.13	–
CSS–Total score	6.58 (1.65)	5.98 (1.75)	6.02 (2.04)	6.89 (2.80)	1.53 (3,189)	0.20	–
Performance IQ	72.93 (20.26)	78.70 (23.57)	76.88 (24.67)	68.33 (22.82)	0.92 (3,182)	0.42	–

TABLE 5 | Clinical comparison of ASD subjects with high scores on CBCL–PDP scale and subjects with low score on CBCL–PDP scale.

		PDP high (*n* = 682) M (*SD*)	PDP low (*n* = 307) M (*SD*)	*t*–test	*p*–value	Effect Size
Age (months)		44.00 (13.40)	44.05 (14.53)	−0.05	0.95	**–**
Performance IQ		76.68 (23.33)	84.82 (22.83)	**−5.02**	**<0.001**	$d = 0.35$
CSS–SA		6.36 (1.87)	5.57 (2.04)	**5.98**	**<0.001**	$d = 0.40$
CSS–RRB		7.09 (2.00)	6.64 (2.21)	**3.13**	**<0.001**	$d = 0.21$
CSS– Total score		6.49 (1.87)	5.73 (2.02)	**5.72**	**<0.001**	$d = 0.39$
Pervasive developmental problems		73.88 (6.21)	57.2 (4.6)	**42.43**	**<0.0001**	$d = 3.05$
Psychiatric Comorbidity				*t*–test or X^2	*p*–value	Effect Size
Affective problems	A	60.94 (9.06)	54.12 (5.84)	**12.09**	**<0.001**	$d = 0.89$
	B	30.6	7.2	**65.19**	**<0.0001**	$\phi = 0.25$
Anxiety problems	A	58.61 (8.70)	52.59 (4.44)	**11.45**	**<0.0001**	$d = 0.87$
	B	22.7	3.3	**57.73**	**<0.0001**	$\phi = 0.24$
Attention deficit/Hyperactivity problems	A	59.22 (7.04)	54.65 (5.67)	**9.98**	**<0.0001**	$d = 0.71$
	B	22.6	5.5	**43.11**	**<0.0001**	$\phi = 0.20$
Oppositional problems	A	55.74 (6.62)	51.81 (3.43)	**9.82**	**<0.0001**	$d = 0.74$
	B	11.1	0.7	**32.08**	**<0.0001**	$\phi = 0.18$

A, mean and SD; B, number of subjects in the borderline/clinical range expressed as percentage of subjects with PDP high or low score.
Significant comparison are highlighted in bold.

well; 38: has trouble getting to sleep; 49: overeating; 74: sleeps less than most kids during day and/or night) are strictly related to neurovegetative symptoms, such as sleeping and eating problems: these features, as well as being part of clinical depression are also disturbances that occurred at a higher rate in ASD individuals than in typically developing (TD) children, independently from the associated PC (76, 77). For example, as far as sleep problems, we found that 56% of our sample had at least one sleep problem (i.e., scored 2 in at least one out of the seven sleep items in the CBCL) and this percentage is close to 53% found by Krakowiak et al. (78) who included in their "sleep problems group" children

with ASD and at least one frequent sleep problem. Therefore, it is possible that the inclusion of eating and sleep problems in the CBCL–AP scale has led to overestimate the rate of affective problems in preschoolers with ASD. Similarly, other AP items, such as the 43 ("looks unhappy without good reason"), the 89 ("underactive, slow moving, or lacks energy"), and the 71 ("shows little interest in things around him/her") could be part of the ASD early presentation in which troubles of affect are frequently reported (79), besides being depressive symptoms. However, previous investigation supports the use of the CBCL 1.5–5 to assess for emotional disorders in preschoolers with

TABLE 6 | Gender differences: comparison of clinical characteristics and of PC in male vs. female.

		Male M (*SD*)	Female M (*SD*)	*t*–test	*p*–value	Effect size
Age		44.08 (13.92)	43.67 (13.06)	0.32	0.74	–
Performance IQ		76.68 (23.17)	76.58 (23.82)	1.61	0.10	–
CSS–SA		6.11 (02.16)	6.15 (1.92)	−0.20	0.84	–
CSS–RRB		7.02 (2.04)	6.57 (2.16)	**2.64**	**0.008**	*d* = 0.21
CSS– Total score		6.27 (1.92)	6.20 (2.01)	0.48	0.62	–
Pervasive developmental problems		68.89 (9.80)	67.44 (9.15)	1.75	0.08	–
Psychiatric comorbidity				*t*–test or X^2	*p*–value	Effect size
Affective problems	A	58.95 (8.86)	58.21 (8.36)	0.99	0.32	–
	B	23.5	22.5	0.08	0.76	–
Anxiety problems	A	56.98 (8.30)	55.97 (7.13)	**2.06**	**0.04**	*d* = 0.09
	B	17.6	12.4	2.65	0.10	–
Attention deficit/ Hyperactivity problems	A	57.75 (6.89)	58.05 (7.33)	−0.50	0.61	–
	B	17.0	18.9	0.37	0.53	–
Oppositional problems	A	54.50 (6.11)	54.59 (6.02)	−0.14	0.88	–
	B	7.9	7.7	0.01	0.91	–

A, mean and SD; B, number of subjects in the borderline/clinical range expressed as percentage of male or female. Significant comparison are highlighted in bold.

ASD (80) and replicated studies have demonstrated the construct validity of the CBCL for evaluating PC in older ASD subjects (29, 30); in particular the AP scale, despite including sleep and eating problems, showed a statistically significant correlation with Depression based on the K–SADS (81). More broadly, the association between AP and ASD should be interpreted with caution, since a considerable phenotypic overlap between these two conditions exists (82): consequently, the accurate diagnosis of depression in toddlers with ASD remains a challenge. Further, our results show that children with Affective Problems had notable association with lower pIQ and more severe autism, in both case we found a small to medium effect size. Previous studies on the relationship between intellectual disability and affective comorbidities in young individuals with ASD have been inconsistent. Some authors fail to find a relationship between intellectual disability and depression in subjects with ASD (83, 84), while others identified a decreased risk of depression in children with ASD and intellectual disability (27, 85, 86). Thus, our clear results of an quite strong association between the presence of AP and a more severe autism with lower pIQ set the stage for a more careful consideration of the relationship among intellectual disability, affective problems and autism.

We detected that 17.3% of participants exceed the cutoff in the ADHD scale, a percentage lower than that observed in previous research (9, 10, 15, 21, 27). Also for this PC our lower percentage may be partly explained by the lower ages in our sample. In fact, symptoms of ADHD may emerge in toddlerhood (87), but generally increase with age: for instance, in a clinically referred sample of children with ASD, 40% of 3–5-year old and over 50% of 6–12-year–old children met DSM-IV criteria for ADHD (88). Nevertheless, our percentages of children with ADHD are only slightly lower than that detected using the CBCL in a recent investigation (51) where it was reported that 22% of their preschoolers had ADHD. Thus, it is possible to suggest that our lower percentage is due to the ADHD–DOS which is more conservative than other instruments to individuate ADHD. Our results show significantly lower pIQ in ASD comorbid with ADHD, with an effect size ranging between small and medium magnitude; while some investigations suggest that rates of comorbid ADHD are high regardless the level of IQ (85, 89), others reported a more severe ASD phenotype when associated with ADHD, not only in terms of lower IQ, but also of higher autistic symptoms and more behavioral problems (90, 91). Our results support these latter findings, since the ASD plus ADHD children had significant lower pIQ as well as

TABLE 7 | Clinical differences between younger (≤36 months) and older (>36 months) subjects.

		≤36 m (n = 333) M (SD)	>36 m (n = 656) M (SD)	t–test	p–value	Effect size
Age (months)		29.30 (4.90)	51.48 (10.36)	−36.97	<0.0001	d = 2.73
Performance IQ		78.94 (22.11)	79.34 (23.88)	−0.24	0.84	–
CSS–SA		**6.43 (2.07)**	**5.96 (1.88)**	**3.58**	**<0.0001**	d = 0.23
CSS–RRB		**7.20 (2.24)**	**6.82 (1.98)**	**2.75**	**0.006**	d = 0.17
CSS– Total score		**6.62 (2.18)**	**6.07 (1.80)**	**4.22**	**<0.0001**	d = 0.27
Pervasive developmental problems		68.29 (9.90)	68.82 (9.61)	−0.81	0.41	–
Psychiatric comorbidity				**t–test or X^2**	**p–value**	**Effect size**
Affective problems	A	58.69 (9.08)	58.89 (8.63)	−0.32	0.74	–
	B	22.9	24.3	1.08	0.60	–
Anxiety problems	A	**55.85 (7.69)**	**57.20 (8.31)**	**−2.47**	**0.01**	d = 0.16
	B	17.7	14.7	1.40	0.23	–
Attention deficit/Hyperactivity problems	A	57.21 (6.83)	58.10 (7.03)	−1.91	0.06	–
	B	18.8	14.4	2.93	0.08	–
Oppositional problems	A	54.24 (6.20)	54.66 (6.04)	−1.00	0.31	–
	B	8.2	7.2	0.31	0.57	–

A, mean and SD; B, number of subjects in the borderline/clinical range expressed as percentage). Significant comparison are highlighted in bold.

considerable rate of multi–comorbidity; this finding indirectly supports the evidence of a specific phenotype characterized by ASD plus ADHD which may increase the risk of further comorbidity (92).

Also the percentage of children affected by anxiety problems (16.7% of the whole sample) is lower than that observed in other researches (9, 21, 27, 92) and meta–analysis (93). The lower ages of our large sample could partly explain this finding: accordingly, a cross–sectional recent study compared the levels of anxiety in different age–ranges and found an increase of anxiety levels from toddlerhood to childhood (94). Similarly, different studies detected a positive association between anxiety levels and chronological age in toddlers (95), children (27) and adolescents with ASD (96). However, higher rates of anxiety problems were detected also in ASD samples with age similar to ours: for example, Llanes et al. (51), using the CBCL in their subgroup of preschoolers, identify an anxiety problem in 31% of the sample that doubles up the percentage in our sample. Crucially, all the enrolled subjects in that research had an IQ on the WPPSI–III of 50 or above, and the mean IQ score of the participants was within average levels, while in our sample also subjects with a pIQ lower than 50 were included. This difference on IQ scores could be the second reason for the lower percentages of AXP, since literature frequently reported that higher levels of anxiety are associated with

better cognitive skills (97, 98). A third factor, related to the impairment in receptive and expressive language skills (94), could be responsible for the low prevalence of anxiety problems in our ASD sample: in fact, we could suppose that the low mean chronological age and the below–average cognitive level impacted on language and consequently on their ability to express anxious symptoms.

A relative small percentage of our subjects, 7.9% of total sample, exceed the cutoff for Oppositional Defiant Problems: this rate is not significantly different from the prevalence estimates of 7% (99) and 10% (40) for preschoolers in the general population. In previous studies higher rates of OP have been reported – 37% in de Bruin et al. (22); 13% in Gadow et al. (100); 30% in Simonoff et al. (21)–and these symptoms seem to increase over time: in fact, samples composed of older ASD children (85, 88, 101) exhibited a more elevated prevalence of these behavioral problems. It was suggested that the increase in social stressors (e.g., academic and peer demands) could have a role in this behavioral modification with age of ASD individuals. Our results show also that OP symptoms are more likely to be present in ASD preschoolers with lower intellective functioning, in accordance with some (101, 102), but not all literature (85, 103). Secondly, it is important to consider that some behavior of ASD children can be interpreted as oppositional by parents (for instance the items "defiant," "disobedient," "stubborn," "uncooperative")

instead of the consequence of the poor attention to social stimuli and/or impairment in social understanding e.g., (104–106) typical of ASD rather than symptoms of a real OP.

It is important to highlight that 31% of our participants does not reach the borderline scores on the PDP scale. Even if the PDP scale showed an high accuracy in distinguishing preschoolers with ASD from peers with typical development (66) and from peers with other psychiatric disorders (67), the sensitivity of this scale to detect ASD subjects is lower than the sensitivity of other CBCL scales to detect the corresponding PC (72, 107). Subjects positive to the PDP scale are, as expected, more impaired in terms of ADOS severity and intellective functioning, but, interestingly, they are also characterized by more frequent PC as highlighted by significantly higher mean scores on all DOS in comparison to subjects below the cut–off score at the PDP scale, with a moderate to strong effect size (all $d > 0.70$), suggesting a significant effect of having borderline scores on PDP scale. Therefore, in accordance with previous studies (108, 109), we could speculate that the PDP scale could be used as a measure not only of the possible presence of an ASD disorder but also of likely different functional impairments.

The comparison of the DOS scores between males and females participants did not reveal any statistically significant difference as far as scores within the borderline/clinical range of these scales are concerned. Some previous studies suggest a different phenotype in terms of PC in female than in male children with ASD (27, 47, 50, 110, 111), whereas other investigations failed to find clear gender differences (112, 113) or gender differences that reflect those found in typical young children (114). Therefore, data are still limited and inconclusive on this theme and further research is needed on this under–explored issue.

Interestingly, the comparison between younger (≤36 months) and older (>36 months) patients of our sample highlighted significantly higher symptoms severity in the younger group, with a small to moderate effect size (all $d > 0.10$). This result is in line with previous investigations in which the severity of ASD symptoms was negatively correlated with age at first ASD diagnosis (115, 116). Nevertheless, the more severe ASD symptoms at an earlier age is not linked to a more severe PC, which seem stable across ages and not influenced by autism *per se*.

CONCLUSIONS

Our findings should be considered in light of some methodological limitations. First, it is important to highlight that, in order to receive one or more specific DSM comorbid diagnoses, ASD patients can be in–depth evaluated by trained clinicians with expertise in childhood psychiatric disorders. In fact, we relied only on the use of the CBCL 1.5–5 for the evaluation of PC in young children with ASD, which has however shown a good ability to assess for emotional and behavioral disorders in preschoolers with ASD (80).

The absence of a subsequent clinical evaluation to confirm a diagnosis of PC may have caused a certain percentage of false positives. On the other hand, the presence of some false negatives cases should be considered. Since the preschoolers of the current study are referred for a diagnostic evaluation to three tertiary centers specific for ASD, it is possible that parents are more focused on ASD–Specific concerns (e.g., communication/language delays, social deficits) than on non–ASD–Specific concerns (e.g., inattention and hyperactivity, eating/feeding, sleep difficulties, tantrums or inappropriate behaviors) (117).

More broadly, parent ratings inevitably involve the risk of several parental bias, including difficulty in interpreting the questions and quantifying the behaviors, reluctance to acknowledge the child's problems, and lack of motivation to complete the instrument accurately. However, literature indicates that parents are generally reliable informants about the behavioral and emotional problems of their child (118), aside from providing valuable and unique information about the child's behaviors in the home environment and in specific situations (e.g., eating and sleeping habits). The lack of parents' history of psychiatric disorders is another limitation of this study. This information is important not only to increase comprehension of PC in ASD children Wiggins et al. (119), but also to accurately interpreting parent–report. For example, the possible negative bias of anxious or depressed parents can lead them to overestimate the amount of symptoms of their own kid (120). On the other hand, parents with externalizing psychopathology, but without insight into their own condition, could underestimate this type of symptoms in their child, considering them as part of a typical behavior.

The cross–sectional design of this study precluded us to draw inferences about the stability of CBCL profiles and their impact on the developmental trajectories of preschoolers with ASD. The few longitudinal studies on this topic detected a low/declining trajectory for internalizing problems in 70% of the sample (50), or an association between low scores on ADHD related traits over time and positive outcome (121). Future longitudinal investigations are therefore necessary and could also help to clarify whether the treatment of PC had a positive impact on adaptive function and core features of ASD patients. Moreover, we do not have a longitudinal evaluation of subjects diagnosed before or at 36 months of chronological age confirming the clinical diagnosis of ASD. However, diagnoses of ASD in toddlers have been found to be accurate and stable across time in studies of high–risk siblings (122), community–based settings (123) and clinic–referred samples (124). Moreover, the high symptoms severity in our younger ASD subjects supports the stability of their diagnosis, since the severity of ASD symptoms has been indicated as a factor contributing to the diagnostic stability of ASD (116).

Finally, the information about the sibling status could be of valuable relevance, since the presence of typical or atypical older siblings can impact on the parents' sensitivity to reliably rate symptoms of their younger child (125, 126).

In conclusion, this study suggests that in persons with ASD, PC occur early in life necessitating the need for their early detection that could improve our capacity for a more tailored intervention.

DATA AVAILABILITY

The anonymized datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of IRCCS Stella Maris committee with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the IRCCS Stella Maris committee.

AUTHOR CONTRIBUTIONS

FM, MT, SV, MP, and SC participated in the design of the work and wrote the first draft of the manuscript. MT analyzed the data. MT, AN, GV, SG, FA, ES, and CL evaluated the patients and collected the data. SV, SG, SC, and CL helped to evaluate, edit the manuscript and performed critical revision. Each Author has seen and approved the submission of this version of the manuscript and takes full responsibility for the manuscript.

FUNDING

REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders-5.* Washington, DC: American Psychiatric Association (2013).

2. Jeste SS, Geschwind DH. Disentangling the heterogeneity of autism spectrum disorder through genetic findings. *Nat Rev Neurol.* (2014) 10:74–81. doi: 10.1038/nrneurol.2013.278

3. Hong SJ, Valk SL, Di Martino A, Milham MP, Bernhardt BC. Multidimensional neuroanatomical subtyping of autism spectrum disorder. *Cereb Cortex.* (2018) 28:3578–88. doi: 10.1093/cercor/bhx229

4. Grzadzinski R, Huerta M, Lord C. DSM-5 and autism spectrum disorders (ASDs): an opportunity for identifying ASD subtypes. *Mol Autism.* (2013) 4:12–12. doi: 10.1186/2040-2392-4-12

5. Mannion AL, G.: Gastrointestinal symptoms in autism spectrum disorder: a literature review. *Rev J Autism Dev Disord.* (2014)1:11. doi: 10.1007/s40489-013-0007-0

6. Vivanti G, Prior M, Williams K, Dissanayake C. Predictors of outcomes in autism early intervention: why don't we know more? *Front Pediatr.* (2014) 2:58. doi: 10.3389/fped.2014.00058

7. Solomon M, Iosif AM, Reinhardt VP, Libero LE, Nordahl CW, Ozonoff S, et al. What will my child's future hold? phenotypes of intellectual development in 2-8-year-olds with autism spectrum disorder. *Autism Res.* (2018) 11:121–32. doi: 10.1002/aur.1884

8. Abdallah MW, Greaves-Lord K, Grove J, Norgaard-Pedersen B, Hougaard DM, Mortensen EL. Psychiatric comorbidities in autism spectrum disorders: findings from a danish historic birth cohort. *Eur Child Adolesc Psychiatry.* (2011) 20:599–601. doi: 10.1007/s00787-011-0220-2

9. Leyfer OT, Folstein SE, Bacalman S, Davis NO, Dinh E, Morgan J, et al. Comorbid psychiatric disorders in children with autism: interview development and rates of disorders. *J Autism Dev Disord.* (2006) 36:849–61. doi: 10.1007/s10803-006-0123-0

10. Skokauskas N, Gallagher L. Mental health aspects of autistic spectrum disorders in children. *J Intellect Disabil Res.* (2012) 56:248–57. doi: 10.1111/j.1365-2788.2011.01423.x

11. Ameis SH, Szatmari P. Imaging-genetics in autism spectrum disorder: advances, translational impact, future directions. *Front Psychiatry.* (2012) 3:46. doi: 10.3389/fpsyt.2012.00046

12. Matson JL, Goldin RL. Diagnosing young children with autism. *Int J Dev Neurosci.* (2014) 39:44–8. doi: 10.1016/j.ijdevneu.2014.02.003

13. Yorke I, White P, Weston A, Rafla M, Charman T, Simonoff E. The association between emotional and behavioral problems in children with autism spectrum disorder and psychological distress in their parents: a systematic review and meta-analysis. *J Autism Dev Disord.* (2018) 48:3393–415. doi: 10.1007/s10803-018-3605-y

14. Reiss S, Levitan GW, Szyszko J. Emotional disturbance and mental retardation: diagnostic overshadowing. *Am J Ment Defic.* (1982) 86:567–74.

15. Levy SE, Giarelli E, Lee LC, Schieve LA, Kirby RS, Cunniff C, et al. Autism spectrum disorder and co-occurring developmental, psychiatric, and medical conditions among children in multiple populations of the United States. *J Dev Behav Pediatr.* (2010) 31:267–75. doi: 10.1097/DBP.0b013e3181d5d03b

16. Mazefsky CA. Psychiatric comorbidity in children and adolescents with high-functioning autism and Asperger's disorder. In: *Organization for Autism Research 7th Annual Autism Research and Intervention Conference*, Arlington, VA (2009).

17. Holtmann M, Bolte S, Poustka F. Attention deficit hyperactivity disorder symptoms in pervasive developmental disorders: association with autistic behavior domains and coexisting psychopathology. *Psychopathology.* (2007) 40:172–7. doi: 10.1159/000100007

18. Vannucchi G, Masi G, Toni C, Dell'Osso L, Marazziti D, Perugi G. Clinical features, developmental course, and psychiatric comorbidity of adult autism spectrum disorders. *CNS Spectr.* (2014) 19:157–64. doi: 10.1017/S1092852913000941

19. Hollocks MJ, Lerh JW, Magiati I, Meiser-Stedman R, Brugha TS. Anxiety depression in adults with autism spectrum disorder: a systematic review and meta-analysis. *Psychol Med.* (2018) 49:559–72. doi: 10.1017/S0033291718002283

20. Nahar A, Thippeswamy H, Shanker MS, Kishore Reddy MT, Chaturvedi SK. Psychiatric comorbidity in persons with high-functioning autism spectrum disorders: Findings from a tertiary care neuropsychiatric hospital. *Asian J Psychiatr.* (2018). doi: 10.1016/j.ajp.2018.09.008. [Epub ahead of print].

21. Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G. Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. *J Am Acad Child Adolesc Psychiatry.* (2008) 47:921–9. doi: 10.1097/CHI.0b013e318179964f

22. de EI, Bruin, Ferdinand RF, Meester S, de Nijs PF, Verheij F. High rates of psychiatric co-morbidity in PDD-NOS. *J Autism Dev Disord.* (2007) 37:877–86. doi: 10.1007/s10803-006-0215-x

23. Mattila ML, Hurtig T, Haapsamo H, Jussila K, Kuusikko-Gauffin S, Kielinen M, et al. Comorbid psychiatric disorders associated with Asperger

syndrome/high-functioning autism: a community- and clinic-based study. *J Autism Dev Disord.* (2010) 40:1080–93. doi: 10.1007/s10803-010-0958-2

24. Mukaddes NM, Herguner S, Tanidir C. Psychiatric disorders in individuals with high-functioning autism and Asperger's disorder: similarities and differences. *World J Biol Psychiatry.* (2010) 11:964–71. doi: 10.3109/15622975.2010.507785

25. Mukaddes NM, Fateh R. High rates of psychiatric co-morbidity in individuals with Asperger's disorder. *World J Biol Psychiatry.* (2010) 11(2 Pt 2):486–92. doi: 10.3109/15622970902789130

26. Gjevik E, Eldevik S, Fjaeran-Granum T, Sponheim E. Kiddie-SADS reveals high rates of DSM-IV disorders in children and adolescents with autism spectrum disorders. *J Autism Dev Disord.* (2011) 41:761–9. doi: 10.1007/s10803-010-1095-7

27. Salazar F, Baird G, Chandler S, Tseng E, O'Sullivan T, Howlin P, et al. Co-occurring psychiatric disorders in preschool and elementary school-aged children with autism spectrum disorder. *J Autism Dev Disord.* (2015) 45:2283–94. doi: 10.1007/s10803-015-2361-5

28. Hanratty J, Livingstone N, Robalino S, Terwee CB, Glod M, Oono IP, et al. Systematic review of the measurement properties of tools used to measure behaviour problems in young children with autism. *PLoS ONE.* (2015) 10:e0144649. doi: 10.1371/journal.pone.0144649

29. Pandolfi V, Magyar CI, Dill CA. An initial psychometric evaluation of the CBCL 6-18 in a sample of youth with autism spectrum disorders. *Res Autism Spectr Disord.* (2012) 6:96–108. doi: 10.1016/j.rasd.2011.03.009

30. Pandolfi V, Magyar CI, Norris M. Validity study of the CBCL 6-18 for the assessment of emotional problems in youth with ASD. *J Ment Health Res Intellect Disabil.* (2014) 7:306–22. doi: 10.1080/19315864.2014. 930547

31. Achenbach TM, Dumenci L, Rescorla LA. DSM-oriented and empirically based approaches to constructing scales from the same item pools. *J Clin Child Adolesc Psychol.* (2003) 32:328–40. doi: 10.1207/S15374424JCCP3203_02

32. Nakamura BJ, Ebesutani C, Bernstein A, Chorpita BF. A psychometric analysis of the child behavior checklist DSM-oriented scales. *J Psychopathol Behav Assess.* (2009) 31:178–89. doi: 10.1007/s10862-008-9119-8

33. Bellina M, Brambilla P, Garzitto M, Negri GA, Molteni M, Nobile M. The ability of CBCL DSM-oriented scales to predict DSM-IV diagnoses in a referred sample of children and adolescents. *Eur Child Adolesc Psychiatry.* (2013) 22:235–46. doi: 10.1007/s00787-012-0343-0

34. Ebesutani C, Bernstein A, Nakamura BJ, Chorpita BF, Higa-McMillan CK, Weisz JR. Concurrent validity of the child behavior checklist DSM-oriented scales: correspondence with DSM diagnoses and comparison to syndrome scales. *J Psychopathol Behav Assess.* (2010) 32:373–84. doi: 10.1007/s10862-009-9174-9

35. Lacalle M, Ezpeleta L, Domenech JM. DSM-oriented scales of the child behavior checklist and youth self-report in clinically referred spanish children. *Span J Psychol.* (2012) 15:377–87. doi: 10.5209/rev_SJOP.2012.v15.n1.37344

36. de la Osa N, Granero R, Trepat E, Domenech JM, Ezpeleta L. The discriminative capacity of CBCL/1(1/2)-5-DSM5 scales to identify disruptive and internalizing disorders in preschool children. *Eur Child Adolesc Psychiatry.* (2016) 25:17–23. doi: 10.1007/s00787-015-0694-4

37. Mesman J, Koot HM. Early preschool predictors of preadolescent internalizing and externalizing DSM-IV diagnoses. *J Am Acad Child Adolesc Psychiatry.* (2001) 40:1029–36. doi: 10.1097/00004583-200109000-00011

38. Shaw DS, Owens EB, Giovannelli J, Winslow EB. Infant and toddler pathways leading to early externalizing disorders. *J Am Acad Child Adolesc Psychiatry.* (2001) 40:36–43. doi: 10.1097/00004583-200101000-00014

39. Egger HL, Angold A. Common emotional and behavioral disorders in preschool children: presentation, nosology, and epidemiology. *J Child Psychol Psychiatry.* (2006) 47:313–37. doi: 10.1111/j.1469-7610.2006.01618.x

40. Bufferd SJ, Dougherty LR, Carlson GA, Rose S, Klein DN. Psychiatric disorders in preschoolers: continuity from ages 3 to 6. *Am J Psychiatry.* (2012) 169:1157–64. doi: 10.1176/appi.ajp.2012.12020268

41. Moseley DS, Tonge BJ, Brereton AV, Einfeld SL. Psychiatric comorbidity in adolescents and young adults with autism. *J Mental Health Res Intellect Disabil.* (2011) 4:229–43. doi: 10.1080/19315864.2011.595535

42. Kerr DC, Lunkenheimer ES, Olson SL. Assessment of child problem behaviors by multiple informants: a longitudinal study from preschool to school entry. *J Child Psychol Psychiatry.* (2007) 48:967–75. doi: 10.1111/j.1469-7610.2007.01776.x

43. Melegari MG, Sacco R, Manzi B, Vittori E, Persico AM. Deficient emotional self-regulation in preschoolers with ADHD: identification, comorbidity, and interpersonal functioning. *J Atten Disord.* (2016). doi: 10.1177/1087054715622015

44. Luby J, Belden A, Sullivan J, Hayen R, McCadney A, Spitznagel E. Shame and guilt in preschool depression: evidence for elevations in self-conscious emotions in depression as early as age 3. *J Child Psychol Psychiatry.* (2009) 50:1156–66. doi: 10.1111/j.1469-7610.2009.02077.x

45. Studts CR, van Zyl MA. Identification of developmentally appropriate screening items for disruptive behavior problems in preschoolers. *J Abnorm Child Psychol.* (2013) 41:851–63. doi: 10.1007/s10802-013-9738-8

46. Hartley SL, Sikora DM, McCoy R. Prevalence and risk factors of maladaptive behaviour in young children with Autistic Disorder. *J Intellect Disabil Res.* (2008) 52:819–29. doi: 10.1111/j.1365-2788.2008.01065.x

47. Hartley SL, Sikora DM. Sex differences in autism spectrum disorder: an examination of developmental functioning, autistic symptoms, and coexisting behavior problems in toddlers. *J Autism Dev Disord.* (2009) 39:1715–22. doi: 10.1007/s10803-009-0810-8

48. Tseng M-H, Fu C-P, Cermak SA, Lu L, Shieh JY. Emotional and behavioral problems in preschool children with autism: relationship with sensory processing dysfunction. *Res Autism Spectr Disord.* (2011) 5:1441–50. doi: 10.1016/j.rasd.2011.02.004

49. Giovagnoli G, Postorino V, Fatta LM, Sanges V, De Peppo L, Vassena L, et al. Behavioral and emotional profile and parental stress in preschool children with autism spectrum disorder. *Res Dev Disabil.* (2015) 45–46:411–21. doi: 10.1016/j.ridd.2015.08.006

50. Vaillancourt T, Haltigan JD, Smith I, Zwaigenbaum L, Szatmari P, Fombonne E, et al. Joint trajectories of internalizing and externalizing problems in preschool children with autism spectrum disorder. *Dev Psychopathol.* (2017) 29:203–14. doi: 10.1017/S0954579416000043

51. Llanes E, Blacher J, Stavropoulos K, Eisenhower A. Parent and teacher reports of comorbid anxiety and ADHD symptoms in children with ASD. *J Autism Dev Disord.* (2018) doi: 10.1007/s10803-018-3701-z [Epub ahead of print].

52. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders. 5th edn.* American Psychiatric Publishing. (2013).

53. American Psychiatric Association. *Diagnostic Statistical Manual of Mental Disorders (DSM).* Washington DC: American Psychiatric Association (1994).

54. Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, et al. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord.* (2000) 30:205–23. doi: 10.1023/A:1005592401947

55. Lord C, Rutter M, DiLavore PC, Risi S, Gotham K, Bishop S. *Autism Diagnostic Observation Schedule.* 2nd ed. Torrance, CA: Western Psychological Services (2012).

56. Gotham K, Pickles A, Lord C. Standardizing ADOS scores for a measure of severity in autism spectrum disorders. *J Autism Dev Disord.* (2009) 39:693–705. doi: 10.1007/s10803-008-0674-3

57. Hus V, Gotham K, Lord C. Standardizing ADOS domain scores: separating severity of social affect and restricted and repetitive behaviors. *J Autism Dev Disord.* (2014) 44:2400–12. doi: 10.1007/s10803-012-1719-1

58. Shumway S, Farmer C, Thurm A, Joseph L, Black D, Golden C. The ADOS calibrated severity score: relationship to phenotypic variables and stability over time. *Autism Res.* (2012) 5:267–76. doi: 10.1002/aur.1238

59. Gotham K, Risi S, Pickles A, Lord C. The autism diagnostic observation schedule: revised algorithms for improved diagnostic validity. *J Autism Dev Disord.* (2007) 37:613–27. doi: 10.1007/s10803-006-0280-1

60. Esler AN, Bal VH, Guthrie W, Wetherby A, Ellis Weismer S, Lord C. The autism diagnostic observation schedule, toddler module: standardized severity scores. *J Autism Dev Disord.* (2015) 45:2704–20. doi: 10.1007/s10803-015-2432-7

61. Roid GH, Miller LJ. Leiter International Performance Scale -Revised: Examiners Manual. Wood Dale, IL: Stoelting (1997).

62. Luiz BD, Knoesen DN. *Administration Manual Griffiths Mental Developmental Scales–Extended Revised: Two to Eight Years. The Test Agency.* Oxford: Hogrefe (2006)

63. Wechsler D. *Wechsler Preschool and Primary Scale of Intelligence–Revised.* San Antonio, TX: Psycological Corporation. (1989).

64. Achenbach TM, Rescorla A. *Manual for the ASEBA Preschool Forms and Profiles.* Burlington, VT: Department of Psychiatry, University of Vermont (2000).

65. Frigerio CP, Pastore AV, Molteni M, Borgatti R, Montirosso R. La valutazione dei problemi emotivo comportamentali in un campione italiano di bambini in età prescolare attraverso la CBCL. *Infanzia e Adolescenza*. (2006) 12:26–33. doi: 10.1710/162.1771

66. Muratori F, Narzisi A, Tancredi R, Cosenza A, Calugi S, Saviozzi I, et al. The CBCL 1.5-5 and the identification of preschoolers with autism in Italy. *Epidemiol Psychiatr Sci.* (2011) 20:329–38. doi: 10.1017/S204579 601100045X

67. Narzisi A, Calderoni S, Maestro S, Calugi S, Mottes E, Muratori F. Child behavior check list 1½−5 as a tool to identify toddlers with autism spectrum disorders: a case-control study. *Res Dev Disabil.* (2013) 34:1179–89. doi: 10.1016/j.ridd.2012.12.020

68. Rescorla L, Kim YA, Oh KJ. Screening for ASD with the Korean CBCL/1(1/2)-5. *J Autism Dev Disord.* (2015) 45:4039–50. doi: 10.1007/s10803-014-2255-y

69. Jensen M, Girirajan S. Mapping a shared genetic basis for neurodevelopmental disorders. *Genome Med.* (2017) 9:109. doi: 10.1186/s13073-017-0503-4

70. Gandal MJ, Haney JR, Parikshak NN, Leppa V, Ramaswami G, Hartl C, et al. Shared molecular neuropathology across major psychiatric disorders parallels polygenic overlap. *Science.* (2018) 359:693–7. doi: 10.1126/science.aad6469

71. van Steensel FJ, Bogels SM, de Bruin EI. Psychiatric comorbidity in children with autism spectrum disorders: a comparison with children with ADHD. *J Child Fam Stud.* (2013) 22:368–376. doi: 10.1007/s10826-012-9587-z

72. Kanne SM, Abbacchi AM, Constantino JN. Multi-informant ratings of psychiatric symptom severity in children with autism spectrum disorders: the importance of environmental context. *J Autism Dev Disord.* (2009) 39:856–64. doi: 10.1007/s10803-009-0694-7

73. White SW, Oswald D, Ollendick T, Scahill L. Anxiety in children and adolescents with autism spectrum disorders. *Clin Psychol Rev.* (2009) 29:216–29. doi: 10.1016/j.cpr.2009.01.003

74. van der Meer JMJ, Lappenschaar MGA, Hartman CA, Greven CU, Buitelaar JK, Rommelse NNJ. Homogeneous combinations of ASD-ADHD traits and their cognitive and behavioral correlates in a population-based sample. *J Atten Disord.* (2017) 21:753–63. doi: 10.1177/1087054714533194

75. Angold A, Costello EJ. The Child and Adolescent Psychiatric Assessment (CAPA). *J Am Acad Child Adolesc Psychiatry.* (2000) 39:39–48. doi: 10.1097/00004583-200001000-00015

76. Cortesi F, Giannotti F, Ivanenko A, Johnson K. Sleep in children with autistic spectrum disorder. *Sleep Med.* (2010) 11:659–64. doi: 10.1016/j.sleep.2010.01.010

77. Bandini LG, Curtin C, Phillips S, Anderson SE, Maslin M, Must A. Changes in food selectivity in children with autism spectrum disorder. *J Autism Dev Disord.* (2017) 47:439–46. doi: 10.1007/s10803-016-2963-6

78. Krakowiak P, Goodlin-Jones B, Hertz-Picciotto I, Croen LA, Hansen RL. Sleep problems in children with autism spectrum disorders, developmental delays, and typical development: a population-based study. *J Sleep Res.* (2008) 17:197–206. doi: 10.1111/j.1365-2869.2008.00650.x

79. Zachor DA, Curatolo P. Recommendations for early diagnosis and intervention in autism spectrum disorders: an Italian-Israeli consensus conference. *Eur J Paediatr Neurol.* (2014) 18:107–18. doi: 10.1016/j.ejpn.2013.09.002

80. Pandolfi V, Magyar CI, Dill CA. Confirmatory factor analysis of the child behavior checklist 1.5-5 in a sample of children with autism spectrum disorders. *J Autism Dev Disord.* (2009) 39:986–95. doi: 10.1007/s10803-009-0716-5

81. Magyar CI, Pandolfi V. Utility of the CBCL DSM-oriented scales in assessing emotional disorders in youth with autism. *Res Autism Spectr Disord.* (2017) 37:11–20. doi: 10.1016/j.rasd.2017.01.009

82. Magnuson KM, Constantino JN. Characterization of depression in children with autism spectrum disorders. *J Dev Behav Pediatr.* (2011) 32:332–40. doi: 10.1097/DBP.0b013e318213f56c

83. Goldin RL, Matson JL, Cervantes PE. The effect of intellectual disability on the presence of comorbid symptoms in children and adolescents with autism spectrum disorder. *Res Autism Spectr Disord.* (2014) 8:1552–6. doi: 10.1016/j.rasd.2014.08.006

84. Tureck K, Matson JL, Cervantes P, Konst MJ. An examination of the relationship between autism spectrum disorder, intellectual functioning, and comorbid symptoms in children. *Res Dev Disabil.* (2014) 35:1766–72. doi: 10.1016/j.ridd.2014.02.013

85. Kurzius-Spencer M, Pettygrove S, Christensen D, Pedersen AL, Cunniff C, Meaney FJ, et al. Behavioral problems in children with autism spectrum disorder with and without co-occurring intellectual disability. *Res Autism Spectr Disord.* (2018) 56:61–71. doi: 10.1016/j.rasd.2 018.09.002

86. Mayes SD, Calhoun SL, Murray MJ, Zahid J. Variables associated with anxiety and depression in children with autism. *J Dev Phys Disabil.* (2011) 23:325–37. doi: 10.1007/s10882-011-9231-7

87. Fodstad JC, Rojahn J, Matson JL. Emergent comorbidity in at risk children with and without autism spectrum disorder—a cross-sectional study. *J Dev Phys Disabil.* (2010) 22:381–400. doi: 10.1007/s10882-010-9202-4

88. Gadow KD, Devincent CJ, Pomeroy J, Azizian A. Comparison of DSM-IV symptoms in elementary school-age children with PDD versus clinic and community samples. *Autism.* (2005) 9:392–415. doi: 10.1177/1362361305056079

89. Mayes SD, Calhoun SL, Mayes RD, Molitoris S. Autism and ADHD: overlapping and discriminating symptoms. *Res Autism Spectr Disord.* (2012) 6:277–85. doi: 10.1016/j.rasd.2011.05.009

90. Craig F, Lamanna AL, Margari F, Matera E, Simone M, Margari L. Overlap between autism spectrum disorders and attention deficit hyperactivity disorder: searching for distinctive/common clinical features. *Autism Res.* (2015) 8:328–37. doi: 10.1002/aur.1449

91. Mansour R, Dovi AT, Lane DM, Loveland KA, Pearson DA. ADHD severity as it relates to comorbid psychiatric symptomatology in children with Autism Spectrum Disorders (ASD). *Res Dev Disabil.* (2017) 60:52–64. doi: 10.1016/j.ridd.2016.11.009

92. Gordon-Lipkin E, Marvin AR, Law JK, Lipkin PH. Anxiety Mood disorder in children with autism spectrum disorder and ADHD. *Pediatrics.* (2018) 141. doi: 10.1542/peds.2017-1377

93. van Steensel FJA, Zegers VM, Bogels SM. Predictors of treatment effectiveness for youth with ASD and comorbid anxiety disorders: it all depends on the family? *J Autism Dev Disord.* (2017) 47:636–45. doi: 10.1007/s10803-016-2956-5

94. Davis TE III, Moree BN, Dempsey T, Hess JA, Jenkins WS, Fodstad JC, et al. The effect of communication deficits on anxiety symptoms in infants and toddlers with autism spectrum disorders. *Behav Ther.* (2012) 43:142–52. doi: 10.1016/j.beth.2011.05.003

95. Green SA, A. Ben-Sasson, Soto TW, Carter AS. Anxiety sensory over-responsivity in toddlers with autism spectrum disorders: bidirectional effects across time. *J Autism Dev Disord.* (2012) 42:1112–9. doi: 10.1007/s10803-011-1361-3

96. Kuusikko S, Haapsamo H, Jansson-Verkasalo E, Hurtig T, Mattila ML, Ebeling H, et al. Emotion recognition in children and adolescents with autism spectrum disorders. *J Autism Dev Disord.* (2009) 39:938–45. doi: 10.1007/s10803-009-0700-0

97. Eussen MLJM, Van Gool AR, Verheij F, De Nijs PFA, Verhulst FC, Greaves-Lord K. The association of quality of social relations, symptom severity and intelligence with anxiety in children with autism spectrum disorders. *Autism.* (2013) 17:723–35. doi: 10.1177/1362361312 453882

98. Mazurek MO, Kanne SM. Friendship internalizing symptoms among children and adolescents with ASD. *J Autism Dev Disord.* (2010) 40:1512–20. doi: 10.1007/s10803-010-1014-y

99. Ezpeleta L, de la Osa N, Domenech JM. Prevalence of DSM-IV disorders, comorbidity and impairment in 3-year-old Spanish preschoolers. *Soc Psychiatry Psychiatr Epidemiol.* (2014) 49:145–55. doi: 10.1007/s00127-013-0683-1

100. Gadow KD, DeVincent CJ, Pomeroy J, Azizian A. Psychiatric symptoms in preschool children with PDD and clinic and comparison samples. *J Autism Dev Disord.* (2004) 34:379–93. doi: 10.1023/B:JADD.0000037415.21458.93

101. Mayes SD, Calhoun SL, Aggarwal R, Baker C, Mathapati S, Anderson R, Petersen C. Explosive, oppositional, and aggressive behavior in children with autism compared to other clinical disorders and typical children. *Res Autism Spect Disord.* (2012) 6:1–10. doi: 10.1016/j.rasd.2011.08.001

102. Estes AM, Dawson G, Sterling L, Munson J. Level of intellectual functioning predicts patterns of associated symptoms in school-age children with autism spectrum disorder. *Am J Ment Retard.* (2007) 112:439–49. doi: 10.1352/0895-8017(2007)112[439:LOIFPP]2.0.CO;2

103. Cervantes PE, Matson JL, Adams HL, Konst MJ. The relationship between cognitive development and conduct problems in young children with autism spectrum disorder. *Res Autism Spectr Disord.* (2014) 8:1287–94. doi: 10.1016/j.rasd.2014.06.015

104. Kimhi Y. Theory of mind abilities and deficits in autism spectrum disorders. *Top Lang Disord.* (2014) 34:4–343. doi: 10.1097/TLD.0000000000000033

105. Klin A, Lin DJ, Gorrindo P, Ramsay G, Jones W. Two-year-olds with autism orient to non-social contingencies rather than biological motion. *Nature.* (2009) 459:257–61. doi: 10.1038/nature07868

106. Wang J, Gao Y, Kwok HHM, Huang WYJ, Li S, Li L. Children with Intellectual Disability Are Vulnerable to Overweight and Obesity: A Cross-Sectional Study among Chinese Children. *Child Obes.* (2018) 14:316–26. doi: 10.1089/chi.2018.0015

107. Duarte CS, Bordin IA, de Oliveira A, Bird H. The CBCL and the identification of children with autism and related conditions in Brazil: pilot findings. *J Autism Dev Disord.* (2003) 33:703–7. doi: 10.1023/B:JADD.0000006005.31818.1c

108. Verhoeff ME, Blanken LME, Kocevska D, Mileva-Seitz VR, Jaddoe VWV, White T, et al. The bidirectional association between sleep problems and autism spectrum disorder: a population-based cohort study. *Mol Autism.* (2018) 9:8. doi: 10.1186/s13229-018-0194-8

109. Whitehouse AJ, Hickey M, Ronald A. Are autistic traits in the general population stable across development? *PLoS ONE.* (2011) 6:e23029. doi: 10.1371/journal.pone.0023029

110. Frazier TW, Georgiades S, Bishop SL, Hardan AY. Behavioral cognitive characteristics of females and males with autism in the Simons Simplex Collection. *J Am Acad Child Adolesc Psychiatry.* (2014) 53:329-40.e1-3. doi: 10.1016/j.jaac.2013.12.004

111. Hull L, Mandy W, Petrides KV. Behavioural cognitive sex/gender differences in autism spectrum condition and typically developing males and females. *Autism.* (2017) 21:706–27. doi: 10.1177/1362361316669087

112. Andersson GW, Gillberg C, Miniscalco C. Pre-school children with suspected autism spectrum disorders: do girls and boys have the same profiles? *Res Dev Disabil.* (2013) 34:413–22. doi: 10.1016/j.ridd.2012.08.025

113. Postorino V, Fatta LM, De Peppo L, Giovagnoli G, Armando M, Vicari S, et al. Longitudinal comparison between male and female preschool children with autism spectrum disorder. *J Autism Dev Disord.* (2015) 45:2046–55. doi: 10.1007/s10803-015-2366-0

114. Zwaigenbaum L, Bryson SE, Szatmari P, Brian J, Smith IM, Roberts W, et al. Sex differences in children with autism spectrum disorder identified within a high-risk infant cohort. *J Autism Dev Disord.* (2012) 42:2585–96. doi: 10.1007/s10803-012-1515-y

115. Mandell DS, Novak MM, Zubritsky CD. Factors associated with age of diagnosis among children with autism spectrum disorders. *Pediatrics.* (2005) 116:1480–6. doi: 10.1542/peds.2005-0185

116. Zwaigenbaum L, Bryson SE, Brian J, Smith IM, Roberts W, Szatmari P, et al. Stability of diagnostic assessment for autism spectrum disorder between 18 and 36 months in a high-risk cohort. *Autism Res.* (2016) 9:790–800. doi: 10.1002/aur.1585

117. Herlihy L, Knoch K, Vibert B, Fein D. Parents' first concerns about toddlers with autism spectrum disorder: effect of sibling status. *Autism.* (2015) 19:20–8. doi: 10.1177/1362361313509731

118. Glascoe FP. Parents' evaluation of developmental status: how well do parents' concerns identify children with behavioral and emotional problems? *Clin Pediatr.* (2003) 42:133–8. doi: 10.1177/000992280304200206

119. Wiggins LD, Rubenstein E, Daniels J, DiGuiseppi C, Yeargin-Allsopp M, Schieve LA, et al. A phenotype of childhood autism is associated with preexisting maternal anxiety and depression. *J Abnorm Child Psychol.* (2018) doi: 10.1007/s10802-018-0469-8. [Epub ahead of print].

120. Richters JE. Depressed mothers as informants about their children: a critical review of the evidence for distortion. *Psychol Bull.* (1992) 112:485–99. doi: 10.1037/0033-2909.112.3.485

121. Visser JC, Rommelse NNJ, Lappenschaar M, Servatius-Oosterling IJ, Greven CU, Buitelaar JK. Variation in the early trajectories of autism symptoms is related to the development of language, cognition, and behavior problems. *J Am Acad Child Adolesc Psychiatry.* (2017) 56:659–68. doi: 10.1016/j.jaac.2017.05.022

122. Ozonoff S, Young GS, Landa RJ, Brian J, Bryson S, Charman T, et al. Diagnostic stability in young children at risk for autism spectrum disorder: a baby siblings research consortium study. *J Child Psychol Psychiatry.* (2015) 56:988–98. doi: 10.1111/jcpp.12421

123. Barbaro J, Dissanayake C. Diagnostic stability of autism spectrum disorder in toddlers prospectively identified in a community-based setting: behavioural characteristics and predictors of change over time. *Autism.* (2017) 21:830–40. doi: 10.1177/1362361316654084

124. Chawarska K, Klin A, Paul R, Volkmar F. Autism spectrum disorder in the second year: stability and change in syndrome expression. *J Child Psychol Psychiatry.* (2007) 48:128–38. doi: 10.1111/j.1469-7610.2006.01685.x

125. Nilsson Jobs E, Bolte S, Falck-Ytter T. Spotting signs of autism in 3-year-olds: comparing information from parents and preschool staff. *J Autism Dev Disord.* (2018) 49:1232–41. doi: 10.1007/s10803-018-3821-5

126. Ozonoff S, Young GS, Steinfeld MB, Hill MM, Cook I, Hutman T, et al. How early do parent concerns predict later autism diagnosis? *J Dev Behav Pediatr.* (2009) 30:367–75. doi: 10.1097/DBP.0b013e3181ba0fcf

Sex-Gender Comparisons in Comorbidities of Children and Adolescents With High-Functioning Autism Spectrum Disorder

Lucia Margari[1]*, Roberto Palumbi[1], Antonia Peschechera[1], Francesco Craig[2], Concetta de Giambattista[1], Patrizia Ventura[1] and Francesco Margari[1]

[1] *Basic Medical Sciences Neuroscience and Sense Organs Department, University of Bari Aldo Moro, Bari, Italy, [2] Unit for Severe Disabilities in Developmental Age and Young Adults, Developmental Neurology and Neurorehabilitation, Scientific Institute IRCCS E. Medea, Brindisi, Italy*

Over the last few years, new studies focused their attention on the gender-related features in high-functioning autism spectrum disorder (HFA), often leading to controversial results. Another interesting aspect of these subtype of patients is linked to the complexity of clinical presentation, where besides core symptoms, other co-occurrence disorders may complicate the diagnostic evaluation. Therefore, we retrospectively studied 159 HFA patients, male and female, investigating their comorbidities and to find any gender difference. For each patient, were evaluated the presence/absence, type and gender distribution of psychopathological comorbidities, according to DSM-5 diagnostic criteria. The total sample was divided in 100 male and 59 female patients, age and intelligence quotient matched. In our sample, the psychiatric comorbidities observed were Attention Deficit Hyperactivity Disorder, Anxiety Disorders, Depressive Disorders, Bipolar Disorder, Obsessive-Compulsive Disorder, and Anorexia Nervosa. No statistical significant differences were found between male and female HFA patients comorbidities except for Anorexia Nervosa. In both male and female patients, attention deficit and hyperactivity disorder and anxiety disorders were found in high percentage. In conclusion, our investigation showed that a statistical significant difference of comorbidity between male and female HFA patients was found only for AN diagnosis. However, the question about the distinction between female and male HFA patients remains quite interesting and an open area of research for future studies.

Keywords: high-functioning, autism spectrum disorder, psychopathological comorbidities, gender distribution, anorexia nervosa, ADHD, anxiety disorders, mood disorders

INTRODUCTION

It is well-known that in Autism Spectrum Disorder (ASD), males are over-represented than females, with an average gender ratio of 4.3 males to 1 female (1). Many theories have emerged in order to explain this difference in gender distribution but no one explanation appears to be conclusive (2–5). Females are different from males both in core symptoms and in comorbidity; this probably causes the difficulty in their detection. Data show that the gender ratio M:F is intelligence quotient (IQ)-related, varying from 5.75:1 in cognitively high-functioning children (HF; full-scale IQ higher than 70) to 1.9:1 in low-functioning children (LF; full-scale IQ lower than 70) (5–9).

The definition of High Functioning Autism Spectrum Disorder (HFA) refers to the category of ASD "without cognitive impairment" specified by the Diagnostic and Statistical Manual of Mental Disorders—Fifth Edition (10).

This variance shows that HF females have been the most difficult to detect, probably because of milder symptom presentation (11–13) or methodological bias (ex. lack of specific diagnostic tools) (14–16). With increased agreement on the definition of ASD and improvement in case detection, recent studies are identifying more HF females (17). Beyond the sex differences in phenotype, males and females could differ in coexisting psychopathology (5) so there has been an emergence of research focusing on gender differences in ASD regarding comorbidity. The most common practice to date for identifying comorbid psychopathologies had been the use of the Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria. Literature on comorbid psychopathologies, based on the new edition of DSM criteria (DSM-5), is beginning to develop, even if with mixed findings, probably caused by different methodologies (related to stratification for IQ and age) and by small samples.

A report described that boys with ASD are more likely to experience externalizing disorders such as ADHD and oppositional defiant disorder (18), above all in childhood, while it is reported that girls with ASD may be at especially high risk for internalizing psychopathology (8).

Moreover, studies have shown more hyperactive behaviors in HF boys than girls with ASD (19), while no gender differences in LF children (20–23) were found.

Nevertheless, differences also emerged with respect to internalizing disorders as HF girls with ASD evidenced significant anxiety symptoms compared to boys, mainly in adolescence (8), suggesting that the typical gender developmental trajectory of anxiety may be present. However, it might be quite difficult to distinguish if anxiety symptoms or repetitive behaviors would belong to the core ASD symptoms or might be signs of a comorbid condition with an anxiety and/or an obsessive-compulsive disorder (24). In the same way as for anxiety symptoms, other affective disorders, such as depressive disorders, are reported in childhood and at levels dramatically higher in adolescent girls (16, 19, 25). Lastly, in ASD patients are largely reported abnormal eating behaviors and/or conducts (26).

However, sex differences in HFA comorbidities is still poorly understood. Therefore, the aim of this retrospective study was to investigate whether male and female HFA patients might develop specific comorbidities phenotype, using well-defined samples regarding factors like age and IQ.

MATERIALS AND METHODS

This retrospective study included children and adolescents admitted to the Child and Adolescent Neuropsychiatry Unit between April 2016 and May 2018 and diagnosed with HF ASD, according to DSM-5 diagnostic criteria. For each patient, were evaluated the presence/absence, type and gender distribution of psychopathological comorbidities. All participants were drug-naïve.

All demographic and clinical variables were subjected to statistical analysis. Descriptive analysis was conducted for sociodemographic and clinical features. Quantitative variables (IQ and age) were presented as mean ± standard deviation (SD); qualitative variables (psychopathological comorbidities and ASD severity level) were expressed as percentages. The chi-square test ($\chi 2$) was used to compare qualitative variables between male and female with ASD. The $\chi 2$ enabled us to compare observed and expected frequencies of dichotomous variables objectively. Statistical significance, in this case, is due to the difference between observed and expected frequencies. Both groups were compared with the independent sample t test for comparison of age and IQ mean score. All the statistical analyses were considered significant with a p-value equal or lower than 0.05. For statistical processing, we used the Statistical Package for Social Science version 20.0.

RESULTS

The sample included 159 patients with HF ASD diagnosis. **Table 1** summarizes patients demographic and clinical data. The total sample included 100 males (mean age: 9.91 ± 4 DS) and 59 females (mean age: 10.97 ± 4.7 DS). No significant statistical differences for age and Intelligent Quotient were found between male and female patients. In 44% of male patients at least one comorbidity was diagnosed, while, in female patients, 72.8% presented at least one comorbidity.

In both male and female patients, ADHD and Anxiety Disorders were the most frequent diagnoses. Anorexia Nervosa diagnosis resulted more frequent among female patients, with a statistical significant difference ($p = 0.04$) (**Table 2**).

DISCUSSION

It is well-established that the most difficult challenge in ASD diagnostic process is based on the recognition and discrimination of the frequent co-occurrence of this disorder with other conditions, such as other neurodevelopment and/or psychiatric disorders. In fact, core symptoms of ASD often mask psychiatric comorbid symptoms and viceversa (27–29). Furthermore, there are potential similarities of how the symptoms of these disorders appear (24).

The purpose of this study was to investigate HF ASD psychopathological comorbidities between male and female patients. In our sample, high rates of comorbidities were found.

TABLE 1 | Demographic and clinical characteristics of ASD patients.

	ASD male (n = 100)	ASD female (n = 59)	p
Age (mean ± SD)	9.91 ± 4	10.97 ± 4.7	0.140
IQ (mean ± SD)	108 ± 16	104 ± 18	0.137
ASD SEVERITY LEVEL			
Level 1	84	46	0.341
Level 2	16	13	0.341

ASD, autism spectrum disorder; IQ, intelligence quotient; SD, standard deviation.

Neuropsychiatric diagnosis	ASD male (%)	ASD female (%)	p
Attention deficit hyperactivity disorder	32	42.7	0.156
Anxiety disorders	18	25.4	0.265
Depressive disorders	6	13.5	0.104
Bipolar disorder	4	3.4	0.845
Obsessive-Compulsive disorder	5	0	0.081
Anorexia nervosa	1	6.8	**0.004**

ASD, autism spectrum disorder. Bold values indicate statistical significant p-value.

Moreover, 55% of the total sample presented at least one comorbidity, with a higher rate in females (72.8%) than males (44%). This finding about the high prevalence of comorbidities in ASD is in accordance with literature data (28). The difference between the comorbidities rates between male and female patients in our sample may be a statistical bias due to the disproportion of the sample sizes. Eventually larger sample studies would contribute to clarify if this percentage difference of comorbidity exists or not. Moreover, in female HFA patients, it is more difficult to recognize psychiatric comorbidities for several reasons. First, diagnostic criteria and/or tools are mainly male-targeted complicating their detection; moreover, females less refer to clinical attention since their impairment is even less clear than in male patients.

In our sample, the psychiatric comorbidities observed (**Table 2**) were ADHD, Anxiety Disorders (AD), Depressive Disorders (DD), Bipolar Disorder (BD), Obsessive-Compulsive Disorder (OCD), and Anorexia Nervosa (AN). No statistical significant differences were found between male and female HFA patients comorbidities except for AN.

The most frequent comorbidity was ADHD with no statistical significant difference between male and female subjects. Over the years, and lastly after the publication of DSM-5, the overlap between ASD and ADHD has been supported by an increasing number of studies (28, 30–35). A recent study estimated that the prevalence of the co-occurring of ASD and ADHD is about 37–85% of children with ASD (35), supporting the hypothesis of a common neurobiological pathogenesis (35, 36). Moreover, comparing male with female, we found that ADHD with "Predominantly inattention presentation" prevails on female subgroup; while in male subgroup "Combined presentation" is the most frequent clinical presentation. Potentially, associated difficulties commonly occurring in HFA, like externalizing/disruptive behaviors, could differ between boys and girls prompting gender differences in the clinical referral. Girls without externalizing behaviors/hyperactivity could be overlooked for assessment and educational support despite largely similar cognitive, academic and behavioral profiles to boys (37, 38).

In our sample, Anxiety Disorders was found in high percentage of both male and female patients, without any statistical significant difference. In a recent review, Tarazi et al. (39) found that people with HFA experience more anxiety symptoms (such as tension, apprehension, panic, attention deficits) compared to other ASD patients. It is suggested that, in this subtype of patients, anxiety is more likely related to their inability to face social interactions, changing in daily routines or modulation of their emotional experiences.

In our sample, Depressive and Bipolar Disorders appeared to be less frequent than other comorbidities. This finding is probably due to the typical adolescent onset of these mood disorders in ASD, and the mean age of our sample was about 10 years old (8, 16).

In our total sample, OCD diagnosis was found in 3%; this finding probably is understandable considering that OCD could appear particularly difficult to identify in the context of an ASD because of their potential similarities only in five male patients. Moreover, OCD was diagnosed only in five male patients; this may be probably explained by the fact that restrictive interests and ritualistic behaviors in female HFA patients appear to be more socially acceptable (40).

Anorexia Nervosa was more frequent among female subjects with a statistical significant difference. This result reflects the epidemiology of AN, considering that this disorder predominantly affects female patients in early adolescence (41). It is suggested that both genetic and psychosocial risk factors may contribute to this gender difference of AN prevalence. Firstly, during puberty, ovarian hormones are directly involved in genetic effects as transcriptional mediators of neural expression of transmission systems disrupted in eating disorder (e.g., serotoninergic system) (42, 43). Secondly, young girls are more exposed than males to sociocultural factors that may contribute to increase the risk of AN (e.g., pressure about weight and body image, thinness and cultural model) (43, 44). Moreover, it is well-known how early testosterone exposure has a huge impact on brain plasticity and organization, and this effect may contribute to protect male subjects from developing an eating disorder (45–48). The association between AN and ASD was firstly described in 1980s when Gillberg observed that three male autistic patients had a familiar history for AN (49, 50). The author hypothesized that common factors may contribute to develop autism in young boys and AN in female relatives. On the other hand, dysfunctional eating behaviors (such as restrictive and/or limited food intake or repertoire) represents one of the main clinical aspects of ASD (51). Moreover, it is reported that abnormal eating features may be precursors of AN in patients with ASD (52). Nevertheless, patients affected by AN share clinical similarity with ASD (e.g., set-shifting deficits, reduced cognitive flexibility, deficits in emotion recognition, and social cognition) and some of these features might be potential risk factors to develop AN (41). It is suggested that these aspects may be explained by a possible neuropsychological overlap between the two disorders (deficit in central coherence, theory of mind and executive functions theories) (53). A recent study investigated gray matter volumetric aspects in female patients affected by AN and autistic traits. The results showed that higher autistic traits correlate with volumetric alterations of brain regions involved in the social cognition, supporting a valuable link of the association between the two disorders (54). In addition, the overlap between ASD and AN may be supported by other common neurobiological basis, including the

involvement of the dopaminergic system. Variations in dopamine transporters and dopamine receptors gene (e.g., DAT1 and DRD4) are strongly linked to ASD (55–58). Nevertheless, it is known that dopamine is one the crucial neuromediator involved in feeding behavior, distortion of body image perception (59, 60). In fact, mesolimbic dopamine pathways play a crucial role in food reward, anticipation of food and social recognition mechanisms (61, 62). Moreover, specific genotype of DRD4 (DRD4 7R/R), leading to a reduced expression of the receptor, appears to be strongly linked to the risk of AN (59, 63). However, the complete neurobiological basis of the overlap between AN and ASD are quite uncertain and still not defined.

In conclusion, our investigation showed that a statistical significant difference of comorbidity between male and female HFA patients was found only for AN diagnosis. However, the question about the distinction between female and male HFA patients remains quite interesting and open. Furthermore, female HFA patients are often overlooked, misdiagnosed and underestimated; therefore, this retrospective study might be preliminary for future and larger-sampled research on this category of patients.

ETHICS STATEMENT

For this study, an ethical review process by the Local Ethics Committee of Azienda Ospedaliero-Universitaria Policlinico di Bari (Italy) was not required, since all the procedures within the study assessment are included in the diagnostic protocol of our Child and Adolescence Neuropsychiatry Unit. All the participants were recruited after obtaining a written informed consent by their parents.

AUTHOR CONTRIBUTIONS

REFERENCES

1. Fombonne E. Epidemiology of pervasive developmental disorders. *Pediatr Res.* (2009) 65:591–8. doi: 10.1203/PDR.0b013e31819e7203
2. Kopp S, Gillberg C. Girls with social deficits and learning problems: Autism, atypical Asperger syndrome or a variant of these conditions. *Eur Child Adolesc Psychiatry.* (1992) 1:89–99. doi: 10.1007/BF02091791
3. Baron-Cohen S. The extreme male brain theory of autism. *Trends Cogn Sci.* (2002) 6:248–254.
4. Baron-Cohen S. About 1% of children in the South Thames region have an autistic spectrum disorder. *Evid Based Ment Health.* (2007) 10:28. doi: 10.1136/ebmh.10.1.28
5. Holtmann M, Bölte S, Poustka F. Autism spectrum disorders: sex differences in autistic behaviour domains and coexisting psychopathology. *Dev Med Child Neurol.* (2007) 49:361–6. doi: 10.1111/j.1469-8749.2007.00361.x
6. Fombonne E. Epidemiological surveys of autism and other pervasive developmental disorders: an update. *J Autism Dev Disord.* (2003) 33:365–82.
7. Yeargin-Allsopp M, Rice C, Karapurkar T, Doernberg N, Boyle C, Murphy C. Prevalence of autism in a US metropolitan area. *JAMA.* (2003) 289:49–55.
8. Solomon M, Miller M, Taylor SL, Hinshaw SP, Carter CS. Autism symptoms and internalizing psychopathology in girls and boys with autism spectrum disorders. *J Autism Dev Disord.* (2012) 42:48–59. doi: 10.1007/s10803-011-1215-z
9. Frazier TW, Georgiades S, Bishop SL, Hardan AY. Behavioral and cognitive characteristics of females and males with autism in the simons simplex collection. *J Am Acad Child Adolesc Psychiatry.* (2014) 53:329–40.e1–3. doi: 10.1016/j.jaac.2013.12.004
10. American Psychiatric Association (APA). *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed. Arlington, VA: American Psychiatric Publishing (2013).
11. Constantino JN1, Todd RD. Autistic traits in the general population: a twin study. *Arch Gen Psychiatry.* (2003) 60:524–30. doi: 10.1001/archpsyc.60.5.524
12. Russell G, Steer C, Golding J. Social and demographic factors that influence the diagnosis of autistic spectrum disorders. *Soc Psychiatry Psychiatr Epidemiol.* (2011) 46:1283–93. doi: 10.1007/s00127-010-0294-z
13. Dworzynski K, Ronald A, Bolton P, Happé F. How different are girls and boys above and below the diagnostic threshold for autism spectrum disorders? *J Am Acad Child Adolesc Psychiatry.* (2012) 51:788–97. doi: 10.1016/j.jaac.2012.05.018
14. Van Wijngaarden-Cremers PJ, van Eeten E, Groen WB, Van Deurzen PA, Oosterling IJ, Van der Gaag RJ. Gender and age differences in the core triad of impairments in autism spectrum disorders: a systematic review and meta-analysis. *J Autism Dev Disord.* (2014) 44:627–35. doi: 10.1007/s10803-013-1913-9
15. Beggiato A, Peyre H, Maruani A, Scheid I, Rastam M, Amsellem F, et al. Gender differences in autism spectrum disorders: Divergence among specific core symptoms. *Autism Res.* (2017) 10:680–689. doi: 10.1002/aur.1715
16. Hull L, Mandy W, Petrides KV. Behavioural and cognitive sex/gender differences in autism spectrum condition and typically developing males and females. *Autism.* (2017) 21:706–27. doi: 10.1177/1362361316669087
17. Zwaigenbaum L, Bryson SE, Szatmari P, Brian J, Smith IM, Roberts W et al. Sex differences in children with autism spectrum disorder identified within a high-risk infant cohort. *J Autism Dev Disord.* (2012) 42:2585–96. doi: 10.1007/s10803-012-1515-y
18. American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders*. 4th edn. Washington DC: American Psychiatric Association.
19. Oswald TM, Winter-Messiers MA, Gibson B, Schmidt AM, Herr CM, Solomon M. Sex Differences in internalizing problems during adolescence in autism spectrum disorder. *J Autism Dev Disord.* (2016) 46:624–36. doi: 10.1007/s10803-015-2608-1.
20. Gadow KD, Devincent CJ, Pomeroy J, Azizian A. Comparison of DSM-IV symptoms in elementary school-age children with PDD versus clinic and community samples. *Autism.* (2005) 9:392–415. doi: 10.1177/1362361305056079
21. Brereton AV, Tonge BJ, Einfeld SL. Psychopathology in children and adolescents with autism compared to young people with intellectual disability. *J Autism Dev Disord.* (2006) 36:863–70. doi: 10.1007/s10803-006-0125-y
22. Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G. Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. *J Am Acad Child Adolesc Psychiatry.* (2008) 47:921–9. doi: 10.1097/CHI.0b013e318179964f
23. Mandy W, Charman T, Gilmour J, Skuse D. Toward specifying pervasive developmental disorder-not otherwise specified. *Autism Res.* (2011) 4:121–31. doi: 10.1002/aur.178
24. Matson JL, Cervantes PE. Commonly studied comorbid psychopathologies among persons with autism spectrum disorder. *Res Dev Disabil.* (2014) 35:952–62. doi: 10.1016/j.ridd.2014.02.012
25. Lugnegård T, Hallerbäck MU, Gillberg C. Psychiatric comorbidity in young adults with a clinical diagnosis of Asperger syndrome. *Res Dev Disabil.* (2011) 32:1910–7. doi: 10.1016/j.ridd.2011.03.025
26. Karjalainen L, Råstam M, Paulson-Karlsson G, Wentz E. Do autism spectrum disorder and anorexia nervosa have some eating

disturbances in common? *Eur Child Adolesc Psychiatry.* (2018) 28:69–78. doi: 10.1007/s00787-018-1188-y

27. Mazzone L, Ruta L, Reale L. Psychiatric comorbidities in asperger syndrome and high functioning autism: diagnostic challenges. *Ann Gen Psychiatry.* (2012) 11:16. doi: 10.1186/1744-859X-11-16

28. Salazar F, Baird G, Chandler S, Tseng E, O'sullivan T, Howlin P et al. Co-occurring psychiatric disorders in preschool and elementary school-aged children with autism spectrum disorder. *J Autism Dev Disord.* (2015) 45:2283–94. doi: 10.1007/s10803-015-2361-5

29. Postorino V, Kerns CM, Vivanti G, Bradshaw J, Siracusano M, Mazzone L. Anxiety disorders and obsessive-compulsive disorder in individuals with autism spectrum disorder. *Curr Psychiatry Rep.* (2017) 19:92. doi: 10.1007/s11920-017-0846-y

30. Taylor MJ, Charman T, Robinson EB, Plomin R, Happé F, Asherson P et al. Developmental associations between traits of autism spectrum disorder and attention deficit hyperactivity disorder: a genetically informative, longitudinal twin study. *Psychol Med.* (2013) 43:1735–46. doi: 10.1017/S003329171200253X

31. Cooper M, Martin J, Langley K, Hamshere M, Thapar A. Autistic traits in children with ADHD index clinical and cognitive problems. *Eur Child Adolesc Psychiatry.* (2014) 23:23–34. doi: 10.1007/s00787-013-0398-6

32. Taylor MJ, Charman T, Ronald A. Where are the strongest associations between autistic traits and traits of ADHD? Evidence from a community-based twin study. *Eur Child Adolesc Psychiatry.* (2015) 24:1129–38. doi: 10.1007/s00787-014-0666-0

33. Antshel KM, Zhang-James Y, Wagner KE, Ledesma A, Faraone SV. An update on the comorbidity of ADHD and ASD: a focus on clinical management. *Exp Rev Neurother.* (2016) 16:279–93. doi: 10.1586/14737175.2016.1146591

34. Craig F, Margari F, Legrottaglie AR, Palumbi R, de Giambattista C, Margari L. A review of executive function deficits in autism spectrum disorder and attention-deficit/hyperactivity disorder. *Neuropsychiatr Dis Treat.* (2016) 12:1191–202. doi: 10.2147/NDT.S104620

35. Leitner Y. The co-occurrence of autism and attention deficit hyperactivity disorder in children—what do we know? *Front Hum Neurosci.* (2014) 8:268. doi: 10.3389/fnhum.2014.00268

36. Lamanna AL, Craig F, Matera E, Simone M, Buttiglione M, Margari L. Risk factors for the existence of attention deficit hyperactivity disorder symptoms in children with autism spectrum disorders. *Neuropsychiatr Dis Treat.* (2017) 15:1559–67. doi: 10.2147/NDT.S132214

37. May T, Cornish K, Rinehart N. Does gender matter? A one year follow-up of autistic, attention and anxiety symptoms in high-functioning children with autism spectrum disorder. *J Autism Dev Disord.* (2014) 44:1077–86. doi: 10.1007/s10803-013-1964-y

38. Mowlem FD, Rosenqvist MA, Martin J, Lichtenstein P, Asherson P, Larsson H. Sex differences in predicting ADHD clinical diagnosis and pharmacological treatment. *Eur Child Adolesc Psychiatry.* (2018). doi: 10.1007/s00787-018-1211-3. [Epub ahead of print].

39. Tarazi FI, Sahli ZT, Pleskow J, Mousa SA. Asperger's syndrome: diagnosis, comorbidity and therapy. *Exp Rev Neurother.* (2015) 15:281–93. doi: 10.1586/14737175.2015.1009898

40. Hiller RM, Young RL, Weber N. Sex differences in autism spectrum disorder based on DSM-5 criteria: evidence from clinician and teacher reporting. *J Abnorm Child Psychol.* (2014) 42:1381–93. doi: 10.1007/s10802-014-9881-x

41. Treasure J, Zipfel S, Micali N, Wade T, Stice E, Claudino A. et al. Anorexia nervosa. *Nat Rev Dis Primers.* (2015) 26:15074. doi: 10.1038/nrdp.2015.74

42. Klump KL, Keel PK, Sisk C, Burt SA. Preliminary evidence that estradiol moderates genetic influences on disordered eating attitudes and behaviors during puberty. *Psychol Med.* (2010) 40:1745–53. doi: 10.1017/S0033291709992236

43. Klump KL, Culbert KM, Slane JD, Burt SA, Sisk CL, Nigg JT. The effects of puberty on genetic risk for disordered eating: evidence for a sex difference. *Psychol Med.* (2012) 42:627–37. doi: 10.1017/S0033291711001541

44. Cafri G, Yamamiya Y, Brannick M, Thompson JK. The influence of sociocultural factors on body image: a meta-analysis. *Clin Psychol.* (2005) 12:421–33. doi: 10.1093/clipsy/bpi053

45. Culbert KM, Breedlove SM, Burt SA, Klump KL. Prenatal hormone exposure and risk for eating disorders: A comparison of opposite- and same-sex twins. *Arch Gene Psychiatry.* (2008) 65:329–336. doi: 10.1001/archgenpsychiatry.2007.47

46. Culbert KM, Burt SA, Sisk CL, Nigg JT, Klump KL. The effects of circulating testosterone and pubertal maturation on risk for disordered eating symptoms in adolescent males. *Psychol Med.* (2014) 44:2271–86. doi: 10.1017/S0033291713003073

47. Smith AR, Hawkeswood SE, Joiner TE. The measure of a man: Associations between digit ration and disordered eating in males. *Int J Eating Disord.* (2010) 43:543–8. doi: 10.1002/eat.20736

48. Gagnidze K, Pfaff DW, Mong JA. Gene expression in neuroendocrine cells during the critical period for sexual differentiation of the brain. *Progress Brain Res.* (2010) 186:97–111. doi: 10.1016/B978-0-444-53630-3.00007-5

49. Gillberg C. Are autism and anorexia nervosa related? *Br J Psychiatry.* (1983) 142:428b. doi: 10.1192/bjp.142.4.428b

50. Gillberg C. Autism and anorexia nervosa: related conditions? *Nordic J Psychiatry.* (1985) 39:307–12. doi: 10.3109/08039488509101911

51. Matson JL, Fodstad JC. The treatment of food selectivity and other feeding problems in children with autism spectrum disorders. *Res Autism Spectrum Disord.* (2009) 3:455–61. doi: 10.1016/j.rasd.2008.09.005

52. Mandy W, Tchanturia K. Do women with eating disorders who have social and flexibility difficulties really have autism? A case series. *Mol Autism.* (2015) 6:6. doi: 10.1186/2040-2392-6-6

53. Zhou ZC, McAdam DB, Donnelly DR. Endophenotypes: a conceptual link between anorexia nervosa and autism spectrum disorder. *Res Dev Disabil.* (2018) 82:153–65. doi: 10.1016/j.ridd.2017.11.008

54. Björnsdotter M, Davidovic M, Karjalainen L, Starck G, Olausson H, Wentz E. Grey matter correlates of autistic traits in women with anorexia nervosa. *J Psychiatry Neurosci.* (2018) 43:79–86. doi: 10.1503/jpn.170072

55. Gadow KD, Roohi J, DeVincent CJ, Hatchwell E. Association of ADHD, tics, and anxiety with dopaminetransporter (DAT1) genotype in autism spectrumdisorder. *J Child Psychol Psychiatry.* (2008) 49:1331–8. doi: 10.1111/j.1469-7610.2008.01952.x

56. Anderson BM, Schnetz-Boutaud N, Bartlett J, Wright HH, Abramson RK, Cuccaro ML, et al. Examination of association to autism of common geneticvariationin genes related to dopamine. *Autism Res.* (2008) 1:364–9. doi: 10.1002/aur.55

57. Sun X, Yue J, Zheng C. Study of dopamine transporterimaging on the brain of children with autism. *J Biomed Eng.* (2008) 25:327–330

58. Baskerville TA, Douglas AJ. Dopamine and oxytocin interactions underlying behaviors: potential contributions to behavioral disorders. *CNS Neurosci Ther.* (2010) 16:e92–123. doi: 10.1111/j.1755-5949.2010.00154.x

59. Gervasini G, Gordillo I, García-Herráiz A, Flores I, Jiménez M, Monge M, Carrillo JA. Influence of dopamine polymorphisms on the risk for anorexia nervosa and associated psychopathological features. *J Clin Psychopharmacol.* (2013) 33:551–5. doi: 10.1097/JCP.0b013e3182970469

60. Södersten P, Bergh C, Leon M, Zandian M. Dopamine and anorexia nervosa. *Neurosci Biobehav Rev.* (2016) 60:26–30. doi: 10.1016/j.neubiorev.2015.11.003

61. Caine SB, Koob GF. Effects of mesolimbic dopaminedepletion on responding maintained by cocaine andfood. *J Exp Anal Behav.* (1994) 61:213–21.

62. Narayanan NS, Guarnieri DJ, DiLeone RJ. Metabolichormones, dopamine circuits, and feeding. *Front Neuroendocrinol.* (2010) 31:104–12. doi: 10.1016/j.yfrne.2009.10.004

63. Schoots O, Van Tol HH. The human dopamine D4 receptor repeat sequences modulate expression. *Pharmacogenomics J.* (2003) 3:343–8. doi: 10.1038/sj.tpj.6500208

The Relationship Among Gastrointestinal Symptoms, Problem Behaviors, and Internalizing Symptoms in Children and Adolescents With Autism Spectrum Disorder

Bradley J. Ferguson [1,2,3*], Kristen Dovgan [4], Nicole Takahashi [2] and David Q. Beversdorf [2,3,5]

[1] Department of Health Psychology, University of Missouri School of Health Professions, Columbia, MO, United States,
[2] University of Missouri Thompson Center for Autism and Neurodevelopmental Disorders, Columbia, MO, United States,
[3] Department of Radiology, University of Missouri School of Medicine, Columbia, MO, United States, [4] Department of
Psychology, Marist College, Poughkeepsie, NY, United States, [5] William and Nancy Thompson Endowed Chair in Radiology,
Departments of Neurology and Psychological Sciences, University of Missouri, Columbia, MO, United States

Background: Many individuals with autism spectrum disorder (ASD) have co-occurring gastrointestinal (GI) symptoms, but the etiology is poorly understood. These GI symptoms often coincide with problem behaviors and internalizing symptoms, which reduces the quality of life for these individuals.

Methods: This study examined the relationships among GI problems, problem behaviors, and internalizing symptoms in a sample of 340 children and adolescents with ASD who are patients at the University of Missouri Thompson Center for Autism & Neurodevelopmental Disorders.

Results: The majority of patients experienced constipation (65%), about half experienced stomachaches or stomach pain (47.9%), and others experienced nausea (23.2%) or diarrhea (29.7%). Young children with aggressive problem behaviors were 11.2% more likely to have co-occurring nausea; whereas, older children showed more complex relationships between internalizing symptoms and GI symptoms. Older children with greater anxiety symptoms were 11% more likely to experience constipation, but 9% less likely to experience stomachaches. Older children with greater withdrawn behavior were 10.9% more likely to experience stomachaches, but 8.7% less likely to experience constipation. Older children with greater somatic complaints were 11.4% more likely to experience nausea and 11.5% more likely to experience stomachaches.

Conclusions: Results suggest that the presentation of externalizing problem behavior and internalizing symptoms associated with GI problems differs between young children and older children with ASD. Therefore, behavior may have different relationships with GI symptoms at different ages, which may have implications for the treatment of and clinical approach to GI disturbances in ASD.

Keywords: autism spectrum disorder, gastrointestinal disorders, problem behavior, internalizing symptoms, anxiety, depression

INTRODUCTION

Autism spectrum disorder (ASD) is characterized by persistent deficits in social communication and social interaction across multiple contexts, as well as restricted and repetitive patterns of behavior, interests, and activities that occur early in life and cause clinically significant impairment (1). A variety of gastrointestinal (GI) issues commonly occur in ASD, including lower GI symptoms (i.e., constipation, diarrhea) and upper GI symptoms (i.e., nausea and vomiting, stomach aches and pains) (2–10), but the etiology is poorly understood.

Children with ASD have been shown to experience a range of GI symptoms, with the prevalence shown to be anywhere from 9 to 91% (2), which is likely due differences in assessment and context. However, it appears that many individuals with ASD suffer from constipation (2, 3, 11, 12). One association with GI issues in ASD may be the response to stress, since some individuals with ASD show an altered stress response (13) and recent research has shown connections between lower GI symptoms, sensory over-responsivity, and anxiety (14), as well as altered psychophysiological (11) and endocrine (12) responses to stress-inducing stimuli. These associations suggest that activation of the sympathetic nervous system and the hypothalamic-pituitary axis may be associated with GI disorders in ASD.

Consistent with the theory that stress is linked to GI symptoms in ASD, co-occurring internalizing symptoms, such as anxiety and depression, are common in ASD (15) and associated with GI symptoms as well. Heightened stress and anxiety, rigid-compulsive behavior, and sleep problems, have been shown to be associated with GI symptoms in ASD, especially constipation (6, 11, 12, 16–19). Children with ASD who experience GI symptoms also have co-occurring externalizing problems, as studies have shown that children with ASD with co-occurring GI symptoms had increased irritability when compared to those with ASD and no GI symptoms (5, 6).

The present study aimed to examine relationships among GI symptoms, externalizing problem behavior, and internalizing symptoms in a large sample of young children and older children and adolescents with ASD. We expected that the associations between GI symptoms and co-occurring conditions would be different across age groups. This study also aimed to determine which internalizing or externalizing problem behaviors would be associated with which GI symptoms. We hypothesized that regardless of age, anxiety would be associated with more constipation and diarrhea but less stomachaches and nausea due to the heightened stress responses association with lower GI symptoms.

METHODS

Participants

This study included 340 children and adolescents with ASD ranging in age from 2 to 18 years old ($M = 5.56$, $SD = 3.67$) that are clinic patients at University of Missouri Thompson Center for Autism & Neurodevelopmental Disorders in Columbia, Missouri. All participants provided written informed consent in accordance with the Declaration of Helsinki, and the study was approved and carried out in accordance with the recommendations of the University of Missouri Health Sciences Institutional Review Board. Written informed consent was obtained from the parents/caregivers for all participants under the age of 18. Diagnosis of ASD was confirmed using the Autism Diagnostic Observation Schedule (20) or the Autism Diagnostic Interview—Revised (21). The sample included only participants from the database who had at least one GI symptom reported at the most recent clinic visit.

The sample was parsed into two age groups based on which version of the Child Behavior Checklist (CBCL) (22) the caregivers had completed for their child: younger (completed the CBCL for ages 2–5) or older (completed the CBCL for ages 6–18). The younger group consisted of 200 children (80% male), ranging in age from 2 to 5 ($M = 3.03$; $SD = 1.07$). The older group consisted of 140 children (77.1% male), ranging in age from 6 to 18 ($M = 9.19$; $SD = 2.94$).

Measures

At each clinic visit, caregivers completed questionnaires about their child's developmental history and milestones. The responses to the questionnaires from the most recent clinic visit were obtained from the Thompson Center database, and the following variables were extracted and analyzed: dietary problems, nutrition problems, GI symptoms, and internalizing and externalizing symptoms.

Dietary Problems

Dietary problems were examined with the sum of 12 dichotomous caregiver-endorsed symptoms, with a total range from 0 to 12. The individual items included whether or not the child experienced the following: feeding issues in infancy, current feeding issues, picky eating, milk aversion, nonfood item cravings, food group aversion, food reactions, special diet, difficulty with solids, difficulty with liquids, lethargy, or dehydration.

Nutrition Problems

Nutrition problems were determined by the caregiver's response to the question, "Is the child's nutrition adequate?." This variable was dummy coded, such that 0 = adequate nutrition and 1 = the child's nutrition was not adequate.

GI Symptoms

Caregivers completed a questionnaire about their concerns now or in the past about the following GI symptoms in their child: constipation, diarrhea, nausea or vomiting, and stomachaches or stomach pain. These were dichotomous variables, dummy coded such that 0 = no concerns, and 1 = concern. A total GI symptoms score was created by summing the four types of concerns, for a range of 1–4. In addition to the score for total GI problems, each individual GI symptom was considered separately in a subsequent analysis.

Internalizing and Externalizing Symptoms

Measures of internalizing and externalizing symptoms were derived from caregiver responses on the CBCL (22). The CBCL

is a parent-report measure assessing behavioral and emotional symptoms in children. Items are rated on a 3-point Likert scale (0 = not true, 1 = somewhat or sometimes true, and 2 = very true or often true). Two versions of the CBCL are available based on the child's age. The CBCL has strong psychometric properties, including test-retest reliability, inter-rater agreement, and internal consistency (23–25).

Covariates

Demographic covariates included age, gender, and household income, which were provided by caregivers at the time of assessment. Intelligence was assessed using a range of different assessments, each normalized to a mean of 100 and standard deviation of 15 (See **Table 1**).

Statistical Analyses

First, a simple bivariate correlation matrix among our variables was produced to determine which demographic or descriptive child and family covariates to include in our main analyses. Then, to evaluate the relationship between internalizing symptoms and externalizing problem behaviors for children with certain GI symptoms, we performed separate logistic regressions with the four GI symptoms as outcome variables for each age group. The primary predictors of interest were the internalizing and externalizing symptom subscales on the CBCL.

RESULTS

The majority of the sample experienced constipation (65%). About half of the children experienced stomachaches or stomach pain (47.9%), and others experienced nausea (23.2%) or diarrhea (29.7%). The average number of total GI symptoms was 1.66 ($SD = 0.880$), with a range from 1 to 4. The vast majority of the sample was not taking medications for GI symptoms (92.9%). However, over half of the children (53.2%) were taking at least one medication for other reasons (e.g., ADHD, aggression, anxiety symptoms, seizures, or sleep problems).

The two age groups did not differ significantly in gender composition [$\chi^2(1) = 0.403$, $p = 0.308$], nonverbal IQ [$t_{(182)} = 0.578$, $p = 0.564$], verbal IQ [$t_{(153)} = -0.989$, $p = 0.324$], or full scale IQ [$t_{(168)} = 0.869$, $p = 0.386$]. Families with younger children did have less household income [$\chi^2(4) = 9.985$, $p = 0.041$]. Younger and older children took similar amounts of GI medications [$\chi^2(1) = 0.830$, $p = 0.242$] and had similar rates of the four types of GI symptoms (p's > 0.05). Older children were taking more total medications than younger children [$t_{(178.05)} = -4.401$, $p < 0.001$]. Younger children had significant more dietary problems [$t_{(224)} = 3.182$, $p = 0.002$], but similarly adequate nutrition as compared to older children [$\chi^2(1) = 0.007$, $p = 0.524$]. See **Table 1** for descriptive statistics by age group.

Covariates were determined by identifying any significant bivariate correlations for each age group. Therefore, in the younger age group, we controlled for dietary problems, total number of medications, GI medications, and nutrition problems. In the older age group, we controlled for gender and dietary problems.

Do Internalizing or Externalizing Symptoms Predict GI Problems?

In younger children, aggressive problem behavior was a significant predictor of nausea, ($B = 0.106$, $SE = 0.052$, $p < 0.05$). Children with more aggression were 11.2% more likely to experience nausea problems.

In older children, several internalizing and externalizing symptoms were predictive of different GI problems. Older children with greater anxiety were 11% more likely to experience constipation problems ($B = 0.094$, $SE = 0.048$, $p < 0.05$), but 9% less likely to experience stomachaches ($B = -0.099$, $SE = 0.050$, $p < 0.01$.) Older children with greater withdrawn behavior were 10.9% more likely to experience stomachaches ($B = 0.086$, $SE = 0.042$, $p < 0.05$), but 8.7% less likely to experience constipation ($B = -0.131$, $SE = 0.046$, $p < 0.01$). Finally, older children with greater somatic complaints were 11.4% more likely to experience nausea ($B = 0.130$, $SE = 0.050$, $p < 0.01$) and 11.5% more likely to experience stomachaches ($B = 0.138$, $SE = 0.049$, $p < 0.01$). See **Table 2** for logistic regression results.

DISCUSSION

In the current study, constipation accounted for 65% of the GI symptoms in a sample of 340 children and adolescents with ASD. This finding corroborates previous reports in the literature showing that constipation accounts for a significant amount of GI complaints in ASD (2, 11, 12). Regarding externalizing problem behavior, presence of aggressive behavior was associated with nausea in children aged 2–5, suggesting that aggression may be an indicator of nausea in young children in this this population. However, no other GI problems were significantly associated with problem behavior and internalizing symptoms in young children with ASD. One explanation for this relationship may be that young children with ASD who are non-verbal use aggression as a means of communicating somatic complaints, such as internal abdominal pain and GI discomfort (2).

Children and adolescents aged 6–18 revealed associations between internalizing symptoms but not externalizing problem behaviors, and GI symptoms. In the older group, presence of anxiety conferred an 11% increase in the presence of constipation symptoms, which corroborates recent reports on an association between anxiety and lower GI tract symptoms (11, 12). Given these findings, future research may wish to examine the effects of behavioral and/or pharmacological stress and anxiety reductions on GI symptoms in ASD. Interestingly, withdrawn and depressed behavior was associated with an 11% increase in stomachaches, but a 9% decrease in constipation. Previous research has also shown a relationship between upper GI tract symptoms, including stomachaches, and depression in ASD, suggesting that depression and anxiety may place an individual with ASD at heightened risk for GI disturbance, but in different manners. Taken together, it is possible that GI disorders and behavioral problems are related in ASD as a means of communicating their discomfort given the core language deficits in those with ASD. Further research is needed to disentangle the relationship between GI symptoms, anxiety, and depression to determine how

TABLE 1 | Statistical comparison of age groups on descriptive statistics.

	Younger group (N = 200)			Older group (N = 140)			Statistical comparison
	Range	M	SD	Range	M	SD	
Age in years	2–5	3.03	1.07	6–18	9.19	2.94	
IQ[#]							
Verbal	57–128	90.02	15.25	50–136	92.71	17.78	$t_{(153)} = -0.989$
Nonverbal	43–127	94.20	15.11	56–137	92.83	16.90	$t_{(182)} = 0.578$
Full scale	71–121	92.34	12.96	49–127	90.30	16.40	$t_{(168)} = 0.869$
Dietary problems	0–12	3.72	2.30	0–10	2.75	2.12	$t_{(224)} = 3.182^{**}$
	n	%		n	%		Statistical comparison
Male	160	80		108	77.1		$\chi^2_{(1)} = 0.403$
Race							$\chi^2_{(6)} = 8.168$
Caucasian	165	82.5		111	79.3		
African	15	7.5		9	6.4		
Native	5	2.5		5	3.6		
Asian	4	2.0		1	0.7		
Unknown	11	5.5		14	10.0		
Household Income							$\chi^2_{(4)} = 9.985^{*}$
0–24,999	99	49.5		51	36.4		
25,000–49,999	50	25.0		29	20.7		
50,000–74,999	30	15.0		31	22.1		
75,000–99,999	8	4.0		11	7.9		
100,000+	10	5.0		12	8.6		
Missing	3	1.5		6	4.3		
Nutrition							$\chi^2_{(1)} = 0.007$
Adequate	121	60.5		89	63.6		
Not adequate	39	19.5		28	20		
Missing	40	20		23	16.4		
Taking GI meds							$\chi^2_{(1)} = 0.830$
Yes	12	6		12	8.6		
No	188	94		128	91.4		
GI symptoms							$t_{(338)} = 0.551$
Constipation	129	64.5		92	65.7		
Diarrhea	65	32.5		36	25.7		
Nausea/vomiting	43	21.5		36	25.7		
Stomachache/pain	90	45		73	52.1		

[#]The following intellectual assessments were used in the study, followed by the percentage that each assessment was used: Differential Ability Scales–Second Edition (26) (20.2%, General Conceptual Ability score), Wechsler Abbreviated Scale of Intelligence–Second Edition (27) (18.0%, Full Scale IQ score), Wechsler Intelligence Scale for Children–Fourth Edition (28) (5.9%, Full Scale IQ score), Wechsler Intelligence Scale for Children–Fifth Edition (29) (2.4%, Full Scale IQ score), Wechsler Preschool and Primary Scale of Intelligence–Third Edition (30) (0.3%, Full Scale IQ score), Wechsler Preschool and Primary Scale of Intelligence–Fourth Edition (31) (1.2%, Full Scale IQ score). A small group (11.7%) was administered a measure of nonverbal intelligence, the Leiter International Performance Scale–Third Edition (32); therefore, a Full-Scale IQ score was not available for this subsample. Intellectual testing could not be completed for many participants (40.3%) due to difficulties participating or understanding task demands.
$^{*}p < 0.05; ^{**}p < 0.01.$

these disorders cluster with upper or lower GI tract disorders in ASD.

This study has a number of limitations that should be considered alongside of the results. First, the sample is largely male and Caucasian in both age groups, making it difficult to generalize the findings to females and non-Caucasian individuals with ASD. Second, the GI symptoms were from caregiver reports and were not further assessed with a gastroenterological evaluation. Future studies of GI disorders in ASD should aim to utilize standardized measurements to assess GI symptoms, especially those which are ASD-specific (33, 34). Last, participants included in this study were selected based on having at least one GI symptom, so the results may not be representative of the general population of those with ASD. Furthermore, the sample of participants was from one clinic in the Midwestern United States, and so data from other clinics in other regions are needed in order to generalize these findings to the larger ASD population. In sum, this study provides further

TABLE 2 | Logistic regression results for internalizing and externalizing symptoms predicting GI symptoms in younger (aged 2–5) and older (aged 6–18) children with ASD.

Predictor	Younger group (N = 200)			Older group (N = 140)		
	B	**SE B**	**e^B**	**B**	**SE B**	**e^B**
CONSTIPATION						
Withdrawn/depressed	–	–	–	−0.131**	0.046	0.877
Social problems	–	–	–	−0.070	0.050	0.932
Thought problems	–	–	–	0.007	0.016	1.007
Rule breaking	–	–	–	0.009	0.057	1.009
Emotionally reactive	0.042	0.065	1.043	–	–	–
Anxious/depressed	−0.030	0.083	0.971	0.094*	0.048	1.098
Somatic complaints	0.105	0.063	1.111	0.030	0.047	1.031
Withdrawn	−0.093	0.050	0.911	–	–	–
Sleep problems	−0.009	0.035	0.991	–	–	–
Attention	−0.003	0.056	0.997	0.076	0.041	1.079
Aggressive	−0.004	0.041	0.996	−0.068	0.048	0.935
DIARRHEA						
Withdrawn/depressed	–	–	–	0.033	0.036	1.034
Social problems	–	–	–	0.049	0.044	1.050
Thought problems	–	–	–	−0.003	0.007	0.997
Emotionally reactive	0.025	0.056	1.026	–	–	–
Anxious/depressed	−0.047	0.076	0.954	−0.074	0.042	0.928
Somatic complaints	−0.035	0.053	0.966	−0.001	0.043	0.999
Withdrawn	0.020	0.044	1.020	–	–	–
Sleep problems	0.012	0.032	1.012	–	–	–
Attention	−0.027	0.055	0.973	−0.019	0.033	0.982
Aggressive	0.039	0.036	1.040	0.059	0.041	1.061
NAUSEA						
Withdrawn/depressed	–	–	–	0.033	0.039	1.034
Social problems	–	–	–	−0.036	0.045	0.964
Thought problems	–	–	–	−0.005	0.012	0.995
Emotionally reactive	0.006	0.067	1.006	–	–	–
Anxious/depressed	−0.167	0.110	0.846	−0.005	0.044	0.995
Somatic complaints	0.084	0.062	1.087	0.130**	0.050	1.139
Withdrawn	−0.009	0.054	0.991	–	–	–
Sleep Problems	−0.065	0.048	0.937	–	–	–
Attention	−0.045	0.063	0.956	0.028	0.037	1.029
Aggressive	0.106*	0.052	1.112	−0.051	0.045	0.950
STOMACHACHES						
Withdrawn/depressed	–	–	–	0.086*	0.042	1.090
Social problems	–	–	–	−0.009	0.050	0.991
Thought problems	–	–	–	0.005	0.011	1.005
Rule breaking	–	–	–	0.094	0.058	1.098
Emotionally reactive	0.053	0.057	1.054	–	–	–
Anxious/depressed	0.011	0.072	1.011	−0.099*	0.050	0.906
Somatic Complaints	0.020	0.048	1.020	0.138**	0.049	1.148
Withdrawn	−0.018	0.040	0.982	–	–	–
Sleep problems	0.011	0.031	1.012	–	–	–
Attention	−0.033	0.047	0.967	−0.009	0.035	0.991
Aggressive	−0.007	0.033	0.993	−0.028	0.047	0.973

Controls for the younger group are GI medications, total medications, dietary problems, and nutrition problems (omitted from the table). Controls for the older group are gender and dietary problems (omitted from the table).

B = The unstandardized beta value represents the slope of the line between the predictor variable and the dependent variable. For example, for older children, for every one unit increase in the Withdrawn/Depressed subscale, Constipation problems decrease by 0.131 units.

SE B = The standard error for the unstandardized beta describes the dispersion of the distribution around the regression line. For example, a larger number indicates the scores are more spread out from the regression line and there is more error in prediction.

e^B = The exponential B is the odds ratio for the predictor. For example, for older children, for every one unit increase in the Withdrawn/Depressed subscale, an individual is 0.877 times more likely to have Constipation than not have Constipation.

*p < 0.05; **p < 0.01.

evidence of the relationship between co-occurring conditions and GI symptoms in ASD, and highlights the need to evaluate age-related developmental differences in the associations among these symptoms, which may prove to be important when developing GI treatments for those with ASD across the lifespan.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Health Sciences Institutional Review Board at the University of Missouri with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Health Sciences Institutional Review Board at the University of Missouri.

AUTHOR CONTRIBUTIONS

BF and DB conceived the aforementioned studies conducted at the University of Missouri Thompson Center for Autism & Neurodevelopmental Disorders. DB provided expertise on ASD and GI disorders in ASD. KD provided the statistical analysis for the study. NT maintained the database from which the data for this study were obtained. All authors contributed to the development of the manuscript and approved the final version that was submitted for review.

FUNDING

ACKNOWLEDGMENTS

REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Washington, DC: American Psychiatric Association (2013).
2. Buie T, Campbell DB, Fuchs GJ. III, Furuta GT, Levy J, van de Water J, et al. Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. *Pediatrics*. (2010) 125S1:S1–18. doi: 10.1542/peds.2009-1878C
3. McElhanon BO, McCracken C, Karpen S, Sharp WG. Gastrointestinal 86 symptoms in autism spectrum disorder: a meta-analysis. *Pediatrics*. (2014) 133:872–83. doi: 10.1542/peds.2013-3995
4. Doshi-Velez F, Ge Y, Kohane I. Comorbidity clusters in autism spectrum disorders: an electronic health record time-series analysis. *Pediatrics*. (2014) 133:e54–63. doi: 10.1542/peds.2013-0819
5. Chaidez V, Hansen RL, Hertz-Picciotto I. Gastrointestinal problems in children with autism, developmental delays or typical development. *J Autism Develop Disord*. (2014) 44:1117–27. doi: 10.1007/s10803-013-1973-x
6. Fulceri F, Morelli M, Santocchi E, Cena H, Del Bianco T, Narzisi A, et al. Gastrointestinal symptoms and behavioral problems in preschoolers with autism spectrum disorder. *Digest Liver Dis*. (2016) 48:248–54. doi: 10.1016/j.dld.2015.11.026
7. Gorrindo P, Williams KC, Lee EB, Walker LS, McGrew SG, Levitt P. Gastrointestinal dysfunction in autism: parental report, clinical evaluation, and associated factors. *Autism Res*. (2012) 5:101–8. doi: 10.1002/aur.237
8. Bauman ML. Medical comorbidities in autism: challenges to diagnosis and treatment. *Neurotherapeutics*. (2010) 7:320–7. doi: 10.1016/j.nurt.2010.06.001
9. Mouridsen SE, Rich B, Isager T. A longitudinal study of gastrointestinal diseases in individuals diagnosed with infantile autism as children. *Child Care Health Develop*. (2010) 36:437–43. doi: 10.1111/j.1365-2214.2009.01021.x
10. Ibrahim SH, Voigt RG, Katusic SK, Weaver AL, Barbaresi WJ. Incidence of gastrointestinal symptoms in children with autism: a populationbased study. *Pediatrics*. (2009) 124:680–6. doi: 10.1542/peds.2008-2933
11. Ferguson BJ, Marler S, Altstein LL, Lee EB, Akers J, Sohl K, et al. Psychophysiological associations with gastrointestinal symptomatology in autism spectrum disorder. *Autism Res*. (2017) 10:276–88. doi: 10.1002/aur.1646
12. Ferguson BJ, Marler S, Altstein LL, Lee EB, Mazurek MO, McLaughlin A, et al. Associations between cytokines, endocrine stress response, and gastrointestinal symptoms in autism spectrum disorder. *Brain Behav Immun*. (2016) 58:57–62. doi: 10.1016/j.bbi.2016.05.009
13. Lopata C, Volker MA, Putnam SK, Thomeer ML, Nida RE. Effect of social familiarity on salivary cortisol and self-reports of social anxiety and stress in children with high functioning autism spectrum disorders. *J Autism Develop Disord*. (2008) 38:1866–77. doi: 10.1007/s10803-008-0575-5
14. Mazurek MO, Vasa RA, Kalb LG, Kanne SM, Rosenberg D, Keefer A, et al. Anxiety, sensory over-responsivity, and gastrointestinal problems in children with autism spectrum disorders. *J Abnor Child Psychol*. (2013) 41:165–76. doi: 10.1007/s10802-012-9668-x
15. Hovarth K, Perman JA. Autism and gastrointestinal symptoms. *Curr Gastroenterol Rep*. (2002) 4:251–8. doi: 10.1007/s11894-002-0071-6
16. Coury DL, Ashwood P, Fasano A, Fuchs G, Geraghty M, Kaul A, et al. Gastrointestinal conditions in children with autism spectrum disorder: developing a research agenda. *Pediatrics*. (2012) 130(Suppl. 2):S160–8. doi: 10.1542/peds.2012-0900N
17. Bishop-Fitzpatrick L, Mazefsky CA, Minshew NJ, Eack SM. The relationship between stress and social functioning in adults with autism spectrum disorder and without intellectual disability. *Autism Res*. (2015) 8:164–73. doi: 10.1002/aur.1433
18. Nikolov RN, Bearss KE, Lettinga J, Erickson C, Rodowski M, Aman MG, et al. Gastrointestinal symptoms in a sample of children with pervasive developmental disorders. *J Autism Develop Disord*. (2009) 39:405–13. doi: 10.1007/s10803-008-0637-8
19. Peters B, Williams KC, Gorrindo P, Rosenberg D, Lee EB, Levitt P, et al. Rigid-compulsive behaviors are associated with mixed bowel symptoms in autism spectrum disorder. *J Autism Develop Disord*. (2014) 44:1425–32. doi: 10.1007/s10803-013-2009-2
20. Lord C, DiLavorne PC, Risi S. *Autism Diagnostic Observation Schedule*. Los Angeles, CA: Western Psychological Services (2002).
21. Lord C, Rutter M, le Couteur A. Autism diagnostic interview-revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Develop Disord*. (1994) 24:659–85. doi: 10.1007/BF02172145
22. Achenbach TM, Rescorla L. *Manual for the ASEBA School-Age Forms & Profiles: An Integrated System of Multi-informant Assessment*. Burlington, VT: University of Vermont, Research Center for Children, Youth & Families. (2001).
23. Achenbach TM, Dumenci L, Rescorla LA. DSM-Oriented and empirically based approaches to constructing scales from the same item pools. *J Clin Child Adoles Psychol*. (2003) 32:328–40. doi: 10.1207/S15374424JCCP3203_02

24. Nakamura, B.J., Ebesutani, C., Bernstein, A., and Chorpita, B.F. (2009). A psychometric analysis of the child behavior checklist DSM-oriented scales. *J. Psychopathol. Behav. Assess.* 31, 178–189. doi: 10.1007/s10862-008-9119-8

25. Frizzo GB, Pedrini JR, de Souza DS, Bandeira DR, Borsa JC. Reliability of child behavior checklist and teacher's report form in a sample of brazilian children. *Universitas Psychologica.* (2014) 14:149–56. doi: 10.11144/Javeriana.upsy14-1.rcbc

26. Elliot CD. *Differntial Ability Scales.* 2nd ed. San Antonio, TX: PsychCorp Harcourt Assessment Inc (2007).

27. Wechsler D. *Wechsler Abbreviated Scale of Intelligence.* 2nd ed (wasi-ii). San Antonio, TX: NCS Pearson (2011).

28. Wechsler D. *Wechsler Intelligence Scale for Children* 4th ed (wisc-iv). San Antonio, TX: NCS Pearson (2003).

29. Wechsler D. *Wechsler Intelligence Scale for Children-fifth edition.* Bloomington, MN: Pearson (2014).

30. Wechsler D. *The Wechsler Preschool and Primary Scale Of Intelligence.* 3rd ed. San Antonio, TX: The Psychological Corporation (2002).

31. Wechsler D. *Wechsler Preschool and Primary Scale of Intelligence.* 4th ed. Bloomington, MN: Pearson (2012).

32. Roid GH, Miller LJ, Pomplun M, Koch C. *Leiter International Performance.* 3rd ed (leiter-3). Wood Dale. IL: Stoelting (2013).

33. Walker LS, Caplan-Dover A, Rasquin-Weber A. *Rome III Diagnostic Questionnaire for the Pediatric Functional GI Disorders.* (2006).

34. Margolis KG, Buie TM, Turner JB, Silberman AE, Feldman AE, Murray, K.F. et al. Development of a brief parent-report screen for common gastrointestinal disorders in autism spectrum disorder. *J Autism Dev Disord.* (2018) 49:349–62. doi: 10.1007/s10803-018-3767-7

Functional Gastrointestinal Disease in Autism Spectrum Disorder: A Retrospective Descriptive Study in a Clinical Sample

María José Penzol [1,2]*, Gonzalo Salazar de Pablo [1], Cloe Llorente [1], Carmen Moreno [1], Patricia Hernández [1], Maria Luisa Dorado [1] and Mara Parellada [1]

[1] Child and Adolescent Psychiatry Department, Hospital General Universitario Gregorio Marañón, School of Medicine, Universidad Complutense, IiSGM, CIBERSAM, Madrid, Spain, [2] Molecular Medicine Ph.D Program, University of Santiago de Compostela, Santiago de Compostela, Spain

Introduction: Autism spectrum disorder (ASD) is a heterogeneous group of neurodevelopmental disorders with complex multifactorial etiologies. Medical comorbidities are common in ASD and include functional gastrointestinal disorders (fGID), which are reported in 30–70% of patients. In this research study, we aimed to systematically assess the prevalence of gastrointestinal problems in ASD and describe their clinical correlates.

Methods: In this retrospective study, we reviewed the medical records of all patients admitted to the Comprehensive Medical Program for ASD (AMITEA) at Gregorio Marañón University General Hospital from January 2012 to December 2015. All patients fulfilled the clinical criteria for ASD (DSM-IV-TR). In addition to fGID, epidemiological and clinical variables were collected at intake. Clinical and demographic features were compared among subjects with and without comorbid gastrointestinal problems.

Results: The analyses included all patients with documented information about presence/absence of fGID ($n = 845$; 95% of patients). Ages ranged from 1 to 53 years (mean $= 10.52$; SD $= 8.92$; 80.4% males). At least one fGID was present in 30.5% of patients, constipation being the most prevalent (47.4% of fGID patients); fGID were significantly associated with intellectual disability (ID) ($p = 0.017$), sleep disorders ($p = 0.012$), and prescription of psychopharmacological treatment ($p = 0.019$).

Conclusions: Almost one-third of ASD patients in our sample had at least one fGID. The presence of fGID was associated with ID, sleep problems and with behavioral problems (as measured by the prescription of psychotropic drugs). This subsample of ASD patients with fGID deserves particular attention in future research projects, focusing on specific phenotypic characteristics and overlapping biological markers that may underlie both pathologies.

Keywords: functional gastrointestinal disease, autism spectrum disorder, clinical comorbidity, prevalence, retrospective study

INTRODUCTION

Autism spectrum disorder (ASD) is a pervasive neurodevelopmental disorder characterized by impairments in social communication and restricted, repetitive patterns of behavior, interests, or activities (1). The neurobiological basis of ASD seems incontrovertible (2) even though the neurobiological mechanisms that result in the clinical phenotype remain to be fully elucidated. These include genetic factors, neuropathology, neurostructure, and brain networks (2). According to previous studies, more than 70% of individuals with ASD have other concurrent medical, developmental, or psychiatric conditions, which are frequently multiple (3–6).

It is widely reported that children with ASD are more likely to experience unmet medical needs compared with typically developing children (7). According to data from a US national survey, among children and adolescents with special health care needs, those with ASD are more likely to require specific health care services. This also holds true when compared with children who have special health care needs and behavioral, emotional, and developmental problems other than autism (8).

After years of debate about the presence of gastrointestinal (GI) symptoms in autism, there is currently a consensus to describe GI symptoms as a common comorbidity in patients with ASD even though the underlying mechanisms are largely unknown (9). A review published in Pediatrics reported that prevalence rates vary widely among studies and range from 9 to 91% in different samples, with great differences between retrospective and prospective studies (10, 11).

The GI problems, most commonly reported in autism, are chronic constipation, abdominal pain with or without diarrhea, and encopresis as a consequence of constipation (12). Furthermore, greater autism symptom severity is associated with increased odds of having GI problems (11, 13). Gastrointestinal symptoms are more likely to be reported by mothers in children with autism early in infancy than in children with typical development or developmental delay (14). Diagnosis of GI problems can be challenging in children with ASD because of their communication difficulties and complex behaviors (15), and therefore may be delayed (16), and problems frequently go undetected. GI symptoms in ASD may contribute to behavioral impairment, complicating clinical management. Gastrointestinal problems have been found to be associated with other behavioral and anxiety problems (17, 18).

Based on Roma Foundation working team, functional gastrointestinal disorders (fGIDs) are described as gut-brain interaction disorders, defined as a resulting on combination of symptoms affecting motility, hypersensitivity, immunity, and other alterations in mucose, causing an illness experience in patient's body; which are not caused by a anatomic or motility disorder (19).

A high prevalence of sleeping problems has been found in children with ASD (20–22), with longer sleep latencies and more difficulty going to bed and falling asleep (23). Co-occurrence of GI and sleep disturbances have been widely described in ASD, suggesting potential common pathophysiological pathways (24).

There are common pathophysiological mechanisms that account for both autism and epilepsy (25). ASD and epilepsy co-occur in approximately 30% of individuals with either condition (26). An epidemiological study detected an association between GID and epilepsy, identifying an increased risk for seizures co-occurring with GI symptoms in ASD patients (27).

It has been suggested that high medical comorbidity in ASD subjects may be due to common etiopathological factors or shared intermediate mechanisms, such as inflammation, immunity, redox status, etc. (16). However, an association of medical comorbidities with fGID in a sample of ASD subjects representative of the ASD general population has not yet been sufficiently explored.

Against this background, the purpose of this work was to explore fGID and associated conditions in a large sample of patients consecutively seen in a medical program for ASD subjects over a representative period of time.

In this study, we specifically evaluated (1) the prevalence of fGID in a large sample of individuals with ASD quasi-representative of the population of ASD patients in the Community of Madrid and (2) the sociodemographic and clinical characteristics and comorbidities in the subgroup of ASD patients with fGID.

MATERIALS AND METHODS

We conducted a retrospective study reviewing clinical records of all patients admitted to the Comprehensive Medical Program for ASD (AMITEA) (28) at Gregorio Marañón University General Hospital during the first four full years after implementing an electronic medical records system: from January 1, 2012 to December 31, 2015. The AMITEA program was founded in 2009 as a specialty care service for ASD patients with specific objectives: (1) to perform routine and symptom- or sign-driven medical assessments, (2) to facilitate access to the appropriate medical care using an individualized case management approach, (3) to centralize all possible medical and nursing procedures, and (4) to assess and treat comorbid psychiatric symptomatology (28). The only criteria for admission to the program are: (1) diagnosis of an ASD (all ages); (2) referral by a physician in the public sector; and (3) residing in the Community of Madrid (thus serving a population of 6,000,000). AMITEA is located in a tertiary hospital with all adult and pediatric specialties and more than 2,000 inpatient beds. Spain has a national health system that covers around 95% of the population. Given that the AMITEA program is open to all residents in the Community of Madrid Community with an ASD diagnosis (the only program of its kind) and admission is not restricted to individuals with health problems, the characteristics of program participants may be considered representative of the ASD general population in Madrid.

All participants fulfilled DSM-IV-TR criteria for ASD. Diagnoses were made by Child and Adolescent Psychiatrists, after a full psychiatric and developmental history, previous reports' review and observation of the child/adolescent. The Autistic Diagnostic Interview-Revised (ADI-R) and Autism Diagnostic

Observation Schedule-Generic ADOS-G were used to support the diagnosis when clinicians deemed it necessary. When needed, ADI-R clinically trained and ADOS research trained child psychiatrists or psychologists did conduct these evaluations. All data was introduced in the electronic medical history of the Hospital at the time of intake, and electronically signed. In order to organize the information from the hospital medical history and report the data herein presented, we built a database in which patients' data were de-identified.

All data were collected as part of routine care, according to a protocol completed for every new participant registered in AMITEA. Sociodemographic and clinical variables were collected systematically by attending physicians (Child and Adolescent Psychiatrists). Data collected included age, sex, ASD diagnosis, type of ASD, and presence of fGID (gastrointestinal reflux, aerophagia, functional diarrhea, functional constipation, functional abdominal pain, cyclic vomiting). Specifics of the fGID, if present, were manually extracted from the electronic medical record and entered verbatim in the study database. Within variables deemed interesting with respect to gastrointestinal comorbidity, we only analyzed data that was included in the initial intake clinical history template. In this way, the data we report herein were available for all patients. GI information was categorized according to the domains in the ROME-III-R questionnaire for fGID (Appendix. Rome Foundation, 2006). Other comorbidities such as epilepsy, estimated intellectual disability (ID), and sleep disorders were also compiled. Behavioral and emotional problems were recorded as part of the psychiatric history, with no standard questionnaire; therefore, prescription of psychotropic treatment was gathered as a proxy for the behavioral problems (with clinical impact). Estimated ID was recorded as a dichotomous (yes/no) clinical variable after reviewing all the information in the medical record including psychometric tests, adaptive functioning questionnaires, and school placement and performance. The Institutional Review Board (IRB) of Gregorio Marañón University General Hospital approved the study.

ANALYSES

We used descriptive statistics to characterize the study population including demographic variables and clinical characteristics. These were compared between patients groups with and without comorbid GI problems. A logistic regression model was then used to estimate odds ratios (ORs) and explore how factors differentially distributed between patients with and without fGID in the bivariate analyses contributed to the association with fGID. Therefore, we included age, sex, intellectual disability and sleep disorder as covariates in the multivariate analysis. Statistical analyses were performed with SPSS 21 for Windows software (IBM).

RESULTS

Our analyses included all patients with available information about presence/absence of fGID ($n = 845$; 95.6%) of a total sample of 879 persons admitted in the AMITEA program during 4 years; therefore, 34 patients were excluded from analysis since data of interest were not fully completed in clinical records. Ages ranged from 1 to 53 years, with a mean age of 10.52 (SD 8.92), and 19.6% were female. Children under 5 years of age comprised 25.2% of the sample ($n = 213$), 43% ($n = 363$) were ages 5–12, 15.9% ($n = 134$) were adolescents ages 13–17, and 16% ($n = 135$) were 18 or older. Four children aged 21–23 months were included in the program (following criteria as in the moderate-to-severe concern in Toddler module of the ADOS). Per DSM-IV criteria, all patients had a diagnosis of Pervasive Developmental Disorder; most patients ($n = 476$; 56.3%) had a diagnosis of Autistic Disorder, 210 (24.9%) had a diagnosis of Pervasive Developmental Disorder Not Otherwise Specified, 116 (13.7%) Asperger's Disorder, 2 patients (0.2%) had Rett's Disorder; and a small group ($n = 41$; 4.9%) did not had an specified subtype in the clinical record.

Thirty percent of subjects ($n = 258$, 30.5%) had an fGID, constipation being the most prevalent, occurring in almost half ($n = 121$; 47.6%) of the sub-sample with fGID; other symptoms identified were functional diarrhea ($n = 29$; 11.4%), gastrointestinal reflux ($n = 20$; 7.9%), functional abdominal pain ($n = 16$; 6.3%), aerophagia ($n = 16$; 6.3%), cyclic vomiting ($n = 12$; 4.7%), and other fGID not specifically reported ($n = 40$; 15.7%). Eighty-four patients (9.9%) of total sample were referred to a gastroenterologist for assessment, in this subgroup 75% presented fGID ($\chi^2 = 87.609$, $p < 0.001$).

With respect to the other comorbidities explored, 34.9% of the patients had a sleep disorder, 9.2% epilepsy, and 44.3% intellectual disability. More than half of the patients in the sample (55%) were prescribed psychopharmacological treatment. Psychotropic medication was more frequently used in patients over 12 years of age ($\chi^2 = 79.184$ $p = 0.015$), with a similar frequency in adolescents (79.9%) and adults (79.5%).

In contrast to the 30.5% of fGID in total sample, within the subsample of patients with ID, fGID was present in 34.8%, while in patients without ID, fGID was present in 65.2% ($p = 0.017$).

With respect to the differential sociodemographic and clinical characteristics of patients with and without fGID (see **Table 1**). The percentage of patients with ID was greater in the group with fGID ($p = 0.017$), as was in the sleep disorder group ($p = 0.012$), and the group with prescription of psychotropic drugs ($p = 0.019$). Other clinical variables did not differ in both groups. No differences concerning age and gender were found between groups with and without fGID.

Most patients with sleep disorders (71.8%) were on psychopharmacological treatment, while only 28.2% of patients with sleep disorders were receiving no psychotropic medication ($\chi^2 = 31.732$, $p < 0.001$).

Exploring eventual predictors of fGID, we examined the variables associated with functional Gastrointestinal Disorders (see **Table 2**), logistic regression analysis showed that intellectual disability was significantly associated with fGID (OR = 1.490, 95% CI: 1.081–2.055; $p = 0.015$) and sleep disorder (OR = 1.502, 95% CI:1.107–1.2036; $p = 0.009$) were significantly associated with fGID. Result remained significant in the multivariate analysis when OR was adjusted for age, sex, and sleep problems;

and also when OR for sleep problems when covariated by age, sex, and intellectual disability. Due to the high association between sleep disorder and psychopharmacological treatment, this last variable was not included in the regression.

DISCUSSION

In this sample of 845 patients with ASD seen in a specialty care program for ASD patients in a tertiary hospital, almost a third had an fGID, constipation being the most prevalent (47%). The presence of fGID was associated with ID, sleep problems and with behavioral problems (as measured by the

higher percentage of children in the ASD group with fGID treated with psychotropic drugs), but it was intellectual disability and sleep problems that stood out as the most significant associations with fGID once other variables were considered. No differences were detected in the prevalence of fGID among age groups or ASD diagnosis category.

In this large ASD sample, relatively representative of ASD subjects in a general population of around 6,000,000, there emerges a subgroup of patients (around 25% of the sample) characterized by GID and ID. This group of patients has concomitant associated health problems such as sleep and behavioral problems. Although parents with ID report fGID only a bit more frequently than parents of children without ID (34.8 vs. 30.5%, $p = 0.017$), the percentage could well be higher, taking into account the difficulties that ASD+ID patients may have communicating symptoms, especially if those include subjective perceptions such as pain or discomfort.

Our finding of nearly one-third of ASD patients presenting with fGID adds to the previous literature. A recent large population-based study (17) showed that 7.5% of ASD participants had a gastrointestinal problem, the most common being constipation. One-third of ASD patients presenting with fGID in a tertiary hospital is a higher figure than the 11.7% found in a recent study that automatically searched a large sample of medical records from four general hospitals (29) for comorbidities. Our data is likely more accurate, as the presence of fGID is systematically recorded at the intake visit in a program specifically designed to meet the medical needs of patients with ASD (2) referred by general practitioners. The nature of this program, designed to improve access to specialist care via a case manager, and the nature of the healthcare system in Spain, which is publicly funded and available to all citizens, results in a case load of patients either seen for any medical (including psychiatric) comorbidity or simply registered in the program should they need any specialty.

TABLE 1 | Sociodemographic and clinical characteristics in the groups with and without fGID.

	fGID N = 258	No fGID N = 587	X² test	p-value
Age groups			0.043	0.998
Age ≤ 5 (infants/toddlers)	24.8%	25.4%		
Age 5–12 (children)	43.4%	42.8%		
Age 13–18 (adolescents)	15.9%	15.8%		
Age ≥18 (adults)	15.9%	16.0%		
Sex (female)	20.9%	19.1%	0.389	0.573
ASD diagnostic category			3.634	0.603
Autistic Disorder	63%	57.5%		
Rett's Disorder	0%	0.4%		
Asperger's Disorder	12.2%	15.4%		
PDD NOS	24.8%	26.7%		
Known medical etiology	19.4%	18.5%	0.099	0.753
Epilepsy	11.8%	9.1%	1.457	0.247
Intellectual disability	51.2%	41.3%	5.946	**0.017***
Sleep disorder	42.7%	33.2%	6.878	**0.009***
Pharmacological treatment	65.3%	56.4 %	5.563	**0.019***

PDD-NOS, Pervasive Developmental Disorder-Not otherwise specified.
*Bold values indicate statistically significant difference (p < 0.05).

TABLE 2 | Functional Gastrointestinal Disorder.

	Unadjusted OR	(95% CI)	p-value	Adjusted OR[a/b]	(95% CI)	p-value
Age	1.002	0.986–1.019	0.801	–	–	–
Sex (male)	0.891	0.619–1.082	0.533	–	–	–
ASD diagnostic category	1	0.457–1.142	0.164	–	–	–
Autistic disorder	0.722		0.361	–	–	–
Asperger's Disorder	0.848	0.595–1.208		–	–	–
PPD NOS				–	–	–
Known medical etiology	0.942	0.649–1.367	0.753	–	–	–
Epilepsy	1.348	0.829–2.192	0.229	–	–	–
Intellectual disability	1.490	1.081–2.055	**0.015***	1.402[a]	1.001–1.962	**0.049***
Sleep disorder	1.502	1.107–2.036	**0.009***	1.551[b]	1.112–2.164	**0.010***
Pharmacological treatment	1.455	1.065–1.988	**0.019***	–	–	–

[a] Adjusting for age, sex, and sleep disorder.
[b] Adjusting for age, sex, and intellectual disability.
PDD-NOS, Pervasive Developmental Disorder-Not otherwise specified.
*Bold values indicate statistically significant difference (p < 0.05).

In our sample, functional gastrointestinal disorders were frequently associated with sleep disorders. According to the literature, GID and sleep problems are prevalent in pediatric samples without autism (around 45%) (30). In samples with autism, sleep problems are more prevalent than in general population; two-thirds of children with ASD have chronic insomnia (21), which may cause daytime behavior, memory, and learning problems in patients, and significant stress in caretakers (20). Medical conditions more frequently overlapped in autism samples; some authors have even suggested that patients with GI and/or sleep problems may represent a differential subtype of ASD with potential common pathophysiological pathways (22, 24, 27). GIDs have been described as a risk factor for sleep problems in children and adolescents with idiopathic autism (31).

Although the direction of the causal relationship between sleep problems and fGID is not known in many of the cases, early identification and treatment of GI disorders could improve sleep and help increase functionality and well-being in patients with ASD. In some cases, sleep problems clearly seem to arise from abdominal discomfort. As an example, a high percentage (40%) of some monogenic forms of autism, such as Phelan-McDermid syndrome, present a specific gastrointestinal disorder, namely gastroesophageal reflux (32). This is frequently associated with sleep problems. Understanding the origin and associations of sleep problems in specific cases may help put them in the appropriate place and determine the specific treatment intervention (in this case, antacid treatment), preventing children taking ineffective and potentially damaging treatments (e.g., psychotropics for sleep disturbances).

GI problems have been described in the literature in association with behavioral problems in patients with ASD (6, 22) and also with autism severity (17, 18, 33). Although our data focused only on the use of psychopharmacological treatment, it could be considered an approximation of the presence of this problem in our sample.

Identifying clinical phenotypes in ASD via medical comorbidities may be a promising approach for improving our understanding of the pathophysiology of subgroups of patients and optimizing therapeutic interventions (34). Wasilewska and Klukowsky suggest comorbidity of GI disorders and ASD as a new endophenotype and propose treating it as an "overlap syndrome" through different mechanisms. These mechanisms include multilevel pathways in the gut-brain axis contributing to alterations in behavior and cognition (16).

There is considerable evidence that the gut-brain axis is involved in the etiology of autism. This is based on a number of studies focusing on permeability of the intestinal mucosa, abnormal gut development, leaky gut, and others, with different mechanisms involving different systemic processes and the intermediate mechanism, many of those pointing toward a systemic chronic pro-inflammatory status. Furthermore, for the last several years, there has been an escalating number of studies showing changes of gut microbiota in patients with ASD (35).

LIMITATIONS

We are aware that this study is subject to some limitations. Above all, it is a retrospective study, based on data recorded in medical records with only part of the clinical data potentially relevant to the topic of this study and systematically gathered. Since this is a transversal study, no data is available concerning how treatment of functional gastrointestinal symptoms could change patients' behavior. In addition, some clinical data potentially relevant for interpreting our results was not systematically recorded and could not therefore be analyzed.

Despite the above limitation, our study has important strengths. The major strength is the large sample size and its particularities, making it a sample quite representative of a population of children and adults with ASD.

In conclusion, a large sample of ASD patients from a tertiary hospital shows a subgroup of patients with fGID, half of them with intellectual disability and frequently with comorbid sleep problems and/or behavioral problems. This is a subgroup of patients with greater medical needs that need to be addressed, on top of their autistic core symptoms, in order to improve their well-being and consequently their adjustment.

ETHICS STATEMENT

This study was conducted following the recommendations of the IRB of our institution and the Spanish Agency of Medicines and Medical Devices (AEMPS). Waiver of individual informed consent was approved by the IRB.

AUTHOR CONTRIBUTIONS

MJP and MP were responsible for conception and design of the study. MJP, GS, CL, PH, CM, MD, and MP were responsible for the clinical interview with patients and recorded data from clinical records. MJP and GS conducted the database generation and drafted the article. MJP and MP performed statistical analysis and data interpretation. MJP, GS, CL, PH, CM, MD, and MP reviewed the manuscript, made substantial contributions to conception and data acquisition and interpretation, making modifications as appropriate to the work in progress. All authors agree with the final content of this work.

ACKNOWLEDGMENTS

REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 5th Edition (DSM-5), Diagnostic and Statistical Manual of Mental Disorders 4th edition TR. American Psychiatric Association (2013).

2. Parellada M, Penzol MJ, Pina L, Moreno C, González-Vioque E, Zalsman G, et al. The neurobiology of autism spectrum disorders. *Euro Psychiatry*. (2014) 29:11–9. doi: 10.1016/j.eurpsy.2013.02.005

3. Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G. Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. *J Am Acad Child Adolesc Psychiatry*. (2008) 47:921–9. doi: 10.1097/CHI.0b013e318179964f

4. Lai M-C, Lombardo MV, Baron-Cohen S. Autism. *Lancet*. (2014) 383:896–910. doi: 10.1016/S0140-6736(13)61539-1

5. Levy SE, Giarelli E, Lee LC, Schieve LA, Kirby RS, Cunniff C, et al. Autism spectrum disorder and co-occurring developmental, psychiatric, and medical conditions among children in multiple populations of the United States. *J Dev Behav Pediatr*. (2010) 31:267–75. doi: 10.1097/DBP.0b013e3181d5d03b

6. Soke GN, Maenner MJ, Christensen D, Kurzius-Spencer M, Schieve LA. Prevalence of co-occurring medical and behavioral conditions/symptoms among 4- and 8-year-old children with autism spectrum disorder in selected areas of the United States in 2010. *J Autism Dev Disord*. (2018) 48:2663–76. doi: 10.1007/s10803-018-3521-1

7. Chiri G, Warfield ME. Unmet need and problems accessing core health care services for children with autism spectrum disorder. *Mater Child Health J*. (2012) 16:1081–91. doi: 10.1007/s10995-011-0833-6

8. Kogan MD, Strickland BB, Blumberg SJ, Singh GK, Perrin JM, van Dyck PC. A national profile of the health care experiences and family impact of autism spectrum disorder among children in the United States, 2005-2006. *Pediatrics*. (2008) 122:e1149–158. doi: 10.1542/peds.2008-1057

9. Mayer EA, Padua D, Tillisch K. (2014). Altered brain-gut axis in autism: comorbidity or causative mechanisms? *BioEssays*. 36:933–9. doi: 10.1002/bies.201400075

10. Buie T, Campbell DB, Fuchs GJ, Furuta GT, Levy J, Vandewater J, et al. Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with asds: a consensus report. *Pediatrics*. (2010) 125(Suppl 1):S1–18. doi: 10.1542/peds.2009-1878C

11. Holingue C, Newill C, Lee LC, Pasricha PJ, Daniele Fallin M. Gastrointestinal symptoms in autism spectrum disorder: a review of the literature on ascertainment and prevalence. *Autism Res*. (2018) 11:24–36. doi: 10.1002/aur.1854

12. Rosenfeld CS. Microbiome disturbances and autism spectrum disorders. *Drug Metab Dispos Biol Fate Chem*. (2015) 43:1557–71. doi: 10.1124/dmd.115.063826

13. Wang LW, Tancredi DJ, Thomas DW. The prevalence of gastrointestinal problems in children across the United States with autism spectrum disorders from families with multiple affected members. *J Develop Behav Pediatr*. (2011) 32:351–60. doi: 10.1097/DBP.0b013e31821bd06a

14. Bresnahan M, Hornig M, Schultz AF, Gunnes N, Hirtz D, Lie KK, et al. Association of maternal report of infant and toddler gastrointestinal symptoms with autism: evidence from a prospective birth cohort. *JAMA Psychiatry*. (2015) 72:466–74. doi: 10.1001/jamapsychiatry.2014.3034

15. Kral TV, Eriksen WT, Souders MC, Pinto-Martin JA. Eating behaviors, diet quality, and gastrointestinal symptoms in children with autism spectrum disorders: a brief review. *J Pediatr Nurs*. (2013) 28:548–56. doi: 10.1016/j.pedn.2013.01.008

16. Wasilewska J, Klukowski M. Gastrointestinal symptoms and autism spectrum disorder: links and risks - a possible new overlap syndrome. *Pediatric Health Med Ther*. (2015) 6:153–66. doi: 10.2147/PHMT.S85717

17. Chaidez V, Hansen RL, Picciotto IH, et al. Gastrointestinal problems in children with autism, developmental delays or typical development. *J Autism Dev Disord*. (2014) 44:1117–27. doi: 10.1007/s10803-013-1973-x

18. Fulceri F, Morelli M, Santocchi E, Cena H, Del Bianco T, Narzisi A, et al. Gastrointestinal symptoms and behavioral problems in preschoolers with autism spectrum disorder. *Dig Liver Dis*. (2016) 48:248–54. doi: 10.1016/j.dld.2015.11.026

19. Drossman DA. Functional gastrointestinal disorders: history pathophysiology clinical features and rome IV. *Gastroenterology*. (2016). doi: 10.1053/j.gastro.2016.02.032

20. Kotagal S, Broomall E. Sleep in children with autism spectrum disorder. *Pediatr Neurol*. (2012) 11:242–51. doi: 10.1016/j.pediatrneurol.2012.05.007

21. Souders MC, Zavodny S, Eriksen W, Sinko R, Connell J, Kernset C, et al. Sleep in children with autism spectrum disorder. *Curr Psychiatry Rep*. (2017) 19:34. doi: 10.1007/s11920-017-0782-x

22. Yang XL, Liang S, Zou MY, Sun CH, Han PP, Jiang XT, et al. Are gastrointestinal and sleep problems associated with behavioral symptoms of autism spectrum disorder? *Psychiatry Res*. (2018) 259:229–35. doi: 10.1016/j.psychres.2017.10.040

23. Goldman SE, Alder ML, Burgess HJ, Corbett BA, Hundley R, Wofford D, et al. Characterizing sleep in adolescents and adults with autism spectrum disorders. *J Autism Dev Disord*. (2017) 47:1682–95. doi: 10.1007/s10803-017-3089-1

24. Neumeyer AM, Anixt J, Chan J, Perrin JM, Murray D, Coury DL, et al. Identifying associations among co-occurring medical conditions in children with autism spectrum disorders. *Acad Pediatr*. (2018). doi: 10.1016/j.acap.2018.06.014

25. Tuchman R, Rapin I. Epilepsy in autism. *Lancet Neurol*. (2002) 352–8. doi: 10.1016/S1474-4422(02)00160-6

26. Tuchman R, Alessandri M, Cuccaro M. Autism spectrum disorders and epilepsy: moving towards a comprehensive approach to treatment. *Brain Dev*. (2010) 31:719–30. doi: 10.1016/j.braindev.2010.05.007

27. Aldinger KA, Lane CJ, Veenstra-VanderWeele J, Levitt P. Patterns of risk for multiple co-occurring medical conditions replicate across distinct cohorts of children with autism spectrum disorder. *Autism Res*. (2015) 8:771–81. doi: 10.1002/aur.1492

28. Parellada M, Boada L, Moreno C, Llorente C, Romo J, Muela C, et al. Specialty Care programme for autism spectrum disorders in an urban population: a case-management model for health care delivery in an ASD population. *Eur Psychiatry*. (2013) 28:102–9. doi: 10.1016/j.eurpsy.2011.06.004

29. Kohane IS, McMurry A, Weber G, MacFadden D, Rappaport L, Kunkel L, et al. The co-morbidity burden of children and young adults with autism spectrum disorders. *PLoS ONE*. (2012) 7:e33224. doi: 10.1371/journal.pone.0033224

30. Schurman JV, Friesen CA, Dai H, Danda CE, Hyman PE, Cocjin JT. (2012). Sleep problems and functional disability in children with functional gastrointestinal disorders: an examination of the potential mediating effects of physical and emotional symptoms. *BMC Gastroenterol*. (2012) 12:142. doi: 10.1186/1471-230X-12-142

31. McCue LM, Flick LH, Twyman KA, Xian H. Gastrointestinal dysfunctions as a risk factor for sleep disorders in children with idiopathic autism spectrum disorder: a retrospective cohort study. *Autism*. (2017) 21:1010–20. doi: 10.1177/1362361316667061

32. Soorya L, Kolevzon A, Zweifach J, Lim T, Dobry Y, Schwartz L, et al. Prospective investigation of autism and genotype-phenotype correlations in 22q13 deletion syndrome and SHANK3 deficiency. *Mol Autism*. (2013) 4:18. doi: 10.1186/2040-2392-4-18

33. Adams JB, Johansen LJ, Powell LD, Quig D, Rubin RA. Gastrointestinal flora and gastrointestinal status in children with autism–comparisons to typical children and correlation with autism severity. *BMC Gastroenterol*. (2011) 11:22. doi: 10.1186/1471-230X-11-22

34. Bauman ML. Medical comorbidities in autism: challenges to diagnosis and treatment. *Neurotherapeutics*. (2010) 7:320–7. doi: 10.1016/j.nurt.2010.06.001

35. Tomova A, Husarova V, Lakatosova S, Bakos J, Vlkova B, Babinska K, et al. Gastrointestinal microbiota in children with autism in Slovakia. *Physiol Behav*. (2015) 138:179–87. doi: 10.1016/j.physbeh.2014.10.033

Novel Contribution of Secreted Amyloid-β Precursor Protein to White Matter Brain Enlargement in Autism Spectrum Disorder

Deborah K. Sokol[1], Bryan Maloney[2], Cara J. Westmark[3] and Debomoy K. Lahiri[2,4]*

[1] Pediatrics Section, Department of Neurology, Indiana University School of Medicine, Indianapolis, IN, United States, [2] Indiana Alzheimers Disease Center, Department of Psychiatry, Stark Neuroscience Research Institute, Indiana University School of Medicine, Indianapolis, IN, United States, [3] Department of Neurology, University of Wisconsin, Madison, WI, United States, [4] Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, United States

The most replicated neuroanatomical finding in autism is the tendency toward brain overgrowth, especially in younger children. Research shows that both gray and white matter are enlarged. Proposed mechanisms underlying brain enlargement include abnormal inflammatory and neurotrophic signals that lead to excessive, aberrant dendritic connectivity via disrupted pruning and cell adhesion, and enlargement of white matter due to excessive gliogenesis and increased myelination. Amyloid-β protein precursor (βAPP) and its metabolites, more commonly associated with Alzheimer's disease (AD), are also dysregulated in autism plasma and brain tissue samples. This review highlights findings that demonstrate how one βAPP metabolite, secreted APPα, and the ADAM family α-secretases, may lead to increased brain matter, with emphasis on increased white matter as seen in autism. sAPPα and the ADAM family α-secretases contribute to the anabolic, non-amyloidogenic pathway, which is in contrast to the amyloid (catabolic) pathway known to contribute to Alzheimer disease. The non-amyloidogenic pathway could produce brain enlargement via genetic mechanisms affecting mRNA translation and polygenic factors that converge on molecular pathways (mitogen-activated protein kinase/MAPK and mechanistic target of rapamycin/mTOR), promoting neuroinflammation. A novel mechanism linking the non-amyloidogenic pathway to white matter enlargement is proposed: α-secretase and/or sAPPα, activated by ERK receptor signaling activates P13K/AKt/mTOR and then Rho GTPases favoring myelination via oligodendrocyte progenitor cell (OPC) activation of cofilin. Applying known pathways in AD to autism should allow further understanding and provide options for new drug targets.

Keywords: amyloid, anabolic, brain overgrowth, biomarker, comorbidity, metabolites, neurodevelopmental, secretase

INTRODUCTION

Autism or Autistic Syndrome Disorder (ASD) is characterized by lack in social communication and interaction and by restricted/repetitive patterns of interests, behaviors or activities (DSM-5) (1). It is considered the fastest growing neurodevelopmental disability. ASD prevalence estimates from the Centers for Disease Control (CDC) in 2014 were 1 in 68 children, which was up 30% from that reported in 2008, and more than double the frequency from 2000 (2). Autism affects more boys than girls. There is no agreed upon biomarker for ASD, however, the most replicated neuroanatomical finding is the tendency for heads and brains to be large, especially in young ASD subjects (3–7). Both macrocephaly—enlarged heads, as measured by occipital-frontal circumference (OFC), and megalencephaly, or enlarged brains, as measured post mortem or by MRI, are reported in autism. It is possible to have macrocephaly without megalencephaly. Unfortunately, the two are at times confused in the literature. Megalencephaly is increased growth of cerebral structures related to anomalies during brain development or because of postnatal abnormal events that cause excessive cerebral growth. On the other hand, macrocephaly, or simple increased head circumference, is linked to various events that are not necessarily neurological, including anomalies of bone skull structures, subdural fluid collections, hydrocephalus, intracranial masses, and arteriovenous malformations (8). However, only 20% of individuals with autism have enlarged brains (9), and the association between macrocephaly and core features of autism remains weak. This opens the door to entertain novel associations or apply knowledge from better-developed fields such as Alzheimer Disease (AD) to the study of autism.

AD comprises up to 80% of all dementias, affects 1 in 10 individuals over the age of 65, and affects more women than men (10). The amyloid hypothesis predicts clinical disease associated with amyloid-β loaded plaques resulting in brain atrophy in individuals with AD. Amyloid-β protein precursor (βAPP) and its metabolites are dysregulated in autism (11–15). βAPP is a large membrane spanning glycoprotein with a long extracellular N-terminus, a transmembrane region and an intracellular C-terminus, the βAPP intracellular domain [AICD; (16)]. After translation in the endoplasmic reticulum, APP undergoes post-translational modification in the Golgi complex before it travels to the cell membrane (17). The mature protein undergoes proteolytic cleavage by a combination of secretase enzymes. β- and γ-secretases generate amyloid-β (Aβ) peptides, primarily Aβ-40 and 42, found in the cerebral amyloid plaques of AD along with secreted APPβ (sAPPβ). α- and γ-secretase produce sAPPα, which is generally considered neurotrophic and neuroprotective, as well as AICD and p3 peptide. Under normal conditions, a small fraction of secreted βAPP is sAPPβ, produced via the amyloidogenic pathway. The majority of secreted product consists of sAPPα, produced constitutively via the non-amyloidogenic pathway. In AD, Aβ peptide is secreted at high levels and accumulates as neurotoxic extracellular aggregates. βAPP localizes to somatodendritic, axonal and the presynaptic active zones (18). βAPP expression is upregulated during inflammation (19) and is activated by proinflammatory cytokines and astrocytes (17, 20), so that APP may be involved in the acute phase protein response to immune stress. In synopsis, the localization of βAPP at dendritic synapses, its role in cell adhesion, its interaction with post-translational pathways and in transcription suggests that βAPP metabolites may play a pivotal role in the development of autism, and its single gene model kin, Fragile X Syndrome (FXS) (21, 22).

In this article we demonstrate how βAPP, particularly the non-amyloidogeneic pathway involving sAPPα contributes to the following mechanisms underlying excessive brain growth in autism: neuroinflammation, including cytokine activation, translational pathways, and increased myelination. These mechanisms were chosen as they are important to βAPP processing in AD, and serve to demonstrate catabolic vs. anabolic forces underlying these disorders.

In two independent studies (11, 21), our group has reported higher sAPPα levels and lower Aβ peptide levels in plasma of children with autism ($n = 26$). The same pattern was seen in autism temporal lobe brain tissue ($n = 7$) (21) of children with autism. The sAPPα results have been replicated by an independent laboratory in autism serum (15) and brain tissue (23). The finding of increased sAPPα also has been reported via confocal microscopy of brain tissue from groups of children with duplication 15q11.2-q13 and idiopathic autism (24). We have found increased levels of sAPPα, βAPP, and Aβ plasma markers in children with FXS ($n = 12$) compared to those with autism ($n = 11$) and typical development ($n = 18$), and in brain tissue from individuals with FXS [$n = 3$; (21)]. One of the purported α-secretase enzymes that cleaves βAPP and generates sAPPα (25), A Disintegrin and Metalloproteinase Domain 17 (ADAM17) also known as tumor necrosis factor-α converting enzyme (TACE), increases in autism brain tissue (21). ADAM17 is a member of the adamalysin family sheddases, and together with ADAMs 9 and 10, cleaves APP at the alpha-secretase site (26). Further, ADAM17 is pro-inflammatory, as it releases the cytokine tumor necrosis factor-α (TNF-α) during inflammation (27). ADAM17 and ADAM10 are essential for development of oligodendrocytes (28, 29), which produce myelin within the CNS. Several studies in the field point to a connection between differences in βAPP processing and autism (**Table 1**).

In total, these findings support the "Anabolic Hypothesis of βAPP in Autism" (14, 32), which postulates that sAPPα contributes to neuronal and glial overgrowth in the brain resulting in neuronal interference contributing to autism symptoms. One direct mechanism has been reported by Westmark and Malter (33) who found that βAPP is regulated via Fragile X Mental Retardation Protein (FMRP) via mGluR-5 in mouse models of FXS. When the FMRP "brake" is removed in translation, βAPP mRNA is increased. Of note, children with FXS often display macrocephaly, with a predominance of white matter (34). βAPP and metabolites play an anabolic role in other translation regulating pathways such as Mammalian target of rapamycin [mTOR; (35)], Ras small GTPase/Extracellular signal regulated kinase [Ras/ERK; (36, 37)] and phosphoinositol 3 kinase/mammalian target of rapamycin [P13k/mTOR; (37, 38)].

TABLE 1 | Studies linking βAPP with autism.

Study	Subjects/materials	Finding
Lahiri et al. (30)	Brain samples from TD, AD, ASD, and FXS human subjects	Elevated βAPP and Aβ in FXS and AD, reduced Aβ and elevated sAPPα in ASD subjects, all vs. age-matched controls.
Ray et al. (21)	Plasma from TD, ASD, and FXS human subjects	Aβ and sAPPβ levels reduced and sAPPα elevated in ASD while Aβ and βAPP elevated in FXS, all vs. controls.
Ray et al. (13)	Plasma from TD, mild/moderate ASD, severe ASD human subjects	Aβ and sAPPβ levels reduced and sAPPα elevated in severe but not mild/moderate ASD vs. controls
Sokol et al. (11)	Plasma from TD, mild/moderate ASD, severe ASD human subjects	Aβ40 and Aβ42 levels diminished according to severity of autism (more severe → lower levels). Secreted APP increased alongside autism severity.
Bailey (15)	Whole blood samples from autism subjects and age-matched TD controls, human umbilical cord blood	sAPPα elevated in whole blood and plasma of autistic children. 7% of undiagnosed cord blood samples had elevated sAPPα
Wegiel et al. (24)	Brain samples from TD, ASD, and dup(15) human subjects	dup(15) subjects accumulated high levels of p3 peptide. ASD subjects had intermediate accumulation of P3, while controls had lowest levels of accumulated p3.
Bailey et al. (31)	Blood from human sAPPα overexpressing mice	sAPPα elevation corresponded to elevated CD8$^+$ T cells and decreased effector memory T cells. Multiple signal and cytokine levels were also perturbed.

This sets the stage for the discussion of gray and white matter enlargement in autism, with the potential contribution from βAPP metabolites.

NORMAL BRAIN GROWTH

Mammalian brain cells include neurons and glia. Neuron cell bodies and dendrites form the outer gray matter, with axons projecting downward into the subcortical white matter. Brain size depends both on the size of the cerebral cortex or gray matter and the underlying white matter (39). The volume of gray cortex is determined by cortical thickness, and cortical surface area, believed to be genetically independent (40). One model to explain cortical thickness and surface area pertains to radial cortical columns (41). Radial cortical columns depend on the number of neural stem cells that symmetrically divide in the ventricular zone before neurogenesis (39). The growth of progenitor cells predicts the number of neurons within the radial columns (42). Cortical thickness is predicted by the number of cells within the columns, whereas surface area is predicted by the number of cortical columns (41). The white matter comprises over half the human brain, in greater proportion than other animals (43). Intensely studied in demyelinating conditions such as multiple sclerosis, myelin, and white matter structure is dynamic, regulated by impulse activity, and essential to cognitive function (5, 43–45). Besides neuronal axons, white matter is composed of glia. Glia are of three types including: (1) astrocytes, which outnumber neurons five to one (46), contribute to synaptic transmission, neuronal processing (46) and form the blood brain barrier; (2) microglia, which act as innate immune cells providing host defense and tissue repair-these release cytokines, nitric oxide and various neurotrophic factors; and (3) oligodendrocytes which manufacture myelin sheaths for axonal insulation. The precursor to oligodendrocytes are oligodendrocyte precursor cells (OPCs), which are the only type of glia to receive synaptic input from neurons (44). OPCs produce neuron/glia antigen 2-NG2, which communicate directly with neurons and demonstrate the newly appreciated importance of glia, which seem to "talk back to neurons" (46). Glia are found in both gray and white matter, but there are more oligodendrocytes in white matter (47).

MACROCEPHALY IN AUTISM
Anatomical Findings

In 1943, Leo Kanner (48) observed that some autistic children have large heads. Subsequent observations showed a strong trend toward enlarged OFC in autism, particularly in younger subjects (3, 49–51). However, individual reports must be evaluated carefully, since evaluations based on standard growth curves can give drastically different results depending on the specific curve. For example, a survey of over 75,000 neonatal OFC from infants in a single US-based primary care network (PCN) found macrocephaly in 8.6% according to CDC curves, in 14% according to the World Health Organization (WHO) curves, and in 5.1% according to National Center of Health Statistics (NCHS) curves. If the norm was the PCN's own distribution, only 4.4% had OFC > 95th percentile (52). Studies that compare autistic and non-autistic groups directly should be considered more reliable than other studies (51, 53, 54). Indeed, use of population norms may have introduced systematic age-dependent bias into nearly 83% of the field's studies on head circumference (HC) vs. autism (54). The effect of autism vs. other known influences on head size also may have been chronically over-stated. A systematic review of ~400,000 HC measures on ~75,000 children found that when models also included covariates known to strongly influence HC regardless of autism, such as parental height and parental HC, the covariates played a large role in child HC even when the parents were not themselves autistic. In addition, the best-replicated aspects

of early brain overgrowth across studies were more reflective of biases in HC norms than specific ASD traits or biomarkers (54). In a specific study, once gender, age, height, weight, and parental contribution were taken into account, the contribution of autism came to an overall effect of +2 millimeters. This small effect was, however, significant (53).

Nevertheless, in properly designed, control-matched studies, autism still associates with macrocephaly, even though most autistic children are not macrocephalic. Compared to typical development, autistic brains show a growth spurt shortly after birth, continuing in the first year of life, suggesting postnatal pathology. Neuropathological evidence of brain overgrowth include increased brain size and weight (55–57) and the early migration of pyramidal cells with more numerous, smaller, and less compact minicolumns (58–60). Minicolumns, described as vertical groups of large neurons flanked by cell-sparse surroundings, would require the growing support of short-range fibers that make up the white matter without affecting neuron density, as the brain grows larger (61). Large brains have been noted in autism, particularly in children <4 years of age, in cross sectional MR brain volumetry studies (3, 4, 62–64). Autism investigators have reported increases in total cerebral volume (64–66), cortical thickness (54), cortical surface area (39, 67), regional gray (60, 65, 68), and white matter (44, 69). A recent volumetric MRI longitudinal study of 200 children with autism identified 15% of this sample as boys with large heads disproportionate to height or "disproportionate megalencephaly" (70). Further study of this megalencephalic cohort revealed increased surface area but not increased cortical thickness (39), as well as increased white matter of the brain (70).

At young ages (2–6 years), brain enlargement occurs seemingly coincidental with the display of autistic symptoms (62, 69). This is followed by abnormal decline in brain growth by 12–16 years (71). This raises the question if genetic growth factors/neuroinflammation can cause early brain enlargement resulting in later destruction. Further, brain enlargement appears to be greatest in the frontal and temporal cortices and amygdala and least in the occipital regions. This corresponds to known deficits in autism in social skills (frontal lobes), language and communication (temporal lobes), and emotional control (amygdala) with sparing of the visual spatial abilities (occipital lobe) (71).

Few studies to date have undertaken cellular analysis to determine the neural underpinnings of brain overgrowth in autism. For example, an increased number of neurons in autism prefrontal gray matter could contribute to brain overgrowth (71). This study raises the possibility that a prenatal cause of autism as neurogenesis is complete before birth in prefrontal cortex and all of cerebral cortex (72) However, no similar studies have been performed on white matter. Myelin and white matter continue to develop until the third decade, which involves processes other than increased number of neurons, such as gliogenesis and myelination, as shown by mutations of the phosphatase and tensin homolog (PTEN) gene associated with autism and macrocephaly (73), as discussed below.

Functional Correlates of Increased Brain Growth in Autism

The functional correlates of enlarged brains in autism have eluded researchers. Early autism studies showed no association between macrocephaly and clinical function such as IQ or seizure frequency (9, 74), whereas in others, macrocephaly was associated with higher function (50, 75). However, IQ and seizure frequency are *not* core traits of autism. Instead, it may be that macrocephaly associates more strongly with *specific endophenotypes within the autism spectrum*, rather than autism in general. For example, within autistic boys (but not girls), greater HC associated with higher (more dysfunctional) scores in the Autism Diagnostic Interview-Revised (ADI-R) social algorithm scale (9). Further evidence that HC may correspond to different autism subtypes is that the HC of boys with regressive autism was significantly greater than non-autistic boys or boys with non-regressive autism. No HC differences were found for girls vs. regression (64).

Regional brain enlargement also is associated with higher function: enlarged amygdala in young children with autism, ages 2–4, was associated with joint attention ability, a core clinical feature of autism (76), and the larger volume of corpus callosum in younger preschool children was associated with lower severity of autism core symptoms (77). However, enlarged amygdala in autism does not necessarily entail macrocephaly (78). In a more comprehensive study of adults with autism with average intelligence (68), both cortical thickness and voxel based volume measurements identified enlarged gray matter regions that are associated with autism core features including the inferior frontal cortex, superior temporal sulcus, cingulate gyrus, middle occipital gyrus, fusiform gyrus, and inferior parietal lobule, which are associated with social abilities; superior temporal sulcus and inferior frontal gyrus, which are associated with communication; and orbital frontal gyrus and anterior cingulate gyrus, which are associated with repetitive behaviors. In particular, boys with disproportionate megalencephaly and autism had more severe disabilities, and a poorer prognosis (65), language deficits (79), and showed more regression (64). Large brains in autism may be responsible for more global deficits such as weak coherence theory, the ability to understand context or the "big picture," instead of more discrete autism core features (44).

MECHANISMS OF BRAIN OVERGROWTH IN AUTISM

Mechanisms of Increased Cortical Gray Matter in Autism

As stated above, cortical thickness is predicted by the number of neurons within the radial cortical columns, whereas surface area is predicted by the number of cortical columns (41). Casanova et al. (58, 60) have reported reduction in minicolumn width and neuropil spacing in layer III neocortex reflecting an increase in minicolumns in autism. β-catenin and caspase 3/9 proteins have been implicated in cortical surface area. Transgenic mice expressing β-catenin develop an increased number of progenitor cells in the ventricular zone which leads to more cortical radial

columns and increased surface area (80). Caspase 3/9 mutations result in decreased apoptosis of progenitor and radial glial cells, which increase surface area (81). Although this paper proposes a relationship between anabolic βAPP and increased white matter in autism, there are mechanisms potentially linking βAPP to increased gray matter as well. For example, βAPP shows reciprocal regulation of the anabolic Wnt/β-catenin signaling pathway, and βAPP physically interacts with β-catenin (82). Furthermore, βAPP decreases apoptosis in part by decreasing Caspase 3/9 by means of the phosphoinositide 3-kinase and Protein kinase B (PI3K/AKT) pathway in AML1-ETO positive (AE) leukemia (83). As described below, the contribution of βAPP to the PI3K/AKT pathway may be involved in white matter growth in autism.

Mechanisms of Increased Cortical White Matter in Autism

Autism has been described as a disorder of brain connectivity (84) and therefore the white matter, subjacent to gray matter cortex, which connects discreet cortical regions, is a region of interest. Increased white matter in autism exists in whole brain and cerebellum (62), in radiate outer zones (6), and in specific brain regions (85–88). Herbert (44) observed larger cerebral white matter volume in children with autism as well as in children with developmental language disorders. This was replicated in a larger sample of children with autism compared to control groups of children with benign macrocephaly and reading disability (89). Newer imaging techniques such as diffusion tensor imaging (DTI) have uncovered differences in white matter tract microstructure in autism (90–92). A review of cross sectional DTI in autism showed results consistent with an abundance of local, short white matter connections within lobes (e.g., temporal lobes) and reduced long distance connections (corpus callosum) between lobes (93, 94). Although white matter tracts appear disrupted in autism, the neuropathology underlying the disruption is not clear from DTI studies (93). Recent review of DTI longitudinal studies showed increased fractional anisotropy, a measure of white matter integrity, at 6 months of age, remaining atypical until 24 months of age and a reduced rate of white matter growth in late childhood-early adolescence in the left parietal, left occipital and bilateral temporal lobes (88). Hypermetabolism, as measured by fluorodeoxyglucose positron emission tomography (PET), was found for white matter within the internal capsule, corpus callosum, frontal, and temporal lobes of young adult subjects with autism, similar to patients with schizophrenia (95). This was explained by disruption of myelination (95). Altogether these results suggest at least a transient increase in white matter volume that may recede over time.

We propose that βAPP metabolites may contribute to mechanisms of increased white matter in autism. Thus, we discuss neuroinflammation, cytokine release, translation pathways and increased myelination. In contrast to increased neurons found in autism gray matter (71), neuroinflammation [including astrogliosis and microgliosis; (96, 97)], along with increased myelination, may contribute more to the white matter enlargement in autism (43, 44, 94). Genetic mechanisms

proposed to cause macrocephaly and autistic traits include genes affecting mRNA translation pathways (98). Many factors, including neuroinflammation and polygenic factors may converge on molecular pathways (mitogen-activated protein kinase -MAPK and mechanistic target of rapamycin- mTOR), which could explain significant features of ASD, including white matter enlargement. In this review, we propose that secreted amyloid precursor protein metabolites, particularly sAPPα, contribute to ASD white matter enlargement, ultimately through MAPK and protein translation pathways that may stimulate increased gliosis and/or myelination This is a novel application of pathways developed for AD which may invite new understanding and treatment for autism.

NEUROINFLAMMATORY EFFECTS

Neuroinflammation in Autism

How can neuroinflammation contribute to brain overgrowth in autism? Up until 2005, it had been assumed that autism did not involve an inflammatory process, as there were no reports of gliosis or replicable inflammation in neuropathologic studies or brain MRI (44). By using immunochemistry and cytokine protein arrays, Vargas first showed an increase in CSF level cytokines (macrophage chemoattractant protein-1 and tumor growth factor β1) and microglia-astroglial activation (increased glial fibrillary acidic protein-GFAP) in the medial frontal gyrus and cerebellum in children and adults with autism (96). Further studies showed microglial pathology in autism (60, 99, 100). Specific decrease in ramified microglia (said to underlie deficits in synaptic pruning) and increase in primed microglia (associated with chronic inflammation) were found in autistic gray and white matter (97). Neuroinflammation in autism appears to be similar to AD (44) showing microscopic but not MRI signs of inflammation. Confocal microscopy study of children with idiopathic autism and dup15q11.2-q13 revealed that p3 peptide (a neurotrophic, non-amyloidogenic βAPP processing product) aggregates in astrocytes and microglia (24). Using confocal microscopy, specific antibodies identified higher equivalents of sAPPα in gray and white matter structures for dup15q11.2-q13, followed by idiopathic autism, followed by typical controls.

Reports exist of peripheral blood abnormalities involving T-cell, B-cell, autoantibody production, and increased pro-inflammatory cytokines (101), but these studies were limited by cross sectional design, small sample size, subjects of different ages, and lack of standardized diagnosis. A recent longitudinal study of serum (with typically developing controls) and cerebrospinal fluid (no controls) from young children with autism (ages 2–8) surprisingly found no evidence of immune mediators supportive of active systemic inflammation in autism (102). Only "modulators of immune function," Epidermal Growth Factor (EGF) and soluble CD40 ligand were increased in autism serum (102). The authors concluded that this was due to a "genetically determined growth or immune-modulatory dysregulation" rather than an active systemic inflammatory response (102). The following CSF immune mediators were elevated: FLT3L, IL-15, CX3CL1, CXCL8, and CCL2, and their role interpreted as homeostatic in support of microglia

rather than an adaptive neuroinflammatory response. The biggest finding was the lack of overlap between peripheral and cerebrospinal markers and of serum markers over time, demonstrating no reliable peripheral biomarker (102).

Given the possibility of a genetically upregulated immunity in autism, how would this produce brain overgrowth? Herbert (44) suggests that white matter volume increase is due to cell swelling/tissue volume increase as a result of activated microglia and astroglia, or via a compensatory increase in vascularization. Neuroinflammation could trigger cytokine or chemokine release (44), as identified by Pardo (102), or other secondary signaling pathways such as mTor-PI3-Akt. Alternatively, neuroinflammation could lead to increased excitotoxicity with increased oligodendrocyte activity leading to an increase in myelination. Persistent, chronic neuroinflammation over time could then lead to cell death. This would explain the longitudinal changes of overgrowth followed by decrease in growth in autism brain MRI studies. Altogether, this sets the stage for the role βAPP metabolites may play in neuroinflammation, cytokine activation, and excessive myelination in autism.

Neuroinflammation and βAPP

AD is a neurodegenerative disorder characterized by ongoing, permanent memory impairment and dementia. It is construed as a multifactorial disease affected by genetic and environmental factors. Neuroinflammation, vascular compromise and free radical damage lead to the abnormal accumulation of cerebral Aβ peptide and ultimately death (103). Alpha secretase, now believed to be ADAM10 or ADAM17, constitutively cleaves βAPP to produce sAPPα, p3 peptide, and AICD along the non-amyloidogenic (neurotrophic) pathway. The β-amyloid hypothesis states that improper cleavage yields Aβ peptides found in the cerebral amyloid plaques of AD. Early in the disease, microglia are protective, increasing the phagocytic clearance of Aβ peptides. However, overtime, microglia become more catabolic producing proinflammatory cytokines IL-1 and TNF-α with the latter being more detrimental, eventually leading to brain atrophy seen in AD. Inflammatory mediators, in turn, cause more Aβ production (104). Evidence supports sAPPα activity on microglial cells, activating release of IL-1 (105), glutamate and inflammatory markers (106, 107). Treatment of neural stem cells with recombinant sAPPα led to increased astrogliogenesis (108). Treatment with sAPPα differentiates neuronal progenitor cells into astroglia (109).

ADAM17is one of the alpha secretases that is active in AD. It is found in higher levels in the CSF of patients with AD and its precursor Mild Cognitive Impairment (MCI) (110). ADAM-17 positive neurons are often found together with amyloid plaques in AD brains, suggestive that ADAM17 is involved in AD pathogenesis (111). ADAM17 produces neurotrophic sAPPα following the non-amyloidogenic pathway, but also contributes to the neuroinflammation in AD microglial activation (112). ADAM17, therefore, has the unique role of contributing to or preventing AD (113).

ADAM17 promotes cellular growth and acts as an extracellular physiological convertase, shedding proteins other than APP, including the EGF family of growth factors. These include TGF-α and its receptor EGF (114). ADAM17 contributes to generation and maturation of TNF-α, EGR, and some cell adhesion molecules [CAMS; (115)]. ADAM17 and ADAM10 cleave Notch proteins to induce Notch signaling (116). Further, acting anabolically, ADAM17 (and its kin, ADAM10) (mal) function in the pathogenesis of cancers, rheumatoid arthritis (117), and spinal cord injury (118). Besides converting EGF (114), the peripheral immune mediator found in the Pardo 2017 study of children with autism (102), ADAM17 converts the other cytokine mediators of autism found in the Pardo study: IL-15 (119), FLT3 (120), CX3CL1(121), and CXCL8 (122).

Key Translation Signaling Pathways in Inflammation

Key biochemical pathways involved in the immune response are of great interest as they also play a central role in growth and metabolism and may be involved in brain overgrowth in autism. mTOR and MAPK pathways regulate immune function (123). mTOR is composed of two structures, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). More is known about mTORC1 and its immediately upstream regulator Ras homolog enriched in brain (RHEB). RHEB is controlled by GTPase-activating protein (GAP), which involves the tuberous sclerosis complex 1 (TSC1 and TSC2). When this complex is phosphorylated by Akt or ERK1/2 (through phosphatidylinositol 3 kinase), this inhibits GAP activity so that RHEB is active, which in turn activates mTORC1. Several cytokine and growth factors activate mTOR including CD28, IL-1, IL-2, IL-4, and IL-12. mTOR is involved in T-cell trafficking as upon recognition of their antigen, $CD8^+$ T cells activate mTOR and switch to anabolism. Upon resolution of the infection, the antigen-specific $CD8^+$ cells contract and mTOR activity is decreased. mTOR promotes differentiation, activation, and function in T-cells, B-cells, and antigen presenting cells (123). The mTOR anabolic biochemical pathway is of great interest in autism (124) and in macrocephaly and autism (125). mTOR deregulation also exists in AD (35). Evidence supports βAPP's role in activating T-lymphocytes. For example, transcription, translation and secretion of βAPP have been induced via T-cell mitogen stimulation of blood leukocytes (126). sAPPα modulation of the immune system has been studied in an overproducing sAPPα mouse model (31). These mice showed increased levels of CD8 T-cells and decreased memory T-cells suggesting that sAPPα activates immunity.

The MAP kinase cascade is composed of three major groups: the p38 MAP kinases, the c-Jun NH2-terminal kinases (JNK) and the extracellular signal-regulated protein kinases (ERK). MAP kinases are important in lymphocyte development. Activation of MAP kinases p38 and JNK produces inflammatory cytokines including TNFα, IL-1, and IL-12. In turn, TNFα and IL-1 activate p38 and JNK MAP kinases. It has been shown that JNK and p38 activation acts catabolically, leading to apoptosis in AD (127). ERK regulates T cell activation and differentiation (128). The growth factor signaling properties of the MAPK pathways act anabolically, and are of interest in autism (124, 129, 130), with convergence of the MAPK pathway with calcium signaling pathways reported as major contributors to autism pathophysiology in a large gene set enrichment analysis (131). We speculate that TNFα, elevated in autism, favors increased

p38 and JNK resulting in increased brain matter, while decreased TNFα promotes apoptosis via p38 and JNK in AD.

REGULATORY FACTORS

Purported mTOR Regulation in Autism and AD

Neurotrophins, including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) bind to and activate tropomyosin-related kinases (TrkA, TrkB, or Trk C) which in turn activate PI3K, phospholipase C (PLC), ERK1/2, and mTOR (132). Neurotrophin signaling at multiple levels (epigenetic, transcriptional, post-transcriptional, and post-translational) determines cell fate, axonal and dendritic growth and pruning, and the connection of neural networks (124). Abnormalities in neurotrophins likewise exist in autism and AD (132).

mTOR is involved in promoting growth by means of protein synthesis, actin cytoskeletal dynamics, energy homeostasis and metabolism (124). Gene defects associated with upstream regulators of mTOR (TSC1, TSC2, and PTEN), are associated with autism and in many cases, macrocephaly. Constitutive activation of upstream PI3K, Akt, and Ras, is anabolic, and associates with macrocephaly, neuronal hypertrophy, increased soma size and dendritic complexity of neurons (133). Products of mTOR that contribute to translation such as p70 ribosomal S6 kinase 1 (Sgk1) and eukaryotic translation initiation factor 4 E-binding proteins (4E-BPs) may play a role in autism (98, 134). Over expression of eIF4E associates with an increase in complex formation, long-term synaptic plasticity, increased dendritic spine density, and behaviors similar to autism (135, 136). 4E-BPs provide a brake on eukaryotic translation initiative factor 4E (EIF4E) which is a primary effector through which proteins are synthesized. Phosphorylation of 4EBP by mTORC1 stops this inhibition favoring EIF4E, and anabolic protein synthesis (135). Huber's view, based on research with FXS, supports a model where FMRP represses a PI3K enhancer (PIKE) so that mTOR is inhibited under basal conditions (137). Lack of FMRP in FXS (and in some individuals with autism), would also favor PIKE activation of mTOR and downstream Rho GTPases including Rac1 and cofilin that in turn would cause actin disassembly and myelination (138). Recently, several studies have identified a catabolic role of mTOR, and the upstream and downstream components of its signaling, in the pathogenesis and progression of AD (35). In some models, activation of mTOR is interpreted as preventing autophagy of Aβ in the lysosomes. However, treatment of AD with mTOR inhibitor rapamycin was not recommended, as rapamycin decreases ADAM10, an alpha secretase that may promote the protective, anabolic, non-amyloidogenic pathway.

Myelination is metabolically demanding, and therefore, the mTOR pathway, particularly the mTORC1 hub coordinating cell metabolism, is a key signal for myelination (139). Besides its main function as a regulator of mRNA translation, mTORC1 activates lipid synthesis. Lipid is a major component of myelin. Further, mTORC1 appears to coordinate protein and lipid synthesis to make the membrane. It differentiates oligodendrocytes from OCPs. Interestingly, hyperactivation of oligodendrocytes

after disruption of the TSC complex by deletion of TSC1 effects *hypomyelination* in the CNS and PNS (140, 141). Hypomyelination and decreased oligodendrocytes were also reported after deletion of TSC2 (142). These changes were unexpected and not in keeping with the clinical picture of macrocephaly and often megalencephaly with white matter increases and tubers seen in tuberous sclerosis caused by TSC1 or TSC2 deletions, a single gene kin to autism. Therefore, other factors besides overactivation of mTOR must lead to excessive white matter in autism. Upstream from mTOR, Akt-1 is phosphorylated by PI3K in response to growth factors such as Neuregulin 1, insulin growth factors and steroids that promote myelination (143) The insulin growth factor 1 (IGF-1), when phosphorylated, stimulates the P13K/Akt and MAPK pathways and has been implicated in AD (144). IGF-1 acts anabolically, stimulating α-secretase and reducing β secretase that has been shown to decrease Aβ formation (145). In contrast, Aβ acts catabolically, resulting in over-expression of PTEN which leads to deactivation of P13K/Akt (146). Deletion of PTEN activates the P13K/Akt pathway which results in increased myelination, especially within the corpus callosum. Individuals with PTEN syndrome show macrocephaly and autism (73). It appears that there are several pathways that lead to myelination, perhaps via activation of the Rho family proteins that in turn activate myelination through sAPPα/α-secretases.

Purported MAPK Regulation in Autism and AD

ERK 1/2, among the MAPK family members are involved with cellular growth, chemokines, oxidative stress, and cytokines. Single gene mutations associated with autistic-like syndromes can also cause ERK1/2 activation including Tuberous sclerosis, FXS, 16p11.2 and Neurofibromatosis. Macrocephaly and increased white matter are often seen in these conditions (98). However, of far greater interest to investigating the much larger proportion of idiopathic autism, this pathway can be activated by multiple stimuli, both internal and environmental. These include interleukin-1β (IL-1β), TNF-α, and EGF (147), and EGF receptor [EGFR; (148)], all which are elevated in autism. The ADAM proteins play critical roles in EGFR signaling (115, 122, 149). Several studies have found an association between MAPK signaling and autistic traits. Indeed, genes from the MAPK pathway were among the most frequent represented in a large gene set analysis in autism (131). This pathway has also been intensely researched in cancer studies, as derangement in MAPK signaling may be important in the development of cancer (150, 151). ERK and MAP kinase p38, which are activated in response to inflammation or stress signals, directly activates ADAM17 (152, 153). Sources of inflammatory stimulation of MAPK include infection by Gram-negative bacteria, and ensuing lipopolysaccharide (LPS) exposure. Several mechanistic studies of LPS exposure in model animals showed that LPS exposure induced both behavioral and neurological autism-like symptoms, and that this induction was stronger in male than female offspring (154–158). The MAPK pathway is less implicated in pathogenesis of AD, although JNK and P38 activation leads to apoptosis in AD (159). The neurogenic properties of this pathway in association with those of the

non-amyloidogenic pathway have been studied and will be discussed below.

ERK1 and ERK2, downstream mediators of MAPK appear to control CNS myelin thickness after oligodendrocyte differentiation and initiation of myelin (160), as demonstrated by *in vivo* loss of function studies (160) and *in vivo* gain of ERK1/2 function studies (161). In the latter, two lines of transgenic mice with sustained activation of OPCs during early development produced transient over-proliferation of OPCs, but resulted in normal numbers of myelinating oligodendrocytes. This was interpreted as ERK 1/2 effecting a biphasic response-first an early expansion of OPC and a later promotion of myelin growth. Another MAPK regulator, P38, directs oligodendrocyte differentiation and myelination by way of gene transcription (162). BDNF increases OPC proliferation and development though the TrkB and MAPK pathway (163).

HYPERMYELINATION—A SOURCE OF INCREASED WHITE MATTER IN AUTISM?

Having discussed how neuroinflammation, potentially causes brain tissue edema and increased perfusion and how activated signaling pathways associated with growth factors could contribute to activation of white matter, we will now turn to the potential contribution of myelination to increased white matter in autism.

Oligodendrocytes ensheath multiple neuronal axons with a lipid-rich myelin membrane. Myelin allows rapid synaptic transmission, provides metabolic support and reduces the cost of neuronal energy (164). There has been much effort to understand myelin formation in the hopes of promoting myelin repair for conditions such as multiple sclerosis. The belief that remyelination "depends on signals that are similar to those occurring in developmental myelination" (143), provides a window into the origins of myelination and allows speculation as to what myelinating processes may be in overdrive in autism. During development, myelinating oligodendrocytes are produced by OPCs within the subventricular zone (SVZ) of the germinal matrix in the cortex. OPCs then undergo migration and proliferation and extend through the entire nervous system. OPCs retain the ability to migrate and to travel within the CNS into adulthood where they continue to generate new oligodendrocytes routinely (165), and after demyelinating injury (166). In recent years it has been discovered that OPCs do more than just give rise to oligodendrocytes, as "they are found throughout the brain in numbers far greater than would be needed for that role" (46). OPCs are the only glia that receive synaptic input from neurons by way of an NG2 protein (28). ADAM10, one of the purported alpha secretases, cleaves this protein in response to neuronal network activity (28). It appears that NG2 cleavage functions to strengthen long-term potentiation (LTP) in the mouse somatosensory cortex, so, if not a growth factor, overexpression of ADAM10 may yield LTP aberration through OPC NG2 cleavage. OPC polarity and directional migration also appears to be under the control of NG2 glia (167), which features RhoA and Rac signaling. It is worth noting here that mTOR regulates cofilin through Rho family member Rac1, important in myelination (see below) and implicated in autism (137). OPC embryonic development is guided along endothelial cells by the powerful anabolic Wnt signaling (168), a pathway associated with autism (169), and regulated by the NOTCH canonical pathway (170) and βAPP (171). There appear to be several paths to increased myelination, and perhaps autism, should OPC N2 glial cells get excited.

Another view is that the consistent, abundant supply of OPCs in the brain may be required for developing novel motor skills (172). Myelination is a developmental process and can continue into the third decade of life (173). Myelination can change according to environmental experience (43). Early studies reported oligodendroctyes increase by 27–33% in the visual cortex of rats raise in enriched environments (174), and the number of myelinated axons within the corpus callosum increases in rats (175) and rhesus monkeys raised in enriched environments (176). Myelination of specific areas of the brain correlate with children's cognitive ability (177) and learning new tasks (178, 179). Indeed, activity dependent communication between axon and oligodendrocytes may cause increased oligodendrocyte production, and thicker and longer myelin on axons (164). However, too much stimulation may be deleterious. In rats, stress during late pregnancy causes hypermyelination in the offspring (180). Maternal IgG antibodies directed against fetal brain, considered immunologic stress, are elevated in mothers of and children with autism (181–183). These human maternal antibodies were injected into pregnant rhesus monkeys and their offspring followed for 2 years (184, 185). Their offspring showed subtle autistic behaviors; neuropathology revealed enlarged brains notable for increased frontal lobe white matter (184).

During OPC differentiation and myelination, OPC processes change from thin membrane extensions to multi-layered, lipid rich tubes ensheathing axons (164). The myelin sheath growth occurs in two steps. First, an actin network supports the leading edge clamping it between the axon and overlying oligodendrocyte. Second, actin disassembly allows the myelin membrane to spread around and along the axon (138). Actin is disassembled by cofilin and gelsolin family proteins. Myelin Basic Protein (MBP), an important component of CNS myelin, is necessary for myelin wrapping. MBP promotes myelination by releasing cofilin and gelsolin from the membrane and deactivating actin. The Rho1/Rac polarity of the OCP NG2 demonstrates how oligodendrocyte differentiation might be paired with axon wrapping (164). Pertinent to APP, MBP is a potent inhibitor of Aβ fibrillary assembly (186). Similar to how MBP functionally disassembles actin, the C-terminal of MBP_{1-64} binds to Aβ40 and Aβ42 peptides to inhibit fibril assembly. This would deter developing AD. These authors Kotarba et al. note that in human brain and in APP transgenic mice, Aβ peptides usually are not seen in MBP rich white matter. Several studies show increased levels of autoantibodies to MBP in the sera of children with autism (187, 188), implying that cerebral MBP levels may be high, although MBP has not been extensively studied in autism brain tissue. In AD brain tissue, MBD was degraded, suggesting destruction of white matter in AD (189).

There is *in vitro* evidence that ADAM8 (not an alpha-secretase) cleaves MBP (190, 191), which may indicate an inflammatory reaction in AD (191). Therefore, reduction of MBP may lead to increased Aβ peptides and brain atrophy in AD, whereas increased MBP favors myelination and brain growth in autism. Could these mechanisms in autism confer protection from AD? This question recently has been discussed (32).

ADAM FAMILY α-SECRETASES

ADAM secretases perform many duties and play a role in CNS myelination. For many years, it was known that ADAM family members, particularly ADAM22, were important in peripheral system myelination (192), although, ADAM17 inhibited Schwann cell myelination (193). However, recently ADAM17 turned out to be "essential for oligodendrocyte development and CNS myelination" (29). ADAM17 modulated OPC cell cycle exit and oligodendrocyte lineage cell survival during subcortical white matter development in transgenic mice. ADAM17 accomplished this by shedding EGFR ligands and performed activation of oligodendrocytes during white matter development by EGFR signaling. EGFR overexpression in ADAM17-deficient OCPs restored cell survival and proliferation and subsequent myelination. Unlike other reports of diseased states, this study was distinguished by the analysis of oligodendrocytes during postnatal CNS myelination. The authors noted a divergence of ADAM17 function between the CNS and PNS (29).

NOTCH

Notch signaling is essential for glial development and CNS myelination (164, 170). Notch receptors are cleaved intracellularly by secretases. In the CNS, Notch 1 receptor is expressed by oligodendrocytes. Studies show that Notch 1 is important for correct OPC temporal and spatial differentiation (164). Contactin-1, a ligand of Notch, may promote OPC differentiation within the expression of CNS myelin genes. The contactin family of Ig cell adhesion molecules harbor several members that have a genetic association with autism (194). Following the canonical pathway, ADAM10 cleaves NOTCH at the membrane which activates a piece that participates in transcription. Activation of NOTCH within the non-canonical pathway leads to OPC maturation and myelination. APP interacts with Notch receptors and the APP gamma secretase that produces the Aβ peptide is a Notch family member (195).

THE CASE FOR sAPPα DIRECTING INCREASED WHITE MATTER IN AUTISM

Typical processing of βAPP greatly favors production of sAPPα over Aβ peptide. Could an increase in α-secretase activation overproduce myelin to the extent of inducing
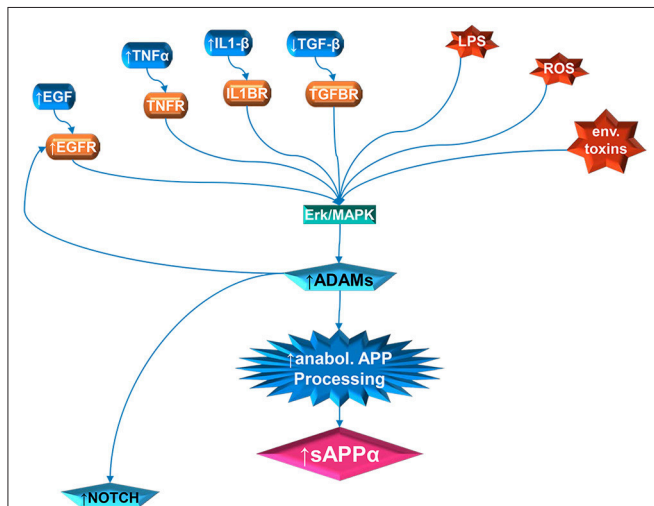

FIGURE 1 | Multiple pathway stimulation of ADAM activity, leading to sAPPα proliferation and notch stimulation. The contribution of white matter overgrowth to autistic symptoms could begin with aberrant signaling of the ERK/MAPK pathway. Multiple extracellular signal molecules have disrupted levels in autism. This includes IL1β, EGF, and TNFα, which are elevated; and TGFβ, which is depressed. In addition, the receptor for EGF (EGFR) is elevated in autism. External stressors, such as LPS and reactive oxidizing species (ROS) produced by oxidative stress, have been implicated in autism. Activity of specific environmental toxins may exist but is still controversial. Once the ERK/MAPK pathway has, by whatever means, been perturbed, this can stimulate the ADAM proteins. The ADAMs not only cleave APP at the anabolic cleavage site, producing sAPPα, but are also necessary for EGFR signaling and stimulate NOTCH enzyme activity.
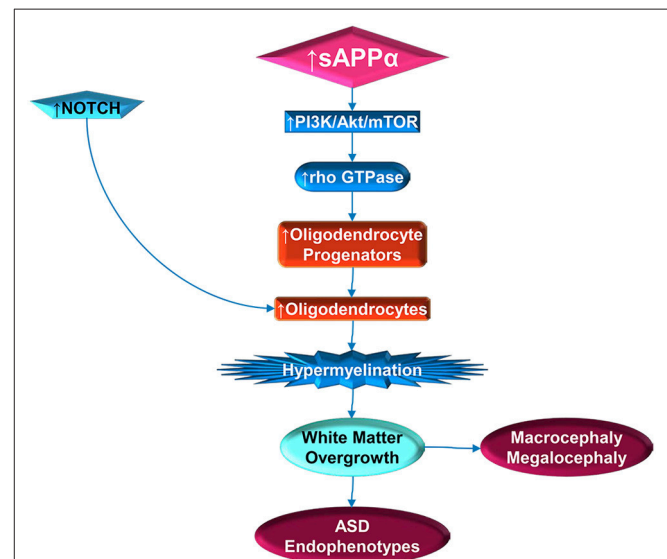

FIGURE 2 | Stimulation of white matter overgrowth by elevated sAPPα (and Notch). Elevated sAPPα enhances PI3K/Act/mTOR pathway activity, which includes rho GTPase. The GTPase stimulates oligodendrocyte progenitors, which require NOTCH activity to mature into oligodendrocytes. Elevated oligodendrocytes would then contribute to hypermyelination, which would lead to white matter overgrowth. The white matter overgrowth would contribute to exacerbation of ASD endophenotypes, such as poor social functioning or regression. Incidentally, this would also be reflected by macrocephaly and megalocephaly.

or exacerbating autism? As previously described, ADAM17 appears to act directly on oligodendrocyte development and CNS myelination and in oligodendrocyte regeneration (29, 149). *In vitro* α-secretase activation produces an increased number of mature oligodendrocytes and increased percentage of myelinated axons with short internodes (196). One clinical study of whole-exome sequencing in children with macrocephaly and/or autism showed a surprising 10/21 patients with likely pathogenic mutations along the PI3K/AKT-mTOR pathway (125). It is possible that similar underlying, closely related genetic mutations could push sAPPα into overdrive, resulting in autism.

Increased sAPPα in autism could be a neuroinflammatory response with resultant upregulation of the non-amyloidogenic pathway. Bailey et al. (23) demonstrated increased sAPPα, neuroinflammatory GFAP and gliosis in transgenic mice designed to overexpress sAPPα in brain tissue. Upregulation of GFAP was correlated with elevations in Interleukin 6 (IL-6), gp130, and Notch1. The IL-6/gp130 pathway is considered anabolic and promotes axonal sprouting of neurons and activated astrocytes after entorhinal cortex lesion in rats (197). Furthermore, ADAM17 participates in the cleavage of inflammatory factors related to microglial activation (113) and in reaction to injury (118). A prenatal insult would also increase the expression of NMDA and glutamate receptors in the offspring. High glutamate receptors activate the ERK signaling cascade. Zeidan-Chulia et al. (198) proposed this model for autism after performing a focused microarray analysis of genes belonging to NOTCH, WNT and AD. They found upregulation of glutamate ionotropic receptor NMDA type subunit 1 (GRIN1), and MAP3K1, which activates the JNK and ERK pathways. Among their conclusions, they proposed that epigenetic stress could lead to increased NMDA receptors and increased calcium that would stimulate ERK-dependent α-secretase activity. Activation of ADAM17 by ERK (and P38 MAP kinase), for example, can activate EGF receptor signaling which leads to enhanced cell proliferation (152, 153). Higher levels of sAPPα would then activate the PI3K/Akt/mTOR pathway also resulting in aberrant brain growth. This model applies to brain cells in general, not just white matter. However, as described above, mTOR pathways favors myelination, except for the findings that disruption of upper mTOR pathways result in hypomyelination instead of hypermyelination (123). Therefore, other pathways such as activation of ERK1/2 may be needed to explain increased white matter in autism (**Figure 1**).

The intersection of sAPPα, its ADAM family secretases and white matter expansion may be most convincingly proposed by merging the above mentioned models (137, 198) with the recent finding that the abundant OPC N2G is regulated by Rho GTPases (164). α-secretase, activated by ERK receptor signaling, in turn may activate PI3K/Akt/mTOR, and then Rho GTPases, which would favor OPC stimulation (**Figure 2**). Subsequent activation of cofilin with the disassembly of actin also favors myelination, again stimulated by α-secretase. Recent findings

that netrin-1 appears to underlie OPC density and turnover (199), and that OPC migration along blood vessels is mediated by WNT signaling (168), further opens the door to APP regulation of myelination. APP regulates Netrin-1 mediated commissural axon outgrowth (200), and Wnt signaling is protective against Aβ peptide akin to the non-amyloidogenic pathway (201). Future study of α-secretase in relation to autism may enable novel treatments and avoid pitfalls in tested treatments for AD. One example would be to determine if ADAM17 antibody could reduce excessive brain growth and autistic symptoms, similarly to the recent success of the drug BAN2401 (202), an antibody that targets Aβ peptide.

CONCLUSION

Brain overgrowth is a consistent endophenotype in 20% of individuals with autism. MRI volumetric studies showed overgrowth for both gray and white matter for young children (ages 2–6) with autism, coincidental to presentation of autistic symptoms. The trajectory of brain growth slows in adolescence and may show decreased growth at older ages. Enlargement of brain matter in autism may be due to a combination of elevated metabolic processes, migrational abnormality, and/or neuroinflammation. Recognizing potential contribution of the non-amyloidogenic pathway of βAPP processing to brain enlargement in autism enables novel adaptation of long-known AD pathway analyses to autism. Increased sAPPα and the ADAM family α-secretases may directly increase oligodendrocyte myelination or the neuroinflammatory response that promotes axonal sprouting of neurons and astrocyte activation. Consequently sAPPα and the ADAM family α-secretases, activated ERK receptor signaling, can activate P13K/AKt/mTOR. Resulting activation of Rho GTPases would favor OPC stimulation, thus enhancing myelination by activation of cofilin. Identification of new roles for AD pathways in autism may lead to new treatments for this enigmatic disorder.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

ACKNOWLEDGMENTS

REFERENCES

1. Battle DE. Diagnostic and statistical manual of mental disorders (DSM). *Codas.* (2013) 25:191–2. doi: 10.1176/appi.books.9780890425596

2. Wright J. *Autism Rates in the United States Explained.* Simons Foundation (2017). Available online at: https://www.spectrumnews.org/news/autism-rates-united-states-explained/ (accessed March 12, 2018).

3. Aylward EH, Minshew NJ, Field K, Sparks BF, Singh N. Effects of age on brain volume and head circumference in autism. *Neurology.* (2002) 59:175–83. doi: 10.1212/WNL.59.2.175

4. Sparks BF, Friedman SD, Shaw DW, Aylward EH, Echelard D, Artru AA, et al. Brain structural abnormalities in young children with autism spectrum disorder. *Neurology.* (2002) 59:184–92. doi: 10.1212/WNL.59.2.184

5. Courchesne E, Carper R, Akshoomoff N. Evidence of brain overgrowth in the first year of life in autism. *JAMA.* (2003) 290:337–44. doi: 10.1001/jama.290.3.337

6. Herbert MR, Ziegler DA, Makris N, Filipek PA, Kemper TL, Normandin JJ, et al. Localization of white matter volume increase in autism and developmental language disorder. *Ann Neurol.* (2004) 55:530–40. doi: 10.1002/ana.20032

7. Sokol DK, Edwards-Brown M. Neuroimaging in autistic spectrum disorder (ASD). *J Neuroimaging.* (2004) 14:8–15. doi: 10.1111/j.1552-6569.2004.tb00210.x

8. Pavone P, Pratico AD, Rizzo R, Corsello G, Ruggieri M, Parano E, et al. A clinical review on megalencephaly: a large brain as a possible sign of cerebral impairment. *Medicine.* (2017) 96:e6814. doi: 10.1097/MD.0000000000006814

9. Lainhart JE, Piven J, Wzorek M, Landa R, Santangelo SL, Coon H, et al. Macrocephaly in children and adults with autism. *J Am Acad Child Adolesc Psychiatry.* (1997) 36:282–90. doi: 10.1097/00004583-199702000-00019

10. Alzheimer's Association. 2018 Alzheimer's disease facts and figures. *Alzheimers Dementia.* (2018) 14:367–429. doi: 10.1016/j.jalz.2018.02.001

11. Sokol DK, Chen D, Farlow MR, Dunn DW, Maloney B, Zimmer JA, et al. High levels of Alzheimer beta-amyloid precursor protein (APP) in children with severely autistic behavior and aggression. *J Child Neurol.* (2006) 21:444–9. doi: 10.1177/08830738060210062201

12. Sokol DK, Long J, Maloney B, Chen D, Lahiri DK. Potential Alzheimer's disease markers for autism? Beta amyloid precursor protein and acetylcholinesterase correlated with aggression in autism. In: *61st Annual Meeting of American-Academy-of-Neurology.* Seattle, WA (2009).

13. Ray B, Long JM, Sokol DK, Lahiri DK. Increased secreted amyloid precursor protein-alpha (sAPPalpha) in severe autism: proposal of a specific, anabolic pathway and putative biomarker. *PLoS ONE.* (2011) 6:e20405. doi: 10.1371/journal.pone.0020405

14. Sokol DK, Maloney B, Long JM, Ray B, Lahiri DK. Autism, Alzheimer disease, and fragile X: APP, FMRP, and mGluR5 are molecular links. *Neurology.* (2011) 76:1344–52. doi: 10.1212/WNL.0b013e3182166dc7

15. Bailey AR, Giunta BN, Obregon D, Nikolic WV, Tian J, Sanberg CD, et al. Peripheral biomarkers in Autism: secreted amyloid precursor protein-alpha as a probable key player in early diagnosis. *Int J Clin Exp Med.* (2008) 1:338–44.

16. Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, et al. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature.* (1987) 325:733–6. doi: 10.1038/325733a0

17. Hefter D, Draguhn A. APP as a Protective Factor in Acute Neuronal Insults. *Front Mol Neurosci.* (2017) 10:22. doi: 10.3389/fnmol.2017.00022

18. Weingarten J, Weingarten M, Wegner M, Volknandt W. APP—a novel player within the presynaptic active zone proteome. *Front Mol Neurosci.* (2017) 10:43. doi: 10.3389/fnmol.2017.00043

19. Herbst-Robinson KJ, Liu L, James M, Yao Y, Xie SX, Brunden KR. Inflammatory eicosanoids increase amyloid precursor protein expression via activation of multiple neuronal receptors. *Sci Rep.* (2015) 5:18286. doi: 10.1038/srep18286

20. Yang X, Sheng W, Ridgley DM, Haidekker MA, Sun GY, Lee JC. Astrocytes regulate alpha-secretase-cleaved soluble amyloid precursor protein secretion in neuronal cells: involvement of group IIA secretory phospholipase A2. *Neuroscience.* (2015) 300:508–17. doi: 10.1016/j.neuroscience.2015.05.052

21. Ray B, Sokol DK, Maloney B, Lahiri DK. Finding novel distinctions between the sAPPalpha-mediated anabolic biochemical pathways in Autism Spectrum Disorder and Fragile X Syndrome plasma and brain tissue. *Sci Rep.* (2016) 6:26052. doi: 10.1038/srep26052

22. Westmark CJ, Sokol DK, Maloney B, Lahiri DK. Novel roles of amyloid-beta precursor protein metabolites in fragile X syndrome and autism. *Mol Psychiatry.* (2016) 21:1333–41. doi: 10.1038/mp.2016.134

23. Bailey AR, Hou H, Song M, Obregon DF, Portis S, Barger S, et al. GFAP expression and social deficits in transgenic mice overexpressing human sAPPalpha. *Glia.* (2013) 61:1556–69. doi: 10.1002/glia.22544

24. Wegiel J, Frackowiak J, Mazur-Kolecka B, Schanen NC, Cook EH Jr, Sigman M, et al. Abnormal intracellular accumulation and extracellular Abeta deposition in idiopathic and Dup15q11.2-q13 autism spectrum disorders. *PLoS ONE.* (2012) 7:e35414. doi: 10.1371/journal.pone.0035414

25. Buxbaum JD, Liu KN, Luo Y, Slack JL, Stocking KL, Peschon JJ, et al. Evidence that tumor necrosis factor alpha converting enzyme is involved in regulated alpha-secretase cleavage of the Alzheimer amyloid protein precursor. *J Biol Chem.* (1998) 273:27765–7. doi: 10.1074/jbc.273.43.27765

26. Asai M, Hattori C, Szabo B, Sasagawa N, Maruyama K, Tanuma S, et al. Putative function of ADAM9, ADAM10, and ADAM17 as APP alpha-secretase. *Biochem Biophys Res Commun.* (2003) 301:231–5. doi: 10.1016/S0006-291X(02)02999-6

27. Kim H, Hwang JS, Woo CH, Kim EY, Kim TH, Cho KJ, et al. TNF-alpha-induced up-regulation of intercellular adhesion molecule-1 is regulated by a Rac-ROS-dependent cascade in human airway epithelial cells. *Exp Mol Med.* (2008) 40:167–75. doi: 10.3858/emm.2008.40.2.167

28. Sakry D, Neitz A, Singh J, Frischknecht R, Marongiu D, Biname F, et al. Oligodendrocyte precursor cells modulate the neuronal network by activity-dependent ectodomain cleavage of glial NG2. *PLoS Biol.* (2014) 12:e1001993. doi: 10.1371/journal.pbio.1001993

29. Palazuelos J, Klingener M, Raines EW, Crawford HC, Aguirre A. Oligodendrocyte regeneration and CNS remyelination require TACE/ADAM17. *J Neurosci.* (2015) 35:12241–7. doi: 10.1523/JNEUROSCI.3937-14.2015

30. Lahiri DK, Maloney B, Ray B, Sokol DK. Novel parallels and distinctions among APP metabolite pathways in Alzheimer's disease and neurodevelopmental disorders. *Alzheimers Dementia.* (2016) 12:P1150. doi: 10.1016/j.jalz.2016.07.048

31. Bailey AR, Hou H, Obregon DF, Tian J, Zhu Y, Zou Q, et al. Aberrant T-lymphocyte development and function in mice overexpressing human soluble amyloid precursor protein-alpha: implications for autism. *FASEB J.* (2012) 26:1040–51. doi: 10.1096/fj.11-195438

32. Lahiri DK, Sokol DK, Erickson C, Ray B, Ho CY, Maloney B. Autism as early neurodevelopmental disorder: evidence for an sAPPalpha-mediated anabolic pathway. *Front Cell Neurosci.* (2013) 7:94. doi: 10.3389/fncel.2013.00094

33. Westmark CJ, Malter JS. FMRP mediates mGluR5-dependent translation of amyloid precursor protein. *PLoS Biol.* (2007) 5:e52. doi: 10.1371/journal.pbio.0050052

34. Williams CA, Dagli A, Battaglia A. Genetic disorders associated with macrocephaly. *Am J Med Genet A.* (2008) 146A:2023–37. doi: 10.1002/ajmg.a.32434

35. Cai Z, Chen G, He W, Xiao M, Yan LJ. Activation of mTOR: a culprit of Alzheimer's disease? *Neuropsychiatr Dis Treat.* (2015) 11:1015–30. doi: 10.2147/NDT.S75717

36. Venezia V, Nizzari M, Repetto E, Violani E, Corsaro A, Thellung S, et al. Amyloid precursor protein modulates ERK-1 and −2 signaling. *Ann N Y Acad Sci.* (2006) 1090:455–65. doi: 10.1196/annals.1378.048

37. Rohe M, Carlo AS, Breyhan H, Sporbert A, Militz D, Schmidt V, et al. Sortilin-related receptor with A-type repeats (SORLA) affects the amyloid precursor protein-dependent stimulation of ERK signaling and adult neurogenesis. *J Biol Chem.* (2008) 283:14826–34. doi: 10.1074/jbc.M710574200

38. Bhaskar K, Miller M, Chludzinski A, Herrup K, Zagorski M, Lamb BT. The PI3K-Akt-mTOR pathway regulates Abeta oligomer induced neuronal cell cycle events. *Mol Neurodegener.* (2009) 4:14. doi: 10.1186/1750-1326-4-14

39. Ohta H, Nordahl CW, Iosif AM, Lee A, Rogers S, Amaral DG. Increased surface area, but not cortical thickness, in a subset of young boys with autism spectrum disorder. *Autism Res.* (2016) 9:232–48. doi: 10.1002/aur.1520

40. Panizzon MS, Fennema-Notestine C, Eyler LT, Jernigan TL, Prom-Wormley E, Neale M, et al. Distinct genetic influences on cortical surface area and cortical thickness. *Cereb Cortex.* (2009) 19:2728–35. doi: 10.1093/cercor/bhp026

41. Rakic P. Specification of cerebral cortical areas. *Science.* (1988) 241:170–6. doi: 10.1126/science.3291116

42. Rakic P. Evolution of the neocortex: a perspective from developmental biology. *Nat Rev Neurosci.* (2009) 10:724–35. doi: 10.1038/nrn2719

43. Fields RD. White matter in learning, cognition and psychiatric disorders. *Trends Neurosci.* (2008) 31:361–70. doi: 10.1016/j.tins.2008.04.001

44. Herbert MR. Large brains in autism: the challenge of pervasive abnormality. *Neuroscientist.* (2005) 11:417–40. doi: 10.1177/0091270005278866

45. Dawson G, Munson J, Webb SJ, Nalty T, Abbott R, Toth K. Rate of head growth decelerates and symptoms worsen in the second year of life in autism. *Biol Psychiatry.* (2007) 61:458–64. doi: 10.1016/j.biopsych.2006.07.016

46. Jones R. Glia talk back. *PLoS Biol.* (2014) 12:e1001990. doi: 10.1371/journal.pbio.1001990

47. Dimou L, Simon C, Kirchhoff F, Takebayashi H, Gotz M. Progeny of Olig2-expressing progenitors in the gray and white matter of the adult mouse cerebral cortex. *J Neurosci.* (2008) 28:10434–42. doi: 10.1523/JNEUROSCI.2831-08.2008

48. Kanner L. Autistic disturbances of affective contact. *Nervous Child.* (1943) 2:217–50.

49. Deutsch CK, Joseph RM. Brief report: cognitive correlates of enlarged head circumference in children with autism. *J Autism Dev Disord.* (2003) 33:209–15. doi: 10.1023/A:1022903913547

50. Dementieva YA, Vance DD, Donnelly SL, Elston LA, Wolpert CM, Ravan SA, et al. Accelerated head growth in early development of individuals with autism. *Pediatr Neurol.* (2005) 32:102–8. doi: 10.1016/j.pediatrneurol.2004.08.005

51. Lainhart JE, Bigler ED, Bocian M, Coon H, Dinh E, Dawson G, et al. Head circumference and height in autism: a study by the Collaborative Program of Excellence in Autism. *Am J Med Genet A.* (2006) 140:2257–74. doi: 10.1002/ajmg.a.31465

52. Daymont C, Hwang WT, Feudtner C, Rubin D. Head-circumference distribution in a large primary care network differs from CDC and WHO curves. *Pediatrics.* (2010) 126:e836–42. doi: 10.1542/peds.2010-0410

53. Chaste P, Klei L, Sanders SJ, Murtha MT, Hus V, Lowe JK, et al. Adjusting head circumference for covariates in autism: clinical correlates of a highly heritable continuous trait. *Biol Psychiatry.* (2013) 74:576–84. doi: 10.1016/j.biopsych.2013.04.018

54. Raznahan A, Wallace GL, Antezana L, Greenstein D, Lenroot R, Thurm A, et al. Compared to what? Early brain overgrowth in autism and the perils of population norms. *Biol Psychiatry.* (2013) 74:563–75. doi: 10.1016/j.biopsych.2013.03.022

55. Bauman M, Kemper TL. Histoanatomic observations of the brain in early infantile autism. *Neurology.* (1985) 35:866–74. doi: 10.1212/WNL.35.6.866

56. Bailey A, Luthert P, Dean A, Harding B, Janota I, Montgomery M, et al. A clinicopathological study of autism. *Brain.* (1998) 121 (Pt 5):889–905. doi: 10.1093/brain/121.5.889

57. Kemper TL, Bauman M. Neuropathology of infantile autism. *J Neuropathol Exp Neurol.* (1998) 57:645–52. doi: 10.1097/00005072-199807000-00001

58. Casanova MF, Buxhoeveden DP, Brown C. Clinical and macroscopic correlates of minicolumnar pathology in autism. *J Child Neurol.* (2002) 17:692–5. doi: 10.1177/088307380201700908

59. Casanova MF, Buxhoeveden DP, Switala AE, Roy E. Minicolumnar pathology in autism. *Neurology.* (2002) 58:428–32. doi: 10.1212/WNL.58.3.428

60. Casanova MF, Van Kooten IA, Switala AE, Van Engeland H, Heinsen H, Steinbusch HW, et al. Minicolumnar abnormalities in autism. *Acta Neuropathol.* (2006) 112:287–303. doi: 10.1007/s00401-006-0085-5

61. Casanova MF. White matter volume increase and minicolumns in autism. *Ann Neurol.* (2004) 56:453; author reply 454. doi: 10.1002/ana.20196

62. Courchesne E, Karns CM, Davis HR, Ziccardi R, Carper RA, Tigue ZD, et al. Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology.* (2001) 57:245–54. doi: 10.1212/WNL.57.2.245

63. Stanfield AC, Mcintosh AM, Spencer MD, Philip R, Gaur S, Lawrie SM. Towards a neuroanatomy of autism: a systematic review and meta-analysis

64. Nordahl CW, Lange N, Li DD, Barnett LA, Lee A, Buonocore MH, et al. Brain enlargement is associated with regression in preschool-age boys with autism spectrum disorders. *Proc Natl Acad Sci USA.* (2011) 108:20195–200. doi: 10.1073/pnas.1107560108

65. Amaral DG, Li D, Libero L, Solomon M, Van de Water J, Mastergeorge A, et al. In pursuit of neurophenotypes: the consequences of having autism and a big brain. *Autism Res.* (2017) 10:711–22. doi: 10.1002/aur.1755

66. Pagnozzi AM, Conti E, Calderoni S, Fripp J, Rose SE. A systematic review of structural MRI biomarkers in autism spectrum disorder: a machine learning perspective. *Int J Dev Neurosci.* (2018) 71:68–82. doi: 10.1016/j.ijdevneu.2018.08.010

67. Hazlett HC, Poe MD, Gerig G, Styner M, Chappell C, Smith RG, et al. Early brain overgrowth in autism associated with an increase in cortical surface area before age 2 years. *Arch Gen Psychiatry.* (2011) 68:467–76. doi: 10.1001/archgenpsychiatry.2011.39

68. Hyde KL, Samson F, Evans AC, Mottron L. Neuroanatomical differences in brain areas implicated in perceptual and other core features of autism revealed by cortical thickness analysis and voxel-based morphometry. *Hum Brain Mapp.* (2010) 31:556–66. doi: 10.1002/hbm.20887

69. Courchesne E, Pierce K. Brain overgrowth in autism during a critical time in development: implications for frontal pyramidal neuron and interneuron development and connectivity. *Int J Dev Neurosci.* (2005) 23:153–70. doi: 10.1016/j.ijdevneu.2005.01.003

70. Libero LE, Nordahl CW, Li DD, Ferrer E, Rogers SJ, Amaral DG. Persistence of megalencephaly in a subgroup of young boys with autism spectrum disorder. *Autism Res.* (2016) 9:1169–82. doi: 10.1002/aur.1643

71. Courchesne E, Mouton PR, Calhoun ME, Semendeferi K, Ahrens-Barbeau C, Hallet MJ, et al. Neuron number and size in prefrontal cortex of children with autism. *JAMA.* (2011) 306:2001–10. doi: 10.1001/jama.2011.1638

72. Lainhart JE, Lange N. Increased neuron number and head size in autism. *JAMA.* (2011) 306:2031–2. doi: 10.1001/jama.2011.1633

73. Frazier TW, Embacher R, Tilot AK, Koenig K, Mester J, Eng C. Molecular and phenotypic abnormalities in individuals with germline heterozygous PTEN mutations and autism. *Mol Psychiatry.* (2015) 20:1132–8. doi: 10.1038/mp.2014.125

74. Miles JH, Hillman RE. Value of a clinical morphology examination in autism. *Am J Med Genet.* (2000) 91:245–53. doi: 10.1002/(sici)1096-8628(20000410)91:4<245::aid-ajmg1>3.3.co;2-u

75. Gillberg C, de Souza L. Head circumference in autism, Asperger syndrome, and ADHD: a comparative study. *Dev Med Child Neurol.* (2002) 44:296–300. doi: 10.1111/j.1469-8749.2002.tb00814.x

76. Mosconi MW, Cody-Hazlett H, Poe MD, Gerig G, Gimpel-Smith R, Piven J. Longitudinal study of amygdala volume and joint attention in 2- to 4-year-old children with autism. *Arch Gen Psychiatry.* (2009) 66:509–16. doi: 10.1001/archgenpsychiatry.2009.19

77. Giuliano A, Saviozzi I, Brambilla P, Muratori F, Retico A, Calderoni S. The effect of age, sex and clinical features on the volume of Corpus Callosum in pre-schoolers with Autism Spectrum Disorder: a case-control study. *Eur J Neurosci.* (2018) 47:568–78. doi: 10.1111/ejn.13527

78. Schumann CM, Barnes CC, Lord C, Courchesne E. Amygdala enlargement in toddlers with autism related to severity of social and communication impairments. *Biol Psychiatry.* (2009) 66:942–9. doi: 10.1016/j.biopsych.2009.07.007

79. Naigles LR, Johnson R, Mastergeorge A, Ozonoff S, Rogers SJ, Amaral DG, et al. Neural correlates of language variability in preschool-aged boys with autism spectrum disorder. *Autism Res.* (2017) 10:1107–19. doi: 10.1002/aur.1756

80. Chenn A, Walsh CA. Increased neuronal production, enlarged forebrains and cytoarchitectural distortions in beta-catenin overexpressing transgenic mice. *Cereb Cortex.* (2003) 13:599–606. doi: 10.1093/cercor/13.6.599

81. Haydar TF, Kuan CY, Flavell RA, Rakic P. The role of cell death in regulating the size and shape of the mammalian forebrain. *Cereb Cortex.* (1999) 9:621–6. doi: 10.1093/cercor/9.6.621

82. Zhang N, Parr CJC, Birch AM, Goldfinger MH, Sastre M. The amyloid precursor protein binds to beta-catenin and

modulates its cellular distribution. *Neurosci Lett.* (2018) 685:190–5. doi: 10.1016/j.neulet.2018.08.044

83. Yu G, Yin C, Jiang L, Zheng Z, Wang Z, Wang C, et al. Amyloid precursor protein cooperates with c-KIT mutation/overexpression to regulate cell apoptosis in AML1-ETO-positive leukemia via the PI3K/AKT signaling pathway. *Oncol Rep.* (2016) 36:1626–32. doi: 10.3892/or.2016.4963

84. Minshew NJ, Williams DL. The new neurobiology of autism: cortex, connectivity, and neuronal organization. *Arch Neurol.* (2007) 64:945–50. doi: 10.1001/archneur.64.7.945

85. Boddaert N, Chabane N, Gervais H, Good CD, Bourgeois M, Plumet MH, et al. Superior temporal sulcus anatomical abnormalities in childhood autism: a voxel-based morphometry MRI study. *Neuroimage.* (2004) 23:364–9. doi: 10.1016/j.neuroimage.2004.06.016

86. Waiter GD, Williams JH, Murray AD, Gilchrist A, Perrett DI, Whiten A. Structural white matter deficits in high-functioning individuals with autistic spectrum disorder: a voxel-based investigation. *Neuroimage.* (2005) 24:455–61. doi: 10.1016/j.neuroimage.2004.08.049

87. Ke X, Tang T, Hong S, Hang Y, Zou B, Li H, et al. White matter impairments in autism, evidence from voxel-based morphometry and diffusion tensor imaging. *Brain Res.* (2009) 1265:171–7. doi: 10.1016/j.brainres.2009.02.013

88. Lainhart JE. Brain imaging research in autism spectrum disorders: in search of neuropathology and health across the lifespan. *Curr Opin Psychiatry.* (2015) 28:76–82. doi: 10.1097/YCO.0000000000000130

89. Bigler ED, Abildskov TJ, Petrie JA, Johnson M, Lange N, Chipman J, et al. Volumetric and voxel-based morphometry findings in autism subjects with and without macrocephaly. *Dev Neuropsychol.* (2010) 35:278–95. doi: 10.1080/87565641003696817

90. Bakhtiari R, Zurcher NR, Rogier O, Russo B, Hippolyte L, Granziera C, et al. Differences in white matter reflect atypical developmental trajectory in autism: a Tract-based Spatial Statistics study. *Neuroimage Clin.* (2012) 1:48–56. doi: 10.1016/j.nicl.2012.09.001

91. Shukla DK, Keehn B, Muller RA. Tract-specific analyses of diffusion tensor imaging show widespread white matter compromise in autism spectrum disorder. *J Child Psychol Psychiatry.* (2011) 52:286–95. doi: 10.1111/j.1469-7610.2010.02342.x

92. Hanaie R, Mohri I, Kagitani-Shimono K, Tachibana M, Azuma J, Matsuzaki J, et al. Altered microstructural connectivity of the superior cerebellar peduncle is related to motor dysfunction in children with autistic spectrum disorders. *Cerebellum.* (2013) 12:645–56. doi: 10.1007/s12311-013-0475-x

93. Travers BG, Adluru N, Ennis C, Tromp Do PM, Destiche D, Doran S, et al. Diffusion tensor imaging in autism spectrum disorder: a review. *Autism Res.* (2012) 5:289–313. doi: 10.1002/aur.1243

94. Aoki Y, Yoncheva YN, Chen B, Nath T, Sharp D, Lazar M, et al. Association of white matter structure with autism spectrum disorder and attention-deficit/hyperactivity disorder. *JAMA Psychiatry.* (2017) 74:1120–8. doi: 10.1001/jamapsychiatry.2017.2573

95. Mitelman SA, Buchsbaum MS, Young DS, Haznedar MM, Hollander E, Shihabuddin L, et al. Increased white matter metabolic rates in autism spectrum disorder and schizophrenia. *Brain Imaging Behav.* (2017) 12:1290–305. doi: 10.1007/s11682-017-9785-9

96. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol.* (2005) 57:67–81. doi: 10.1002/ana.20315

97. Lee AS, Azmitia EC, Whitaker-Azmitia PM. Developmental microglial priming in postmortem autism spectrum disorder temporal cortex. *Brain Behav Immun.* (2017) 62:193–202. doi: 10.1016/j.bbi.2017.01.019

98. Ebert DH, Greenberg ME. Activity-dependent neuronal signalling and autism spectrum disorder. *Nature.* (2013) 493:327–37. doi: 10.1038/nature11860

99. Morgan JT, Chana G, Pardo CA, Achim C, Semendeferi K, Buckwalter J, et al. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biol Psychiatry.* (2010) 68:368–76. doi: 10.1016/j.biopsych.2010.05.024

100. Morgan JT, Barger N, Amaral DG, Schumann CM. Stereological study of amygdala glial populations in adolescents and adults with autism spectrum disorder. *PLoS ONE.* (2014) 9:e110356. doi: 10.1371/journal.pone.0110356

101. Pardo CA, Vargas DL, Zimmerman AW. Immunity, neuroglia and neuroinflammation in autism. *Int Rev Psychiatry.* (2005) 17:485–95. doi: 10.1080/02646830500381930

102. Pardo CA, Farmer CA, Thurm A, Shebl FM, Ilieva J, Kalra S, et al. Serum and cerebrospinal fluid immune mediators in children with autistic disorder: a longitudinal study. *Mol Autism.* (2017) 8:1. doi: 10.1186/s13229-016-0115-7

103. Shamim D, Laskowski M. Inhibition of inflammation mediated through the tumor necrosis factor alpha biochemical pathway can lead to favorable outcomes in Alzheimer disease. *J Cent Nerv Syst Dis.* (2017) 9:1179573517722512. doi: 10.1177/1179573517722512

104. Ge YW, Lahiri DK. Regulation of promoter activity of the APP gene by cytokines and growth factors: implications in Alzheimer's disease. *Ann N Y Acad Sci.* (2002) 973:463–7. doi: 10.1111/j.1749-6632.2002.tb04684.x

105. Li Y, Liu L, Kang J, Sheng JG, Barger SW, Mrak RE, et al. Neuronal-glial interactions mediated by interleukin-1 enhance neuronal acetylcholinesterase activity and mRNA expression. *J Neurosci.* (2000) 20:149–55. doi: 10.1523/JNEUROSCI.20-01-00149.2000

106. Barger SW, Harmon AD. Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E. *Nature.* (1997) 388:878–81. doi: 10.1038/42257

107. Barger SW, Basile AS. Activation of microglia by secreted amyloid precursor protein evokes release of glutamate by cystine exchange and attenuates synaptic function. *J Neurochem.* (2001) 76:846–54. doi: 10.1046/j.1471-4159.2001.00075.x

108. Kwak YD, Brannen CL, Qu T, Kim HM, Dong X, Soba P, et al. Amyloid precursor protein regulates differentiation of human neural stem cells. *Stem Cells Dev.* (2006) 15:381–9. doi: 10.1089/scd.2006.15.381

109. Baratchi S, Evans J, Tate WP, Abraham WC, Connor B. Secreted amyloid precursor proteins promote proliferation and glial differentiation of adult hippocampal neural progenitor cells. *Hippocampus.* (2012) 22:1517–27. doi: 10.1002/hipo.20988

110. Sun Q, Hampel H, Blennow K, Lista S, Levey A, Tang B, et al. Increased plasma TACE activity in subjects with mild cognitive impairment and patients with Alzheimer's disease. *J Alzheimers Dis.* (2014) 41:877–86. doi: 10.3233/JAD-140177

111. Skovronsky DM, Fath S, Lee VM, Milla ME. Neuronal localization of the TNFalpha converting enzyme (TACE) in brain tissue and its correlation to amyloid plaques. *J Neurobiol.* (2001) 49:40–6. doi: 10.1002/neu.1064

112. Sastre M, Walter J, Gentleman SM. Interactions between APP secretases and inflammatory mediators. *J Neuroinflammation.* (2008) 5:25. doi: 10.1186/1742-2094-5-25

113. Qian M, Shen X, Wang H. The distinct role of ADAM17 in APP proteolysis and microglial activation related to Alzheimer's disease. *Cell Mol Neurobiol.* (2016) 36:471–82. doi: 10.1007/s10571-015-0232-4

114. Lee HJ, Chang JH, Kim YS, Kim SJ, Yang HK. Effect of ets-related transcription factor (ERT) on transforming growth factor (TGF)-beta type II receptor gene expression in human cancer cell lines. *J Exp Clin Cancer Res.* (2003) 22:477–80.

115. Blobel CP. ADAMs: key components in EGFR signalling and development. *Nat Rev Mol Cell Biol.* (2005) 6:32–43. doi: 10.1038/nrm1548

116. Matthews AL, Noy PJ, Reyat JS, Tomlinson MG. Regulation of A disintegrin and metalloproteinase (ADAM) family sheddases ADAM10 and ADAM17: the emerging role of tetraspanins and rhomboids. *Platelets.* (2017) 28:333–41. doi: 10.1080/09537104.2016.1184751

117. Huang Y, Benaich N, Tape C, Kwok HF, Murphy G. Targeting the sheddase activity of ADAM17 by an anti-ADAM17 antibody D1(A12) inhibits head and neck squamous cell carcinoma cell proliferation and motility via blockage of bradykinin induced HERs transactivation. *Int J Biol Sci.* (2014) 10:702–14. doi: 10.7150/ijbs.9326

118. Wei Z, Yu D, Bi Y, Cao Y. A disintegrin and metalloprotease 17 promotes microglial cell survival via epidermal growth factor receptor signalling following spinal cord injury. *Mol Med Rep.* (2015) 12:63–70. doi: 10.3892/mmr.2015.3395

119. Khawam K, Giron-Michel J, Gu Y, Perier A, Giuliani M, Caignard A, et al. Human renal cancer cells express a novel membrane-bound interleukin-15 that induces, in response to the soluble interleukin-15 receptor alpha chain, epithelial-to-mesenchymal transition. *Cancer Res.* (2009) 69:1561–9. doi: 10.1158/0008-5472.CAN-08-3198

120. Horiuchi K, Morioka H, Takaishi H, Akiyama H, Blobel CP, Toyama Y. Ectodomain shedding of FLT3 ligand is mediated by TNF-alpha converting enzyme. *J Immunol.* (2009) 182:7408–14. doi: 10.4049/jimmunol.08 01931

121. Wong HS, Jaumouille V, Heit B, Doodnauth SA, Patel S, Huang YW, et al. Cytoskeletal confinement of CX3CL1 limits its susceptibility to proteolytic cleavage by ADAM10. *Mol Biol Cell.* (2014) 25:3884–99. doi: 10.1091/mbc.e13-11-0633

122. Ovrevik J, Refsnes M, Totlandsdal AI, Holme JA, Schwarze PE, Lag M. TACE/TGF-alpha/EGFR regulates CXCL8 in bronchial epithelial cells exposed to particulate matter components. *Eur Respir J.* (2011) 38:1189–99. doi: 10.1183/09031936.001 71110

123. Powell JD, Pollizzi KN, Heikamp EB, Horton MR. Regulation of immune responses by mTOR. *Annu Rev Immunol.* (2012) 30:39–68. doi: 10.1146/annurev-immunol-020711-075024

124. Subramanian M, Timmerman CK, Schwartz JL, Pham DL, Meffert MK. Characterizing autism spectrum disorders by key biochemical pathways. *Front Neurosci.* (2015) 9:313. doi: 10.3389/fnins.2015.00313

125. Yeung KS, Tso WWY, Ip JJK, Mak CCY, Leung GKC, Tsang MHY, et al. Identification of mutations in the PI3K-AKT-mTOR signalling pathway in patients with macrocephaly and developmental delay and/or autism. *Mol Autism.* (2017) 8:66. doi: 10.1186/s13229-017-0182-4

126. Monning U, Konig G, Prior R, Mechler H, Schreiter-Gasser U, Masters CL, et al. Synthesis and secretion of Alzheimer amyloid beta A4 precursor protein by stimulated human peripheral blood leucocytes. *FEBS Lett.* (1990) 277:261–6. doi: 10.1016/0014-5793(90)80861-C

127. Kim EK, Choi EJ. Compromised MAPK signaling in human diseases: an update. *Arch Toxicol.* (2015) 89:867–82. doi: 10.1007/s00204-015-1472-2

128. Dong C, Davis RJ, Flavell RA. MAP kinases in the immune response. *Annu Rev Immunol.* (2002) 20:55–72. doi: 10.1146/annurev.immunol.20.091301.131133

129. Parikshak NN, Luo R, Zhang A, Won H, Lowe JK, Chandran V, et al. Integrative functional genomic analyses implicate specific molecular pathways and circuits in autism. *Cell.* (2013) 155:1008–21. doi: 10.1016/j.cell.2013.10.031

130. Faridar A, Jones-Davis D, Rider E, Li J, Gobius I, Morcom L, et al. Mapk/Erk activation in an animal model of social deficits shows a possible link to autism. *Mol Autism.* (2014) 5:57. doi: 10.1186/2040-2392-5-57

131. Wen Y, Alshikho MJ, Herbert MR. Pathway network analyses for autism reveal multisystem involvement, major overlaps with other diseases and convergence upon MAPK and calcium signaling. *PLoS ONE.* (2016) 11:e0153329. doi: 10.1371/journal.pone.0153329

132. Chao MV, Rajagopal R, Lee FS. Neurotrophin signalling in health and disease. *Clin Sci.* (2006) 110:167–73. doi: 10.1042/CS20050163

133. Kwon DS, Kwon CH, Kim JH, Woo JS, Jung JS, Kim YK. Signal transduction of MEK/ERK and PI3K/Akt activation by hypoxia/reoxygenation in renal epithelial cells. *Eur J Cell Biol.* (2006) 85:1189–99. doi: 10.1016/j.ejcb.2006.06.001

134. Klann E, Dever TE. Biochemical mechanisms for translational regulation in synaptic plasticity. *Nat Rev Neurosci.* (2004) 5:931–42. doi: 10.1038/nrn1557

135. Gkogkas CG, Khoutorsky A, Ran I, Rampakakis E, Nevarko T, Weatherill DB, et al. Autism-related deficits via dysregulated eIF4E-dependent translational control. *Nature.* (2013) 493:371–7. doi: 10.1038/nature11628

136. Santini E, Huynh TN, Macaskill AF, Carter AG, Pierre P, Ruggero D, et al. Exaggerated translation causes synaptic and behavioural aberrations associated with autism. *Nature.* (2013) 493:411–5. doi: 10.1038/nature11782

137. Huber KM, Klann E, Costa-Mattioli M, Zukin RS. Dysregulation of mammalian target of rapamycin signaling in mouse models of autism. *J Neurosci.* (2015) 35:13836–42. doi: 10.1523/JNEUROSCI.2656-15.2015

138. Nawaz S, Sanchez P, Schmitt S, Snaidero N, Mitkovski M, Velte C, et al. Actin filament turnover drives leading edge growth during myelin sheath formation in the central nervous system. *Dev Cell.* (2015) 34:139–51. doi: 10.1016/j.devcel.2015.05.013

139. Figlia G, Gerber D, Suter U. Myelination and mTOR. *Glia.* (2018) 66:693–707. doi: 10.1002/glia.23273

140. Lebrun-Julien F, Bachmann L, Norrmen C, Trotzmuller M, Kofeler H, Ruegg MA, et al. Balanced mTORC1 activity in oligodendrocytes is required for accurate CNS myelination. *J Neurosci.* (2014) 34:8432–48. doi: 10.1523/JNEUROSCI.1105-14.2014

141. Jiang M, Rao R, Wang J, Wang J, Xu L, Wu LM, et al. The TSC1-mTOR-PLK axis regulates the homeostatic switch from Schwann cell proliferation to myelination in a stage-specific manner. *Glia.* (2018) 66:1947–59. doi: 10.1002/glia.23449

142. Carson RP, Kelm ND, West KL, Does MD, Fu C, Weaver G, et al. Hypomyelination following deletion of Tsc2 in oligodendrocyte precursors. *Ann Clin Transl Neurol.* (2015) 2:1041–54. doi: 10.1002/acn3.254

143. Taveggia C, Feltri ML, Wrabetz L. Signals to promote myelin formation and repair. *Nat Rev Neurol.* (2010) 6:276–87. doi: 10.1038/nrneurol.2010.37

144. Jiang G, Wang C, Zhang J, Liu H. Mediation of insulin growth factor-1 in Alzheimer's disease and the mechanism of PRNP genetic expression and the PI3K/Akt signaling pathway. *Exp Ther Med.* (2017) 13:2763–6. doi: 10.3892/etm.2017.4320

145. Zhang H, Gao Y, Dai Z, Meng T, Tu S, Yan Y. IGF-1 reduces BACE-1 expression in PC12 cells via activation of PI3-K/Akt and MAPK/ERK1/2 signaling pathways. *Neurochem Res.* (2011) 36:49–57. doi: 10.1007/s11064-010-0260-x

146. Huang HC, Tang D, Xu K, Jiang ZF. Curcumin attenuates amyloid-beta-induced tau hyperphosphorylation in human neuroblastoma SH-SY5Y cells involving PTEN/Akt/GSK-3beta signaling pathway. *J Recept Signal Transduct Res.* (2014) 34:26–37. doi: 10.3109/10799893. 2013.848891

147. Russo AJ. Increased epidermal growth factor receptor (EGFR) associated with hepatocyte growth factor (HGF) and symptom severity in children with autism spectrum disorders (ASDs). *J Cent Nerv Syst Dis.* (2014) 6:79–83. doi: 10.4137/JCNSD.S13767

148. Iseri E, Guney E, Ceylan MF, Yucel A, Aral A, Bodur S, et al. Increased serum levels of epidermal growth factor in children with autism. *J Autism Dev Disord.* (2011) 41:237–41. doi: 10.1007/s10803-010-1046-3

149. Palazuelos J, Crawford HC, Klingener M, Sun B, Karelis J, Raines EW, et al. TACE/ADAM17 is essential for oligodendrocyte development and CNS myelination. *J Neurosci.* (2014) 34:11884–96. doi: 10.1523/JNEUROSCI.1220-14.2014

150. McGivern N, El-Helali A, Mullan P, Mcneish IA, Paul Harkin D, Kennedy RD, et al. Activation of MAPK signalling results in resistance to saracatinib (AZD0530) in ovarian cancer. *Oncotarget.* (2018) 9:4722–36. doi: 10.18632/oncotarget.23524

151. Slattery ML, Mullany LE, Sakoda LC, Wolff RK, Samowitz WS, Herrick JS. The MAPK-signaling pathway in colorectal cancer: dysregulated genes and their association with microRNAs. *Cancer Inform.* (2018) 17:1176935118766522. doi: 10.1177/1176935118766522

152. Xu P, Derynck R. Direct activation of TACE-mediated ectodomain shedding by p38 MAP kinase regulates EGF receptor-dependent cell proliferation. *Mol Cell.* (2010) 37:551–66. doi: 10.1016/j.molcel.2010.01.034

153. Arkun Y, Yasemi M. Dynamics and control of the ERK signaling pathway: sensitivity, bistability, and oscillations. *PLoS ONE.* (2018) 13:e0195513. doi: 10.1371/journal.pone.0195513

154. Oskvig DB, Elkahloun AG, Johnson KR, Phillips TM, Herkenham M. Maternal immune activation by LPS selectively alters specific gene expression profiles of interneuron migration and oxidative stress in the fetus without triggering a fetal immune response. *Brain Behav Immun.* (2012) 26:623–34. doi: 10.1016/j.bbi.2012.01.015

155. Doenni VM, Gray JM, Song CM, Patel S, Hill MN, Pittman QJ. Deficient adolescent social behavior following early-life inflammation is ameliorated by augmentation of anandamide signaling. *Brain Behav Immun.* (2016) 58:237–47. doi: 10.1016/j.bbi.2016.07.152

156. Pang Y, Dai X, Roller A, Carter K, Paul I, Bhatt AJ, et al. Early postnatal lipopolysaccharide exposure leads to enhanced neurogenesis and impaired communicative functions in rats. *PLoS ONE.* (2016) 11:e0164403. doi: 10.1371/journal.pone.0164403

157. Fernandez de Cossio L, Guzman A, van der Veldt S, Luheshi GN. Prenatal infection leads to ASD-like behavior and altered synaptic pruning in the mouse offspring. *Brain Behav Immun.* (2017) 63:88–98. doi: 10.1016/j.bbi.2016.09.028

158. Schaafsma SM, Gagnidze K, Reyes A, Norstedt N, Mansson K, Francis K, et al. Sex-specific gene-environment interactions underlying

ASD-like behaviors. *Proc Natl Acad Sci USA.* (2017) 114:1383–8. doi: 10.1073/pnas.1619312114

159. Kim M, Suh J, Romano D, Truong MH, Mullin K, Hooli B, et al. Potential late-onset Alzheimer's disease-associated mutations in the ADAM10 gene attenuate {alpha}-secretase activity. *Hum Mol Genet.* (2009) 18:3987–96. doi: 10.1093/hmg/ddp323

160. Ishii A, Fyffe-Maricich SL, Furusho M, Miller RH, Bansal R. ERK1/ERK2 MAPK signaling is required to increase myelin thickness independent of oligodendrocyte differentiation and initiation of myelination. *J Neurosci.* (2012) 32:8855–64. doi: 10.1523/JNEUROSCI.0137-12.2012

161. Ishii N, Harada N, Joseph EW, Ohara K, Miura T, Sakamoto H, et al. Enhanced inhibition of ERK signaling by a novel allosteric MEK inhibitor, CH5126766, that suppresses feedback reactivation of RAF activity. *Cancer Res.* (2013) 73:4050–60. doi: 10.1158/0008-5472.CAN-12-3937

162. Haines JD, Fulton DL, Richard S, Almazan G. p38 mitogen-activated protein kinase pathway regulates genes during proliferation and differentiation in oligodendrocytes. *PLoS ONE.* (2015) 10:e0145843. doi: 10.1371/journal.pone.0145843

163. Van't Veer A, Du Y, Fischer TZ, Boetig DR, Wood MR, Dreyfus CF. Brain-derived neurotrophic factor effects on oligodendrocyte progenitors of the basal forebrain are mediated through trkB and the MAP kinase pathway. *J Neurosci Res.* (2009) 87:69–78. doi: 10.1002/jnr.21841

164. Hughes EG, Appel B. The cell biology of CNS myelination. *Curr Opin Neurobiol.* (2016) 39:93–100. doi: 10.1016/j.conb.2016.04.013

165. Yeung MS, Zdunek S, Bergmann O, Bernard S, Salehpour M, Alkass K, et al. Dynamics of oligodendrocyte generation and myelination in the human brain. *Cell.* (2014) 159:766–74. doi: 10.1016/j.cell.2014.10.011

166. Tripathi RB, Rivers LE, Young KM, Jamen F, Richardson WD. NG2 glia generate new oligodendrocytes but few astrocytes in a murine experimental autoimmune encephalomyelitis model of demyelinating disease. *J Neurosci.* (2010) 30:16383–90. doi: 10.1523/JNEUROSCI.3411-10.2010

167. Biname F, Sakry D, Dimou L, Jolivel V, Trotter J. NG2 regulates directional migration of oligodendrocyte precursor cells via Rho GTPases and polarity complex proteins. *J Neurosci.* (2013) 33:10858–74. doi: 10.1523/JNEUROSCI.5010-12.2013

168. Tsai HH, Niu J, Munji R, Davalos D, Chang J, Zhang H, et al. Oligodendrocyte precursors migrate along vasculature in the developing nervous system. *Science.* (2016) 351:379–84. doi: 10.1126/science.aad3839

169. Bae SM, Hong JY. The Wnt signaling pathway and related therapeutic drugs in autism spectrum disorder. *Clin Psychopharmacol Neurosci.* (2018) 16:129–35. doi: 10.9758/cpn.2018.16.2.129

170. Mathieu P, Adami PV, Morelli L. Notch signaling in the pathologic adult brain. *Biomol Concepts.* (2013) 4:465–76. doi: 10.1515/bmc-2013-0006

171. Chen Y, Bodles AM. Amyloid precursor protein modulates beta-catenin degradation. *J Neuroinflammation.* (2007) 4:29. doi: 10.1186/1742-2094-4-29

172. McKenzie IA, Ohayon D, Li H, De Faria JP, Emery B, Tohyama K, et al. Motor skill learning requires active central myelination. *Science.* (2014) 346:318–22. doi: 10.1126/science.1254960

173. Yakovlev PL, Lecours AR. The myelogenetic cycles of regional maturation of the brain. In: Minkowski A, editor. *Resional Development of the Brain in Early Life.* Oxford: Blackwell (1967). p. 3–70.

174. Sirevaag AM, Greenough WT. Differential rearing effects on rat visual cortex synapses. III Neuronal and glial nuclei, boutons, dendrites, and capillaries. *Brain Res.* (1987) 424:320–32. doi: 10.1016/0006-8993(87)91477-6

175. Juraska JM, Kopcik JR. Sex and environmental influences on the size and ultrastructure of the rat corpus callosum. *Brain Res.* (1988) 450:1–8. doi: 10.1016/0006-8993(88)91538-7

176. Sanchez I, Hassinger L, Paskevich PA, Shine HD, Nixon RA. Oligodendroglia regulate the regional expansion of axon caliber and local accumulation of neurofilaments during development independently of myelin formation. *J Neurosci.* (1996) 16:5095–105. doi: 10.1523/JNEUROSCI.16-16-05095.1996

177. Deoni SC, O'muircheartaigh J, Elison JT, Walker L, Doernberg E, Waskiewicz N, et al. White matter maturation profiles through early childhood

predict general cognitive ability. *Brain Struct Funct.* (2016) 221:1189–203. doi: 10.1007/s00429-014-0947-x

178. Bengtsson SL, Nagy Z, Skare S, Forsman L, Forssberg H, Ullen F. Extensive piano practicing has regionally specific effects on white matter development. *Nat Neurosci.* (2005) 8:1148–50. doi: 10.1038/nn1516

179. Scholz J, Klein MC, Behrens TE, Johansen-Berg H. Training induces changes in white-matter architecture. *Nat Neurosci.* (2009) 12:1370–1. doi: 10.1038/nn.2412

180. Wiggins RC, Gottesfeld Z. Restraint stress during late pregnancy in rats elicits early hypermyelination in the offspring. *Metab Brain Dis.* (1986) 1:197–203. doi: 10.1007/BF01001781

181. Zimmerman AW, Connors SL, Matteson KJ, Lee LC, Singer HS, Castaneda JA, et al. Maternal antibrain antibodies in autism. *Brain Behav Immun.* (2007) 21:351–7. doi: 10.1016/j.bbi.2006.08.005

182. Braunschweig D, Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Croen LA, et al. Autism: maternally derived antibodies specific for fetal brain proteins. *Neurotoxicology.* (2008) 29:226–31. doi: 10.1016/j.neuro.2007.10.010

183. Braunschweig D, Van de Water J. Maternal autoantibodies in autism. *Arch Neurol.* (2012) 69:693–9. doi: 10.1001/archneurol.2011.2506

184. Bauman MD, Iosif AM, Ashwood P, Braunschweig D, Lee A, Schumann CM, et al. Maternal antibodies from mothers of children with autism alter brain growth and social behavior development in the rhesus monkey. *Transl Psychiatry.* (2013) 3:e278. doi: 10.1038/tp.2013.47

185. Bauman MD, Iosif AM, Smith SE, Bregere C, Amaral DG, Patterson PH. Activation of the maternal immune system during pregnancy alters behavioral development of rhesus monkey offspring. *Biol Psychiatry.* (2014) 75:332–41. doi: 10.1016/j.biopsych.2013.06.025

186. Kotarba AE, Aucoin D, Hoos MD, Smith SO, Van Nostrand WE. Fine mapping of the amyloid beta-protein binding site on myelin basic protein. *Biochemistry.* (2013) 52:2565–73. doi: 10.1021/bi4001936

187. Singh VK, Warren RP, Odell JD, Warren WL, Cole P. Antibodies to myelin basic protein in children with autistic behavior. *Brain Behav Immun.* (1993) 7:97–103. doi: 10.1006/brbi.1993.1010

188. Connolly AM, Chez M, Streif EM, Keeling RM, Golumbek PT, Kwon JM, et al. Brain-derived neurotrophic factor and autoantibodies to neural antigens in sera of children with autistic spectrum disorders, Landau-Kleffner syndrome, and epilepsy. *Biol Psychiatry.* (2006) 59:354–63. doi: 10.1016/j.biopsych.2005.07.004

189. Zhan X, Jickling GC, Ander BP, Stamova B, Liu D, Kao PF, et al. Myelin basic protein associates with AbetaPP, Abeta1-42, and amyloid plaques in cortex of Alzheimer's disease brain. *J Alzheimers Dis.* (2015) 44:1213–29. doi: 10.3233/JAD-142013

190. Roghani M, Becherer JD, Moss ML, Atherton RE, Erdjument-Bromage H, Arribas J, et al. Metalloprotease-disintegrin MDC9: intracellular maturation and catalytic activity. *J Biol Chem.* (1999) 274:3531–40. doi: 10.1074/jbc.274.6.3531

191. Naus S, Reipschläger S, Wildeboer D, Minai Y, Lichtenthaler SF, Mitterreiter S, et al. Identification of ADAM proteinase substrates in neurodegeneration and neuroinflammation. *BMC Neurosci.* (2007) 8:P30. doi: 10.1186/1471-2202-8-S1-P30

192. Sagane K, Hayakawa K, Kai J, Hirohashi T, Takahashi E, Miyamoto N, et al. Ataxia and peripheral nerve hypomyelination in ADAM22-deficient mice. *BMC Neurosci.* (2005) 6:33. doi: 10.1186/1471-2202-6-33

193. La Marca R, Cerri F, Horiuchi K, Bachi A, Feltri ML, Wrabetz L, et al. TACE (ADAM17) inhibits Schwann cell myelination. *Nat Neurosci.* (2011) 14:857–65. doi: 10.1038/nn.2849

194. Zuko A, Kleijer KT, Oguro-Ando A, Kas MJ, Van Daalen E, Van Der Zwaag B, et al. Contactins in the neurobiology of autism. *Eur J Pharmacol.* (2013) 719:63–74. doi: 10.1016/j.ejphar.2013.07.016

195. Groot AJ, Habets R, Yahyanejad S, Hodin CM, Reiss K, Saftig P, et al. Regulated proteolysis of NOTCH2 and NOTCH3 receptors by ADAM10 and presenilins. *Mol Cell Biol.* (2014) 34:2822–32. doi: 10.1128/MCB.00206-14

196. Llufriu-Daben G, Carrete A, Chierto E, Mailleux J, Camand E, Simon A, et al. Targeting demyelination via alpha-secretases promoting sAPPalpha release to enhance remyelination in central nervous system. *Neurobiol Dis.* (2018) 109:11–24. doi: 10.1016/j.nbd.2017.09.008

197. Xia XG, Hofmann HD, Deller T, Kirsch M. Induction of STAT3 signaling in activated astrocytes and sprouting septal neurons following entorhinal cortex lesion in adult rats. *Mol Cell Neurosci.* (2002) 21:379–92. doi: 10.1006/mcne.2002.1180

198. Zeidan-Chulia F, De Oliveira BH, Salmina AB, Casanova MF, Gelain DP, Noda M, et al. Altered expression of Alzheimer's disease-related genes in the cerebellum of autistic patients: a model for disrupted brain connectome and therapy. *Cell Death Dis.* (2014) 5:e1250. doi: 10.1038/cddis.2014.227

199. Birey F, Aguirre A. Age-dependent netrin-1 signaling regulates NG2+ glial cell spatial homeostasis in normal adult gray matter. *J Neurosci.* (2015) 35:6946–51. doi: 10.1523/JNEUROSCI.0356-15.2015

200. Rama N, Goldschneider D, Corset V, Lambert J, Pays L, Mehlen P. Amyloid precursor protein regulates netrin-1-mediated commissural axon outgrowth. *J Biol Chem.* (2012) 287:30014–23. doi: 10.1074/jbc.M111.324780

201. Tapia-Rojas C, Burgos PV, Inestrosa NC. Inhibition of Wnt signaling induces amyloidogenic processing of amyloid precursor protein and the production and aggregation of Amyloid-beta (Abeta)42 peptides. *J Neurochem.* (2016) 139:1175–91. doi: 10.1111/jnc.13873

202. Lannfelt L, Moller C, Basun H, Osswald G, Sehlin D, Satlin A, et al. Perspectives on future Alzheimer therapies: amyloid-beta protofibrils—a new target for immunotherapy with BAN2401 in Alzheimer's disease. *Alzheimers Res Ther.* (2014) 6:16. doi: 10.1186/alzrt246

Infant Social Avoidance Predicts Autism but Not Anxiety in Fragile X Syndrome

Jane E. Roberts[1]*, Hayley Crawford[2], Elizabeth A. Will[1], Abigail L. Hogan[1], Samuel McQuillin[1], Bridgette L. Tonnsen[3], Shannon O'Connor[1], Douglas A. Roberts[4] and Alexis M. Brewe[1]

[1] Department of Psychology, University of South Carolina, Columbia, SC, United States, [2] Centre for Innovative Research Across the Life Course, Coventry University, Coventry, United Kingdom, [3] Department of Psychological Science, Purdue University, West Lafayette, IN, United States, [4] Georgia State University, Atlanta, GA, United States

Objective: Autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), and anxiety are three of the most common childhood psychiatric disorders. Early trajectories of social avoidance have been linked with these psychiatric disorders in previous studies, but it remains unclear how social avoidance differentially predicts comorbid disorders in a high-risk genetic subgroup. Here, we delineate the association between trajectories of social avoidance from infancy and subsequent ASD, ADHD, and anxiety outcomes at preschool in children with fragile X syndrome (FXS), a well-characterized single-gene disorder highly associated with social avoidance as well as elevated rates of ASD, ADHD, and anxiety.

Method: Males with FXS ($n = 78$) aged 4–62 months participated in a longitudinal study resulting in 201 assessments. The Social Avoidance Scale (SAS) documented socially avoidant behaviors from infancy in three domains—physical movement, facial expression, and eye contact during both the first minute and the last hour of an interaction. ASD, ADHD, and anxiety symptom outcomes at preschool were measured via parent-report questionnaires.

Results: Increased social avoidance across infancy and preschool predicted elevated ASD symptom severity but reduced ADHD and anxiety symptom severity in males with FXS.

Conclusion: ASD, ADHD, and anxiety symptoms relate inconsistently to social avoidance behaviors, providing new insight toward the debate of independence or overlap among these disorders in FXS and other disorders (i.e., ASD). The results suggest that the nuanced profile of the developmental and temporal aspects of social avoidance may inform more the accuracy of differential diagnoses of comorbid psychiatric disorders in FXS.

Keywords: social avoidance, FMR1, fragile X syndrome, autism, anxiety

INTRODUCTION

Psychiatric disorders are debilitating and common, affecting more than one in five children (1). Autism spectrum disorder (ASD) is one of the most common and impairing disorders affecting nearly 2% of the general population (2). The diagnosis of ASD is complicated, however, given the common co-occurrence of other disorders. Attention-deficit/hyperactivity disorder (ADHD) and anxiety are two psychiatric disorders that often present in children with ASD. Differential diagnosis of ASD, ADHD, and anxiety in young children is challenging given the subtlety of behavioral features in early development coupled with the presence of features that represent typical variation or overlap across multiple disorders. Adopting a developmental trajectory approach to capture variation linked to the emergence of psychiatric disorders will increase understanding of the timing and targets for both prevention and intervention (3). As such, studies that identify precursors or symptoms of ASD, ADHD, and anxiety in infancy and track these symptoms over time until the age of diagnosis hold great promise to advance psychiatric research. Of interest to this study, social avoidance is a core or associated feature of ASD, ADHD, and anxiety that often presents early in development (4, 5). Identifying developmental trajectories of social avoidance in groups at high risk for ASD, ADHD and anxiety is likely to advance our understanding of both the emergence and independence of features across these common and impairing psychiatric disorders.

While mean levels of symptom impairment may index risk for psychiatric disorders, clear evidence suggests that developmental trajectories representing change or stability can be uniquely predictive (4, 6). For example, infants later diagnosed with ASD display reduced avoidance at 6 months-of-age followed by increased levels of avoidance that emerge at 12 months-of age and continue through preschool (7, 8). Similarly, trajectories of change in avoidance and reactivity across the first years, but *not* mean levels at any specific age, predict ASD outcomes (9). Also, a stable trajectory of reduced avoidance from infancy through preschool characterize children later diagnosed with ADHD (10, 11). Finally, both high and stable levels, as well as steep increases of social avoidance and stranger fear from 6 to 36 months-of-age, has been linked to elevated risk for anxiety in preschool (6, 12) through to middle childhood (4).

Whereas most studies of social avoidance have focused on "typically developing" children, examining social avoidance trajectories in genetic subgroups at elevated risk for ASD, ADHD, and anxiety may inform biological influences on symptom emergence, as well as the generalizability of social avoidance research conducted in non-clinical samples to clinical groups. Fragile X syndrome (FXS) is a well-characterized single-gene disorder highly associated with co-occurring features of ASD, ADHD, and anxiety, making the disorder an ideal candidate for studying social avoidance trajectories in a "high risk," genetically-defined sample. FXS is the most common inherited cause of intellectual disability (ID), affecting ∼1 in 4,000 males and 1 in 8,000 females (13). FXS is caused by a cytosine-guanine-guanine (CGG) expansion and subsequent methylation of *FMR1* gene on the Xq27.3 site resulting in reduced

FMRP, the protein associated with FXS. Males with FXS are typically more severely affected given random X inactivation in females associated with elevated FMRP. Along with moderate ID, 50–70% of males with FXS meet criteria for ASD (14, 15) and 53–73% meet criteria for ADHD (16, 17). Anxiety is also highly prevalent, affecting over 85% of males (18). In addition, social avoidance—a feature also associated with ASD, ADHD, and anxiety—is a hallmark characteristic of 82–98% of males with FXS (19–21). The phenotypic overlap of FXS with ASD, ADHD, and anxiety offers an ideal model for understanding gene-brain-behavior relationships in a simplified genetic context (22).

The validity and independence of ASD, ADHD, and anxiety disorders in FXS has been challenged, not surprisingly, with debate regarding whether they represent "true" comorbidities or whether co-occurring symptomology shared across these disorders results in artifactual diagnostic categorization (21, 23). For example, poor eye contact is a nearly universal feature of FXS (24–26); yet, it is not clear whether poor eye contact indexes the presence of ASD or anxiety, both ASD and anxiety or neither ASD nor anxiety in FXS. These questions of "true" diagnoses and the confounding association of anxiety symptomatology and social avoidance are also debated in the non-syndromic ASD field (27, 28). Exploring the trajectory of specific traits that are common across disorders can provide valuable insight into the distinction and potential overlap among multiple psychiatric outcomes.

Social avoidance is a core or associated feature of ASD, ADHD, and anxiety and is a hallmark characteristic of males with FXS (25, 26). In a recent study (29), we confirmed initial reports that social avoidance in males with FXS is elevated throughout infancy and early childhood relative to age-matched typically-developing control subjects, with 73% of males with FXS exhibiting social avoidance. Furthermore, this work suggests that social avoidance in males with FXS emerges in infancy and increases in severity across early childhood, becoming more stable in adolescence and early adulthood. However, little work has focused on the relationship of social avoidance to the symptoms of ASD, ADHD, and anxiety across development. In the current study, we extend previous work (25, 26) to characterize the association between trajectories of social avoidance and subsequent ASD, ADHD, and anxiety outcomes in FXS from infancy through preschool. ASD and ADHD are two of the earliest emerging childhood disorders, typically presenting before 5 years of age (30–32). In contrast, anxiety disorders are most commonly identified during late childhood and adolescence (33). However, features of anxiety are evident during the infant and preschool years, and anxiety can be diagnosed in preschool-aged children (34). Given clear evidence that ASD, ADHD, and anxiety have optimal outcomes when recognized and treated early (35–37), our focus on the very early years of life is critical for maximal translational effects.

Using a prospective longitudinal design, we examined the relationship between trajectories of social avoidance from infancy to the severity of ASD, ADHD, and anxiety outcomes at preschool in males with FXS. We hypothesize that males with FXS who exhibit higher ASD and anxiety symptoms will

exhibit higher levels of social avoidance that will intensify across age. This relationship will not be present in ADHD outcomes. This hypothesis is based on the fact that social avoidance is a core feature of both ASD and anxiety, whereas in ADHD, social avoidance may present as a secondary product of attention-related difficulties in managing social situations. Distinguishing the relationship of the level and trajectories of social avoidance to ASD, ADHD, and anxiety in FXS will provide novel information about their differential impact on these three psychiatric disorders that occur frequently and are very impairing in this population. As such, this work is critical to improve differential diagnostic processes and direct targeted treatment.

METHODS

Participants

Participants were drawn from two sites that conducted longitudinal studies and included the measures reported here (University of South Carolina and University of North Carolina, Chapel Hill). Participant's parents at all sites provided written informed consent prior to participation. All of these studies focused on documenting the phenotype of FXS across early development with identical procedures and a subset of common measures. The age of the samples, however, varied across studies with some focused on infants while others focused on preschoolers. Participants were drawn from these studies if they were male, under 6 years of age and had at least one assessment that included all of the measures listed below. The advantage of including data from multiple studies allows us to generalize across contexts so our findings are not restricted to one discrete age group and to increase the sample size given the relatively low prevalence of FXS. All participants with FXS were recruited from past studies, national parent listservs, social media, the National Fragile X Foundation, and the Research Participant Registry Core of the Carolina Institute for Developmental Disabilities at the University of North Carolina at Chapel Hill. The full mutation of the *FMR1* gene (>200 CGG repeats) was confirmed through genetic testing. Subsets of these data have been published previously (25, 26); however, data including this large sample for the SAS and the inclusion of three psychiatric outcomes have not been published.

From the potential pool of participants, 74 males with FXS representing 201 assessments met criteria for study inclusion. Chronological age at the first assessment ranged from 4 to 62 months ($M = 27.31$ months, $SD = 15.12$) while chronological age at the outcome assessment ranged from 23.28 to 70.10 months ($M = 51.81$, $SD = 13.22$). The average time between the first and final assessment was 29.39 months (range of 11.04 to 59.64 and SD = 12.10). Nine participants only had one assessment, whereas the rest ($n = 65$) had 2 or more assessments.

Measures

Social Avoidance Scale

The Social Avoidance Scale [SAS; (25, 26)] is an experimental observation scale used to document socially avoidant behaviors in three domains: physical movement, facial expression, and eye contact. Higher ratings represent more socially avoidant

behaviors. Two ratings are assigned for each of the three SAS subscales, the first rating is based on the first *minute* of social interaction and the second rating is based on the *last hour* of structured social interaction. Parents were present for both periods of interaction. This results in six SAS ratings (three first-minute and three last hour). Intra-class correlation coefficients (ICCs) reflect a moderate to high degree of reliability between raters on all of the SAS scales, with a range from 0.82 to 0.90. The capacity for the SAS to capture dynamic aspects of social avoidance is central given evidence that a decrease in avoidance between the two ratings is a core feature of FXS and one that differentiates those with and without comorbid ASD (25, 26). The SAS was completed at every assessment point, and the ratings taken at the first minute and last hour for each of the three domains were used as the independent variables to predict ASD, ADHD, and anxiety symptom severity at the final assessment within each age cohort.

Autism Spectrum Disorder (ASD) Symptom Severity

ASD severity was measured using the Childhood Autism Rating Scale [CARS; (38)], an observational rating scale comprised of 15 items that measures specific ASD features, such as non-verbal and verbal communication and adaptation to change, for individuals from 2 years of age. While the Autism Diagnostic Observation Scale-2 [ADOS-2; (39)] is recognized as the most robust diagnostic measure of ASD, we utilized the CARS for this study as we were focused on ASD symptoms and not diagnoses and only a subset (~40%) had an ADOS-2 while all participants had a CARS. Also, we have documented a strong relationship between CARS ratings and ADOS-2 continuous severity scores in males with FXS [$r = 0.90$; (40)]. In our sample, 48.72% had CARS raw scores of 30 or above suggesting the presence of ASD. The total raw score of the CARS at the final assessment was used as the dependent variable in the ASD models.

Attention Deficit/Hyperactivity Disorder (ADHD) Symptom Severity

The ADHD Problems scale of the CBCL was utilized to measure ADHD symptoms. The ADHD subscale assesses symptoms including inability to sit still, talking too much, and failing to concentrate for long periods of time. In our sample, 56.41% had scores above the clinical cutoff suggesting a high likelihood of ADHD. Due to potential floor and ceiling effects in standardized scores and the fact that the t-scores are restricted to 50 and above, the raw score of the CBCL ADHD Problems at the final assessment was used as the dependent variable in the ADHD models.

Anxiety Symptom Severity

Anxiety symptoms were measured using the DSM Anxiety Problems scale of the Child Behavior Checklist [CBCL; (40)], a parent questionnaire assessing emotional and behavioral problems in children. Parents completed the CBCL version appropriate for their child's age (CBCL 1.5–5 Years; CBCL 6–18 Years). The DSM Anxiety Problems scale assesses symptoms associated with anxiety, such as worries, fears about going to school, and clinging to adults. In our sample, 7.70% had scores

above the clinical cutoff suggesting a high likelihood of having anxiety. Due to potential floor and ceiling effects in standardized scores and the fact that the *t*-scores are restricted to 50 and above, the raw score of the CBCL Anxiety Problems scale at the final assessment was used as the dependent variable in the anxiety models.

Developmental Level

The Mullen Scales of Early Learning (41) is a standardized developmental measure of abilities for children from birth to 68 months. The Early Learning Composite (ELC), an overall estimate of cognitive functioning, was used to analyze the effect of developmental level on measures of social avoidance, ASD, ADHD, and anxiety.

Procedure

A standard battery of direct child assessments that included developmental and temperament assessments and parent/caregiver rating scales was administered by trained personnel over the course of 2 days [see (42, 43) for more details]. To control for potential effects associated with variation in the assessments across sites and given our primary interest on initial social avoidance, this study focused on SAS ratings for Day 1 only. Across all sites, the SAS and developmental measures were completed at each assessment. All available SAS ratings were included to generate the trajectories of social avoidance, and the ASD, ADHD, and anxiety outcome data were restricted to the single assessment at the child's oldest age. All procedures were implemented with approval from the institutional review boards at each respective site.

Data Analytic Plan

Analyses were conducted using SPSS 24 (44), R Core Team (45), and Mplus Version 8 (46). As preliminary analyses, we calculated Pearson correlations between ASD, anxiety, and ADHD symptoms, controlling for developmental level, to coarsely assess overlap among symptom profiles and inform structure of subsequent models. Results indicated no relationship between CARS and both anxiety ($r = -0.004$; $p = 0.972$) and ADHD ($r = 0.170$; $p = 0.162$). Anxiety and ADHD were moderately related ($r = 0.240$; $p = 0.047$). Thus, separate models were used for each outcome. We analyzed our primary research questions using random effect latent variable models, which account for variable assessment intervals. Specifically, we used 2-level random slope and intercept model with continuous child-level outcomes. The two levels of our model correspond to (1) variation within child (i.e., child observations across time nested within child) and (2) variation between children. Our model is represented in **Figure 1**.

In the within portion of our model (i.e., Level 1), we regressed social avoidance measures (i.e., SAS) on the age of the child to estimate a) a latent slope (i.e., **S,** the solid circle on top of the line seen in **Figure 1** the within portion of the model) corresponding to the average unit increment in social avoidance per standard deviation unit increase in age, and b) a latent intercept (i.e., **I**, the solid circle within the SAS box in **Figure 1**) corresponding to the social avoidance at the average age. We grand mean centered

FIGURE 1 | Model of analytic plan.
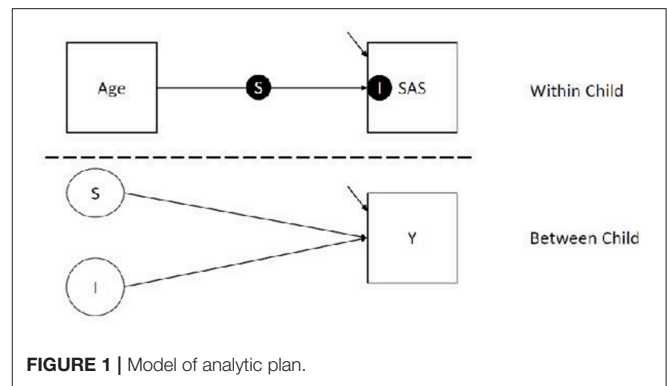
and scaled Age to a variance of 1. We used the grand mean to center because children were measured at different times, and some children completed more assessments than others; because of this, interpretation of child centered (i.e., centering on level 2 clusters) age means would be difficult to interpret. In our models, the intercept is interpreted as the predicted social avoidance for a child at the average age of our sample, which is 36.12 months.

In the between portion of our model, we regressed the aforementioned random slope (the unfilled circle S in **Figure 1**) and intercept (the unfilled circle I in **Figure 1**) on the child-level outcome (square box Y in **Figure 1**), which includes measures of ASD, ADHD, or anxiety at the final assessment. In these models, the regression lines correspond to the average difference in our outcome measures per unit difference in the slope and intercept. This portion of the model answers both of our research questions, with question 1 (testing the influence of levels) being answered by regression of child-level outcome on the latent intercept, and question 2 (testing the influence of linear trajectory) being answered by the regressing child-level outcomes on the latent slope of SAS score by Age.

RESULTS

Describing Intercepts and Slopes

Prior to completing the full models, we first estimated global (i.e., independent of outcome) slopes and intercepts for each SAS predictor (physical movement, facial expression, and eye contact) for the first minute and last hour of interaction. These global scores are found in **Table 1**. These scores are constructed using month-unit measures of age (i.e., as opposed to scaled age as in our full model). The interpretation of the intercept is the average SAS score at 36.12 months, which is the average age of the sample. The slope is the average linear difference in social avoidance for every month increase in Age. Base model results are presented in **Table 2** and base model trajectories are presented in **Figure 2**.

Estimating the Influence of Slopes and Intercepts on Outcomes

In our full models, age and SAS scores were scaled to a mean of zero and variance of one. Results from the full models supported our hypotheses that SAS levels and trajectories would predict ASD outcomes. However, our hypothesis that SAS levels and

TABLE 1 | Demographics table.

	Mean (SD)
Age at initial assessment	27.31 (15.12)
Mullen early learning scales composite standard score	58.34 (13.93)
Autism symptoms: childhood autism rating scale-II total raw score	28.64 (6.62)
ADHD[†] symptoms: child behavior checklist, DSM scale, T score	63.16 (8.67)
Anxiety symptoms: child behavior checklist, DSM scale, T score	54.23 (6.23)

Race	%
Caucasian	77.00
African American	5.12
Hispanic or Latino	1.30
Asian	0
American Indian/Alaska Native	1.30
Bi-Racial	15.40

[†]ADHD, attention deficit hyperactivity disorder.

TABLE 2 | Base model results.

	Intercept	95% CI		Slope	95% CI	
		Lower	Upper		Lower	Upper
FIRST MINUTE						
Physical movement	2.24	2.10	2.40	0.01	0.01	0.02
Facial expression	1.67	1.50	1.84	0.01	0.004	0.02
Eye contact	2.02	1.74	2.30	0.02	0.01	0.04
LAST HOUR						
Physical movement	0.90	0.67	1.10	−0.003	−0.013	0.01
Facial expression	0.70	0.55	0.85	0.001	−0.01	0.01
Eye contact	1.33	1.10	1.60	0.01	−0.001	0.02

trajectories would predict anxiety and ADHD outcomes was only partially supported (see **Table 3** for full results).

ASD Outcomes

Physical avoidance in the last hour but not the first minute significantly predicted ASD outcomes. We found that both a higher mean level ($B = 5.84(1.67)$, $z = 3.51$, $p < 0.001$, CI[2.58, 9.12]), and a trajectory of increasing physical avoidance ($B = 7.93(3.66)$, $z = 2.17$, $p = 0.030$, CI[0.76,15.12]) during the last hour of interaction predicted more severe ASD symptomology at outcome. We also found that both a higher mean level ($B = 3.16(0.31)$, $z = 10.12$, $p < 0.001$, CI[2.55,3.77]) and a trajectory of increasing facial expressions of social wariness ($B = 51.47(0.15)$, $z = 355.02$, $p < 0.001$ CI[51.19, 51.75]) during the first minute of interaction predicted more severe ASD symptomology at outcome. However, only the mean level, not the trajectory, of facial expression during the last hour of interaction ($B = 8.51(2.50)$, $z = 3.41$, $p = 0.001$, CI[3.61,13.40]) predicted ASD symptomology. Finally, a higher mean level ($B = 8.15(2.52)$,

$z = 3.23$, $p < 0.001$, CI[3.20, 13.10]) of avoidant eye contact during the first minute of interaction predicted more severe ASD symptomology at outcome, whereas the trajectory did not. However, both a higher mean level ($B = 10.05(2.02)$, $z = 4.97$, $p < 0.001$, CI[6.08, 14.02]) and a trajectory of increasing avoidance of eye contact ($B = 8.22(2.21)$, $z = 3.72$, $p < 0.001$, CI[3.90, 12.55]) during the last hour of interaction predicted more severe ASD symptomology at outcome.

ADHD Outcomes

For ADHD, only the trajectory of increasing physical avoidance during the first minute of social interaction predicted decreased ADHD symptomology at outcome ($B = -10.79(3.73)$, $z = -2.89$, $p = 0.004$, CI[−18.10, −3.47]). No other SAS variables predicted ADHD outcomes.
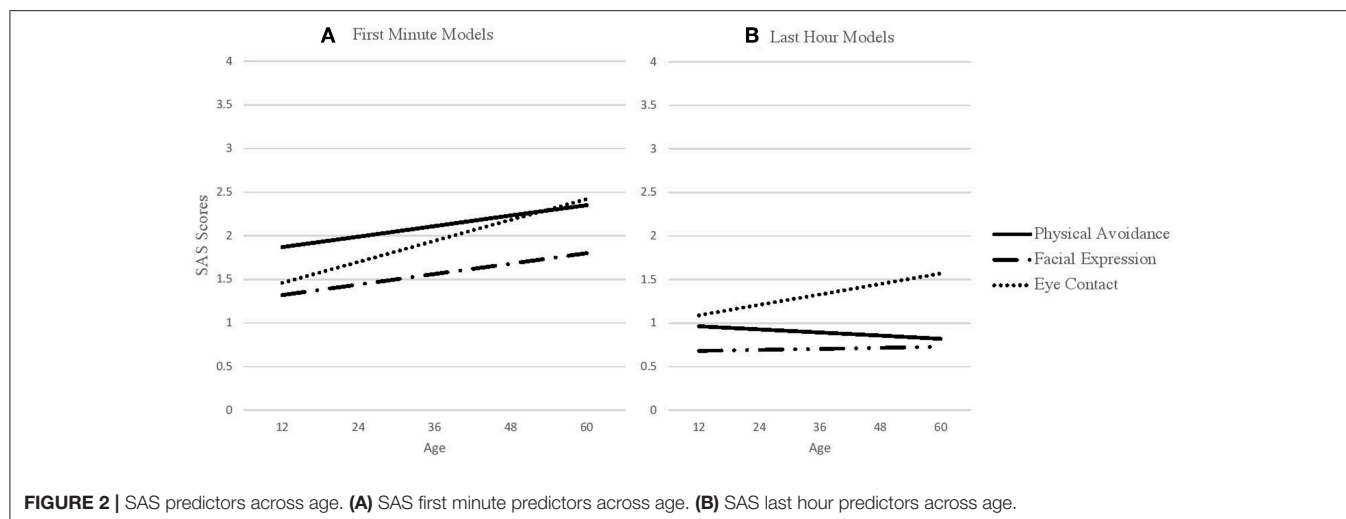
Anxiety Outcomes

For Anxiety, only the trajectory of increasing physical avoidance during the first minute of social interaction predicted decreased anxiety symptomology at outcome ($B = -11.33(5.47)$, $z = 2.07$, $p = 0.038$, CI[−22.1, −0.61]), No other SAS domains predicted Anxiety Outcomes.

RESULTS SUMMARY

Collectively, elevated physical avoidance, increased facial expressions of avoidance, and reduced eye contact were all predictors of ASD, ADHD, or anxiety outcomes. However, the relationships were dependent on the timing of the interaction (i.e., first minute or last hour) and parameter (i.e., average level or trajectory) with increased social avoidance predicting elevated ASD severity and, surprisingly, it also predicted reduced ADHD and anxiety severity at outcome. For ASD outcomes, we found that elevated physical avoidance, increased facial expressions of avoidance and reduced eye contact all signaled risk for the later onset of more severe ASD symptoms. Specifically, elevated levels of physical movement during the final hour and elevated levels of facial shyness and avoidant eye contact during both the first minute and final hour were associated with increased severity of ASD features. A trajectory of increased avoidance in physical movement and avoidant eye contact during the last hour and for the first minute for facial shyness also were associated with increased severity of ASD outcomes. And, while social avoidance during initial social encounters provided some signal for the later onset of ASD symptoms, it was elevated social avoidance during social encounters with familiar people that reflected the strongest indicator of more severe ASD symptomology. In contrast, only trajectories of increasing social avoidance during initial interactions were associated with reduced severity of ADHD and anxiety outcomes.

DISCUSSION

Psychiatric disorders are common and impairing in children, and efforts to increase understanding of the prodromal and predictive features of these disorders represent significant impact

FIGURE 2 | SAS predictors across age. **(A)** SAS first minute predictors across age. **(B)** SAS last hour predictors across age.

given the known benefits of early intervention. Given the high rates of ASD, ADHD and anxiety in males with FXS, studies of the developmental trajectories of symptoms associated with these psychiatric disorders can impact the timing and targets for intervention. This work has implications not only for individuals with FXS but also for those who exhibit features of ASD, ADHD and anxiety of known or unidentified etiology. Because of the high rates of comorbidity and the monogenic etiology of FXS, this population provides a saturated model for risk that includes more "signal" for detecting meaningful associations than can be observed in studies of the general population (47). The overall aim of this study was to document the relationship of social avoidance to ASD, ADHD and anxiety outcomes in males with FXS across a critical developmental period when symptoms and diagnoses are known to emerge. To accomplish this, we employed the SAS, an experimental observation scale that incorporates multiple dimensions of social avoidance within a temporal framework.

Based on our results, increased social avoidance across infancy and preschool predicted elevated severity of ASD symptoms but reduced ADHD and anxiety symptoms in males with FXS. A nuanced set of relationships was apparent with ASD predicted by specific social avoidance features that were entirely unique from those that predicted ADHD and anxiety outcomes. Surprisingly, identical predictors (trajectories of physical avoidance during initial interaction) emerged for both ADHD and anxiety outcomes. The trajectory of increasing social avoidance appears to be one of the most salient features as it predicted ASD, ADHD, and anxiety outcomes whereas an elevated level of social avoidance only predicted ASD features. However, it was the trajectory of initial interactions with unfamiliar people at the beginning of the assessment (first minute) that predicted reduced ADHD and anxiety whereas the trajectory of interactions with both unfamiliar people (beginning of the assessment) and people who had become more familiar by the end of the assessment (last hour) predicted ASD. These findings highlight the importance of capturing social avoidance across multiple domains and the value

of including both initial responses to unfamiliar people as well as responses to people who have become familiar over the course of the assessment. Overall, these results suggest that elevated social avoidance emerging during infancy may be a salient marker for the onset for ASD and of reduced risk for ADHD and anxiety in males with FXS.

The finding that multiple indicators of social avoidance across the infant and preschool years' signal risk for the emergence of elevated ASD symptoms is of great importance. ASD is one of the most prevalent and impairing psychiatric disorders associated with FXS, and FXS is the leading identified genetic cause of ASD (22). Despite the importance of studying the association of ASD and FXS, few studies have identified early markers of ASD in FXS and most of these are cross-sectional. This focus of research is becoming increasingly studied, however, with recent reports indicating that social communication deficits (43, 48), motor atypicalities and delays (42, 49) along with gaze avoidance (50) and reduced escape during social challenges (51) may be detectable within the first years of life and serve as a signal for ASD risk in males with FXS. Here, we add that elevated levels and trajectories of increasing social avoidance also serve as risk markers for ASD in the largest longitudinal sample of infants and preschool-aged males with FXS to date.

Trajectories of social avoidance across infancy and preschool were also associated with ADHD and anxiety symptom outcomes, however, in the opposite direction to that expected with trajectories of elevated physical avoidance during initial social interactions predicting lower ADHD and anxiety symptom severity at outcome. These relationships were unexpected as we anticipated no relationship with ADHD as social impairment is an associated, but not core, feature of ADHD. The unexpected direction of the relationship could signal strong emotion regulation skills in these children. Per this interpretation, children who have less severe ADHD and anxiety symptom outcomes may have learned to manage their emotions by physically retreating from the source of stress, in this case, the approach of an unfamiliar person. This finding could also suggest

TABLE 3 | Slopes and intercepts models.

	Intercept	z	CI lower	CI upper	Slope	z	CI lower	CI upper
CARS								
First minute								
Physical movement	−2.05	−0.23	−19.44	15.34	39.24	1.14	−28.12	106.61
Facial expression	3.16**	10.12**	2.55	3.77	51.47**	355.02**	51.19	51.75
Eye contact	8.15*	3.23*	3.20	13.10	21.39	1.40	−8.46	51.24
Last hour								
Physical movement	5.84**	3.51**	2.58	9.12	7.93^	2.17^	0.76	15.12
Facial expression	8.51*	3.41*	3.61	13.40	15.39	1.10	−11.92	42.70
Eye contact	10.05**	4.97**	6.08	14.02	8.22**	3.73**	3.90	12.55
ADHD								
First minute								
Physical movement	−4.68	−1.64	−10.28	0.92	−10.787^	−2.89^	−18.10	−3.47
Facial expression	−0.48	−0.45	−2.55	1.60	−0.80	−0.09	−18.86	17.27
Eye contact	−0.30	−0.38	−1.83	1.24	−0.58	−0.37	−3.70	2.53
Last hour								
Physical movement	−0.03	−0.06	−1.08	1.01	1.95	1.55	−1.08	5.00
Facial expression	0.36	0.53	−0.96	1.67	1.45	0.321	−7.42	10.33
Eye contact	0.39	0.54	−1.01	1.79	1.61	0.50	−4.66	7.88
ANXIETY								
First minute								
Physical movement	0.83^	0.47^	−2.61	4.26	−11.33	−2.07	−22.06	−0.61
Facial expression	0.86	0.73	−1.44	3.17	−0.005	−0.001	−13.66	13.65
Eye contact	0.18	0.29	−1.01	1.36	1.31	0.61	−2.88	5.50
Last hour								
Physical movement	−0.03	−0.07	−0.77	0.72	0.00	0.00	−2.15	2.15
Facial expression	−0.08	−0.14	−1.25	1.08	0.50	0.14	−6.61	7.61
Eye contact	0.16	0.30	−0.90	1.21	0.60	0.18	−5.98	7.18

**p < .0001; *p < .001; ^p < .05

a developmental effect in that children with less severe ADHD and anxiety outcomes may respond to social challenge differently across age with increasing physical avoidance of novel social partners more likely at preschool age with less physical avoidance during the infant and toddler years (52).

The results reported here are consistent with a recent study, which identified unique and distinct early-life predictors of ASD and ADHD, but are inconsistent with the same study documenting overlap in predictors of ASD and anxiety (53). Specifically, this recent study reported that shyness was associated with both anxiety and ASD. Here, differences in the relationship of social avoidance to the three psychiatric outcomes likely reflect the nature of the different disorders and the developmental period we have focused on. Increased severity of ASD features was associated with a number of aspects of social avoidance including all three dimensions (physical movement, facial expression, and eye contact) as well as during both initial and sustained social interactions (first minute and last hour). The pervasiveness of social avoidance associated with ASD likely reflects an aloof presentation which is often associated with non-syndromic ASD profiles (54). In contrast, reduced ADHD and anxiety symptoms align with an acute, and potentially

adaptive, pattern of social avoidance only during initial social encounters. Children who do not have elevated ADHD or anxiety symptoms are more likely to have strong emotion regulation skills and for those skills to develop and increase across the preschool period (55, 56). Thus, the pattern of walking away from a potentially stressful social encounter as an adaptive response aligns with the nature of these disorders in that children with elevated ADHD might have little social avoidance and more impulsivity (57). And, those with elevated anxiety might be more likely to demonstrate initial social avoidance but to "warm up" over time.

One of the most important findings of this study is that ASD, ADHD and anxiety symptoms relate inconsistently to social avoidance behaviors, providing new insight toward the debate of independence or overlap among these disorders in FXS and other disorders (i.e., ASD). As noted above, ASD was the only disorder in which elevated social avoidance predicted increased severity of symptoms. In fact, increased social avoidance predicted reduced symptom severity for ADHD and anxiety. Thus, elevated social avoidance is a trait that uniquely predicts ASD and not ADHD or anxiety despite social avoidance being a feature of all three disorders. Our preliminary correlational analyses confirm this

conclusion as ASD was not related to either ADHD or anxiety. This finding regarding the apparent independence across these disorders is particularly striking considering the high prevalence of 48.72 and 56.41% for ASD and ADHD who are above the clinical cut-offs, respectively. These rates are generally consistent with extant literature (15, 17). In contrast, only 7.70% of males with FXS were above the clinical cutoff for anxiety which may have constrained our power to detect a relationship between anxiety and trajectories of social avoidance. The rate of anxiety in our sample appears low compared to the fact that over 85% of older males with FXS meet diagnostic criteria for anxiety (18). However, no studies exist that document the early presentation of anxiety in FXS so the validity of its prevalence in our sample is unknown.

This study has a number of important clinical implications. First, we demonstrated that both the level and trajectory of social avoidance show meaningful individual differences in the expression of ASD, ADHD, and anxiety symptom outcomes in males with FXS. This suggests that the nature and function of social avoidance is complex, and caution should be taken to attend to the nuanced profile of social avoidance to ensure diagnostic accuracy with developmental and temporal aspects of social avoidance carefully considered. Specifically, both initial and prolonged social avoidance should be integrated into differential diagnoses, and this practice rests on clinical knowledge and observation as some tools (e.g., ADOS-2, CBCL) may not be sufficiently sensitive to these clinical factors. Second, our findings confirm that social avoidance manifests very early in development, and we extend initial work in this area by demonstrating that early trajectories of social avoidance may index risk for ASD while signaling resilience or a protective factor for ADHD and anxiety in young males with FXS. This information is critical to inform the timing and targets for treatment.

Despite inclusion of the largest sample of males with FXS to date in a study focused on social avoidance and integration of multiple outcomes with repeated measures, there are a number of limitations. These include failure to include females and reliance on behavioral ratings without biomarkers. We also targeted the first years of life as a critical developmental period as our focus was on early signs and symptoms vs. diagnoses. However, the focus on this early childhood period likely constrained our ability to fully detect the relationship of social avoidance to anxiety given that symptoms of anxiety emerge later in development than ASD and ADHD symptoms. Also, with regard to our

models, the average amount of assessments available across children was three (SD = 1.04), with some children having as many as five assessments, and some children having as few as one assessment. Because of this range, we modeled our trajectories using simple linear trends; however, in reality, these trends are not likely entirely linear. Thus, our understanding of how developmental changes in social avoidance influences later outcomes is constrained to linear trends (i.e., the expected outcome if children's social avoidance improves or declines, on average, over time), rather than more nuanced profiles that could be estimated with additional data. These are important factors for future work, as is inclusion of a contrast group to see if these findings generalize to other clinical or non-clinical groups (e.g., non-syndromic ASD or low-risk controls).

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Human Subjects Research University of South Carolina Institutional Review Board and the Institutional Review Board at University of North Carolina at Chapel Hill, with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the University of South Carolina Institutional Review Board and the Institutional Review Board at the University of North Carolina at Chapel Hill.

AUTHOR CONTRIBUTIONS

JR conceptualized the study and oversaw data collection and provided leadership for producing the paper. EW and SM analyzed the data with consultation from BT and AH. HC played a key role in writing the paper with input from AH. SO, DR, and AB wrote sections of the paper.

FUNDING

ACKNOWLEDGMENTS

REFERENCES

1. Giedd JN, Keshavan M, Paus T. Why do many psychiatric disorders emerge during adolescence? *Nat Rev Neurosci.* (2008) 9:947–57. doi: 10.1038/nrn2513

2. Centers for Disease Control and Prevention. Prevalence of autism spectrum disorders among children aged 8 years – autism and developmental disabilities monitoring network, 11 sites, United States, 2010. *Morbidity Mortal Weekly Rep.* (2014) 63:1–21.

3. Insel TR. Mental disorders in childhood: shifting the focus from behavioral symptoms to neurodevelopmental trajectories. *J Am Med Assoc.* (2014) 311:1727–8. doi: 10.1001/jama.2014.1193

4. Van Hulle CA, Moore MN, Lemery-Chalfant K, Goldsmith HH, Brooker RJ. Infant stranger fear trajectories predict anxious behaviors and diurnal cortisol rhythm during childhood. *Dev Psychopathol.* (2017) 29:1–12. doi: 10.1017/S0954579417000311

5. Visser JC, Rommelse NN, Greven CU, Buitelaar JK. Autism spectrum disorder and attention-deficit/hyperactivity disorder in early childhood: a review of

unique and shared characteristics and developmental antecedents. *Neurosci Biobehav Rev.* (2016) 65:229–63. doi: 10.1016/j.neubiorev.2016.03.019

6. Brooker RJ, Buss KA, Lemery-Chalfant K, Aksan N, Davidson RJ, Goldsmith HH. The development of stranger fear in infancy and toddlerhood: normative development, individual differences, antecedents, and outcomes. *Dev Sci.* (2013) 16:864–78. doi: 10.1111/desc.12058

7. Clifford SM, Hudry K, Elsabbagh M, Charman T, Johnson MH. Temperament in the first 2 years of life in infants at high-risk for autism spectrum disorders. *J Autism Dev Disord.* (2013) 43:673–86. doi: 10.1007/s10803-012-1612-y

8. Del Rosario M, Gillespie-Lynch K, Johnson S, Sigman M, Hutman T. Parent-reported temperament trajectories among infant siblings of children with autism. *J Autism Dev Disord.* (2014) 44:381–93. doi: 10.1007/s10803-013-1876-x

9. Zwaigenbaum L, Bryson S, Rogers T, Roberts W, Brian J, Szatmari P. Behavioral manifestations of autism in the first year of life. *Int J Devl Neurosci.* (2005) 23:143–52. doi: 10.1016/j.ijdevneu.2004.05.001

10. Auerbach JG, Atzaba-Poria N, Berger A, Landau R. Emerging developmental pathways to ADHD: possible path markers in early infancy. *Neural Plasticity.* (2004) 11:29–43. doi: 10.1155/NP.2004.29

11. Miller AC, Keenan JM, Betjemann RS, Willcutt E, Pennington BF, Olson RK. Reading comprehension in children with ADHD: cognitive underpinnings of the centrality deficit. *J Abnormal Child Psychol.* (2013) 41:473–83. doi: 10.1007/s10802-012-9686-8

12. Brooker RJ, Kiel EJ, Buss KA. Early social fear predicts kindergartners' socially anxious behaviors: direct associations, moderation by inhibitory control, and differences from nonsocial fear. *Emotion.* (2016) 16:997–1010. doi: 10.1037/emo0000135

13. Crawford DC, Acuna JM, Sherman SL. FMR1 and the fragile X syndrome: human genome epidemiology review. *Genet Med.* (2001) 3:359–71. doi: 10.1097/00125817-200109000-00006

14. Clifford S, Dissanayake C, Bui QM, Huggins R, Taylor AK, Loesch DZ. Autism spectrum phenotype in males and females with fragile X full mutation and premutation. *J Autism Dev Disord.* (2007) 37:738–47. doi: 10.1007/s10803-006-0205-z

15. Lee M, Martin GE, Berry-Kravis E, Losh M. A developmental, longitudinal investigation of autism phenoyptic profiles in fragile X syndrome. *J Neurodev Disord.* (2016) 8:47. doi: 10.1186/s11689-016-9179-0

16. Baumgardner TL, Reiss AL, Freund LS, Abrams MT. Specification of the neurobehavioral phenotype in males with fragile X syndrome. *Pediatrics.* (1995) 955:744–52.

17. Sullivan K, Hatton D, Hammer J, Sideris J, Hooper S, Ornstein P, et al. ADHD symptoms in children with FXS. *Am J Med Genet Part A.* (2006) 140:2275–88. doi: 10.1002/ajmg.a.31388

18. Cordeiro L, Ballinger E, Hagerman R, Hessl D. Clinical assessment of DSM-IV anxiety disorders in fragile X syndrome: prevalence and characterization. *J Neurodev Disord.* (2011) 3:57–67. doi: 10.1007/s11689-010-9067-y

19. Lachiewicz AM, Dawson DV, Spiridigliozzi GA. Physical characteristics of young boys with fragile X syndrome: reasons for difficulties in making a diagnosis in young males. *Am J Med Genet.* (2000) 92:229–36. doi: 10.1002/(SICI)1096-8628(20000605)92:4<229::AID-AJMG1>3.0.CO;2-K

20. Merenstein SA, Sobesky WE, Taylor AK, Riddle JE, Tran HX, Hagerman RJ. Molecular-clinical correlations in males with an expanded FMR1 mutation. *Am J Med Genet.* (1996) 64:388–94.

21. Roberts JE, Ezell JE, Fairchild AJ, Klusek J, Thurman AJ, McDuffie A, et al. *Biobehavioral composite of social aspects of anxiety in young adults with fragile X syndrome contrasted to autism spectrum disorder.* Am J Med Genet B Neuropsychiatr Genet. (2018) 177:665–75. doi: 10.1002/ajmg.b.32674

22. Hagerman R, Hoem G, Hagerman P. Fragile X and autism: intertwined at the molecular level leading to targeted treatments. *Mol Autism* 1:12. doi: 10.1186/2040-2392-1-12

23. Abbeduto L, McDuffie A, Thurman AJ. The fragile X syndrome-autism comorbidity: what do we really know? *Front Genet.* (2014) 5:1–10. doi: 10.3389/fgene.2014.00355

24. Hall SS, Frank MC, Pusiol GT, Farzin F, Lightbody AA, Reiss AL. Quantifying naturalistic social gaze in fragile X syndrome using a novel eye tracking paradigm. *Am J Med Genet Part B Neuropsychiatr Genet.* (2015) 9999:1–9. doi: 10.1002/ajmg.b.32331

25. Roberts JE, Weisenfeld LAH, Hatton DD, Heath M, Kaufman WE. Social approach and autistic behavior in children with fragile X syndrome. *J Autism Dev Disord.* (2007) 37:1748–60. doi: 10.1007/s10803-006-0305-9

26. Roberts JE, Clarke MA, Alcorn K, Carter JC, Long AC, Kaufman WE. Autistic behavior in boys with fragile X syndrome: social approach and HPA-axis dysfunction. *J Neurodev Disord.* (2009) 1:283. doi: 10.1007/s11689-009-9028-5

27. White SW, Mazefsky CA, Dichter GS, Chiu PH, Richey JA, Ollendick TH. Social-cognitive, physiological, and neural mechanisms underlying emotion regulation impairments: understanding anxiety in autism spectrum disorder. *Int J Dev Neurosci.* (2014) 39:22–36. doi: 10.1016/j.ijdevneu.2014.05.012

28. Kerns CM, Kendall PC. The presentation and classification of anxiety in autism spectrum disorders. *Clin Psychol Sci Pract.* (2013) 19:323–47. doi: 10.1111/cpsp.12009

29. Roberts JE, Crawford, H, Hogan AL, Fairchild A, Tonnsen B, Brewe A, et al. Social avoidance emerges in infancy and persists into adulthood in males with fragile X syndrome. *J Autism Dev Dis.* In press

30. Ozonoff S, Iosif A, Baguio F, Cook IC, Hill MM, Hutman T, et al. A prospective study of the emergence of early behavioral signs of autism. *J Am Acad Child Adolesc Psychiatry.* (2010) 49:256–66.e1-2. doi: 10.1097/00004583-201003000-00009

31. Egger HL, Kondo D, Angold A. The epidemiology and diagnostic issues in preschool attention-deficit/hyperactivity disorder: a review. *Infants Young Child.* (2006) 19:109–22. doi: 10.1097/00001163-200604000-00004

32. Posner K, Melvin GA, Murray DW, Gugga SS, Fisher P, Skrobala A, et al. Clinical presentation of attention-deficit/hyperactivity disorder in preschool children: the preschoolers with attention-deficit/hyperactivity disorder treatment study (PATS). *J Child Adolesc Psychopharmacol.* (2007) 17:547–62. doi: 10.1089/cap.2007.0075

33. Lijster JM, Dierckx B, Utens EMWJ, Verhulst FC, Zieldorff C, Dieleman GC, et al. The age of onset of anxiety disorders. *Can J Psychiatry.* (2017) 62:237–46. doi: 10.1177/0706743716640757

34. Egger HL, Angold A. Common emotional and behavioral disorders in preschool children: presentation, nosology, and epidemiology. *J Child Psychol Psychiatry.* (2006) 47:313–37. doi: 10.1111/j.1469-7610.2006.01618.x

35. Halperin JM, Bedard ACV, Curchack-Lichtin JT. Preventive interventions for ADHD: a neurodevelopmental perspective. *Neurotherapeutics.* (2012) 9:531–41. doi: 10.1007/s13311-012-0123-z

36. Koegel LK, Koegel RL, Ashbaugh K, Bradshaw J. The importance of early identification and intervention for children with or at risk for autism spectrum disorders. *Int J Speech Lang Pathol.* (2014) 16:50–6. doi: 10.3109/17549507.2013.861511

37. Rapee RM, Kennedy S, Ingram M, Edwards S, Sweeney L. Prevention and early intervention of anxiety disorders in inhibited preschool children. *J Consult Clin Psychol.* (2005) 73:488–97. doi: 10.1037/0022-006X.73.3.488

38. Schopler E, Reichler R, Rochen Renner B. *The Childhood Autism Rating Scale.* Los Angeles: Western Psychological Services (1988).

39. Lord C, Rutter M, DiLavore PC, Risi S, Gotham K, Bishop SL, et al. *Autism Diagnostic Observation Schedule.* 2nd ed. Torrance, CA: Western Psychological Services (2012).

40. Achenbach TM, Rescorla LA. *Manual for the ASEBA Preschool Forms and Profiles: An Integrated System of Multi-Informant Assessment.* Burlington: University of Vermont, Research Center for Children, Youth, and Families (2000).

41. Mullen EM. *Mullen Scales of Early Learning: AGS Edition.* Circle Pines, MN: American Guidance Service (1995).

42. Roberts JE, McCary LM, Shinkareva SV, Bailey Jr DB. Infant development in fragile X syndrome: cross-syndrome comparisons. *J Autism Dev Disord.* (2016) 46:2088–99. doi: 10.1007/s10803-016-2737-1

43. Hogan AL, Caravella KE, Ezell J, Rague L, Hills K, Roberts JE. Autism spectrum disorder symptoms in infants with fragile X syndrome: a prospective case series. *J Autism Dev Disord.* (2017) 47:1628–44. doi: 10.1007/s10803-017-3081-9

44. IBM Corp. *IBM SPSS Statistics for Windows (Version 24.0).* Armonk, NY: IBM Corp (2016).
45. R Core Team. *R: A Language and Environment for Statistical Computing.* (2017). Available online at: https://www.R-project.org/
46. Muthén LK, Muthén BO. *Mplus User's Guide.* 8th ed. Los Angeles, CA: Muthén and Muthén (2017).
47. Tonnsen BL, Roberts JE. Characterizing emergent anxiety through the lens of fragile X. In *International Review of Research in Developmental Disabilities*, Vol. 51, Academic Press (2016). 41–83.
48. Hahn LJ, Brady NC, McCary L, Rague L, Roberts JE. Early social communication in infants with fragile X syndrome and infant siblings of children with autism spectrum disorder. *Res Dev Disabil.* (2017) 71:169–80. doi: 10.1016/j.ridd.2017.10.004
49. Roberts JE, Tonnsen BL, McCary LM, Caravella KE, Shinkareva SV. Brief report: autism symptoms in infants with fragile X syndrome. *J Autism Dev Disord.* (2016) 46:3830–7. doi: 10.1007/s10803-016-2903-5
50. Scherr JF, Hogan AL, Hatton D, Roberts JE. Stranger fear and early risk for social anxiety in preschoolers with fragile X syndrome contrasted to autism spectrum disorder. *J Autism Dev Disord.* (2017) 47:3741–55. doi: 10.1007/s10803-017-3059-7
51. Tonnsen B, Scherr J, Reisinger D, Roberts J. Behavioral markers of emergent stranger anxiety in infants and toddlers with fragile X syndrome. *J Autism Dev Disord.* (2017) 47:3646–58. doi: 10.1007/s10803-017-3270-6
52. Charman T, Taylor E, Drew A, Cockerill H, Brown J, Baird G. Outcome at 7 years of children diagnosed with autism at age 2: predictive validity of assessments conducted at 2 and 3 years of age and pattern of symptom change over time. *J Child Psychol Psychiatry.* (2005) 46:500–13. doi: 10.1111/j.1469-7610.2004.00377.x
53. Shephard E, Bedford R, Milosavljevic B, Gliga T, Jones EJH, Pickles A, et al. *Early developmental pathways to childhood symptoms of attention-deficit hyperactivity* disorder, anxiety and autism spectrum disorder. *J Child Psychol Psychiatry.* (2018). doi: 10.1111/jcpp.12947
54. Wing L, Gould J. Severe impairments of social interaction and associated abnormalities in children: epidemiology and classification. *J Autism Dev Disord.* (1979) 9:11–29. doi: 10.1007/BF01531288
55. Carlson SM. Developmentally sensitive measures of executive function in preschool children. *Dev Neuropsychol.* (2005) 28:595–616. doi: 10.1207/s15326942dn2802_3
56. Carlson SM, Wang TS. Inhibitory control and emotion regulation in preschool children. *Cogn Dev.* (2007) 22:489–510. doi: 10.1016/j.cogdev.2007.08.002
57. Luteijn EF, Serra M, Jackson S, Steenhuis L, Althaus M, Volkmar F, et al. How unspecified are disorders of children with a pervasive developmental disorder not otherwise specified? *A study of social problems in children with PDD-NOS and ADHD. Eur Child Adolesc Psychiatry.* (2000) 9:168–79. doi: 10.1007/s007870070040

Co-Occurrence of Autism Spectrum Disorder and Achondroplasia

Angel Belle Cheng Dy[1,2]* and Lourdes Sumpaico Tanchanco[1,2]

[1] School of Medicine and Public Health, Ateneo de Manila University, Pasig City, Philippines, [2] MedMom Institute for Human Development, Pasig City, Philippines

Autism spectrum disorder (ASD) and achondroplasia are common disorders on their own. However, this case of co-occurrence in the same patient has not yet been reported in literature except for a hypothesized statistical probability based on prevalence studies stating that two to five in 10 million children could have the probability of having both conditions occurring simultaneously. Achondroplasia typically presents with motor delays and difficulties that are related to musculoskeletal impairments that can affect self-care, mobility, and social cognition; however, the presence of delays in other domains of development, particularly in social communication, raises a suspicion of a co-occurring autism spectrum disorder. The content of this report reviews the common delays and difficulties seen in children with achondroplasia and those with autism spectrum disorder and describes the presence of both in the child presented in this case.

Keywords: achondroplasia, autism spectrum disorder, neurodevelopmental disorders, developmental delays

BACKGROUND

Autism spectrum disorder (ASD) is a developmental disorder that manifests as difficulties with social communication and interactions and restrictive or repetitive behaviors or interests (1). Achondroplasia is a genetic disorder with characteristic phenotypic presentation of disproportionate short stature, craniofacial and skeletal abnormalities, and motor developmental delays (2). Autism spectrum disorder and achondroplasia are common disorders on their own. However, the co-occurrence in the same patient has not yet been reported in literature except for a hypothesized statistical probability based on prevalence studies stating that two to five in 10 million children could have the probability of having both conditions occurring simultaneously (3).

Case Presentation

The case is a female, Filipino child born at 37 weeks' age of gestation. Her skeletal dysplasia was identified on fetal anomaly scanning during pregnancy and was diagnosed with achondroplasia after birth.

She was the third and youngest child to a 36-year-old mother and a 37-year-old father. She has a healthy and neurotypical older sister and brother. The parents were not consanguineous and have no known genetic abnormalities in their family history. Her birth weight was 1,390 g (less than the third percentile, World Health Organization (WHO)), birth length was 40 cm (less than the third percentile, WHO), and head circumference was 32 cm (third to 15th percentile, WHO). Her physical features at birth were described as having proportionately small frontal bossing and dolichocephaly with infraorbital creases. She had a relatively small thoracic cage, which was proportionate to her size. There were no gross rhizomelia or acromelic shortening of limbs. She had bilateral simian creases,

bilateral fifth digit midphalanx hypoplasia, and hypoplastic toenails. External genitalia were grossly female.

Peri-natal care included 17-day confinement in the neonatal intensive care unit for hyperbilirubinemia and antibiotic treatment with amikacin and ampicillin for suspected sepsis. Newborn screening and otoacoustic emission tests were normal. She was breastfed for 2 months, after which she was started on formula milk. She has frequent respiratory infections but does not have a history of recurrent ear infections. She also had an exposure to tuberculosis, which was managed with isoniazid for 6 months. Bone aging reports were normal.

At the age of 6 months, delays in motor, language, socio-emotional, and cognitive development were observed. Her fine motor skills included visually tracking, grasping a rattle, and holding her hands together but not yet regarding and reaching for objects. Her gross motor skills were almost of a newborn wherein she was not able to hold up or lift her head. Her language skills included vocalizations, turning to sounds and voices, and laughing but did not yet imitate speech sounds or babbled. Her personal-social skills included regarding faces and smiling responsively and spontaneously but not yet regarding her own hands or feeding herself. Anthropometric measurements were as follows: body weight was 4,100 g (less than the third percentile, WHO), body length was 53 cm (less than the third percentile, WHO), and head circumference was 42.5 cm (50th percentile, WHO). Physical examination revealed universal hypotonia, with a preference for the right hand.

At the age of 17 months, she had improvements in her developmental skills with more motor imitations, vocalizations, and babbling and the ability to follow simple commands. Physical examination continued to show poor muscle tone, flat foot, and joint laxity. She had difficulty feeding as she choked on solid food and had frequent regurgitation. A gastroenterologist assessed her to have gastroesophageal reflux due to hypotonia. She was advised to have slower, smaller feeding to help in her digestion. The issues with reflux resolved by 30 months of age.

In the next 2 years, the child continued receiving physical, occupational, and speech therapy. **Table 2** presents her developmental skills acquisition. It is compared with the skills acquisition of other children with achondroplasia in a study by Ireland et al.

By the age of 27 months, her parents began noticing her poor social skills such as fleeting eye contact, not turning to her name being called, and not pointing or requesting for her needs. Despite undergoing early intervention services, she continued to demonstrate delays in all developmental domains. A summary

of her progress and developmental quotient using the Griffith Mental Development Scales is presented in **Table 1**.

At 39 months, she meets the DSM-5 criteria for autism spectrum disorder, manifested by her delays in communication and social skills. She also presented with repetitive and stereotypic behaviors such as a fascination of shadows and hand flapping and would demonstrate repetitive tapping of objects (i.e., pencil) and her hands against hard surfaces like the table.

The Autism Diagnostic Observation Schedule (ADOS-2), Module 1, was administered at 39 months, and she received an additional diagnosis of autism spectrum disorder. The Autism Diagnostic Observation Schedule is a semi-structured standardized assessment of communication, reciprocal social interaction, play, and imaginative use of materials. Module 1 of the assessment was used, which consisted of 10 activities that focused on playful use of toys and other concrete materials. The child's communication score was 6 (cut-off = 4), and social interaction was 8 (cut-off = 7), which both reached the cut-off for autism.

Management of her developmental delays and behavioral concerns continues to be targeted through speech and language therapies that focus on pre-language skills, pragmatics, and communication of her needs and wants. She also continues occupational therapy and physical therapy to address her motor skills, activities of daily living, and sensory processing concerns. She receives special education with focus on self-regulation and engagement, and behavior modifications to allow her to be involved in learning activities.

DISCUSSION

Predispositions and Risk Factors

Although genetic studies were not performed in this patient, achondroplasia may be a cryptogenic cause for her presentation of autism. Approximately 40% of children with autism are diagnosed with other co-morbidities such as epilepsy, neuropsychiatric disorders, attention deficit/hyperactivity disorder, and Tourette syndrome. A term "secondary" ASD has been coined to refer to cases of identifiable syndromes or medical disorders known to be associated with ASD, and these are seen in approximately 6% of confirmed cases. Aside from these, dysmorphic features are seen in some cases of ASD that may indicate genetic syndromes as well (4, 5).

There are a number of genes that are associated with ASD presentation. A study by M.R. Herbert et al. (2006) listed 135 environmentally susceptible genes that showed an overlap with

TABLE 1 | Patient's performance on the Griffith Mental Development Scales.

	18 months of age	Developmental quotient	39 months of age	Developmental quotient
Locomotor	12 months	67	19 months	49
Personal–social	10 months	56	12 months	30
Hearing and speech	8 months	44	10 months	27
Eye–hand coordination	10 months	55	11 months	28
Performance	11 months	61	15 months	38

autism linkage regions that may not have been studied yet in relation to autism itself. One of these included the FGFR3 gene for achondroplasia (6). Even though it is merely part of the extensive listing of genes, it highlights that multiple genetic and environmental mechanisms could be playing a role in the presentation of autism in this case.

The parents of this child are both in their mid-30s and may be additional risk factors for her condition. Increased paternal age has been associated as a contributing factor to some diseases and disorders, including both achondroplasia and ASD (7, 8). Increased maternal age over 35 years has also been associated as a risk for having a child with ASD (9).

Abnormal presentation, fetal distress, small for gestational age, low birth weight, feeding difficulties, and hyperbilirubinemia are among the associated risk factors for ASD (10). These risk factors were present in the case.

Developmental Trajectories

A cohort study by Ireland et al. (11, 12) developed a projection of milestones for children with achondroplasia. Children with achondroplasia commonly have developmental delays in gross motor skills such as lifting their head, rolling over, crawling, and walking. These children develop other pre-ambulation strategies to compensate for their limb shortening and hypotonia such as forward and reverse snowplow or commando crawling as demonstrated by the child in the case (11, 12). In the comparison table with the children diagnosed with achondroplasia in the Ireland et al. (11, 12) study, her motor performance is at par with that of the cohort of children from the study (**Table 2**).

This case presents with delays in fine motor and social skills with slow progress in these developmental domains despite interventions initiated at an early age. The development of fine motor and social skills in children with achondroplasia is expected to be similar with typical development, achieving the 90th percentile for age similar to that of typically developing children (11, 12). The delays in fine motor skills and social skills in our case are better explained by the presence of autism (13).

Early communication skills such as babbling and saying "mama" are likewise expected within typically developing age ranges for children with achondroplasia. However, they take a longer time to achieve the use of single words and two-word combinations but eventually learn them (11, 12). At 39 months of age, our case is expected to use simple sentences, but she is still unable to say or imitate words. She still uses jargons and is only able to follow one-step requests. Therefore, the language delays seen in this patient are uncommon in achondroplasia by her current age; but they can be attributed to the impairments in ASD.

Based on the Ireland et al., (14) study, skills for independence emerge by the fourth and fifth years of life. By 7 years old, children can be independent or have modified independence for self-care skills such as eating and toileting. They are also completely mobile, either independently or with modifications. Social cognition also improves, showing difficulty in comprehension only (11, 12, 14). The musculoskeletal impairments of achondroplasia seen

TABLE 2 | Age of achievement (months) for gross motor, fine motor, communication, and feeding skills (11, 12).

Milestone	Patient's age of achievement	Achondroplasia 90th percentile Ireland et al.	Typical development 90th percentile Denver II Test
Gross motor			
Lift head when lying on stomach	9	7	3.5
Roll over	9	9.9	5.2
Reverse snow plough	12	13.8	
Traditional crawling	16	18.3	
Into sitting from lying	15	18.5	10
Into sitting from standing	18	22.8	
Into standing from sitting	20	20	10
Stand holding on	16	20	12
Stand unsupported	18	24	14
Walk holding on	18	22	12.7
Walk independently	20	26	15
Fine motor			
Reach for object	6	6.5	5.5
Pass objects	12	10	7.5
Bang objects together	18	11.1	11
Scribble with crayon	26	20	17
Draw circle	—	36	45
Build tower two blocks	24	20.9	20
Build tower eight blocks	—	32.9	42
Communication			
Smile	6	2.8	2
Babble	18	11.3	9
Wave	18	14.3	14
Say "mama"	—	14	13
Shake head	—	20	
Peek-a-boo	24	13	9.7
1-step request	36	23.5	
Short sentences	—	38.4	
Feeding			
Cup drinking	36	15.6	17
Puree/smooth solids	12	6.5	
Mashed solids	18	10.2	
Finger feeding	36	12.3	7
Self-feed with spoon	—	22	20

in this patient increase the need for a caregiver's assistance. It is possible to predict that she will continue to have difficulties in independence for a number of years. However, these may be overcome through continuous intervention in her therapy sessions as well as creation of modifications that allow for independence.

Barriers in the medical and social life course of individuals with achondroplasia include unequal opportunities in education and employment and increase in social isolation, which then influence their financial situation and quality of life (15). It is important to recognize that other difficulties of individuals with achondroplasia would be social in nature, particularly public perceptions that lead to teasing and discrimination. Individuals may have lower self-esteem, less education, lower annual income,

and less likely to marry. In their transitions through different life stages, interventions should also include psychotherapy in fostering a positive self-concept to increase quality of life (16).

Anticipatory guidance and regular health supervision will assist in earlier identification of known complications in achondroplasia and includes confirming the diagnosis through radiographic studies, documentation of measurements, medical evaluations, and determining social adjustment (17). This patient benefited from early detection and appropriate monitoring for her disorder through access to a pediatrician and geneticist. She has also been followed up by a developmental-behavioral pediatrician in order to monitor her development, leading to early detection of her developmental delays, which are being addressed through a number of interventions, including speech therapy, occupational therapy, and physical therapy. Multidisciplinary approaches in caring for these types of special cases are necessary to optimize management and care (11, 12).

CONCLUSION

Achondroplasia is a genetic disorder with characteristic phenotypic presentation of disproportionate short stature, craniofacial and skeletal abnormalities, and motor developmental delays. Its co-occurrence with autism spectrum disorder has not been reported before. This patient presents with physical features of achondroplasia with the concomitant delays in social communication and stereotypic behaviors seen in autism spectrum disorder. Early intervention is critical in addressing the unique needs of this patient. Collaboration among the intervention team is necessary to optimize her development.

This case report has its limitations. It is unable to generalize to other populations, and the association does not imply

there is a cause–effect relationship between autism and achondroplasia. It could be that the observation is merely a coincidence. Achondroplasia in this case was based on clinical features of the child. More intensive work-up including radiographic studies, documentation of measurements, extensive medical evaluations, and determination of social adjustment, when available, should be used to establish baseline findings. It is recommended that her developmental trajectories be monitored as she grows up.

ETHICS STATEMENT

A written informed consent for writing and publishing this report was obtained from the parent and legal guardian of the individual included.

AUTHOR CONTRIBUTIONS

LT performed the clinical assessment and follow-up of the case. AD reviewed related literature. LT and AD reviewed the subject's medical history and physical presentation. AD drafted the manuscript. LT provided critical revisions to the manuscript and provided the final approval for the document to be submitted for publication.

FUNDING

REFERENCES

1. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders*. Arlington, VA: American Psychiatric Association (2013). doi: 10.1176/appi.books.9780890425596
2. Jones K, Jones M, Casanelles MD. *Smith's recognizable patterns of human malformation*. 7th ed. Philadelphia, PA: Elsevier Saunders (2013).
3. Steiner C, Guerreiro M, Marques-de-Faria A. Brief report: acrocallosal syndrome and autism. *J Autism Dev Disord* (2004) 34(6):723–6. doi: 10.1007/s10803-004-5292-0
4. Gabis LV, Pomeroy J. An etiologic classification of autism spectrum disorders. *Isr Med Assoc J* (2014) 16:295–8. Retrieved from: https://www.ima.org.il/FilesUpload/IMAJ/0/79/39892.pdf.
5. Johnson C, Myers SM. Identification and evaluation of children with autism spectrum disorders. *Pediatrics* (2007) 230:1183–215. doi: 10.1542/peds.2007-2361
6. Herbert M, Russo J, Yang S, Roohi J, Blaxill M, Kahler S, et al. Autism and environmental genomics. *Neurotoxicology* (2006) 27:671–84. doi: 10.1016/j.neuro.2006.03.017
7. Wiener-Megnazi Z, Auslender R, Dirnfeld M. Advanced paternal age and reproductive outcome. *Asian J Androl* (2012) 14:69–76. doi: 10.1038/aja.2011.69
8. Wilkin DJ, Szabo JK, Cameron R, Henderson S, Bellus GA, Mack ML, et al. Mutations in fibroblast growth-factor receptor 3 in sporadic cases of

achondroplasia occur exclusively on the paternally derived chromosome. *Am J Hum Genet* (1998) 63:711–6. doi: 10.1086/302000
9. Dirkin MS, Maenner MJ, Newschaffer CJ, Lee L-C, Cunniff CM, Daniels JL, et al. Advanced parental age and the risk of autism spectrum disorder. *Am J Epidemiol* (2008) 168:1268–76. doi: 10.1093/aje/kwn250
10. Gardener H, Spiegelman D, Buka SL. Perinatal and neonatal risk factors for autism: a comprehensive meta-analysis. *Pediatrics* (2011) 128:344. doi: 10.1542/peds.2010-1036
11. Ireland PJ, Johnson S, Dognahey S, Johnstan L, Ware RS, Zankl A, et al. Medical management of children with achondroplasia: evaluation of an Australasian cohort aged 0–5 years. *J Paediatr Child Health* (2012a) 48:443–9. doi: 10.1111/j.1440-1754.2011.02255.x
12. Ireland P, Donaghey S, McGill J, Zankl A, Ware RS, Pacey V, et al. Development in children with achondroplasia: a prospective clinical cohort study. *Dev Med Child Neurol* (2012b) 54(6):532–7. doi: 10.1111/j.1469-8749.2012.04234.x
13. Provost B, Lopez B, Heimerl S. A comparison of motor delays in young children: autism spectrum disorder, developmental delay, and developmental concerns. *J Autism Dev Disord* (2007) 37:321–8. doi: 10.1007/s10803-006-0170-6
14. Ireland P, McGill J, Zankl A, Ware RS, Pacey V, Ault J, et al. Functional performance in young Australian children with achondroplasia. *Dev Med Child Neurol* (2011) 53:944–50. doi: 10.1111/j.1469-8749.2011.04050.x

15. Thompson S, Shakespeare T, Wright M. Medical and social aspects of the life course for adults with a skeletal dysplasia: a review of current knowledge. *Disabil Rehabil* (2008) 30(1):1–12. doi: 10.1080/09638280701192857

16. Gollust SE, Thompson RE, Gooding HC, Biesecker BB. Living with achondroplasia in an average-sized world: an assessment of quality of life. *Am J Med Genet* (2003) 120A:447–58. doi: 10.1002/ajmg.a.20127

17. Trotter TL, Hall JG, Committee on Genetics. Health supervision for children with achondroplasia. *Pediatrics* (2005) 116(3):771–82. doi: 10.1542/peds.2005-1440

A Personalized Autism Diagnosis CAD System Using a Fusion of Structural MRI and Resting-State Functional MRI Data

Omar Dekhil[1], Mohamed Ali[1], Yaser El-Nakieb[1], Ahmed Shalaby[1], Ahmed Soliman[1], Andrew Switala[1], Ali Mahmoud[1], Mohammed Ghazal[1,2], Hassan Hajjdiab[2], Manuel F. Casanova[3], Adel Elmaghraby[4], Robert Keynton[5], Ayman El-Baz[1]*[†] and Gregory Barnes[6][†]

[1] Bioimaging Lab, Bioengineering Department, University of Louisville, Louisville, KY, United States, [2] Department of Electrical and Computer Engineering, Abu Dhabi University, Abu Dhabi, United Arab Emirates, [3] Department of Biomedical Sciences, University of South Carolina, Greenville, SC, United States, [4] Computer Engineering and Computer Science Department, University of Louisville, Louisville, KY, United States, [5] Bioengineering Department, University of Louisville, Louisville, KY, United States, [6] Department of Neurology, University of Louisville, Louisville, KY, United States

Autism spectrum disorder is a neuro-developmental disorder that affects the social abilities of the patients. Yet, the gold standard of autism diagnosis is the autism diagnostic observation schedule (ADOS). In this study, we are implementing a computer-aided diagnosis system that utilizes structural MRI (sMRI) and resting-state functional MRI (fMRI) to demonstrate that both anatomical abnormalities and functional connectivity abnormalities have high prediction ability of autism. The proposed system studies how the anatomical and functional connectivity metrics provide an overall diagnosis of whether the subject is autistic or not and are correlated with ADOS scores. The system provides a personalized report per subject to show what areas are more affected by autism-related impairment. Our system achieved accuracies of 75% when using fMRI data only, 79% when using sMRI data only, and 81% when fusing both together. Such a system achieves an important next step towards delineating the neurocircuits responsible for the autism diagnosis and hence may provide better options for physicians in devising personalized treatment plans.

Keywords: structural magnetic resonance imaging, functional magnetic resonance imaging, autism, personalized diagnosis, Computer-Added Diagnostics (CAD) systems, machine learning

INTRODUCTION

Autism spectrum disorder (ASD) is a neuro-developmental disorder that has three main associated characteristics (1): i) social functioning disorders, ii) communication impairments, and iii) restricted and repetitive behaviors (RRBs). In many previous research projects, correlation was reported between autism and both anatomical abnormalities and functional activation abnormalities. For studying anatomical abnormalities, the most commonly used imaging modality is structural MRI (sMRI) (2), while functional MRI (fMRI) is the most commonly used modality for studying brain activation (3).

The relationships between MRI parameters and the autism diagnosis play a key role in defining the impaired neurocircuits in an individual ASD subject. When studying anatomical sMRI, there are two main categories of features, and each study either uses features driven from one of them or a combination of both i) shape features and ii) volumetric features. With regard to volumetric analysis, Courchesne et al. (4) conducted a study on 60 autistic and 52 typically developed individuals (ages between 2 and 16 years) to explore the anatomical abnormalities in cerebral and cerebellar volume of autistic brains with 50% of the autistic participants being aged 5 or more years and 50% between 2 and 4 years old. In the age group between 2 and 4 years old, 90% of the participants were found to have brain volumes larger than normal. This result reinforced the hypothesis that the brain volume in autistic infants was larger in size than in typically developed infants. This hypothesis was also supported by results in the study of Hazlett et al. (5), where 51 autistic and 25 typically developed individuals (ages between 1.5 and 3 years) were examined and it was found that the cerebellar white matter volume in autistic subjects between 2 and 4 years old were larger than normal size. Geschwind and Levitt (6) also emphasized the same assumption during infancy interval, but found additionally that the cerebral hemisphere can remain enlarged during adulthood. Meanwhile, other investigators (7–9) suggested that the main areas of the brain with enlarged white matter and gray matter were the frontal, temporal, and parietal lobes (ages between 2 and 12 years). A voxel-based morphometry (VBM) study was conducted by Toal et al. (10) to study the brain anatomy of both autistic and typically developed adults (mean age is 32 years with 9 years standard deviation), and found that the brain of the autistic individuals had significant increased gray matter involving both the frontal and temporal lobes.

Instead of studying the cortical volume (CV) as a single parameter, and based on the fact that CV is the product of two parameters, cortical thickness (CT) and surface area (SA), Ecker et al. (11) analyzed these three parameters together in order to attain a more insightful observation about anatomical abnormalities in the autistic brains (age mean is 26 years with 7 years standard deviation). This study observed differences in the three parameters (CV, CT, and SA) between the two groups with the CT in autistic subjects being significantly larger than that in typically developed individuals in the frontal lobe regions, while SA in the orbitofrontal cortex and posterior cingulum in autistic subjects was less than that in the typically developed. Using the same three parameters, a more comprehensive study by Haar et al. (12) was conducted (ages between 6 and 65 years). While more detailed results were obtained from this study, the results were still in line with previous studies and anatomical hypotheses. Specifically, in autistic individuals, larger ventricular volumes, smaller corpus callosum volume (central segment only), and several cortical areas with increased thickness were detected. Another approach for studying anatomical abnormalities in autistic subjects was to study the longitudinal changes in CT (13) (ages between 3 and 36 years), which allows the identification of specific regional differences in the CT. In their study, Zielinski et al. (13) discovered that the most significant differences in CT between autistic and typically

developed individuals of the same mean age was in the bilateral inferior frontal gyrus, pars opercularis, pars triangularis, right caudal middle frontal, and left rostral middle frontal regions. Other studies addressed different brain regions. For example, autistic subjects displayed larger amygdala than normal subjects (14). Waiter et al. (15) (age mean is 15.4 years with 2.24 years standard deviation) examined the areas believed to be responsible for the social cognitive functions: in particular, 1) facial recognition (right fusiform gyrus), 2) perception and eye gaze (superior temporal gyrus), and 3) mental state attribution (anterior cingulate and superior temporal sulcus). In these areas, a significant increase in gray matter was observed. Another VBM study was performed by Salmond et al. (16) (ages between 8 and 18 years) to examine the cerebellum, fusiform gyrus, and frontal cortex. Similarly, increased gray matter volume was observed in the regions of the cerebellum in the participants located near the high functioning end of the autism spectrum, which is consistent with the anatomical hypothesis that it relates increased brain size to autism.

Another major approach explored for discriminating between autistic and typically normal developed brains was shape-based analysis of sMRI. As a quantitative measure for shape analysis, the gyrification index (GI) (17) was used by Hardan et al. (18) (age mean is 12.7 years with 2.2 years standard deviation). GI is a measure of cortical folding that is calculated as the ratio between total contour length and outer contour length from coronal sMRI slices. The GI in the left frontal area was noticed to be larger in autistic children and adolescents than corresponding typically developed children and adolescents. In this study, the GI decreased with age in autistic subjects but not in typically developed subjects. In line with the increased GI finding reported by Hardan et al. (18), Wallace et al. (19) (age mean is 16.7 years with 2.8 years standard deviation) also reported increased gyrification in the bilateral posterior cortices in autistic subjects. Furthermore, a positive correlation between vocabulary knowledge and gyrification in the left inferior parietal cortex in typically developed individuals was noted while no correlation was found for autistic subjects. One of the most commonly used shape-based analysis techniques was folding analysis. For example, Awate et al. (20) (ages between 7.5 and 31 years) used six folding measures for cortical curvature analysis between both groups (autistic and typically developed). The folding measures used were i) intrinsic curvature index, ii) mean curvature norm, iii) convexity ratio, iv) isoperimetric ratio, v) shape index S, and vi) curvedness. The Awate et al. (20) study found increased folding in the ASD frontal, parietal, and temporal lobes when compared to typically developed individuals. This increased folding was more prominent in children than in adults. A more recent study by Katuwal et al. (21) also addressed the curvature abnormalities using seven features extracted from a reconstructed brain mesh. The features used were i) Gaussian curvature, ii) mean curvature, iii) folding index, iv) thickness, v) thickness standard deviation, vi) SA, and vii) volume. Another study by Nordahl et al. (22) (ages between 7.5 and 18 years) addressed the cortical shape abnormalities in both high-functioning and low-functioning ASDs plus TDs using surface-based morphometry. Sulcal depth was used as a quantitative measure to analyze morphological

abnormalities. For the low-functioning autistic subjects, the abnormalities in sulcal depth were mainly noticed in the anterior insula and frontal operculum in addition to shape abnormalities in the inferior frontal gyrus. The same abnormalities were noticed in high-functioning autistic patients, but with relatively smaller size. They were centered near the parietal operculum and ventral postcentral gyrus. Sulcal depth differences were also reported by Dierker et al. (23) (age mean is 11.4 years with 1.9 years standard deviation), in which differences in sulcal depth were noted in the anterior insula and temporoparietal junction between the two groups. The areas having the most significant abnormalities were the frontal and temporal areas, particularly from social and language regions, which were highly implicated in autism. Brain shape differences between autistic and typically developed individuals were explored by Ecker et al. (24) (ages between 18 and 43 years), where GI in gray matter was studied. The experimental outcome was a prominent increase in gyrification around the left pre- and post-central gyrus in autistic individuals.

Regarding fMRI analysis, there are two major types of experiments to examine brain functional activity: i) resting-state fMRI (RfMRI) and ii) task-based fMRI (25) (age mean is 24 years with 10 years standard deviation).

In Just et al. (26), the underconnectivity theory was first proposed. This theory states that ASD is due to both cognitive and neurobiological disorders. The cognitive disorder mainly appears as reduced synchronized brain activity in integrative processing demanding tasks, for example, forming a sentence from a set of words (27) (age mean is 27.1 years with 11.9 years standard deviation).

Researchers conducted different studies to study the brain connectivity using task-based approaches. For example, less activation in the left dorsolateral prefrontal and inferior parietal areas was identified, and more activation was reported in the right occipital (visuospatial) areas and bilateral superior parietal using a figures task experiment in Damarla et al. (28) (age mean is 19 years with 5.5 years standard deviation). In Weng et al. (29) (age mean is 14.36 years with 1.7 years standard deviation), the response to facial expressions was studied, where autistic individuals were reported to have higher activation in the amygdala, ventral prefrontal cortex, and striatum. Another example of using task-based experiments is the rewards task, where subjects are given either monetary or social reward and their brain activity in response to this reward is recorded (30, 31) (age mean is 12.3years with 1.76 years standard deviation). In Dichter et al. (32), less activation in the right nucleus accumbens and more activation in left midfrontal and anterior cingulate gyrus were reported in ASDs than in TDs in response to social and monetary rewards. Another study by Cox et al. (33) (age mean is 24.11 years with 4.16 years standard deviation) supported less connectivity in autistic subjects in response to rewards.

To study the alterations in connectivity between TDs and ASDs, Deshpande et al. (34) (age mean is 21.14 years with 1 year standard deviation) applied a machine learning algorithm based on a multivariate autoregressive model trying to find the most logical end to a story shown to them.

In the study by Itahashi et al. (35) (age mean is 31 years with 8 years standard deviation), researchers found that functional connectivity of ASDs is less than that of TD subjects. These results were also supported by Alaerts et al. (36) (age mean is 13.7 years with 4.64 years standard deviation), which is providing more evidence for the underconnectivity theory.

In Rausch et al. (37) (age mean is 16.23 years with 3.218 years standard deviation), reduced functional connectivity in visuospatial and superior parietal areas was reported on ASDs when compared to TDs. Also in another study by Tyszka et al. (38) (age mean is 27.4 years with 2.4 years standard deviation), reduced connectivity was reported in local areas of both the frontal and temporal cortex, but no global abnormalities were detected. In Plitt et al. (39) (age mean is 17.5 years with 5.5 years standard deviation), dysfunction in the functional networks was reported, and this dysfunction was more obvious in social information processing related networks. The altered connectivity result was also supported by Di Martino et al. (40), where both hypoconnectivity and hyperconnectivity were reported in ASD circuits.

Not only underconnectivity was reported for ASDs in the previous studies. A study by Hahamy et al. (41) (ages between 18 and 44 years) reported alterations in functional connectivity patterns, where the interhemispheric connectivity analysis in autistic subjects showed areas of decreased connectivity while other areas showed increased connectivity compared to healthy control subjects. The hyperconnectivity was also reported in autistic children in Supekar et al. (42) (ages between 7 and 13 years), where autistic children with more severe social dysfunction were found to be functionally hyperconnected.

In addition to reporting global differences between ASDs and TDs, resting-state connectivity patterns demonstrated promising results in diagnosing many diseases, e.g., Alzheimer's disease (43), schizophrenia (44) (age mean is 35.9 years with 13.5 years standard deviation), and autism. For example, the approaches in Kim et al. (44) achieved high accuracy in schizophrenia diagnosis.

A deep neural network and functional connectivity analysis have been used in the recent study by Heinsfeld et al. (45) and Dvornek et al. (46) for autism diagnosis where the functional connectivity correlation matrix was the input to the classification network.

The heterogeneity of autism among individuals according to symptoms and severity has raised the need for a more personalized approach to predict and analyze the behavior and functionality of each autistic subject. Hence, we could then design an optimum treatment plan for every autistic subject. In this study, we aim to answer two main research questions: i) Can fMRI and sMRI be used for autism diagnosis in an objective way? ii) Are fMRI and sMRI features associated with ADOS scores? The hypothesis of this study is that combined sMRI and fMRI parameters are more likely to correlate more closely with behavior and yield high diagnostic accuracy, sensitivity, and specificity. The proposed system uses machine learning to define global and local features of ASD regardless of age or gender. Finally, we again analyze our results to be sure that it fits nicely within research domain criteria (RDoC)-defined neurocircuits related to ASD. Such criteria are important for the generalization of this model to highly heterogeneous ASD populations that present to the physician's office.

MATERIALS AND METHODS

In this study, both fMRI and sMRI data are obtained from the National Database of Autism Research (NDAR). The data for both experiments are obtained from a single study (NDAR study ID 2021). Imaging data provided by NDAR are fully anonymized and they are linked with other records (diagnostic, behavioral, demographic, etc.) using a unique identifier, the NDAR globally unique identifier (GUID). The total number of subjects used is 185 subjects. The selected data were collected at George Washington University. All the selected subjects have both high-resolution T1-weighted structural images and RfMRI images (7 subjects out of the 185 have corrupted RfMRI imaging files, so they were included in the sMRI analysis and excluded from fMRI analysis and from sMRI–fMRI modalities fusion). Out of the used 185 subjects, 61 subjects have autism diagnostic observation schedule (ADOS) reports.

All neuroimages were produced by a Siemens Magnetom TrioTim with a 3-T magnet. Structural scans used an MPRAGE pulse sequence with TR = 2,530 ms, TE = 3.31 ms, TI = 1,100 ms, and flip angle 7°. Volumes were acquired in 3D with isotropic 1-mm voxel spacing. For the functional scans, they have TR = 2,000 ms, TE = 30 ms, and flip angle 90° in a two-dimensional acquisition sequence to produce images with 3-mm pixel spacing and 4-mm slice spacing. Time to acquire 33 coronal slices spanning the entire brain was 2.01 s, and the resting-state data were recorded for approximately 6 min.

sMRI Experiment

In this experiment, both morphological and volumetric features are extracted and studied. Prior to extracting any of these features, there are some mandatory data preprocessing steps to be applied followed by the segmentation of the brain cortex. These preprocessing steps could be summarized as follows:

Brain Data Preprocessing

The preprocessing is a vital requirement to remove the variability between subjects that may stem from data acquisition, different scanners, artifacts, or partial volume effects. Moreover, the preprocessing step removes non-brain tissues such as skull. The following steps are applied to preprocessing sequentially.

1. Intensity normalization (47): In this step, intensity non-uniformities are corrected using a non-parametric model. It does not require any prior knowledge about existing tissue classes in the image.
2. Brain extraction and skull stripping (48): In this step, an algorithm combining both watershed algorithm and deformable surface model is used for skull stripping. The used algorithm starts by localizing a voxel belonging to the white matter, creating local minimum in the white matter, and then applying watershed algorithm with a pre-flooding height. This creates an initial estimate about the brain volume. To overcome any inaccuracies that might lead to cortical surface erosion, a deformable surface model is then applied. This allows the integration of geometric constraints into the skull stripping process.

Brain Segmentation and Area Labeling

The atlas-based brain segmentation task (49, 50) is formulated as a joint model using the given atlas and an affine transformation with 12 degrees of freedom, $\omega = f$ that maps the input volume to the atlas domain.

Let $\mathbf{R} = \{r = (x, y, z): 0 \leq x \leq X - 1, 0 \leq y \leq Y - 1, 0 \leq z \leq Z - 1\}$; $\mathbf{Q} = \{0, 1,\ldots, Q - 1\}$; and $\mathbf{L} = \{0,\ldots,l\}$ denote a finite arithmetic lattice of the size XYZ supporting gray scale images and their region (segmentation) maps, a finite set of Q integer gray values, and a labeled set of objects ("0"), non-brain tissue ("1"), cerebrospinal fluid (CSF) ("2") for gray matter, and so on. Let $g = \{g_r: r \in \mathbf{R}; g_r \in \mathbf{Q}\}$ and $m = \{m_r: r \in \mathbf{R}; m_r \in \mathbf{L}\}$ be a gray-scale image having values from $\mathbf{L}$, i.e., $\mathbf{m}: \mathbf{R} \mapsto \mathbf{L}$, respectively.

First, the brain atlas, $\mathbf{A} = \{a_i = (g_i, m_i): i = 1, 2,\ldots, N\}$, contains 3D MRI scans of different brains and their manually labeled volumes. Given the atlas function, f, that co-aligns a_i to the atlas domain (preselected template). This atlas is constructed in such a way that retains each anatomical label information at each voxel. The prior probability for each label m to occur at atlas location r is:

$$P(\mathbf{m}(r) = m) \approx \frac{\# \text{ of times label } m \text{ occurred at location } \mathbf{f}(r)}{\# \text{ of voxels that map to } r \text{ in the training set}} \quad (1)$$

Since each location r can be mapped to different labels, the intensity distribution of each label m at r is modeled as a Gaussian distribution. The mean and variance of such distribution are calculated as:

$$\mu_m(r)) = \frac{1}{N} \sum_{i=1}^{N} \mathbf{g}_i(\mathbf{f}(r)) \quad (2)$$

where g_i are the set of N images for which label m occurs at location $f(r)$ in the corresponding manually labeled image S_i. The variance for label m at location r is given by:

$$\sigma_m(r)^2 = \frac{1}{N} \sum_{i=1}^{N} (\mathbf{g}_i(\mathbf{f}(r)) - \mu_m(r))^2 \quad (3)$$

Having both prior information and conditional probability for each class at each atlas location, the segmentation problem for a new input subject, given its affine transformation, ω, for the atlas domain is modeled using MAP estimate:

$$P(\mathbf{m}|\mathbf{g}, \omega) = P(\mathbf{g}|\mathbf{m}, \omega) P(\mathbf{m}) \quad (4)$$

with the assumption that the noise is independent at r, $P(\mathbf{g}|\mathbf{m}, \omega)$ can be written as:

$$P(g|m, \omega) = \prod_{r \in R} P(g(\omega_r)|m(r)) \quad (5)$$

Using the atlas information, Eqs. 2 and 3, the conditional probability for each label at each voxel is given by:

$$P(\mathbf{g}(\omega_r)|\mathbf{m}(r) = m) = \frac{1}{\sqrt{2\pi\sigma_m(r)}} \exp(-0.5\sigma_m(r)^{-2}(\mathbf{g}(\omega_r) - \mu_m(r))$$

$$(6)$$

To get the prior probability ($P(\mathbf{m})$) for Eq. 4, a Markov random field model is used to encode the label's relationship as a function of location within the brain in addition to the local direction. Taking into account 6 voxels in the positive and negative cardinal directions at each location in the atlas space, the $P(\mathbf{m})$ is expressed as:

$$P(\mathbf{m}) = \prod_{r \in \rho} P(\mathbf{m}_r) \prod_{i=1}^{m} P(\mathbf{m}(r_i) | \mathbf{m}(r), r_i) \qquad (7)$$

where ρ is the neighborhood system of r. The values of $P(\mathbf{m}(r))$ are computed and stored in the atlas using Eq. 1. The spatial relationship between different labels is encoded in $P(\mathbf{m}(r_i) | \mathbf{m}(r), r_i)$. However, for simplicity and computation efficiency, the MAP estimate assumes $P(\mathbf{m}(r_i) | \mathbf{m}(r), r_i)$ as uniform as no labels have been assigned yet. After obtaining the initial segmentation, it is sequentially updated using iterated conditional modes (ICMs) algorithm. For more details about the segmentation algorithm, the reader is referred to Sled et al. (47).

After completing the preprocessing and the segmentation steps, the following steps are applied for 3D surface reconstruction and brain parcellation to an anatomical atlas from the segmented volume.

1. Tessellation of the gray–white matter boundary (51, 52): In this step, the spherical topology of the surface is accurately corrected. The used technique constructs a mapping between the original surface onto a sphere. Topological defects are detected as the minimal nonhomeomorphic regions. Each topological defect is then corrected by opening and sealing along the set of non-separating loops.
2. Surface inflation and spherical atlas registration (53, 54): In order to establish a spherical-based cortical surface, three steps are applied: i) inflate the cortical surface to visualize hidden structures in the sulci, ii) cut and flatten the entire hemisphere, and iii) parameterize using a sphere. The parameterized surface is then used to create a spherical surface-based coordinate system. To define such coordinate system, the average folding pattern of a large population is used as an atlas. Each individual subject is then aligned to this atlas.
3. Cortical surface parcellation to the Desikan–Killiany (DK) atlas (55): In this step, each hemisphere is parcellated into 34 cortical labels.

Figure 1 shows a typical sample of (a) an original volume, (b) intensity normalization, (c) brain extraction, (d) segmentation, and (e) DK atlas parcellation

After completing the above steps, eight features are calculated for each of the 34 hemisphere areas. The eight calculated features for each DK atlas area are as follows:
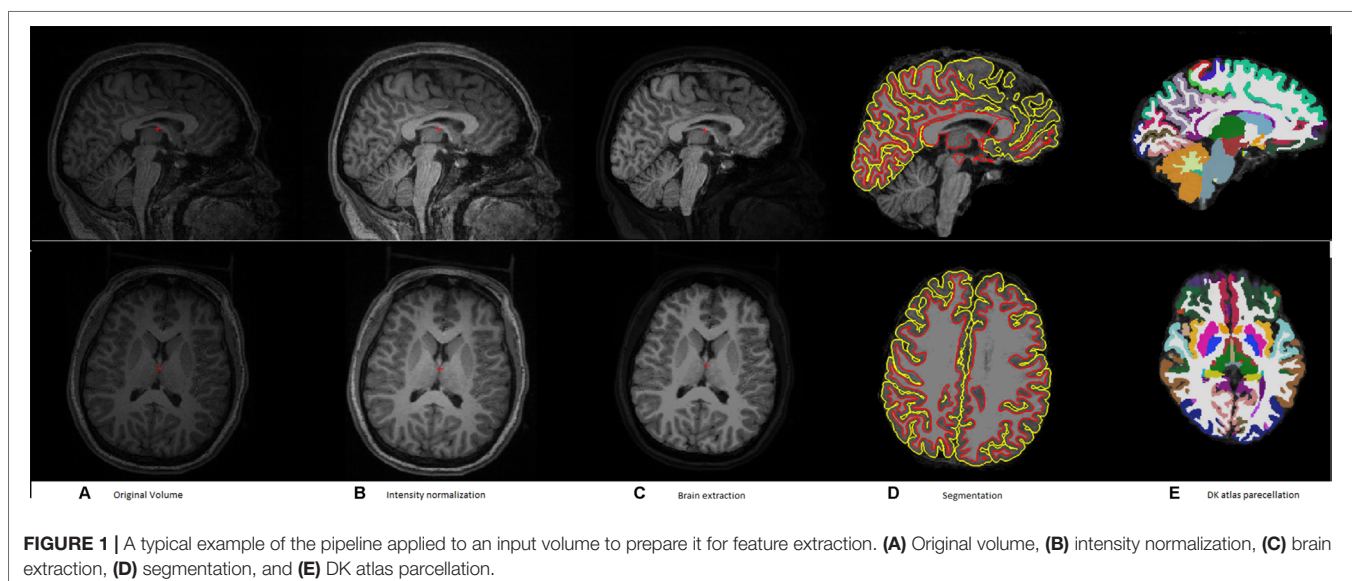
1. Surface area (A).
2. Volume (V).
3. Average thickness (T).
4. Standard deviation of the thickness (T).
5. Average of mean curvature (MCI), defined as:
   $MCI = \dfrac{K1 + K2}{2}$, where $K1$, $K2$ are the two principle curvatures calculated at each vertex.
6. Average of Gaussian curvature (K), defined as:
   $K = K1 * K2$.
7. The average intrinsic curvature index (ICI), defined as:
   $ICI = MAX(K, 0)$.
8. The average folding index (FI), defined as:
   $FI = ABS(K1) * (ABS(K1) - ABS(K2))$.

In this study, we used the publicly available and widely used brain MRI analysis software FreeSurfer pipeline, available at: http://surfer.nmr.mgh.harvard.edu/ for all preprocessing and feature extraction steps mentioned above.

To overcome the problem of variability in the utilized eight features between subjects due to confounding variables like age,

FIGURE 1 | A typical example of the pipeline applied to an input volume to prepare it for feature extraction. **(A)** Original volume, **(B)** intensity normalization, **(C)** brain extraction, **(D)** segmentation, and **(E)** DK atlas parcellation.

IQ, or gender, a normalized form of these features is used. For each subject and for every feature, a 68 areas × 68 areas delta matrix is created, where each element in this matrix is the difference in the feature value between two different areas. In this way, the interaction between the feature values at different areas is studied instead of using the individual feature value per area. Using this technique adds more robustness to the system against variability between subjects due to any confounding variables like age, gender, or IQ. The pipeline of the sMRI experiment is shown in **Figure 2**.

In order to assess how the extracted features are associated with autism severity, a correlation analysis is performed between the difference in feature values among all subjects having ADOS report and the ADOS overall score.

fMRI Experiment

In the fMRI experiment, the features used are the functional connectivity coefficients between each couple of areas in the DK atlas. The first step in the fMRI analysis is the data preprocessing. The preprocessing in this experiment was applied using the FSL-5.0 nuero-imaging toolbox. The preprocessing steps applied in this study are as follows:

1. Slice timing correction: In an interleaved order, to correct for the effect of acquiring 2D slices at different time shifts.
2. Motion correction (56): To correct for unintended subject motion in the scanner.
3. Normalization to MNI-152 space: The normalization is applied using two-step reregistration. The first step is to register the fMRI subject to its structural image. The second step it to register the sMRI image to the MNI-152 space.
4. Spatial smoothing: A Gaussian filter of full width at half maximum (FWHM) of 6 mm is applied to remove the spatial noise.
5. High-pass filtering: To remove the low-frequency drifts effect.

The main purpose of this experiment is to study the functional connectivity within each subject and how it is capable of diagnosing autism. The functional connectivity was selected to be the used feature as it gives an indication about the coherence of activation between the different brain areas. Hence, it is useful in identifying the brain functional networks and how these networks' connectivity could be altered between (57) autistic and typically developed subjects. Since we are concerned in this study with DK cortical parcellation, the functional connectivity matrix is constructed between each pair of these atlas areas.
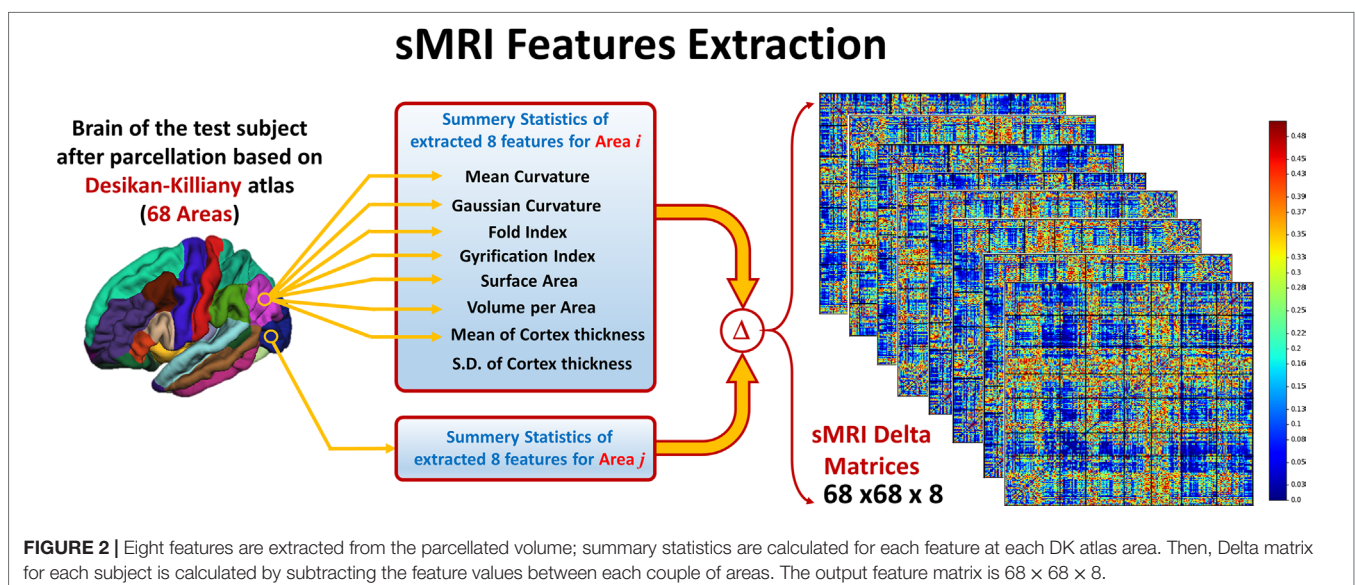
After calculating the preprocessing, the subject 4-D volume is masked with each of the DK atlas areas to calculate the mean time course of this area. The Pearson correlation coefficient (ρ) is used to calculate the functional connectivity between each pair of areas in the atlas. **Figure 3** shows how feature matrix of the functional connectivity is calculated in this experiment. After calculating the connectivity matrix and assessing how the altered connectivity pattern could reflect autism severity, a regression model is used to fit the functional connectivity coefficients with the overall ADOS severity score in the same way as in the sMRI experiment.
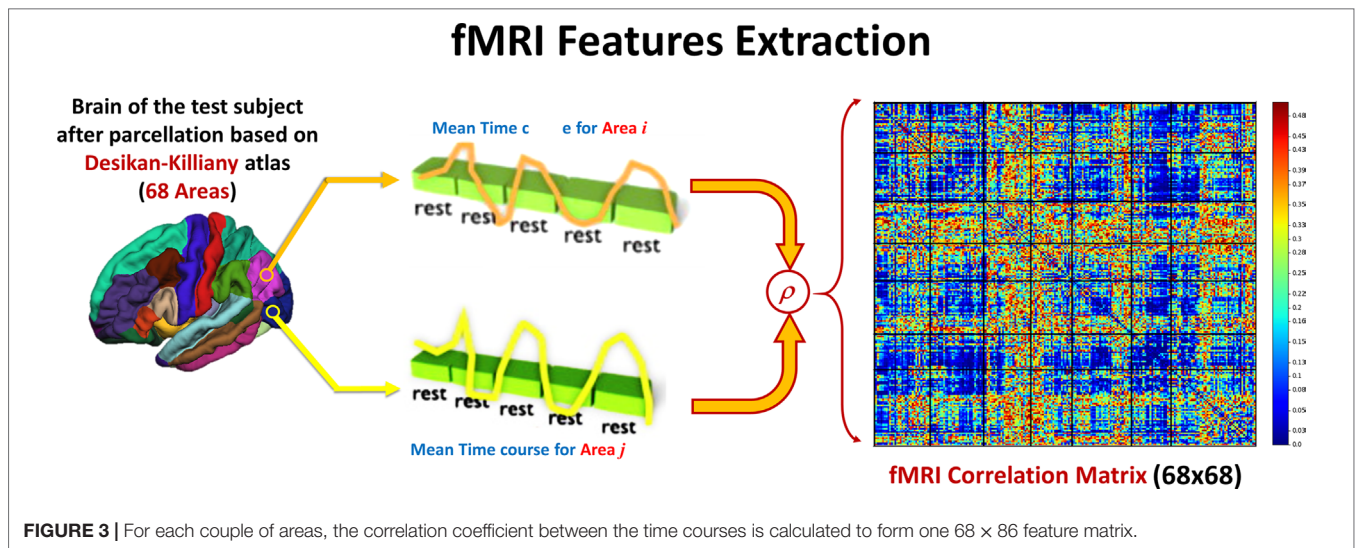
Local and Global Diagnosis

Having the sMRI and fMRI features ready, the same classification pipeline is applied to both of them. As mentioned above, the fMRI features are a 68 × 68 connectivity matrix, F per subject.

$$F = \begin{bmatrix} \rho_{1,1} & \rho_{1,2} & \cdots \\ \vdots & \ddots & \\ \rho_{n,1} & & \rho_{68,68} \end{bmatrix} \tag{8}$$

where $\rho_{i,j}$ is the Pearson correlation coefficient between time courses in area i and area j and n is the index of areas ($n = 68$). For the sMRI features S, they are a 68 × 68 × 8 matrix per subject and

FIGURE 2 | Eight features are extracted from the parcellated volume; summary statistics are calculated for each feature at each DK atlas area. Then, Delta matrix for each subject is calculated by subtracting the feature values between each couple of areas. The output feature matrix is 68 × 68 × 8.

FIGURE 3 | For each couple of areas, the correlation coefficient between the time courses is calculated to form one 68 × 86 feature matrix.

each element in the difference in each of the eight features values between each couple of areas.

$$
S_f = \begin{bmatrix} \Delta_{1,1} & \Delta_{1,2,f} & \cdots \\ \vdots & \ddots & \\ \Delta_{68,1,f} & & \Delta_{68,68,f} \end{bmatrix} \tag{9}
$$

where S_f is the feature matrix of the feature f and $S_{i,j,f}$ is the difference in the values of feature f between areas i and j.

With these feature matrices, one for each modality, a local classifier is applied for each element in each of the two matrices. Both the accuracy and the output probability of each feature to be belonging to the autism class are recorded. The local classifiers used for both sMRI and fMRI are KNN classifiers with number of neighbors = 7. After finishing the local classification phase, the features for each modality are sorted according to the local classification accuracy they achieved in the first step.

KNN is a non-parametric, distance-based classifier. The KNN algorithm assigns a membership score to each new sample based on the number of closest K-neighbors samples from this sample belonging to each of the classes. Based on a majority voting, the sample is assigned to one of the classes (58).

The second step in this diagnosis system is to use the sorted feature vector of both sMRI and fMRI for the per-modality diagnosis. In this step, an incremental approach was used by adding one feature at a time to the used feature vector and recording the cross-validation accuracy until reaching the optimal feature vector length per modality. In this step, a random forest classifier is used. To adjust the hyperparameters of the random forest (number of estimators and maximum depth of the tree), a grid search is used.

Random forest is an ensemble machine learning algorithm combining multiple decision trees using bootstrap aggregating. Each decision tree is fed with a bootstrap of the data with replacement. In order to calculate the feature selection when using random forest, GINI impurity is used (59, 60).

Once the optimal cutoff threshold is obtained for each modality and the optimal feature vector is determined for sMRI and fMRI, these two feature vectors are concatenated and fed to another random forest classifier for the global diagnosis decision. The two-step classification approach used is illustrated in **Figure 4**. Also, **Figure 5** illustrates the whole pipeline of the proposed methodology in this study.
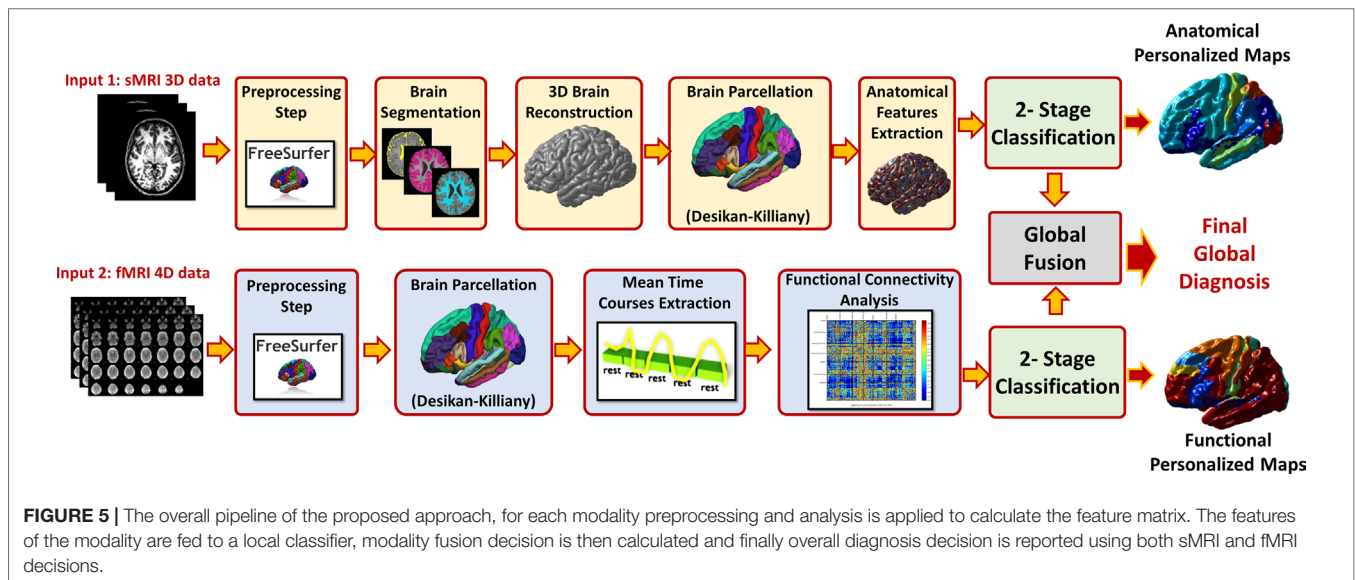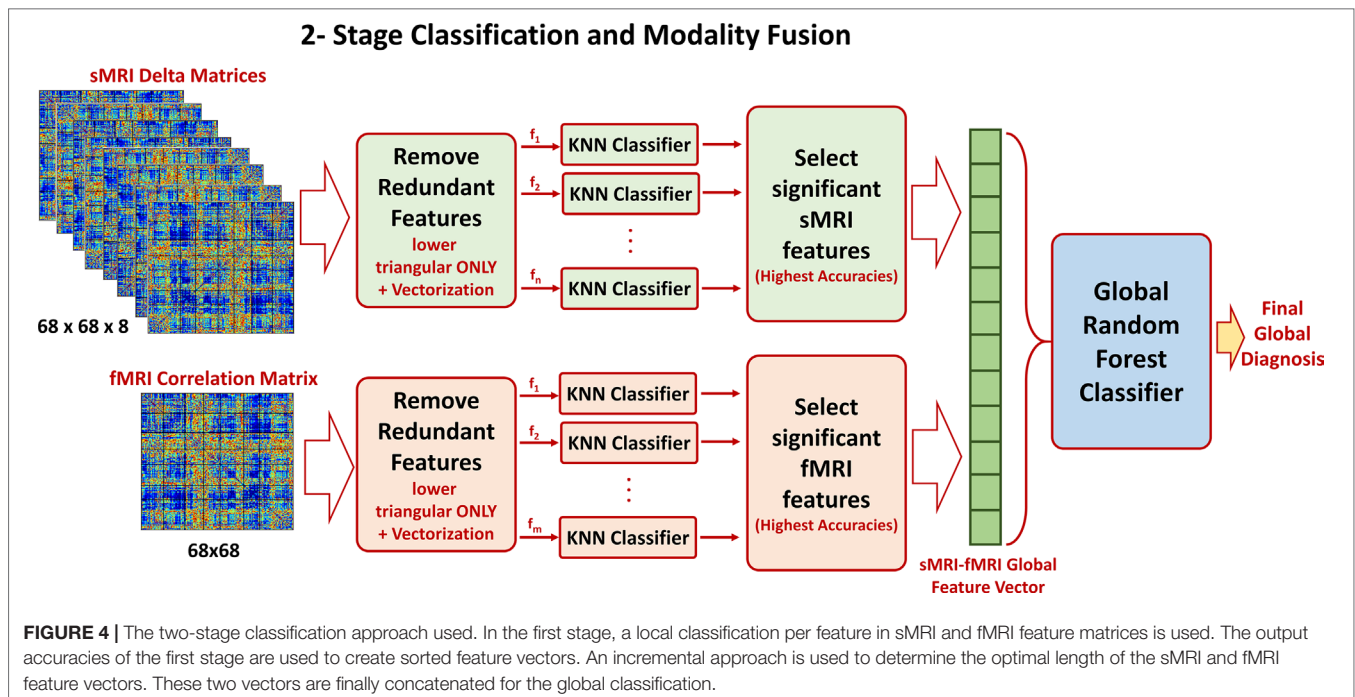
EXPERIMENTAL RESULTS

Subjects Demographics and Cohort Summary Statistics

Out of the 185 subjects used, 7 subjects were excluded from the fMRI analysis and hence from the sMRI–fMRI fusion because they have corrupted fMRI volumes. The dataset contains 72 autistic subjects (33 males and 39 females) and 113 typically developed subjects (49 males and 64 females). The gender was statistically tested using chi-squared test, and it was found statistically insignificant, $\chi^2 = 0.01088$, $P = 0.744$. For the ASD group, the males' mean age is 13.07 years while the females mean age is 13.53. In the TD group, the males' mean age is 13.04 and the females' mean age is 12.8125. The age difference between the two groups is also statistically insignificant ($t = 0.95$, $P = 0.343$). The ADOS scores for 61 are available. The social affect (SA) ADOS varied between 0 and 19 with a median of 9, the RRBs varied between 0 and 6 with median of 2.5, while the cumulative ADOS varied between 1 and 24 with a median 11.5. **Table 1** shows the entire summary statistics of the used cohort.

Correlation Analysis With ADOS Total Score

For each of the features used in the two modalities, the correlation between the feature values in the 61 subjects having ADOS overall score and the corresponding ADOS score was studied.

2- Stage Classification and Modality Fusion

FIGURE 4 | The two-stage classification approach used. In the first stage, a local classification per feature in sMRI and fMRI feature matrices is used. The output accuracies of the first stage are used to create sorted feature vectors. An incremental approach is used to determine the optimal length of the sMRI and fMRI feature vectors. These two vectors are finally concatenated for the global classification.

FIGURE 5 | The overall pipeline of the proposed approach, for each modality preprocessing and analysis is applied to calculate the feature matrix. The features of the modality are fed to a local classifier, modality fusion decision is then calculated and finally overall diagnosis decision is reported using both sMRI and fMRI decisions.

The selected correlation thresholded at a correlation of 0.32, which corresponds to a P value of 0.01.

In the fMRI experiment, 31 features have correlation coefficients above the significance threshold. In the sMRI experiment, there are 345 features above the selected threshold. The number of features meeting the significance criteria in the sMRI is much higher than that in fMRI as the number of features used in sMRI is eight times the number of features in fMRI.

In the sMRI feature matrix, the distribution of the features above the significance level is found to be as follows (**Figure 6**): volume: 62 times, thickness standard deviation: 44 times,

TABLE 1 | The used cohort summary statistics.

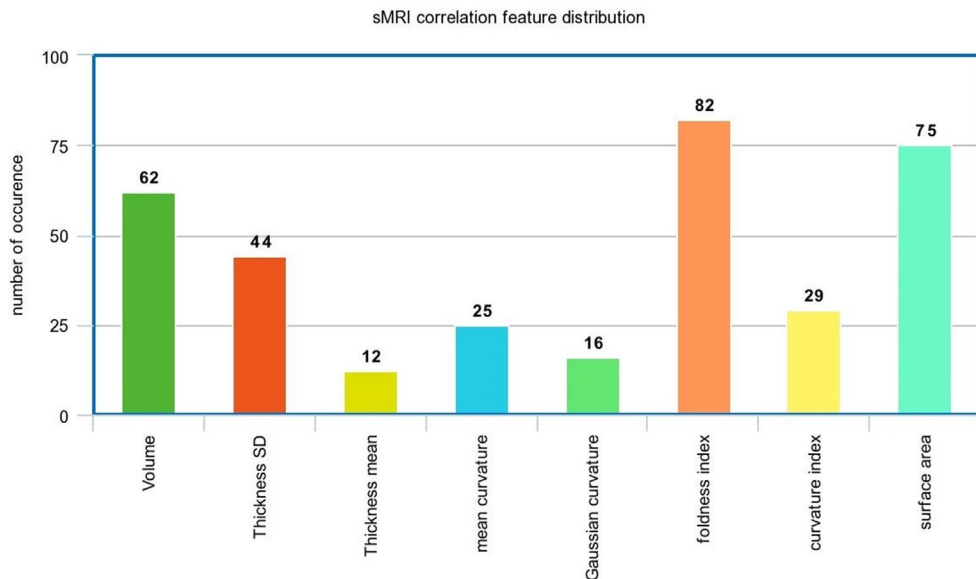|	ASD		TD		
	Males	**Females**	**Males**	**Female**	
Count	33	39	49	64	$p = 0.744$
Age mean	13.07	13.53	13.04	12.81	$p = 0.34$
Age SD	2.75	2.58	2.68	3.17	
	Median	**Range**			
SA	9	0–19			
RRB	2.5	0–6			
Cumulative	11.5	1–24			

FIGURE 6 | The frequency of occurrence of each of the sMRI features in the significantly correlated features list with ADOS overall score.
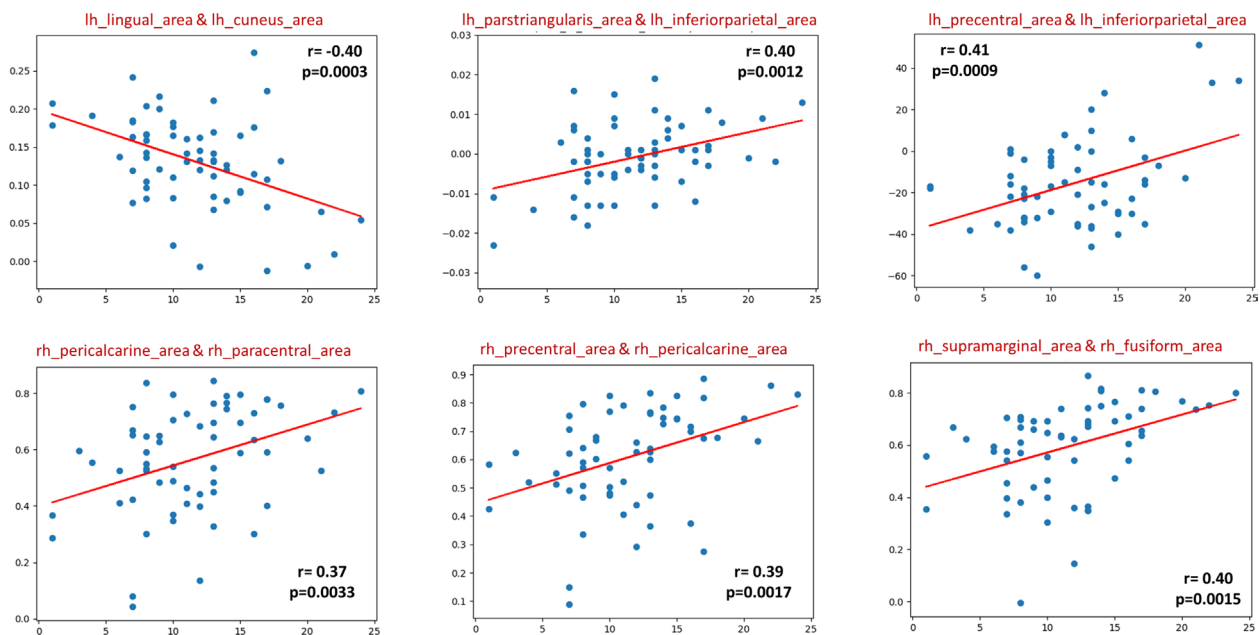

FIGURE 7 | Six selected samples to show the correlation between the feature values in sMRI (upper row), fMRI (lower row), and the ADOS overall score. For each subplot, the figure title shows the couple of area numbers in the DK atlas, the feature name in the case of sMRI, the correlation coefficient, and the *P* value.

thickness: 12 times, mean curvature: 25 times, Gaussian curvature: 16 times, foldness index: 82 times, curvature index: 29 times, and SA: 75 times. **Figure 7** shows sample of correlation of both sMRI and fMRI with ADOS overall score. Also, **Figures 8** and **9** show the most frequent areas associated with features having significantly correlated features with overall ADOS score.

Local and Global Diagnosis

For each subject, the local probabilities for both sMRI and fMRI feature matrices are calculated. These probabilities are used to generate the personalized brain maps. The output of the local classification is two matrices P_S and P_F with the same size as the feature matrices F and S.
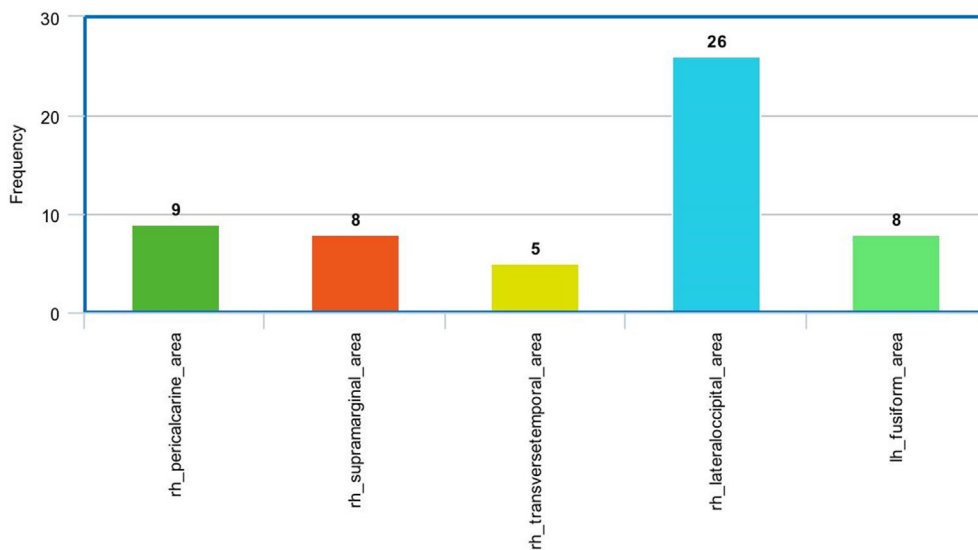
FIGURE 8 | The most frequent areas in the fMRI experiment found to be associated with significantly correlated features with ADOS overall score.
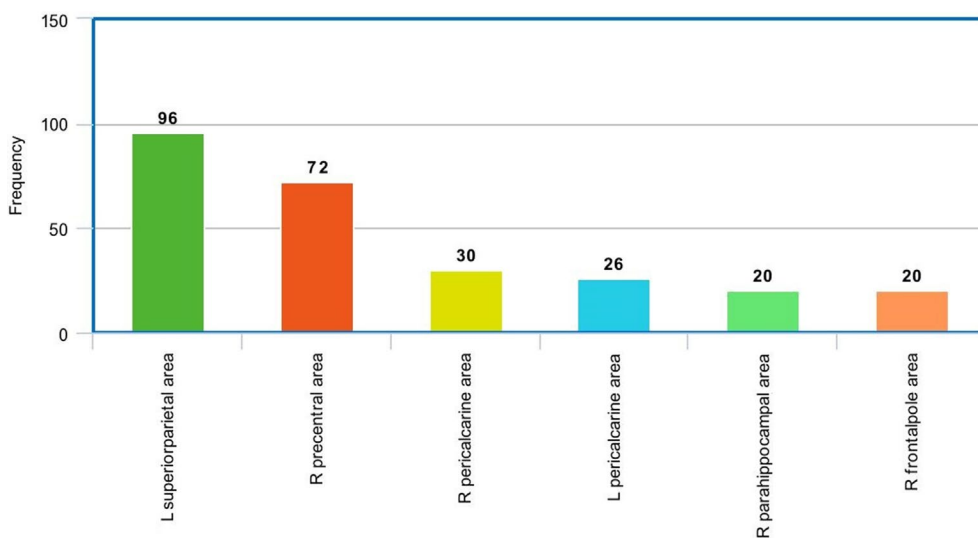

FIGURE 9 | The most frequent areas in the sMRI experiment found to be associated with significantly correlated features with ADOS overall score.

$$
P_F = \begin{bmatrix} pf_{1,1} & pf1,2 & \cdots \\ \vdots & \ddots & \\ pf & & pf\,68,68 \end{bmatrix} \tag{10}
$$

$$
P_S f = \begin{bmatrix} Ps_{1,1,f} & Ps_{1,2,f} & \cdots \\ \vdots & \ddots & \\ Ps_{68,1,f} & & Ps_{68,68,f} \end{bmatrix} \tag{11}
$$

where pf_{ij} if the probability of the functional connectivity between areas i and j to belong to the autism class and $Ps_{i,j,f}$ is the probability that the difference in the structural feature f between areas i and j belong to the autism class.

To obtain the personalized maps, two vectors, V_f and V_s, are calculated for fMRI and sMRI, respectively.

$$
V_f(i) = \max_{0 \le j \le 68} P_F(i,j) \tag{12}
$$

$$
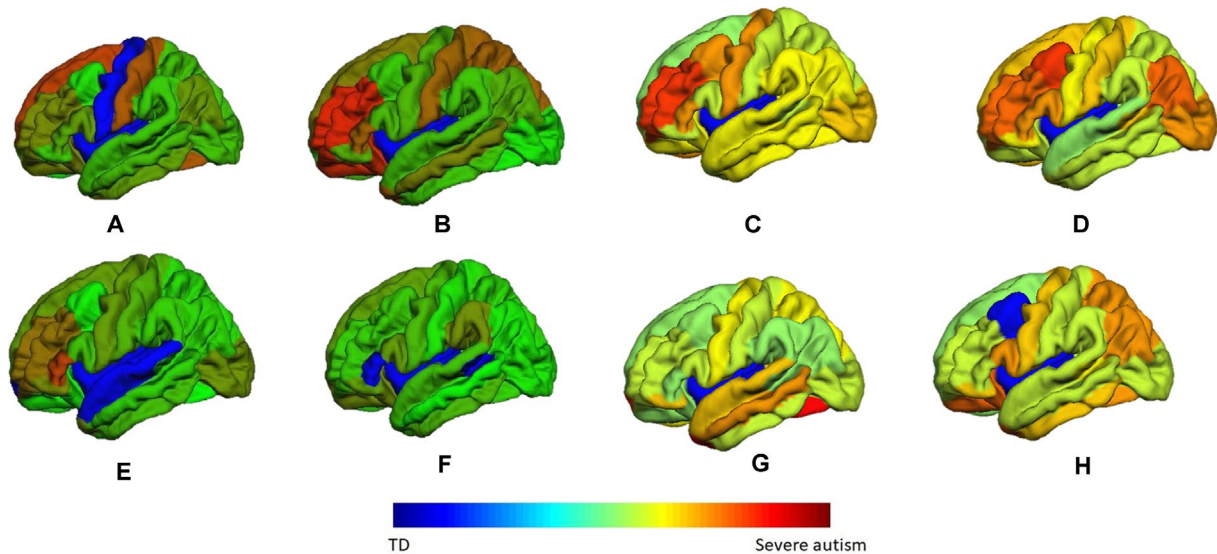V_s(i) = \max_{i \le j \le 68, 1 \le f \le 8} P_S(i,j,f) \tag{13}
$$

FIGURE 10 | A sample of the generated personalized maps for eight subjects: **(A, B)** are the personalized maps of two ASD subjects obtained from sMRI local classification, **(C, D)** are the personalized maps of two ASD subjects obtained from fMRI local classification, **(E, F)** are the personalized maps of two TD subjects obtained from sMRI local classification, and **(G, H)** are the personalized maps of two TD subjects obtained from fMRI local classification.

These two vectors indicate the highest probability of an area to be belonging to the autism class. A sample of these color coded maps is shown in **Figure 10**.

Per-Modality Diagnosis Results

After calculating the local probabilities, P_S and P_F, they were sorted according to the obtained accuracy. A linear scan is done to find the optimal number of features to concatenate for sMRI and fMRI. From this scan, it is found that by fusing the first 34 sorted features in sMRI and the first 4 features, the highest sMRI and fMRI accuracies are achieved. To show the effect of changing the number of selected features, **Figures 11** and **12** show the accuracy, sensitivity, specificity, and area under the curve (AUC) when using different numbers of features (from 1 to 100 areas). **Table 2** shows the accuracy, sensitivity, specificity, and AUC obtained for sMRI and fMRI when selecting the first 34 and 4 features, respectively. The reported results used random forest classifier with fourfold cross validation.

Global Diagnosis Results

After knowing the optimal number of features to be used from both sMRI and fMRI, these features are concatenated together to form a global feature vector. The output global feature vector contains 38 features. These 38 features are then fed to a random forest classifier to obtain global accuracy, sensitivity, specificity, and AUC. These results are 80.8%, 84.9%, 79.2%, and 81.92% for the accuracy, sensitivity, specificity, and AUC, respectively. In addition, a comparison between different classifiers in the global diagnosis is reported in **Table 3**.

DISCUSSION

The challenge of understanding the child's individual neural circuitry is daunting. Multiple reports in general suggest hypoconnectivity (35) in most studies. However, neurophysiological and MRI evidence does suggest local hyperconnectivity in some brain regions (28). This report extends our previous fMRI findings (61–63) and suggests that particular MRI parameters related to the expanded neuropil in mini-columns including foldness index, SA, and volume are more relevant to defining ASD-related neural circuits (64). These parameters make it possible to link between two adjacent Brodmann areas (BAs) or brain regions, which directly increase the correlation to behavior. Again, the local diagnosis of our algorithm identified ASD-related brain regions that fit into RDoC neural circuits and are similar circuits found to be predictive of ASD diagnosis at 24 months.

Computer-Aided Diagnostic System for ASD

The current dataset suggests that it is possible to define a localized diagnosis, which is the key to defining each relevant ASD neurocircuit within an individual. The algorithm provides high accuracy, sensitivity, and specificity when sMRI or fMRI are analyzed separately. Our current algorithm also fuses the sMRI and fMRI datasets, which provides a greater estimate of 80% accuracy, 85% sensitivity, and 79% specificity. The ability of the algorithm to estimate a whole-brain diagnosis was validated by a cross-validation technique using fourfolds. The principle of dataset fusion is to handle the individual variability of brain structure and function that is impacted by various genetic and environmental factors.
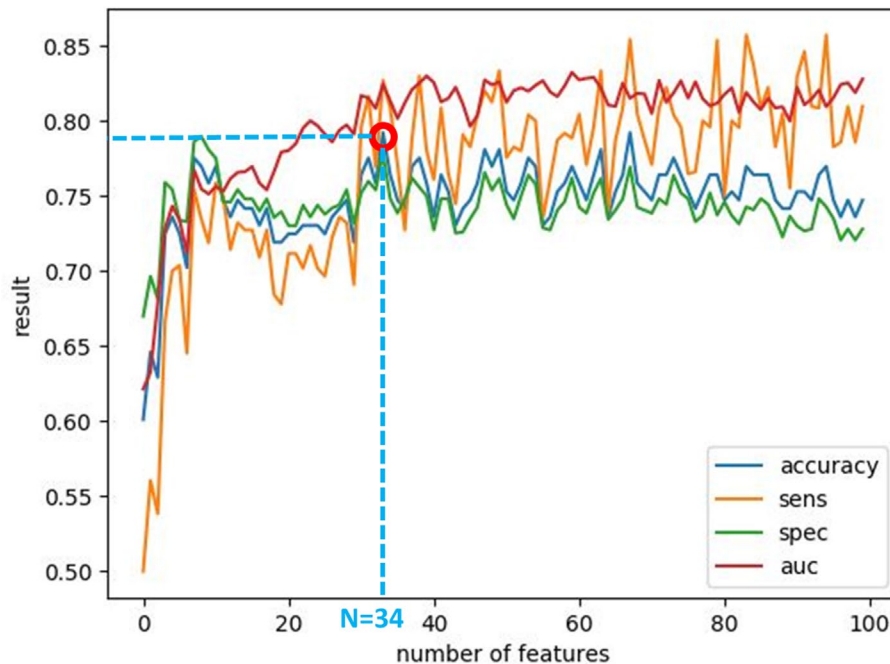
FIGURE 11 | The accuracy, sensitivity, specificity, and AUC obtained by changing the number of selected features for sMRI between 1 and 100 features. The maximum accuracy achieved is obtained when using 34 features.

Further, the principle of data fusion by Big Data techniques will delineate key circuits that correlate with behavioral output of those circuits. Our careful approach has studied the different MRI parameters that are inputs into the machine learning algorithm. While it may be possible that, as a whole, global diagnosis may be better *via* fMRI vs. sMRI, our study suggests that the addition of fMRI or sMRI parameters in regions specific for the ASD diagnosis/classification of regions linked in neural circuits gives an even higher significant Pearson correlation (0.37–0.45) at *P* = 0.001 than previous Rs-MRI (0.25) (62) data with ADOS total score. Similarly to what was found to be predictive in high-risk infants who then developed ASD at 24 months, we found that MRI parameters related to the neuropil expansion (volume, SA, and folding index) and fMRI parameters (functional connectivity measure) were features that mediated the significant Pearson correlation between any two brain regions and ADOS total scores. These significant correlations between brain regions were most significantly frequent in the posterior brain regions. Such observations would be consistent with the overall increased functional connectivity observed in the posterior cortex (28). Whether the predominance of right hemispheric correlation over left hemispheric correlation is significant will await the input of further data. Thus, the current data suggest that the approach of a localized diagnosis with fusion of multi-model datasets will greatly improve accuracy, sensitivity, and specificity while linking two or more adjacent BAs or brain regions to directly increase the correlation to behavior.

LIMITATIONS OF THE APPROACH

While neuroimaging is an attractive and easily obtainable piece of clinical data, the experiments here are limited by different sources of data including harmonization of scans for head motion, different MRI scanners and sequences, plus fMRI data obtained under different conditions. Such variables could limit the utility of our data in building a personalized medicine model. Further the drawback of the current data and MRI methods includes defining the developmental trajectory, impact of age/gender, development of clinically applicable techniques for scanning across ages, and the unknown nature of the relationship between modern psychology diagnostics/behavioral testing and MRI/genetic data. The current findings may be only applicable to older ages (8–18 years old) and higher-functioning ASD subjects. However, the current data link multiple BA regions in RDoC neurocircuits implicated in ASD, thereby suggesting the scalability of this approach to larger, more heterogeneous ASD populations.

The lack of longitudinal fMRI data in the under 8-year population of typically developing children may limit the approach (65). The number, diversity of subject pool (age/gender), design of MRI protocols, and preprocessing/methods of analyses are still variables under study. Additionally, the methods for analyses and selections of datasets for our machine learning algorithms are still not standardized and must yield biologically relevant information. The generalization and feasibility of a system will be improved by increasing the number of subjects and the intra-variability between subjects, including age/sex, multiple scanner data, and other factors.
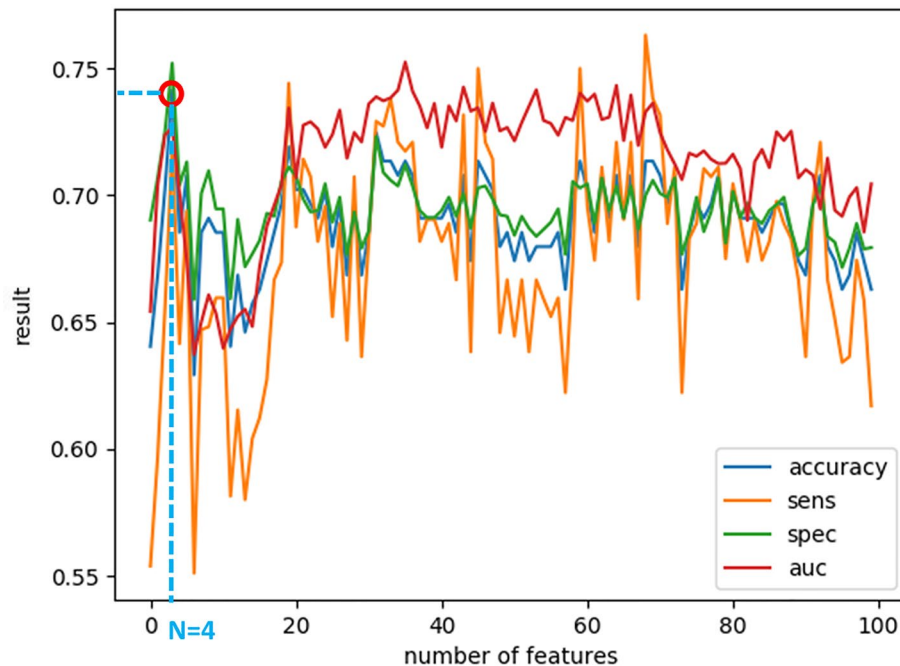
FIGURE 12 | The accuracy, sensitivity, specificity, and AUC obtained by changing the number of selected features for fMRI between 1 and 100 features. The maximum accuracy achieved is obtained when using four features.

TABLE 2 | The comparison between random forest, SVM, naive Bayes, and neural network results for the global fusion.

	sMRI	fMRI
Accuracy	0.79	0.74
Sensitivity	0.82	0.72
Specificity	0.77	0.75
AUC	0.824	0.72

TABLE 3 | The accuracy, sensitivity, specificity, and AUC obtained for sMRI and fMRI when selecting the first 34 and 4 features, respectively.

	Random Forest	SVM	Naïve Bayes	Neural network
Accuracy	0.808	0.71	0.75	0.68
Sensitivity	0.849	0.72	0.77	0.7
Specificity	0.792	0.67	0.71	0.64
AUC	0.819	0.73	0.73	0.67

FUTURE DIRECTIONS: MULTI-MODEL FUSION LINKS TO RDOC NEUROCIRCUITS

Our previous data using fMRI alone from the NDAR dataset identified frontopolar and temporal parietal junction functional networks as key regions correlated with ADOS scores and mapped to RDoC neurocircuits (62). The new extended list of ADOS-associated brain regions (pericalcarine cortex, lateral occipital cortex, supramarginal gyrus, transverse temporal, fusiform gyrus, superior parietal, precentral gyrus, parahippocampal gyrus, and frontal polar regions) includes the previous regions but greatly expands the RDoC neurocircuits identified through as localized diagnosis in ASD brains (65).

The greater mapping of behaviors over an anatomical and functionally linked circuit is more likely to map a cluster of ASD individuals whose behaviors and characteristics are more similar than different. Further, the division of behavioral clusters across specific neurocircuits may identify not only traditional autism susceptibility genes like those in the SFARI database but also ASD modifier genes that subtly impact the structure and function of circuits, thereby influencing behavioral output of the circuit. Such ASD modifier genes (in the genetic background of an individual) may mediate the gene–environment interactions responsible for 50% of ASD etiology. This group of genes may be quite large but have low effect size and therefore would not be picked up in traditional autism genetic studies (66). This group of genes may be represented among the transcriptome studies reported in ASD where altered transcripts may not necessarily correspond to a specific autism susceptibility gene but may define specific developmental trajectories (42). Such genes or traditional ASD susceptibility genes may also define specific MRI clusters. In a recent report (67), three groups of MRI phenotypic clusters were defined during an fMRI survey of mouse models of autism. Group 1 (SGSH, TREM1, FMR1, and CNT2) had hypoconnectivity involving the PFC, BG, retrosplenium of CC, and thalamus plus hyperconnectivity of the ventral striatum/

nucleus accumbens. Group 2 (CDKL5, EN2, MECP2, and CHD8) had whole cortex/BG hypoconnectivity plus increased functional connectivity of the lateral septum. Group 3 (Syn2, BTBR, and 16p11 deletion) had increased functional connectivity in PFC, insula, parietal cortex, amygdala, midbrain and hypoconnectivity in sensory cortex, ventral striatum, and thalamus. Further, some mutations (EN2, FMR1, MECP2, and SGSH) had highly correlated changes between hemispheres while other mutations had a low correlation between hemispheres (16p11 deletion and Syn2). The MRI evidence supports the hypothesis that multiple behavioral clusters may map onto distinct MRI phenotypes involving the frontal, temporal, and parietal cortices identified here as well as important subcortical structures (nucleus accumbens, striatum, and thalamus). While distinct ASD susceptibility genes may define global MRI phenotypes, our careful comparisons of fused datasets with genomics are likely to identify more subtle modifier ASD genes that finally influence the local sculpting and function of neural circuits during specific developmental periods, thereby producing more distinct behavioral clusters. In summary, the advancement of new technologies in psychology, radiology, and genetics has allowed never before interrogation of datasets that further delineate human biology. The use of Big Data technology is the only current realistic experimental methodology that could define the variability of neural circuits and linking genetics with behavior in such a polygenic disease such as ASD. This study demonstrates that fusion of MRI data and machine learning could refine diagnostic accuracy, especially at the local neurocircuit level. Such data could define clinically distinct endophenotypes from particular affected neural networks and therefore amenable to targeted pharmacological and/or behavioral interventions. The goal of this project is to ultimately develop personalized treatments for ASD. The next phase of this study will focus on the full integration of genomic, behavioral, and MRI datasets to further define the feasibility, robustness, and generalizability of

our systems. In addition, more data will be included for subjects at younger ages and infants; also, some other phenotypes, like ADHD for example, will be included. In this way, the system will be more comprehensive with higher diagnosis ability for ASD.

AUTHOR CONTRIBUTIONS

OD has the primary responsibility for the conduct of the research and wrote the bulk of the manuscript. MA, YE-N, and AM contributed to the guidance in data collection and preparation and manuscript writing. ASh contributed to the guidance in data preparation, manuscript writing, and figure preparation. ASo contributed to the guidance in sMRI experiment, manuscript writing, and figure preparation. ASw performed statistical analyses and reporting, and contributed to the guidance in algorithm validation and results interpretation. MG and HH contributed to the guidance in fMRI experiment and guidance in literature review and manuscript writing. MC contributed to the guidance in medical-related points and was responsible for results interpretation and validation. AE and RK contributed to the supervision, manuscript revision and editing, and results interpretation and verification. AE-B contributed to the project initiation and idea preparation, supervision, manuscript revision, and editing and results interpretation and verification. GB, the medical collaborator, guided in all medical-related points and also was responsible for results interpretation and validation. He was also responsible for the writing, editing, and revision of the manuscript.

FUNDING

REFERENCES

1. Frith U, Happé F. Autism spectrum disorder. *Curr Biol* (2005) 15:R786–R790. doi: 10.1016/j.cub.2005.09.033
2. Christensen DL, Bilder DA, Zahorodny W, Pettygrove S, Durkin MS, Fitzgerald RT, et al. Prevalence and characteristics of autism spectrum disorder among 4-year-old children in the autism and developmental disabilities monitoring network. *J Dev Behav Pediatr* (2016) 37:1–8. doi: 10.1097/DBP.0000000000000235
3. Strakowski SM, Fleck DE, Welge J, Eliassen JC, Norris M, Durling M, et al. fMRI brain activation changes following treatment of a first bipolar manic episode. *Bipolar Disord* (2016) 18:490–501. doi: 10.1111/bdi.12426
4. Courchesne E, Karns C, Davis H, Ziccardi R, Carper R, Tigue Z, et al. Unusual brain growth patterns in early life in patients with autistic disorder an MRI study. *Neurology* (2001) 57:245–54. doi: 10.1212/WNL.57.2.245
5. Hazlett HC, Poe M, Gerig G, Smith RG, Provenzale J, Ross A, et al. Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. *Arch Gen Psychiatry* (2005) 62:1366–76. doi: 10.1001/archpsyc.62.12.1366
6. Geschwind DH, Levitt P. Autism spectrum disorders: developmental disconnection syndromes. *Curr Opin Neurobiol* (2007) 17:103–11. doi: 10.1016/j.conb.2007.01.009
7. Herbert MR, Ziegler DA, Makris N, Filipek PA, Kemper TL, Normandin JJ, et al. Localization of white matter volume increase in autism and

developmental language disorder. *Ann Neurol* (2004) 55:530–40. doi: 10.1002/ana.20032
8. Carper RA, Courchesne E. Localized enlargement of the frontal cortex in early autism. *Biol Psychiatry* (2005) 57:126–33. doi: 10.1016/j.biopsych.2004.11.005
9. Hardan AY, Muddasani S, Vemulapalli M, Keshavan MS, Minshew NJ. An MRI study of increased cortical thickness in autism. *Am J Psychiatry* (2006) 163:1290–2. doi: 10.1176/ajp.2006.163.7.1290
10. Toal F, Daly E, Page L, Deeley Q, Hallahan B, Bloemen O, et al. Clinical and anatomical heterogeneity in autistic spectrum disorder: a structural MRI study. *Psychol Medicine* (2010) 40:1171–81. doi: 10.1017/S0033291709991541
11. Ecker C, Ginestet C, Feng Y, Johnston P, Lombardo MV, Lai M-C, et al. Brain surface anatomy in adults with autism: the relationship between surface area, cortical thickness, and autistic symptoms. *JAMA Psychiatry* (2013) 70:59–70. doi: 10.1001/jamapsychiatry.2013.265
12. Haar S, Berman S, Behrmann M, Dinstein I. Anatomical abnormalities in autism? *Cereb Cortex* (2014) 26:1440–52. doi: 10.1093/cercor/bhu242
13. Zielinski BA, Prigge MB, Nielsen JA, Froehlich AL, Abildskov TJ, Anderson JS, et al. Longitudinal changes in cortical thickness in autism and typical development. *Brain* (2014) 137:1799–812. doi: 10.1093/brain/awu083
14. Bellani M, Calderoni S, Muratori F, Brambilla P. Brain anatomy of autism spectrum disorders. II. Focus on amygdala. *Epidemiol Psychiatr Sci* (2013) 22:309–12. doi: 10.1017/S2045796013000346

15. Waiter GD, Williams JH, Murray AD, Gilchrist A, Perrett DI, Whiten A. A voxel-based investigation of brain structure in male adolescents with autistic spectrum disorder. *Neuroimage* (2004) 22:619–25. doi: 10.1016/j. neuroimage.2004.02.029

16. Salmond CH, Vargha-Khadem F, Gadian DG, de Haan M, Baldeweg T. Heterogeneity in the patterns of neural abnormality in autistic spectrum disorders: evidence from ERP and MRI. *Cortex* (2007) 43:686–99. doi: 10.1016/S0010-9452(08)70498-2

17. Zilles K, Armstrong E, Schleicher A, Kretschmann H-J. The human pattern of gyrification in the cerebral cortex. *Anat Embryol* (1988) 179:173–9. doi: 10.1007/BF00304699

18. Hardan AY, Jou RJ, Keshavan MS, Varma R, Minshew NJ. Increased frontal cortical folding in autism: a preliminary MRI study. *Psychiat Res Neuroim* (2004) 131:263–8. doi: 10.1016/j.pscychresns.2004.06.001

19. Wallace GL, Robustelli B, Dankner N, Kenworthy L, Giedd JN, Martin A. Increased gyrification, but comparable surface area in adolescents with autism spectrum disorders. *Brain* (2013) 136:1956–67. doi: 10.1093/brain/awt106

20. Awate SP, Win L, Yushkevich P, Schultz RT, Gee JC (2008). 3d cerebral cortical morphometry in autism: increased folding in children and adolescents in frontal, parietal, and temporal lobes. In *International Conference on Medical Image Computing and Computer-Assisted Intervention* (Springer), 559–67. doi: 10.1007/978-3-540-85988-8_67

21. Katuwal GJ, Cahill ND, Baum SA, Michael AM (2015). The predictive power of structural MRI in autism diagnosis. In Engineering in Medicine and Biology Society (EMBC), *2015 37th Annual International Conference of the IEEE* (IEEE), 4270–3. doi: 10.1109/EMBC.2015.7319338

22. Nordahl CW, Dierker D, Mostafavi I, Schumann CM, Rivera SM, Amaral DG, et al. Cortical folding abnormalities in autism revealed by surface-based morphometry. *J Neurosci* (2007) 27:11725–35. doi: 10.1523/JNEUROSCI.0777-07.2007

23. Dierker DL, Feczko E, Pruett JR, Jr., Petersen SE, Schlaggar BL, Constantino JN, et al. Analysis of cortical shape in children with simplex autism. *Cereb Cortex* (2013) 25:1042–51. doi: 10.1093/cercor/bht294

24. Ecker C, Andrews D, Dell'Acqua F, Daly E, Murphy C, Catani M, et al. Relationship between cortical gyrification, white matter connectivity, and autism spectrum disorder. *Cereb Cortex* (2016) 26:3297–309. doi: 10.1093/cercor/bhw098

25. Cherkassky VL, Kana RK, Keller TA, Just MA. Functional connectivity in a baseline resting-state network in autism. *Neuroreport* (2006) 17:1687–90. doi: 10.1097/01.wnr.0000239956.45448.4c

26. Just MA, Cherkassky VL, Keller TA, Minshew NJ. Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. *Brain* (2004) 127:1811–21. doi: 10.1093/brain/awh199

27. Just MA, Cherkassky VL, Keller TA, Kana RK, Minshew NJ. Functional and anatomical cortical underconnectivity in autism: evidence from an fMRI study of an executive function task and corpus callosum morphometry. *Cereb Cortex* (2006) 17:951–61. doi: 10.1093/cercor/bhl006

28. Damarla SR, Keller TA, Kana RK, Cherkassky VL, Williams DL, Minshew NJ, et al. Cortical underconnectivity coupled with preserved visuospatial cognition in autism: evidence from an fMRI study of an embedded figures task. *Autism Res* (2010) 3:273–9. doi: 10.1002/aur.153

29. Weng S-J, Carrasco M, Swartz JR, Wiggins JL, Kurapati N, Liberzon I, et al. Neural activation to emotional faces in adolescents with autism spectrum disorders. *J Child Psychol Psychiatry* (2011) 52:296–305. doi: 10.1111/j.1469-7610.2010.02317.x

30. Zeeland S-V, Ashley A, Dapretto M, Ghahremani DG, Poldrack RA, Bookheimer SY. Reward processing in autism. *Autism Res* (2010) 3:53–67. doi: 10.1002/aur.122

31. Dekhil O, Ismail M, Shalaby A, Switala A, Elmaghraby A, Keynton R, et al. (2017). A novel cad system for autism diagnosis using structural and functional MRI. In *Biomedical Imaging (ISBI 2017), 2017 IEEE 14th International Symposium on* (IEEE), 995–8. doi: 10.1109/ISBI.2017.7950683

32. Dichter GS, Richey JA, Rittenberg AM, Sabatino A, Bodfish JW. Reward circuitry function in autism during face anticipation and outcomes. *J Autism Dev Disord* (2012) 42:147–60. doi: 10.1007/s10803-011-1221-1

33. Cox A, Kohls G, Naples AJ, Mukerji CE, Coffman MC, Rutherford HJ, et al. Diminished social reward anticipation in the broad autism phenotype as revealed by event-related brain potentials. *Soc Cogn Affect Neurosci* (2015) 10:1357–64. doi: 10.1093/scan/nsv024

34. Deshpande G, Libero L, Sreenivasan KR, Deshpande H, Kana RK. Identification of neural connectivity signatures of autism using machine learning. *Front Hum Neurosci* (2013) 7:670. doi: 10.3389/fnhum.2013.00670

35. Itahashi T, Yamada T, Watanabe H, Nakamura M, Jimbo D, Shioda S, et al. Altered network topologies and hub organization in adults with autism: a resting-state fMRI study. *PLoS One* (2014) 9:e94115. doi: 10.1371/journal.pone.0094115

36. Alaerts K, Swinnen SP, Wenderoth N. Sex differences in autism: a resting-state fMRI investigation of functional brain connectivity in males and females. *Soc Cogn Affect Neurosci* (2016) 11:1002–16. doi: 10.1093/scan/nsw027

37. Rausch A, Zhang W, Haak KV, Mennes M, Hermans EJ, van Oort E, et al. Altered functional connectivity of the amygdaloid input nuclei in adolescents and young adults with autism spectrum disorder: a resting state fMRI study. *Mol Autism* (2016) 7:13. doi: 10.1186/s13229-015-0060-x

38. Tyszka JM, Kennedy DP, Paul LK, Adolphs R. Largely typical patterns of resting-state functional connectivity in high-functioning adults with autism. *Cereb Cortex* (2013) 24:1894–905. doi: 10.1093/cercor/bht040

39. Plitt M, Barnes KA, Martin A. Functional connectivity classification of autism identifies highly predictive brain features but falls short of biomarker standards. *Neuroimage Clin* (2015) 7:359–66. doi: 10.1016/j.nicl.2014.12.013

40. Di Martino A, Yan C-G, Li Q, Denio E, Castellanos FX, Alaerts K, et al. The autism brain imaging data exchange: towards a large-scale evaluation of the intrinsic brain architecture in autism. *Mol Psychiatry* (2014) 19:659. doi: 10.1038/mp.2013.78

41. Hahamy A, Behrmann M, Malach R. The idiosyncratic brain: distortion of spontaneous connectivity patterns in autism spectrum disorder. *Nat Neurosci* (2015) 18:302. doi: 10.1038/nn.3919

42. Supekar K, Uddin LQ, Khouzam A, Phillips J, Gaillard WD, Kenworthy LE, et al. Brain hyperconnectivity in children with autism and its links to social deficits. *Cell Rep* (2013) 5:738–47. doi: 10.1016/j.celrep.2013.10.001

43. Suk H-I, Lee S-W, Shen D, The Alzheimer's Disease Neuroimaging Initiative. Latent feature representation with stacked auto-encoder for AD/MCI diagnosis. *Brain Struct Funct* (2015) 220(2):841–59. doi: 10.1007/s00429-013-0687-3

44. Kim J, Calhoun VD, Shim E, Lee J-H. Deep neural network with weight sparsity control and pre-training extracts hierarchical features and enhances classification performance: evidence from whole-brain resting-state functional connectivity patterns of schizophrenia. *Neuroimage* (2016) 124:127–46. doi: 10.1016/j.neuroimage.2015.05.018

45. Heinsfeld AS, Franco AR, Craddock RC, Buchweitz A, Meneguzzi F. Identification of autism spectrum disorder using deep learning and the abide dataset. *Neuroimage Clin* (2018) 17:16–23. doi: 10.1016/j.nicl.2017.08.017

46. Dvornek NC, Ventola P, Duncan JS (2018). Combining phenotypic and resting-state fMRI data for autism classification with recurrent neural networks. In *2018 IEEE 15th International Symposium on Biomedical Imaging (ISBI 2018)* (IEEE), 725–8. doi: 10.1109/ISBI.2018.8363676

47. Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging* (1998) 17:87–97. doi: 10.1109/42.668698

48. Ségonne F, Dale AM, Busa E, Glessner M, Salat D, Hahn HK, et al. A hybrid approach to the skull stripping problem in MRI. *Neuroimage* (2004) 22:1060–75. doi: 10.1016/j.neuroimage.2004.03.032

49. Fischl B, Salat DH, Van Der Kouwe AJ, Makris N, Ségonne F, Quinn BT, et al. Sequence-independent segmentation of magnetic resonance images. *Neuroimage* (2004) 23:S69–S84. doi: 10.1016/j.neuroimage.2004.07.016

50. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* (2002) 33:341–55. doi: 10.1016/S0896-6273(02)00569-X

51. Fischl B, Liu A, Dale AM. Automated manifold surgery: constructing geometrically accurate and topologically correct models of the human cerebral cortex. *IEEE Trans Med Imaging* (2001) 20:70–80. doi: 10.1109/42.906426

52. Ségonne F, Pacheco J, Fischl B. Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. *IEEE Trans Med Imaging* (2007) 26:518–29. doi: 10.1109/TMI.2006.887364

53. Fischl B, Sereno MI, Dale AM. Cortical surface-based analysis: Ii: inflation, flattening, and a surface-based coordinate system. *Neuroimage* (1999a) 9:195–207. doi: 10.1006/nimg.1998.0396

54. Fischl B, Sereno MI, Tootell RB, Dale AM. High-resolution intersubject averaging and a coordinate system for the cortical surface. *Hum Brain Mapp* (1999b) 8:272–84. doi: 10.1002/(SICI)1097-0193(1999)8:4<272::AID-HBM10>3.0. CO;2-4

55. Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* (2006) 31:968– 80. doi: 10.1016/j.neuroimage.2006.01.021

56. Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage* (2002) 17:825–41. doi: 10.1006/nimg.2002.1132

57. Van Den Heuvel MP, Pol HEH. Exploring the brain network: a review on resting-state fMRI functional connectivity. *Eur Neuropsychopharmacol* (2010) 20:519–34. doi: 10.1016/j.euroneuro.2010.03.008

58. Liao Y, Vemuri VR. Use of k-nearest neighbor classifier for intrusion detection. *Computers & Security* (2002) 21:439–48. doi: 10.1016/S0167-4048(02)00514-X

59. Gastwirth JL. The estimation of the Lorenz curve and Gini index. *Rev Econ Stat* (1972) 54(3):306–16. doi: 10.2307/1937992

60. Liaw A, Wiener M. Classification and regression by randomforest. *R News* (2002) 2(3):18–22.

61. Dekhil O, Hajjdiab H, Ayinde B, Shalaby A, Switala A, Sosnin D, et al. (2018b). Using resting state functional MRI to build a personalized autism diagnosis system. In *Biomedical Imaging (ISBI 2018), 2018 IEEE 15th International Symposium on* (IEEE), 1381–5. doi: 10.1109/ISBI.2018.8363829

62. Dekhil O, Hajjdiab H, Shalaby A, Ali MT, Ayinde B, Switala A, et al. Using resting state functional MRI to build a personalized autism diagnosis system. *PLoS ONE* (2018c) 13:1–22. doi: 10.1371/journal.pone.0206351

63. Dekhil O, Ali M, Shalaby A, Mahmoud A, Switala A, Ghazal M, et al. (2018a). Identifying personalized autism related impairments using resting functional MRI and ADOS reports. In *International Conference on Medical Image Computing and Computer-Assisted Intervention* (Springer), 240–8. doi: 10.1007/978-3-030-00931-1_28

64. Piven J, Elison JT, Zylka MJ. Toward a conceptual framework for early brain and behavior development in autism. *Mol Psychiatry* (2017) 22:1385. doi: 10.1038/mp.2017.131

65. Hull JV, Jacokes ZJ, Torgerson CM, Irimia A, Van Horn JD. Resting-state functional connectivity in autism spectrum disorders: a review. *Front Psychiatry* (2017) 7:205. doi: 10.3389/fpsyt.2016.00205

66. Beversdorf DQ, Consortium MAS. Phenotyping, etiological factors, and biomarkers: toward precision medicine in autism spectrum disorders. *J Dev Behav Pediatr* (2016) 37:659. doi: 10.1097/DBP.0000000000000351

67. Zerbi V, Markicevic M, Pagani M, Rudin M, Matteoli M, Fagiolini M, et al. The autism mouse brain connectome project. *Soc Neurosci* (2018).

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

in Psychiatry

Problem Behavior in Autism Spectrum Disorder: Considering Core Symptom Severity and Accompanying Sleep Disturbance

Ebony Lindor*, Carmel Sivaratnam, Tamara May, Nicole Stefanac, Katherine Howells and Nicole Rinehart

Faculty of Health, School of Psychology, Deakin Child Study Centre, Deakin University, Geelong, VIC, Australia

In addition to the core symptoms that define autism spectrum disorder (ASD), many individuals experience broader problem behavior at a level significant enough for families to seek further clinical assessment and intervention. We define "problem behavior" as any significant emotional or behavioral issue captured by the Child Behavior Checklist (CBCL) including anxiety, depression, withdrawal, somatic complaints, problems with socialization, thought or attention, rule-breaking, and aggression. While greater ASD symptom severity and accompanying sleep disturbance have each been linked with more severe problem behavior, there is little understanding about how these two key factors interact; that is, it is unclear whether the severity and type of sleep disturbance an individual experiences differentially influences the relationship between ASD symptom severity and problem behavior. The aim of the current study was, thus, to explore whether the link between greater ASD symptom severity and clinically elevated problem behavior is moderated by the presence/degree of accompanying sleep disturbance. Forty males with ASD, aged 5–12, participated in the study. The Social Responsiveness Scale, CBCL, and Children's Sleep Habits Questionnaire were administered to obtain information about ASD symptom severity, problem behavior, and sleep habits, respectively. Results indicated that the relationship between ASD symptom severity and problem behavior differed among individuals with ASD depending on the degree of sleep disturbance they experienced. Specifically, there was a significant positive relationship between ASD symptom severity and problem behavior for individuals with no sleep disturbance or milder sleep disturbance (i.e., in these cases, individuals with severe ASD symptoms experienced clinically elevated problem behavior, while those with milder ASD symptoms experienced milder problem behavior). In contrast, there was no significant relationship between ASD symptom severity and problem behavior for individuals with moderate-to-severe sleep disturbance; rather, clinically significant problem behavior was apparent across all individuals irrespective of ASD symptom severity. Follow-up analyses indicated that disturbances in sleep duration, disordered breathing, and daytime sleepiness were related to clinically elevated problem behavior even among those with milder ASD symptoms. These findings emphasize the importance of routinely assessing for accompanying sleep disturbance in this population regardless of whether individuals present with mild, moderate, or severe ASD.

Keywords: autism spectrum disorder, symptom severity, sleep, accompanying disturbance, problem behavior

INTRODUCTION

Autism spectrum disorder (ASD) is a heterogenous neurodevelopmental disorder characterized by social deficits and restricted, repetitive patterns of behavior (1). Core symptoms typically manifest as poor social-emotional reciprocity, abnormal non-verbal communication, difficulty developing and maintaining social relationships, repetitive movements, intense fixations/interests, and atypical sensory sensitivity. In addition, a significant proportion of individuals with ASD exhibit a broader range of problem behaviors that extend beyond the core symptoms that define the disorder (2). For example, there is existing research to suggest that many individuals with ASD experience clinically significant internalizing and externalizing problems including anxiety, depression, somatization, rule-breaking, aggression, self-harm, inattention, hyperactivity, impulsivity, and abnormal thought (3–9). In this paper, the term "problem behavior" encompasses any significant emotional or behavioral issue captured by the Child Behavior Checklist (CBCL) including problems with anxiety, depression, withdrawal, somatization, socialization, thought, attention, rule-breaking, and aggression. As is common in ASD research, the nature and severity of these broader problem behaviors vary considerably among individuals on the spectrum; there are some individuals who experience relatively few of these problem behaviors, while many others exhibit one or more problem behaviors that are significant enough to warrant clinical concern (3, 10, 11) and may even result in comorbid diagnoses such as anxiety, depression, conduct disorder, oppositional defiant disorder (ODD), and/or attention deficit hyperactivity disorder (ADHD). Being able to predict who on the spectrum (i.e., individuals of which severities and clinical presentations) is more likely to exhibit clinically significant problem behavior is particularly important, as those with persistent difficulties in this area are at greater risk of long-term mental health issues, poor academic achievement, and future crime and violence (12, 13).

Researchers have, thus, become increasingly interested in studying the factors related to clinically elevated problem behavior in different individuals with ASD. While a range of factors have been examined in the literature, the relevance and contribution of accompanying sleep disturbance have emerged as an area of particular interest (14, 15). Sleep plays an important role in memory consolidation (16) and, more generally, brain plasticity, which in turn supports typical cognitive, emotional, and behavioral development (17). Sleep problems are prevalent in ASD and reportedly affect 40% to 80% of children; these estimates are significantly greater than that of the typically developing population and place those who are already developmentally vulnerable at a greater disadvantage (18, 19). In ASD, the exact etiology of accompanying sleep disturbance is unclear; however, researchers commonly argue that sleep problems are contributed to by a complex combination of biological, psychological, and social/environmental factors (18). While neurophysiological and neurochemical abnormalities can promote chronic sleep–wake disturbance and abnormal timing of melatonin secretion in this population, comorbid medical conditions and current medications also need to be considered, as do other potential factors such as core ASD symptoms, child-rearing practices, and family stress (14, 18, 20). In ASD, common types

of sleep disturbance include delayed sleep latency, reduced sleep efficiency, decreased total sleep duration, poor sleep maintenance/night waking, bedtime resistance, and daytime sleepiness (14, 21). Improving our understanding of how the nature and severity of these common sleep problems vary among individuals with ASD may help us conceptualize the possible consequences they can have on the behavior and broader functioning of these individuals. Given that sleep disturbance has been found to be amenable to intervention, there are also tangible benefits to focusing our efforts on sleep in ASD.

In terms of existing research, there is evidence to suggest that sleep disturbance in ASD may exacerbate problem behavior. For example, poor sleep has been linked to increased internalizing and externalizing problems, including tantrums, oppositional behavior, physical aggression, irritability, self-injury, depression, anxiety, mood variability, inattention, and hyperactivity (15, 22–30). Recently, Cohen et al. (31) found direct evidence that greater variation in sleep timing and duration can predict subsequent problematic daytime behavior (e.g., aggression, self-injury, tantrums, and property destruction), and there is also longitudinal evidence to suggest that sleep problems may predict later anxiety (32). Sleep researchers frequently acknowledge that, although highly prevalent, sleep problems do not affect all individuals with ASD. As such, a number of studies have categorized ASD participants as "poor" sleepers and "good" sleepers, based on actigraphy, polysomnography, and parent-report measures, to examine behavioral differences among those with and without significant sleep problems. A large-scale study conducted by Goldman et al. (25) indicated that poor sleepers with ASD exhibited a significantly higher percentage of problem behavior than did good sleepers with ASD. Researchers relying on parent-report measures have revealed that individuals with ASD and accompanying sleep disturbance demonstrate greater externalizing and internalizing problem behaviors than do those without accompanying sleep disturbance (27, 29). Others who have taken advantage of objective measures have demonstrated similar relationships; for example, Malow et al. (33) reported more significant affective and social problems in individuals identified as poor sleepers, while Goldman et al. (23) noted greater levels of inattention, hyperactivity, and restricted, repetitive behavior.

The severity of sleep disturbance in ASD and the particular type of sleep disturbance that individuals experience have also been identified as important considerations. In terms of severity, Adam et al. (29) revealed different behavioral profiles in those with mild versus severe sleep problems, whereby individuals with more severe sleep problems exhibited significantly greater externalizing and overall problem behavior; surprisingly, internalizing behaviors were not sensitive to sleep severity in this study. This is somewhat in contrast to other researchers who have found both significantly greater internalizing and externalizing problems in individuals with moderate-to-severe sleep disturbance relative to those with milder sleep disturbance (28). In terms of identifying particularly relevant types of sleep disturbance, Hirata et al. (19) reported that insomnia was strongly associated with problem behavior; Mazurek and Sohl (15) identified night wakings as having the most consistent link with behavior problems, and Fadini et al. (34) noted relationships

between behavioral problems and disorders of arousal (e.g., sleepwalking, sleep terrors, and nightmares) and excessive somnolence (e.g., difficulties rising and daytime sleepiness). These studies highlight the importance of considering the severity and type of accompanying sleep disturbance when attempting to understand problem behavior in ASD.

While the above research outlines that both the presence and severity of sleep problems may be importantly linked to problem behavior in ASD, there is broader research to suggest that ASD symptom severity is another key factor to consider. Notably, there is accumulating research to suggest a strong positive association between ASD symptom severity and problem behavior. Researchers have indicated that those with more severe ASD symptoms are likely to experience significantly more problem behaviors that are also of greater severity than those with moderate or mild ASD symptoms (24, 35–37). There is also longitudinal evidence to suggest that ASD symptom severity is a key predictor of later emotional and behavioral problems in children and adolescents with high-functioning ASD (38). Studies such as these emphasize that ASD severity itself is related to increased problem behavior. Complicating the picture, there is also evidence to suggest that more severe ASD symptoms are associated with more significant sleep disturbance (23, 39–41). While the research in this area is sound and it is often the case that sleep problems are more pronounced in individuals with severe ASD presentations, it is important to note that sleep problems do not exclusively present in this subgroup and can affect individuals across the full spectrum (14).

Although there is a large body of evidence indicating that ASD severity and accompanying sleep disturbance are important factors each associated with more severe problem behavior in ASD, it remains unclear whether the presence/severity of an accompanying sleep disturbance differentially influences the relationship between ASD severity and problem behavior. In other words, more research is required to understand whether the positive relationship between ASD symptom severity and problem behavior (which suggests that those with greater ASD symptoms exhibit clinically elevated problem behavior while those with milder ASD symptoms exhibit significantly milder problem behavior) changes depending on the degree and type of sleep disturbance an individual experiences. The current study, therefore, aimed to examine whether the presence/degree of accompanying sleep disturbance moderates the relationship between ASD severity and problem behavior and to further explore the relevance of different types of sleep disturbance. While we expected ASD severity and degree of sleep disturbance to each show an association with problem behavior, there was limited literature available on which to base a hypothesis on how these two key factors would interact. Although somewhat exploratory from this perspective, the findings from this study were expected to improve our ability to identify those on the spectrum who may be more likely to experience clinically significant problem behavior.

MATERIALS AND METHODS

Participants

We recruited 40 children with ASD to the study *via* a range of methods. Advertisement flyers were distributed to community institutions, networking websites, and social media (e.g., Autism Victoria, Deakin Child Study Centre Facebook, early intervention services, and private pediatric clinics). There were also a number of primary schools and special development schools who expressed interest in supporting the study and agreed to send letters of invitation to families in their community containing advertising information about the study. Because this particular study was contained within a broader longitudinal project that aimed to promote the inclusion of children of all abilities into physical activity, the NAB AFL Auskick database was also used as an avenue of recruitment.

For inclusion in the study, participants were required to have a pre-existing formal diagnosis of ASD. To meet formal diagnosis in Victoria, Australia, an individual must satisfy DSM criteria, have undergone assessment by a multidisciplinary panel (e.g., medical, psychology, and speech clinicians), and had the diagnosis confirmed by a pediatrician or child psychiatrist. Participants were required to be aged between 5 and 12 and attend primary school (or equivalent special education). The intention was to capture a broad range of functioning levels; as such, no specific requirement was enforced around level of functioning or comorbidities. Testing locations were offered throughout metropolitan and regional Melbourne, which permitted a broad, representative community sample.

Measures
Demographics and Level of Functioning
Basic demographic information including age, gender, and handedness was obtained through parent report. Level of adaptive functioning was measured using the Vineland Adaptive Behavior Scale—Third Edition (VABS-3) (42). The domain-level parent/caregiver form was selected for use in this study; it is appropriate for ages 3 to 90+ and consists of three core domains (i.e., communication, daily living, and social skills and relationships), each of which contains 40 items. An overall adaptive behavior composite score was calculated from the 120 core items ($M = 100$, $SD = 15$). Scores between 86 and 114 reflect "adequate" adaptive level. Scores between 115 and 129 can be considered "moderately high," and those above 130 as "high." In comparison, scores from 71 to 85 can be considered "moderately low" and those at or below 70 as "low." In terms of psychometric properties, this form has an internal consistency coefficient of .97 and test–retest reliability of .87. Validity has previously been established and is further supported by intercorrelation data, assessment against other measure of adaptive functioning, and specific standardization for clinical samples [see Ref. (42)].

The Social Responsiveness Scale—Second Edition (SRS-2) School Age Form
The SRS-2 was administered to measure ASD symptom severity (43). The school age form consists of 65 items relevant to children aged 4 to 18, which measures five core areas of functioning: social awareness, social cognition, social communication, social motivation, and restricted interests and repetitive behavior. Using a four-point Likert scale (i.e., 1 = not true, 2 = sometimes true, 3 = often true, and 4 = almost always true), parents were required to select the degree to which each of the items applied to

their child in the past 6 months. Parent ratings were summed to obtain an SRS-2 total score ($M = 50$, $SD = 10$). Total scores at or below 59 are considered to be within normal limits; total scores placed between 60 and 65 indicate mild deficiencies in reciprocal social behavior; scores between 66 and 75 suggest moderate deficiencies; and those at 76 or above reflect severe deficiencies in reciprocal social behavior.

The Children's Sleep Habits Questionnaire (CSHQ)

The CSHQ was administered to measure a range of behaviorally based and medically based sleep problems (44). It consists of 33 core items that relate to eight sleep domains: bedtime resistance, sleep onset delay, sleep duration, sleep anxiety, night waking, parasomnias, sleep-disordered breathing, and daytime sleepiness. Parents were required to rate each of the items on the basis of a typical week using a three-point Likert scale (i.e., 1 = usually, defined as occurring five to seven times per week; 2 = sometimes, defined as occurring two to four times per week; and 1 = rarely, defined as occurring zero to one time per week). Normative data (i.e., means and standard deviations) are available for each of the domains, from which T-scores can be calculated for ease of interpretation. Generally, T-scores between 60 and 64 are considered higher than average and may indicate slightly more concerns than is typical; those between 65 and 69 are notably elevated and border clinical significance, while those above 70 warrant clinical concern. For total sleep problems, a cutoff point of 41 has been established; scores over 41 indicate clinically significant sleep problems, while scores below 41 reflect sleep behavior within normal limits (44). With the use of this cutoff point, psychometric properties are acceptable, with a sensitivity estimate of .80 and a specificity estimate of .72. Assigning severity to total sleep problems has been inconsistently performed across studies. Of note, however, a previous large-scale study (i.e., $N > 1,000$) described scores from 41 up to 55 as mild to moderate and scores above 56 as moderate to severe, which were obtained by calculating the 75th percentile/fourth quartile (28). While all variables in this study were analyzed in continuous form, these descriptive categories were adopted to describe variability among individuals where significant interactions were revealed (i.e., to interpret the "pick a point" output from simple slopes analyses and zones of significance produced by the Johnson–Neyman technique at a clinically meaningful level; see Data Analysis section for further details).

The Child Behavior Checklist (CBCL)

The CBCL was administered to measure problem behavior. Given that primary-school-aged children were recruited to the study, two forms were required to capture the age ranges, the 1.5–5-year-old form (45) and the 6–18-year-old form (46). The 1.5–5-year-old form consists of 100 age-relevant items, whereas the 6–18-year-old form consists of 113 age-relevant items. Parents were required to rate the degree to which each of the items applied to their child over the past 6 months using a three-point Likert scale (i.e., 0 = not true, 1 = somewhat/ sometimes true, and 2 = very/often true). Each of these forms summarizes child emotional and behavioral problems into measures of internalizing problems, externalizing problems, and total problems. The total problem T-score was used for the

purposes of this study with T-scores at or above 70 taken to reflect "clinically significant" problem behavior. Given the broad range of emotional and behavioral problems captured by the CBCL, it should be noted that individuals with problems at this level may meet criteria for comorbid diagnoses such as anxiety, depression, conduct disorder, ODD, and/or ADHD. In terms of psychometrics, the CBCL is a widely used well-validated measure that has been adequately shown to discriminate between clinical and non-clinical behavioral problems (87% accuracy for school-aged forms; 84% accuracy for pre-school form).

Procedure

Ethics approval was obtained through Deakin University Human Research Ethics Committee and the Victorian Department of Education and Training. In accordance with the Declaration of Helsinki, parents formally provided informed consent, while children gave verbal assent. Following the consent process, parents were asked to complete a brief survey consisting of demographic questions (e.g., date of birth) and medical history (e.g., details of diagnoses and health services attended). They then completed the VABS-3 to provide information on their child's level of functioning. Given that the data for this study were collected as part of a larger longitudinal project involving direct child measures, parents and children physically attended testing sessions. In most cases, parents completed the SRS-2, CSHQ, and CBCL during the session, while their child engaged in motor and cognitive tasks relevant to the broader project. If this was not possible (e.g., school-based testing sessions or insufficient time to complete measures), parents were permitted to complete the questionnaires in their own time and return them to the researchers, either at the initial session or after the session by reply-paid envelope. In cases where parents were unable to complete the questionnaires prior to or at the session, they were encouraged to complete the questionnaires and return by post at their earliest convenience.

Data Analysis

To characterize the sample, descriptive statistics were conducted for 1) level of adaptive functioning (VABS-3), 2) ASD severity (SRS-2), 3) problem behavior (CBCL), and 4) sleep disturbance (CSHQ). Correlations among these variables were also conducted. A moderation analysis was then run to examine whether the relationship between ASD symptom severity and problem behavior varied as a function of the participants' level of accompanying sleep disturbance. Follow-up moderation analyses were conducted to explore the most relevant types of sleep disturbance. All data were analyzed using IBM SPSS Statistics Version 21, with the assistance of the PROCESS macro (47). All variables were mean centered for analysis, and the HC3 (Davidson–MacKinnon) heteroscedasticity-consistent inference applied. Where moderation was found, the main analysis was followed by simple slopes analysis and then the Johnson–Neyman technique to identify the true zone of significance. Given the relatively small sample, a power analysis was conducted to ensure our approach for the main moderation analysis. Assuming the following parameters - large effect size, α error probability of .05, and power of .80 - a total sample size of 36 was required.

RESULTS

Sample Characteristics and Correlations

The sample was exclusively male, and ages ranged from 5.02 to 12.87, with an M of 8.23 and SD of 2.12. **Table 1** includes detailed information on participant characteristics, including level of functioning (VABS-2), ASD severity (SRS-2), problem behavior (CBCL), and sleep (CSHQ). **Table 2** further displays correlations among these variables.

Total Sleep Problems as a Moderator

The overall linear model revealed that ASD symptom severity (SRS-2 total score), total sleep problems (CHSQ total score), and their interaction significantly predicted level of problem behavior (CBCL total score), $F(3, 36) = 10.90$, $p < .001$, $R^2 = .55$. Individually, ASD symptom severity predicted level of problem behavior, $b = .36$, $t = 2.90$, $p = .006$ [.11, .62], as did total sleep problems, $b = .48$, $t = 4.45$, $p = .001$ [.26, .69]. Most importantly, the interaction between ASD symptom severity and total sleep problems bordered significance, $b = -.02$, $t = -2.00$, $p = .05$ [−.04, −.0003], indicating that the relationship between ASD symptom severity and problem behavior differs depending on the degree of accompanying sleep disturbance present.

A follow-up simple slopes analysis revealed that the relationship between ASD symptom severity and problem

TABLE 1 | Participant characteristics.

	M	**SD**	**Range**
Age	8.23	2.12	5.02–12.87
Adaptive functioning			
ABC composite	72.98	8.85	54–89
Communication	75.85	12.02	46–105
Daily living	74.75	11.95	52–95
Social skills	72.35	10.17	49–91
ASD severity			
SRS-2 total	76.93	9.30	56–90
Social awareness	73.40	10.41	48–90
Social cognition	73.53	10.77	53–90
Social communication	75.18	10.51	51–90
Social motivation	68.75	10.71	48–90
RRB index	77.70	8.68	53–90
SCI index	75.55	9.56	54–90
Problem behavior			
Internalizing	63.93	9.89	41–82
Externalizing	61.78	11.43	33–83
Total CBCL problems	66.75	9.38	46–83
Sleep disturbance			
Bedtime resistance	57.65	15.41	44–97
Sleep onset delay	62.58	16.88	45–83
Sleep duration	59.53	19.33	46–110
Sleep anxiety	60.88	16.38	44–99
Night wakings	62.75	18.97	44–100
Parasomnias	67.60	19.38	41–113
Sleep-disordered breathing	53.98	17.99	46–141
Daytime sleepiness	56.90	10.08	44–91
Total CSHQ	48.18	9.68	36–72

All of the above, with the exception of age and total CSHQ, have been calculated based on T-scores. Total CSHQ is based on raw data with a clinical cutoff point of 41 (see Measures section for further information).

TABLE 2 | Correlations.

	Age	**ABC composite**	**SRS-2 Total**	**Total CBCL**	**Total CSHQ**
Age	1				
ABC Composite	−.32*	1			
SRS-2 total	.04	−.54**	1		
Total CBCL	.06	−.12	.57**	1	
Total CSHQ	.01	−.19	.43**	.62**	1

$**p < .01$, $*p < .05$.

behavior was only significant for those in the sample with no sleep problems (i.e., estimated at a score of 38.50), $b = .57$, $t = 3.04$ $p = .004$ [.19, .95], or mild sleep problems (i.e., estimated at a score of 48.18), $b = .36$, $t = 2.90$, $p = .006$ [.11, .62]. For individuals with moderate-to-severe sleep problems (i.e., estimated at a score of 57.86), the relationship between ASD symptom severity and problem behavior was not significant, $b = .16$, $t = 1.20$, $p = .24$ [−.11, .43]; rather, the degree of problem behavior was high at all severities of ASD. See **Figure 1**. The Johnson–Neyman technique further clarified the zone of significance was from a total sleep score of 36.00 (lower bound of data) to 53.92. The relationship between ASD severity and problem behavior was no longer significant for individuals in the sample with total sleep scores above 53.92.

Contribution of Specific Sleep Problems

Table 3 summarizes the linear models examining the relationship between ASD symptom severity and problem behavior as moderated by each of the specific sleep domains: 1) bedtime resistance, 2) sleep onset delay, 3) sleep duration, 4) sleep anxiety, 5) night waking, 6) parasomnias, 7) disordered breathing, and 8) daytime sleepiness.

As is evident from **Table 3**, sleep duration, disordered breathing, and daytime sleepiness were found to significantly moderate the relationship between ASD symptom severity and problem behavior. To detail the nature of these interactions, the sections below outline the simple slopes analyses and Johnson–Neyman results.

Moderating Effect of Sleep Duration

Simple slopes analysis revealed that the relationship between ASD symptom severity and problem behavior was only significant for those who had no problems with sleep duration (i.e., estimated at a T-score of 46.00), $b = .55$, $t = 3.21$ $p = .003$ [.20, .89], or few problems with sleep duration (i.e., estimated at a T-score of 59.53), $b = .35$, $t = 2.62$, $p = .01$ [.08, .62]. For individuals who had clinically significant problems with sleep duration (i.e., estimated at a T-score of 78.86), the relationship between ASD symptom severity and problem behavior was not significant, $b = .07$, $t = .43$, $p = .67$ [−.27, .41]; rather, the degree of problem behavior was high at all severities of ASD. The Johnson–Neyman technique further clarified the zone of significance was from a T-score of 46.00 (lower bound of data) to a T-score of 65.15. The relationship between ASD symptom severity and problem behavior was no longer significant for individuals in the sample with a T-score above 65.15.
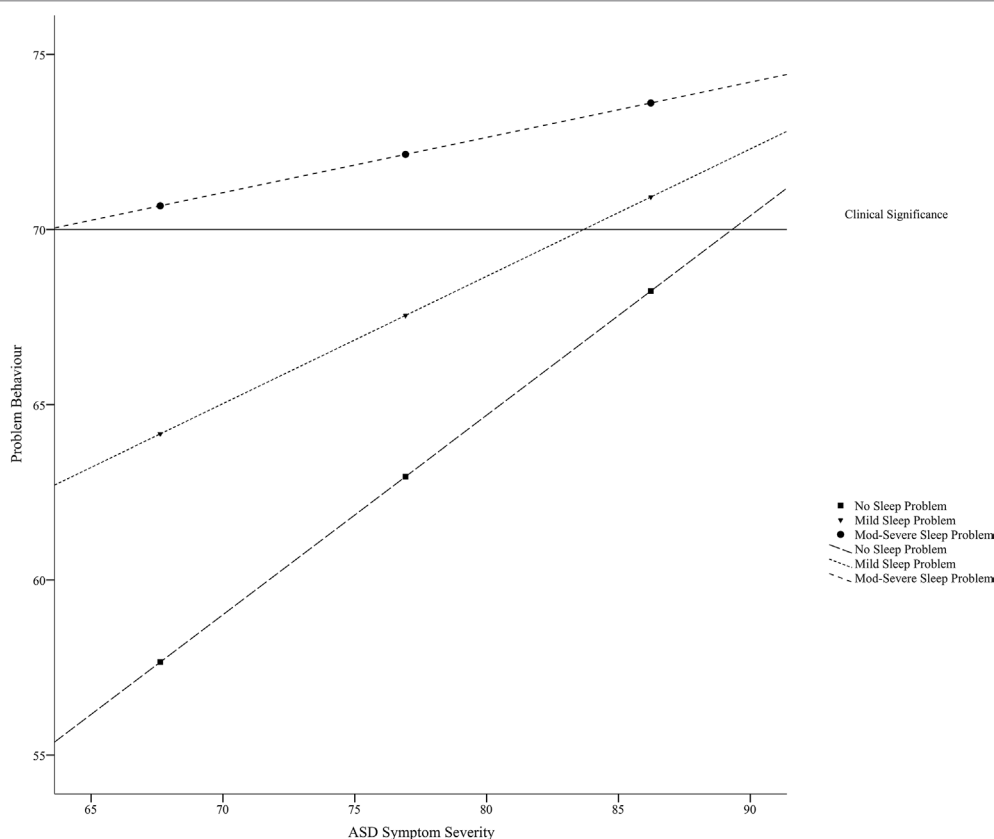
FIGURE 1 | Relationship between ASD symptom severity (i.e., SRS-2 score) and problem behavior (i.e., total CBCL score) as moderated by degree of sleep disturbance (i.e., total CSHQ score).

Moderating Effect of Disordered Breathing

A similar pattern of findings was evident for disordered breathing. Simple slopes analysis revealed that the relationship between ASD symptom severity and problem behavior was only significant for those who had no problems with disordered breathing (i.e., estimated at a T-score of 46.00), $b = .68$, $t = 4.45$, $p < .001$ [.37, .99], or very few problems with disordered breathing (i.e., estimated at a T-score of 53.97), $b = .51$, $t = 3.89$, $p < .001$ [.24, .77]. For individuals who had clinically significant problems with disordered breathing (i.e., estimated at a T-score of 71.96), the relationship between ASD symptom severity and problem behavior was not significant, $b = .12$, $t = .77$, $p = .45$ [−.20, .44]; again, the degree of problem behavior was high at all severities of ASD. The Johnson–Neyman technique further clarified that the zone of significance was from a T-score of 46.00 (lower bound of data) to a T-score of 64.90. The relationship between ASD symptom severity and problem behavior was not significant for individuals in the sample with a T-score above 64.90. It was, however, noted that the relationship became significant again for those with very high T-scores above 117.26.

Moderating Effect of Daytime Sleepiness

As per sleep duration and disordered breathing, simple slopes analysis revealed that the relationship between ASD symptom severity and problem behavior was only significant for those who had no problems with daytime sleepiness (i.e., estimated

at a T-score of 46.82), $b = .75$, $t = 4.59$, $p < .001$ [.42, 1.08], or few problems with daytime sleepiness (i.e., estimated at a T-score of 56.90), $b = .45$, $t = 3.59$, $p = .001$ [.20, .71]. For individuals who had borderline clinically significant daytime sleepiness (i.e., estimated at a T-score of 66.98), the relationship between ASD severity and problem behavior was not significant, $b = .16$, $t = .86$, $p = .40$ [−.22, .53]; rather, the degree of problem behavior was high at all severities of ASD. The Johnson–Neyman technique revealed a zone of significance from a T-score of 44.00 (lower bound of data) to a T-score of 62.15. The relationship between ASD severity and problem behavior was no longer significant for individuals in the sample with a T-score above 62.15.

DISCUSSION

This study aimed to examine the influence of accompanying sleep disturbance on the established relationship between ASD symptom severity and problem behavior in ASD. Although a significant literature exists on the relationships between ASD severity, sleep disturbance, and problem behavior, relatively little is known about whether ASD severity is related to problem behavior comparably across individuals with varying degrees of accompanying sleep disturbance (i.e., none, mild, and moderate to severe). The current study employed a series of moderation

TABLE 3 | Linear models summarizing the relationship between ASD symptom severity and problem behavior as moderated specific sleep disturbances.

	Model	b	SE	t	p	CI
1	Constant	66.94	1.10	61.08	<.001	[64.72, 69.16]
	ASD severity	.50	.13	3.83	<.001	[.23, .76]
	Bedtime resistance	.24	.06	3.82	<.001	[.11, .37]
	ASD severity × bedtime resistance	−.01	.01	−.90	.37	[−.02, .01]
	Note. $F_{(3,36)} = 12.60$, $p < .001$, $R^2 = .51$					
2	Constant	67.06	1.41	47.58	<.001	[64.20, 69.92]
	ASD severity	.63	.13	4.73	<.001	[.36, .90]
	Sleep onset delay	−.09	.09	−1.04	.31	[−.26, .09]
	ASD severity × sleep onset delay	−.01	.01	−.48	.63	[−.03, .02]
	Note. $F_{(3,36)} = 7.89$, $p < .001$, $R^2 = .36$					
3	Constant	68.00	1.22	55.66	<.001	[65.52, 70.48]
	ASD severity	.35	.13	2.62	.01	[.08, .62]
	Sleep duration	.22	.08	2.90	.006	[.07, .38]
	ASD severity × sleep duration	−.01	.01	−2.26	.03	[−.03, −.002]
	Note. $F_{(3,36)} = 9.32$, $p < .001$, $R^2 = .42$					
4	Constant	66.84	1.19	56.40	<.001	[64.43, 69.24]
	ASD severity	.54	.13	4.10	<.001	[.27, .81]
	Sleep anxiety	.18	.06	2.86	.007	[.05, .30]
	ASD severity × sleep anxiety	−.01	.01	−1.50	.14	[−.02, .004]
	Note. $F_{(3,36)} = 8.54$, $p < .001$, $R^2 = .45$					
5	Constant	66.81	1.13	59.22	<.001	[64.52, 69.10]
	ASD severity	.50	.16	3.21	.003	[.18, .82]
	Night waking	.23	.05	4.42	<.001	[.12, .34]
	ASD severity × night waking	−.002	.01	−.28	.78	[−.02, .01]
	Note. $F_{(3,36)} = 11.44$, $p < .001$, $R^2 = .54$					
6	Constant	67.27	1.25	53.86	<.001	[64.74, 69.81]
	ASD severity	.45	.14	3.08	.004	[.15, .74]
	Parasomnias	.20	.08	2.38	.02	[.03, .37]
	ASD severity × parasomnias	−.01	.01	−1.12	.27	[−.03, .01]
	Note. $F_{(3,36)} = 7.62$, $p < .001$, $R^2 = .48$					
7	Constant	67.88	1.22	55.48	<.001	[65.39, 70.36]
	ASD severity	.51	.13	3.89	<.001	[.24, .77]
	Disordered breathing	.24	.09	2.69	.01	[.06, .41]
	ASD severity × disordered breathing	−.02	.01	−3.16	.003	[−.04, −.01]
	Note. $F_{(3,36)} = 9.32$, $p < .001$, $R^2 = .42$					
8	Constant	67.60	1.28	52.61	<.001	[65.00, 70.21]
	ASD severity	.45	.13	3.59	.001	[.20, .71]
	Daytime sleepiness	.31	.15	2.12	.04	[.01, .60]
	ASD severity × daytime sleepiness	−.03	.01	−2.46	.02	[−.05, −.005]
	Note. $F_{(3,36)} = 9.66$, $p < .001$, $R^2 = .43$					

analyses to explore this and revealed that the relationship between ASD severity and problem behavior varied significantly depending on the degree of accompanying sleep disturbance present. For individuals with no sleep disturbance or mild sleep disturbance, ASD symptom severity and problem behavior were positively related. For these individuals, having milder ASD symptoms was associated with significantly fewer problem behaviors; it was only those with the most severe ASD symptoms who experienced problem behavior that reached clinical levels. In contrast, ASD symptom severity and problem behavior were not related in individuals with moderate-to-severe sleep disturbance; rather, these individuals exhibited clinically significant problem behavior regardless of whether they had mild, moderate, or severe ASD symptoms. This suggests that individuals with moderate-to-severe sleep disturbance in addition to an ASD, of any severity, are likely to experience problem behavior at levels high enough to warrant clinical attention. This is in contrast to those with

milder sleep disturbance or no sleep disturbance who appear incrementally more likely to experience clinically significant problem behavior as ASD symptom severity increases.

These findings complement the broader literature in this area and, importantly, offer novel information regarding the complex interaction between ASD symptom severity and accompanying sleep disturbance. Many researchers have previously acknowledged that there is a relationship between ASD symptom severity and problem behavior (24, 35–38), and while our results mirror this argument in individuals with no significant sleep disturbance and milder sleep disturbance, we did not find evidence of this relationship for those with moderate-to-severe sleep disturbance. This revelation is novel and emphasizes that the relationship between ASD symptom severity and problem behavior may be more nuanced than previously described.

To extend our findings and gain further insight into whether specific types of sleep problems influence the relationship between

ASD symptom severity and problem behavior, we conducted a series of follow-up moderation analyses, considering each sleep domain in turn: bedtime resistance, sleep onset delay, sleep duration, sleep anxiety, night waking, parasomnias, disordered breathing, and daytime sleepiness. Some sleep domains revealed a similar pattern of results to the overall sleep model, whereby the relationship between ASD symptom severity and problem behavior was only apparent for those with no sleep problems or milder sleep problems, whereas other domains revealed that the relationship between ASD symptom severity and problem behavior was similar across all levels of sleep disturbance (i.e., none, mild, and moderate to severe). More specifically, there was evidence that ASD symptom severity and degree of sleep disturbance in each of the following areas—bedtime resistance, sleep anxiety, night waking, and parasomnias—individually predicted level of problem behavior. The relationships were all positive and indicated that individuals with more severe ASD symptoms or a higher degree of sleep disturbance in these specific domains were likely to experience greater problem behaviors. There was no evidence of moderation in these domains, which suggests that having milder ASD may be of some benefit when sleep problems manifest as bedtime resistance, sleep anxiety, night waking, and parasomnias.

Sleep onset delay showed a similar pattern to domains described above; however, for this domain, only ASD symptom severity predicted problem behavior. The severity of sleep onset delay itself did not reach significance, nor was there any evidence of moderation. One consideration here is that delayed sleep can occur for a variety of reasons, which may affect the overall quantity and quality of sleep to different degrees. Sleep latency may be extended as a consequence of other problems such as bedtime resistance, sleep anxiety, or sleep associations (18), and when evaluating how problematic delayed sleep may be for an individual, it is important to consider not only the cause but also whether sleep is adequately maintained after onset and whether overall duration is sufficient.

The results from the remaining three sleep domains revealed sleep duration, disordered breathing, and daytime sleepiness as significant moderators of the relationship between ASD symptom severity and problem behavior. Unlike the total sleep score on the CSHQ, means and standard deviations are available for the individual domains, and thus conversion to T-scores was performed for ease of interpretation (see Measures section for further details). For sleep duration, disordered breathing, and daytime sleepiness, the relationships between ASD severity and problem behavior were positive and significant up to a T-score of 65, 64, and 62 on the sleep scales, respectively.[1] When the degree of sleep disturbance in these domains exceeded these T-scores, the relationship between ASD severity and problem behavior was no longer significant. Notably, each of these values is above normative limits and approaching clinical significance. These findings suggest that when problems with sleep duration,

disordered breathing, and daytime sleepiness reach more significant levels, ASD symptom severity appears less relevant. While there was also some evidence of significance at very high levels of disordered breathing (T-scores exceeding 117), further investigation is required to examine the nature of this relationship given that relatively few participants scored in this range in the current cohort.

As per the broader results, the domain analyses both complement and extend the existing literature in this area. In particular, the bedtime resistance, sleep anxiety, night waking, and parasomnias results align closely with studies that have presented ASD severity (24, 35–38) or accompanying sleep disturbance (23, 25, 27, 29, 33) as key factors influencing problem behavior. They also align closely with studies that have indicated a relationship between severity of sleep disturbance and degree of problem behavior [e.g., Refs. (28, 29)]. As for sleep duration, disordered breathing, and daytime sleepiness, the results do not necessarily contradict the existing literature, but they do clarify that the association typically observed between ASD symptom severity and problem behavior varies as a function of how severe an individual's difficulties are with sleep duration, disordered breathing, or daytime sleepiness. They further clarify that high levels of these specific sleep problems are associated with clinically significant problem behavior even in individuals with milder ASD. Research into the types of sleep disturbance that may be most important to consider in the context of problem behavior is still being investigated. While some connection can be drawn between the domains that emerged in this study and those of existing studies [e.g., Refs. (15, 19, 34)], direct comparison and further progress are limited by the way in which researchers conceptualize and measure sleep disturbance. There appears to be little consensus among methods of determining severity and significant overlap in the breakdown of sleep domains captured both within and between different measures.

While the results and implications of this study are novel and clinically relevant, they do need to be interpreted with some degree of caution until they are able to be replicated. The study is limited by its relatively small sample size and large age span. Due to recruitment methods, and the nature of the broader study, the sample was exclusively male, which, on the one hand, may be considered a strength given the frequent differences in abilities and behaviors observed between males and females with ASD but, on the other hand, limits generalizability. This factor also limited how much information we were able obtain around medications and interventions that may have been prescribed to help manage sleep disturbance in the sample. The potential influence of these factors is important to consider and should be examined in future related research wherever feasible. It is also important to note that while ASD symptom severity and accompanying sleep disturbance are important factors that appear to explain a very significant portion of variance, they are not the only factors that have the potential to influence problem behavior. Another broad consideration to keep in mind with research of this nature is that the relationships between these variables are complex and unlikely to be unidirectional. While the rhetoric developed in this paper focused on the effect that ASD symptom severity and sleep disturbance may have on problem behavior, there is also literature to indicate

---

[1]As previously indicated, generally, T-scores between 60 and 64 are considered higher than average and may indicate slightly more concerns than is typical; those between 65 and 69 are notably elevated and border clinical significance, while those above 70 warrant clinical concern.

that sleep disturbance may elevate ASD symptom severity (23, 39–41) and a possibility that problem behavior may exacerbate difficulties with sleep. Future research aimed at capturing the broad range of (dis)abilities across the spectrum will enable us to further our understanding of how core factors and comorbidities affect different individuals and may improve our ability to predict outcomes and long-term functioning. We suggest sleep as a key avenue to continue this endeavor, particularly given that problems are potentially treatable with behavioral sleep interventions, light therapy, and/or pharmacological options, and when identified for treatment can have reaching, positive consequences for an individual's functioning [e.g., Ref. (48)].

In sum, key findings from this study indicated a positive relationship between ASD severity and problem behavior for individuals with no sleep disturbance or milder sleep disturbance, yet no significant relationship between ASD symptom severity and problem behavior for those with moderate-to-severe sleep disturbance. Clinically significant problem behavior was apparent across all individuals with moderate-to-severe sleep disturbance regardless of their ASD symptom severity, and this pattern was particularly apparent for sleep duration, disordered breathing, and daytime sleepiness. Overall, the findings highlight the variability among individuals with ASD and demonstrate that profiling individuals on the basis of their sleep habits may help to identify those who are most likely to experience clinically significant problem behavior. More broadly, the findings also seem to suggest that those with one clinically significant issue (i.e., sleep disturbance) may be more vulnerable to other clinically significant issues or comorbid conditions (e.g., psychopathologies such as anxiety and depression) and reinforce the need for careful assessment and early intervention.

ETHICS STATEMENT

AUTHOR CONTRIBUTIONS

All authors, EL, CS, TM, NS, KH and NR, participated in the conception and design of the study. EL, CS, KH, and NS were involved in recruitment and data collection. EL and NS analyzed the data. All authors were involved in data interpretation and manuscript drafting. All authors read and approved the final manuscript.

FUNDING

ACKNOWLEDGMENTS

REFERENCES

1. APA (2013). *Diagnostic and statistical manual of mental disorders*. Washington, DC: American Psychiatric Publishing.
2. Schroeder J, Weiss J, Bebko J. CBCL profiles of children and adolescents with Asperger syndrome: a review and pilot study. *J Dev Disabil* (2011) 17(1):26–37. http://oadd.org/wp-content/uploads/2011/01/41009_JoDD_17-1_26-37_Schroeder_et_al.pdf
3. Hartley SL, Sikora DM, McCoy R. Prevalence and risk factors of maladaptive behaviour in young children with autistic disorder. *J Intellect Disabil Res* (2008) 52(10):819–29. doi: 10.1111/j.1365-2788.2008.01065.x
4. Solomon M, Ozonoff S, Carter C, Caplan R. Formal thought disorder and the autism spectrum: relationship with symptoms, executive control, and anxiety. *J Autism Dev Disord* (2008) 38(8):1474–84. doi: 10.1007/s10803-007-0526-6
5. Bauminger N, Solomon M, Rogers SJ. Externalizing and internalizing behaviors in ASD. *Autism Res* (2010) 3(3):101–12. doi: 10.1002/aur.131
6. Chandler S, Howlin P, Simonoff E, O'Sullivan T, Tseng E, Kennedy J, et al. Emotional and behavioural problems in young children with autism spectrum disorder. *Dev Med Child Neurol* (2016) 58(2):202–8. doi: 10.1111/dmcn.12830
7. Chen C, Shen YD, Xun GL, Cai WX, Shi LJ, Xiao L, et al. Aggressive behaviors and treatable risk factors of preschool children with autism spectrum disorder. *Autism Res* (2017) 10(6):1155–62. doi: 10.1002/aur.1751
8. McClain MB, Hasty Mills AM, Murphy LE. Inattention and hyperactivity/impulsivity among children with attention-deficit/hyperactivity-disorder, autism spectrum disorder, and intellectual disability. *Res Dev Disabil* (2017) 70:175–84. doi: 10.1016/j.ridd.2017.09.009

9. Summers J, Shahrami A, Cali S, D'Mello C, Kako M, Palikucin-Reljin A, et al. Self-injury in autism spectrum disorder and intellectual disability: exploring the role of reactivity to pain and sensory input. *Brain Sci* (2017) 7(11):1–16. doi: 10.3390/brainsci7110140
10. Tonge B, Brereton A, Gray KM, EInfield SL. Behavioural and emotional disturbance in high-functioning autism and Asperger syndrome. *Autism* (1999) 3(2):117–30. doi: 10.1177/1362361399003002003
11. Wijnhoven L, Creemers DHM, Vermulst AA, Granic I. Prevalence and risk factors of anxiety in a clinical Dutch sample of children with an autism spectrum disorder. *Front Psychiatry* (2018) 9:1–10. doi: 10.3389/fpsyt.2018.00050
12. Bub KL, McCartney K, Willett JB. Behavior problem trajectories and first-grade cognitive ability and achievement skills: a latent growth curve analysis. *J Educ Psychol* (2007) 99(3):653–70. doi: 10.1037/0022-0663.99.3.653
13. Langstrom N, Grann M, Ruchkin V, Sjostedt G, Fazel S. Risk factors for violent offending in autism spectrum disorder: a national study of hospitalized individuals. *J Interpers Violence* (2009) 24(8):1358–70. doi: 10.1177/0886260508322195
14. Cohen S, Conduit R, Lockley SW, Rajaratnam SM, Cornish KM. The relationship between sleep and behavior in autism spectrum disorder (ASD): a review. *J Neurodev Disord* (2014) 6(44):1–10. doi: 10.1186/1866-1955-6-44
15. Mazurek MO, Sohl K. Sleep and behavioral problems in children with autism spectrum disorder. *J Autism Dev Disord* (2016) 46(6):1906–15. doi: 10.1007/s10803-016-2723-7
16. Stickgold R. A memory boost while you sleep. *Neuroscience* (2006) 444:559–60. doi: 10.1038/nature05309

17. Graven SN, Browne JV. Sleep and brain development: the critical role of sleep in fetal and early neonatal brain development. *Newborn Infant Nurs Rev* (2008) 8(4):173–9. doi: 10.1053/j.nainr.2008.10.008

18. Cortesi F, Giannotti F, Ivanenko A, Johnson K. Sleep in children with autistic spectrum disorder. *Sleep Med* (2010) 11(7):659–64. doi: 10.1016/j.sleep.2010.01.010

19. Hirata I, Mohri I, Kato-Nishimura K, Tachibana M, Kuwada A, Kagitani-Shimono K, et al. Sleep problems are more frequent and associated with problematic behaviors in preschoolers with autism spectrum disorder. *Res Dev Disabil* (2016) 49–50:86–99. doi: 10.1016/j.ridd.2015.11.002

20. Mazzone L, Postorino V, Siracusano M, Riccioni A, Curatolo P. The relationship between sleep problems, neurobiological alterations, core symptoms of autism spectrum disorder, and psychiatric comorbidities. *J Clin Med* (2018) 7(5):1–12. doi: 10.3390/jcm7050102

21. Hollway JA, Aman MG. Sleep correlates of pervasive developmental disorders: a review of the literature. *Res Dev Disabil* (2011) 32(5):1399–421. doi: 10.1016/j.ridd.2011.04.001

22. Patzold LM, Richdale AL, Tonge BJ. An investigation into sleep characteristics of children with autism and Asperger's disorder. *J Paediatr Child Health* (1998) 34:528–33. doi: 10.1046/j.1440-1754.1998.00291.x

23. Goldman SE, Surdyka K, Cuevas R, Adkins K, Wang L, Malow BA. Defining the sleep phenotype in children with autism. *Dev Neuropsychol* (2009) 34(5):560–73. doi: 10.1080/87565640903133509

24. Mayes SD, Calhoun SL. Variables related to sleep problems in children with autism. *Res Autism Spectr Disord* (2009) 3(4):931–41. doi: 10.1016/j.rasd.2009.04.002

25. Goldman SE, McGrew S, Johnson KP, Richdale AL, Clemons T, Malow BA. Sleesp is associated with problem behaviors in children and adolescents with autism spectrum disorders. *Res Autism Spectr Disord* (2011) 5(3):1223–9. doi: 10.1016/j.rasd.2011.01.010

26. Henderson JA, Barry TD, Bader SH, Jordan SS. The relation among sleep, routines, and externalizing behavior in children with an autism spectrum disorder. *Res Autism Spectr Disord* (2011) 5(2):758–67. doi: 10.1016/j.rasd.2010.09.003

27. Park S, Cho S-C, Cho IH, Kim B-N, Kim J-W, Shin M-S, et al. Sleep problems and their correlates and comorbid psychopathology of children with autism spectrum disorders. *Res Autism Spectr Disord* (2012) 6(3):1068–72. doi: 10.1016/j.rasd.2012.02.004

28. Sikora DM, Johnson K, Clemons T, Katz T. The relationship between sleep problems and daytime behavior in children of different ages with autism spectrum disorders. *Pediatrics* (2012) 130(Suppl 2):S83–S90. doi: 10.1542/peds.2012-0900F

29. Adams HL, Matson JL, Jang J. The relationship between sleep problems and challenging behavior among children and adolescents with autism spectrum disorder. *Res Autism Spectr Disord* (2014) 8(9):1024–30. doi: 10.1016/j.rasd.2014.05.008

30. Nadeau JM, Arnold EB, Keene AC, Collier AB, Lewin AB, Murphy TK, et al. Frequency and clinical correlates of sleep-related problems among anxious youth with autism spectrum disorders. *Child Psychiatry Hum Dev* (2015) 46(4):558–66. doi: 10.1007/s10578-014-0496-9

31. Cohen S, Fulcher BD, Rajaratnam SMW, Conduit R, Sullivan JP, St Hilaire MA, et al. Sleep patterns predictive of daytime challenging behavior in individuals with low-functioning autism. *Autism Res* (2018) 11(2):391–403. doi: 10.1002/aur.1899

32. May T, Cornish K, Conduit R, Rajaratnam SM, Rinehart NJ. Sleep in high-functioning children with autism: longitudinal developmental change and associations with behavior problems. *Behav Sleep Med* (2013) 13(1):2–18. doi: 10.1080/15402002.2013.829064

33. Malow BA, Marzec ML, McGrew SG, Wang L, Henderson LM, Stone WL. Characterizing sleep in children with autism spectrum disorders: a multidimensional approach. *Sleep* (2006) 29(12):1563–71. doi: 10.1093/sleep/29.12.1563

34. Fadini CC, Lamonica DA, Fett-Conte AC, Osorio E, Zuculo GM, Giacheti CM, et al. Influence of sleep disorders on the behavior of individuals with autism spectrum disorder. *Front Hum Neurosci* (2015) 9:1–8. doi: 10.3389/fnhum.2015.00347

35. Matson JL, Wilkins J, Macken J. The relationship of challenging behaviors to severity and symptoms of autism spectrum disorders. *J Ment Health Res Intellect Disabil* (2008) 2(1):29–44. doi: 10.1080/19315860802611415

36. Jang J, Dixon DR, Tarbox J, Granpeesheh D. Symptom severity and challenging behavior in children with ASD. *Res Autism Spectr Disord* (2011) 5(3):1028–32. doi: 10.1016/j.rasd.2010.11.008

37. Tudor ME, Hoffman CD, Sweeney DP. Children with autism: sleep problems and symptom severity. *Focus Autism Other Dev Disabl* (2012) 27(4):254–62. doi: 10.1177/1088357612457989

38. Andersen PN, Hovik KT, Skogli EW, Oie MG. Severity of autism symptoms and degree of attentional difficulties predicts emotional and behavioral problems in children with high-functioning autism: a two-year follow-up study. *Front Psychol* (2017) 8:1–9. doi: 10.3389/fpsyg.2017.02004

39. Schreck K. Sleep problems as possible predictors of intensified symptoms of autism. *Res Dev Disabil* (2004) 25(1):57–66. doi: 10.1016/j.ridd.2003.04.007

40. Hundley RJ, Shui A, Malow BA. Relationship between subtypes of restricted and repetitive behaviors and sleep disturbance in autism spectrum disorder. *J Autism Dev Disord* (2016) 46(11):3448–57. doi: 10.1007/s10803-016-2884-4

41. Thenhausen N, Kuss M, Wiater A, Schlarb AA. Sleep problems in adolescents with Asperger syndrome or high-functioning autism. *Somnologie* (2016) 21(S1):28–36. doi: 10.1007/s11818-016-0078-0

42. Sparrow SS, Cicchetti DV, Saulnier CA. *Vineland adaptive behavior scales, third edition (Vineland -3)*. Bloomington: NCS Pearson (2016).

43. Constantino JN, Gruber CP. *Social responsiveness scale—second edition (SRS-2)*. Torrance, CA: Western Psychological Services (2012).

44. Owens JA, Spirito A, McGuinn M. The children's sleep habits questionnaire (CSHQ): psychometric properties of a survey instrument for school-aged children. *Sleep* (2000) 23(8):1–9. doi: 10.1093/sleep/23.8.1d

45. Achenbach TM, Rescorla LA. *Manual for the ASEBA preschool forms & profiles*. Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families (2000).

46. Achenbach TM, Rescorla LA. (2001). *Manual for the ASEBA School-Age Forms & Profiles*. Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families.

47. Hayes AF. (2013). Introduction to mediation, moderation, and conditional process analysis: a regression-based approach. New York: The Guildford Press.

48. Papadopoulos N, Sciberras E, Hiscock H, Mulraney M, McGillivray J, Rinehart N. The efficacy of a brief behavioral sleep intervention in school-aged children with ADHD and comorbid autism spectrum disorder. *J Atten Disord* (2019), 23(4):341–50. doi: 10.1177/1087054714568565

Heterogeneities in Cognitive and Socio-Emotional Development in Children With Autism Spectrum Disorder and Severe Intellectual Disability as a Comorbidity

Marie-Anna Bernard Paulais[1,2], Camilla Mazetto[1,3,4], Eric Thiébaut[5], Maria Clara Nassif[3], Maria Thereza Costa Coelho De Souza[4], Ana Paula Stefani[3], Romuald Blanc[1,6], Maria Pilar Gattegno[1,7], Fethia Aïad[8], Nadia Sam[8], Lina Belal[9], Laid Fekih[10], Kelley Kaye[11], Yves Contejean[11], Jaqueline Wendland[1], Catherine Barthélémy[6], Frédérique Bonnet-Brilhault[6] and Jean-Louis Adrien[1]*

[1] Laboratory of Psychopathology and Health Processes (EA n°4057), Institute of Psychology, University of Paris, Paris, France, [2] Psychology Office ESPAS-IDDEES, Pont-Ste-Maxence and Bordeaux, France, [3] CARI Psichology and Education, São Paulo, Brazil, [4] Institute of Psychology, University of São Paulo, São Paulo, Brazil, [5] Lorrain Laboratory of Psychology and Neurosciences of Behaviors' Dynamics (2LPN), University of Lorraine, Nancy, France, [6] UMR 1253, iBrain, University of Tours, INSERM, Tours, France, [7] Psychology Office ESPAS-IDDEES, Bordeaux, France, [8] Language, Cognition and Interaction Laboratory, University of Blida 2 Lounici Ali, Blida, Algeria, [9] Faculty of Social and Human Sciences, University Abdelhamid Ibn Badis, Mostaganem, Algeria, [10] Laboratory of Psychometric and Its Applications, University Abou Beker Belkaid, Tlemcen, Algeria, [11] Child Psychiatry Department of Sainte Anne Hospital, CREDAT, Paris, France

Introduction: Intellectual disability (ID) is frequently associated as a comorbidity in autism spectrum disorders (ASD). This study investigated a) how similar the heterogeneity in the cognitive and socio-emotional developmental profiles was for children with ASD and ID, b) the difference between the subjects' profiles and those of typically developing children (TD) matched for developmental levels, c) the skills existing with the lowest and highest developmental levels, and d) the relationship between developmental profiles in ASD and the severity of autism, ID, and the overall developmental level.

Participants: The sample was comprised of 119 children (101 boys and 18 girls) who ranged in chronological age (CA) from 21 months to 14 years (M = 5 years 2 months; SD = 2 years 6 months) with developmental levels lower than 24 months. They came from three countries (France = 40, Brazil = 40, and Algeria = 39). The control group was comprised of 40 TD children from these same countries who ranged in CA from 4 to 24 months (M = 1 year 3 months; SD = 5 months). The ASD diagnosis was carried out according to International Statistical Classification of Diseases and Related Health Problems-10th Edition (ICD-10), Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR), Diagnostic and Statistical Manual of Mental Disorders-5th ed (DSM-5) criteria and the Childhood Autism Rating Scale (CARS).

Measures: Children were tested using the Social Cognitive Evaluation Battery (SCEB; Adrien, 2007) by trained psychologists from public and private institutions specialized in the diagnosis of autism and interventions in this field. The SCEB explores 16 functional

abilities, in both cognitive and socio-emotional areas, and allows the calculation of domain and area developmental levels and heterogeneity indices for the global, cognitive, and socio-emotional areas.

Results: Children with ASD developmental profiles show very high heterogeneity as opposed to TD children. Regardless of the country of origin, there are similarities between the heterogeneous cognitive and socio-emotional developmental profiles of the children with ASD, whose profiles are characterized by lower developmental levels of language and vocal imitation skills, and a relationship between these developmental heterogeneities and the degree of severity of autistic symptomatology, intellectual disability, and overall development level. The implications of this study are presented for clinical assessment and intervention purposes in ASD and ID.

Keywords: autism spectrum disorder, intellectual disability, Comorbidity, heterogeneities, cognitive and socioemotional developmental profiles, The Social Cognitive Evaluation Battery

INTRODUCTION

Autistic spectrum disorder (ASD) is characterized by disturbances in social interaction, communication and repetitive activity Diagnostic and Statistical Manual of Mental Disorders-5th ed. (DSM-5) (1). The continuum of autism is identified and diagnosed by assessing not only the nature of the disorder (including hyper- and hypo-sensory reactivity) but also its severity. Intellectual disability (IQ less than 70), previously referred to as "mental retardation" (2) and with a wide variability in profiles (3) affects more than half of the children with autism (4, 5) but Christensen et al. (6) showed that the percentage of children with an intellectual disability (ID) varied widely across 9 out of 11 geographic areas, ranging from 20 to 50%, and significantly more girls had ASD associated with ID (37%) than boys (30%). Using the criteria of DSM-5, Brown et al. (7) reported IQ prevalence and indicated that about 40% of the ASD population are likely to present ID. In the present study, differences and similarities in the cognitive and socio-emotional developmental profiles of children with ASD and comorbid ID as compared with those of typical children were examined across three countries (France, Brazil, and Algeria).

Clinical heterogeneity is a notable characteristic of ASD. Lombardo et al. (8) noted that this heterogeneity is present at several levels of analysis such as genetics, neural systems, cognition, behavior, and development, as well as in clinical features (e.g., response to treatment, outcome). From a developmental psychopathology approach, the heterogeneity in question encompasses different variations between and within individuals relative to their developmental, cognitive, and behavioral characteristics. Between individuals with ASD, developmental heterogeneity may be characterized by some different developmental trajectories (9) and outcomes relative to verbal and nonverbal abilities (10) and intellectual and socio-adaptive development (11). Within individuals, heterogeneity may be defined at a first level in terms of functional dysregulation in using mental representation, symbolic play, social communication, and sensory-motor abilities (12–21). Moreover, at a second level, heterogeneity is defined by some

atypical differences in developmental stages between several abilities, explained by the changes in the timing and rates of infant and child development, corresponding to the unevenness or developmental heterochrony (22).

This type of heterogeneity was already attested in children with autism by a large number of studies centered on verbal and nonverbal communication, sensorimotor, and cognitive functions profiles, showing evidence of atypical patterns, discrepancies, and unusual correlates between developmental levels of various cognitive and socio-communicative skills (23–28). Moreover, in the intellectual domain, the profiles of children with autism are usually characterized by a discrepancy between nonverbal abilities and verbal abilities (NVIQ > VIQ). This discrepancy lessened with age in children with functional language and overall cognitive abilities in the mildly impaired range or above (29). Moreover, it was specifically associated with a high level of symptoms in the social domain (30) and correlated to the intensity of autistic symptomatology (31). Most of these works described this developmental heterogeneity in children with ASD whose developmental ages were above 2 years of age (29, 32, 33). However, only a few studies describe the cognitive and socio-emotional profiles of children with developmental levels under 2 years of age with a moderate or severe ID. Thiébaut et al. (34) showed evidence of heterogeneous developmental cognitive and socio-emotional profiles in these children with strong inter- and intraindividual variability and developmental delay contrary to what is observed in children with ID without ASD (35).

Cross-cultural studies in ASD are still recent and have focused on different ways of thinking and understanding autism as a disorder (36, 37) and on the diversity of symptoms, characteristics, or traits of ASD using screening tools (38–40) and on social skills interventions (41). The heterogeneity of the developmental profile of children with ASD across several countries in North and South America, North Africa, and Europe (42–44) was pointed out. While no significant differences in overall, cognitive and socio-emotional development levels between two groups of young (1 year and 6 months to 3 years of age) and older children

(8–14 years of age) with ASD from the same countries were observed, the youngest group of children exhibited a greater socio-emotional heterogeneity (45). However, the samples of children in each of the seven countries were small and very different from one another. Therefore, in this study, using larger and similar-sized samples of children, we sought to examine whether the heterogeneity of the cognitive and socio-emotional developmental profile in children with ASD and comorbid ID from three countries was different from typical children, had the same intensity, was independent of the country of origin, and correlated to the severity of autistic symptomatology and of ID and the overall developmental level.

METHOD

Participants

The total sample of participants with ASD included 119 children (101 boys and 18 girls; gender ratio = 5.61:1; mean age = 5 years 3 months, from 1 year 9 months to 14 years of age; SD = 2 years 6 months) from three countries, Brazil, Algeria, and France. Clinical data were collected for 40 Brazilian children with ASD at the CARI Psychology and Education Clinic and at the Centro Pró-Autista, both in the city of São Paulo, where children received a diagnostic assessment and a neuropsychological and developmental program (46). There were also 39 Algerian children who were referred by several private and public institutions providing behavioral, integrative, and psycho-educational interventions to children with autism. Clinical data for an equivalent sample of 40 French children with ASD were taken from databases of different clinical services and used in some previous studies (34, 42, 47). The French children with ASD were treated in the Child and Adolescent Psychiatry Department of the University Hospital Center Bretonneau in Tours, in the Psychology Offices ESPAS-IDDEES, and in the Child Psychiatry Department of Sainte Anne Hospital in Paris. As a control group, data from 40 children with typical development (TD) was gathered (Algeria $N = 13$, Brazil $N = 13$, and France $N = 14$) randomly from the same database. The subjects were recruited from public nurseries and in the professional or social environment of psychologists. All the children were assessed in the country where they lived and in their native language. In an analysis of variance, with four groups of 40 participants, the probability of detecting an average effect (48) at the α threshold of 0.05 is 0.98. For a correlational study, with 40 participants per group, the probability of detecting an average effect ($r = 0.3$) at the α threshold of 0.05 is 0.90. Groups of 40 participants would then seem sufficient to detect an average effect with reasonable success.

To obtain background information about each participant, information on age, gender, and diagnostic status (where, when, and by whom a diagnosis had been made) was used. The diagnosis for children younger than 3 years ($N = 20$) was confirmed some months or years later. All children included in our clinical sample were diagnosed with autism disorder according to the criteria of International Statistical Classification of Diseases and Related Health Problems-10th Edition (ICD-10) (49) and Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text

Revision (DSM-IV) and confirmed as ASD subjects based on DSM-5 criteria (1) and assessed with the Childhood Autism Rating Scale (CARS) (51). Intellectual disability (ID) diagnosis was established from developmental assessments with the Socio-emotional and Cognitive Evaluation Battery (SCEB) (47) for all the children, thus avoiding possible variations in the data due to the use of several different tests. Developmental Quotient (DQ) was calculated based upon the existence of very high correlations between the overall scores (not corrected for unreliability) of the SCEB and the psychomotor development ages calculated with the Psychomotor Development Scale of Brunet–Lézine Revised (52), a French adaptation of Gesell developmental scales (53), which included assessments on postural, verbal, nonverbal, and sociability domains.

The diagnoses of ASD and ID as a comorbidity were performed by child psychiatrists and psychologists experienced in ASD and other neurodevelopmental disorders. Demographic information of participants is shown in **Table 1**.

Some differences (two-sided *p* value) appeared between ASD groups for chronological age [$F(2, 115) = 6.44$, $p = 0.002$]. The Brazilian group was slightly younger (from 4 to approximately 6 years of age) than the other two groups (from 5 to 14 years of age).

Although the children were recruited from a variety of settings, the developmental evaluation of each child was organized at the start for a diagnostic decision or for monitoring his/her evolution during overall and intensive psycho-educational care. Psychological evaluations were recorded in a written report. The study was carried out in accordance with official laws[1] and standards of ethics, biomedical, and clinical research in France. In Algeria, it was done with the University Charter of Ethics and Deontology of the Algerian Ministry for Higher Education and Research[2], and in Brazil, the study was approved by the National Commission for Research Ethics (CONEP) under the aegis of the Brazilian Ministry of Health[3]. All data were anonymized. Written and informed consent was obtained from the legal guardians who were assured of the noninvasive nature of the research and the confidentiality of the data. Furthermore, the systematic use of video recordings during evaluations was subject to written consent from the families.

Material

The Childhood Autism Rating Scale (CARS) (51) was rated by trained psychologists experienced in ASD who carried out the developmental assessments of the children participating in the study. Rating was carried out at the end of the examination.

All assessments of the development of children in the study were performed using the Socio-emotional Cognitive Evaluation Battery (SCEB) (47), an instrument specifically created for the examination of preschool and school-aged children with autism and intellectual disability and recommended by the French High

---

[1] Order no 2017-884 of 9 May 2017 modifying some regulatory dispositions relative to research implying human people. *Official Journal of French Republic*, 10 May 2017, Text 84 on 396.

[2] Universitary Ethic and Deontological Chart. *Superior Teaching and Research Ministry*, 2010. Algeria.

[3] Process No. 306.264, National Commission for Research Ethics (CONEP). *Brazilian Ministry of Health*, 2015, Brazil.
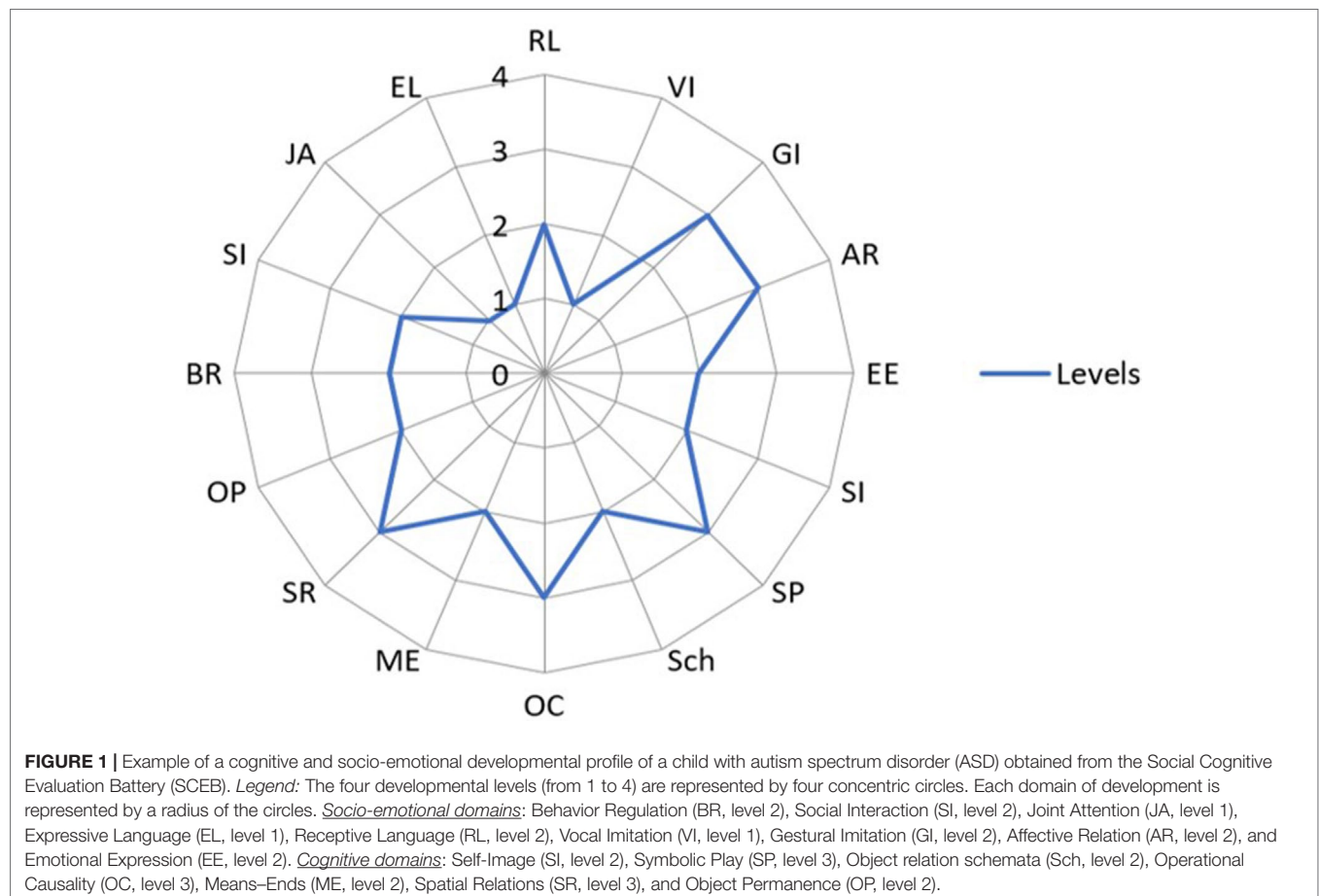
TABLE 1 | Chronological age (year-yr and month-mth) in autism spectrum disorder (ASD) groups (N = 119) and typical development group (N = 40).

Country	Chronological age (years and months)			
	Mean	SD	Min.	Max.
Algeria (*N* = 39)	6 yr, 3 mth	2 yr, 3 mth	2 yr, 2 mth	12 yr, 4 mth
Brazil (*N* = 40)	4 yr, 4 mth.	1 yr, 2 mth	2 yr, 9 th.	6 yr, 7 mth
France (*N* = 40)	5 yr, 3 mth	3 yr, 3 mth	1 yr, 9 mth	14 yr, 0 mth
Total (*N* = 119: 101 male, 18 female)	5 yr, 3 mth	2 yr, 6 mth	1 yr, 9 mth	14 yr, 0 mth
Typical development (*N* = 40)	1 yr, 3 mth	5 mth	4 mth	2 yr., 0 mth

Health Authority (54, 55). This battery can be used to examine children who have a developmental level between 4 and 24 months and to assess both the cognitive area, including seven domains such as self-image, symbolic play, object-relation schemata, operational causality, means–ends relations, spatial relations and object permanence, and the socio-emotional area, including nine domains such as behavior regulation, social interaction, joint attention, expressive language, receptive language, vocal imitation, gesture imitation, affective relations, and emotional expression. Based on the Piaget and Fisher models of child development (56, 57), this assessment tool determines the developmental level in each of 16 domains, according to a hierarchical list of items for each developmental level: level 1 (4–8 months), level 2 (8–12 months), level 3 (12–18 months), and level 4 (18–24 months).

Each item was rated, either as grade 2 (= complete success), grade 1 (= emergence or relative success with a bit of help and a demonstration), or grade 0 (= failure in spite of some help and a demonstration). The developmental level reached by the child in a domain corresponds to a level in which at least one of the items among the higher level was graded 1. A developmental level score from 1 to 4 was determined for each of the 16 domains, and this provides a developmental profile for each child (**Figure 1**).

The overall average levels, as well as both cognitive and socio-emotional development and indices of heterogeneity of profile for overall, cognitive and socio-emotional domains, were calculated. These indices corresponded to the mean difference (in absolute values) between all the level scores (1–4) of each domain multiplied by 10. They ranged from 0 (no heterogeneity) to 16

FIGURE 1 | Example of a cognitive and socio-emotional developmental profile of a child with autism spectrum disorder (ASD) obtained from the Social Cognitive Evaluation Battery (SCEB). *Legend:* The four developmental levels (from 1 to 4) are represented by four concentric circles. Each domain of development is represented by a radius of the circles. *Socio-emotional domains*: Behavior Regulation (BR, level 2), Social Interaction (SI, level 2), Joint Attention (JA, level 1), Expressive Language (EL, level 1), Receptive Language (RL, level 2), Vocal Imitation (VI, level 1), Gestural Imitation (GI, level 2), Affective Relation (AR, level 2), and Emotional Expression (EE, level 2). *Cognitive domains*: Self-Image (SI, level 2), Symbolic Play (SP, level 3), Object relation schemata (Sch, level 2), Operational Causality (OC, level 3), Means–Ends (ME, level 2), Spatial Relations (SR, level 3), and Object Permanence (OP, level 2).

(maximum heterogeneity). An example of index calculation is presented in **Table 2**.

The transformation of the development level score into a developmental quotient (DQ) is based here on two empirical findings, namely, the existence of very high correlations on the one hand between the scores (not corrected for unreliability) of the SCEB and those of Brunet–Lézine Revised (52) for both a sample of children with a diagnosis of ASD [$r(91) = 0.87$, p < 0.001] and an international sample of young typical children [$r(71) = 0.90$, p < 0.001], and on the other hand, between SCEB scores and the chronological age observed for a sample of young typical French children [$r(104) = 0.94$, p < 0.001] and of young typical Brazilian children [$r(20) = 0.70$, p < 0.0001] (unpublished data). As no Algerian children of this study were tested with the Brunet–Lézine Scale and no typical young Algerian children were tested with the SCEB, correlations between these variables could not be calculated. However, as the correlations' coefficients for children with ASD and for international samples of young typical children were high, it was assumed that these relationships might be applied to this group of children.

These observations rely on different processes to determine a developmental quotient from a SCEB overall developmental score: 1) according to the parameters (slope and intercept) of the regression equation between the sum of 16 SCEB developmental scores and the Brunet–Lézine Developmental Age (the equation is the following: developmental age, DA (in month) = 0.414 × the sum of the scores on SCEB scales + 2.21) (47) or 2) by simply rescaling the SCEB scores (level 0–4) on a scale from 0 to 24 months (ratio, 6). The DAs, as defined with the equation or rescaling, are perfectly correlated. This DA is divided by the chronological age (CA) in months. The product of this ratio is then multiplied by 100 to obtain a developmental quotient [DQ = (DA/CA) × 100]. It is this last possibility that is being applied in the current study. Thus, Development Quotients are: overall scores SCEB (0–4) rescaling on 0–24 (months) (DA) divided by chronological age (CA) and multiplied by 100 (**Table 3**).

All scores of the SCEB presented appropriate reliability and validity according to the usual psychometric criteria (34, 47).

The mean sample score of ASD groups on overall level of development was 2.83 corresponding to the 12–18 months development stage. On Developmental Quotients, it was 32, corresponding to severe ID, and on the CARS scale, it was 39.00, which falls within the "severely autistic" degree (**Table 3**).

There were no significant differences between the TD and the ASD groups for number of subjects per gender [$\chi^2(1, N = 159) = 3.07$, p = 0.079], country [$\chi^2(2, N = 159) = 0.03$, p = 0.985], and the overall development level score on SCEB [$F(1, 157) = 0.94$, p = 0.334].

TABLE 2 | An example of the calculation of Heterogeneity Index: for the Cognitive Heterogeneity Index (CHI).

Domains	Cognitive	OP	SR	ME	OC	Sch	SP	SI	Sum of differences (Σ) In absolute value
Cognitive	Levels	2	3	2	3	2	3	2	
OP	2		1	0	1	0	1	0	3
SR	3			1	0	1	0	1	3
ME	2				1	0	1	0	2
OC	3					1	0	1	2
Sch	2						1	0	1
SP	3							1	1
SI	2								
						Total			12
						Mean = Σ ÷ 21			12 ÷ 21 = 0.57
						CHI = MOY × 10			0.57 × 10 = 5.7

Cognitive, cognitive domains; OP, object permanence; SR, spatial relations; ME, means–ends; OC, operational causality; Sch, object relation schemata; SP, symbolic play; SI, self-image.

TABLE 3 | Mean overall level of development (SCEB) and development quotients and CARS scores of children with ASD by country and of children with typical development (TD).

Country	Overall level of development		Development quotients		CARS scores			
	Mean	SD	Mean	SD	Mean	SD	Min	Max
Algeria N = 39	2.73	0.91	24.58	7.22	42.56	7,22	30	57
Brazil N = 40	3.11	0.75	38.52	6.08	37.89	6,08	30	56
France N = 40	2.66	0.46	34.24	3.33	36.95	3,33	30	42.5
Total (ASD) N = 119 (101 male; 18 female)	2.83	0.75	32.51	6.22	39.11	6.22	30	57
TD N = 40	2.97	0.88	119.97	14.93	–	–	–	–

Some differences (two-sided p value) were noticeable between ASD groups: the CARS score [$F(2, 115) = 10.3$, $p < 0.001$], the developmental quotient [$F(2, 115) = 8.9$, $p < 0.001$], and the overall level of development (SCEB) [$F(2, 115) = 4.15$, $p = 0.02$]. There was no significant sex difference for age [$F(1, 117) = 1.38$, $p = 0.243$], CARS scores [$F(1, 117) = 0.31$, $p = 0.578$], overall levels of development [$F(1, 117) = 1.05$, $p = 0.308$], or developmental quotients [$F(1, 117) = 0.85$, $p = 0.359$].

Procedure

Each participant was accompanied by his/her parents to the medical or psychological clinical service in order to perform the examination. The child was examined in a single 30–45-min session in a suitable room by a psychologist experienced with children presenting ASD and ID and familiar with the SCEB material. The SCEB was administered in the same naturalistic manner to all participants, and assessment protocols were respected at all times. As such, different children had comparable experiences during testing. There was no strict order for the presentation of the material, and the examiner chose toys and objects according to child's interest. With patience and determination, he/she captured the child's attention by showing the material (one activity at a time) and by inviting the child to use it. The psychologist interacted with the child by inviting him/her to make verbal and nonverbal contact with him/her and to manipulate the test material. He/she observed the child's behaviors, including response and initiating interactions, objects and toy manipulation, vocalizations, words and sentences of two or more words, facial expressions, imitation, and joint attention behaviors. Since each child's assessment session was videotaped, the examiner was able to review the recordings in order to rate the items corresponding to the observed behaviors. Complementary information was obtained from the family or educators after the examination, in particular for the Emotional Expression and Affective Relations domains. In fact, these domains included behaviors mainly expressed in the presence of parents or teachers, such as "He/she can recognize and differentiate between his/her parents" or "He/she delights in provoking the favorite person."

The study protocol resulted in the establishment of research agreements with universities and specialized clinical services for the assessment and/or psycho-educational support of children with autism. The retrospective data collection from experienced psychologists, already specifically trained to use the SCEB instrument, was carried out in France. Concerning the recruitment and assessment of other children in the clinical population (in Brazil and Algeria), research university collaborations were developed in the use of the SCEB in clinical services in each country. Before we started the study in Algeria and Brazil, and to control for the cross-cultural contexts, we ensured that the SCEB was easily usable for all children regardless of their culture. As a first step, psychologists from each of these countries assessed a few children with ASD and then were able to confirm that the SCEB could be easily and correctly used without adaptation. On the one hand, the material is very common and familiar to children (toys such as cars, dolls and blocks…), and on the other hand, the vocal, verbal (e.g., "give me the car," "look at pictures," "show me the ball"), and nonverbal instructions (e.g., "point to a picture or an object," "imitate a gesture of clapping," "look at the mirror," "hide an object under a box") are simple and very accessible to and understood by children across these cultures. Moreover, psychologists were specifically trained (individual and group seminars directed by the first and last authors) on the use of the SCEB and continuously supervised throughout the duration of the research (ratings of SCEB sessions from videotapes). Double ratings of five Brazilian and five Algerian SCEB protocols were made by the first author to obtain a complete interagreement for each (99%). Each of these five protocols was initially rated respectively by the Brazilian and Algerian psychologists who had examined the child using the SCEB in his/her native country's clinical service. The second rater (first author) then watched the videotapes and rated the child's behaviors independently in the Laboratory of Psychopathology and Health Processes (Paris). During these double ratings, the Brazilian or the Algerian psychologist was present during viewings with instructions to help only for the understanding of verbal words and sentences the child pronounced. The two domains of Emotional Expression and Affective Relations could not be double-rated because their items were mainly rated through information from parents or teachers. However, the information was discussed by both psychologists and approved by the second rater who could then validates the rating of items and the scoring of developmental levels.

Analysis

Developmental levels on the 16 SCEB scales correspond to developmental age periods: level 1 (4–8 months), level 2 (8–12 months), level 3 (12–18 months), and level 4 (18–24 months). Nevertheless, for the present samples, the distributions of the SCEB scores are not random samples of a Gaussian distribution. This is not the case with the overall level of development. The heterogeneity index, CARS score, overall level of development, Developmental Quotient, and age are continuous variables, and the Kolmogorov–Smirnov test shows no significant difference with normal distribution. No outliers were observed that were more than 3.5 standard deviations away for each group. When the variables could not be considered as normally distributed continuous variables, nonparametric tests were used such as the Spearman rank correlation, the Wilcoxon signed-rank test that is a nonparametric test equivalent to the dependent t-test, and also the Friedman rank sum test (58). The latter is a nonparametric alternative to the one-way ANOVA with repeated measures. It is used to test for differences between repeated measures when the dependent variable being measured is ordinal. It can also be used for continuous data that has gone against the assumptions necessary to run the one-way ANOVA with repeated measures. Pairwise comparisons were made with probability adjustment for the number of tests according to the Bonferroni method. Data analyses were performed with R Development Core Team Software (59). The comparison of heterogeneity levels of development profiles between ASD and TD groups uses global,

cognitive, and socio-emotional heterogeneity indices and is also based on a more basic level of information. The most detailed information is obtained, group by group, with the identification and counting of all significant differences that appear for 120 possible comparisons [(16 × 15)/2] between the scores on the 16 SCEB scales (within-subjects factor). The number of significant differences (according to an alpha threshold of .05) is then compared between ASD and TD groups. The identification of the functions that collect the lowest and the highest scores makes it possible to search for regularities according to country.

Analysis steps

1. We first tested the hypothesis of differences between ASD and TD groups on the variability on SCEB scores (within-subjects factor). We assume that the number of significant differences between the scores on the 16 scales will be greater for the ASD groups than for the TD group.
2. We checked for regularity between three ASD groups regarding development scales that show the lowest and highest levels. We postulate that some differences will be noticeable in a few cognitive and socio-emotional domains.
3. We tested the null difference hypothesis between groups from different countries in mean indices of heterogeneity profiles, but significant differences are expected between the ASD and TD groups.

In order to test this assumption, a hypothesis of no difference in indices of heterogeneity profiles by country was tested using analysis of variance. There was an overall index of heterogeneity for everyone. The difference between heterogeneity indices on the "cognitive" and "socio-emotional" scales was also computed; a positive value of this difference for a given subject indicates greater heterogeneity in cognitive aspects than in socio-emotional aspects. The range of the socio-emotional and cognitive heterogeneity scales was the same. A difference between socio-emotional and cognitive heterogeneity is relevant, and intergroup analysis with a single dependent variable (score difference) was performed because it is simpler and more powerful (the difference score reduces intragroup variability) than an interaction analysis with two dependent variables (within variable) and an intermediate variable.

4. We tested the hypothesis of no differences between groups in difference of cognitive and socio-emotional heterogeneity indices. We expected no significant difference between all the groups.
5. We checked if there was a relationship among heterogeneity, chronological age, degree of severity of autism (CARS), degree of severity of ID (Development Quotient), and overall level of development. We assumed significant correlations for ASD groups but no significant relationship between heterogeneity, chronological age, and overall level of development for the TD group. It was expected that correlation coefficients would be similar according to nationality for the ASD groups.
6. We tested the hypothesis of a relationship between heterogeneity indices, each domain's developmental levels, and the severity of autistic symptomatology.

RESULTS

Testing the Hypothesis of Differences Between the ASD and the TD Groups Corresponding to Differences on the SCEB Scores (Within-Subjects Factor)

A comparison of differences on the 16 domains SCEB scores was conducted independently for the four groups. Then, the number of cases in which a difference was observed was compared between the groups. Median developmental level scores in domains on the SCEB for four groups are presented in **Figure 2.**

The results show significant differences between scores on the 16 SCEB domains for each group, with lower effect for the typical group [typical: Friedman χ^2(15, $N = 40$) = 90.41, $p < 0.001$; Algeria: Friedman χ^2(15, $N = 39$) = 162.01, $p < 0.001$; France: Friedman χ^2(15, $N = 40$) = 244.27, $p < 0.001$; Brazil: Friedman χ^2(15, $N = 40$) = 150.83, $p < 0.001$].

The results of the pairwise comparisons ($n = 120$) using the Wilcoxon signed-rank test with a probability adjustment (for the number of tests) according to the Bonferroni method are used to compare the number of significant differences [alpha (two-sided): 0.05] between the SCEB scores between the groups. The number of cases in which a difference was observed was compared between the groups using the χ^2 test (**Table 4**).

The χ^2 in the table shows significant variations [χ^2 (3, $N = 480$) = 40.96, $p < 0.001$]. The main contributions to χ^2 appear with the typical group, as expected, with a lower number of significant differences, while the French group showed the highest number of significant differences. The heterogeneity of profiles is therefore much more significant for all of the ASD groups than for the TD group of children.

Testing the Hypothesis of No Differences Between Developmental Levels in All 16 Domains

The results of the pairwise comparisons (two-tailed probability of a Z value of Wilcoxon signed-rank test adjusted with the Bonferroni method) showed some regularity in the three ASD groups. The lowest average developmental levels were systematically observed in the Expressive Language and Vocal Imitation domains. Thus, they differed more significantly ($\alpha < 0.05$) than other SCEB domains.

— In the Algerian group, Expressive Language differed significantly from all the other domains except for Vocal Imitation, Gestural Imitation, and Receptive Language; Vocal Imitation did not differ from the Expressive Language and Gestural Imitation domains.
— In the French group, Expressive Language differed significantly from 7 of the other domains, and Vocal Imitation differed from 9 of the 15 other domains.
— In the Brazilian group, Expressive Language differed significantly from 6 of the other domains, and Vocal Imitation differed from 5 of the 15 other domains.

The domains that showed the highest developmental levels varied according to the groups, but they always involved the cognitive area,
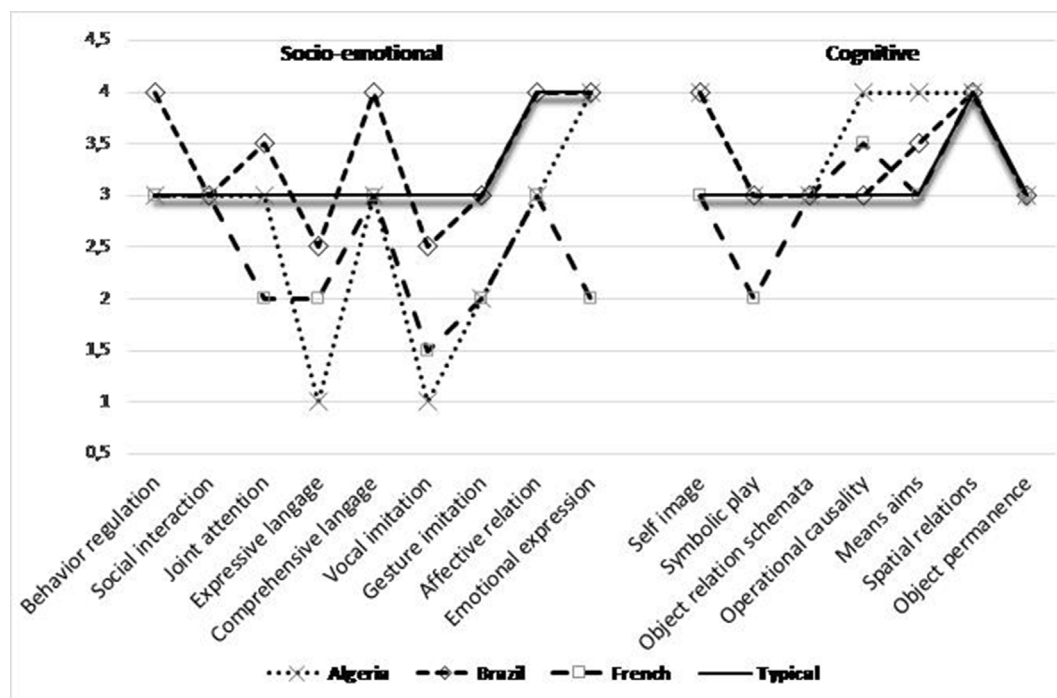
FIGURE 2 | Profiles of median developmental level scores (from 1 to 4) in all the 16 cognitive and socio-emotional domains on SCEB, for the three groups and for French children with ASD as well as the typical development groups from Algeria and Brazil. *Legend:* <u>Socio-emotional domains</u>: BR, Behavior Regulation, SI, Social Interaction, JA, Joint Attention, EL, Expressive Language; RL, Receptive Language; VI, Vocal Imitation; GI, Gestural Imitation; AR, Affective Relation; EE, Emotional Expression. <u>Cognitive domains</u>: SI, Self-Image; SP, Symbolic Play; Sch, Object relation schemata; OC, Operational Causality; ME, Means–Ends; SR, Spatial Relations; OP, Object Permanence.

TABLE 4 | Size of significant (α = .05) and insignificant differences on 16 SCEB scores by group.

Group	n.s.	Significant differences
Algeria	82	38
χ² components	0.23	0.60
Brazil	83	37
χ² components	0.14	0.37
France	69	51
χ² components	3.54	9.14
Typical	112	8
χ² components	7.52	19.41

n.s, non-significant.

with Means–Ends in the Algerian group (differing significantly from 6 of the other domains), Operational Causality and Spatial Relations in the French group (differing respectively from 11 of the other domains), and Spatial Relations also in the Brazilian group which differed significantly from 11 of the other domains.

Testing the Hypothesis of No Differences Between Groups in Mean Indices of Heterogeneity Profiles

ANOVA results on the overall scores of heterogeneity indices of the four groups (**Table 5**) showed that there were significant differences $F(3, 155) = 17.9, < 0.001$. Pairwise comparisons using t tests with a p value adjusted with the Bonferroni method

TABLE 5 | Overall, cognitive, and socio-emotional heterogeneity indices of the three ASD groups and the TD group.

Groups	Overall heterogeneity		Cognitive heterogeneity		Socio-emotional heterogeneity	
	Mean	SD	Mean	SD	Mean	SD
Algeria (*n* = 39)	10.95	5.59	7.25	5.71	10.65	5.73
Brazil (*n* = 40)	9.49	2.45	8.07	2.77	9.07	3.63
France (*n* = 40)	8.25	4.89	7.97	4.37	8.42	6.64
Total ASD group (*n* = 119)	9.55	4.60	7.77	4.41	9.37	5.51
TD group (*n* = 40)	4.47	2.74	4.64	2.81	3.76	3.59

showed differences between each ASD group and the TD group [Algeria: $t(77) = 6.57$, $p < 0.001$; Brazil: $t(78) = 4.26$, $p < 0.001$; France: $t(78) = 8.63$, $p < 0.001$]. However, the results also showed a significant difference between the Algerian and the Brazilian ASD groups [$t(77) = 2.29$, $p = 0.025$].

Testing the Hypothesis of No Differences Between Groups Regarding the Difference of Cognitive and Socio-Emotional Heterogeneity Indices

The analysis of variance showed a significant difference between the groups [$F(3, 155) = 4.74$, $p = 0.003$]. Pairwise comparisons using t tests with p value adjusted with the Bonferroni method showed a difference only between the Algerian ASD group and the TD group [$t(77) = -3.68$, $p = 0.002$].

Testing the Relationship Between Heterogeneity, Chronological Age, Degree of Severity of Autism, Degree of Severity of Intellectual Disability, and Overall Level of Development

To estimate the homogeneity of correlations between heterogeneity and chronological age, degree of severity of autism, degree of severity of ID, and overall level of development across groups, we considered the existence of overlaps between the confidence intervals (**Table 6**). The analysis did not indicate significant differences in correlations except between the French ASD and TD groups for the overall developmental level and between both the Algerian and Brazilian ASD and TD groups for the DQ.

Moreover, we noted that heterogeneity indices were not related to chronological age. However, there is evidence of significant relationships between developmental profile heterogeneity indices and both the severity of autistic symptomatology, assessed with CARS, and overall developmental level assessed with the SCEB (negative relationship), except for the TD group. There is also a

negative and significant correlation between the degree of severity of ID (DQ) and the heterogeneity, for the Brazilian group, and a positive and significant correlation for the TD group.

Testing the Relationship Between Heterogeneity Indices and Developmental Levels of Each Domain and the Severity of Autistic Symptomatology

In Table 7, we can note Spearman's rank correlations show that overall heterogeneity links are the closest to verbal skills: Vocal Imitation, Receptive Language, and Expressive Language in ASD children. We also observe moderate links between heterogeneity and these same skills in DT children: Expressive Language and Vocal Imitation, and the cognitive domain: Operational Causality.

We also note that the closest links between CARS scores and SCEB domains occur in verbal skills, Expressive Language, Receptive Language, and Vocal Imitation, but they do not occur at all in Spatial Relations and Means–Ends.

DISCUSSION

The present study investigated whether the cognitive and socio-emotional developmental profile of children presenting ASD with comorbid ID is different from that of a group of young typical children. Moreover, we sought to identify the skills that had the lowest and the highest developmental levels and to explore whether the heterogeneity of the profiles in children with ASD is a common characteristic (intraheterogeneity), regardless of the country of origin (interheterogeneity), but is correlated to the severity of autistic symptomatology and intellectual disability and to the overall development level.

Results showed that children with ASD and ID developmental profiles were heterogeneous as opposed to typical children in all the developmental heterogeneity indices. Overall, cognitive, and

TABLE 6 | Correlations between heterogeneity, chronological age, degree of severity of autism, degree of severity of intellectual disability, and overall level of development by groups.

Country	Criteria	Heterogeneity	Adjusted two-sided p values (Holm's method)	C.I. [0.95] of Pearson r	
		Pearson r		Lower	Upper
Algeria	Age	−0.38	0.064	−0.62	−0.08
Brazil		0.05	1.000	−0.27	0.36
France		−0.16	1.000	−0.45	0.16
Typical		−0.34	0.103	−0.59	−0.03
Algeria	CARS	0.48	0.014	0.19	0.69
Brazil		0.66	<0.0001	0.44	0.80
France		0.46	0.027	0.17	0.67
Algeria	Overall level of development	−0.80	<0.0001	−0.89	−0.65
Brazil		−0.88	<0.0001	−0.93	−0.78
France		−0.44	0.036	−0.66	−0.15
Typical		−0.20	0.212	−0.48	0.12
Algeria	DQ	−0.26	0.228	−0.53	0.06
Brazil		−0.63	<0.0001	−0.79	−0.40
France		0.02	1.000	−0.29	0.33
Typical		0.44	0.020	0.14	0.66

TABLE 7 | Spearman's rank correlations between heterogeneity and overall level of development in the 16 SCEB domains for the ASD and the TD group and CARS scores for the ASD group.

	Heterogeneity (global)		CARS scores
	ASD	**TD**	**ASD**
Behavior Regulation	−0.34***	−0.18	−0.21*
Social Interaction	−0.65***	−0.22	−0.40***
Joint Attention	−0.58***	−0.16	−0.31***
Expressive Language	−0.79***	−0.48**	−0.50***
Receptive Language	−0.80***	−0.23	−0.52***
Vocal Imitation	−0.85***	−0.54***	−0.43***
Gestural Imitation	−0.75***	−0.21	−0.44***
Affective Relation	−0.25**	−0.11	−0.23*
Emotional Expression	−0.49***	−0.05	−0.22*
Self-Image	−0.34***	−0.18	−0.25**
Symbolic Play	−0.48***	−0.21	−0.22*
Object Relation Schemata	−0.51***	−0.22	−0.40***
Operational Causality	−0.34***	−0.32*	−0.34***
Means–Ends	−0.32***	−0.21	−0.08
Spatial Relations	−0.17	−0.10	−0.12
Object Permanence	−0.28**	−0.23	−0.28**

*Two-sided p values: *p < 0.05, **p < 0.01, ***p < 0.001.*

socio-emotional were significantly higher than in the typical group of children. These findings confirm Bernard's results (42) on a larger group of 119 typical children from seven countries on three continents (America, Europe, and Africa). In fact, the low heterogeneity mean indices and the limited differences between developmental levels of all the cognitive and socio-emotional domains confirm that children with typical development have a cognitive (60–62) and social-communicative (63, 64) homogeneous developmental profile. In children with ASD, atypical developmental patterns were mainly explained by relative weaknesses and developmental delays in some abilities such as joint attention, expressive and receptive language, and symbolic play (23, 25–27, 28, 65).

Moreover, we found a similar cognitive and socio-emotional development intraheterogeneity in children with ASD and ID from samples in three different countries but only a few moderate differences between them that affected mean heterogeneity indices profiles between the Algerian and Brazilian ASD groups. This heterogeneity was also evidenced by several differences between SCEB developmental level scores in ASD that turned out to be even more numerous in the French group and not only in cognitive areas but also in socio-emotional areas. Significant differences were found for Expressive Language (EL) and Vocal Imitation (VI) median developmental levels, which were the lowest. The EL and VI median developmental level scores (from 1 to 2.5 = 8–12 months of age) correspond to the prelinguistic phase when the infant can only imitate his/her own vocal productions and express monosyllabic ("ba") and bisyllabic ("tata") sounds and a few new sounds and when he/she begins to pronounce one or two words ("dad," "mum"). Language and vocal imitation deficits may be related to motor difficulties (66), to early oro-motor anticipation deficits (67), and to very early atypical vocal productions (68, 69). They may impact social communication (21) and can be explained by early hypoactivity in language-sensitive superior temporal cortices implying different outcomes for language, which is poorer in preschool children with ASD (70). Moreover,

cognitive domains demanding mainly nonverbal actions, such as using means to reach a goal or establishing causal and spatial relationships between objects, showed better developmental levels compared to expressive language and vocal imitation domains. Thus, these domain-specific developmental strengths and weaknesses might reflect the developmental heterogeneity corresponding to the nonverbal and verbal discrepancy noted in the most intellectually disabled children with ASD presenting the lowest verbal development levels (31).

In addition, there is evidence of some deficits and delays in verbal expression, which are also known to be present in toddler siblings of children with ASD (71). They are predictors of ASD and later-diagnosed ASD in infants at high risk, even though the social communication developmental pathways are variable during the 6–36-month period (72). These linguistic deficits that were common in children with other neurodevelopmental disorders without social communication deficits, such as specific language impairment (73) and intellectual disability with genetic syndromes (74, 75), might be related to common cerebral developmental deficits or dysfunctions (70, 76, 77).

Unexpectedly, a positive correlation between heterogeneity and DQ was observed in the typical group of young children. The typical children's mean DQ was superior to the normative benchmark of 100. This may be due to sampling bias given that the recruitment of typical children was done from among psychologists' acquaintances. Thus, for children with the most advanced development in our sample, there is a developmental heterochrony.

This absence of statistical differences between cognitive and socio-emotional heterogeneity indices in any of these three ASD groups shows a pattern across the three countries, although in the Algerian group, the difference between these indices appears significantly higher than in the typical group, showing a higher developmental intraheterogeneity. This could be explained by a high socio-emotional heterogeneity in this group of children, being reflected by lower developmental levels in both Expressive

Language (=1) and Vocal Imitation (=1) domains. In a transcultural cross-sectional study (39), two samples of autistic children were compared in two large Arab countries: Egypt (N = 20) and Saudi Arabia (N = 28). With regard to the behaviors and development of both groups assessed with Gilliam subscales (78), although there were significant differences in the stereotypical and developmental characteristics (Saudi children showing significantly more stereotypical and lower developmental abilities than Egyptian children), there was no significant difference between both groups regarding level of intelligence. Moreover, the Saudi group showed significantly more severe and profound communication defects as assessed with the Vineland communication subscale.

Furthermore, overall developmental heterogeneity was positively correlated with the degree of severity of autism (CARS score) and negatively correlated with the level of development for all ASD groups. Thus, the higher the developmental heterogeneity, the more severe the autistic symptomatology and the lower the overall developmental level are. A significant relationship between the degree of severity of ID (DQ) and heterogeneity was only observed for the Brazilian group. This specific relationship for the Brazilian group might be explained by intergroup age differences, the severity of autism, and the level of development, given that correlations between heterogeneity with ID controlled by differences in age, severity of autism, and levels of development were not significant for each group. Thus, these results indicate that overall developmental heterogeneity was not an effect of severity of ID but rather a characteristic of the development of children with ASD. This seems to prove the universality of this atypical development in children with ASD and comorbid ID, which is characterized by socio-cognitive developmental heterogeneity correlated to linguistic function delay, for example, in expressive and receptive language and vocal imitation.

Furthermore, while this correlation was also noted in typical children, the link was higher in ASD children and was even more intense in children with severe autistic symptomatology and with low levels of development in these language skills. The results observed in children with ASD and severe ID (25 < DQ < 35), low developmental levels (4–24 months) and important differences in chronological age (1 year and 9 months to 14 years) confirm results obtained in children with ASD without ID by Joseph et al. (30) and Ankenman et al. (31). In fact, these researchers noted that the cognitive heterogeneous profile characterized by the discrepancy between nonverbal > verbal abilities was related to a high level of autistic symptomatology and that it was higher in low developmental level children. Thus, while their manipulations and spatial skills grow with age, their functional language does not develop, so the gap between these abilities and intraindividual heterogeneity increases. In a longitudinal study, Vivanti et al. (79) showed that the more "autism specific" symptoms young children have, the more at risk they are of poor cognitive outcome. In addition, Baghdadli et al. (11) found that the developmental trajectory of socialization and communication disorders in children with ASD was associated with the worst outcomes in lower functioning children, with absence of language abilities, higher severity of autistic symptoms, and lower levels of cognitive functioning related to objects and to people.

Our result shows that heterogeneity, which is observed at the individual level, is also found at the normative level. This indicates that heterogeneity at the individual level is not random and is a true marker of the development of children with ASD. This heterogeneity appears to be stable whatever the country of origin. Thus, at the interheterogeneity level, it was shown that the SCEB function rankings according to the developmental levels are relatively similar across cultural groups, as shown by between-group correlations.

This study was essentially empirical, descriptive, and exploratory. Although psychological assessment with the SCEB is founded on theoretical and well-known models of cognitive and socio-emotional development in young children (56, 57) and based on robust data on cognitive and communicative skills in children with ASD and ID (23–27), the absence of theoretical models of typical psychological developmental cross-cultural differences prevented us from developing psychological and/or social explanations of potential differences or similarities in children with atypical development such as those presenting ASD. These differences and similarities might be explained by more specific variables, for example, the genetic, biological, cerebral, and developmental trajectory (10, 11, 70, 76) characterizing each child with ASD. Further cross-national studies should be carried out on these variables to investigate the presence and/or absence of differences and similarities in developmental profiles between children with ASD from various countries (38, 39).

No great variability in the cognitive and socio-emotional developmental profiles of children with ASD and ID was found as a function of their culture of origin. This result confirms the relevance of comparative and intercountry studies dealing with neurodevelopmental disorders such as ASD and the development of assessment instruments adapted both to this clinical subgroup of children with ASD and severe ID as a comorbidity and to each country. Some studies have already been carried out on this topic with the SCEB in other countries, such as Belgium (35) and Italy (80, 81).

Moreover, given that the developmental heterogeneity in children presenting with ASD, severe ID, and low developmental levels in expressive language and vocal imitation skills has been shown to be a major feature across these countries, this dimension must be assessed and analyzed, mainly to facilitate the development of early and appropriate interventions that are focused on these specific disabilities and are based on cognitive and socio-communicative developmental profiles. It is also important to involve the parents in early intervention (82–86) in order to decrease adaptive impairments that are mainly explained by socio-communicative ASD symptoms severity (87).

AUTHOR'S NOTE

This work was carried out based upon four doctoral theses, from FA (88), LB (89), M-AB (42), and CM (90).

AUTHOR CONTRIBUTIONS

Supervision and double rating of videotapes: M-AB, J-LA. Developmental and quantitative diagnostic data collection: M-AB, CM, MN, AS, FA, MG, RB, KK, NS, LB, LF, YC, CB, FB-B. Study design: J-LA, M-AB, ET, CM, MC. Data analysis: ET, J-LA, M-AB. Writing: J-LA, M-AB, ET, KK, MC, CM, JW.

FUNDING

ACKNOWLEDGMENTS

REFERENCES

1. APA. *Diagnostic and Statistical Manual of Mental Disorders*. 5th Edn. Washington, D.C: American Psychiatric Association (2013).

2. Schalock RL, Luckasson RA, Shogren KA, Borthwick-Duffy S, Bradley V, Buntinx WHE, et al. The renaming of mental retardation: understanding the change to the term intellectual disability. *Intellect Dev Disab* (2007) 45(2):116–24. doi: 10.1352/1934-9556(2007)45[116:TROMRU]2.0.CO;2

3. Munson J, Dawson G, Sterling L, Beauchaine T, Zhou A, Koehler E, et al. Evidence for latent classes of IQ in young children with autism spectrum disorder. *Am J Ment Retard* (2008) 113(6):439–52. doi: 10.1352/2008.113:439-452

4. Chakrabarti S, Fombonne E. Pervasive developmental disorders in preschool children: confirmation of high prevalence. *Am J Psychiat* (2005) 162(6):1133–41. doi: 10.1176/appi.ajp.162.6.1133

5. Charman T, Pickles A, Simonoff E, Chandler S, Loucas T, Baird G. IQ in children with autism spectrum disorders: data from the Special Needs and Autism Project (SNAP). *Psychol Med* (2011) 41(3):619–27. doi: 10.1017/S0033291710000991

6. Christensen DL, Baio J, Van Naarden Braun K, Bilder D, Charles J, Constantino JN, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2012. *Morb Mortal Wkly Rep Surveill Summ* (2018) 65(13):1–23. doi: 10.15585/mmwr.ss6513a1

7. Brown AC, Chouinard PA, Crewther SG. Vision research literature may not represent the full intellectual range of autism spectrum disorder. *Front Hum Neurosci* (2017) 11:57. doi: 10.3389/fnhum.2017.00057

8. Lombardo MV, Lai MC, Baron-Cohen S. Big data approaches to decomposing heterogeneity across the autism spectrum. *Mol Psychiatr* (2019). doi: 10.1038/s41380-018-0321-0

9. Fountain C, Winter AS, Bearman PS. Six developmental trajectories characterize children with autism. *Pediatrics* (2012) 129(5):1112–20. doi: 10.1542/peds.2011-1601

10. Delehanty AD, Stronach S, Guthrie W, Slate E, Wetherby AM. Verbal and nonverbal outcomes of toddlers with and without autism spectrum disorder, language delay, and global developmental delay. *Autism Dev Lang Impair* (2018) 3:1–19. doi: 10.1177/2396941518764764

11. Baghdadli A, Assouline B, Soni S, Pernon E, Darrou C, Michelon C, et al. Developmental trajectories of adaptive behaviors from early childhood to adolescence in a cohort of 152 children with autism spectrum disorders. *J Autism Dev Disord* (2012) 42:1314–25. doi: 10.1007/s10803-011-1357-z

12. Adrien JL, Tanguay P, Barthélémy C, Martineau J, Perrot A, Hameury L, et al. Autistic children and the object permanence task. *Acta Paedopsychiatr* (1993) 56:556–60.

13. Adrien JL, Martineau J, Barthélémy C, Bruneau N, Garreau B, Sauvage D. Disorders of regulation of cognitive activity in autistic children. *J Autism Dev Disord* (1995) 25(3):249–63. doi: 10.1007/BF02179287

14. Adrien JL, Rossignol N, Martineau J, Roux S, Couturier G, Barthélémy C. Regulation of cognitive activity and early communication development in young autistic, mentally retarded and young normal children. *Dev Psychobiol* (2001) 39(2):124–36. doi: 10.1002/dev.1036

15. Blanc R, Tourrette C, Delétang N, Roux S, Barthélémy C, Adrien JL. Regulation of symbolic activity and development of communication in children with autism. *Eur Rev Appl Psychol* (2000) 50(4):369–81.

16. Blanc R, Adrien JL, Roux S, Barthélémy C. Dysregulation of pretend play and symbolic communication in children with autism. *Autism* (2005) 9(3):229–45. doi: 10.1177/1362361305053253

17. Whitman TL. *The development of autism: a self-regulatory perspective*. London: Jessica Kingsley Publishers (2004).

18. Seynhaeve I, Nader-Grosbois N. Sensorimotor development and dysregulation of activity in young children with autism and with intellectual disabilities. *Res Autism Spect Dis* (2008) 2(1):46–59. doi: 10.1016/j.rasd.2007.03.001

19. Nader-Grosbois N. Self-regulation, dysregulation, emotion regulation and their impact on cognitive and socio-emotional abilities in children and adolescents with autism spectrum disorders. In: Williams T, editor. *Autism Spectrum Disorders: From Genes to Environment*. Rijeka, Croatia: Intech Open access Publishers (2011). p. 243–86.

20. Dowd AM, McGinley JL, Taffe JR, Rinehart NJ. Do planning and visual integration difficulties underpin motor dysfunction in autism? A kinematic study of young children with autism. *J Autism Dev Disord* (2012) 42:1539–48. doi: 10.1007/s10803-011-1385-8

21. Hannant P, Cassidy S, Tavassoli T, Mann F. Sensorimotor difficulties are associated with the severity of autism spectrum conditions. *Front Integr Neurosci* (2016) 10:28. doi: 10.3389/fnint.2016.00028

22. Crespi B. Developmental heterochrony and the evolution of autistic perception, cognition and behavior. *BMC Med* (2013) 11:119. doi: 10.1186/1741-7015-11-119

23. Curcio F. Sensorimotor functioning and communication in mute autistic children. *J Autism Child Schiz* (1978) 8(3):281–92. doi: 10.1007/BF01539631

24. Sigman M, Ungerer JA. Cognitive and language skills in autistic, mentally retarded, and normal children. *Dev Psychol* (1984) 20(2):293–302. doi: 10.1037/0012-1649.20.2.293

25. Mundy P, Sigman M, Ungerer J, Sherman T. Nonverbal communication and play correlates of language development in autistic children. *J Autism Dev Disord* (1987) 17(3):349–64. doi: 10.1007/BF01487065

26. Landry SH, Loveland KA. Communication behaviors in autism and developmental language delay. *J Child Psychol Psyc* (1988) 29(5):621–34. doi: 10.1111/j.1469-7610.1988.tb01884.x

27. Wetherby A, Prutting C. Profiles of communicative and cognitive–social abilities in autistic children. *J Speech Hear Res* (1984) 27(3):364–77. doi: 10.1044/jshr.2703.364

28. Wetherby AM, Prizant BM, Hutchinson TA. Communicative, social/affective, and symbolic profiles of young children with autism and pervasive developmental disorders. *Am J Speech-Lang Pat* (1998) 7:79–91. doi: 10.1044/1058-0360.0702.79

29. Nowell KP, Schanding GT, Jr., Kanne SM, Goin-Kochel RP. Cognitive profiles in youth with autism spectrum disorder: an investigation of base rate discrepancies using the differential ability scales-second edition. *J Autism Dev Disord* (2015) 45(7):1978–88. doi: 10.1007/s10803-014-2356-7

30. Joseph RM, Tager-Flusberg H, Lord C. Cognitive profiles and social-communicative functioning in children with autism spectrum disorder. *J Child Psychol Psyc* (2002) 43(6):807–21. doi: 10.1111/1469-7610.00092

31. Ankenman K, Elgin J, Sullivan K, Vincent L, Bernier R. Nonverbal and verbal cognitive discrepancy profiles in autism spectrum disorders: influence of age and gender. *Am J Intellect and Dev Disabil* (2014) 119(1):84–99. doi: 10.1352/1944-7558-119.1.84

32. Siegel DJ, Minshew NJ, Goldstein G. Wechsler IQ profiles in diagnosis of high-functioning autism. *J Autism Dev Disord* (1996) 26(4):389–406. doi: 10.1007/BF02172825

33. Mecca TP, Orsati FT, Coutinho de Macedo E. Non-verbal cognitive profile of young children with autism spectrum disorders. *Psychology* (2014) 5:1404–17. doi: 10.4236/psych.2014.511151

34. Thiébaut E, Adrien JL, Blanc R, Barthélémy C. The Social Cognitive Evaluation Battery for children with autism: a new tool for the assessment of cognitive and social development in children with autism spectrum disorders. *Autism Res Treat* (2010) 875037. doi: 10.1155/2010/875037

35. Nader-Grosbois N, Seynhaeve I. Étude des profils multidimensionnels d'enfants à déficience intellectuelle et à trouble autistique. In: Adrien J-L, editor. *BECS Pratiques psychologiques et recherches cliniques auprès d'enfants atteints de TED*. Brussels: De Boeck (2008). p. 171–91.

36. Kim HU. Autism across cultures: rethinking autism. *Disabil Soc* (2012) 27(4):535–45. doi: 10.1080/09687599.2012.659463

37. Norbury CF, Sparks A. Difference or disorder? Cultural issues in understanding neurodevelopmental disorders. *Dev Psychol* (2013) 49(1):45–58. doi: 10.1037/a0027446

38. Matson JL, Worley JA, Fodstad JC, Chung KM, Suh D, Jhin HK, et al. A multinational study examining the cross-cultural differences in reported symptoms of autism spectrum disorders: Israel, South Korea, the United Kingdom, and the United States of America. *Res Autism Spect Dis* (2011) 5(4):1598–604. doi: 10.1016/j.rasd.2011.03.007

39. Hussein H, Tahal GR, Almanasef A. Characteristics of autism spectrum disorders in a sample of Egyptian and Saudi patients: transcultural cross-sectional study. *Child Adol Psych Men* (2011) 5:34. doi: 10.1186/1753-2000-5-34

40. Carruthers S, Kinnaird E, Rudra A, Smith P, Allison C, Auyeung B, et al. A cross-cultural study of autistic traits across India, Japan and the UK. *Mol Autism* (2018) 9:52. doi: 10.1186/s13229-018-0235-3

41. Davenport M, Mazurek M, Brown A, McCollom E. A systematic review of cultural considerations and adaptation of social skills interventions for individuals with autism spectrum disorder. *Res Autism Spect Dis* (2018) 52:23–33. doi: 10.1016/j.rasd.2018.05.003

42. Bernard MA (2015). *International study of cognitive and socio-emotional development of children with autism spectrum disorder, with the Socio-emotional and Cognitive Evaluation Battery (SCEB)*. Dissertation thesis. Paris: University Paris Descartes Sorbonne Paris City, France.

43. Bernard MA, De la Iglesia Gutierrez M, Olivar Parra JS, Thiébaut E, Kaye K, Contejean Y, et al. Cognitive and socio-emotional developmental assessment of children with autism from France, Belgium, Canada, Brazil, Spain, Greece and Algeria with the Social Cognitive Evaluation Battery (SCEB): a comparative analysis. In: *Book of Abstracts of 1st International Congress of Clinical and Health Psychology with Children and Adolescents*. Spain: Ediciones Pirámide (2015). p. p.143.

44. Bernard MA, Thiébaut E, Mazetto C, Nassif MC, de Souza MTCC, Nader-Grosbois N, et al. L'hétérogénéité du développement cognitif et socio-émotionnel d'enfants atteints d'autisme en lien avec la sévérité des troubles. *Neuropsychiatr Enfance Adolesc* (2016) 64:376–82. doi: 10.1016/j.neurenf.2016.05.002

45. Adrien JL, Bernard MA, Thiébaut E, Gattegno MP, Blanc R, Kaye K, et al. Profils de développement dans le Trouble du Spectre Autistique (TSA), avec ou sans déficience intellectuelle sévère. Implications pour l'évaluation et l'intervention. (Developmental profiles in Autism Spectrum Disorder (ASD) children with and without severe intellectual deficiency. Implications for assessment and intervention). *Devenir* (2016) 28(3):255–72. doi: 10.3917/dev.164.0255

46. Nassif MC. *Inovações em Psicologia Clínica—O Programa Abrangente Neurodesenvolvimental (P.A.N)*. Rio de Janeiro: Editora Atheneu, Brésil (2017).

47. Adrien JL. *Manuel de la Batterie d'Evaluation Cognitive et Socio-émotionnelle (BECS)*. Paris: Pearson France-ECPA (2007).

48. Cohen J. *Statistical power analysis for the behavioral sciences*. 2nd ed. Hillsdale, NJ: Erlbaum (1988).

49. WHO. *The ICD-10 classification of mental and behavioural disorders: Clinical descriptions and diagnostic guidelines*. Geneva: World Health Organization (1992).

50. APA. *Diagnostic and Statistical Manual of Mental Disorders*. Text Revision, 4th Edn. Washington, D.C: American Psychiatric Association (2000).

51. Schopler E, Reichler R, Renner B. *The Childhood Autism Rating Scale (CARS)*. Los Angeles: Western Psychological Services (1988), adaptation française, B. Rogé, 1989, Echelle d'évaluation de l'autisme infantil), Issy-les-Moulineaux: Editions d'Application Psychotechnique (EAP).

52. Brunet O, Lézine I. *Echelle de développement psychomoteur de la première enfance*. Montreuil: Pearson France-ECPA (2001).

53. Gesell A, Amatruda CS. *Developmental diagnosis. Normal and abnormal child development: clinical methods and pediatric applications*. New York: P.B. Hoeber (1947).

54. HAS, Haute Autorité de Santé/High Health Authority. Service des bonnes pratiques professionnelles, Autisme et autres troubles envahissants du développement. Etat des connaissances. (2010),http://www.has-sante.fr/portail/jcms/c_935617/fr/autisme-et-autres-troubles-envahissants-du-developpement. *Service of good Professional practices. Autism and pervasive developmental disorders. State of knowledges*. Accessed January 2010. www.has-sante.fr.

55. HAS, Haute Autorité de Santé/High Health Authority, (2018). Trouble du spectre de l'autisme. Signes d'alerte, repérage, diagnostic et évaluation chez l'enfant et l'adolescent. Argumentaire scientifique. *Autism spectrum disorder. Alarm signs, identification signs, diagnosis and assessment in children and adolescent. Scientific Argumentary.* February 2018. www.has-sante.fr.

56. Piaget J. *La naissance de l'intelligence chez l'enfant*. 9th edition. Neuchâtel: Delachaux et Niestlé (1977).

57. Fischer KWA. theory of cognitive development: the control and construction of hierarchies of skills. *Psychol Rev* (1980) 87(6):477–531. doi: 10.1037/0033-295X.87.6.477

58. Friedman M. The use of ranks to avoid the assumption of normality implicit in the analysis of variance. *J Am Stat Assoc* (1937) 32(200):675–701. doi: 10.1080/01621459.1937.10503522

59. R Development Core Team, (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

60. Uzgiris IC, Hunt JMV. *Assessment in infancy: ordinal scales of psychological development*. Urbana: University of Illinois Press (1975).

61. Nader-Grosbois N. *Le développement cognitif et communicatif précoce (Early cognitive and communicative development)*. Bruxelles: De Boeck Supérieur (2006). doi: 10.3917/dbu.nader.2006.01

62. Nader-Grosbois N. *Les Échelles d'évaluation du développement cognitif précoce EEDCP. (Assessment scales of early cognitive development). Manuel illustré d'administration (Illustrated manual for scoring)*. Louvain, Belgium: UCL, Presses Universitaires de Louvain (2009).

63. Seibert JM, Hogan AE, Mundy PC. Assessing interactional competencies: the early social communication scales. *Inf Mental Hlth J* (1982) 3(4):244–58. doi: 10.1002/1097-0355(198224)3:4<244::AID-IMHJ2280030406>3.0.CO;2-R

64. Guidetti M, Tourette C. An original tool to assess communicative competencies in young children. The ESCS. *Eur Rev Appl Psychol* (1992) 42(3):185–92.

65. Thiemann-Bourque K, Johnson LK, Bra NC. Similarities in functional play and differences in symbolic play of children with autism spectrum disorder. *Am J Intellect* (2019) 124(1):77–91. doi: 10.1352/1944-7558-124.1.77

66. Page J, Boucher J. Motor impairments in children with autistic disorder. *Child Lang Teach The* (1998) 14(13):233–59. doi: 10.1191/026565998673400901

67. Brisson J, Serres J, Warreyn P, Foussier S, Adrien JL. Motor anticipation failure in infants with autism: a retrospective analysis of feeding situations. *Autism* (2012) 16(4):420–29. doi: 10.1177/1362361311423385

68. Brisson J, Martel K, Serres J, Sirois S, Adrien JL. Acoustic analysis of vocal/verbal productions of infants later diagnosed with autism and their mother. *Inf Mental Hlth J* (2014) 35(3):285–95. doi: 10.1002/imhj.21442

69. English MS, Tenenbaum EJ, Levine TP, Lester BM, Sheinkopf SJ. Perception of cry characteristics in 1-month-old infants later diagnosed with autism spectrum disorder. *J Autism Dev Disord* (2019) 49(3):834–44. doi: 10.1007/s10803-018-3788-2

70. Lombardo MV, Pierce K, Eyler LT, Barnes CC, Ahrens-Barbeau C, Solso S, et al. Different functional neural substrates for good and poor language outcome in autism. *Neuron* (2015) 86(2):567–77. doi: 10.1016/j.neuron.2015.03.023

71. Marrus N, Hall LP, Paterson SJ, Elison JT, Wolff JJ, Swanson MR, et al. Language delay aggregates in toddler siblings of children with autism spectrum disorder. *J Neurodev Disord* (2018) 10:29. doi: 10.1186/s11689-018-9247-8

72. Franchini M, Duku E, Armstrong V, Brian J, Bryson SE, Garon N, et al. Variability in verbal and nonverbal communication in infants at risk for autism spectrum disorder: predictors and outcomes. *J Autism Dev Disord* (2018) 48(10):3417–31. doi: 10.1007/s10803-018-3607-9

73. Kjelgaard MM, Tager-Flusberg H. An investigation of language impairment in autism: implications for genetic subgroups. *Lang Cognitive Proc* (2001) 16(2–3):287–308. doi: 10.1080/01690960042000058

74. Carvey JS, May Bernhardt B. Communicative acts of a child with Rubinstein-Taybi syndrome during early communicative development. *Child Lang Teach The* (2009) 25(2):172–90. doi: 10.1177/0265659009102976

75. Roberts JER, Price J, Malkin C. Language and communication development in Down syndrome. *Ment Retard Dev D R* (2007) 13(1):26–35. doi: 10.1002/mrdd.20136

76. Hodge SM, Makris N, Kennedy DN, Caviness VS, Howard J, McGrath L, et al. Cerebellum, language, and cognition in autism and specific language

impairment. *J Autism Dev Disord* (2010) 40(3):300–16. doi: 10.1007/s10803-009-0872-7

77. Menghini D, Costanzo F, Vicari S. Relationship between brain and cognitive processes in Down syndrome. *Behav Genet* (2011) 41(3):381–93. doi: 10.1007/s10519-011-9448-3

78. Abdelrahman ME, Hasan MKA. *Gilliam Autism Rating Scale Arabic version.* Cairo: Dar El-Sahab Press (2004).

79. Vivanti G, Barbaro J, Hudry K, Dissanayake C, Prior M. Intellectual development in autism spectrum disorders: new insights from longitudinal studies. *Front Hum Neurosci* (2013) 7:354. doi: 10.3389/fnhum.2013.00354

80. Nardini C, Monti A, Pieraccin C, Guerrieri C (2006). La "Batteria di valutazione Cognitivo-Sociale (B.E.C.S)., un ulteriore strumento di valutazione nei bambini con disturbo pervasivo dello sviluppo". Atti del XXIII Congresso Nazionale SINPIA, Abano Terme. 25–28 ottobre 2006.

81. Pieraccini C, Monti A, Nardini C, Armellini M, Ongaro S, Centini G, et al. (2009). *Ados and Becs in early diagnosis of children with pervasive developmental disorders.* Abstract in Acts of International Conference on Innovative Research in Autism (pp)., Tours, France.

82. Aldred C, Green J, Adams C. A new social communication Intervention for children with autism: pilot randomised controlled treatment study suggesting effectiveness. *J Child Psychol Psyc* (2004) 45(8):1420–30. doi: 10.1111/j.1469-7610.2004.00338.x

83. Dawson G, Rogers SJ, Smith M, Munson J, Winter J, Greenson J, et al. Randomized controlled trial of the Early Start Denver Model: a relationship-based developmental and behavioral intervention for toddlers with autism spectrum disorders: effects on IQ, adaptive behavior and autism diagnosis. *Pediatrics* (2010) 125(1):17–23. doi: 10.1542/peds.2009-0958

84. Estes A, Munson J, Rogers SJ, Greenson J, Winter J, Dawson G. Long-term outcomes of early intervention in 6-year-old children with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry* (2015) 54(7):580–87. doi: 10.1016/j.jaac.2015.04.005

85. Schreibman L, Dawson G, Stahmer AC, Landa R, Rogers SJ, McGee GG, et al. Naturalistic developmental behavioral interventions: empirically validated treatments for autism spectrum disorder. *J Autism Dev Disord* (2015) 45(8):2411–28. doi: 10.1007/s10803-015-2407-8

86. Pickles A, Le Couteur A, Leadbitter K, Salomone E, Cole-Fletcher R, Tobin H, et al. Parent-mediated social communication therapy for young children with autism (PACT): long-term follow-up of a randomised controlled trial. *The Lancet* (2016) 388(10059):2501–09. doi: 10.1016/S0140-6736(16)31229-6

87. Tillmann J, San José Cáceres A, Chatham CH, Crawley D, Holt R, Oakley B, et al. Investigating the factors underlying adaptive functioning in autism in the EU-AIMS Longitudinal European Autism Project. *Autism Res* (2019) 12(4):645–57. doi: 10.1002/aur.2081

88. Aïad F (2014). *Assessment of cognitive and socio-emotional development in Algerian children with ASD.* Dissertation thesis. Blida, University Ali Lounici, El-Affroun Blida City, Algeria.

89. Belal L (2015). S*tudy of the developmental trajectories of children with autism spectrum disorder and followed by integrative therapy. Study done on a sample of 20 children with autism spectrum disorder with the Socio-emotional and Cognitive Evaluation Battery (SCEB) and the children autism rating scale (CARS).* Dissertation thesis (2016). Tlemcen, University Abou Baker Belkaid, Tlemcen, Algeria.

90. Mazetto CTM (2015). A criança com autismo: trajetórias desenvolvimentais atípicas à luz da teoria piagetiana da equilibração. *The child with autism: atypical developmental trajectories in light of Piaget's equilibration theory.* Tese de Doutorado. São Paulo (SP): Universidade de São Paulo.

Lack of Associations Between Dietary Intake and Gastrointestinal Symptoms in Autism Spectrum Disorder

Bradley J. Ferguson[1,2,3]*, Kristen Dovgan[4], Danielle Severns[5], Shannon Martin[6], Sarah Marler[7], Kara Gross Margolis[8], Margaret L. Bauman[9], Jeremy Veenstra-VanderWeele[10], Kristin Sohl[2,11] and David Q. Beversdorf[2,3,12]

[1] Department of Health Psychology, School of Health Professions, University of Missouri, Columbia, MO, United States, [2] Thompson Center for Autism & Neurodevelopmental Disorders, Columbia, MO, United States, [3] Department of Radiology, University of Missouri School of Medicine, Columbia, MO, United States, [4] Department of Psychology, Marist College, Poughkeepsie, NY, United States, [5] School of Medicine, University of Missouri, Columbia, MO, United States, [6] Department of Psychological Sciences, University of Missouri, Columbia, MO, United States, [7] Medical Exploration of Neurodevelopmental Disorders Clinic (MEND), Vanderbilt University Medical Center, Nashville, TN, United States, [8] Department of Pediatrics, Division of Pediatric Gastroenterology, Hepatology, & Nutrition & Morgan Stanley Children's Hospital, Columbia University Medical Center, Columbia, MO, United States, [9] Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA, United States, [10] Department of Psychiatry, Columbia University, Columbia, MO, United States, [11] Department of Child Health, University of Missouri, Columbia, MO, United States, [12] Departments of Neurology & Psychological Sciences, William and Nancy Thompson Endowed Chair in Radiology, University of Missouri, Columbia, MO, United States

Background: Many individuals with autism spectrum disorder (ASD) have significant gastrointestinal (GI) symptoms, but their etiology is currently unknown. Dietary interventions are common in children and adolescents with ASD, including diets with increased omega-3 fatty acids or diets free of gluten and/or casein, which may also impact GI symptoms and nutrition. However, little is known about the relationship between nutritional intake and GI symptomatology in ASD. The objective of this study was to assess the relationships between GI symptoms, omega-3 intake, micronutrients, and macronutrients in children with ASD.

Methods: A total of 120 children diagnosed with ASD participated in this multisite study. A food frequency questionnaire was completed by the patient's caretaker. The USDA Food Composition Database was utilized to provide nutritional data for the food items consumed by each participant. GI symptomatology was assessed using a validated questionnaire on pediatric gastrointestinal symptoms.

Results: There were no significant associations between GI symptoms and the amount of omega-3 fatty acids and/or other micro- and macronutrients contained in the diet.

Conclusions: This study suggests that dietary variations do not appear to drive GI symptoms, nor do GI symptoms drive dietary variations in those with ASD, although causation cannot be determined with this observational assessment. Furthermore, there may be other factors associated with lower GI tract symptoms in ASD, such as increased stress response.

Keywords: autism spectrum disorder (ASD), gastrointestinal symptoms, dietary intake, omega-3 fatty acids, micro- and macronutrients

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by persistent deficits in social communication and social interaction, as well as restricted and repetitive patterns of behavior that present during early development and result in clinically significant impairment (1). Research has shown that children with ASD tend to have more gastrointestinal (GI) symptoms than their typically developing peers (2–6), especially for constipation, diarrhea, and abdominal pain (2, 7–9). A review of the literature in 2010 indicated that the proportion of ASD individuals having co-occurring GI problems may range from 9 to 91% (10), the reported variations in prevalence rates potentially resulting from differences in diagnostic methods used to assess GI symptoms in this population. Despite the relatively high rates of GI symptoms in ASD, the etiology is poorly understood. Therefore, it is important to explore the role of dietary associations with GI symptoms in ASD given the potential for certain diets to be associated with GI dysfunction in ASD.

Many individuals with ASD have used complementary and alternative medicine (CAM) approaches, including dietary changes, as part of their treatment for the core ASD symptoms, as well as GI disturbance, sleep problems, or in the promotion of general health (11). As such, changes in diet may affect GI functioning in ASD. Some studies have shown that children with ASD may be deficient in micro- and macronutrients (12–15), as well as iron (16), which could result from altered GI function and/or potentially impact GI symptoms. Furthermore, many parents and caretakers have employed the use of gluten- and casein-free (GFCF) diets (17) that seem to have mixed effects on core ASD symptoms (18) and GI symptoms (19–23) in ASD. In addition, many families also administer omega-3 fatty acids in the hope of deriving benefit, but the results from randomized, placebo-controlled clinical trials of omega-3 supplementation in ASD are also mixed in most cases (24, 25). In addition, limited dietary intake and selective food preferences, common among individuals with ASD (26), can result in nutritional deficiencies or other problems that could potentially interact with GI symptoms, either contributing to these symptoms or emerging in response to them.

As such, a better understanding of the association between dietary intake on GI functioning in ASD is of interest, especially given the implications for treatment. The focus of the present study is to assess the associations between approximate omega-3 intake and micro- and macronutrient intake over the prior month and self- and parent-reported GI symptoms in individuals with ASD with the goal of determining whether dietary factors may be related to GI symptomatology.

METHODS

A total of 120 patients with ASD (mean age = 11.8, SD = 3.8, range = 6–18, 108 male, 92.5% Caucasian, mean full-scale intelligence

quotient = 84, SD = 22.6, range = 36–130) participated in this study. Patients were recruited sequentially from individuals enrolled in the Autism Speaks Autism Treatment Network (AS-ATN) registries at the University of Missouri Thompson Center for Autism & Neurodevelopmental Disorders in Columbia, Missouri, and at the Vanderbilt Kennedy Center and Monroe Carell Jr. Children's Hospital at Vanderbilt University in Nashville, Tennessee. To expand the sample, additional patients who were not enrolled in the AS-ATN were recruited from clinic patients at each site. Diagnosis of ASD was made based on *Diagnostic and Statistical Manual for Mental Disorders IV-TR* criteria (27) and the administration of the Autism Diagnostic Observation Schedule (ADOS) (28). Patients with known genetic or metabolic disorders or bleeding disorders were excluded from this study, as an associated portion of this project involved drawing blood. A more detailed explanation of the inclusionary and exclusionary criteria can be found elsewhere (29, 30). This study was carried out in accordance with the recommendations of the Institutional Review Boards at the University of Missouri and Vanderbilt University, with written informed consent from all participants over the age of 18 and consent from the parent/guardian and assent from those under the age of 18. All participants gave written informed consent in accordance with the Declaration of Helsinki. The study protocol was approved by the Institutional Review Boards at the University of Missouri and Vanderbilt University.

Assessment of Gastrointestinal Symptoms

Gastrointestinal symptoms were assessed based on parent or self-report using the Questionnaire of Pediatric Gastrointestinal Symptomatology-Rome III (QPGS-RIII) (31). Patients who were over the age of 18 and able to provide an accurate account of their GI symptoms, as determined by asking the caretaker and/or the patient if they could reliably report their GI symptoms over the month before participation in the study, completed the self-report version of the QPGS-RIII. Otherwise, the QPGS-RIII was completed by a parent or caretaker that could provide a reliable account of the patient's GI functioning over the month before their participation in the study. A scoring rubric previously created by the research team was used to create continuous variables for upper and lower GI tract symptoms over the past month (29). Briefly, items from the QPGS-RIII were sorted into upper and lower GI tract symptoms, and the scores for each were summed to reflect an overall upper and lower GI score for each patient. Greater QPGS-RIII scores indicate greater frequency, severity, and duration of GI symptoms.

Assessment of Nutritional Intake

Omega-3 nutritional intake during this same period of time (1 month before entering the study) for each patient was assessed using a food frequency questionnaire (FFQ) that was designed to assess omega-3 fatty acid intake as well as information that could be utilized to calculate micro- and macronutrient intake (32). The 152-item FFQ was developed based on foods that contain 10 mg of n-3 fatty acids/medium serving from fish, animal, and plant sources. The FFQ was either completed by the patient's parent or caregiver or by the patient. Responses were analyzed

---

Abbreviations: ASD, autism spectrum disorder; GI, gastrointestinal; AS-ATN, Autism Speaks Autism Treatment Network.

for nutritional intake using the online, publicly available United States Department of Agriculture Food Composition Database (33), which provides nutrient information for specific foods. A total monthly estimate of the patient's nutritional intake was calculated by summing the nutrient information for each food based on the serving size and frequency of consumption. Total nutrient scores were created for both University of Missouri and Vanderbilt AS-ATN sites to determine if differences exist between midwestern and southern region diets. The individuals scoring the FFQs were blinded to the patient's GI status.

RESULTS

Gastrointestinal Symptoms

Both sites had similar number of patients with upper and lower GI tract problems [$t(113) = 1.608$, $p = 0.111$]. Therefore, the two populations were pooled for the primary comparisons. In addition, upper GI tract problems were significantly correlated with lower GI tract problems ($r = 0.411$, $p < 0.001$). The most common GI disorder reported by the participants was functional constipation (42.5%), followed by irritable bowel syndrome (11.7%) and lower bowel pain associated with bowel symptoms (9.2%). A more detailed description of the GI disorders experienced by this study population as well as how the GI scores were calculated can be found in previous reports (29, 30).

Omega-3 and Dietary Nutrient Intake

See **Table 1** for approximate mean monthly nutrient intake values across both the University of Missouri and Vanderbilt sites. First, as the nutrient variables were significantly skewed and non-normal, nonparametric Spearman rank correlations were conducted on the full dataset (i.e., without removing outliers), which do not make assumptions about the normal distribution of the data. In this way, we could assess the potential contribution of any extreme diets and picky eaters. Total GI tract symptoms were not significantly correlated with fatty acids ($r_s = 0.145$, $p = 0.20$), gluten ($r_s = 0.114$,

TABLE 1 | Mean monthly nutrient intake values for the full sample.

	Mean	SD	Range (with outliers)[a]	N
Rome III Upper GI Score	4.5	4.6	0–20	116
Rome III Lower GI Score	17.6	11.8	0–50	118
Casein (g)	39.0	35.2	0–247	116
Gluten (g)	21.6	19.7	0–96	110
Water (g)	15,157.3	8,678.9	1,395.36–79,019.23	116
Energy (kcal)	19,410.6	8,187.4	6,700–72,793	116
Protein (g)	1,082.9	439.4	395.42–3,929.90	114
Total lipid fat (g)	766.6	352	169.59–2,897.07	115
Carbohydrate (by difference; g)	2,021.5	970.5	296.92–8,394.04	114
Dietary fiber (total; g)	234	147.3	2.20–865.95	117
Sugars (total; g)	779.3	538.3	25.14–4,447.32	114
Calcium (mg)	16,710	11,842	1,067–107,330	116
Iron (mg)	113.3	64.4	26.65–386.99	109
Magnesium (mg)	3,805.8	1,990.4	818–17,700	117
Phosphorous (mg)	20,161	10,452.2	4,614–91,666.50	117
Potassium (mg)	40,703.2	20,063.2	7,936–196,649	117
Sodium (mg)	24,292.4	10,906.4	6,224–90,283	113
Zinc (mg)	143.4	66.8	28.03–544.18	115
Vitamin C (total ascorbic acid; mg)	1,178.6	1,067.8	2.80–9,534.95	113
Thiamin (mg)	16.5	7.5	3.73–61.41	115
Riboflavin (mg)	26.3	15.8	3.54–137.25	117
Niacin (mg)	250.2	107.0	72.74–714.32	115
Vitamin B6 (mg)	28.7	15.5	6.84–189.69	112
Folate (DFE; µg)	3,436.4	2,094.2	384–23,399	114
Vitamin B12 (µg)	68.6	39.2	1.26–305.51	113
Vitamin A (RAE; µg)	8,091.1	5,379.1	126–46,020	115
Vitamin A (IU)	57,991.1	44,091.6	726–470,724.50	112
Vitamin E (alpha tocopherol; mg)	98.3	52.6	7.83–486.53	115
Vitamin D2 + D3 (µg)	75.7	67.6	2.50–339	114
Vitamin D (IU)	2,984.2	2,694.5	82.50–13,212.56	114
Vitamin K (phylloquinone; µg)	981.3	714.1	37–5,649.75	115
Total saturated fatty acids (g)	258.9	130.7	34.59–1,391.45	115
Total monounsaturated fatty acids (g)	263.7	128.6	43.68–1,173.70	112
Total polyunsaturated fatty acids (g)	161.4	79.1	16.68–537.98	115
Total trans fatty acids (g)	6.5	4.9	0.54–84.90	110
Cholesterol (mg)	3,139.3	1,535.5	50–10,945	111

[a]Note that the range reflects the full sample, including outliers.

$p = 0.336$), casein ($r_s = -0.104$, $p = 0.357$), water ($r_s = -0.059$, $p = 0.605$), calories ($r_s = 0.137$, $p = 0.225$), protein ($r_s = 0.113$, $p = 0.319$), fats ($r_s = 0.147$, $p = 0.193$), carbohydrates ($r_s = 0.088$, $p = 0.438$), sugar ($r_s = 0.093$, $p = 0.412$), vitamins ($r_s = 0.005$, $p = 0.962$), minerals ($r_s = 0.068$, $p = 0.550$), or cholesterol ($r_s = 0.132$, $p = 0.244$). However, fiber was positively correlated with upper and lower GI tract symptoms ($r_s = 0.243$, $p = 0.030$) when outliers were included. Furthermore, it is not unusual for extreme points to increase the strength of a correlation, and the sample included three participants who consumed over 656 g of fiber in the past month.

Next, we wished to reanalyze the data excluding the outliers. As such, 173 outlier values (3.7% of the data) were removed using the interquartile range rule (i.e., values >1.5 times the interquartile range), creating a normally distributed dataset that could be analyzed using Pearson correlations. See **Table 1** for the number of patients remaining for each micro- and macronutrient.

TABLE 2 | Correlation matrix for nutrient intake values and Questionnaire of Pediatric Gastrointestinal Symptomatology-Rome III (QPGS-RIII) upper and lower gastrointestinal (GI) tract symptom scores.

	Rome III Upper GI Score	Rome III Lower GI Score
Rome III Upper GI Score	1	0.411**
Rome III Lower GI Score	0.411**	1
Casein (g)	−0.182	−0.084
Gluten (g)	0.003	0.122
Water (g)	−0.034	−0.032
Energy (kcal)	0.071	0.107
Protein (g)	−0.004	0.131
Total lipid fat (g)	0.095	0.142
Carbohydrate (by difference; g)	0.067	0.121
Dietary fiber (total; g)	0.180	0.166
Sugars (total; g)	0.088	0.096
Calcium (mg)	−0.148	−0.020
Iron (mg)	0.155	0.150
Magnesium (mg)	0.021	0.091
Phosphorous (mg)	−0.036	0.026
Potassium (mg)	−0.022	0.041
Sodium (mg)	0.017	0.134
Zinc (mg)	0.093	0.176
Vitamin C (total ascorbic acid; mg)	0.176	0.093
Thiamin (mg)	0.008	0.055
Riboflavin (mg)	−0.087	−0.015
Niacin (mg)	0.037	0.081
Vitamin B6 (mg)	−0.017	−0.006
Folate (DFE; µg)	0.096	0.126
Vitamin B12 (µg)	−0.016	0.012
Vitamin A (RAE; µg)	−0.068	−0.002
Vitamin A (IU)	−0.011	0.084
Vitamin E (alpha tocopherol; mg)	0.096	0.153
Vitamin D2 + D3 (µg)	−0.086	−0.040
Vitamin D (IU)	−0.112	−0.046
Vitamin K (phylloquinone; µg)	0.127	0.107
Total saturated fatty acids (g)	−0.009	0.039
Total monounsaturated fatty acids (g)	0.119	0.137
Total polyunsaturated fatty acids (g)	0.140	0.112
Total trans fatty acids (g)	0.111	0.123
Cholesterol (mg)	0.033	0.076

**$p < 0.001$.

Estimated omega-3 fatty acids were not significantly correlated with upper or lower GI tract symptoms across both sites ($r = 0.10$, $p = 0.304$). Furthermore, upper and lower GI tract symptoms were not significantly correlated with the consumption of gluten, casein, water, calories, protein, fats, carbohydrates, fiber, sugar, or any vitamins, minerals, or cholesterol. See **Table 2** for Pearson correlations between upper and lower GI tract problems and each nutrient intake value.

DISCUSSION

Previous research from this multidisciplinary, investigative team found associations between the stress response and GI symptoms among those with ASD (29, 30). General nutritional intake as well as consuming foods high in n3-PUFA may also, however, affect GI symptoms, or GI symptoms might affect diet. Thus, we sought to examine the association between nutritional intake and GI symptoms in the same group of individuals from the aforementioned study. The present study was conducted to specifically examine the effects of diets that are high in n-3 PUFA on GI problems in those with ASD. The results from this multisite study indicate no association between consumption of a diet that is high in n-3 PUFA and upper or lower GI tract symptoms in the study sample. Furthermore, micro- and macronutrients contained in the diet were also not significantly associated with upper and/or lower GI tract symptoms in the sample. These results suggest that previous relationships between stress reactivity and GI symptomatology are not due to dietary factors, at least those assessed herein, and begins to provide evidence against the concept of dietary factors impacting GI symptomatology or of GI symptomatology impacting diet in children with ASD. The isolated finding of a positive correlation between fiber intake and GI symptomatology before excluding outliers may result from unsuccessful attempts to manage the GI symptoms with high fiber intake. Indeed, dietary fiber has been shown to be associated with GI symptoms of abdominal pain, bloating, and constipation, flatulence, and diarrhea (34, 35). Therefore, parents, caratakers, and clinicians should be aware of this finding as well as recommended fiber intake (36, 37) when considering treatment of abdominal pain and constipation in children with ASD.

There are a number of limitations in this study that should be addressed. First, the study did not examine the effects of altered diets on GI functioning in the sample. As many individuals with ASD have altered diets, it is a possibile that a subgroup of autistic individuals with altered diets may have concomitant alterations in GI functioning. Thus, future research should examine the effects of altred diets on GI symptoms in ASD. Second, the present study utilized a food frequency questionnaire that contained a limited number of food items. While the questionnaire contains a wide range of food items, it is not exhaustive. Furthermore, it is not clear if taking dietary supplements by participants could be related to their GI symptoms. Future research may wish to utilize a food diary to log food items and amounts consumed per day as well as assess whether or not the participant is taking dietary supplements in an attempt to reduce ASD symptoms. Third, the sample was largely male and Caucasian, and so it is not clear if the results

transfer to female and other ethnicities. Fourth, the QPGS depends on an informant, usually a parent, to answer a questionnaire regarding their child's GI functioning, including identification of the location of abdominal discomfort. Given that many children with ASD are nonverbal or have limited verbal abilities, it is possible that the GI scores may not be accurate for all participants. Future GI investigations should utilize formal gastroenterological evaluations or, at minimum, consider the use of ASD-specific measures of GI symptoms (38). Finally, the results presented herein will need to be replicated before drawing conclusions regarding the relationship between diet and GI disorders in ASD in the broader population. Larger samples would also allow incorporation of other co-occurring conditions to examine their relationships with the results from this study, as well as a better ability to recognize subtypes in the heterogenous ASD population.

CONCLUSION

The results from this study indicate no significant associations between dietary omega-3 and GI symptoms as well as dietary micro- and macronutrient intake and GI symptoms in a sample of 120 individuals with ASD, in whom relationships were previously observed and reported between stress reactivity and GI symptoms. These findings suggest that dietary changes do not appear to be driving GI symptoms nor do GI symptoms appear to impact dietary behavior among those with ASD.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Institutional Review Boards at the University of Missouri and Vanderbilt University with written informed consent from all participants under the age of 18. All participants gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Institutional Review Boards at the University of Missouri and Vanderbilt University.

AUTHOR CONTRIBUTIONS

BF conceptualized and designed the study, coordinated and supervised data collection, drafted the initial manuscript, and reviewed and revised the manuscript. ShM and DS processed and entered data, conducted a literature review, and assisted with preparation of the manuscript. KD carried out the statistical analyses and assisted with preparation of the manuscript. SaM collected the GI and diet data from patients at Vanderbilt University. JV-V supervised data collection at Vanderbilt University and revised the manuscript. KG, KS, and MB provided their expertise and guidance regarding autism spectrum disorder and revised the manuscript. DB supervised the research team and provided expertise and guidance regarding autism spectrum disorder and gastrointestinal disorders in autism and revised the manuscript. All authors approved of the final manuscript as submitted.

FUNDING

REFERENCES

1. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders (4th edition text revision) (DSM-IV-TR)*. Washington: American Psychiatric Association (2000).
2. McElhanon BO, McCracken C, Karpen S, Sharp WG. Gastrointestinal symptoms in autism spectrum disorder: a meta-analysis. *Pediatrics* (2014) 133:872–83. doi: 10.1542/peds.2013-3995
3. Chandler S, Carcani-Rathwell I, Charman T, Pickles A, Loucas T, Meldrum D. Parent-reported gastrointestinal symptoms in children with autism spectrum disorders. *J Autism Dev Disord* (2013) 43:2737–47. doi: 10.1007/s10803-013-1768-0
4. Chaidez V, Hansen RL, Hertz-Picciotto I. Gastrointestinal problems in children with autism, developmental delays or typical development. *J Autism Dev Disord* (2014) 44:1117–27. doi: 10.1007/s10803-013-1973-x
5. Bresnahan M, Hornig M, Schultz AF, Gunnes N, Hirtz D, Lie KK, et al. Association of maternal report of infant and toddler gastrointestinal symptoms with autism: evidence from a prospective birth cohort. *JAMA Psychiatry* (2015) 72:466–74. doi: 10.1001/jamapsychiatry.2014.3034

6. Fulceri F, Morelli M, Santocchi E, Cena H, Bianco TD, Narzisi A, et al. Gastrointestinal symptoms and behavioral problems in preschoolers with autism spectrum disorder. *Dig Liver Dis* (2016) 48(3):248–54. doi: 10.1016/j.dld.2015.11.026
7. Gorrindo P, Williams KC, Lee EB, Walker LS, McGrew SG, Levitt P. Gastrointestinal dysfunction in autism: parental report, clinical evaluation, and associated factors. *Autism Res* (2012) 5:101–8. doi: 10.1002/aur.237
8. Ibrahim SH, Voigt RG, Katusic SK, Weaver AL, Barbaresi WJ. Incidence of gastrointestinal symptoms in children with autism: a population-based study. *Pediatrics* (2009) 124:680–6. doi: 10.1542/peds.2008-2933
9. Kang V, Wagner GC, Ming X. Gastrointestinal Dysfunction in Children with autism spectrum disorders. *Autism Res* (2014) 7(4):501–6. doi: 10.1002/aur.1386
10. Buie T, Campbell DB, Fuchs G.J. III, Furuta GT, Levy J, Van de Water J, et al. Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. *Pediatrics* (2010) 125(S1):S1–8. doi: 10.1542/peds.2009-1878C
11. Wong HH, Smith RG. Patterns of complementary and alternative medical therapy use in children diagnosed with autism spectrum disorders. *J Autism Dev Disord* (2006) 36:901–9. doi: 10.1007/s10803-006-0131-0

12. Herndon AC, Diguiseppi C, Johnson SL, Leiferman J, Reynolds A. Does nutritional intake differ between children with autism spectrum disorders and children with typical development? *J Autism Dev Disord* (2008) 39(2):212–22. doi: 10.1007/s10803-008-0606-2

13. Saad K, Abdel-Rahman AA, Elserogy YM, Al-Atram AA, Cannell JJ, Bjørklund G, et al. Vitamin D status in autism spectrum disorders and the efficacy of vitamin D supplementation in autistic children. *Nutr Neurosci* (2016) 19(8):346–51. doi: 10.1179/1476830515Y.0000000019

14. Barnhill K, Gutierrez A. Analysis of dietary intake and nutritional status in children with autism spectrum disorder. *Autism-Open Access* (2015) 5:3. doi: 10.4172/2165-7890.1000154

15. Moore E, Crook T, James J, Gonzales D, Hakkak R. Nutrient intake among children with autism. *J Nutr Disord Ther* (2012) 2:3. doi: 10.4172/2161-0509.1000115

16. Latif A, Heinz P, Cook R. Iron deficiency in autism and Asperger syndrome. *Autism* (2002) 6:103–14. doi: 10.1177/1362361302006001008

17. Rubenstein E, Schieve L, Bradley C, Diguiseppi C, Moody E, Thomas K, et al. The prevalence of gluten free diet use among preschool children with autism spectrum disorder. *Autism Res* (2017) 11(1):185–93. doi: 10.1002/aur.1896

18. Hyman SL, Stewart PA, Foley J, Cain U, Peck R, Morris DD, et al. The gluten-free/casein-free diet: a double-blind challenge trial in children with autism. *J Autism Dev Disord* (2016) 46(1):205–20. doi: 10.1007/s10803-015-2564-9

19. Pusponegoro HD, Ismael S, Firmansyah A, Sastroasmoro S. & Vandenplas Y. Gluten and casein supplementation does not increase symptoms in children with autism spectrum disorder. *Acta Paediatr* (2015) 104(11):e500–5. doi: 10.1111/apa.13108

20. Johnson CR, Handen BL, Mayer-Costa M, Sacco K. Eating habits and dietary status in young children with autism. *J Dev Phys Disabil* (2008) 20:437–48. doi: 10.1007/s10882-008-9111-y

21. Harris C, Card B. A pilot study to evaluate nutritional influences on gastrointestinal symptoms and behavior patterns in children with autism spectrum disorder. *Complement Ther Med* (2012) 20(6):437–40. doi: 10.1016/j.ctim.2012.08.004

22. Ghallchi F, Ghaemmaghami J, Ostadrahimi A. Effect of gluten free diet on gastrointestinal and behavioral indices for children with autism spectrum disorders: a randomized clinical trial. *World J Pediatr* (2016) 12(4):436–42. doi: 10.1007/s12519-016-0040-z

23. Alessandria C, Caviglia GP, Campion D, Nalbone F, Sanna C, Musso A, et al. HLA-DQ genotyping, duodenal histology, and response to exclusion diet in autistic children with gastrointestinal symptoms. *J Pediatr Gastroenterol Nutr* doi: 10.1097/MPG.0000000000002293. [Epub ahead of print].

24. Mankad D, Dupuis A, Smile S, Roberts W, Brian J, Lui T, et al. A randomized, placebo controlled trial of omega-3 fatty acids in the treatment of young children with autism. *Mol Autism* (2015) 6(18):1–11. doi: 10.1186/s13229-015-0010-7

25. Mazahery H, Stonehouse W, Delshad M, Kruger MC, Conlon CA, Beck KL, et al. Relationship between long chain n-3 polyunsaturated fatty acids and autism spectrum disorder: systematic review and meta-analysis of case-control and randomised controlled trials. *Nutrients* (2017) 9(2):155. doi: 10.3390/nu9020155

26. Bandini LG, Anderson SE, Curtin C, Cermak S, Evans EW, Scampini R, et al. Food selectivity in children with autism spectrum disorders and typically developing children. *J Pediatr* (2010) 157(2):259–64. doi: 10.1016/j.jpeds.2010.02.013

27. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders.* 5th ed. Arlington, VA: American Psychiatric Association (2013). doi: 10.1176/appi.books.9780890425596

28. Lord C, Rutter M, Goode S, Heemsbergen J, Jordan H, Mawhood L, et al. Autism diagnostic observation schedule: a standardized observation of communicative and social behavior. *J Autism Dev Disord* (1989) 19:185–212. doi: 10.1007/BF02211841

29. Ferguson BJ, Marler S, Altstein LL, Batey Lee E, Akers J, Sohl K, et al. Psychophysiological associations with gastrointestinal symptomatology in autism spectrum disorder. *Autism Res* (2017) 10(2):276–88. doi: 10.1002/aur.1646

30. Ferguson BJ, Marler S, Altstein LL, Lee EB, Mazurek MO, McLaughlin A, et al. Associations between cytokines, endocrine stress response, and gastrointestinal problems in autism spectrum disorder. *Brain Behav Immun* (2016) 58:7–62. doi: 10.1016/j.bbi.2016.05.009

31. Walker LS, Caplan-Dover A, Rasquin-Weber A. *Rome III Diagnostic Questionnaire for the Pediatric Functional GI Disorders [Measurement instrument].* Raleigh, NC: Rome Foundation (2006).

32. Ritter-Gooder PK, Lews NM, Heidal KB, Eskridge KM. Validity and reliability of a quantitative food frequency questionnaire measuring n-3 fatty acid intakes in cardiac patients in the Midwest: a validation pilot study. *J Am Diet Assoc* (2006) 106(8):1251–5. doi: 10.1016/j.jada.2006.05.005

33. US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. 0000 USDA National Nutrient Database for Standard Reference, Release 28. Current: April 2018. Internet: /nea/bhnrc/ndl.

34. Ho KS, Tan CY, Mohd Daud MA, Seow-Choen F. Stopping or reducing dietary fiber intake reduces constipation and its associated symptoms. *World J Gastroenterol* (2012) 18(33):4593–6. doi: 10.3748/wjg.v18.i33.4593

35. Briet F, Achour L, Flourie B, Beaugerie L, Pellier P, Franchisseur C, et al. Symptomatic response to varying levels of fructo-oligosaccharides consumed occasionally or regularly. *Eur J Clin Nutr* (1995) 49(7):501–7.

36. Dahl WJ, Stewart ML. Position of the Academy of Nutrition and Dietetics: health implications of dietary fiber. *J Acad Nutr Diet* (2015) 115(11):1861–70. doi: 10.1016/j.jand.2015.09.003

37. Trumbo P, Schlicker S, Yates AA, Poos M. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. *J Acad Nutr Diet* (2002) 102(11):1621–30. doi: 10.1016/S0002-8223(02)90346-9

38. Margolis KG, Buie TM, Turner B, Silberman AE, Feldman JF, Murray K.F., et al. Development of a brief parent-report screen for common gastrointestinal disorders in autism spectrum disorder. *J Autism Dev Disord* (2019) 49(1):349–62. doi: 10.1007/s10803-018-3767-7

Assessment of Psychopathological Comorbidities in Children and Adolescents With Autism Spectrum Disorder Using the Child Behavior Checklist

Silvia Guerrera, Deny Menghini, Eleonora Napoli, Silvia Di Vara, Giovanni Valeri and Stefano Vicari*

Child and Adolescence Neuropsychiatry Unit, Department of Neuroscience, Children Hospital Bambino Gesù, Rome, Italy

Autism spectrum disorder (ASD) is characterized by psychiatric and behavioral comorbidities. The Child Behavior Checklist (CBCL) provides valid and well-established measures of emotional, behavioral, and social problems in children and adolescents. The aim of the present study was to verify whether emotional, behavioral, and social problems were modulated by ASD symptom severity, cognitive development, gender, and age by analyzing the CBCL in a large group of children and adolescents with ASD. The results show that around 30% of participants with ASD exhibited internalizing problems and only 6% externalizing problems, with males exhibiting more internalizing problems than females. No correlation was found between CBCL scores and indices of ASD severity. However, higher CBCL Total Problems scores were found in older children and in children with lower cognitive abilities. The detection of behavioral and emotional problems allows children with ASD to undergo specific and individualized treatment that takes into account their psychopathological problems.

Keywords: neurodevelopmental disorders, emotional problems, behavioral problems, psychiatric comorbidity, autism spectrum disorder, Child Behavior Checklist

INTRODUCTION

Autism spectrum disorder (ASD) is characterized by persistent deficits in social communication and social interaction across multiple contexts as well as restricted, repetitive patterns of behavior, interests, or activities (1).

Psychiatric symptoms and behavioral disorders are frequently documented in people with ASD: about two-thirds of them are indeed reported to have at least one associated mental health condition (1). Although in the past, psychiatric symptoms in children and adults with ASD have been attributed to ASD itself, an increasing number of reports suggest that additional behavior disorders in people with ASD potentially indicate the presence of psychiatric and behavioral comorbidities and demand an additional diagnosis. Attention deficit and hyperactivity disorder (ADHD), anxiety and obsessive–compulsive disorder, and emotional disorders are just some examples of psychiatric comorbidities often reported in people with ASD (2–5). It has been observed that psychiatric and behavioral comorbidities generally lead to more severe impairments because of the cumulative effects

of having more than one disorder (6). Moreover, psychiatric and behavioral comorbidity can often cause more distress to caregivers than the ASD symptoms (7), interfering with help-seeking behavior and thereby affecting the long-term prognosis (8). In the field of ASD, acceptance and help-seeking behavior lead to a more positive outcome. Indeed, diagnosis and early intervention before emotional, behavioral, and social problems are firmly ingrained have significant benefits for parental mental health, such as maximizing family acceptance and adjustment to their child's disability, and an impact on child functioning (9).

According to the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (*DSM-5*) (1), additional neurodevelopmental, mental, or behavioral conditions should be specified in ASD, raising the need for a behavioral, emotional, and psychiatric evaluation. The evaluation of co-occurring disorders and follow-up examinations are then recommended and will help to determine the level of care needed.

The Child Behavior Checklist (CBCL) is a well-established and widely used parent-completed measure of emotional, behavioral, and social problems in children and adolescents aged 1.5–18 years (10, 11). It was developed to assess a range of problem behaviors (10, 11) and comprises several subscales, including Withdrawn Somatic Complaints, Anxious/Depressed, Rule-Breaking Behavior, Social Problems, Thought Problems, Attention Problems, and Aggressive Behavior. The measure provides scores for three summary scales (Internalizing, Externalizing, and Total Problems), seven or eight syndrome scales (for the age groups of 1.5–5 years and 6–18 years, respectively) representing different patterns of co-occurring emotional and behavioral problems, and five or six *DSM*-oriented scales (for the two age groups) derived through expert consensus. Each *DSM*-oriented scale reflects a broad emotional or behavioral problem that corresponds to a broad diagnostic category of the *DSM* (10). The power of the CBCL lies in a guided description of the child by the parents, whose fidelity in reporting symptoms is also widely recognized for symptoms of ASD (12).

There are a number of studies that provide data for the CBCL as a valid tool to assess co-occurring emotional and behavioral problems in children with ASD (13–17). The study by Pandolfi and colleagues (18) provides evidence to support the inclusion of the CBCL in screenings and diagnostic assessments for emotional and behavioral problems in a group of 76 youths with ASD. However, with respect to the factor validity of the CBCL, the sample size was too small to test the true hierarchical structure, and each subscale was therefore analyzed separately. The results suggest that the Total Problems scale is a valid measure for both emotional and behavioral problems, while the Internalizing Problems scale, with the Anxious/Depressed and the Withdrawn/Depressed scales, is a sensitive measure for emotional problems. Nevertheless, the authors emphasized that the CBCL had lower specificity for assessing emotional problems in their group of youths with ASD than diagnostic outcomes based on categorical classification systems (*DSM*). Recently, Magyar and Pandolfi (2) evaluated the validity of the *DSM*-oriented CBCL scales for assessing depression and anxiety in 93 children and adolescents with ASD with or without intellectual disability. The results provide strong support for using the *DSM*-oriented Affective and

Anxiety Problem scales to screen emotional disorders including depression and anxiety conditions in ASD.

However, in the cited studies, the numbers of participants are small, and the patient samples constitute only a minor proportion of the general population with ASD (19–21). Moreover, the CBCL has been proposed primarily for screening of ASD in clinical settings (13, 14, 16, 17, 22, 23), rather than for evaluating psychopathological conditions and behavioral and emotional problems in comorbidity with ASD.

The aim of the present study was to assess emotional, behavioral, and social problems in relation to ASD symptom severity, cognitive development, gender, and age by analyzing the CBCL in a large group of children and adolescents with ASD.

MATERIAL AND METHODS

Participants

Seven hundred thirty-five children and adolescents were recruited for this study, between January 2008 and December 2017, at the Child and Adolescence Neuropsychiatry Unit of the Children Hospital Bambino Gesù, Rome, Italy. All children and adolescents were outpatients attending the unit for clinical assessments and rehabilitative follow-ups.

The chronological age of all participants ranged from 2.6 to 17.8 years (mean age ± sd: 10.7 ± 2.4).

Neuropsychological and psychopathological evaluations were conducted by trained developmental psychiatrists and neuropsychologists. Patients recruited earlier than 2013 received a diagnosis of ASD according to the *DSM Fourth Edition, Text Revised* (*DSM-IV-TR*) criteria (24), while those recruited later were diagnosed according to the *DSM-5* criteria (1).

Measures

Cognitive development was assessed by the nonverbal intelligence quotient (IQ) obtained from the Leiter-R (25) or Leiter-3 (26), or by the general quotient (GQ) obtained from the Griffiths Mental Development Scales—Extended Revised for age 2–8 (GMDS-ER 2-8) (27). The Leiter-R and Leiter-3 offer a completely nonverbal measure of intelligence and evaluate the ability to reason by analogy, by matching and perceptual reasoning in general, irrespective of language and formal schooling. The brief IQ composite obtained from the Leiter-R is based on four subtests: Figure Ground, Form Completion, Sequential Order, and Repeated Patterns. Similarly, the complete IQ composite obtained from the Leiter-3 is based on four subtests: Figure Ground, Form Completion, Classification and Analogies, and Sequential Order.

The GMDS-ER 2-8 was administered when a child failed to complete the Leiter scales because of his/her reduced attentional resources. The GMDS-ER 2-8 was completed by 249 children (mean age ± sd: 4.2 ± 1.1), while the Leiter scales were completed by 153 children (mean age ± sd: 7.4 ± 3.1).

The gold-standard instruments used in this study to assess ASD symptoms were the following: the Autism Diagnostic Interview—Revised (ADI-R) (28), the Autism Diagnostic Observation Schedule—Generic (ADOS-G) (29), and the revised version ADOS-2 (30). The ADI-R is a parent-report

semi-structured interview for establishing a clinical diagnosis of ASD. It follows the *DSM-IV-TR* diagnostic criteria for ASD in children with a mental age of 18 months and above. The ADI-R generates algorithm scores for each of the three subdomains of autistic symptoms: qualitative impairments in reciprocal social behavior; qualitative abnormalities in communication; and restricted range of interests and/or stereotypic behaviors. The ADOS (29, 30) is a semi-structured direct assessment of communication, social interaction, and play or imaginative use of materials for individuals with a suspected diagnosis of ASD. The ADOS consists of four (29) or five (30) modules designed for children and adults with different language levels, ranging from nonverbal to verbally fluent. The ADOS was administered and scored by licensed clinicians who have demonstrated clinical proficiency on the instrument. In the analyses, raw total scores and comparison scores (CS) were considered for the ADOS-G and ADOS-2, respectively. For the ADOS-G, the calibrated severity score (31) (renamed "CS" in the ADOS-2) was not included in the analyses, given that the present study started prior to the development of this measure.

Behavioral problems were assessed by the Achenbach System of Empirically Based Assessment (ASEBA) questionnaire. The ASEBA family of instruments is well validated to study global psychopathology in children and adolescents (32). Several ASEBA measures that allow a comprehensive approach to identify functioning in youths include the CBCL 1.5–5 years and the CBCL 6–18 years. The parents completed the CBCL; they were requested to evaluate their child's behavior during the preceding 6 months on a 3-point Likert scale for each item (0 = not true; 1 = somewhat or sometimes true; 2 = very true or often true). All scales of the CBCL have a *t*-score mean of 50 and a standard deviation of 10, and different norms are provided for gender across age groups. The CBCL 1.5–5 comprises 100 problem items identified on several subscales, including Emotionally Reactive, Anxious/Depressed, Somatic Complaints, Withdrawn, Sleep Problems, Attention Problems, and Aggressive Behavior. Moreover, scores on Internalizing, Externalizing, and Total Problems can be obtained. The Internalizing domain is a broad measure of emotional problems. It is an aggregate of anxiety and depression symptoms that subsumes four more narrowly focused syndrome scales: Emotionally Reactive, Anxious/Depressed, Somatic Complaints, and Withdrawn. The Externalizing domain is an aggregate measure of behavioral problems and includes Attention Problems and Aggressive Behavior. The Total Problems score quantifies the overall extent of both emotional and behavioral problems, based on responses to all CBCL items including those on the one remaining syndrome scale: Sleep Problems. In the CBCL 6–18, the 113-item scale is also subdivided into several subscales, namely Withdrawn/Depressed, Somatic Complaints, Anxious/Depressed, Rule-Breaking Behavior, Social Problems, Thought Problems, Attention Problems, and Aggressive Behavior. As for the CBCL 1.5–5, scores on Internalizing, Externalizing, and Total Problems can be obtained. The Internalizing domain here subsumes three syndrome scales: Anxious/Depressed, Withdrawn/Depressed, and Somatic Complaints. The Externalizing domain includes the Rule-Breaking Behavior and Aggressive Behavior syndrome

scales. The Total Problems is based on responses to all CBCL items including those on the three remaining syndrome scales: Social Problems, Thought Problems, and Attention Problems.

In the present study, Total Problems, Internalizing Problems, and Externalizing Problems scores were used as an estimate of behavioral and emotional problems. According to the normative data of the CBCL, a *t*-score ≤ 59 indicates non-clinical symptoms, a *t*-score between 60 and 64 indicates that the child is at risk for problem behaviors, and a *t*-score ≥ 65 indicates clinical symptoms (for demographical, cognitive, and psychopathological measures of participants, see **Table 1**).

Data Analysis

Student's *t*-tests were used to compare Total, Internalizing, and Externalizing Problems *t*-scores of male and female children and adolescents. To correct for multiple comparisons, the level of significance was set at $p \leq 0.008$, using the Bonferroni correction (6 comparisons).

Pearson's correlation coefficient was used to study the relationship between Total, Internalizing, and Externalizing Problems scores as well as measures derived from the ADOS, cognitive development, and age. To correct for multiple comparisons, the level of significance was set at $p \leq 0.003$, using the Bonferroni correction (15 comparisons).

RESULTS

Out of 735 patients with ASD, the parents of 472 children completed the CBCL. Concerning Total Problems of the CBCL, the results show that 49.2% of all participants ($N = 232$) who obtained scores in the non-clinical range (*t*-score ≤ 59), 16.1% ($N = 76$) were at risk ($60 \geq$ *t*-score ≥ 64), and 34.7% ($N = 164$) had clinical symptoms (*t*-score ≥ 65). In sum, around 50% of all children and adolescents had a score in a clinical range or were at risk, according to their Total Problems scores. Concerning Internalizing and Externalizing Problems of the CBCL, 32.6% of all participants ($N = 154$) obtained a non-clinical score. However, as for Internalizing Problems, 13.6% ($N = 64$) of individuals were at risk, and 16.3% ($N = 77$) had clinical symptoms. Regarding Externalizing Problems, 4.2% ($N = 20$) of

TABLE 1 | Demographic characteristics and cognitive and psychopathological measures of children and adolescents with autism spectrum disorder.

	N	M (SD)
Age	472	5.45 (2.94)
GQ	249	67.63 (21.27)
IQ	153	92.99 (23.29)
ADOS-G	315	13.90 (4.21)
ADOS-2	132	6.11 (1.53)
CBCL INT	472	60.50 (9.36)
CBCL EXT	472	61.34 (9.10)
CBCL TOT	472	56.47 (9.00)

GQ, general quotient; IQ, intelligence quotient; ADOS-G, Autism Diagnostic Observation Schedule—Generic total raw score; ADOS-2, Autism Diagnostic Observation Schedule-2 comparative score; CBCL INT, Child Behavior Checklist Internalizing Problems; CBCL EXT, Child Behavior Checklist Externalizing Problems; CBCL TOT, Child Behavior Checklist Total Problems.

children were at risk, and 1.7% (N = 8) showed clinical scores. In sum, around 30% of participants with ASD scored in the clinical range or were at risk regarding their Internalizing Problems score, while only around 6% of participants showed Externalizing Problems (clinical or at-risk scores).

With regard to gender, 48.6% of male children with ASD (N = 186) obtained scores in the non-clinical range for Total Problems, 14.9% (N = 57) were at risk, and 36.6% (N = 140) showed clinical symptom scores. Of the female children, 51.7% with ASD (N = 46) obtained scores in the non-clinical range for Total Problems, 21.3% (N = 19) were at risk, and 27% (N = 24) showed clinical symptom scores. As for Total Problems mean scores, male and female children and adolescents did not differ (see **Table 2**).

For Internalizing Problems, 35.2% of the male patients with ASD (N = 135) showed scores in the non-clinical range, 22.5% were at risk (N = 86), and 42.3% showed significant symptoms (N = 162). Of the female patients with ASD, 52.8% (N = 47) showed scores in the non-clinical range for Internalizing Problems, 16.9% were at risk (N = 15), and 30.3% showed clinical symptom scores (N = 27). Male patients showed significantly higher scores on the Internalizing Problems scale than female patients (see **Table 2**).

As for Externalizing Problems, 62.9% of male patients with ASD (N = 241) obtained scores in the non-clinical range, 17.2% were at risk (N = 66), and 19.8% (N = 76) showed clinical problems. Of the female patients, 60.7% (N = 54) obtained scores in the non-clinical range, 21.3% were at risk (N = 19), and 18% (N = 16) showed clinical problems. Male and female patients did not differ regarding their Externalizing Problems mean scores.

Analyses of the relationship between CBCL and ADOS scores did not show any significant correlation. Specifically, the Total, Internalizing, and Externalizing Problems scores of the CBCL did not correlate with the raw total score of the ADOS-G or the CS of the ADOS-2. With regard to cognitive developmental measures, the GQ was found to be inversely correlated with Total Problems scores, with participants with lower GQ showing more symptoms. The IQ was not found to correlate with Total Problems scores. No correlation was found between cognitive developmental measures (GQ or IQ) and Internalizing and Externalizing Problems scores (see **Table 3**).

Concerning the relationship between age and CBCL scores, Total Problems scores were found to correlate with age, with older children exhibiting more symptoms. No correlation was found between age and Internalizing or Externalizing Problems (see **Table 3**).

DISCUSSION

The present study aimed at investigating whether emotional, behavioral, and social problems were modulated by ASD symptom severity, cognitive development, gender, and age by analyzing the CBCL in a large group of 472 children and adolescents with ASD.

The CBCL is one of the most widely investigated instruments to detect emotional and behavioral problems in children and adolescents. Many studies support its reliability and validity across different clinical groups [see, for example, Ref. (33)]. Earlier investigations have corroborated its factor structure, the consistency of its major scales and subscales, and its sensitivity for identifying emotional and behavioral problems. A number of studies using the CBCL have evaluated emotional and behavioral symptoms (34, 35) as well as psychopathological comorbidities in children with neurodevelopmental disorders by examining the correlations between CBCL scores, IQ, and gender. However, the CBCL has generally been proposed for supporting an ASD diagnosis in a clinical setting (18, 36), while very little is known about the development of internalizing and externalizing problems in isolation, and nothing is known about their joint development (37).

In the present study, CBCL scores were correlated with indices of ASD severity, cognitive developmental measures, and age. Differences between male and female patients regarding Total,

TABLE 2 | Comparisons between males and females with autism spectrum disorder on Child Behavior Checklist scales.

	Males	Females	
	M (SD)	M (SD)	t-value (p)
CBCL INT	61.88 (8.94)	59.06 (9.49)	2.65 (0.008)*
CBCL EXT	56.60 (8.92)	55.87 (9.35)	0.70 (0.48)
CBCL TOT	60.83 (9.37)	59.10 (9.24)	1.57 (0.12)

CBCL INT, Child Behavior Checklist Internalizing Problems; CBCL EXT, Child Behavior Checklist Externalizing Problems; CBCL TOT, Child Behavior Checklist Total Problems.
* The level of significance was set at p ≤ 0.008 by using Bonferroni correction (6 comparisons).

TABLE 3 | Correlation between Child Behavior Checklist measures and indices of autism spectrum disorder, cognitive developmental measures, and age.

	CBCL TOT		CBCL INT		CBCL EXT	
	r	p	r	P	r	p
ADOS-G	0.02	0.67	0.03	0.57	0.02	0.75
ADOS-2	−0.09	0.32	−0.07	0.45	−0.12	0.19
GQ	−0.22	0.001*	−0.17	0.007	−0.15	0.015
IQ	0.06	0.44	0.17	0.04	0.05	0.53
Age	0.2	<0.001*	0.12	0.012	0.11	0.02

CBCL TOT, Child Behavior Checklist Total Problems; CBCL INT, Child Behavior Checklist Internalizing Problems; CBCL EXT, Child Behavior Checklist Externalizing Problems; ADOS-G, Autism Diagnostic Observation Schedule—Generic total raw score; ADOS-2, Autism Diagnostic Observation Schedule-2 comparative score; GQ, general quotient; IQ, intelligence quotient.
* The level of significance was set at p ≤ 0.003 by using Bonferroni correction (15 comparisons).

Internalizing, and Externalizing Problems scores of the CBCL were also evaluated.

Our results show that out of the 472 examined children, around 30% exhibited Internalizing Problems, with CBCL scores in the clinical or at-risk range, and with significantly higher scores for male than female children. However, concerning Externalizing Problems, only 6% of all participants had clinical or at-risk scores.

Similarly, an earlier study by Hartley and colleagues (22) reported that 29.6% of children with ASD had significant Internalizing Problems. However, the authors reported a higher percentage for Externalizing Problems (27.2%) than what we found in the current study. This discrepancy could be due to differences in participant characteristics, with younger children (mean age 3.5 years) involved in the study by Hartley and colleagues (20) and older children in our study (mean age 10.7 years old). Indeed, the Externalizing Problems scale is derived from Attention Problems and Aggressive Behavior items that may be displayed more prominently at younger ages.

A previous report (38) studied phenotypic expressions of behavioral problems in 215 preschool children (3 years old) by using CBCL in different clinical populations and typically developing children. The authors evidenced that children with ASD or cerebral palsy had more total symptoms and more internalizing problems than children with Down syndrome or typically developing children. Moreover, children with ASD showed more externalizing symptoms than typically developing children. A reduction of externalizing symptoms during early childhood in ASD was also documented. This result could explain why a small percentage of externalizing symptoms was found in our participants with mean age of 10.7 years old.

Psychiatric and behavioral comorbidities were also examined in studies that assessed the *DSM*-oriented subscale of CBCL (39) or single clinical subscales (40, 41). In particular, Georgiades and colleagues (40) explored in 335 preschool children with ASD the following CBCL subscales: Emotionally Reactive, Anxious/Depressed, Somatic Complaints, Withdrawn, Attention Problems, and Aggressive Behavior. Results showed that 39.2% of children with ASD were in the "clinical range" on the Withdrawn subscale, whereas 11.7% and 7.2% scored above the clinical cutoff on the Attention Problems and Emotionally Reactive subscales, respectively. In this preschool-age population, the study (40) documented higher prevalence of internalizing symptoms than externalizing symptoms.

Llanes and colleagues (41) used the CBCL (Parent and Teacher Report Forms) to evaluate the prevalence of ADHD symptoms and anxiety in 180 preschools (age range 4–5 years) and school-aged children (age range 6–7 years) with ASD. In the study, parents reported anxiety symptoms in 31% of preschool children and in 50% of school-aged children and ADHD symptoms in 22% of preschool children and 45% of school-aged children. Even if authors did not report specific scores for Internalizing and Externalizing Problems scales, the study (41) showed more anxiety symptoms than ADHD symptoms at each age investigated, documenting a high percentage of internalizing problems. Moreover, in our study, children with ASD were older (mean age 10.7 years) than those in the study by Llanes and colleagues (41), and it could be that internalizing symptoms are more present in older than in younger children.

There are studies that did not use CBCL to investigate behavioral and emotional problems in individuals with ASD but adopted different diagnostic approaches and assessment batteries. Thus, it is difficult to compare our results with these studies, when researchers use different tools to assess comorbid psychopathology symptoms and diagnose psychiatric disorders.

For example, concerning an internalizing disorder such as anxiety, the prevalence rates of anxiety in children with ASD ranged from 11% to 84% in a literature review (42, 43).

The review by Skokauskas and Callager (44) highlighted that anxiety disorders represent the most common psychiatric comorbidity in individuals with ASD. For example, Caamaño and colleagues (45) found that 76% of children and adolescents with ASD presented anxiety symptoms and 56% attention problems; Amr and colleagues (46) found that 58.3% of children with ASD showed anxiety disorders and 13.3% a major depressive disorder (while only 31.6% and 23.3% presented ADHD and conduct disorders, respectively).

Although internalizing symptoms were frequently found associated with ASD, a wide range of externalizing symptoms were also documented in ASD. Sizing and colleagues (47) carried out a study on ADHD-like symptoms using 3 rating scales. Results revealed a high phenotypical overlap between ASD and ADHD (53%) that was interpreted by authors as the effect of difficulties in communicating needs and in attracting adult attention in children with ASD. Another study (5) used a parent interview (the Child and Adolescent Psychiatric Assessment) to assess emotional and behavioral problems in 112 children with ASD (age range 10–14 years). Results evidenced that the most common diagnoses were social anxiety disorder (29.2%), ADHD (28.2%), and oppositional defiant disorder (28.1%). Even if authors (5) found a high percentage of internalizing disorders (such as social anxiety disorder), it is difficult to compare their results with our results obtained by CBCL, which did not consider *DSM*-oriented scales and individual subscales.

Despite the different method adopted (the Problem Behavior scale of the Scales of Independent Behavior—Revised) (48), Shattuck and colleagues (49) examined internalizing and externalizing problems across 4.5 years in 241 individuals with ASD with a wide age range (from 10 years to 52 years). Similarly to our results, the authors found a higher prevalence of internalizing behaviors (hurtful to self, unusual or repetitive habits, withdrawal or inattentive behavior) than externalizing behaviors (hurtful to others, destructive to property, disruptive behavior) in individuals with ASD.

In our study, no correlation was found between CBCL scores and indices of ASD severity (ADOS-G raw total score and ADOS-2 CS), similarly to previous results that showed no relation (20).

Conversely, a correlation was observed between age and CBCL scores, with older children exhibiting more symptoms for Total Problems than younger ones. The higher percentage of behavioral problems in older children with ASD could be interpreted in the light of increasing social and environmental demands and resulting difficulties of older children in adjusting their behavior to changing environmental demands. Since the CBCL is completed by the parents, another possible interpretation is that

parents are more likely to have concerns regarding emotional and behavioral problems when their children are older. It is difficult, however, to compare our results with previous studies on the relation between age and CBCL scores, due to differences in analysis methods between studies. For example, the study by Hartley and colleagues (20) did not find a significant correlation between age and CBCL Externalizing Problems scores, but it documented a positive correlation between age and Internalizing Problems scores. Since the authors did not consider Total Problems scores in their analyses, it is difficult to compare our results with their findings (20).

A negative correlation was also found between CBCL Total Problems scores and cognitive developmental measures as evaluated by the GMDS-ER 2-8, since children with ASD with lower GQ had higher Total Problems scores. However, no correlation was found between IQ measured by the Leiter scales and CBCL Total Problems scores. This result could be explained by differences in the mean age of children evaluated using the GMDS-ER 2-8 and the Leiter scales. Indeed, the former group evaluated using the GMDS-ER 2-8 was younger (mean age 4.2 years) than the second group (mean age 7.4 years) evaluated using the Leiter scales. An early study conducted by Bölte and colleagues (19) in 77 children with ASD (54 male and 23 female children, with a mean age of 11.3 years) found a significant influence of age and IQ on CBCL scores, with no gender differences in CBCL scores. It is, however, difficult to compare our results with the findings by Bölte and colleagues (19), since their CBCL scores were not normalized and their analyses were conducted on raw scores. More recently, Hartley and colleagues (20) studied correlations between CBCL Externalizing Problems, Internalizing Problems, and syndrome scale scores and characteristics such as cognitive functioning, gender, age, ethnicity, expressive language, and severity of autistic and adaptive behaviors in 169 children with ASD (mean age 3.5 years). Similar to our results in a younger group of participants, they found that CBCL scores correlated with cognitive functioning scores, with a significant negative correlation between Internalizing and Externalizing Problems scores and cognitive functioning measures. Mensi and colleagues (21) also evaluated correlations between emotional and behavioral problems (derived from the CBCL 1.5–5 years) and the GQ (derived from the GMDS-ER 2-8), age, and ADOS and ADI-R scores in a group of 90 children with ASD with a mean age of 3.9 years who were diagnosed according to the *DSM-IV* criteria. Mothers and fathers completed the CBCL separately, and the authors showed a significant low-grade positive correlation between the children's age and their CBCL scores on the Pervasive Developmental Disorders scale of the questionnaires completed by the mothers. No correlation with age was found in the questionnaires completed by the fathers. In the CBCL completed by both mothers and fathers, some positive correlations were identified between the Internalizing, Withdrawal, and Pervasive Developmental Disorders scores and the subscales relating to social interactions and behavior of the ADI-R. Beside these measures to confirm a diagnosis of ASD, the findings by Mensi and colleagues (21) on a relationship between CBCL scores and GMDS-ER 2-8 measures are in line with our results. An inverse correlation between the GMDS-ER 2-8 Language subscale and Total and Externalizing Problems scores for the fathers and

Total Problems scores for the mothers was found. Furthermore, the study by Mensi and colleagues (21) emphasizes the importance of considering the different perspectives of the mother and the father to obtain a complete representation of the emotional and behavioral problems of the child.

The present study underlines the need to use instruments such as the CBCL in children with ASD, not just for screening and forming a diagnosis, but also for detecting emotional and behavioral problems. Indeed, our results point out that psychopathological comorbidities (especially internalizing symptoms) are present in a significant number of children with ASD (in 30% of the population investigated) and that they are related to gender (more present in males), age (more present in older children), and cognitive development (more present in children with lower GQ).

The detection of related conditions in ASD is a major concern for experts in the field (50). The inherent heterogeneity of ASD, with a variety of different symptom clusters, complicates the goal of identifying specific treatments. Several factors make it difficult to assess emotional and behavioral problems in many individuals with ASD. These include apparent symptom overlap between characteristics of ASD and symptoms of comorbidities (51). In recent years, there is a growing recognition of the need for individualization of treatment and approaches in ASD (52), and the detection of behavioral and emotional problems allows children with ASD to undergo more specific and individualized treatments that take into account their psychopathological problems.

We believe this study with a large sample size helps move the field toward more precise and valid measurements of psychopathologies in young children and adolescents with ASD, and our findings have implications for the quality of life and long-term outcomes of those individuals. The present study has several limitations. First, since this is a retrospective study, a cause-and-effect relationship cannot be determined, but it is useful for providing preliminary data and in guiding the development of future prospective studies. Clinical assessments of ASD were conducted over a period of 9 years, using different instruments to asses ASD such as the ADOS-G and the revised version ADOS-2. Therefore, analyses required us to include two different scores: raw total scores for the ADOS-G and comparison scores for the ADOS-2. Moreover, we analyzed data that were collected prior to the development of the calibrated severity score (included only in the ADOS-2), and it was thus not possible to consider this single valid measure to more accurately capture the core symptom severity of ASD.

Second, our analyses examined only three main areas of the CBCL questionnaire (Total Problems, Internalizing Problems, Externalizing Problems). By examining only these three scales, we did not analyze more discrete aspects of emotional and behavioral problems, such as individual subscales or *DSM*-oriented scales. Further studies are needed to assess the emotional and behavioral profile of children with ASD by using more CBCL scales and more objective assessment of psychiatric comorbidity.

Moreover, the CBCL questionnaire was completed by only one parent, while studies that analyze reports from both parents may be more informative.

Future studies should also examine emotional and behavioral problems of children with ASD against a typically developing

control group or other group with neurodevelopmental disorders to control for the nonspecific psychopathological comorbidity in ASD.

Finally, it's difficult to distinguish how much of clinical scores on CBCL are affected by core ASD symptoms or by comorbid psychiatric, emotional, and behavioral problems. However, Hess and colleagues (53) documented that internalizing symptoms (worry/depressed, undereating, overeating, avoidant behavior, and repetitive behavior) should be taken into account in ASD since they were significantly different between individuals with and without ASD, whereas externalizing symptoms (conduct behavior and tantrum behavior) were not significantly different among those with and without ASD. Noordhof and colleagues (54) found that ASD-related problems assessed by CBCL constituted a specific domain of psychopathology that could be distinguished from the internalizing and externalizing.

In conclusion, our study assessed psychopathological comorbidities in a large group of children with ASD. Since psychiatric disorders can worsen symptoms of ASD, interfere with education, and reduce the benefits of treatment, more studies on the detection of these problems are needed and will allow children with ASD to receive appropriate therapies that take into account their emotional and behavioral problems.

ETHICS STATEMENT

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. For this type of study, formal consent is not required. Written informed consent was obtained from parents of each participant included in the study.

AUTHOR CONTRIBUTIONS

SG participated in the design and coordination of the study and interpretation of the data, performed the measurement, and drafted and revised the manuscript. DM conceived of the study, participated in its design and interpretation of the data, performed the statistical analysis, and helped to draft and revise the manuscript. EN participated in the design of the study, performed the measurement, and helped to draft and revise the manuscript. SD participated in the design of the study, performed the measurement, and helped to draft and revise the manuscript. GV participated in the design of the study and helped to draft the manuscript. SV participated in the design of the study, coordination, and interpretation of the data, and helped to draft the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

REFERENCES

1. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders (DSM-5®)*. Washington: American Psychiatric Publishing (2013). doi: 10.1176/appi.books.9780890425596

2. Magyar CI, Pandolfi V. Utility of the CBCL *DSM*-oriented scales in assessing emotional disorders in youth with autism. *Res Autism Spectr Disord* (2017) 37:11–20. doi: 10.1016/j.rasd.2017.01.009

3. Romero M, Aguilar JM, Del-Rey-Mejías Á, Mayoral F, Rapado M, Peciña M, et al. Psychiatric comorbidities in autism spectrum disorder: a comparative study between *DSM-IV-TR* and *DSM-5* diagnosis. *Int J Clin Health Psychol* (2016) 16:266–75. doi: 10.1016/j.ijchp.2016.03.001

4. van Steensel FJ, Bögels SM, Perrin S. Anxiety disorders in children and adolescents with autistic spectrum disorders: a meta-analysis. *Clin Child Fam Psychol Rev* (2011) 14:302. doi: 10.1007/s10567-011-0097-0

5. Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G. Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. *J Am Acad Child Adolesc Psychiatry* (2008) 47:921–9. doi: 10.1097/CHI.0b013e318179964f

6. Gadow KD, Guttmann-Steinmetz S, Rieffe C, DeVincent CJ. Depression symptoms in boys with autism spectrum disorder and comparison samples. *J Autism Dev Disord* (2012) 42:1353–63. doi: 10.1007/s10803-011-1367-x

7. Lecavalier L, Leone S, Wiltz J. The impact of behaviour problems on caregiver stress in young people with autism spectrum disorders. *J Intellect Disabil Res* (2003) 50:172–83. doi: 10.1111/j.1365-2788.2005.00732.x

8. Horner RH, Diemer SM, Brazeau KC. Educational support for students with severe problem behaviors in Oregon: a descriptive analysis from the 1987–88 school year. *J Assoc Pers Sev Handicaps* (1992) 17:154–69. doi: 10.1177/154079699201700304

9. Daley TC. From symptom recognition to diagnosis: children with autism in urban India. *Soc Sci Med* (2004) 58:1323–35. doi: 10.1016/S0277-9536(03)00330-7

10. Achenbach TM, Rescorla L. *Manual for the ASEBA preschool forms and profiles*. Burlington, VT: University of Vermont, Research center for children, youth, & families (2000).

11. Achenbach TM, Rescorla L. *Manual for the ASEBA school-age forms & profiles*. Burlington, VT: University of Vermont, Research center for children, youth, & families (2001).

12. So P, Greaves-Lord K, van der Ende J, Verhulst FC, Rescorla L, de Nijs PF. Using the Child Behavior Checklist and the teacher's report form for identification of children with autism spectrum disorders. *Autism* (2013) 17:595–607. doi: 10.1177/1362361312448855

13. Biederman J, Petty CR, Fried R, Wozniak J, Micco JA, Henin A, et al. Child Behavior Checklist clinical scales discriminate referred youth with autism spectrum disorder: a preliminary study. *J Dev Behav Pediatr* (2010) 31:485–90. doi: 10.1097/DBP.0b013e3181e56eddd

14. Ooi YP, Rescorla L, Ang RP, Woo B, Fung DSS. Identification of autism spectrum disorders using the Child Behavior Checklist in Singapore. *J Autism Dev Disord* (2011) 41:1147–56. doi: 10.1007/s10803-010-1015-x

15. Achenbach TM, Rescorla L. Achenbach System of Empirically Based Assessment. In: Volkmar FR, editor. *Encyclopedia of autism spectrum disorders*. NY: Springer (2013). p. 31–9. doi: 10.1007/978-1-4419-1698-3_219

16. Muratori F, Narzisi A, Tancredi R, Cosenza A, Calugi S, Saviozzi I, et al. The CBCL1.5–5 and the identification of preschoolers with autism in Italy. *Epidemiol Psychiatr Sci* (2011) 20:329–38. doi: 10.1017/S204579601100045X

17. Narzisi A, Calderoni S, Maestro S, Calugi S, Mottes E, Muratori F. Child behavior check list 1½–5 as a tool to identify toddlers with autism spectrum disorders: a case–control study. *Res Dev Disabil* (2013) 34:1179–89. doi: 10.1016/j.ridd.2012.12.020

18. Pandolfi V, Magyar CI, Norris M. Validity study of the CBCL 6–18 for the assessment of emotional problems in youth with ASD. *J Ment Health Res Intellect Disabil* (2014) 7:306–22. doi: 10.1080/19315864.2014.930547

19. Bölte S, Dickhut H, Poustka F. Patterns of parent-reported problems indicative in autism. *Psychopathology* (1999) 2:93–7. doi: 10.1159/000029072

20. Hartley SL, Sikora DM, McCoy R. Prevalence and risk factors of maladaptive behaviour in young children with autistic disorder. *J Intellect Disabil Res* (2008) 52:819–29. doi: 10.1111/j.1365-2788.2008.01065.x

21. Mensi MM, Carigi T, Chiappedi M, Balottin U. Correlazioni tra CBCL e variabili cliniche e socio-demografiche in bambini con Disturbi Pervasivi dello Sviluppo. *Boll Soc Med Chir di Pavia* (2013) 126:571–7. doi: 10.6092/2039-1404.126.1585

22. Duarte CS, Bordin IA, de Oliveira A, Bird H. The CBCL and the identification of children with autism and related conditions in Brazil: pilot findings. *J Autism Dev Disord* (2003) 33:703–7. doi: 10.1023/B:JADD.0000006005.31818.1c

23. Havdahl KA, von Tetzchner S, Huerta M, Lord C, Bishop SL. Utility of the Child Behavior Checklist as a screener for autism spectrum disorder. *Autism Res* (2016) 9:33–42. doi: 10.1002/aur.1515

24. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders 4ᵗʰ ed., text revised (DSM-IV-TR)*. Washington, DC: American Psychiatric Association (2000).

25. Roid GH, Miller LJ. *Leiter International Performance Scale—Revised*. Woodale, IL: Stoelting C (1997). doi: 10.1037/t05120-000

26. Roid GH, Miller LJ, Pomplun M, Koch C. *Leiter International Performance Scale, Third Edition (Leiter-3)*. Los Angeles: Western Psychological Services. (2013). Italian edition Cornoldi C, Giofrè D, Belacchi C, Editors. Firenze: Giunti Organizzazioni Speciali.

27. Luiz D, Barnard A, Knoesen N, Kotras N, Horrocks S, McAlinden P, et al. *GMDS-ER 2-8—Griffiths Mental Development Scales—Extended Revised: 2 to 8 years (2006)*. Italian edition Cianchetti C, Fancello GS, Editors. Firenze: Giunti Organizzazioni Speciali.

28. Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview—Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord* (1994) 24:659–85. doi: 10.1007/BF02172145

29. Lord C, Risi S, Lambrecht L, Cook EH, Leventhal BL, DiLavore PC, et al. The Autism Diagnostic Observation Schedule—Generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord* (2000) 30:205–23. doi: 10.1023/A:1005592401947

30. Lord C, Rutter M, DiLavore PC, Risi S, Gotham K, Bishop S. *Autism Diagnostic Observation Schedule—Second Edition (ADOS-2)*. Los Angeles: Western Psychological Service Italian edition. Colombi C, Tancredi R, Persico A, Faggioli A, Editors. Firenze: Hogrefe Editore (2012).

31. Gotham K, Pickles A, Lord C. Standardizing ADOS scores for a measure of severity in autism spectrum disorders. *J Autism Dev Disord* (2009) 39:693–705. doi: 10.1007/s10803-008-0674-3

32. Rescorla LA. Assessment of young children using the Achenbach System of Empirically Based Assessment (ASEBA). *Dev Disabil Res Rev* (2005) 11:226–37. doi: 10.1002/mrdd.20071

33. Bérubé RL, Achenbach TM. *Bibliography of published studies using the Achenbach System of Empirically Based Assessment (ASEBA)*. Burlington, VT: University of Vermont, Research Center for Children, Youth, and Families (2010).

34. Osório AA, Rossi NF, Gonçalves ÓF, Sampaio A, Giacheti CM. Psychopathology and behavior problems in children and adolescents with Williams syndrome: distinctive relationships with cognition. *Child Neuropsychol* (2017) 23:631–41. doi: 10.1080/09297049.2016.1183607

35. Rietman AB, Oostenbrink R, van Noort K, Franken MCJ, Catsman-Berrevoets CE, Aarsen FK, et al. Development of emotional and behavioral problems in neurofibromatosis type 1 during young childhood. *Am J Med Genet A* (2017) 173:2373–80. doi: 10.1002/ajmg.a.38323

36. Pandolfi V, Magyar CI, Dill CA. An initial psychometric evaluation of the CBCL 6–18 in a sample of youth with autism spectrum disorders. *Res Autism Spectr Disord* (2012) 6:96–108. doi: 10.1016/j.rasd.2011.03.009

37. Vaillancourt T, Haltigan JD, Smith I, Zwaigenbaum L, Szatmari P, Fombonne E, et al. Joint trajectories of internalizing and externalizing problems in preschool children with autism spectrum disorder. *Dev Psychopathol* (2017) 29:203–14. doi: 10.1017/S0954579416000043

38. Eisenhower AS, Baker BL, Blacher J. Preschool children with intellectual disability: syndrome specificity, behaviour problems, and maternal well-being. *J Intellect Disabil Res* (2005) 49:657–71. doi: 10.1111/j.1365-2788.2005.00699.x

39. Kanne SM, Abbacchi AM, Constantino JN. Multi-informant ratings of psychiatric symptom severity in children with autism spectrum disorders: the importance of environmental context. *J Autism Dev Disord* (2009) 39:856–64. doi: 10.1007/s10803-009-0694-7

40. Georgiades S, Szatmari P, Duku E, Zwaigenbaum L, Bryson S, Roberts W, et al. Phenotypic overlap between core diagnostic features and emotional/behavioral problems in preschool children with autism spectrum disorder. *J Autism Dev Disord* (2011) 41:321–9. doi: 10.1007/s10803-010-1158-9

41. Llanes E, Blacher J, Stavropoulos K, Eisenhower A. Parent and teacher reports of comorbid anxiety and ADHD symptoms in children with ASD. *J Autism Dev Disord* (2018) 1–12. doi: 10.1007/s10803-018-3701-z

42. Grondhuis SN, Aman MG. Assessment of anxiety in children and adolescents with autism spectrum disorders. *Res Autism Spectr Disord* (2012) 6:1345–65. doi: 10.1016/j.rasd.2012.04.006

43. White SW, Oswald D, Ollendick T, Scahill L. Anxiety in children and adolescents with autism spectrum disorders. *Clin Psychol Rev* (2009) 29:216–29. doi: 10.1016/j.cpr.2009.01.003

44. Skokauskas N, Gallagher L. Mental health aspects of autistic spectrum disorders in children. *J Intellect Disabil Res* (2012) 56:248–57. doi: 10.1111/j.1365-2788.2011.01423.x

45. Caamaño M, Boada L, Merchán-Naranjo J, Moreno C, Llorente C, Moreno D, et al. Psychopathology in children and adolescents with ASD without mental retardation. *J Autism Dev Disord* (2013) 43:2442–9. doi: 10.1007/s10803-013-1792-0

46. Amr M, Raddad D, El-Mehesh F, Bakr A, Sallam K, Amin T. Comorbid psychiatric disorders in Arab children with autism spectrum disorders. *Res Autism Spectr Disord* (2012) 6:240–8. doi: 10.1016/j.rasd.2011.05.005

47. Sinzig J, Walter D, Doepfner M. Attention deficit/hyperactivity disorder in children and adolescents with autism spectrum disorder: symptom or syndrome? *J Atten Disord* (2009) 13:117–26. doi: 10.1177/1087054708326261

48. Bruininks RH, Woodcock RW, Weatherman RF. *Hill BK Scales of Independent Behavior—Revised*. USA: Riverside Publishing (1996).

49. Shattuck PT, Seltzer MM, Greenberg JS, Orsmond GI, Bolt D, Kring S, et al. Change in autism symptoms and maladaptive behaviors in adolescents and adults with an autism spectrum disorder. *J Autism Dev Disord* (2007) 37:1735–47. doi: 10.1007/s10803-006-0307-7

50. Committee on Children with Disabilities. The pediatrician's role in the diagnosis and management of autistic spectrum disorder in children. *Pediatrics* (2001) 107:1221–6. doi: 10.1542/peds.107.5.1221

51. Stewart ME, Barnard L, Pearson J, Hasan R, O'Brien G. Presentation of depression in autism and Asperger syndrome: a review. *Autism* (2006) 10:103–16. doi: 10.1177/1362361306062013

52. Sherer MR, Schreibman L. Individual behavioral profiles and predictors of treatment effectiveness for children with autism. *J Consult Clin Psychol* (2005) 73:525. doi: 10.1037/0022-006X.73.3.525

53. Hess JA, Matson JL, Dixon DR. Psychiatric symptom endorsements in children and adolescents diagnosed with autism spectrum disorders: a comparison to typically developing children and adolescents. *J Dev Phys Disabil* (2010) 22:485–96. doi: 10.1007/s10882-009-9185-1

54. Noordhof A, Krueger RF, Ormel J, Oldehinkel AJ, Hartman CA. Integrating autism-related symptoms into the dimensional internalizing and externalizing model of psychopathology. The TRAILS Study. *J Abnorm Child Psychol* (2014) 43:577–87. doi: 10.1007/s10802-014-9923-4

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Examining the Association Between Electrodermal Activity and Problem Behavior in Severe Autism Spectrum Disorder: A Feasibility Study

Bradley J. Ferguson[1,2,3], Theresa Hamlin[4], Johanna F. Lantz[4], Tania Villavicencio[4], John Coles[4,5,6] and David Q. Beversdorf[2,3,7]*

[1] Department of Health Psychology, University of Missouri School of Health Professions, Columbia, MO, United States, [2] Thompson Center for Autism & Neurodevelopmental Disorders, University of Missouri, Columbia, MO, United States, [3] Department of Radiology, University of Missouri School of Medicine, Columbia, MO, United States, [4] The Center for Discovery, Harris, NY, United States, [5] CUBRC, Inc. Information Exploitation Sector, Buffalo, NY, United States, [6] Department of Industrial and Systems Engineering, University at Buffalo, Buffalo, NY, United States, [7] University of Missouri Departments of Neurology & Psychological Sciences, Columbia, MO, United States

Background: Many individuals with autism spectrum disorder (ASD) engage in problem behavior, presenting significant challenges for those providing care and services for this population. Psychophysiological measures of arousal, such as electrodermal activity (EDA), may provide an early indication of subsequent problem behavior. However, variability in EDA patterns associated with behaviors may limit this predictive ability.

Methods: EDA data was sampled from eight individuals with severe ASD in a naturalistic setting, while participating in educational programming in a school setting at a residential facility for severely affected individuals with developmental disabilities, to examine variability in EDA patterns.

Results: An anticipatory rise in EDA only occurred 60% of the time prior to the problem behavior. Additionally, EDA after a problem behavior returned to median baseline levels only 45% of the time.

Conclusions: Heterogeneity of EDA responses in those with the most severe forms of ASD will be an important consideration in future studies utilizing psychophysiological tools such as EDA to anticipate problem behavior, including the need for monitoring of return to baseline after problem behaviors. Incorporation of this consideration may lead to greater reliability of these approaches to help anticipate and manage problem behaviors.

Keywords: autism spectrum disorder (ASD), electrodermal activity (EDA), skin conductance, problem behavior, stress, anxiety, intellectual disability

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental condition that is characterized by persistent deficits in social communication and restricted, repetitive patterns of behavior that occur early in development (1). Research has demonstrated that many individuals with ASD engage in aggression and other problem behaviors such as self-injurious behavior and irritability. For example, one study found that in a sample of 1,380 children with ASD, up to two-thirds became aggressive toward their caregivers, and

nearly half became aggressive toward others (2). Those individuals with ASD with greater intellectual impairment, often with significantly limited communication and social engagement skills, are at greater risk for the development of problem behaviors (3). Problem behavior poses many challenges for caregivers and service providers alike, with the potential to become worse over time (4). In order to attempt to reduce the occurrence of problem behavior, research has begun to explore the use of psychophysiological markers preceding problem behavior so that an intervention may occur prior to the onset of the behavior.

Among those with ASD, high levels of stress may manifest as problem behaviour (5). Additionally, emotional regulation appears to be impacted in general in ASD (6). Knowing the physiological stress state of individuals with ASD may allow caretakers and service providers to intervene prior to the occurrence of problem behavior. Furthermore, better understanding environmental correlates of physiological responses can help to develop more precise treatments through control of or manipulation of these factors. One psychophysiological technique to assess an individuals' internal stress state is the measurement of electrodermal activity (EDA). Increases in EDA indicate activation of the sympathetic nervous system (the "fight-or-flight" response of the autonomic nervous system), which is measured by assessing changes in electrical conductivity between two electrodes placed in close proximity to each other on the skin. Activation of the sympathetic nervous system results in secretion of sweat, which conducts electricity, from eccrine sweat glands throughout the body. Eccrine sweat glands are only innervated by the sympathetic branch of the autonomic nervous system, so increases in EDA can be, in part, attributed to increases in physiological arousal. EDA is typically measured from areas of the body with a high density of eccrine sweat glands, such as the palms of the hands or soles of the feet, but can also reliably measure sweat secretion when placed on other areas of the body such as the wrist or immediately above ankle (7). Measurement of EDA has been shown to be well-tolerated in ASD and is sensitive to changes in arousal and emotional states in this population (8–10). However, changes in EDA in response to an arousing stimulus, vary widely in ASD. For example, abnormal baseline EDA as well as both hypo- and hyperactivities in response to human faces have been shown in ASD (11). Typically, EDA returns to baseline shortly after the application of an arousing stimulus. However, some children with ASD fail to return to their baseline EDA after the occurrence of an environmental stimulus (11), suggesting that a large stress response may continue to affect behavior long after the occurrence of a stressful event, including the engagement in problem behavior. A recent study found a relationship between EDA and externalizing behavior problems during compliance-oriented play tasks (12). However, EDA was relatively low while the individual engaged in the problem behavior, suggesting heterogeneity in the autonomic nervous system response to task demands in ASD. Furthermore, greater variability in EDA in response to a battery of naturalistic and

structured parent–child, child alone, and direct testing tasks has been shown to be associated with overall ASD severity (13), suggesting that EDA may be a promising predictor of problem behavior in ASD. In light of this, research is now beginning to explore the utility of psychophysiological markers to anticipate problem behaviors in ASD. One recent study has utilized multimodal psychophysiological arousal to successfully predict aggression in minimally verbal ASD patients in a naturalistic setting (14).

A great amount of heterogeneity exists in ASD, which can complicate research efforts when examining data at the group level. As such, identification of subtypes within ASD may lead to more effective treatments (15, 16). Psychophysiological data has recently been used to identify relationships between co-occurring symptoms in ASD, such as gastrointestinal symptoms, irritability, and sleep problems (8). Therefore, while psychophysiological data may also be useful to assess the internal stress state of individuals with ASD in a variety of settings, it is important to examine the heterogeneity of this response. Understanding this variability will be important in the development of tools to anticipate the onset of behaviors, which may help lead to more individualized treatment approaches. This will be of a particularly acute need in the most severely affected individuals, for whom problem behaviors are more frequent (3).

The present study first examined the feasibility of collecting EDA data from individuals with severe ASD in a naturalistic setting, while participating in skill acquisition in a school setting at a residential facility for severely affected individuals with developmental disabilities. The lab school at The Center for Discovery (TCFD) utilizes discreetly mounted video cameras and microphones in classrooms to collect behavioral data, while students wear physiological data collection sensors to gain a better understanding of the physiological correlates associated with learning and behavior in individuals with ASD. As such, we wished to examine the feasibility of examining psychophysiological variables in individuals with severe ASD as they are related to problem behavior. The beginning and end of problem behaviors were identified through video recordings and were confirmed by trained staff at TCFD. The associated EDA (i.e., time locked to the EDA recordings) was monitored prior to the occurrence of the initial problem behaviors as well as immediately after the cessation of the problem behavior, to examine individual variability in this particular psychophysiological variable as it related to problem behavior.

METHODS

Participants

Eight individuals with ASD (age = 15.9 years ± 2.5 std. dev., range = 13–20, all male, 6 Caucasians, 2 Hispanics) were examined (see **Table 1** for descriptive statistics). Diagnosis of ASD was made by a licensed psychologist and corroborated with scores from the Autism Spectrum Rating Scales (ASRS) DSM-IV-TR scale (17), a 70-item parental report of how often their child displayed each behavior associated with ASD in the past 4 weeks derived from DSM-IV-TR criteria for ASD (17). The ASRS is administered annually for all students. ASRS scores for this study correspond to the year of EDA data collection. The students' scores from the ASRS DSM-IV-TR scale all fell within the elevated (65–69) or

TABLE 1 | Participant demographics and descriptive statistics.

ID	Age	Gender	Ethnicity	Intelligence quotient (type) (M = 100, SD = 15)	Adaptive functioning	ASRS T-score (M = 50, SD = 10)	Co-occurring conditions
S01	18	M	Caucasian	44 (NV)	41 (ABAS)	82	OCD, ADHD, constipation
S06	15	M	Caucasian	52 (SB abbreviated)	45 (VAB)	84	OCD, ADHD, vomiting
S07	13	M	Caucasian	42 (NV)	35 (VAB)	74	Constipation
S08	15	M	Caucasian	NS	38 (VAB)	85	Movement disorder
S09	13	M	Hispanic	NS	59 (VAB)	68	None
S11	15	M	Caucasian	NS	35 (VAB)	76	Constipation, ADHD, GERD
S12	20	M	Caucasian	53 (NV)	48 (ABAS)	70	Constipation
S13	18	M	Hispanic	42 (NV)	43 (ABAS)	73	Constipation

NV, non-verbal; SB, Stanford–Binet; NS, attempted but unable to obtain score; ABAS, Adaptive Behavior Assessment System General Adaptive Composite; VAB, Vineland Adaptive Behavior Scales Adaptive Behavior Composite; OCD, obsessive-compulsive disorder; ADHD, attention-deficit hyperactivity disorder; ODD, oppositional defiant disorder; ASRS, Autism Spectrum Rating Scales; GERD, gastroesophageal reflux disease.

very elevated (70–85) ranges (mean 76.5 ± 6.0 std. dev.) (**Table 1**), supporting that the students all had symptoms directly related to the DSM-IV-TR diagnostic criteria for ASD. Intelligence quotient (IQ) scores were obtained from administration of either the Stanford–Binet Intelligence Scales—5th Edition for students with verbal abilities (18), or the Comprehensive Test of Non-Verbal Intelligence—2nd Edition (CTONI-2) for those who were non-verbal (19). IQ scores were able to be obtained from only five of the eight students due to compliance issues. For those five students, IQ scores were 3.6 standard deviations below the mean (mean IQ 46.6 ± 5.5 std. dev.). Furthermore, adaptive behaviors were assessed by the Adaptive Behavior Assessment System (ABAS) General Adaptive Composite (20) score or the Vineland Adaptive Behavior Scales (VABS) Adaptive Behavior Composite (21) score. As both measures were normalized to a mean of 100, an average score was calculated as an overall estimate of adaptive behavior. On average, the adaptive behavior scores for the students were 3.8 standard deviations below the mean (mean 43.0 ± 7.4 std. dev.).

The study was approved by the Institutional Review Board at TCFD. All data were collected from students with ASD enrolled at the TCFD, located in Hurleyville, NY. Due to the severity of the patient population, consent was provided by the parent/legal guardian, and assent was also obtained for those with the capacity to respond after explanation through an explicit social story. The data were processed and analyzed at the University of Missouri, which was approved by the Health Sciences Institutional Review Board through a reliance agreement with TCFD. Research staff at TCFD recorded problem behavior and EDA data for the eight students over 1 year in the lab school setting at TCFD.

Behavior Monitoring

Monitoring was done while students were engaged in a classroom-based behavior intervention plan, where interventions were targeting specific behaviors. Occurrences of each problem behavior were noted by educational and residential staff trained to document these behaviors, as part of the standard procedure. The onset and offset of the each problem behavior was notated in Noldus The Observer XT software (Noldus Information Technology, Leesburg,

VA). The resulting onset and offset of problem behaviors were then used as endpoints for the EDA analysis.

For the EDA analysis, only the first occurrence of a problem behavior was analyzed per session. This was implemented to prevent the occurrence of additive effects of previous problem behaviors on EDA, which may confound the results. Thus, if a participant engaged in problem behavior at 10 min into a session, then EDA analyses were only carried out on that problem behavior, and subsequent problem behaviors during the session were not analyzed. Overall behavioral profile for each student was also tracked, as documented by staff, for all behaviors at any point during the session (see **Table 2** for distribution of behaviors among students).

Electrodermal Activity Procedures

EDA data were obtained using a Q-Sensor pod wristband (Affectiva Inc., Cambridge, MA), with a sampling rate of 32Hz, which was placed with Ag/AgCl dry electrodes, for extended use, to the wrist of the non-dominant hand. If the student would not tolerate a wrist-worn sensor, then the sensor was applied to the student's ankle (22). Once the EDA site was selected (i.e., wrist or ankle), the sensor was always applied to the same site for each child and across time. Though the ankle is not the most optimal location from which to collect EDA (23), this location has been used in previous research in individuals with neurodevelopmental disorders when tolerance to a biosensor is an issue (24). All Q-sensor data were obtained while the students were in a temperature-controlled classroom with the classroom thermostat kept at a consistent temperature for the duration of the study. During baseline data collection, all students were expected to stationary and in their seats performing classroom activities. The Q-sensor devices were time-synchronized daily with the same computer system that has the video recording software to ensure synchronization. At the start of each video session, the Q-sensor button was pressed at the time of recording as an additional time-stamp. Next, EDA data from the Q-Sensor were downloaded onto a computer and processed using Affectiva Software (Affectiva Inc., Cambridge, MA). The EDA data were visually inspected by the first author,

TABLE 2 | Frequency and percentage of the occurrence of an anticipatory rise in EDA prior to the problem behavior, mean anticipatory rise time, number of times that a student returned to baseline EDA after engaging in problem behavior, and the mean time for EDA to return to baseline. Note for S13, no statistics for anticipatory rise and return to baseline were able to be calculated as the student's repetitive behavior was continuous for the duration of each session.

ID	PB assessed	Valid EDA records	Number of times anticipatory rise prior to PB (%)	Mean EDA prior to PB (µS) (SD)	Mean anticipatory rise time (s) (SD)	Number of times returned to BL after PB (%)	Mean time to return to BL (s) (SD)
S1	Jumping in seat	9	6 (67%)	0.99 (0.85)	590 (466)	5 (56%)	2,165(673)
S6	Repetitive body hitting	11	6 (55%)	1.09 (1.66)	1,076 (1099)	6 (55%)	2,436 (1, 919)
S7	General classroom disruption	9	8 (89%)	2.22 (1.92)	945 (1, 201)	2 (22%)	4,939 (2, 742)
S8	Aggression	8	1 (13%)	0.74 (0.81)	681 (0)	2 (25%)	3,759 (356)
S9	Out of seat	9	5 (56%)	0.48 (0.42)	403 (399)	8 (89%)	6,389 (4, 289)
S11	Self-injurious behavior	9	8 (89%)	0.89 (0.61)	490 (385)	5 (56%)	3,536 (4, 138)
S12	Agitation	7	3 (43%)	0.18 (0.20)	95 (86)	0 (0%)	X
S13	Repetitive motor movement	X	X	X	X	X	X
TOTAL	8	62	37 (60%)	0.94 (0.64)	611 (306)	28 (45%)	3,870 (1, 586)

s, seconds; SD, standard deviation; BL, median baseline EDA level; PB, problem behavior; µS, microsiemens.

who has over a decade of experience working with EDA data, for significant motion artifacts within 5 min of the start of the problem behavior, during the problem behavior, and immediately after each problem behavior. Visual inspection of the EDA data was critical for this particular study due to the severity of the patient population resulting in frequent issues with behaviors resulting in the potential for significant artifacts due to motion. EDA data were excluded from the final analysis if visual inspection of the accelerometer data from the Q-sensor indicated a significant amount of motion corresponded with significant motion artifacts in the EDA data during the timepoints of interest. Consistent with previous research from our team (8, 9), baseline EDA was determined by taking the median of 5 min of EDA data during the beginning of each session. Baseline periods did not include problem behaviors. Median baseline EDA was used instead of mean baseline EDA as the data were typically skewed in a negative direction which would influence mean scores. Next, the anticipatory EDA rise time was determined by examining EDA data 5 min prior to the onset of the problem behavior. An anticipatory rise in EDA was defined by the presence of at least two skin conductance responses of at least 0.03µS, followed by a gradual increase in the slope of EDA after the SCR during the 5-min period prior to the problem behavior. This method was chosen as it involves both tonic and phasic elements of skin conductance such that a skin conductance response that was due to motion was unlikely to satisfy the criteria for an anticipatory rise in EDA as it likely wouldn't be followed by an increase in the slope of the EDA. A threshold of 0.03µS and even as low as 0.01µS has been shown to be common in the literature as a skin conductance response (23, 25). Next, latency to return to baseline EDA after the student engaged in problem behavior was determined by examining the amount of time between the point in time where the student stopped engaging in the problem behavior and the point where the EDA returned to the median baseline score. EDA records were also monitored for whether or not they returned to

their median baseline EDA level, with an average duration of monitoring for return to baseline of at least 20 min (average duration = 134 min, ± 72 min. std. dev., range 20–242 min.) after engaging in problem behavior, and so frequency of this occurrence was tracked within each individual and for the overall group of students.

Analysis

Frequencies of BIP and EDA associated with the behaviors were reported overall for each student. Incidence of anticipatory rise prior to behaviors and recovery of EDA after behaviors was also documented.
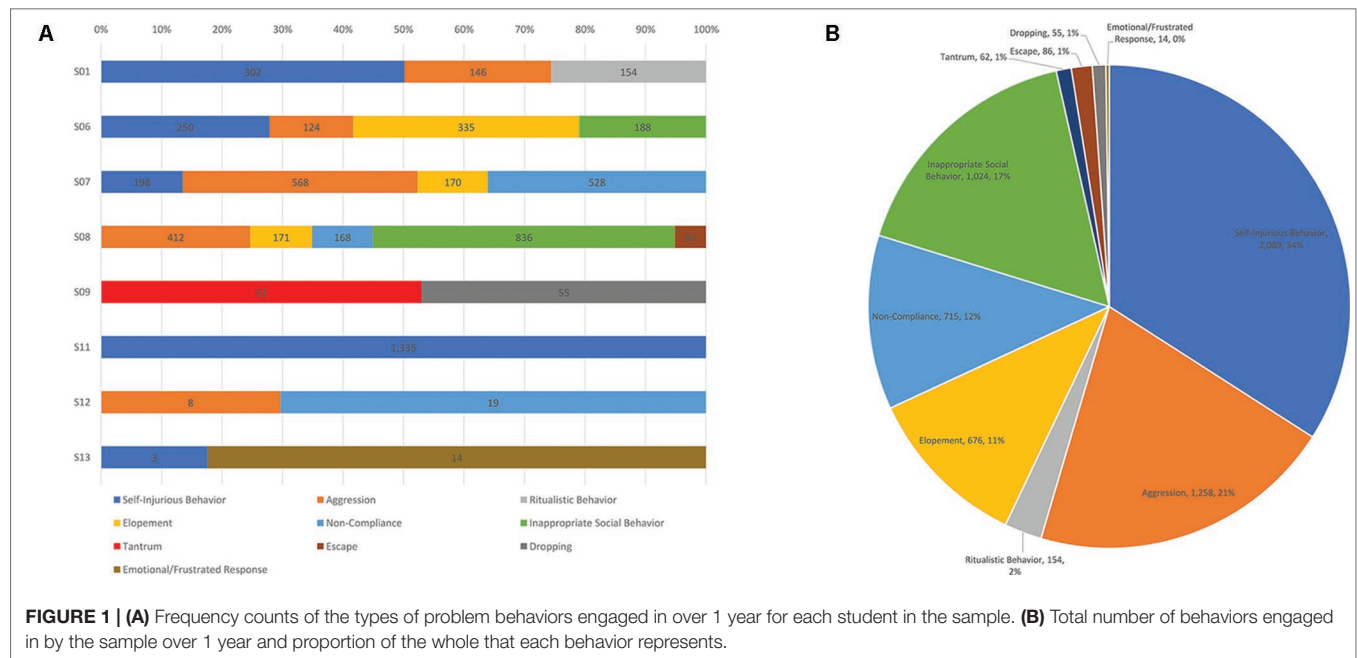
RESULTS

Frequency of Problem Behaviors

To demonstrate the range of behaviors among the students, behavior frequency each participant engaged in over the year-long period assessed is depicted in **Figure 1**. Overall, most of the 8 students engaged in more than one type of behavior, except for student 11 who only engaged in self-injurious behavior. For all of the documented behaviors across all of the students, the most common problem behavior was self-injurious behavior, followed by aggression, inappropriate social behavior, non-compliance, and elopement.

Quality of EDA Signal

Initially, the EDA data were visually inspected by an experienced psychophysiology researcher (BF) for artifacts as the Q-sensors were placed either on the wrist or the ankle of the students. Any cases with significant artifacts in EDA during the times that were analyzed, as defined by a visual analysis of motion from accelerometer data in the x, y, and z directions at the same time as significant EDA artifacts were noted, or DC shift, indicating either loose or no contact between the skin and the Q-sensor (25), were excluded from

FIGURE 1 | (A) Frequency counts of the types of problem behaviors engaged in over 1 year for each student in the sample. **(B)** Total number of behaviors engaged in by the sample over 1 year and proportion of the whole that each behavior represents.

the final analysis. Significant EDA artifacts were defined as an immediate drop in EDA to 0, indicating DC shift, or when "spikes" or rapid increases in EDA data appeared and were at least three standard deviations above the mean. This typically corresponded with rapid fluctuations in accelerometer data in x, y, and z directions and was present during periods when the student engaged in problem behavior that resulted in movement of the arms and/or legs. Artifacts in the EDA data were easily detected as they tended to be characterized by quick "spikes" in the EDA data that aren't physiologically likely, followed by DC shift, indicating that contact between the EDA sensor and the skin was broken. Visual inspection of each EDA record yielded 22 records that contained a significant amount of artifact, according to the visual analysis mentioned above, that rendered the data unreliable, and so they were excluded from the final analysis. Each of these 22 records were associated with participant behaviors. This yielded a total of 62 valid records that were suitable for analysis.

Anticipatory Rise Time Prior to Problem Behavior

The presence or absence of an anticipatory rise in EDA prior to a student engaging in problem behavior was analyzed. On average, across all episodes of problem behavior documented, across all students, the students displayed an anticipatory rise in EDA prior to engaging in problem behavior 60% of the time. However, individuals varied in the frequency with which an anticipatory rise in EDA was observed before the onset of the problem behavior (see **Table 2**). Of note, the students with the greatest incidence of anticipatory rise in EDA had primarily engaged in general classroom disruption behaviors and self-injurious behavior, while the student with the least amount of times of an anticipatory rise had primarily engaged in aggression (see **Table 2**).

Recovery of EDA to Baseline After Problem Behavior

On average, across all problem behaviors documented, the students' EDA returned to median baseline values 45% of the time after engaging in problem behavior. Individuals also varied in the frequency with which EDA returned to median baseline levels after cessation of the problem behavior. Of note, the student who returned the most frequently to their median baseline EDA primarily engaged in out-of-seat behavior, while the student who returned the least amount of times to their median baseline EDA primarily displayed agitation (see **Table 2**).

Examples of the different types of EDA responses are illustrated in **Figure 2**. For some problem behavior, the EDA pattern was characterized by a gradual increase in EDA leading up to the problem behavior, a peak in EDA while the individual was engaged in the problem behavior, and then a gradual decrease in EDA following discontinuation of the problem behavior. For some problem behavior, the EDA pattern was characterized by no build-up in EDA prior to the engagement of the problem behavior, with a gradual increase in EDA during engagement in the problem behavior, followed by a decrease in EDA following discontinuation in the behavior. For other episodes of problem behavior, the EDA pattern was characterized by an increase in EDA prior to engagement in the problem behavior which was followed by a decrease in EDA during engagement of the problem behavior (see **Figure 2** for examples of each response pattern).

DISCUSSION

The findings from this exploratory study suggest that examining the relationship between EDA and problem behaviors is feasible in
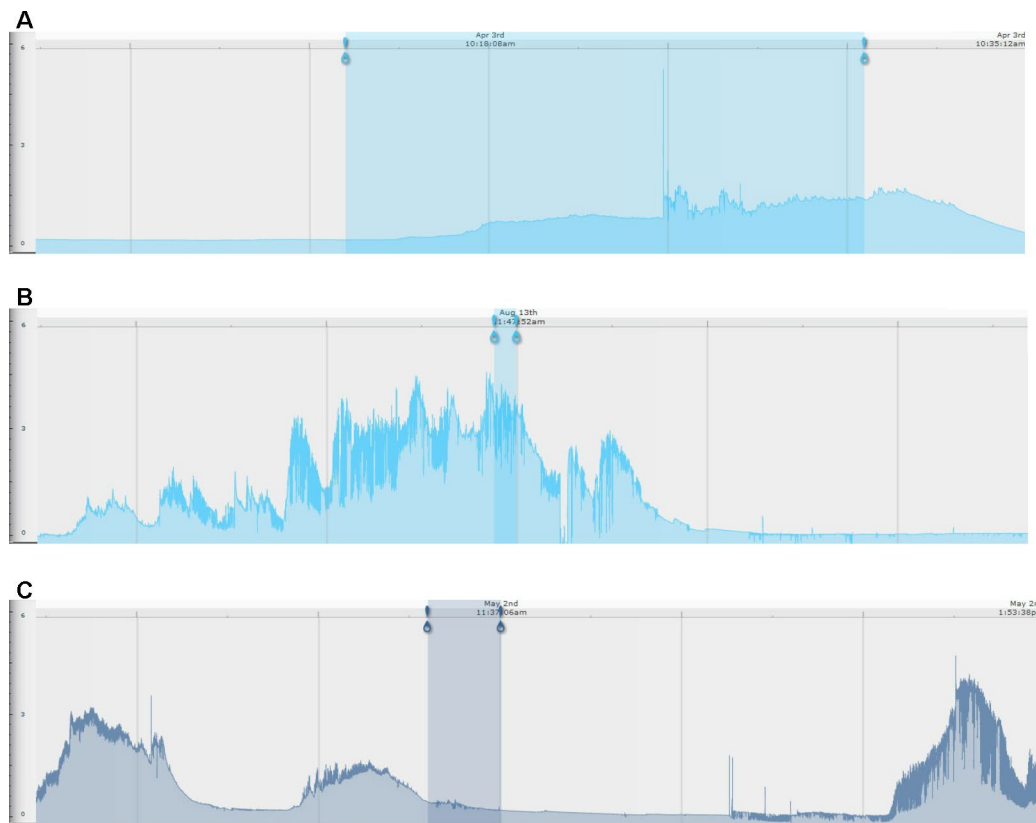
FIGURE 2 | Examples of skin conductance level traces by behavioral subtype. For all images, the blue-shaded area represents the time period of an individual engaging in problem behavior. The left-most blue pin represents the beginning of the problem behavior, and the right-most pin represents the end of the problem behavior. **(A)** No EDA build-up prior to engaging in problem behavior (in this example, jumping in seat); **(B)** EDA build-up prior to engaging in problem behavior (in this example, jumping in seat); **(C)** EDA build-up prior to engaging in problem behavior (in this example, repetitive body hitting) and subsequent reduction with engagement in problem behavior. The x-axis is EDA in microsiemens, and the y-axis is time (marked at the top of each EDA record).

a naturalistic setting in a severely affected population with ASD. There was some loss of data due to artifact, which was expected due to the level of functioning of the students and the assessment of EDA prior to and during the engagement of problem behavior that involves motion, rather than in a well-controlled laboratory setting. However, given the exploratory nature of the study, the findings suggest that the collection of EDA in a population with severe ASD is possible, but investigators should be aware of a number of limitations that are likely to occur in this population.

Variability was found in the incidence of changes in EDA prior to the occurrence of problem behavior, during the problem behavior, and after the occurrence of a problem behavior, targeting a unique set of severely affected individuals in a residential setting. The results indicate that 60% of the total episodes of problem behavior documented were associated with an anticipatory rise in EDA prior to engaging in problem behavior. This has important implications for efforts to utilize psychophysiological markers as a predictor of problem behaviors (14). This finding could also have significant implications for the prediction and management of problem behavior in a classroom setting of individuals who are severely affected by ASD. For those cases where an anticipatory rise was found, the average amount of time that EDA rose prior to engaging in problem behavior was over 10 min, providing a

window of opportunity for intervention to occur prior to the occurrence of problem behavior. However, this would not be helpful in all cases, as many behaviors were not associated with an anticipatory rise in EDA. Further monitoring of these individuals may be helpful to understand other factors that might predict behaviors, and in how many of these individuals do other psychophysiological variables contribute predictive information. Given the variability in behavioral profiles of individuals with ASD in this study, future work will need to determine whether specific behavioral profiles are associated with certain patterns of EDA change, allowing improved specificity for the psychophysical prediction efforts. Many have suggested that understanding the psychophysiological underpinnings of problem behavior in ASD is important for predicting when an individual with ASD is likely to engage in problem behaviour (12, 26), but the distinct patterns of EDA suggest that different treatment strategies may be more effective for each type of EDA response pattern. For example, individuals with ASD that display a steady increase in EDA prior to engaging in problem behavior may respond best from cognitive and/or behavioral interventions to reduce anxiety. Additionally, pharmacological treatment with agents targeting stress reactivity may also provide benefit in this subtype. For example, propranolol, an agent utilized in other conditions associated with altered

stress reactivity (27), has been shown to increase conversational reciprocity in ASD (9), making it a potential candidate drug for the treatment of anxiety-related behaviors in ASD. However, those without an increase in EDA prior to engaging in problem behavior may benefit from behavioral or pharmacological interventions targeting impulse control.

In this sample, we also identified that over half of the students did not reliably return to their initial resting baseline EDA after engaging in problem behavior. This suggests that, in future work examining the relationship between EDA and behaviors, it will be critical to account for the potential confound of a lack of returning to baseline from previous behaviors for EDA, and which behavioral profiles are associated with lack of return to baseline. Additionally, this raises the possibility that interventions after the cessation of behavior might be beneficial in some individuals, targeting de-escalation.

A number of limitations to the present study should be noted, as they affect the generalization of the results across a broad range of individuals with ASD. First, the students in this study ranged in age from 13 to 20 years, with an average age of 16, were all male and have severe ASD. As such, it is not clear how this generalizes to all individuals with ASD, and so future studies will need to examine data from a more diverse sample of individuals. Second, detailed data from only eight individuals were analyzed for this study, and so future research should aim to examine these results in a much larger group of individuals with ASD, which would also allow the examination of the impact of co-occurring conditions and medications on the EDA/problem behavior relationship. However, this remains of interest for the management of those with the most severe problem behaviors, who were studied herein, and the variability that will be important in future psychophysiological monitoring in this type of setting.

Finally, when planning psychophysiological experiments in those with severe ASD, careful consideration should be given to the testing environment, the problem behaviors engaged in by the individual(s) to be studied, and the tolerance of biosensors on the individual. For instance, if data collection will be indoors and outdoors, EDA would not be an appropriate measure given that EDA readings can be influenced by changes in hydration status, relative humidity, or sweat, for example (23, 28, 29). In this case, it may be better to collect electrocardiogram (ECG) to analyze heart rate variability (HRV), which provides information about sympathetic as well as parasympathetic nervous system functioning. Further, if an individual engages in arm flapping or elopement—for example, collection of data from the wrist or leg may not be appropriate given the high probability that the data will contain motion artifact. To this point, EDA data from student 13

in this study was unable to be analyzed due to significant motion artifact from repetitive motor movements (**Table 2**). In this case, investigators may consider the use of a physiological apparatus that is affixed to the trunk of the body that is less susceptible to motion artifact. As such, there are a number of limitations of collecting EDA data from those with severe ASD, but with careful planning, such studies are possible and add a wealth of knowledge on those more severely affected.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Institutional Review Board (IRB) at The Center for Discovery with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the IRB at The Center for Discovery. Data analyses were conducted at the University of Missouri under a reliance agreement between the University of Missouri Health Sciences Institutional Review Board and IRB at The Center for Discovery.

AUTHOR CONTRIBUTIONS

BF conceptualized and designed the study, coordinated and supervised data management, analyzed the EDA data, drafted the initial manuscript, and reviewed and revised the manuscript. JL and TV collected and processed the initial data, and assisted with preparation of the manuscript. JC advised on the data handling and assisted with preparation of the manuscript. TH developed the program for data collection, supervised data collection at The Center for Discovery, and revised the manuscript. DB conceptualized the overall plan of the study with BF, supervised the research team and provided expertise and guidance regarding autism spectrum disorder and revised the manuscript. All authors approved of the final manuscript as submitted.

FUNDING

REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders IV—Text Revision (DSM-IV-TR)*. Washington, DC: APA (2000).
2. Kanne SM, Mazurek MO. Aggression in children and adolescents with ASD: prevalence and risk factors. *J Autism Dev Disord* (2011) 41:926–37. doi: 10.1007/s10803-010-1118-4
3. Koegel LK, Koegel RL, Surratt A. Language intervention and disruptive behavior in preschool children with autism. *J Autism Dev Disord* (1992) 22:141–53. doi: 10.1007/BF01058147
4. Horner RH, Carr EG, Strain PS, Todd AW, Reed HK. Problem behavior interventions for young children with autism: a research synthesis. *J Autism Dev Disord* (2002) 32(5):423–46. doi: 10.1023/A:1020593922901
5. Groden J, Cautela J, Prince S, Berryman J. The impact of stress and anxiety on individuals with autism and developmental disabilities. In: Schopler E,

Mesibov GB, editors. *Behavioral Issues in Autism*. Springer (2009). p. 177–94. doi: 10.1007/978-1-4757-9400-7

6. Mazefsky CA, Herrington J, Siegel M, Scarpa A, Maddox BB, Scahill L, et al. The role of emotion regulation in autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry* (2013) 52:679–88. doi: 10.1016/j.jaac.2013.05.006

7. van Dooren M, de Vries JJ, Janssen JH. Emotional sweating across the body: comparing 16 different skin conductance measurement locations. *Physiol Behav* (2012) 106(2):298–304. doi: 10.1016/j.physbeh.2012.01.020

8. Ferguson BJ, Marler S, Altstein LL, Lee EB, Akers J, Sohl K, et al. Psychophysiological associations with gastrointestinal symptomatology in autism spectrum disorder. *Autism Res* (2017) 10:276–88. doi: 10.1002/aur.1646

9. Zamzow RM, Ferguson BJ, Stichter JP, Porges EC, Ragsdale AS, Lewis ML, et al. Effects of propranolol on conversational reciprocity in autism spectrum disorder: a pilot, double-blind, single-dose psychopharmacological challenge study. *Psychopharmacology (Berl)* (2016) 233(7):1171–8. doi: 10.1007/s00213-015-4199-0

10. O'Haire ME, McKenzie SJ, Beck AM, Slaughter V. Animals may act as social buffers: skin conductance arousal in children with autism spectrum disorder in a social context. *Dev Psychobiol* (2015) 57(5):584–95. doi: 10.1002/dev.21310

11. Hirstein W, Iversen P, Ramachandran VS. Autonomic responses of autistic children to people and objects. *Proc R Soc B* (2001) 268(1479):1883–8. doi: 10.1098/rspb.2001.1724

12. Baker JK, Fenning RM, Erath SA, Baucom BR, Moffitt J, Howland MA. Sympathetic under-arousal and externalizing behavior problems in children with autism spectrum disorder. *J Abnormal Child Psychol* (2018) 46:895–906. doi: 10.1007/s10802-017-0332-3

13. Fenning RM, Baker JK, Baucom BR, Erath SA, Howland MA, Moffitt J. Electrodermal variability and symptom severity in children with autism spectrum disorder. *J Autism Dev Disord* (2017) 47(4):1062–72. doi: 10.1007/s10803-016-3021-0

14. Goodwin MS, Özdenizci O, Cumpanasoiu C, Tian P, Guo Y, Stedman A, et al. Predicting imminent aggression onset in minimally-verbal youth with autism spectrum disorder using preceding physiological signals. *Int Conf Pervasive Comput Technol Healthc* (2018) 2018:201–7. doi: 10.1145/3240925.3240980

15. Lai MC, Lombardo MV, Chakrabarti B, Baron-Cohen S. Subgrouping the autism "spectrum": reflections on DSM-5. *PLoS Biol* (2013) 11(4):e1001544. doi: 10.1371/journal.pbio.1001544

16. Beversdorf DQ, Wang P, Barnes G, Weisskoph M, Hardan A, Hu VW, et al. Phenotyping, etiological factors, and biomarkers: toward precision medicine in autism spectrum disorders. *J Dev Pediatrics* (2016) 37(8):659–73. doi: 10.1097/DBP.0000000000000351

17. Goldstein S, Naglieri JA. *Autism Spectrum Rating Scales (ASRS)*. Tonawanda, NY: Multi-Health Systems (2009).

18. Roid GH. *Stanford-Binet Intelligence Scales, Fifth Edition*. Itasca, IL: Riverside Publishing (2003).

19. Hammill DD, Pearson NA, Wierderholt JL. *Comprehensive Test of Nonverbal Intelligence—Second Edition*. Bloomington, MN: Pearson (2009).

20. Harrison PL, Oakland T. *Adaptive Behavior Assessment System—Second Edition*. Torrance, CA: Western Psychological Services (2000).

21. Sparrow SS, Cicchetti DV, Balla DA. *Vineland Adaptive Behavior Scale—Second Edition*. Bloomington, MN: Pearson (2006). doi: 10.1037/t15164-000

22. Hedman E, Miller L, Schoen S, Nielsen D, Goodwin M, Picard R. Measuring autonomic arousal during therapy. In *Proc of Design and Emotion* 2012 September 11–14.

23. Boucsein W. *Electrodermal activity*. New York, NY: Springer Science & Business Media (2012). doi: 10.1007/978-1-4614-1126-0

24. Betancourt MA, Dethorne LS, Karahalios K, Kim JG. Skin conductance as an *in situ* marker for emotional arousal in children with neurodevelopmental communication impairments: methodological considerations and clinical implications. *ACM Trans Access Comput (TACCESS)* (2017) 9(3):8. doi: 10.1145/3035536

25. Society for Psychophysiological Research Ad Hoc Committee on Electrodermal Measures, Boucsein W, Fowles DC, Grimnes S, Ben-Shakhar G, Roth WT, Dawson ME, et al. Publication recommendations for electrodermal measurements. *Psychophysiology* (2012) 49(8):1017-34. doi: 10.1111/j.1469-8986.2012.01384.x

26. Prince EB, Kim ES, Wall CA, Gisin E, Goodwin MS, Simmons ES, et al. The relationship between autism symptoms and arousal level in toddlers with autism spectrum disorder, as measured by electrodermal activity. *Autism* (2017) 21(4):504–8. doi: 10.1177/1362361316648816

27. Faigel HC. The effect of beta blockade on stress-induced cognitive dysfunction in adolescents. *Clin Pediatr* (1991) 30(7):441–5. doi: 10.1177/000992289103000706

28. Bundy RS, Mangan SM. Electrodermal indices of stress and cognition: possible hydration artifacts. *Psychophysiology* (1979) 16(1):30–3. doi: 10.1111/j.1469-8986.1979.tb01433.x

29. Turpin G, Shine P, Lader MH. Ambulatory electrodermal monitoring: effects of ambient temperature, general activity, electrolyte media, and length of recording. *Psychophysiology* (1983) 20(2):219–24. doi: 10.1111/j.1469-8986.1983.tb03291.x

Comorbidity Burden in Adults With Autism Spectrum Disorders and Intellectual Disabilities—A Report From the EFAAR (Frailty Assessment in Ageing Adults With Autism Spectrum and Intellectual Disabilities) Study

Stéphanie Miot[1,2,3]*, Tasnime Akbaraly[2,4,5], Cecile Michelon[2], Sylvie Couderc[2], Sophie Crepiat[2], Julie Loubersac[2], Marie-Christine Picot[6], Éric Pernon[2], Véronique Gonnier[2], Claude Jeandel[3], Hubert Blain[3] and Amaria Baghdadli[1,2]

[1] *CESP, INSERM U1178, Centre de recherche en Epidemiologie et Santé des Populations, Paris, France,* [2] *Autism Resources Centre of Languedoc-Roussillon, University Hospital of Montpellier, CHRU de Montpellier, Univ. Montpellier, Montpellier, France,* [3] *Gerontology Centre, Antonin Balmès, University Hospital of Montpellier, CHRU de Montpellier, Univ. Montpellier, Montpellier, France,* [4] *MMDN, Univ. Montpellier, EPHE, INSERM, U1198, Montpellier, France,* [5] *Department of Epidemiology and Public Health, University College London, London, United Kingdom,* [6] *Biostatistic Department, University Hospital of Montpellier, CHRU de Montpellier, Univ. Montpellier, Montpellier, France*

Background: Autism spectrum disorder (ASD) is an early-onset and lifelong neurodevelopmental condition frequently associated with intellectual disability (ID). Although emerging studies suggest that ASD is associated with premature ageing and various medical comorbidities, as described for ID, data are scarce.

Objectives: To determine the comorbidity burden and its association with distinct clinical presentation in terms of ASD severity, adaptive skills, level of autonomy, and drug exposure in a well-phenotyped sample of individuals with ASD-ID—the EFAAR (Frailty Assessment in Ageing Adults with Autism Spectrum and Intellectual Disabilities) cohort.

Methods: A total of 63 adults with ASD-ID, with a mean age of 42.9 ± 15.1 years, were recruited from 2015 to 2017 from nine specialized institutions. They underwent detailed clinical examinations, including screening for comorbidities, ASD severity [Childhood Autism Rating Scale (CARS)], adaptive functioning [Vineland Adaptive Behavior Scale II (VABS-II)], autonomy [activities of daily living (ADLs)], and drug use [polypharmacy and the Drug Burden Index (DBI)]. The comorbidity burden was evaluated using the Cumulative Illness Rating Scale (CIRS-G) and its sub-scores [the severity index (CIRS-SI) and severe comorbidity (CIRS-SC)].

Results: We found a large range of comorbidities, including gastrointestinal disorders and mental and neurological diseases. Overall, 25% of our ASD-ID sample had chronic kidney disease with the associated increased cardiovascular risk factors. The comorbidity burden was high (mean CIRS-G total score of 10.6 ± 4.8), comparable with that observed

among patients older than those in our population hospitalized in geriatric departments. Furthermore, the comorbidity burden positively correlated with age, decreased autonomy, and polypharmacy.

Conclusion: The severity of the comorbidity burden associated with premature ageing in adults with ASD and ID highlight their crucial need of personalized medical care.

Keywords: autism spectrum disorder, intellectual disability, ageing, comorbidity burden, CIRS

INTRODUCTION

Ageing is a dynamic process, resulting in decreased physiological reserves that can lead to impaired adaptive capacities in elderly individuals. In the general population, ageing results in increased multimorbidity (defined as two or more chronic conditions) (1), leading to disability (2), polypharmacy (defined as five or more medications per day) (3), and mortality (4).

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social and communication impairment associated with repetitive and restrictive behaviors (5). One individual in 68 has an ASD in the United States (6) and one in 100 in France (7), making it a relatively common condition (8). Its clinical presentation is heterogeneous, and psychiatric and somatic comorbidities are both variable and frequent (9).

Aside from ASD patients having a higher mortality rate than that of the general population, little is known about ageing in ASD (9). Several studies have hypothesized a pathological ageing trajectory in ASD (10, 11), related to a high rate of comorbidities, particularly feeding (12, 13) and gastrointestinal disorders (14, 15), which have been reported in almost 90% of cases. Seizure disorders (16), immune dysregulation (17), and cardiovascular diseases (18) are also common and reported in one third of individuals.

In addition, intellectual disability (ID), found in 32% of ASD individuals (9), is commonly associated with a large range of medical comorbidities, such as nutritional deficiencies, cardiovascular diseases (18), polypharmacy (19), and multi-morbidity (20), and may contribute to the increased risk of premature ageing of ASD patients (11, 21, 22).

We hypothesized that the cumulative weight of comorbidity associated with ASD and ID may lead to premature ageing of adult patients with ASD and comorbid ID (ASD-ID). However, there have been few observational studies to investigate the impact of comorbidities on ageing trajectories in adults with ASD. Here, we aimed to determine the comorbidity burden in a well-phenotyped cohort of adults with ASD-ID, the EFAAR (Frailty Assessment in Ageing adults with Autism Spectrum and Intellectual Disabilities) cohort, using the Cumulative Illness Rating Scale (CIRS-G) and its sub-scores [the severity index (CIRS-SI) and severe comorbidity (CIRS-SC)]. We explored the predictive factors of such a comorbidity burden in terms of age, ASD severity, adaptive functioning, autonomy, and drug use. Secondary objectives were to better characterize the medical comorbidities associated with ASD-ID during adulthood and the pre-elderly period and determine those

comorbidities that are more frequently associated with each clinical feature.

MATERIALS AND METHODS

Study Design and Population

The EFAAR study is an ongoing prospective multicentric study. Participants were recruited from nine medico-social institutions in the south of France between 2015 and 2018. These institutions are the place of both residence and care of participants. Participants with a diagnosis of ASD [according to the *Diagnostic and Statistical manual of Mental Disorders* (DSM-5) criteria] and an ID [established according to the American Psychiatric Association (APA, 2013)] were invited to participate in the EFAAR study. Inclusion criteria included being over the age of 20 years and being institutionalized in a medico-social institution of Languedoc-Roussillon (South of France). The exclusion criterion was having Down syndrome, known to be a cause of premature ageing (23). Among the 65 participants (recruited in nine centers), two aged 65 years were excluded (one declined and one dropped out after moving away from Languedoc Roussillon). In total, the EFAAR cohort included 63 participants who underwent a thorough clinical examination at baseline focused on frailty assessment. They will be followed up over 5 years, during which time certain health events will be recorded annually through phone interviews with the health workers (falls, hospitalizations, and death). The present study is based on baseline examination data.

Baseline Examinations

Baseline examinations were carried out within the medico-social institution of the participant to reduce anxiety due to the assessment and evaluate each patient during a stable phase of their disease.

ASD severity was assessed using the Childhood Autism Rating Scale (CARS) (24), a standardized scale that evaluates the intensity of autism symptoms across 15 domains, each scored from 1 to 4. The total score is the sum of each of the 15 sub-scores (range 15–60, with a higher score indicating higher severity). This evaluation was completed by three of the authors (SM, SC, or SC). The three investigators reached a consensus to determine the CARS total score.

The intellectual quotient (IQ) was assessed using the Raven Progressive Matrices. However, none of the participants could understand the test instructions or requirements. A developmental

quotient (DQ) was calculated to confirm the ID, according to Stern's formula (25): developmental age (defined based on the daily life sub-score of the VABS-II)/chronological age * 100.

Adaptive functioning was assessed by the Vineland Adaptive Behavior Scale II (VABS-II) (26), a semi-structured interview conducted with the health worker of the participant. Three sub-scores (communication, daily life, and social skills) (Vineland II, 2004) were calculated, with a higher score indicating a less severe impairment of adaptive functioning (27).

Autonomy was assessed using the Katz index of independence for six activities of daily living (ADLs), which included bathing, dressing, toileting, transferring, continence, and feeding (28). A score of 1 (if the patient needs no assistance for the specific ADL), 0.5 (if the patient needs supervision, direction, or assistance), or 0 (if the patient needs total care) was attributed for each activity. A total score of 6 represents full autonomy, 4 a moderate impairment of autonomy, and <2 a severe impairment of autonomy (29).

Baseline Treatment Record

Data on daily treatment were collected from the medical records. Polypharmacy was defined as the prescription of ≥5 medications daily (30). The Drug Burden Index (DBI) was used to assess the sedative and anticholinergic burden of medication (31). The DBI was calculated using the anticholinergic burden calculator developed by the Instituto de BIomedicina de Sevilla (IBIS), available on the Internet (http://www.anticholinergicscales.es/calculate). The DBI is the sum of anticholinergic and sedative effects of every treatment taken by the participant. This effect is calculated using the formula $D/(\delta + D)$, in which D is the daily dose taken by the participant and δ the minimum efficacious daily dose approved by the Food and Drug Administration (FDA) and ranges from 0 to 1 for each drug (32). The DBI score is higher if participants take high doses and multiple drugs with sedative and anticholinergic effects.

Assessment of Baseline Comorbidities

Screening for 49 diseases (listed in **Table 2**) was performed.

The Dementia Screening Questionnaire for Individuals with Intellectual Disabilities (DSQIID) (33) was completed by the referent health worker of the participant to screen for neurocognitive conditions. The first part of this questionnaire targets the participant's abilities, the second targets the behavior and symptoms usually associated with dementia in people with ID, and the third includes 10 comparative questions. Dementia is suspected for a total score (sum of the second and third parts) of ≥20.

Mental health conditions were assessed using the Reiss Scale (34), designed to screen for mental disorders in people with ID and aged over 12 years. This 38-item scale includes eight sub-scales: aggressive behavior, autism, psychosis, paranoia, depression (behavior symptoms), depression (physiological symptoms), dependence, and avoidance. There are also six maladaptive items, including drug abuse, hyperactivity, self-harm, sexual disorders, suicidal tendencies, and theft. The

questionnaire was completed by the referent health worker. Each item is scored from 0 to 2 (0, no problem; 1, problem; 2, severe problem). The presence of co-occurring mental disorders is considered for a score ≥9 for the 26 selected items. The Reiss Scale is used to determine whether the presence of a mental problem for an ID patient has been sufficiently demonstrated. Each sub-scale shows good internal validity (between 0.72 and 0.81), and the French version, developed by Lecavalier and Tassé, shows satisfactory adequacy with the original version of the Reiss Scale (35). A mental disorder was suspected when a current co-occurring psychiatric disease was diagnosed, except ASD and ID. The diagnosis of a mental disorder was established on the basis of a body of evidence: psychiatric symptoms detected by the Reiss Scale, in particular depression and hyperactivity, and the clinical evaluation of investigators (a practitioner and a psychologist).

Other comorbidities were evaluated by examining the medical record (in collaboration with the participant's general practitioner), the last biological checkup (completed in the year of inclusion), and medical examinations of the participant carried out by one of the authors (SC or SM).

Among the 49 diseases, 44 were grouped into 14 categories of chronic health conditions (detailed in **Table 2**) to provide an overview of the prevalence of the comorbidity categories.

Determination of the Comorbidity Burden

The revised CIRS-G (36) is the gold standard to evaluate the presence of comorbidities and their medical burden. A total of 14 organ-specific categories are assessed (cardiac, vascular, hematopoietic, respiratory, eye–ear–nose–throat, upper gastrointestinal, lower gastrointestinal, liver, genitourinary system, musculo-skeletal system, neurology, endocrine/ metabolic and breast, and psychiatry) (37), with a score between 0 and 4 for each category. 0 indicates no problem, 1 a mild or past significant problem, 2 a moderate problem requiring regular first-line treatment, 3 a severe and chronic problem requiring second-line treatment, and 4 an extremely severe problem requiring acute treatment and involving severe disability. The CIRS-G total score is the sum of each organ-system score.

The severity index (CIRS-SI) is defined as the CIRS-G total score divided by the total number of categories with a score > 1. The participants were separated into two groups according to the CIRS-SI: the *low-severity index group* (CIRS-SI ≤2) and the *high-severity index group* (CIRS-SI >2), as previously described in other studies using these scores (37, 38).

Statistical Analysis

For analyses of the clinical characteristics associated with the comorbidity burden (CIRS), regression models were used to estimate the association between clinical factors and the CIRS-G score, and CIRS-SI and CIRS-SC components. For analyses of the CIRS-G score, linear regression models were used in which the CIRS-G score was normalized by logarithmic transformation. For the binary components of the CIRS-SC score, logistic regression models were generated. The predictive ability of the models, that

is, the concordance rates between the predicted and observed responses, were calculated. The alpha-to-enter was set at 0.2 and alpha-to-exit at 0.10. The significance of adding or removing a variable from the multivariate models was determined by the maximum likelihood ratio test. The goodness of fit of the models was assessed using the Hosmer and Lemeshow test.

First, we described the comorbidities by calculating the prevalence of each in our population. An overview was provided by categorizing the comorbidities into 14 chronic health conditions and calculating the prevalence of each.

Second, we examined the association between comorbidities and clinical characteristics (ASD severity (CARS), level of adaptive functioning (VABS-II scores), level of autonomy (ADL), polypharmacy, and sedative and anticholinergic burden (DBI)). The ADL was analyzed using three sub-groups: low autonomy for a score of 0, 1, or 2; moderate autonomy for 3 or 4; and preserved autonomy for 5 or 6. Analysis of variance (ANOVA), χ^2, Student–Fisher, or Mann–Whitney tests were applied, depending on the nature of the variables (continuous, dichotomous, or categorized in three levels).

Third, we used multivariate analysis to determine which comorbidity significantly associated with a clinical feature had a dominant effect on this clinical characteristic. The models were adjusted for age. Analysis of covariance (ANCOVA) tests were used for continuous variables (CARS, VABS-II sub-scores, and DBI), polytomous logistic regression for ADL, and logistic regression for dichotomous variables (polypharmacy).

All values are expressed as a percentage or mean ± standard error. The significance level used was 5%. Statistical analyses were performed using SAS version 9.3 (SAS institute, Cary, NC, USA).

RESULTS

Patient Characteristics

Overall, 63 adults, with a mean age of 43±15.1 years, were included in our study. The male-to-female ratio was 3.7. Their clinical characteristics are shown in **Table 1**. They had a severe ASD, according to the CARS score (38.9 ± 6.6) and a profound ID, according to the DQ score (57 participants had a DQ score < 20,

and 5 had a DQ score between 20 and 30). Gender had no effect on the clinical characteristics ($p > 0.05$).

The comorbidity rates are listed in **Table 2**, with the three most frequent being constipation (54%), epilepsy (28.6%), and chronic kidney disease, essentially chronic renal failure (25.4%). Chronic health conditions are also shown in **Table 2**, the most prevalent being gastrointestinal disorders, essentially constipation (55.56%), mental diseases, essentially hyperactivity and depression (39.68%), and neurological diseases, essentially epilepsy (36.51%). In addition, 28.5% of participants had at least one cardiovascular risk factor (hypertension, diabetes, obesity, or dyslipidemia).

Association Between Clinical Characteristics and Comorbidities in ASD-ID Patients

We examined the extent to which the seven clinical characteristics of ASD-ID patients [ASD severity (CARS), adaptive functioning (VABS-II sub-scores), autonomy (ADL), polypharmacy, and sedative and anticholinergic burden (DBI)] are associated with each comorbidity and performed multivariate analyses adjusted for age to determine the weight of such comorbidity on these seven clinical characteristics (**Table 2**).

A more severe ASD was associated with epilepsy, whereas lower ASD severity was associated with chronic kidney disease and chronic liver disease, as well as cardiovascular risk factors (p value of 0.03). Multivariate analysis showed that only epilepsy was correlated with ASD severity (p value of 0.0128, adjusted R^2 of 0.217; having epilepsy increased the CARS score by 5.13).

Lower VABS-II communication sub-scores were associated with chronic kidney disease, dyslipidemia, and chronic anemia. Higher scores were associated with psoriasis and eczema. After multivariate analysis, psoriasis and eczema still were correlated with the VABS-II communication sub-score (p value of 0.0036, adjusted R^2 of 0.189; having psoriasis or eczema increased the communication sub-score by 9.8). Constipation was the only comorbidity associated with lower VABS-II social skills sub-scores (p value of 0.001). Lower VABS-II daily-life sub-scores were

TABLE 1 | Characteristics of the population in the EFAAR study.

		Total sample	Women (n = 17)	Men (n = 46)	Gender effect (p value)
Age (years)		42.9 ± 15.1 (21–68)	47.5 ± 14 (23–63)	41.3 ± 15.2 (21–68)	0.21
ASD severity (CARS)		38.9 ± 6.6 (25–52)	37.6 ± 7 (25–51.5)	39.4 ± 6.5 (25–52)	0.36
Adaptive functioning (VABS-II)	**SS communication**	23.1 ± 7.2 (20–73)	25.4 ± 12.8 (20–73)	22.2 ± 3.2 (21–38)	0.42
	SS daily life	23.6 ± 5.7 (20–47)	22.5 ± 2.9 (20–33)	24 ± 6.4 (21–47)	0.92
	SS social skills	20.7 ± 2.9 (20–37)	20 ± 0 (20–20)	20.1 ± 3.4 (20–37)	0.22
Autonomy level (ADL)		4.2 ± 1.6 (0–6)	3.8 ± 1.6 (0–6)	4.4 ± 1.6 (0–6)	0.13
Polypharmacy		58.7%	70.6%	54.3%	0.25
Sedative and anticholinergic burden (DBI)		2 ± 1 (0-5.5)	1.9 ± 1 (0–4.1)	2.1 ± 1.1 (0–5.5)	0.73

Values are expressed as percentages or the means ± standard deviation (minimum–maximum).
For the gender effect, the association between gender and every clinical characteristic was assessed using the mean comparison for continuous variables and the χ^2 test for dichotomous variables.
ADL, activities of daily living; CARS, Childhood Autism Rating Scale; DBI, Drug Burden Index; SS, sub-scores at the VABS-II; VABS-II, Vineland Adaptive Behavior Scale II.

TABLE 2 | Prevalence of the 14 chronic health conditions and the 49 chronic diseases and their association with clinical characteristics (values depict those without comorbidity vs those with comorbidity).

Chronic health condition	Prevalence (%)	Comorbidity	Prevalence (%)	ASD severity (CARS)	Adaptive functioning (VABS-II)			ADL	Polypharmacy	DBI
					SS communication	SS daily life	SS social skills	Low category (0–2)		
Hypertension	13.56	Hypertension	13.56	39.6 ± 6.3 vs 35.9 ± 7	23.1 ± 7.7 vs 23.5 ± 5.5	20.8 ± 3.3 vs 20 ± 0	23.9 ± 6.3 vs 22.9 ± 2	19.6% vs 0%	54.9% vs 75%	2 ± 1.1 vs 1.9 ± 0.8
Eye disease	17.46	Glaucoma	0	–	–	–	–	–	–	–
		Blindness and low vision	17.46	39 ± 6.8 vs 38.5 ± 6	23.1 ± 7.7 vs 23 ± 4.7	20.8 ± 3.3 vs 20 ± 0	24 ± 6.2 vs 21.6 ± 0.5	15.4% vs 18.2%	61.5% vs 45.5%	2.1 ± 1.1 vs 1.8 ± 1
Cardiovascular disease	15.87	Coronary heart disease	0	–	–	–	–	–	–	–
		Atrial fibrillation	0	–	–	–	–	–	–	–
		Heart failure	7.94	38.9 ± 6.7 vs 38.6 ± 6	23.2 ± 7.5 vs 21.8 ± 0.5	20.8 ± 3.3 vs 20 ± 0	23.7 ± 5.9 vs 22.6 ± 1.3	15.5% vs 20%	60.3% vs 40%	2.1 ± 1.1 vs 1.6 ± 0.8
		Orthostatic hypotension	17.86	37.9 ± 6.2 vs 38.2 ± 8.3	22.8 ± 4.4 vs 21.8 ± 0.5	21.9 ± 4.7 vs 20 ± 0	26.9 ± 8.4 vs 21.8 ± 0.5	4.3% vs 0%	**39.1% vs 100%***	1.7 ± 1 vs 2.2 ± 1
		Peripheral vascular disease	4.76	39.3 ± 6.6 vs 31.7 ± 1.5	23.1 ± 7.4 vs 22 ± 0	20.7 ± 3 vs 20 ± 0	23.7 ± 5.8 vs 22 ± 0	16.7% vs 0%	58.3% vs 66.7%	2 ± 1.1 vs 2.3 ± 0.8
Endocrine disorder	26.98	Diabetes	3.17	39.1 ± 6.7 vs 34.3 ± 3.2	23.1 ± 7.3 vs 22 ± 0	20.7 ± 3 vs 20 ± 0	23.7 ± 5.8 vs 20 ± 0	16.4% vs 0%	57.4% vs 100%	2 ± 1.1 vs 2.2 ± 0.7
		Thyroid disorders	11.11	39 ± 6.9 vs 38.7 ± 4.5	23 ± 7.4 vs 23.7 ± 5.9	20.8 ± 3.1 vs 20 ± 0	23.9 ± 6 vs 21.6 ± 0.5	**16.1% vs 14.3%***	55.4% vs 85.7%	2 ± 1 vs 2.5 ± 1.6
		Obesity	4.76	39.1 ± 6.6 vs 35.8 ± 8.5	22.7 ± 7 vs 30.3 ± 8.3	**20.5 ± 2.6 vs 24.3 ± 7.5***	23.4 ± 5.2 vs 29 ± 13	16.7% vs 0%	58.3% vs 66.7%	2 ± 1.1 vs 2.5 ± 0.7
		Dyslipidemia	12.7	39.3 ± 6.6 vs 36.4 ± 6.6	**23 ± 7.6 vs 23.3 ± 4***	20.6 ± 2.7 vs 21.6 ± 4.6	23.6 ± 5.7 vs 25 ± 5.7	**16.4% vs 12.5%***	58.2% vs 62.5%	2 ± 1.1 vs 2.3 ± 1
		Other endocrine disease	3.17	38.9 ± 6.6 vs 40 ± 8.5	23.1 ± 7.3 vs 21 ± 0	20.7 ± 3 vs 20 ± 0	23.6 ± 5.7 vs 25 ± 5.7	14.8% vs 50%	59% vs 50%	2 ± 1.1 vs 1.9 ± 0.2
Joint disease	15.87	Rheumatoid arthritis. Other inflammatory polyarthropathies and systematic connective tissue disorders	0	–	–	–	–	–	–	–
		Arthrosis	1.59	39 ± 6.6 vs 32.5	23.1 ± 7.3 vs 22	20.7 ± 3 vs 20	23.7 ± 5.7 vs 22	16.1% vs 0%	59.7% vs 0%	2 ± 1.1 vs 1.8
		Osteoporosis with fracture	3.17	38.8 ± 6.5 vs 43.3 ± 11.7	23.1 ± 7.3 vs 21 ± 1.4	20.7 ± 3 vs 20 ± 0	23.7 ± 5.8 vs 21 ± 1.4	14.8% vs 50%	57.4% vs 100%	2 ± 1 vs 2.9 ± 1.7
		Other chronic joint disease	11.11	39.1 ± 6.3 vs 37.5 ± 9.2	23.3 ± 7.6 vs 21.4 ± 0.5	20.8 ± 3.1 vs 20 ± 0	23.9 ± 6 vs 21.4 ± 0.5	16.1% vs 14.3%	57.1% vs 71.4%	2 ± 1.1 vs 1.8 ± 1

(Continued)

TABLE 2 | Continued

Chronic health condition	Prevalence (%)	Comorbidity	Prevalence (%)	ASD severity (CARS)	Adaptive functioning (VABS-II)			ADL	Polypharmacy	DBI
					SS communication	SS daily life	SS social skills	Low category (0–2)		
Lung disease	7.94	Chronic obstructive pulmonary disease	3.17	38.9 ± 6.6 vs 39.5 ± 9.9	22.9 ± 7.1 vs 29.5 ± 10.6	20.7 ± 3 vs 20 ± 0	23.7 ± 5.8 vs 22 ± 0	16.4% vs 0%	57.4% vs 100%	2 ± 1.1 vs 1.6 ± 0.3
		Asthma	4.76	38.9 ± 6.7 vs 38.8 ± 6.7	23.2 ± 7.4 vs 21 ± 0	20.7 ± 3 vs 20 ± 0	23.5 ± 5.8 vs 25.7 ± 4.2	16.7% vs 0%	58.3% vs 66.7%	2 ± 1 vs 2.2 ± 1.5
		Bronchiectasis	0	–	–	–	–	–	–	–
Gastrointestinal disease	55.56	Inflammatory bowel disease	0	–	–	–	–	–	–	–
		Diverticular disease of intestine	1.59	39.1 ± 6.6 vs 30	23.1 ± 7.3 vs 22	20.7 ± 3 vs 20	23.7 ± 5.7 vs 22	16.1% vs 0%	58.1% vs 100%	2 ± 1.1 vs 2.6
		Dyspepsia	11.11	39.3 ± 6.4 vs 36.5 ± 7.9	23.1 ± 7.7 vs 22.9 ± 3.8	20.6 ± 2.7 vs 21.4 ± 4.3	23.5 ± 5.4 vs 24.1 ± 7.5	14.8% vs 22.2%	53.7% vs 88.9%	2 ± 1.1 vs 2.2 ± 0.7
		Irritable bowel syndrome	0	–	–	–	–	–	–	–
		Constipation	53.97	38.1 ± 6.7 vs 39.6 ± 6.6	23.2 ± 4.7 vs 22.9 ± 8.9	**21.5 ± 4.2 vs 20 ± 0***	**26 ± 7.7 vs 21.6 ± 0.8****	**13.8% vs 17.6%***	**27.6% vs 85.3%*****	**1.6 ± 0.8 vs 2.3 ± 1.1****
Mental disease	39.68	Depression	11.11	38.8 ± 6.5 vs 39.4 ± 8	**22.1 ± 2.9 vs 30.7 ± 19.6***	20.8 ± 3.1 vs 20 ± 0	23.6 ± 5.9 vs 23.6 ± 4.4	16.1% vs 14.3%	57.1% vs 71.4%	2 ± 1 vs 2.1 ± 1.4
		Anxiety and other neurotic stress-related and somatoform disorders	3.17	39 ± 6.7 vs 36.5 ± 5	23.1 ± 7.3 vs 21.5 ± 0.7	20.7 ± 3 vs 20 ± 0	23.7 ± 5.8 vs 21.5 ± 0.7	16.4% vs 0%	57.4% vs 100%	2 ± 1 vs 2.6 ± 1.2
		Alcohol problems	0	–	–	–	–	–	–	–
		Other psychoactive substance misuse	0	–	–	–	–	–	–	–
		Schizophrenia. Related non-organic psychosis	3.17	39.2 ± 6.5 vs 30.3 ± 6.7	22.3 ± 3.4 vs 47.5 ± 36.1	20.7 ± 3 vs 20 ± 0	23.7 ± 5.8 vs 22 ± 0	16.4% vs 0%	57.4% vs 100%	2 ± 1.1 vs 1.6 ± 0.3
		Hyperactivity	22.22	38.2 ± 6.7 vs 41.4 ± 5.7	22.5 ± 3.7 vs 25 ± 13.8	20.9 ± 3.3 vs 20 ± 0	24 ± 6.3 vs 22.2 ± 2.2	10.2% vs 35.7%	51% vs 85.7%*	1.9 ± 1 vs 2.4 ± 1.1
		Anorexia or bulimia	9.52	39.1 ± 6.5 vs 37 ± 7.8	23.1 ± 7.5 vs 22.8 ± 3.1	20.6 ± 2.8 vs 21.7 ± 4.1	23.4 ± 5.1 vs 25.8 ± 10.4	15.8% vs 16.7%	59.6% vs 50%	2 ± 1.1 vs 1.9 ± 0.5
Stroke	3.17	Stroke and transient ischemic attack	3.17	38.9 ± 6.7 vs 38.5 ± 2.8	23.1 ± 7.3 vs 22 ± 0	20.7 ± 3 vs 20 ± 0	23.7 ± 5.8 vs 22 ± 0	16.4% vs 0%	57.4% vs 100%	2 ± 1.1 vs 2.2 ± 0.6
Cancer	3.17	Cancer in last 5 years	3.17	38.9 ± 6.7 vs 38.3 ± 4.6	22.3 ± 3.4 vs 47.5 ± 36.1	20.7 ± 3 vs 20 ± 0	23.7 ± 5.8 vs 22	16.4% vs 0%	57.4% vs 100%	2 ± 1.1 vs 2.1 ± 0.4
Kidney disease	25.39	Chronic kidney disease	25.39	40.6 ± 6.4 vs 35.2 ± 6.9**	22.1 ± 3.1 vs 26.7 ± 13.2**	20.8 ± 3.3 vs 20.8 ± 3.3	24 ± 6.3 vs 23.3 ± 5.5	22.2% vs 6.3%	58.3% vs 68.8%	2 ± 1.2 vs 2.1 ± 0.8

(Continued)

TABLE 2 | Continued

Chronic health condition	Prevalence (%)	Comorbidity	Prevalence (%)	ASD severity (CARS)	Adaptive functioning (VABS-II)			ADL	Polypharmacy	DBI
					SS communication	SS daily life	SS social skills	Low category (0–2)		
Neurological disease	36.51	Parkinson's disease	7.94	39.1 ± 6.7 vs 36.6 ± 5.2	23.2 ± 7.5 vs 21.6 ± 0.6	20.7 ± 3.1 vs 20 ± 0	23.7 ± 5.9 vs 22.4 ± 1.5	17.2% vs 0%	58.6% vs 60%	2 ± 1.1 vs 2.4 ± 1
		Epilepsy	28.57	37.2 ± 6.3 vs 43.3 ± 5.3***	23.4 ± 8.2 vs 22.2 ± 4	20.6 ± 2.4 vs 20.9 ± 4	24 ± 5.9 vs 22.7 ± 5.2	11.1% vs 27.8%	57.8% vs 61.1%	2 ± 1.1 vs 2.1 ± 0.9
		Dementia	3.17	39.2 ± 6.4 vs 30 ± 7.1	22.3 ± 3.4 vs 47.5 ± 36.1	20.7 ± 3 vs 20 ± 0	23.7 ± 5.8 vs 22 ± 0	16.4% vs 0%	57.4% vs 100%	2 ± 1.1 vs 2.4 ± 0.8
		Migraine	3.17	38.9 ± 6.6 vs 38 ± 8.5	23.1 ± 7.3 vs 21.5 ± 0.7	20.7 ± 3 vs 20 ± 0	23.7 ± 5.8 vs 21.5 ± 0.7	16.4% vs 0%	59% vs 50%	2 ± 1.1 vs 1.9 ± 0.3
		Multiple sclerosis	0	–	–	–	–	–	–	–
Liver disease	7.94	Viral hepatitis	0	–	–	–	–	–	–	–
		Chronic liver disease	7.94	39.4 ± 6.1 vs 33.4 ± 10.4*	22.9 ± 7.3 vs 25.2 ± 5.5	20.3 ± 2.3 vs 24.6 ± 6.4**	3 ± 4.2 vs 31 ± 13.3	15.5% vs 20%	56.9% vs 80%	2 ± 1 vs 2.5 ± 1.1
Immune dysfunction	23.81	Allergy	9.84	38.6 ± 6.6 vs 41.1 ± 6.5	22.4 ± 3.6 vs 27 ± 17.3	20.8 ± 3.2 vs 20 ± 0	23.9 ± 6.1 vs 22 ± 1.9	16.7% vs 11.1%	57.4% vs 66.7%	2 ± 1.1 vs 1.6 ± 1
		Psoriasis or eczema	1.59	38.7 ± 6.8 vs 40.9 ± 4.2	22 ± 2.8 vs 33.2 ± 20.5**	20.5 ± 2.2 vs 22.8 ± 6.9	23.4 ± 5.4 vs 25.5 ± 8.6	14% vs 33.3%	61.4% vs 33.3%	2 ± 1.1 vs 2 ± 0.5
Others		Undernutrition	4.76	38.6 ± 6.7 vs 44.3 ± 1.8	23.2 ± 7.4 vs 21.3 ± 0.6	20.7 ± 3 vs 20 ± 0	23.7 ± 5.8 vs 21.3 ± 0.6	16.7% vs 0%	56.7% vs 100%	**2.1 ± 1 vs 0.9 ± 0.8***
		Hearing loss	9.52	39 ± 6.6 vs 33	23.1 ± 7.3 vs 22	20.7 ± 3 vs 20	23.7 ± 5.7 vs 22	16.1% vs 0%	58.1% vs 100%	2 ± 1 vs 3.2
		Chronic anemia	17.54	38.4 ± 6.9 vs 42.1 ± 5.44	**23.7 ± 8.3 vs 21.2 ± 0.6***	20.9 ± 3.4 vs 20 ± 0	24 ± 6.3 vs 21.6 ± 1.4	19.1% vs 10%	57.4% vs 90%	2 ± 1 vs 2.1 ± 1.3
		Painful condition	4.76	38.9 ± 5.8 vs 43.6 ± 8.8	23.3 ± 7.7 vs 21.3 ± 0.8	20.8 ± 3.1 vs 20 ± 0	23.9 ± 6 vs 21.3 ± 0.8	14.5% vs 33.3%	56.4% vs 66.7%	1.9 ± 1.1 vs 2.7 ± 0.9
		Prostate disorders	14.29	38.8 ± 6.7 vs 40.5 ± 4.6	23.2 ± 7.4 vs 21.3 ± 0.6	20.7 ± 3 vs 20 ± 0	23.7 ± 5.8 vs 21.3 ± 0.6	16.7% vs 0%	58.3% vs 66.7%	2 ± 1.1 vs 2.2 ± 0.9

Among 49 chronic diseases, 44 are placed into 14 chronic health conditions detailed in the first column. Prevalence is expressed as a percentage.

The results for continuous variables for the group without comorbidities versus that with are expressed as the means ± standard error.

The results for dichotomous variables for the group without comorbidities versus that with are expressed as percentages. For polypharmacy, results are expressed in percentage for the group without versus with the comorbidity. For example, 54.9% of patient without hypertension have polypharmacy, whereas 75% of patients with hypertension have polypharmacy. For ADL category, results are expressed in percentage of patients without versus with the comorbidity only for the "low autonomy" category (ADL score between 0 and 2). For example, 19.6% of patients without hypertension have a low score at ADL, whereas 0% of patients with hypertension have a low score at ADL.

ANOVA, χ², Student–Fisher, or Mann–Whitney tests were applied, depending on the nature of the variables (continuous, dichotomous, or categorized in three levels). p values are expressed as ranges. No symbol: nonsignificant (p value > 0.05), *0.05 ≤ p < 0.01, **0.01 ≤ p < 0.001, ***p ≤ 0.001.

ADL, activities of daily living; CARS, Childhood Autism Rating Scale; DBI, Drug Burden Index; SS, sub-scores at the VABS-II; VABS-II, Vineland Adaptive Behavior Scale II.

TABLE 3 | ANCOVA analysis of CIRS total scores (log CIRS tot) by covariable [selected forward with the best Akaike information criterion (AIC)].

	log CIRS-G		
	Beta (SE)	**[95% CI]**	**p value**
Intercept	2.1 (0.2)	[1.3; 2.7]	<0.0001
Age	0.009 (0.003)	[0.003; 0.01]	0.001
SS daily life	–	–	–
SS social skills	–	–	–
ADL	–0.1 (0.02)	[–0.1; –0.04]	<0.0001
Polypharmacy (1/0)	0.4 (0.08)	[0.1; 0.5]	<0.0001
DBI	–	–	–
R² total	0.55		

Linear regressions were used when the CIRS-G score was normalized by logarithmic transformation because of its distribution. Dashes indicate that the variable was not entered into the model.
SE, standard error; CI, confidence interval; ADL, activities of daily living; DBI, Drug Burden Index; SS, sub-score at VABS-II (Vineland Adaptive Behavior Scale II).

associated with constipation, whereas higher scores were associated with obesity and chronic liver disease. After multivariate analyses, chronic liver disease and age still were correlated with daily-life sub-scores (respectively $p = 0.02$ and 0.004, respectively, adjusted R^2 of 0.256; having chronic liver disease increased the daily-life sub-score by 4.4, and being older decreased the daily-life sub-score by 0.05).

The ADL score was associated with thyroid disorders, dyslipidemia, and constipation (see **Supplementary Table 1** for details). No associations remained after multivariate analyses.

Higher polypharmacy was associated with orthostatic hypotension, constipation, and hyperactivity symptoms. Logistic regression showed that polypharmacy is associated with an 11.8-fold increased risk of constipation (OR = 11.8; 95% CI 3.25–42.97). Similarly, a high DBI was associated with constipation, whereas a low DBI was associated with undernutrition. The variable "undernutrition" could not be entered into the multivariate model because only three patients showed undernutrition.

Determination of Comorbidity Burden

The mean CIRS-G total score was 10.6±4.8. In univariate analyses, the log(CIRS-G total score) was significantly associated with age ($p < 0.0001$), low Vineland II daily-life and social-skills sub-scores ($p = 0.03$ and 0.01, respectively), a low level of autonomy assessed by the ADL ($p < 0.001$), polypharmacy ($p = 0.0001$), and a sedative and anticholinergic burden assessed by the DBI ($p = 0.005$). The results of multivariate analyses for the log(CIRS-G

TABLE 4 | Logistic regression analysis of factors related to CIRS-SC categories.

Risk factors	Unit	ORa*	95% CI	p value
Age	5	1.1	(1.1; 1.7)	0.0025
DBI	1	3.1	(1.4; 6.6)	0.002

*Adjusted odds ratio; concordance rate: 83.5%; Hosmer and Lemeshow test = 0.17.
The UNITS statement makes it possible to specify the units of change for continuous explanatory variables so that customized odds ratios can be estimated.
DBI, Drug Burden Index.

total score) are shown in **Table 3**. The CIRS-G total score was significantly predicted by age ($p = 0.001$), polypharmacy ($p < 0.0001$), and a low level of autonomy assessed by the ADL ($p < 0.0001$) (R^2 of 0.55, $p < 0.0001$). Univariate and multivariate analyses performed for the CIRS-G total score without logarithmic transformation gave similar results. Furthermore, inflammation (defined as a C-reactive protein concentration >5 mg/mL) was significantly associated with the log(CIRS-G total score) ($p = 0.004$), but not age ($p = 0.17$).

The mean CIRS-SI was 2.46 ± 0.5, with 73% of participants in the *high-severity index group*. Univariate analyses showed no significant associated factors for the CIRS-SI.

The mean CIRS-SC score was 1.79 ± 1.03, with 49% of participants in the *high-severity comorbidity group*. Univariate analyses showed that the *high-severity comorbidity group* was older ($p = 0.005$) and had lower Vineland II daily-life and social-skills sub-scores ($p = 0.02$ and 0.04, respectively), a lower level of autonomy assessed by the ADL ($p = 0.02$), more frequent polypharmacy ($p = 0.003$), and a higher sedative and anticholinergic burden assessed by the DBI ($p = 0.001$). The results of logistic regressions are shown in **Table 4**. They showed that the older the participants and the higher their DBI, the higher the CIRS-SC score (OR = 1.1, $p = 0.0025$, and OR = 3.1, $p = 0.002$, respectively).

Given the high and unexpected prevalence of chronic kidney diseases, we explored the possible causes of such kidney impairment. There was a positive correlation between age and cardiovascular risk factors ($p = 0.02$), age and chronic kidney disease ($p = 0.005$), and cardiovascular risk factors and chronic kidney disease ($p = 0.0009$).

DISCUSSION

We provide a detailed qualitative and quantitative description of comorbidities in a well-phenotyped cohort of adult patients with ASD and ID. Our analyses provide new information concerning the weight of such comorbidities by showing that the comorbidity burden is associated with age, autonomy, polypharmacy, and sedative and anticholinergic burden. Our study is the first to explore the comorbidity burden in ageing ASD-ID patients using the CIRS-G. The distribution of comorbidities shows the extent to which they are common in ASD-ID patients during adulthood and the pre-elderly period. Analyses of the associations between such comorbidities and the clinical characteristics of ASD could indicate future directions to promote personalized medicine for ageing ASD patients.

Potential Shortcomings and Limitations of the Interpretations

The EFAAR study is the first with a multicentric and prospective design carried out on adult patients with ASD-ID in France. With only 63 patients, our ASD-ID cohort may not be representative of all people with ASD-ID in France. In addition, our patients were recruited from medico-social institutions. They were not hospitalized at the time of the assessment and were considered to be stable. Nevertheless, we may have selected individuals with more severe ASD-ID, as shown by the mean DQ. Thus, the

high comorbidity burden and rates found in our study should be interpreted with caution, because it refers to a very specific population with a very severe ASD-ID disorder. The severe ID observed in our population could be the most important cause of the observed high comorbidity burden.

The homogeneity of the profound ID prevented us from using the level of ID as a variable in univariate and multivariate analyses. Thus, the results of this preliminary study need to be confirmed in a larger cohort of ageing people with ASD, with or without ID, to better understand the effect of ID on the comorbidity burden.

The colinearity of the clinical characteristics and certain comorbidities also make interpretation of the univariate analyses difficult.

Furthermore, there are no previous studies concerning ageing with ASD-ID. Thus, we can compare our results only with those obtained for ageing people with ID.

Mental disorders were diagnosed on the basis of a screening scale (Reiss Scale) and clinical evaluation. Although there are no standardized tools to diagnose mental disorders, such as depression or hyperactivity, in the ASD-ID population, underdiagnosis or overdiagnosis of mental disorders could have been made, introducing a measurement bias.

Integration of the Discovery Into Current Understanding of the Problem

The comorbidity burden, assessed by the CIRS-G total score, of our ASD-ID population, with a mean age of 42.9 years, was comparable with that of an older population (with a mean age of 79 years) from the general hospitalized population in a geriatric department (37). The CIRS-SI of our sample was also higher than that of a population with a mean age of approximately 80 years, supporting the hypothesis of premature ageing in ASD-ID, partially due to a high comorbidity burden. Furthermore, elderly people from the general population often show chronic and low-level inflammation, due to an imbalance between proinflammatory and anti-inflammatory cytokines, called inflamm-ageing, which is associated with multimorbidity and frailty (40). A specific serum inflammation profile has been observed in ASD (41), and we observed a significant association between the CIRS-G score and elevated CRP levels in our ASD-ID cohort (data not shown). This inflamm-ageing process could thus partially explain such a comorbidity burden and be an indirect cause of pathological and/or premature ageing in ASD. However, more precise tools for assessing inflammation and, in particular, microinflammation, such as the measurement of serum orosomucoid or interleukin 6 (IL-6) serum levels, would be useful to further explore this hypothesis.

In multivariate analyses, the comorbidity burden (assessed by the CIRS-G total score) correlated with higher age, lower autonomy, and higher polypharmacy. The level of autonomy assessed by the ADL is significantly associated with higher age in the general population (42). Polypharmacy is associated with multimorbidity in the general population (3) and can increase the risk of decreased autonomy in the geriatric population (43). Thus, these three factors (age, autonomy, and polypharmacy) could synergize to increase the comorbidity burden in ageing ASD-ID people. Focusing on promoting autonomy and reducing polypharmacy in older ASD-ID

patients could reduce their comorbidity burden and thus reduce the impact of pathological ageing. Comprehensive geriatric assessment (CGA) is a multidimensional and multidisciplinary process used to identify the needs of patients to reduce morbidity and mortality and promote their autonomy (44). Given the factors associated with the comorbidity burden in our study, CGA could be an interesting basis from which to propose the medical management of ageing ASD-ID patients. In light of the associations observed between these three clinical characteristics (age, autonomy, and polypharmacy) and certain comorbidities in our study, courses of action could be proposed for daily clinical practice to reduce the comorbidity burden. Autonomy was not associated with any specific comorbidity in multivariate analyses. Thus, its management must be more global than a targeted action on one associated disease of ASD-ID patients. Multivariate analyses revealed an association between polypharmacy and constipation. Thus, special attention towards treating constipation in connection with reducing polypharmacy could have a positive impact on the comorbidity burden.

In the general population, the CIRS-SC score correlates with the multimorbidity prognosis (45) and reflects the number of comorbidity categories with a severe degree of illness. Multivariate analyses showed the CIRS-SC score to positively correlate with age and sedative and anticholinergic burden assessed by the DBI and the DBI to be associated with polypharmacy. An increase of the DBI by 1 point increased the CIRS-SC score by 3.1 points, showing the important weight of the sedative and anticholinergic burden in the severity of comorbidity, probably due to higher polypharmacy. Furthermore, our ASD-ID population had a higher DBI score (2 ± 1.1) than those of an ID population aged over 50 years (1.1 ± 1.73) (18) and general population patients hospitalized in a medical service with a mean of age of 85 years (between 0.53 and 0.64) (46). Thus, the higher DBI score we observed could be due to the severe ID of our population, the co-occurrence of ASD, or simply the resulting high comorbidity burden. A high DBI was associated with constipation, probably because of the side effects of the psychotropic medications in our sample, which needs to be more precisely evaluated. The DBI could thus be a useful tool to improve pharmacological treatment in ASD-ID, all the more since the misuse of psychotropic drugs has been demonstrated for approximately one third of ASD patients due to the lack of a consensus on pharmacological treatment for ASD (47).

The three most common chronic health conditions in our ASD-ID population, with an average age of 43 years, were gastrointestinal (56%), mental (40%), and neurological disorders (37%). There are no data concerning the frequency of these chronic health conditions in ASD patients with ID. The reported prevalence of these chronic health conditions is heterogeneous, depending on whether the ASD or ID population was considered.

The general reported prevalence of gastrointestinal disorders varies between 30% and almost 90% in ASD (14, 15) and has been estimated to be 17% in ID patients (48), suggesting that gastrointestinal disorders are a comorbid condition of ASD, rather than ID (49).

The reported prevalence of mental disorders in ASD children varies between 26% and 70% (50), is approximately 34% in young adults (51), and reaches 54% in ASD adults with an average age of 39 years (17), whereas 16.6% to 48% of ID adults have mental disorders (52–54) and only 9.2% of those of the general

population (51). Thus, the prevalence of mental disorders appears to be comparable between the ASD and ID population, and the rate observed in our study is concordant with that of the literature. Studies exploring mental comorbidities in ASD adults of approximately 40 years of age have reported depression rates between 10% and 69% (55–58), similar to the prevalence found in our study. A recent meta-analysis concluded that the prevalence of current depression in ASD adults is 23% (59), whereas a prevalence of 14.7% to 39% has been reported for an ID population aged over 50 years (48). The rate of 11% observed in our study appears to be low relative to the prevalence of depression previously reported for ASD and ID. A recent study in young adults showed depression in 24.1% of ASD patients without ID, 9.1% in ASD patients with ID, and 6% in patients without ASD or ID (60). The authors emphasized the difficulty of diagnosing depression in ASD-ID patients to explain the reduced prevalence of depression when ASD was associated with ID. It is possible that depression was also underdiagnosed in our study because of the difficulty for patients with severe ASD and ID to verbalize their symptoms. The moderate significant association between depression and high VABS-II communication sub-scores in our study reinforces this argument, leading us to believe that we can detect depression only in mild or moderate ID patients. Depression is also influenced by the level of ID and was shown to be 10% lower in the ASD-ID population in the recent meta-analysis conducted by Hollocks et al. (59), and our population showed profound ID, reflected by the very low DQ scores. Here, we used a standardized tool to detect psychiatric comorbidities, in particular depression, for which two aspects were screened by the Reiss Scale: behavioral and physiological depressive symptoms. Although this scale is only a screening tool, the use of behavioral and physiological indicators appears to be well adapted for ASD-ID patients. Nevertheless, the complexity of diagnosing mental disorders in ASD-ID patients highlights the necessity to develop specific scales to detect these overlapping diseases (61).

Neurological disorders were the third most common chronic health condition in our ASD-ID population. Epilepsy was found in 29% of participants. This disorder has a general prevalence of between 11% and 39% in ASD (62), with no increase with age (63), whereas it occurs in 24.1% of the ID population aged over 50 years (48), compared with only 1% in the general population (64). These data suggest comparable epilepsy rates in ASD and ID, without any additive effect of ASD and ID in our cohort.

In conclusion, the heterogeneity of the assessment methods used can at least partially explain the large range of the prevalence of these three chronic health conditions in ASD reported in the literature (59).

Our study highlights a surprisingly high rate of chronic kidney disease (25%) in ASD-ID patients, whereas only 15% of ID patients with an average age of 62 years have been reported to have this condition (65). We thus explored the possible causes. We observed a positive correlation between age and cardiovascular risk factors, age and chronic kidney disease, and cardiovascular risk factors and chronic kidney disease. Thus, chronic kidney disease was associated with age, probably due to a higher frequency of cardiovascular risk factors in older

participants, which is commonplace in the general population (66). Chronic kidney disease was also more common in women in our sample (data not shown), without any physiological explanation.

In multivariate analyses, ASD severity positively correlated with epilepsy, as already described in literature (67). The IQ level appears to be the most dominant risk factor of epilepsy in the ASD population, more than ASD severity (68). However, the DQ of our cohort showed a profound and homogeneous ID in our population, which prevented us from evaluating the association between ID level and epilepsy. A high VABS-II communication sub-score positively correlated with psoriasis and eczema in multivariate analysis. This association could be explained by the underdiagnosis of dermatological affections in more severe ASD-ID patients, who cannot notify the general practitioner of their symptoms or for whom a complete clinical examination can be more difficult. In multivariate analyses, a lower VABS-II social skills sub-score was associated with constipation, which is consistent with the common observation of an association between ASD severity and gastrointestinal disorders (49, 69). A higher VABS-II daily-life sub-score was associated with chronic liver diseases in multivariate analyses, without any explanation. This association needs to be tested in a larger cohort to develop a pathophysiological hypothesis. Finally, polypharmacy and a high DBI were associated with constipation in multivariate analyses, likely due to the over-prescription of psychotropic drugs. These associations could be used for the promotion of personalized medical care of ASD-ID patients to assess their comorbidities according to clinical features in daily practice.

Future Directions

The ageing of people with ASD-ID could have an additive effect on their comorbidity burden and its prevalence, likely resulting in pathological ageing. Our results highlight the necessity of assessing gastrointestinal, mental, and neurological disorders, as well as chronic kidney disease and cardiovascular risk factors in ageing ASD-ID patients. Comorbidities need to be evaluated to reduce conflicting treatment and prevent polypharmacy and its iatrogenic effects. The use of the CIRS-G in clinical practice could help practitioners to reduce the comorbidity burden and promote autonomy. The research of specific comorbidities, such as epilepsy, cutaneous diseases, and constipation, based on the clinical characteristics of the ASD-ID patient, should be generalized.

Polypharmacy, multimorbidity and its associated problems, and frailty, three major geriatric concerns, must be investigated to propose personalized geriatric medical care for ASD-ID patients.

Because our population had profound ID, we also need to investigate geriatric syndromes in a large cohort of ASD patients, with and without ID, to evaluate the influence of ID on the comorbidity burden, as well as the prevalence of geriatric syndromes. Data sharing with a general population cohort of adults and pre-elderly people, such as that of CONSTANCES, could also help us to compare the prevalence of comorbidities and reinforce the hypothesis of premature ageing in the ASD-ID population.

ETHICS STATEMENT

AUTHOR CONTRIBUTIONS

SM, TA, and AB drafted the manuscript. SM, CM, and AB revised the manuscript. SCo, SCr, and SM collected the data. CM and SM conducted the statistical analyses. M-CP, AB, SM, ÉP, VG, CJ, and HB designed the EFAAR study. JL and CM monitored the data of the EFAAR study. AB and SM coordinated the EFAAR study. AB is the principal investigator of the EFAAR study.

FUNDING

ACKNOWLEDGMENTS

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2019.00617/full#supplementary-material

REFERENCES

1. Uijen AA, van de Lisdonk EH. Multimorbidity in primary care: prevalence and trend over the last 20 years. *Eur J Gen Pract* (2008) 14 (Suppl 1):28–32. doi: 10.1080/13814780802436093

2. Marengoni A, Angleman S, Fratiglioni L. Prevalence of disability according to multimorbidity and disease clustering: a population-based study. *J Comorb* (2011) 1:11–8. doi: 10.15256/joc.2011.1.3

3. Marengoni A, Angleman S, Melis R, Mangialasche F, Karp A, Garmen A, et al. Aging with multimorbidity: a systematic review of the literature. *Ageing Res Rev* (2011) 10(4):430–9. doi: 10.1016/j.arr.2011.03.003

4. Gijsen R, Hoeymans N, Schellevis FG, Ruwaard D, Satariano WA, van den Bos GA. Causes and consequences of comorbidity: a review. *J Clin Epidemiol* (2001) 54(7):661–74. doi: 10.1016/S0895-4356(00)00363-2

5. Lai MC, Lombardo MV, Baron-Cohen S. Autism. *Lancet* (2014) 383(9920):896–910. doi: 10.1016/S0140-6736(13)61539-1

6. CDC data, https://www.cdc.gov/media/releases/2014/p0327-autism-spectrum-disorder.html.

7. Inserm data, https://www.inserm.fr/information-en-sante/dossiers-information/autisme.

8. Developmental Disabilities Monitoring Network Surveillance Year Principal I, Centers for Disease C, Prevention. Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2010. *MMWR Surveill Summ* (2014) 63(2):1–21.

9. Bilder D, Botts EL, Smith KR, Pimentel R, Farley M, Viskochil J, et al. Excess mortality and causes of death in autism spectrum disorders: a follow up of the 1980s Utah/UCLA autism epidemiologic study. *J Autism Dev Disord* (2013) 43(5):1196–204. doi: 10.1007/s10803-012-1664-z

10. Hwang YI, Foley KR, Trollor JN. Aging well on the autism spectrum: an examination of the dominant model of successful aging. *J Autism Dev Disord* (2018). doi: 10.1007/s10803-018-3596-8

11. Miot S, Crepiat S, Courderc S, Jeandel C, Blain H, Baghdadli A. Vieillissement et trouble du spectre autistique. *NPG* (2018) 18:78–85. doi: 10.1016/j.npg.2018.01.003

12. Cermak SA, Curtin C, Bandini LG. Food selectivity and sensory sensitivity in children with autism spectrum disorders. *J Am Diet Assoc* (2010) 110(2):238–46. doi: 10.1016/j.jada.2009.10.032

13. Sharp WG, Berry RC, McCracken C, Nuhu NN, Marvel E, Saulnier CA, et al. Feeding problems and nutrient intake in children with autism spectrum disorders: a meta-analysis and comprehensive review of the literature. *J Autism Dev Disord* (2013) 43(9):2159–73. doi: 10.1007/s10803-013-1771-5

14. Horvath K, Perman JA. Autism and gastrointestinal symptoms. *Curr Gastroenterol Rep* (2002) 4(3):251–8. doi: 10.1007/s11894-002-0071-6

15. Buie T, Campbell DB, Fuchs GJ, 3rd, Furuta GT, Levy J, Vandewater J, et al. Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. *Pediatrics* (2010) 125 Suppl 1:S1–18. doi: 10.1542/peds.2009-1878C

16. Maski KP, Jeste SS, Spence SJ. Common neurological co-morbidities in autism spectrum disorders. *Curr Opin Pediatr* (2011) 23(6):609–15. doi: 10.1097/MOP.0b013e32834c9282

17. Croen LA, Zerbo O, Qian Y, Massolo ML, Rich S, Sidney S, et al. The health status of adults on the autism spectrum. *Autism* (2015) 19(7):814–23. doi: 10.1177/1362361315577517

18. O'Connell J, Burke E, Mulryan N, O'Dwyer C, Donegan C, McCallion P, et al. Drug Burden Index to define the burden of medicines in older adults with intellectual disabilities: an observational cross-sectional study. *Br J Clin Pharmacol* (2018) 84(3):553–67. doi: 10.1111/bcp.13479

19. O'Dwyer M, Peklar J, McCallion P, McCarron M, Henman MC. Factors associated with polypharmacy and excessive polypharmacy in older people with intellectual disability differ from the general population: a cross-sectional observational nationwide study. *BMJ Open* (2016) 6(4):e010505. doi: 10.1136/bmjopen-2015-010505

20. McCarron M, Swinburne J, Burke E, McGlinchey E, Carroll R, McCallion P. Patterns of multimorbidity in an older population of persons with an intellectual disability: results from the intellectual disability supplement to the Irish longitudinal study on aging (IDS-TILDA). *Res Dev Disabil* (2013) 34(1):521–7. doi: 10.1016/j.ridd.2012.07.029

21. Schoufour JD, Mitnitski A, Rockwood K, Evenhuis HM, Echteld MA. Development of a frailty index for older people with intellectual disabilities: results from the HA-ID study. *Res Dev Disabil* (2013) 34(5):1541–55. doi: 10.1016/j.ridd.2013.01.029

22. Schoufour JD, Mitnitski A, Rockwood K, Evenhuis HM, Echteld MA. Predicting 3-year survival in older people with intellectual disabilities using a Frailty Index. *J Am Geriatr Soc* (2015) 63(3):531–6. doi: 10.1111/jgs.13239

23. Zigman WB. Atypical aging in Down syndrome. *Dev Disabil Res Rev* (2013) 18(1):51–67. doi: 10.1002/ddrr.1128

24. Schopler E, Reichler RJ, Renner BR. *The Childhood Autism Rating Scale-CARS*. New York: Irvington (1986).

25. Stern W. *The psychological methods of intelligence testing*. Whipple, G, editor. Trans. Baltimore: Warwick and York (1912).

26. Sparrow S, Balla D, Cicchetti D. *Vineland Adaptive Behavior Scales*. Circle Pines, MN: American Guidance Service (1984).

27. Carter AS, Volkmar FR, Sparrow SS, Wang JJ, Lord C, Dawson G, et al. The Vineland Adaptive Behavior Scales: supplementary norms for individuals with autism. *J Autism Dev Disord* (1998) 28(4):287–302. doi: 10.1023/A:1026056518470

28. Katz S, Downs TD, Cash HR, Grotz RC. Progress in development of the index of ADL. *Gerontologist* (1970) 10(1):20–30. doi: 10.1093/geront/10.1_Part_1.20

29. Katz S. Assessing self-maintenance: activities of daily living, mobility, and instrumental activities of daily living. *J Am Geriatr Soc* (1983) 31(12):721–7. doi: 10.1111/j.1532-5415.1983.tb03391.x

30. Fulton MM, Allen ER. Polypharmacy in the elderly: a literature review. *J Am Acad Nurse Pract* (2005) 17(4):123–32. doi: 10.1111/j.1041-2972.2005.0020.x

31. Cardwell K, Hughes CM, Ryan C. The association between anticholinergic medication burden and health related outcomes in the 'oldest old': a systematic review of the literature. *Drugs Aging* (2015) 32(10):835–48. doi: 10.1007/s40266-015-0310-9

32. Hilmer SN, Mager DE, Simonsick EM, Cao Y, Ling SM, Windham BG, et al. A Drug Burden Index to define the functional burden of medications in older people. *Arch Intern Med* (2007) 167(8):781–7. doi: 10.1001/archinte.167.8.781

33. Deb S, Hare M, Prior L, Bhaumik S. Dementia screening questionnaire for individuals with intellectual disabilities. *Br J Psychiatry* (2007) 190:440–4. doi: 10.1192/bjp.bp.106.024984

34. Reiss S. *The Reiss Screen Test manual*. Orlando Park, Illinois: IDS, Inc. (1988).

35. Lecavalier L, Tassé MJ. Traduction et adaptation transculturelle du Reiss screen for maladaptive behavior, revue francophone de la déficience intellectuelle. (2001) 12: (1):31–44.

36. Linn BS, Linn MW, Gurel L. Cumulative illness rating scale. *J Am Geriatr Soc* (1968) 16(5):622–6. doi: 10.1111/j.1532-5415.1968.tb02103.x

37. Miller MD, Paradis CF, Houck PR, Mazumdar S, Stack JA, Rifai AH, et al. Rating chronic medical illness burden in geropsychiatric practice and research: application of the Cumulative Illness Rating Scale. *Psychiatry Res* (1992) 41(3):237–48. doi: 10.1016/0165-1781(92)90005-N

38. Kirkhus L, Jordhoy M, Saltyte Benth J, Rostoft S, Selbaek G, Jensen Hjermstad M, et al. Comparing comorbidity scales: attending physician score versus the Cumulative Illness Rating Scale for Geriatrics. *J Geriatr Oncol* (20162016) 7(2):90–8. doi: 10.1016/j.jgo.2015.12.003

39. Martocchia A, Indiano I, Tafaro L, Frugoni P, Amici A, Cacciafesta M, et al. The evaluation of the presence of comorbidity by the Marigliano-Cacciafesta polypathology scale (MCPS) and the cumulative illness rating scale (CIRS) in elderly subjects with disability. *Arch Gerontol Geriatr* (2009) 49(1):150–2. doi: 10.1016/j.archger.2008.06.006

40. Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. *Nat Rev Cardiol* (2018) 15(9):505–22. doi: 10.1038/s41569-018-0064-2

41. Masi A, Quintana DS, Glozier N, Lloyd AR, Hickie IB, Guastella AJ. Cytokine aberrations in autism spectrum disorder: a systematic review and meta-analysis. *Mol Psychiatry* (2015) 20(4):440–6. doi: 10.1038/mp.2014.59

42. Mlinac ME, Feng MC. Assessment of activities of daily living, self-care, and independence. *Arch Clin Neuropsychol* (20162016) 31(6):506–16. doi: 10.1093/arclin/acw049

43. Dauphinot V, Jean-Bart E, Krolak-Salmon P, Mouchoux C. A multi-center, randomized, controlled trial to assess the efficacy of optimization of drug prescribing in an elderly population, at 18 months of follow-up, in the evolution of functional autonomy: the OPTIM study protocol. *BMC Geriatr* (2017) 17(1):195. doi: 10.1186/s12877-017-0600-7

44. Parker SG, McCue P, Phelps K, McCleod A, Arora S, Nockels K, et al. What is comprehensive geriatric assessment (CGA)? *Umbrella Rev Age Ageing* (2018) 47(1):149–55. doi: 10.1093/ageing/afx166

45. Gronberg BH, Sundstrom S, Kaasa S, Bremnes RM, Flotten O, Amundsen T, et al. Influence of comorbidity on survival, toxicity and health-related quality of life in patients with advanced non-small-cell lung cancer receiving platinum-doublet chemotherapy. *Eur J Cancer* (2010) 46(12):2225–34. doi: 10.1016/j.ejca.2010.04.009

46. Dauphinot V, Faure R, Bourguignon L, Goutelle S, Krolak-Salmon P, Mouchoux C. Factors associated with changes in exposure to anticholinergic and sedative medications in elderly hospitalized patients: multicentre longitudinal study. *Eur J Neurol* (2017) 24(3):483–90. doi: 10.1111/ene.13228

47. Houghton R, Ong RC, Bolognani F. Psychiatric comorbidities and use of psychotropic medications in people with autism spectrum disorder in the United States. *Autism Res* (2017) 10(12):2037–47. doi: 10.1002/aur.1848

48. Peklar J, Kos M, O'Dwyer M, McCarron M, McCallion P, Kenny RA, et al. Medication and supplement use in older people with and without intellectual disability: an observational, cross-sectional study. *PLoS One* (2017) 12(9):e0184390. doi: 10.1371/journal.pone.0184390

49. Neuhaus E, Bernier RA, Tham SW, Webb SJ. Gastrointestinal and psychiatric symptoms among children and adolescents with autism spectrum disorder. *Front Psychiatry* (2018) 22(9):515. doi: 10.3389/fpsyt.2018.00515

50. DeFilippis M. Depression in children and adolescents with autism spectrum disorder. *Children (Basel)* (2018) 5(9). doi: 10.3390/children5090112

51. Davignon MN, Qian Y, Massolo M, Croen L. Psychiatric and medical conditions in transition-aged individuals with ASD. *Pediatrics* (2018) 141(S4):S335–45. doi: 10.1542/peds.2016-4300K

52. Cooper SA, Smiley E, Morrison J, Williamson A, Allan L. Mental ill-health in adults with intellectual disabilities: prevalence and associated factors. *Br J Psychiatry* (2007) 190:27–35. doi: 10.1192/bjp.bp.106.022483

53. Morgan VA, Leonard H, Bourke J, Jablensky A. Intellectual disability co-occurring with schizophrenia and other psychiatric illness: population-based study. *Br J Psychiatry* (2008) 193(5):364–72. doi: 10.1192/bjp.bp.107.044461

54. O'Dwyer M, Peklar J, Mulryan N, McCallion P, McCarron M, Henman MC. Prevalence, patterns and factors associated with psychotropic use in older adults with intellectual disabilities in Ireland. *J Intellect Disabil Res* (2017) 61(10):969–83. doi: 10.1111/jir.12391

55. Moss P, Howlin P, Savage S, Bolton P, Rutter M. Self and informant reports of mental health difficulties among adults with autism findings from a long-term follow-up study. *Autism* (2015) 19(7):832–41. doi: 10.1177/1362361315585916

56. Crane L, Goddard L, Pring L. Autobiographical memory in adults with autism spectrum disorder: the role of depressed mood, rumination, working memory and theory of mind. *Autism* (2013) 17(2):205–19. doi: 10.1177/1362361311418690

57. Jones L, Goddard L, Hill EL, Henry LA, Crane L. Experiences of receiving a diagnosis of autism spectrum disorder: a survey of adults in the United kingdom. *J Autism Dev Disord* (2014) 44(12):3033–44. doi: 10.1007/s10803-014-2161-3

58. Charlot L, Deutsch CK, Albert A, Hunt A, Connor DF, McIlvane WJ, Jr. Mood and anxiety symptoms in psychiatric inpatients with autism spectrum disorder and depression. *J Ment Health Res Intellect Disabil* (2008) 1(4):238–53. doi: 10.1080/19315860802313947

59. Hollocks MJ, Lerh JW, Magiati I, Meiser-Stedman R, Brugha TS. Anxiety and depression in adults with autism spectrum disorder: a systematic review and meta-analysis. *Psychol Med* (2018) 49(4):559–572. doi: 10.1017/S0033291718002283

60. Rai D, Heuvelman H, Dalman C, Culpin I, Lundberg M, Carpenter P, et al. Association between autism spectrum disorders with and without intellectual disability and depression in young adulthood. *JAMA Netw Open* (2018) 1(4):e181465. doi: 10.1001/jamanetworkopen.2018.1465

61. Jopp DA, Keys CB. Diagnostic overshadowing reviewed and reconsidered. *Am J Ment Retard* (2001) 106(5):416–33. doi: 10.1352/0895-8017(2001)106<0416:DORAR>2.0.CO;2

62. Tuchman R, Rapin I. Epilepsy in autism. *Lancet Neurol* (2002) 1(6):352–8. doi: 10.1016/S1474-4422(02)00160-6

63. Wise EA, Smith MD, Rabins PV. Aging and autism spectrum disorder: a naturalistic, longitudinal study of the comorbidities and behavioral and neuropsychiatric symptoms in adults with ASD. *J Autism Dev Disord* (2017) 47(6):1708–15. doi: 10.1007/s10803-017-3095-3

64. Forsgren L, Beghi E, Oun A, Sillanpaa M. The epidemiology of epilepsy in Europe—a systematic review. *Eur J Neurol* (2005) 12(4):245–53. doi: 10.1111/j.1468-1331.2004.00992.x

65. de Winter CF, Echteld MA, Evenhuis HM. Chronic kidney disease in older people with intellectual disability: results of the HA-ID study. *Res Dev Disabil* (2014) 35(3):726–32. doi: 10.1016/j.ridd.2013.11.005

66. Jousilahti P, Vartiainen E, Tuomilehto J, Puska P. Sex, age, cardiovascular risk factors, and coronary heart disease: a prospective follow-up study of 14 786 middle-aged men and women in Finland. *Circulation* (1999) 99(9):1165–72. doi: 10.1161/01.CIR.99.9.1165

67. Keller R, Basta R, Salerno L, Elia M. Autism, epilepsy, and synaptopathies: a not rare association. *Neurol Sci* (2017) 38(8):1353–61. doi: 10.1007/s10072-017-2974-x

68. Viscidi EW, Johnson AL, Spence SJ, Buka SL, Morrow EM, Triche EW. The association between epilepsy and autism symptoms and maladaptive behaviors in children with autism spectrum disorder. *Autism* (2013) 18(8):996–1006. doi: 10.1177/1362361313508027

69. Adams JB, Johansan LJ, Powell LD, Quig D, Rubin RA. Gastrointestinal flora and gastrointestinal status in children with autism—comparisons to typical children and correlation with autism severity. *BMC Gastroenterol* (2011) 11:22. doi: 10.1186/1471-230X-11-22

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read
for greatest visibility
and readership

FAST PUBLICATION
Around 90 days
from submission
to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative,
and constructive
peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers
acknowledged by name
on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data
and methods to enhance
research reproducibility

DIGITAL PUBLISHING
Articles designed
for optimal readership
across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics
track visibility across
digital media

EXTENSIVE PROMOTION
Marketing
and promotion
of impactful research

LOOP RESEARCH NETWORK
Our network
increases your
article's readership