AUTISM SPECTRUM DISORDERS: FROM GENOTYPES TO PHENOTYPES

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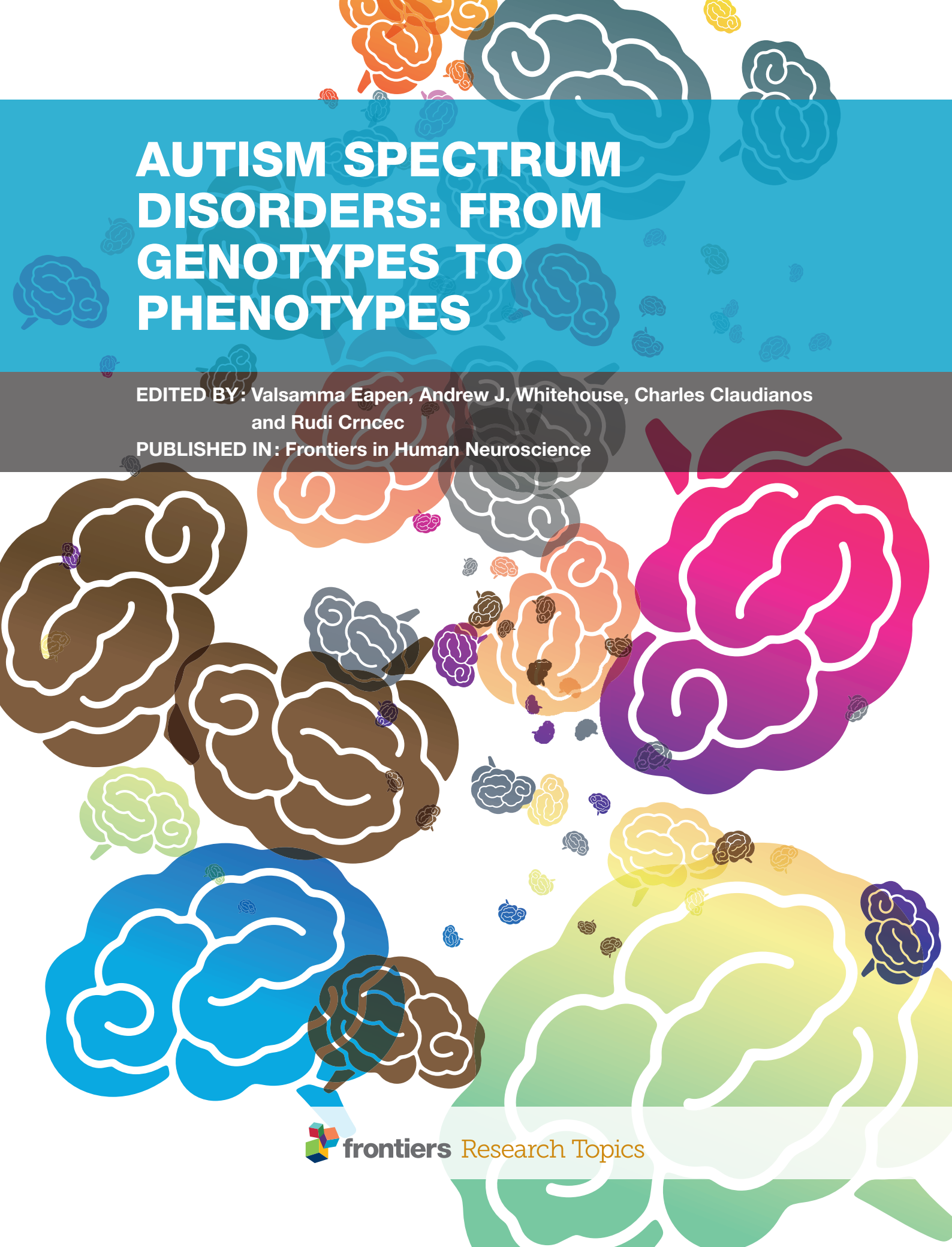

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AUTISM SPECTRUM DISORDERS: FROM GENOTYPES TO PHENOTYPES

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This Research Topic covers the pathogenetic processes in Autism Spectrum Disorder (ASD) that underpin the translation of genetic vulnerability to clinically significant symptoms. Available research data in ASD suggests that it is a neural connectivity disorder and that the social communication and related neurobehavioural symptoms result from reduced synchronization between key "social brain" regions. These interconnected neural systems can be understood through the relationship between functionally relevant anatomic areas and neurochemical pathways, the programming of which are genetically modulated during neurodevelopment and mediated through a range of epigenetic and environmental modulators. Elucidating the underlying molecular mechanisms can provide an invaluable window for understanding the neural wiring that regulates higher brain functions and consequent clinical phenotypes.

In keeping with the multi modal and diverse origins of ASD, this Research Topic explores the genetic underpinnings and environmental modulation in the aetiology; neural substrates, biomarkers and endophenotypes that underlie clinical characteristics; as well as neurochemical pathways and pathophysiological mechanisms that pave the way for therapeutic interventions. Furthermore, since genetically mediated deficits and consequent functional impairments involve activity-dependent synapse development that depends on postnatal learning and experience, the trajectory towards the final clinical expression could be modulated by early interventions that exploit the neuronal maturation and brain plasticity. However, identifying these diverse pathogenetic processes and tailoring interventions would require subtyping ASD into homogeneous subgroups. In this regard, this topic covers the current state of evidence in the literature through topic reviews as well as ongoing original work that provides tangible hypotheses and directions for future research.

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Autism spectrum disorders: from genotypes to phenotypes

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Keywords: Autism spectrum disorder, pathogenesis, etiology, genotype, phenotypic variability, review

Autism spectrum disorder (ASD) is appropriately named due to the broad variability or clinical heterogeneity, which in turn is linked to genetic heterogeneity. Such clinical heterogeneity is difficult to understand and difficult to treat under the auspices of a single disorder. The logical approach to this problem is to try to reduce the heterogeneity by stratifying ASD patients into smaller more homogeneous subgroups. However, this task is difficult as the stratification of ASD on the basis of behavioral differences would require clinicians to make evermore "new" categories of behavioral disorders when the core neurobiological deficits may be the same or similar. This difficulty is further amplified when the extreme genetic heterogeneity associated with ASD is considered and where some comparable genetic lesions are associated with different behavioral profiles and in some cases with different neuropsychiatric conditions. This Research Topic of Frontiers addresses these problems from a diversity of perspectives with the aim of clarifying these issues and promoting novel solutions.

The review by Eapen et al. (2013) sets the tone for this Research Topic of Frontiers by putting clinical heterogeneity in perspective with ASD genotypes, phenotypes, and potentially corresponding treatment outcomes necessitating the need to search for useful endophenotypes to help improve early intervention. The authors provide a perspective to the continuum of genetic variants (rare and common) present within the general population that has the potential to impact social-cognition and behavior. This also provides the ideal introduction to the genome-wide association study reported by Jones et al. (2013) that identified two ASD candidate genes (*PRKCB1* and *CBLN1*) from the general population with nominal association to autistic-like traits. *PRKCB1* (the gene encoding Protein kinase C-B1 involved in signal transduction and the regulation of gene expression) has been reported previously in linkage and association studies for ASD; while CBLN1 is just one of the many ligands for the neurexins – neuronal cell adhesion molecules involved in synaptogenesis and neural circuitry.

Neurexin mutations are common in ASD and all of the gene families encoding neurexin trans-synaptic ligands have been implicated previously in ASD. Together, the neurexins (NRXNs 1–4) and their different trans-synaptic ligands (CBLNs/GRIDs, LRRTMs, and NLGNs) are referred to collectively as the neurexin trans-synaptic connexus (NTSC). Here, Clarke and Eapen (2014) review the NTSC in detail as the basis for the molecular stratification of ASD. As common as NTSC mutations are in ASD, they are even more common in Tourette syndrome including recurrent disruptions of the intergenic region around the CBLN2 gene. NTSC mutations are also found in patients with Schizophrenia and intellectual disability. As such, Clarke et al. argue that the NTSC should represent the primary determinate for the molecular stratification of ASD and related neurodevelopmental disorders, from where characterization of the genetic architecture should provide a window for understanding how the NTSC and secondary variants function to specify behavior.

Recapitulating ASD models in rodents for mutations like those within the NTSC provide probably the best approach for improving drug development for ASD. The study by Argyropoulos et al. (2013) takes this one step further by describing the use of rodent models to identify and characterize endophenotypes that might be useful for the stratification of ASD patients as indicators of the biological pathways affected. This bottom-up approach is taken further by Unwin et al. (2013) in their study that suggests that the risk factors of "low birth weight" and "*in utero* exposure" to selective serotonin reuptake inhibitors give rise to some of the novel endophenotypes such as "sleep disturbance" in the pregnant mother and "gastrointestinal complaints in children with ASD" that could be useful for stratifying patients on the basis of cause and effect. Further, Billeci et al. (2013) have reviewed the use of advanced EEG techniques that has the potential to find distinctive patterns of abnormalities in ASD subjects, paving the way for the development of tailored intervention strategies. Similarly, the use of neuroimaging techniques to explore the heterogeneity in ASD is the focus of the review by Lenroot and Yeung (2013).

It appears that the phenotypic variability within ASD and the phenotypic overlap between ASD and other neurodevelopmental disorders such as Tourette syndrome, attention deficit hyperactivity disorder (ADHD), schizophrenia, language disorder, and intellectual disability could be due to the fact that the genes converge toward a core set of dysregulated biological processes that affect distinct neurodevelopmental pathways involved in synapse development/maintenance and circuitry formation through effects on neurogenesis, axon guidance in dendritic projections, and/or neuronal migration. Thus, defects in synaptic development can result in abnormal development across disorders and broad domains but yet carry distinct neurocognitive and behavioral profiles. The penetrance of the different comorbidities may in turn be related to the dose effects of gene abnormality or the timing of events when different neuronal regions and circuitry are being formed, as may be the influence of gender, intrauterine and perinatal events, epigenetics, and other environmental modulators. In this regard, accumulating evidence supports the notion that immune cells

play important roles in normal brain function, outside of neuroinflammation. Voineagu and Eapen (2013) have reviewed recent data demonstrating the involvement of synaptic dysfunction and abnormal immune responses in ASD, and in particular the role of microglia in synaptic pruning during postnatal brain development, a period that coincides with the onset of ASD symptoms.

Given the multi modal and diverse origins of ASD, including the genetic as well as environmental modulation in the etiology, therapeutic interventions should also reflect such diversity. Furthermore, it seems that genetically mediated deficits and consequent functional impairments involve activity-dependent synapse development that depends on postnatal learning and experience. In this regard, the paper by Vivanti et al. (2013) suggests that intellectual disability in ASD might emerge as a consequence of severe social-communication deficits on the experience-dependent mechanisms underlying neurocognitive development. Such a model would predict that early intervention will prevent or reduce the risk of these deficits cascading into a trajectory toward full expression of the disorder by exploiting the neuronal maturation and brain plasticity. Thus, identifying homogeneous subgroups within ASD and matching appropriate interventions remain the key challenge for future research.

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Exploring links between genotypes, phenotypes, and clinical predictors of response to early intensive behavioral intervention in autism spectrum disorder

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Autism spectrum disorder (ASD) is amongst the most familial of psychiatric disorders. Twin and family studies have demonstrated a monozygotic concordance rate of 70–90%, dizygotic concordance of around 10%, and more than a 20-fold increase in risk for first-degree relatives. Despite major advances in the genetics of autism, the relationship between different aspects of the behavioral and cognitive phenotype and their underlying genetic liability is still unclear. This is complicated by the heterogeneity of autism, which exists at both genetic and phenotypic levels. Given this heterogeneity, one method to find homogeneous entities and link these with specific genotypes would be to pursue endophenotypes. Evidence from neuroimaging, eye tracking, and electrophysiology studies supports the hypothesis that, building on genetic vulnerability, ASD emerges from a developmental cascade in which a deficit in attention to social stimuli leads to impaired interactions with primary caregivers. This results in abnormal development of the neurocircuitry responsible for social cognition, which in turn adversely affects later behavioral and functional domains dependent on these early processes, such as language development. Such a model begets a heterogeneous clinical phenotype, and is also supported by studies demonstrating better clinical outcomes with earlier treatment. Treatment response following intensive early behavioral intervention in ASD is also distinctly variable; however, relatively little is known about specific elements of the clinical phenotype that may predict response to current behavioral treatments. This paper overviews the literature regarding genotypes, phenotypes, and predictors of response to behavioral intervention in ASD and presents suggestions for future research to explore linkages between these that would enable better identification of, and increased treatment efficacy for, ASD.

Keywords: autism spectrum disorder, genotype, phenotype, early intervention, treatment response

GENETIC BASIS OF AUTISM SPECTRUM DISORDER

It has been suggested that autism spectrum disorder (ASD) is one of the most familial of psychiatric disorders, with a heritability of 80%, a monozygotic concordance rate of 70–90%, dizygotic concordance of around 10%, and more than a 20-fold increase in risk for first-degree relatives (Bailey et al., 1995; O'Roak, 2008). Although there have been some significant advances in the recent past (Wang et al., 2009; Pinto et al., 2010), the rate of progress in gene discovery has been modest (Abrahams and Geschwind, 2010). Also, genomic analyses indicate extreme genetic heterogeneity and so far, over 100 genes have been reported in ASD with a conservative estimate of between 380 and 820 loci implicated (Betancur, 2011; Clarke and Eapen, in press), and with considerable overlap with other disorders such as intellectual disability, epilepsy, schizophrenia, and attention deficit hyperactivity disorder (ADHD). These findings suggest that ASD is not a single-gene disorder with Mendelian inheritance but rather a complex disorder resulting from simultaneous genetic variations in multiple genes (Dawson et al., 2002; El-Fishawy, 2010) as well as complex interactions between genetic, epigenetic, and environmental factors (Eapen, 2011).

It has been reported that some of the associated sequence variations noted in ASD are common in the general population although it is unclear as to whether the ASD phenotype results from the involvement of single genes in combination with non-genetic factors, or multiple genes through locus heterogeneity (multiple rare variations in the same gene), or multiple genes through allelic heterogeneity (variations in multiple and different genes). Furthermore, it has been proposed that multiple genes in combination with non-genetic factors may be necessary to result in the ASD phenotype or that ASD may be a collection of rare disorders, that is, a shared phenotype resulting from several different genetic defects. Thus it would seem that there are at least three major pathogenetic processes (Eapen, 2011) resulting in three different subgroups: (1) ASD-Plus group or Syndromic ASD resulting from rare single-gene disorders where ASD is a behavioral phenotype of the associated disorder; (2) Broad ASD group resulting from common variants distributed continually in

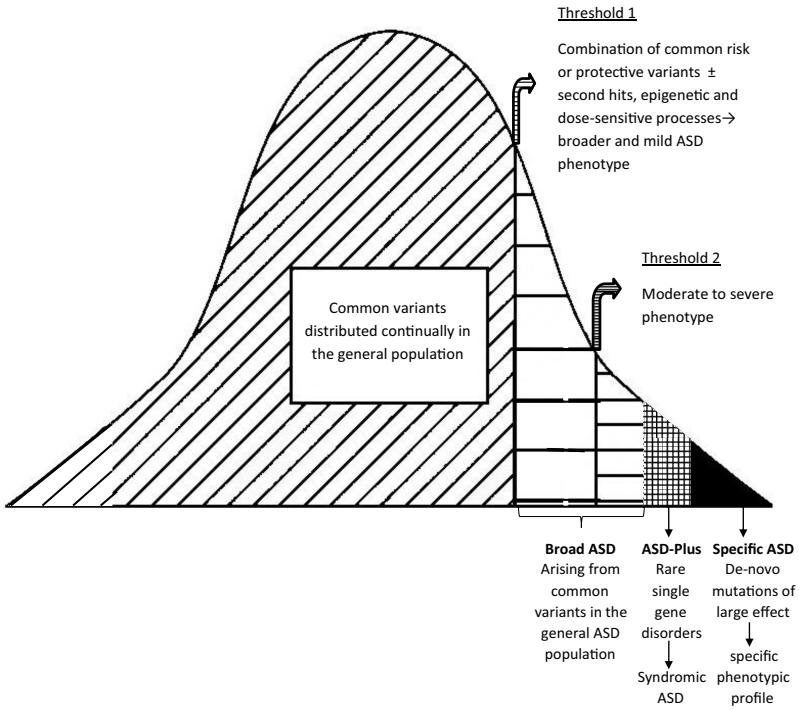

FIGURE 1 | Genetic and phenotypic heterogeneity in ASD. Adapted from Eapen (2011).

the general population but following a gene-environment diathesis model, when it passes the first threshold (threshold 1) due to other gene or environmental additive effects or "second hits" including epigenetic and dose-sensitive processes, it results in the broader and mild ASD phenotype which may be observed in other family members of affected individuals and when it passes a second threshold (threshold 2) it results in a moderate to severe ASD phenotype that is clinically significant; and (3) specific ASD group due to "*de novo* mutations" of large effect resulting in ASD presentations but carrying unique phenotypic profiles based on the specific site and nature of the *de novo* mutation (see **Figure 1**).

BROAD AUTISM PHENOTYPE

The term "broad autism phenotype" (BAP) refers to the presence of subclinical levels of ASD symptoms among individuals who do not meet criteria for a diagnosis of ASD (Bolton et al., 1994; Piven and Palmer, 1999). BAP characteristics correspond to the primary features of ASD, including traits that are social, such as socially reticent or inappropriate behavior, or non-social, such as rigidity and ritualistic or repetitive behaviors (Losh et al., 2009). Twin and family studies have shown that genetic liability to autism is expressed in unaffected relatives of people with ASD through features that are milder but qualitatively similar to the defining characteristics of ASD, including social abnormalities, communication impairments, and repetitive behaviors (Bailey et al., 1998; Goussé et al., 2002; Losh et al., 2009). Previous studies suggest that around 25% of first-degree relatives of children with ASD show impairment in one of the three diagnostic domains for ASD: sociability, communication, and cognitive or behavioral flexibility (for a review, see Goussé et al., 2002). Bailey et al. (1998) conclude that the BAP features observed in relatives of individuals with ASD appear to have a genetic rather than environmental basis.

While early work on the BAP focused on examining ASD-related traits in first and second degree relatives of individuals with ASD (for a review, see Bailey et al., 1998), subsequent studies have demonstrated that the characteristics comprising the BAP exist within the general population as well (Baron-Cohen et al., 2001; Constantino and Todd, 2003, 2005). Features of ASD that have been found to be continuously distributed within the general population include restricted interests (Baron-Cohen et al., 2001), atypical visuospatial and cognitive performance (Grinter et al., 2009; Stewart et al., 2009; Richmond et al., 2013), abnormal speech perception (Stewart and Ota, 2008), reduced gaze reciprocity (Chen and Yoon, 2011), an impaired ability to recognize affect from facial expressions and body language (Ingersoll, 2010b), and reductions in social skill and social-cognitive ability (Sasson et al., 2012). These findings may be consistent with the suggestion that some of the genetic sequence variations found in ASD are common in the general population.

HETEROGENEITY OF AUTISM

Despite major advances in the genetics of ASD, the relationship between different aspects of the behavioral and cognitive phenotype of ASD and their underlying genetic liability is still unclear (Bailey et al., 1998; Klin et al., 2002). This is complicated by the heterogeneity of ASD, which exists at both genetic and phenotypic levels (Charman et al., 2011). Further, it has been suggested that there may be gender dependent differences in the ASD phenotype

(Eapen, 2011). For example, Lai et al. (2012) observed that while performance in the social-cognitive domain was equally impaired in male and female adults with ASD, in the specific non-social-cognitive domains of attention to detail and dexterity involving executive function, there were differences based on gender. Losh et al. (2009) argue that the BAP may provide an important complementary approach for detecting the genes involved in ASD by narrowing the highly heterogeneous phenotype of an ASD diagnosis to particular features that are likely to be more conducive to genetic investigation (Wheelwright et al., 2010; Spencer et al., 2011, 2012a,b; Sucksmith et al., 2012).

Due to its heterogeneity, ASD is no longer viewed as a narrowly defined, categorical disorder, but instead as a spectrum of conditions that affect individuals differently (Wing, 1996). Some researchers have suggested that there are probably many "autisms" with different underlying biological processes and developmental pathways (Elsabbagh, 2012). The term ASD is now commonly used to describe a range of neurodevelopmental conditions that show considerable phenotypic heterogeneity at any one age and across development, and that are likely to differ in underlying etiology (Geschwind and Levitt, 2007). However, they all generally share a primary impairment in social relatedness and reciprocity, an "insistence on sameness," and impairments in the use of language for communication, which is in keeping with Kanner's (1943) description of classically "autistic" children.

It is noteworthy that genetic heterogeneity leads to clinical heterogeneity. For example, similar or identical mutations can result in very broad phenotypic variations as is evident from studies investigating endophenotypes exhibited by patients expressing mutations in the CNTNAP2 gene (Eapen, 2011). Such studies demonstrate a role for CNTNAP2 in schizophrenia, epilepsy, Tourette's syndrome, and obsessive compulsive disorder (Verkerk et al., 2003; Friedman et al., 2008). Alternatively, ASD cases resulting from different genetic lesions can have clinically distinct presentations (Bruining et al., 2010). However, such distinct phenotypic presentations are masked by the limitations of diagnostic categories. Therefore, future studies exploring risk alleles should examine homogenous and heritable endophenotypic traits rather than diagnostic groups. Thus, given the significant genotype to phenotype heterogeneity, one method to find homogeneous entities and link these with specific genotypes would be to pursue endophenotypes.

ENDOPHENOTYPES IN ASD

Neurocognitive profiles and neurophysiological changes observed using neuroimaging, eye tracking, and electrophysiological techniques are commonly reported in individuals with ASD. Studies of head circumference and imaging studies of brain morphometry have found evidence of increased brain growth beginning within the first year of life (Courchesne et al., 2005), while functional brain imaging in older children and adults has shown abnormal patterns of interactions between brain regions, possibly related to aberrant connections being laid down during earlier stages of development (Courchesne et al., 2011). One model relating these early abnormalities in brain development to the characteristic socio-communicative impairments has hypothesized that early low-level deficits in recognition and orientation toward social stimuli lead to a lack of social engagement with primary caregivers during infancy, resulting in decreased exposure to the reciprocal social interactions critical for healthy development of brain circuits responsible for normal social behavior (Dawson, 2008).

Basic, low-level impairments of social attention and reciprocity are thought to relate to the socio-communicative impairments characteristic of ASD and are evident in children with ASD from as early as the first year of life. For example, home videos of 12-month-olds later diagnosed with ASD demonstrate reduced visual attention to people and failure to respond to vocal approaches (Werner et al., 2000; Osterling et al., 2002; Werner and Dawson, 2005), while other studies have shown poor verbal imitation (Sallows and Graupner, 2005). Prospective studies of children at high risk of ASD show similar results (Nadig et al., 2007). Young children with ASD also show a lack of joint attention and failure to coordinate attention and share their experiences with caregivers (Charman, 2003). Researchers using preferential looking techniques have identified a reduction in autistic toddlers' preference for viewing biological motion (Klin et al., 2009) and hearing the human voice (e.g., Klin, 1991; Dawson et al., 1998, 2004).

Similarly, eye gaze abnormalities have been described as indicative of later development of ASD (Bedford et al., 2012; Elsabbagh et al., 2012). Using eye tracking technology, Jones et al. (2008) found that 2-year-olds with ASD lacked the normal bias to attend to the eyes when watching videos of people, replicating earlier studies with autistic adolescents (Klin et al., 2003; see also Norbury et al., 2009) and confirming clinical reports of reduced eye contact in ASD (Zwaigenbaum et al., 2005). Psychophysical evidence suggests that differences in spatial localization between individuals with ASD and controls begins at an early cortical stage of visual processing (Latham et al., 2013). Further evidence comes from electrophysiology. Pre-school and school-aged children with ASD produce atypical cortical event-related potentials (ERPs) in response to deviations in streams of speech stimuli, despite normal responses to deviants in streams of non-speech stimuli (Kuhl et al., 2005; Lepistö et al., 2005; Ceponiene et al., 2005; Whitehouse and Bishop, 2008). Kuhl et al. (2013) compared brain responses to word stimuli between typically developing children and children with ASD, categorized into two groups according to the severity of their social symptoms. They found that the brain activity of children with ASD with less severe social symptoms resembled that of the typically developing controls, while children with ASD with more severe social symptoms showed a clearly atypical brain response. Furthermore, the ERP response among children with ASD at time 1 (when they were 2 years old) was found to predict receptive language, cognitive ability, and adaptive behaviors at two follow-up time points, when the children were 4 and 6 years old (Kuhl et al., 2013). Similarly, school-aged children are reported to show abnormal brainstem evoked responses (ABR) to trains of speech stimuli but not click sounds (Russo et al., 2009), and these abnormalities are linked to clinical assessments of language abilities.

Recognition of facial emotions has also been found to be impaired in children and adults with ASD compared to controls (Sucksmith et al., 2012; Oerlemans et al., 2013). The reduced activation in brain regions associated with facial processing in people with ASD relative to control subjects has been shown to

be correlated with the clinical severity of their impairment in reciprocal social interaction (Spencer et al., 2012b). Finally, studies using electromyography (EMG) to measure facial muscle activity have shown a reduction or delay in the normal tendency to (subconsciously) mimic emotional expressions when viewing pictures of faces (McIntosh et al., 2006; Oberman et al., 2009).

Further, it is widely argued that many of the symptoms of ASD are caused by aberrant neural connectivity (e.g., Brock et al., 2002; Geschwind and Levitt, 2007), including specific findings such as reduced functional connectivity within and between "social" resting state networks in ASD (von dem Hagen et al., 2013) as well as significantly increased gray matter volume in the anterior temporal and dorsolateral prefrontal regions and significant reductions in the occipital and medial parietal regions compared with controls (Ecker et al., 2012). These findings imply that a key component of behavioral intervention may be to compensate for such early deficits and that behavioral intervention should occur as early as possible to normalize the developmental trajectory and avoid downstream effects. Thus baseline performance on neurocognitive responses to socially relevant stimuli might predict the magnitude of clinical and cognitive improvement following behavioral intervention. Recent research suggests that early behavioral intervention may be associated with normalized brain activity in young children with ASD. Previous studies have demonstrated that children receiving the Early Start Denver Model (ESDM), a behavioral intervention for children with ASD, showed gains in IQ and adaptive behavior and decreases in ASD-specific symptoms after intervention (Dawson et al., 2010; Eapen et al., 2013; Vivanti et al., 2013). In a recent study, Dawson et al. (2012) found that typically developing children and children with ASD who had been treated with the ESDM showed more cortical activation and allotted greater attentional and cognitive resources to social stimuli than to non-social stimuli, while children with ASD who had received community-based behavioral intervention showed the reverse pattern.

BEHAVIORAL AND COGNITIVE PHENOTYPES IN ASD

There are increasing efforts to determine and refine subtypes within the ASD behavioral phenotype (e.g., Ingram et al., 2008; Munson et al., 2008a,b; Frazier et al., 2010), with contemporary studies using large samples and sophisticated statistical approaches such as taxometric and latent variable models. To date, however, few distinct behavioral subtypes have been identified, and none is yet well replicated – frustrating efforts to "carve nature at the joints." Ingram et al. (2008) provided the first taxometric analysis of ASD and sought to test putative ASD subgrouping paradigms based on seven phenotypes which vary within the ASD population: social interaction/communication, intelligence, adaptive functioning, insistence on sameness, repetitive sensory motor actions, language acquisition, and essential/complex physical phenotype. The "complex" physical phenotype was defined according to the presence of physical dysmorphology and/or microcephaly, indicating some abnormality of early morphogenesis, whereas the "essential" physical phenotype referred to the remainder of individuals with ASD without these features (Miles et al., 2005). The authors indicated that valid subgroups could be constructed using the social interaction/communication, intelligence, and essential/complex paradigms, whereas the other phenotypes were found to exhibit results consistent with a dimensional structure. Given intelligence is consistently described as one of the primary aspects of heterogeneity in ASD, Munson et al. (2008a) sought to explore whether there were distinct ASD subtypes based upon IQ. Four latent classes were ultimately identified that represented different levels of intellectual functioning as well as different patterns of relative verbal versus non-verbal abilities. Moreover, group membership was related to adaptive functioning and social impairment, above and beyond the direct relationship of verbal and non-verbal IQ (Munson et al., 2008a). In a different study, Munson et al. (2008b) reported that specific aspects of neurocognitive functioning appear to be important predictors of developmental variability during the pre-school years in children with ASD. In particular, learning of reward associations and imitation from memory and novelty preference were significantly related to Vineland socialization and communication growth rates above and beyond non-verbal problem solving ability. A review of factor analytic studies showed that, of the seven studies included, six found evidence for multiple factors underlying autistic features (Mandy and Skuse, 2008). The majority of studies reported at least one social-communication factor and all but one also reported at least one distinct non-social factor comprising repetitive interests, behaviors and activities, however, the total number of factors reported varied.

In a large scale study employing taxometric and latent variable models, Frazier et al. (2010) concluded that the available literature and study results implied a categorical model of ASD, with two to three subdimensions – social communication, repetitive/perseverative behavior, and possibly social motivation – best reflecting the structure of ASD symptoms. Related work by the same group yielded similar results and provided broad support for DSM5 ASD criteria (Frazier et al., 2012). This finding is somewhat at odds with the related body of literature that has concluded that ASDs represent the severe end of a quantitative trait or continuum of social behavior, and the differing conclusions may reflect differing theoretical and statistical approaches. It is of course also possible that both viewpoints are correct and that categorical and dimensional aspects of ASD symptoms should be considered in the conceptualization of ASD.

There is increasing momentum within the literature to conceive of the core ASD symptomatology as distinct, or "fractionable." That is, that while the core features may regularly co-occur, these features may have distinct causes at genetic, cognitive, and neural levels. In their seminal review paper, Happe et al. (2006) argue that "*it is time to give up on the search for a monolithic cause or explanation for the three core aspects of autism*" (p. 1219). This claim was based in part on Ronald and colleagues' work on a large UK general population twin sample which found that correlations between continuous measures of social, communication and repetitive behavior were lower than expected (Happe et al., 2006). Happe et al. describe several implications following from their thesis, including that at the behavioral level each aspect of the ASD behavioral triad needs to be assessed separately rather than using global rating scales. The authors also claim that "*heterogeneity in ASD, on our account, is not simply due to noise or the complex unfolding of development, but is an unavoidable consequence of*

variation along at least three largely independent (although of course interacting) dimensions of impairment" (p. 1220).

A large number of studies have also explored the ASD cognitive phenotype and a number of cognitive models of ASD have been proposed over time. These include the theory of mind account (Baron-Cohen et al., 1985); the executive dysfunction account (Ozonoff et al., 1991); the weak central coherence account (Happe and Frith, 2006); the enhanced perceptual functioning account (Mottron et al., 2006); the theory of reduced generalization and enhanced discrimination ability (Plaisted, 2001); and the empathizing – systematizing theory (Baron-Cohen, 2010; Grove et al., 2013). Each of these cognitive characteristics has been successfully linked to specific aspects of the ASD behavioral phenotype (Taylor et al., 2012) although none would appear to provide a parsimonious account of features observed in ASD. Charman et al. (2011) provide an excellent review of studies in this area and also a compelling account of the potential benefits of articulating ASD cognitive phenotypes with respect to advancing both treatment and genetic research. Charman also highlights the challenges involved in conducting high quality research in this area from statistical and methodological perspectives.

VARIABILITY IN AND PREDICTORS OF RESPONSE TO BEHAVIORAL TREATMENT IN ASD

The heterogeneity of ASD may also underlie the variability in response to treatment that is observed among individuals with ASD. Meta-analyses conducted in recent years have tended to conclude that Early Intensive Behavioral Intervention (EIBI), incorporating the principles of applied behavior analysis (ABA), is the treatment of choice for young children with ASD (Vismara and Rogers, 2010; Reichow, 2012), and that superior outcomes are associated with entry into EIBI at the earliest possible age (Granpeesheh et al., 2009; Wallace and Rogers, 2010). Despite the efficacy of EIBI for some children, there is tremendous variation in treatment response in ASD, with other children who receive EIBI failing to have a dramatic response (Dawson et al., 2002). A systematic review of controlled studies of EIBI showed that, while EIBI resulted in improved outcomes for children with ASD compared to comparison groups at a group level, there was marked variability in outcome at an individual level (Howlin et al., 2009). This differential response to treatment is common across all of the evidence-based approaches for treatment of ASD, with up to 50% of children showing substantial positive gains, and the other 50% making variable progress, some with extremely limited skills development (Stahmer et al., 2011).

Therefore, research aimed at methods of individualizing treatment is important. Such research requires an understanding of the pre-treatment characteristics associated with differential response to treatment, including child and family variables, and how specific behavioral intervention techniques address each of these characteristics (Stahmer et al., 2011). The goal of this line of research is to allow treatments to be tailored to individual children and thereby increase the overall rate of positive outcomes for children with ASD (Stahmer et al., 2011). In a recent systematic review of EIBI for ASD, however, Warren et al. (2011) concluded that the ability to predict children's response to treatment and outcome was very limited and warranted further investigation. The genetic and phenotypic heterogeneity inherent in ASD may also imply that no single EIBI can be universally effective and that, in a sense, many nuanced treatment approaches may ultimately be required for the many autisms in existence.

Nonetheless, available evidence indicates that a number of pre-treatment factors may be associated with response to treatment across various EIBI models. These include overall IQ (McEachin et al., 1993; Harris and Handleman, 2000; Eldevik et al., 2006; Magiati et al., 2007; Remington et al., 2007; Perry et al., 2011), language and communication abilities (Sallows and Graupner, 2005; Eldevik et al., 2006; Eikeseth et al., 2007; Magiati et al., 2007; Remington et al., 2007), adaptive skills (Remington et al., 2007; Makrygianni and Reed, 2010; Flanagan et al., 2012), imitation (Sallows and Graupner, 2005; Vivanti et al., 2013), play skills (Kasari et al., 2008, 2012; Ingersoll, 2010a), joint attention (Yoder and Stone, 2006; Kasari et al., 2008), interest in objects (Yoder and Stone, 2006; Schreibman et al., 2009; Carter et al., 2011), functional use of objects (Vivanti et al., 2013), symptom severity (Smith et al., 2000; Sallows and Graupner, 2005; Remington et al., 2007; Vivanti et al., 2013), and younger age (Harris and Handleman, 2000; Perry et al., 2011). Some studies, however, have failed to find relationships between these factors and treatment response. For example, Eldevik et al. (2006) found that age at intake was not a predictor of children's response to a low-intensity behavioral treatment, while Sallows and Graupner (2005) found that initial IQ did not predict children's response to an intensive behavioral intervention. Furthermore, the direction of relationships between these pre-treatment factors and intervention response is sometimes inconsistent. For example, Remington et al. (2007) found that higher ASD symptom scores at intake were associated with improved EIBI outcomes, while Smith et al. (2000) found that children with milder symptoms (i.e., a diagnosis of Pervasive Developmental Disorder-NOS) tended to have a better response to EIBI than children with more severe symptoms (i.e., a diagnosis of ASD).

INCREASING TREATMENT EFFICACY FOR ASD BY IDENTIFYING INDIVIDUAL DIFFERENCES

Given the heterogeneity of ASD, it is likely that a personalized medicine approach, considering individual differences in etiologic and phenotypic characteristics, would result in increased treatment efficacy (Perrin et al., 2012). Georgiades et al. (2013) suggest that, rather than conducting studies that compare individuals with a diagnosis of ASD with typically developing individuals, future research should focus on understanding the meaning of individual and subgroup differences within the autism spectrum on treatment outcomes. The identification of such differences, and an understanding of how these might impact on response to treatment, has implications for the ability to individually tailor treatment programs and thereby improve their effectiveness.

As an example, this type of approach has been found to be useful in explaining differences in presentation and treatment response in children with diagnoses of oppositional defiant disorder and conduct disorder. There is increasing support for the subtyping of childhood conduct problems based on whether children exhibit high versus low levels of callous-unemotional (CU) traits, such as a lack of guilt and empathy (Hawes et al., 2013). Research suggests

that the conduct problems of children with high levels of CU traits are more severe and less responsive to established psychological interventions than those of children without CU traits (Hawes and Dadds, 2005; Waschbusch et al., 2007). This has allowed research into interventions that may contribute to reductions in the CU traits of young children (Hawes and Dadds, 2007; McDonald et al., 2011). Furthermore, articulating these phenotypic differences has contributed to a better understanding of the etiology of conduct disorder (e.g., Viding et al., 2007).

One method to potentially identify subgroups among children with ASD would be to investigate phenotypic characteristics that may predict response to treatment, which has important implications for guiding choice of treatment. Furthermore, using a longitudinal design, it would be beneficial to compare the developmental trajectory of young children with ASD receiving a standardized treatment with those in "waitlist" conditions as well as with healthy control groups. This would provide a significant contribution to the sparse body of knowledge about developmental changes in brain function during this period of development. Even more importantly, determining which characteristics correspond with observable changes in the treatment group would allow us to identify specific individual characteristics and relevant biomarkers sensitive to behavioral intervention, with implications for assessing response to intervention in clinical and research settings (see **Figure 2**). This would also reveal whether and which variations in the baseline measures of brain function predict response to treatment. Given the significant investment represented by EIBI programs, their overall utility could be greatly enhanced by determining whether there are measurable characteristics at baseline capable of predicting response. Finally, if such phenotypic

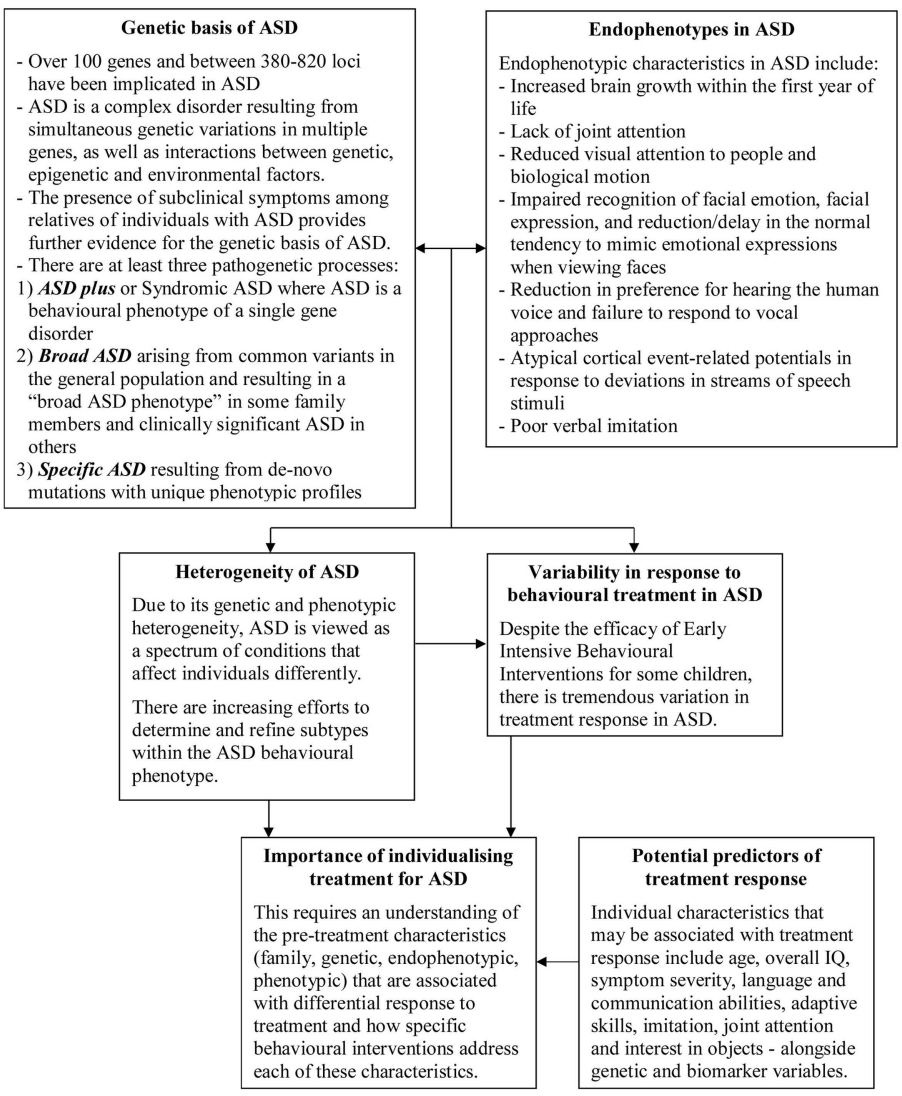

FIGURE 2 | Summary of key points and links between genotypes, endophenotypes, and clinical predictors of response to behavioral intervention in ASD.

predictors were established, this could further our understanding of the genetic basis of ASD, by allowing future research to attempt to link these predictors to specific underlying genetic causes. The task ahead is certainly great, but also tantalizing with respect to the potential refinements in our understanding and also benefits to affected individuals that are possible.

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Genome-wide association study of autistic-like traits in a general population study of young adults

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Lay abstract: It has been proposed that autistic-like traits in the general population lie on a continuum, with clinical Autism Spectrum Disorder (ASD), representing the extreme end of this distribution. The current study undertook a genome-wide association (GWA) scan of 965 young Western Australian adults to identify novel risk variants associated with autistic-like traits. No associations reached genome-wide significance; however, a review of nominally associated single nucleotide polymorphisms (SNPs) indicated two positional candidate loci that have been previously implicated in autistic-like trait etiology.

Scientific abstract: Research has proposed that autistic-like traits in the general population lie on a continuum, with clinical ASD representing the extreme end of this distribution. Inherent in this proposal is that biological mechanisms associated with clinical ASD may also underpin variation in autistic-like traits within the general population. A GWA study using 2,462,046 SNPs was undertaken for ASD in 965 individuals from the Western Australian Pregnancy Cohort (Raine) Study. No SNP associations reached genome-wide significance ($p < 5.0 \times 10^{-8}$). However, investigations into nominal observed SNP associations ($p < 1.0 \times 10^{-5}$) add support to two positional candidate genes previously implicated in ASD etiology, PRKCB1, and CBLN1. The rs198198 SNP ($p = 9.587 \times 10^{-6}$), is located within an intron of the protein kinase C, beta 1 (PRKCB1) gene on chromosome 16p11. The PRKCB1 gene has been previously reported in linkage and association studies for ASD, and its mRNA expression has been shown to be significantly down regulated in ASD cases compared with controls. The rs16946931 SNP ($p = 1.78 \times 10^{-6}$) is located in a region flanking the Cerebellin 1 (CBLN1) gene on chromosome 16q12.1. The CBLN1 gene is involved with synaptogenesis and is part of a gene family previously implicated in ASD. This GWA study is only the second to examine SNPs associated with autistic-like traits in the general population, and provides evidence to support roles for the PRKCB1 and CBLN1 genes in risk of clinical ASD.

Keywords: autistic-like traits, genome-wide association, PRKCB1, autism spectrum disorder, autism spectrum quotient, CBLN1

INTRODUCTION

Autism Spectrum Disorders (ASD) represent a group of neurodevelopmental conditions characterized by impairments in social interaction and communication, and repetitive interests and behaviors. A recent review estimated the median global prevalence of ASD at 62 cases per 10,000 children (Elsabbagh et al., 2012). The overall heritability of ASD is estimated at 90%, which is amongst the highest for any neuropsychiatric disorder (Hollander et al., 2003; Skuse et al., 2005). Evidence suggests that ASD have a complex inheritance where different, likely overlapping, groups of genetic variants may cause susceptibility to disease (Veenstra-VanderWeele et al., 2004). Increasing numbers of epidemiological and genetic studies have deepened the understanding of genetic contribution to ASD, and show that a variety of genetic mechanisms may be involved in the etiology (Li et al., 2012).

Autistic-like traits are sub-threshold deficits in socialization, communication, and restricted interests that do not meet formal criteria for ASD (Constantino and Todd, 2003). Several authors have suggested that ASD can be conceptualized as the conditions arising in individuals found at the extreme end of a normal distribution of autistic-like traits (Gillberg, 1992; Constantino and Todd, 2003; Ronald et al., 2005). Population-based studies have supported this by finding that, in addition to individuals with ASD, many others exhibit sub-threshold autistic or autistic-like traits (Constantino and Todd, 2003; Posserud et al., 2006; Lundström et al., 2012). Sub-threshold autistic-like traits have been examined in general population twin studies, with heritability estimates ranging from 36 to 87% in these studies (Ronald and Hoekstra, 2011).

With the discovery of common genetic variants associated with ASD, the question is raised whether common risk loci also contribute to variation in phenotypes such as autistic-like traits (St Pourcain et al., 2010). One methodological approach that is gaining influence in ASD research is the examination of the quantitative distribution of autistic-like traits in the broader community, rather than a dichotomy of ASD cases and controls. Many authors have postulated that common genetic variants that are present in a significant proportion of the general population may play a role in the etiology of ASD (Campbell et al., 2006; Alarcón et al., 2008; Chakrabarti et al., 2009; Wang et al., 2009; Anney et al., 2010; Ronald et al., 2010). Further to this, it is thought that understanding the etiology of individual differences in autistic-like traits in the general population will help in understanding the causes of ASD (Ronald and Hoekstra, 2011).

The aim of the current study was to use genome-wide association (GWA) methods to search for novel risk variants associated with autistic-like traits in a Western Australian population sample.

MATERIALS AND METHODS

ETHICS STATEMENT

Participant recruitment of the study families were approved by the Human Ethics Committee at King Edward Memorial Hospital. The 20 year follow-up ethics approval was received from the Human Research Ethics Committee at the University of Western Australia. Participants provided written informed consent for data collection of autistic-like trait outcomes at approximately 20 years of age.

GWA STUDY SAMPLE POPULATION

Data were obtained from the Western Australian Pregnancy Cohort (Raine) Study based at the Telethon Institute for Child Health Research. The Raine Study is a longitudinal investigation of pregnant women and their offspring, who were recruited from King Edward Memorial Hospital, Perth, Western Australia or nearby private practices between 1989 and 1991 (Newnham et al., 1993). From the 2,900 pregnancies recruited into the Raine Study, 2,868 live-born children have been followed since the commencement of the study. The final sample consisted of 965 individuals from the Raine study with both genotype and outcome measures.

MEASURE OF AUTISTIC-LIKE TRAITS

At the 20 year follow-up, Raine Study participants who did not have a diagnosis of intellectual disability or ASD were asked to complete the Autism Spectrum Quotient (AQ) (Baron-Cohen et al., 2001). The AQ is a self-report questionnaire that provides a quantitative measure of autistic-like traits in the general population (Baron-Cohen et al., 2001). Individuals are provided with 50 statements and asked to indicate on a 4-point scale how well that statement applies to them (strongly agree, agree, disagree, strongly disagree). The items were scored on a scale ranging from 1 to 4, based on previous research that this scoring method retains more information about responses than the dichotomous scoring first proposed for this instrument (Baron-Cohen et al., 2001; Austin, 2005; Stewart and Austin, 2009; Russell-Smith et al., 2011). Scores on each item are summed to provide a Total AQ, with higher scores indicating greater autistic-like traits. The Total AQ is known to have good test-retest reliability ($r = 0.7$), and validation studies have found that scores in the general population follow a normal quantitative distribution (Baron-Cohen et al., 2001; Whitehouse et al., 2011). Factor analyses of the AQ in several countries have consistently identified three clear factors, relating to social ability, attention to detail/patterns, and the understanding of others. In the current study, we divided items into the subscales identified in a study of Western Australian adults (Russell-Smith et al., 2011), who were highly similar to the sample under investigation here: Social Skills, Details/Patterns, and Communication/Mindreading. There is minimal difference between the items pertaining to these subscales, and those reported in other factor analyses. For the current data set, internal reliability for the scales ranged from moderate (Communication/Mindreading: $\alpha = 0.63$) to good (Details/Patterns: $\alpha = 0.78$) and excellent (Social Skills: $\alpha = 0.85$). In this study, Total AQ scores and the scores of the three subscales were used as four outcome measures.

GWA STUDY GENOTYPING

In the Raine Study, DNA was collected using standardized procedures from 74% of adolescents who attended the 14 year follow-up, and a further 5% at the 16 year follow-up. Of the 1,593 offspring for whom genome-wide data were available, 99 individuals were excluded for the following reasons: 16 had low genotyping success (>3% missing); 3 had excessive heterozygosity, 68 were in high identical-by-descent with another Raine Study participant (related), 7 whose sex was ambiguous, and 5 individuals had mislabeled samples. There were 1,494 individuals whose DNA sample passed quality control criteria and were eligible for the genetic analyses (768 males, 726 females), see **Figure 1**.

Genome-wide data were generated using an Illumina Human 660W Quad array at the Center for Applied Genomics (Toronto,

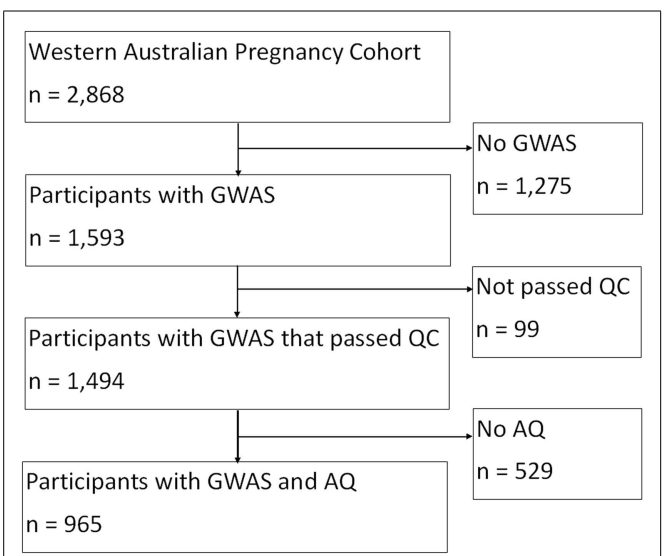

FIGURE 1 | Flow chart of study sample selection.

ON, Canada). The 660W Quad Array includes 657,366 genetic variants including ∼560,000 single nucleotide polymorphisms (SNPs) and ∼95,000 copy number variants. SNPs were excluded based on the Wellcome Trust Case Control Consortium thresholds:

HWE p-value must be $>5.7 \times 10^{-7}$ (919 markers excluded)
Call rate must be >0.95 (95%) (97,718 markers excluded)
MAF must be >0.01 (1%) (119,246 markers excluded – includes copy number variants)
A/T and G/C SNPs were removed due to possible strand ambiguity.

In total, 535,632 SNPs passed genotype quality control before genotype imputation. Imputation was performed using MACH (Li and Abecasis, 2006) software on the 22 autosomes. Once the data were cleaned, a two-step process was carried out using the CEU samples from HapMap phase2 build 36 release 22 as a reference panel. This imputation process imputed both individual genotypes for SNPs that were typed but not called for a particular individual, and SNPs that were not typed in all individuals. After imputation, genotype information was available for 2,543,887 SNPs.

DATA ANALYSIS

Power calculations

Power calculations were performed using the statistical software package Quanto (Gauderman, 2002) for a sample size of 965 and MAF ranging from 0 to 0.5. The means and standard deviations for Total AQ and each subscale were used for the power calculations. Power was calculated to detect a minimum change of one unit assuming an additive genetic model. An alpha level of 1.0×10^{-8} was used for the GWA studies.

Methods for genome-wide association

Allelic dosage scores generated from MACH were used in analyses. The GWA studies were run adjusting for both age and sex. To account for potential population substructure, first and second principal components were also included in the multivariate GWA model. According to the Eigenvectors the first principal component accounted for 6.5% of variation, and the second component accounted for 2.3% of variation. Analysis was run using the ProbABEL package (Aulchenko et al., 2010). The combined results and SNP details file were then filtered to remove SNPs with MAF <1% and an imputation quality value of <0.3 (suggesting poor imputation quality).

A quantile–quantile (Q–Q) plot depicting –log10 transformations of the observed p-values as a function of the expected p-values was generated using "*qqunif*" function of the R Genetic Analysis Package (GAP) (R Development Core Team, 2008). The genomic inflation factor, λ, was calculated from the observed p-values. A Manhattan plot displaying a –log10 transformation of the observed p-values was generated using "*mhtplot*" function of the R GAP (R Development Core Team, 2008).

Genome-wide significance of results was defined as a p-value $\leq 5.0 \times 10^{-8}$ (Panagiotou and Ioannidis, 2011). Tables of SNPs were generated, based on a p-value $< 1.0 \times 10^{-5}$, which has been used by the National Human Genome Research Institute for the identification and archiving of suggestive associations (National Human Genome Research Institute, 2012). Information regarding genes and function for SNPs included in these tables were sourced from SNP Annotation and Proxy Search (SNAP, 2008). Genes and/or regions that appeared in the generated tables were searched using the Simon's Foundation Autism Research Initiative (SFARI) database (Simons Foundation Autism Research Initiative, 2012) for any previous associations with ASD and biological plausibility. LocusZoom (Pruim et al., 2010) was used to generate a regional association plot for loci of interest (±400 kb) based on hg18 genome build and HapMap Phase II CEU133 as the linkage disequilibrium (LD) population.

RESULTS

POWER CALCULATIONS

Total AQ, Social Skills, and Details/Patterns did not reach sufficient power (>80%) to detect a change by one unit. Communication/Mindreading reached sufficient power at 47% MAF.

NORMAL DISTRIBUTION OF SCORES

Autism Spectrum Quotient scores have been described in previous literature to provide a quantitative measurement of autistic-like traits in the general population (Baron-Cohen et al., 2001). Histograms and Q–Q plots indicate that the scores of the total AQ and each subscale were normally distributed. The histograms and Q–Q plots can be found in Figures S1–S4 in Supplementary Material.

DESCRIPTIVE STATISTICS

Characteristics of the current study sample can be found in **Table 1**. About 51.3% of the sample were female and the mean age at AQ completion was 19.68 (SD = 0.70). Mean total AQ score was 103.2 (SD = 12.60).

GENOME-WIDE ASSOCIATION RESULTS

After filtering for effect allele frequencies ≤1 and ≥99% and imputation quality R squared values <0.3, 2,462,046 successfully genotyped or imputed SNPs remained for analysis. No SNP associations in any outcome reached genome-wide significance, defined as p-value $< 5.0 \times 10^{-8}$ (Panagiotou and Ioannidis, 2011). Putative associations, defined as $p < 1.0 \times 10^{-5}$, were examined with respect to candidate genes in the SFARI Gene database. None of the genes that were putatively associated with Total AQ, Details/Patterns and Communication/Mindreading were found to be represented in this gene database. One putative gene association found in the Social Skills GWA results was found to be reported in the SFARI Gene database, prompting further investigation.

Social skills genome-wide association results

In the set of 965 Raine Study participants for Social Skills scores, the observed distribution of p-values for the SNPs exhibited minimal deviation from the expected distribution (see **Figure 2**) indicating minimal test statistic bias or underlying population structure (λ = 0.994). The Manhattan plot –log10 transformation of observed p-values across the genome are displayed in **Figure 3**.

Table 2 displays association results for Social Skills, selected on a p-value below 1.0×10^{-5}. None of these associations reached genome-wide significance. However, the rs198198

Table 1 | Characteristics of Raine study participants with AQ and GWA data available.

Continuous	***n***	**Mean (SD)**
Maternal age at conception	965	28.66 (5.65)
Paternal age at conception	646	31.68 (6.18)
Maternal BMI at conception	956	22.25 (4.00)
Paternal BMI at conception	827	24.44 (3.28)
Age at AQ completion	965	19.68 (0.70)
Total AQ score	965	103.2 (12.60)
Categorical	***n***	***n* (%)**
Season of birth	965	
Summer		301 (31.2)
Autumn		222 (23.0)
Winter		196 (20.3)
Spring		246 (25.5)
Sex	965	
Male		470 (48.7)
Female		495 (51.3)
Gestational age	955	
<32 weeks		13 (1.4)
32–37 weeks		157 (16.4)
38–40 weeks		624 (65.3)
>40 weeks		161 (16.9)
Family income	937	
Income < $24 k		293 (31.3)
Income > $24 k		644 (68.7)
Maternal education	956	
Secondary school not completed		501 (52.4)
Secondary school completed		455 (47.6)

SNP (p-value $= 9.587 \times 10^{-6}$, effect size $= -1.411$) is located within the *protein kinase C, beta 1* (*PRKCB1*) gene that has been previously reported to be associated with ASD. Exploratory analysis of data revealed a nominal association of rs198198 with Total AQ score (p-value $= 0.002$), but no association with Details/Patterns (p-value $= 0.434$) or Communication/Mindreading (p-value $= 0.202$). In addition, the rs16946931 SNP ($p = 1.78 \times 10^{-6}$, effect size $= 1.681$) is located in a region flanking the *Cerebellin 1* (*CBLN1*), a gene previously implicated in ASD. This SNP was nominally associated with the Total AQ score (p-value $= 4.27 \times 10^{-4}$), but no association with Details/Patterns (p-value $= 0.472$), or Communication/Mindreading (p-value $= 0.138$). When association analysis was done conditional on rs16946931 for Social Skills, p-values for the 20 additional *CBLN1* SNPs became insignificant (p-value > 0.05), demonstrating that high LD between these SNPs was responsible for their inclusion in the model.

Figures 4 and **5** show regional association plots of SNPs rs198198 and rs16946931, respectively.

BIOINFORMATIC EVALUATION

All SNPs with p-value below 1.0×10^{-5} were evaluated for possible functional potential. As all SNPs were in non-coding regions they were assessed for regulatory potential using available ENCODE data through Regulome DB (Boyle et al., 2012) and the UCSC Genome (2012) browser. No SNP was shown to be part of a transcription factor binding site or be located in a region of the genome likely to have regulatory potential except the nominally associated SNP rs198198 located within an intron of *PRKCB1*. The major allele (T) was found to be embedded within a near-consensus CCAAT/enhancer binding protein (C/EBP) gamma binding site whereas the minor allele (A) is predicted to ablate C/EBP gamma binding. Further evidence for function comes from ENCODE ChIP-seq data indicating that rs198198 is located in a region subject to histone H3K9 modification.

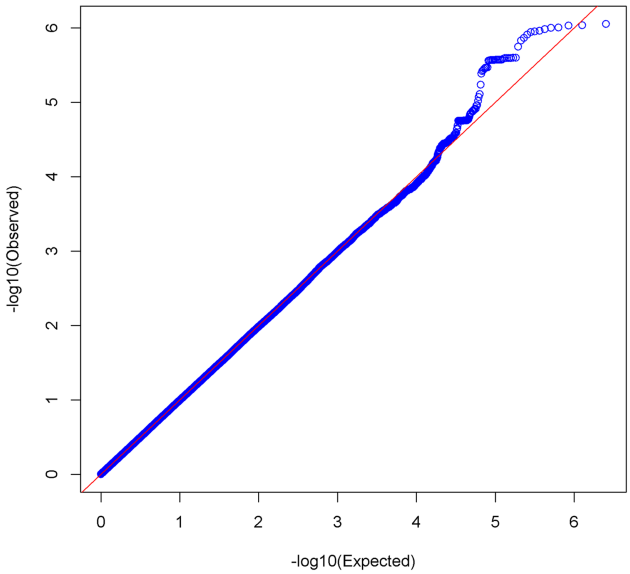

FIGURE 2 | Quantile–Quantile Plot of association results for social skills. Q–Q plots compare the observed p-values [−log10(Observed)] to expected p-values. [−log10(Expected)] on the logarithmic scale under the null hypothesis of no significant association.

DISCUSSION

In this paper we report only the second contemporary GWAS for autistic-like traits in the broader community and the first to use a quantitative distribution measured by the AQ. Although we did not find any SNP associations reaching genome-wide significance (defined as p-value $< 5.0 \times 10^{-8}$) this GWAS has provided some evidence to support the further investigation of two previously identified candidate genes, namely *PRKCB1* and *CBLN1*.

PRKCB1 GENE

Protein kinase C enzymes play an important role in signal transduction, regulation of gene expression, and control of cell division and differentiation (Lintas et al., 2009; NCBI, 2012). The alternative splicing of *PRKCB1* generates two mRNA isoforms named PRKCB1-1 and PRKCB1-2, coding for two isoenzymes βI and βII. These mRNA isoforms are expressed in a variety of tissues within the central nervous system, including the hippocampus, striatum,

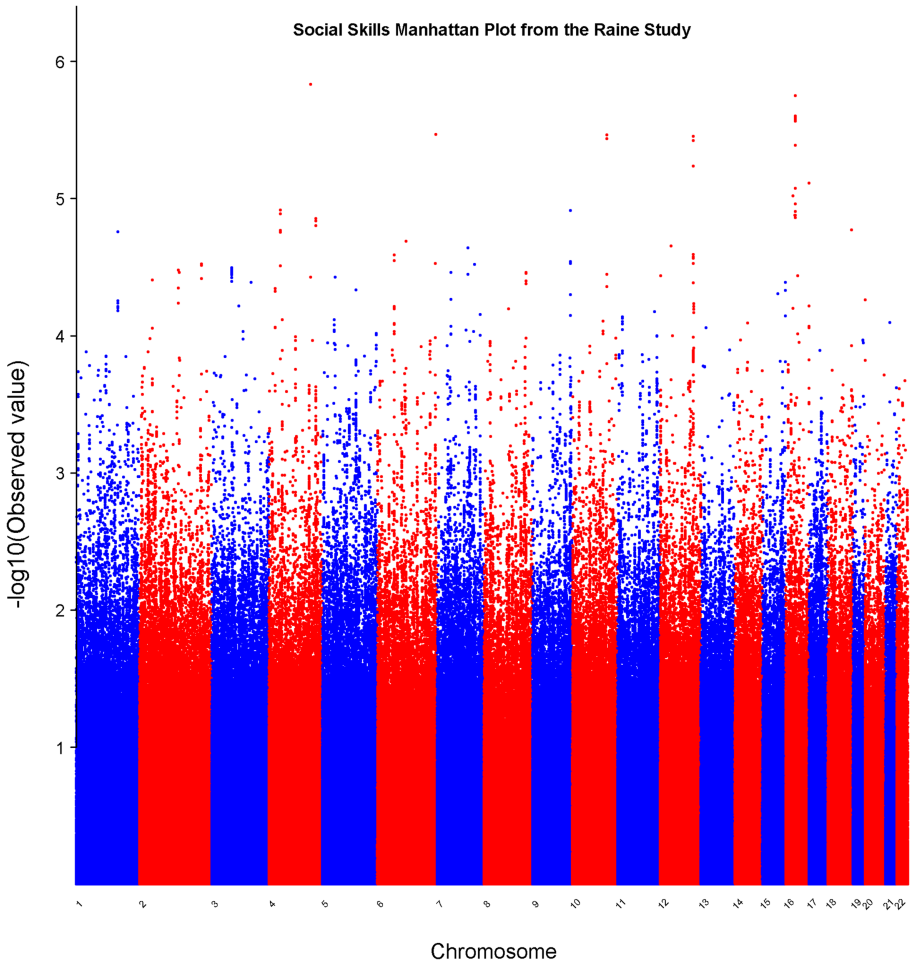

FIGURE 3 | Manhattan plot for social skills. Statistical significance of each SNP on the −log10 scale as a function of the chromosome position.

suprachiasmatic nucleus, and cerebellar granule cells (Lintas et al., 2009).

Several previous genetic studies have supported a role for *PRKCB1* in ASD. A genome-wide linkage scan of 116 families from the Autism Genetic Resource Exchange cohort (Philippi et al., 2005) applied direct physical identity-by-descent mapping using a strict ASD phenotype. Their results demonstrated linkage to a region on chromosome 16p (p-value = 0.0027). Subsequent high-resolution SNP genotyping and analysis of this region showed that haplotypes in the *PRKCB1* gene were strongly associated with ASD (Philippi et al., 2005), a finding that was independently confirmed in two other family studies (Philippi et al., 2005) (Lintas et al., 2009) but not in a study on 171 Irish ASD trios (Yang et al., 2007). These haplotypes were not significantly associated with ASD in the current study (results not shown).

These genetic association studies have been supported by a study of PRKCB1 gene expression in post mortem brain tissue from 11 autistic patients and controls (Lintas et al., 2009). The superior temporal gyrus (BA 41/42) region was chosen for study because of a known role in processing socially relevant information and well documented structural and functional abnormalities seen in ASD (Zilbovicius et al., 2006). A significant down regulation of PRKCB1 gene expression was found to be associated with ASD (Lintas et al., 2009) providing additional strong evidence in support of a role for the *PRKCB1* gene in the etiology of ASD.

CBLN1 GENE

The *CBLN1* gene encodes the cerebellum-specific precursor protein, precerebellin and is involved along with *Cerebellin 2* (*CBLN2*) in encoding ligands for the neurexin-neuroligin trans-synaptic complex (NTSC) (Clarke et al., 2012; Cristino et al., 2013). *CBLN1* belongs to the CBLN subfamily of the C1q/tumor necrosis factor superfamily, which plays a role in intercellular signaling, neuronal cell adhesion, brain development, and formation of synapses (Clarke et al., 2012). In a mouse study, Uemura et al. (2010) found that post-synaptic GluRδ2/GRID2 (a member of the δ-type glutamate receptor) interacts with pre-synaptic neurexins through *CBLN1* to mediate parallel fiber-Purkinje cell synaptic formation in the cerebellum. A recent study has identified rare copy number variants in NTSC gene family members and proposed

Table 2 | Association results for social skills (p-value $< 1.0 \times 10^{-5}$).

Chr	SNP	Position	Imputation	Function	Gene	Alleles	R^2	Effect size	SE	p-Value
4	rs11947645	151321122	imp	Intronic	*DCLK2*	A/G	0.859	5.329	1.100	1.47E-06
16	rs16946931	47700007	imp	Intergenic	*N4BP1/CBLN1*	C/T	0.973	1.681	0.350	1.78E-06
16	rs7499215	47680431	gen	Intergenic	*N4BP1/CBLN1*	G/T	1	−1.639	0.346	2.51E-06
16	rs11860027	47679816	imp	Intergenic	*N4BP1/CBLN1*	C/T	1	−1.639	0.346	2.52E-06
16	rs16946881	47677288	imp	Intergenic	*N4BP1/CBLN1*	C/T	0.999	−1.639	0.346	2.53E-06
16	rs16946880	47676950	imp	Intergenic	*N4BP1/CBLN1*	C/T	0.999	−1.639	0.346	2.53E-06
16	rs16946876	47675600	imp	Intergenic	*N4BP1/CBLN1*	C/T	0.998	1.638	0.346	2.54E-06
16	rs9635530	47674912	imp	Intergenic	*N4BP1/CBLN1*	A/C	0.998	−1.638	0.346	2.54E-06
16	rs2883805	47693566	imp	Intergenic	*N4BP1/CBLN1*	G/T	0.995	−1.640	0.347	2.59E-06
16	rs4785161	47686589	imp	Intergenic	*N4BP1/CBLN1*	A/C	0.997	1.636	0.346	2.66E-06
16	rs753858	47685051	imp	Intergenic	*N4BP1/CBLN1*	C/T	0.998	1.635	0.346	2.66E-06
16	rs11859884	47670664	imp	Intergenic	*N4BP1/CBLN1*	A/G	0.995	−1.633	0.346	2.67E-06
16	rs1861572	47684489	imp	Intergenic	*N4BP1/CBLN1*	A/T	0.999	1.635	0.346	2.67E-06
16	rs1009302	47670836	imp	Intergenic	*N4BP1/CBLN1*	C/T	0.995	−1.633	0.346	2.68E-06
16	rs1009301	47670978	imp	Intergenic	*N4BP1/CBLN1*	G/T	0.995	1.633	0.346	2.68E-06
16	rs1345404	47681670	imp	Intergenic	*N4BP1/CBLN1*	A/G	1	−1.633	0.346	2.69E-06
16	rs1345406	47681539	gen	Intergenic	*N4BP1/CBLN1*	A/C	1	−1.633	0.346	2.69E-06
16	rs1345405	47681629	gen	Intergenic	*N4BP1/CBLN1*	A/G	1	−1.633	0.346	2.69E-06
16	rs1420612	47672854	imp	Intergenic	*N4BP1/CBLN1*	G/T	0.996	−1.631	0.346	2.72E-06
16	rs10521175	47673463	imp	Intergenic	*N4BP1/CBLN1*	A/C	0.997	1.629	0.345	2.75E-06
6	rs11575088	167477024	imp	Intergenic	*CCR6/GPR31*	A/C	0.998	1.620	0.347	3.40E-06
6	rs11575089	167477189	gen	Intergenic	*CCR6/GPR31*	A/G	0.999	1.620	0.347	3.41E-06
10	rs927821	104197789	gen	Intergenic	*MIR146B/LOC100505761*	A/C	0.985	1.7024	0.365	3.44E-06
12	rs10444533	105753835	gen	Intronic	*RIC8B*	C/T	0.996	1.458	0.313	3.54E-06
10	rs7086205	104192719	gen	Intergenic	*MIR146B/LOC100505761*	C/T	0.998	−1.688	0.362	3.67E-06
12	rs10778511	105749461	imp	Intronic	*RIC8B*	A/T	0.993	1.454	0.313	3.80E-06
16	rs1362594	47682845	imp	Intergenic	*N4BP1/CBLN1*	C/G	0.943	−1.648	0.356	4.09E-06
12	rs4964491	105746645	imp	Intronic	*RIC8B*	A/G	0.985	−1.429	0.314	5.79E-06
16	rs533581	87494938	imp	Intronic	*CBFA2T3*	C/T	0.859	1.363	0.303	7.75E-06
16	rs4785276	47678880	imp	Intergenic	*N4BP1/CBLN1*	A/G	0.974	−1.529	0.341	8.46E-06
16	rs198198	24035898	imp	Intronic	*PRKCB1*	A/T	0.777	−1.411	0.317	9.59E-06

Position: base pair. Imputation: imputed (imp) or genotyped (gen). Gene: for intergenic SNPs, 5′/3′ flanking genes listed. Alleles: effect allele/non-effect allele. R^2: imputation quality from MACH. Effect size: regression coefficient. SE, Standard Error.

their potential biological function with ASD and Tourette syndrome (TS) (Clarke et al., 2012) and mutations in these and other synaptic genes are common in ASD (Arons et al., 2012; Clarke et al., 2012). Therefore, it is plausible that long-range genetic variants affecting *CBLN1* expression may be associated with ASD traits.

While no previous genetic linkage or association studies have detected significant associations between *CBLN1* and ASD, *CBLN2* (belonging to the same CBLN subfamily) on Chromosome 18q has been deleted in TS and is located near two TS translocation breakpoints (Clarke et al., 2012). There is evidence to suggest an overlap between TS and ASD from epidemiological perspectives, with an increased prevalence of TS among autistic patients of 6.5% (Baron-Cohen et al., 1999), compared to between 2 and 3% in the general population (Mason et al., 1998). Common susceptibility genes (Sundaram et al., 2010; Fernandez et al., 2012) have also been reported between ASD and TS.

This study was conducted on a well characterized, population-based cohort of adolescents and ASD measurements were collected without ascertainment for autism risk. Despite this study's small sample size ($n = 965$), we were independently able to identify two regions on chromosome 16 that have been previously implicated in the etiology of ASD. While no variants in these regions reached genome-wide significance, they do demonstrate suggestive evidence for association and provide good candidates for future follow-up studies. The difference in observed signal between the two regions is due to high LD around the *CBLN1* SNP, rs16946931, which disappears with conditional association analysis. The lack of replication for the previously identified *PRKCB1* haplotype is potentially due to different underlying LD structure between populations, as the previous study focused on families (Philippi et al., 2005) whereas our study is composed of unrelated individuals. These observed associations are either intergenic or intronic variants suggesting that they are tagging nearby rare variants that may be causal. In most GWAS, the SNPs showing

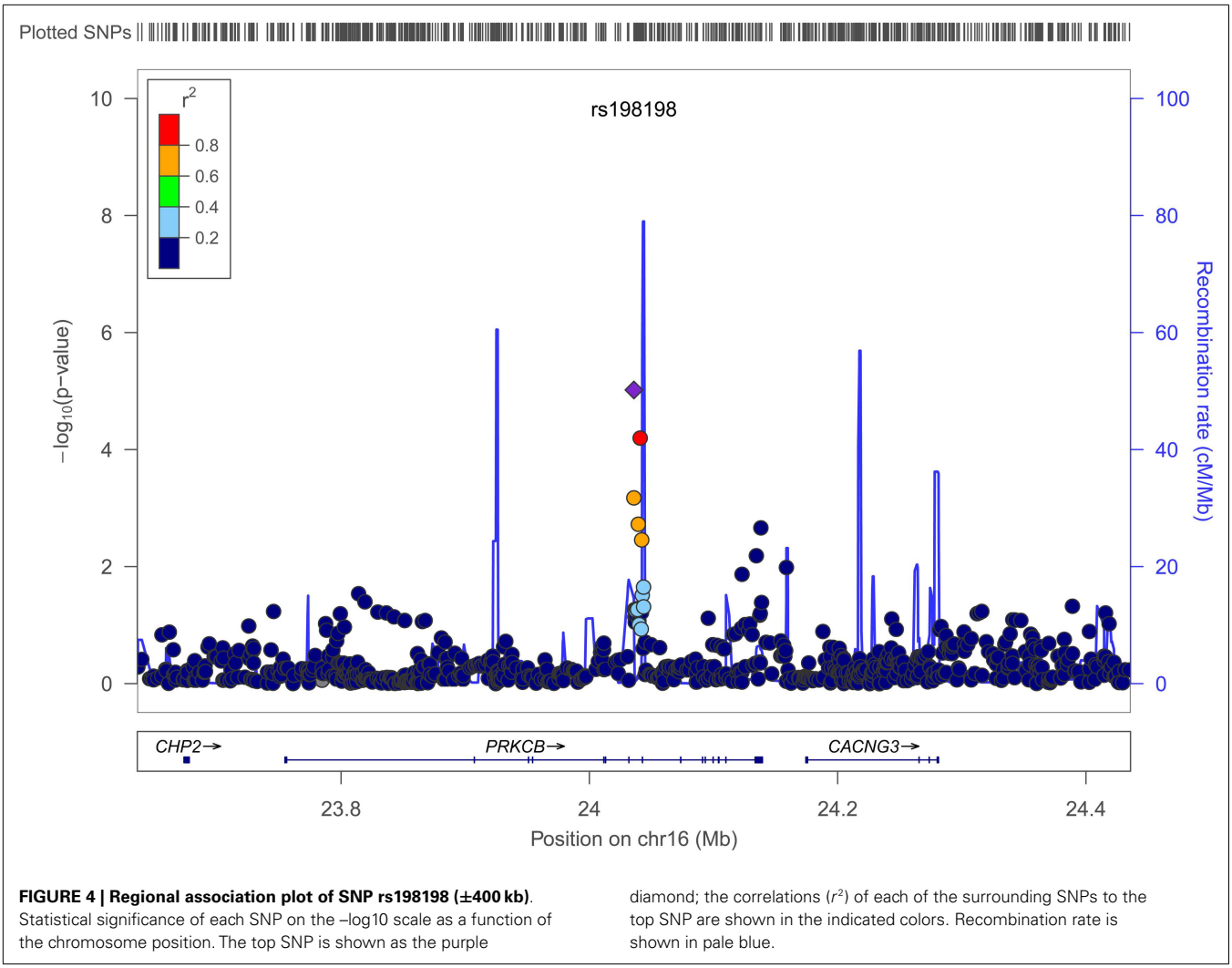

FIGURE 4 | Regional association plot of SNP rs198198 (±400 kb). Statistical significance of each SNP on the –log10 scale as a function of the chromosome position. The top SNP is shown as the purple diamond; the correlations (r^2) of each of the surrounding SNPs to the top SNP are shown in the indicated colors. Recombination rate is shown in pale blue.

evidence of association are not usually the actual causal variants at play but rather are in LD with such variants. The failure to replicate the previously reported haplotype associations with ASD and PRKCB1 and any specific SNP associations in both PRKCB1 and CBLN1 does not invalidate the findings in this study. Over the last 5 years, considerable attention has been paid to the potential reasons why true associations may not replicate across independent data sets and genetic heterogeneity, environmental interactions, age-dependent effects, epistasis, and inadequate statistical power have all been cited as possible explanations (Chanock et al., 2007; Shriner et al., 2007; Williams et al., 2007; Greene et al., 2009).

The identification and characterization of the causal variants at play in common complex human traits/diseases is presenting to be a great challenge in human genetics, in particular as a large proportion are not in coding regions directly altering protein structure/function but rather are in non-coding regions (i.e., intronic and intergenic regions) having a likely regulatory function, such as may be the case for the *PRKCB1* intronic rs198198 SNP identified in this study. The further characterization of this and other genetic variation within the *PRKCB1* and *CBLN1* gene regions is beyond the scope of this study possibly requiring exhaustive DNA sequencing to identify all variants within the regions of association followed by a variety of detailed molecular biological analyses, like those we have recently described (Karimi et al., 2009; Kaskow et al., 2013).

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/Human_Neuroscience/10.3389/fnhum.2013.00658/abstract

Figure S1 | Histogram and Quantile–Quantile Plot of Total AQ Scores. Histograms depict the frequency of the Total AQ observations. Q–Q plots compare the quantiles from the current study (sample quantiles) to the quantiles from a Normal distribution (theoretical quantiles).

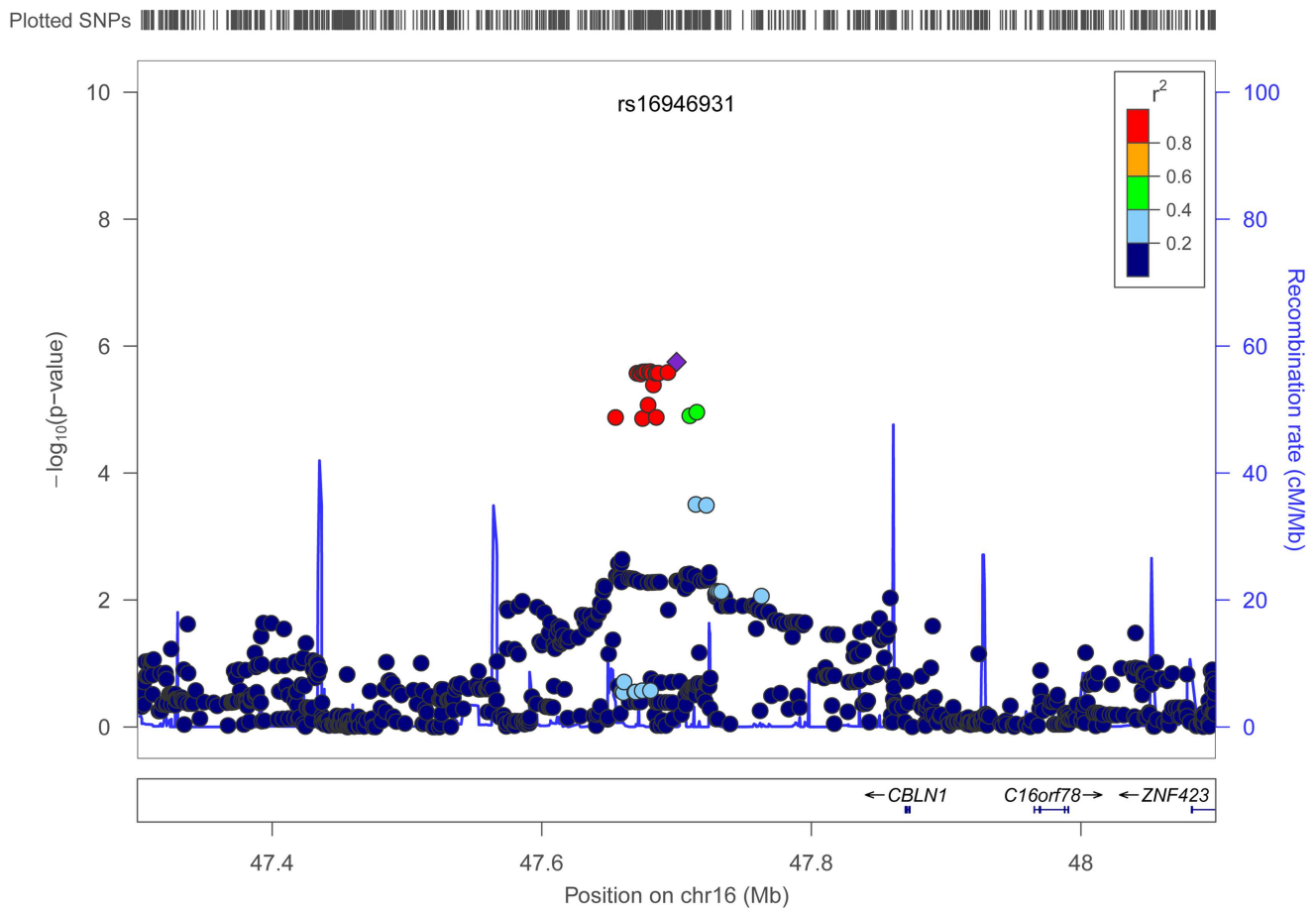

FIGURE 5 | Regional association plot of SNP rs16946931 (±400 kb). Statistical significance of each SNP on the –log10 scale as a function of the chromosome position. The top SNP is shown as the purple diamond; the correlations (r^2) of each of the surrounding SNPs to the top SNP are shown in the indicated colors. Recombination rate is shown in pale blue.

Figure S2 | Histogram and Quantile–Quantile Plot of Social Skills Scores. Histogram depicts the frequency of the Social Skills observations. Q–Q plots compare the quantiles from the current study (sample quantiles) to the quantiles from a Normal distribution (theoretical quantiles).

Figure S3 | Histogram and Quantile–Quantile Plot of Details/Patterns Scores. Histogram depicts the frequency of the Details/Patterns observations. Q–Q plots compare the quantiles from the current study (sample quantiles) to the quantiles from a Normal distribution (theoretical quantiles).

Figure S4 | Histogram and Quantile–Quantile Plot of Communication/Mindreading Scores. Histogram depicts the frequency of the Communication/Mindreading observations. Q–Q plots compare the quantiles from the current study (sample quantiles) to the quantiles from a Normal distribution (theoretical quantiles).

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Balance within the neurexin trans-synaptic connexus stabilizes behavioral control

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Autism spectrum disorder (ASD) is characterized by a broad spectrum of behavioral deficits of unknown etiology. ASD associated mutations implicate numerous neurological pathways including a common association with the neurexin trans-synaptic connexus (NTSC) which regulates neuronal cell-adhesion, neuronal circuitry, and neurotransmission. Comparable DNA lesions affecting the NTSC, however, associate with a diversity of behavioral deficits within and without the autism spectrum including a very strong association with Tourette syndrome. The NTSC is comprised of numerous post-synaptic ligands competing for trans-synaptic connection with one of the many different neurexin receptors yet no apparent association exists between specific NTSC molecules/complexes and specific behavioral deficits. Together these findings indicate a fundamental role for NTSC-balance in stabilizing pre-behavioral control. Further molecular and clinical characterization and stratification of ASD and TS on the basis of NTSC status will help elucidate the molecular basis of behavior – and define how the NTSC functions in combination with other molecular determinates to strengthen behavioral control and specify behavioral deficits.

Keywords: neurexin, NLGN, LRRTM, CBLN, GRID, LRRN, Autism, Tourette

INTRODUCTION

Autism presents within a broad group of neurodevelopmental disabilities known as autism spectrum disorders (ASDs). ASDs are characterized by impaired social interaction and communication and by restricted interests and repetitive behaviors with high heritability estimates. Over 70% of individuals with autism present with intellectual disability (ID) and ~25% with epilepsy indicating overlapping etiologies in addition to secondary molecular determinates of behavior (Baird et al., 2006). There is currently no reliable biomarker, pathology, anatomical finding, or neuroimaging correlate that is specific for or predictive of ASD (Lord et al., 2000; Bauman and Kemper, 2005; Courchesne et al., 2007; Anagnostou and Taylor, 2011). Furthermore, precious little has been established regarding the precise neurological basis of ASD with many brain regions and circuits implicated (Bauman and Kemper, 2005; Courchesne et al., 2007; Amaral et al., 2008; Anagnostou and Taylor, 2011). Several competing hypotheses have been proposed to account for core behavioral deficits and ancillary symptomatic domains in ASD, but none have been widely accepted (Zoghbi, 2003; Belmonte et al., 2004; Courchesne et al., 2007; Geschwind and Levitt, 2007; Rubenstein, 2010). Genomic analyses indicate extreme genetic heterogeneity in ASD with a conservative estimation of between 380 and 820 loci implicated (Abrahams and Geschwind, 2008; O'Roak et al., 2012; Cristino et al., 2013), where many of the loci are associated with overlapping biological pathways (O'Roak et al., 2012; Cristino et al., 2013; Kenny et al., 2013; Yadav et al., 2013). Pathway overlap also extends to neuropsychiatric disorders with behavioral profiles outside the autism spectrum. In this respect, the neurexin trans-synaptic connexus (NTSC) (Clarke et al., 2012), which regulates development and maintenance of neuronal circuitry and neurotransmission is of particular relevance given its high mutation rate in ASD and other neuropsychiatric disorders (Clarke et al., 2012; O'Roak et al., 2012; Cristino et al., 2013; Kenny et al., 2013; Yadav et al., 2013).

Global genomic studies have identified numerous genes/variants and pathways implicated in the behavioral deficits associated with ASD. Cristino et al. (2013) used copy number variations (CNV) and SNP variant analyses to define 13 distinct protein modules involved in ASD including the NTSC. In addition, O'Roak et al. (2012) found high-density of mutations in the β-catenin and p53 signaling pathways consistent with the influence of both *de novo* and extremely rare inherited single nucleotide variations (SNVs) and CNVs contributing to the overall genetic risk. The wnt/β-catenin and Notch signaling pathways in neuronal development are also implicated commensurate with the importance of neuronal circuitry/boundaries and neurotransmission during development as intersecting determinates for ASD (Griswold et al., 2012; Kenny et al., 2013).

The *neurexins* (*NRXNs*) are one of the gene families most commonly mutated in ASD (Missler et al., 2003; Belloso et al., 2007; Gauthier et al., 2011; Clarke et al., 2012; Vaags et al., 2012). NRXNs are single-pass transmembrane proteins concentrated on the pre-synaptic side of the synapse which facilitate neuronal cell-adhesion through the formation of NRXN trans-synaptic cell-adhesion complexes which together comprise the NTSC (Laurén et al., 2003; Missler et al., 2003; Chen et al., 2006; Ko et al., 2009; Linhoff et al., 2009; Wright and Washbourne, 2011; Clarke et al., 2012). The extracellular domain of pre-synaptic NRXNs binds to one of a range of post-synaptic ligands including neuroligins (NLGNs),

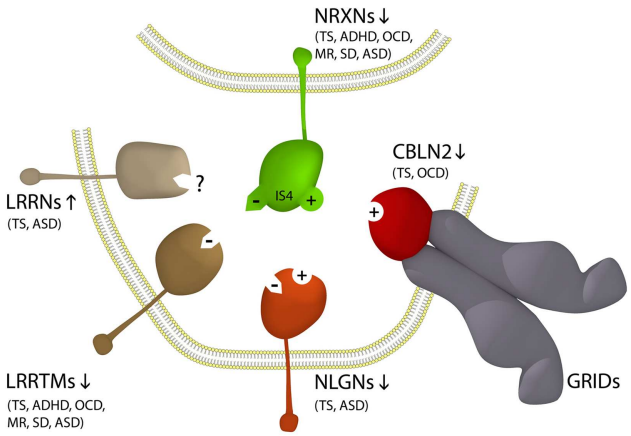

FIGURE 1 | NTSC model for ASD and Tourette Syndrome (Clarke et al., 2012) implicates the full complement of known neurexin (NRXN – green) trans-synaptic cell-adhesion ligand gene families through multiple means of enquiry including the Neuroligin (NLGN – orange); Leucine-rich repeat transmembrane protein (LRRTM – brown); Cerebellin precursor (CBLN – grey); and glutamate receptor delta (GluD/GRID – red) gene families. The pre-synaptic NRXNs form trans-synaptic complexes with post-synaptic ligands NLGNs, LRRTMs, and CBLNs-GRIDs in the formation and/or maintenance of neuronal circuitry within the brain. Vertical arrows indicate putative pathogenic dose effects. Neurexin isoforms with (+) and without (−) the 30 amino acid insert at splice site 4 (IS4) dictate the different/competitive binding of NRXNs between ligands. Co-morbidities listed are those associated with the TS translocations and CNVs affecting the respective genes (Clarke et al., 2012).

leucine-rich repeat transmembrane proteins (LRRTMs), or cerebellin precursor (CBLN) glutamate receptor delta (Glu/GRID) complexes (**Figure 1**) (Missler et al., 2003; Varoqueaux et al., 2006; Ko et al., 2009; Linhoff et al., 2009; Mondin et al., 2011; Wright and Washbourne, 2011; Clarke et al., 2012; Yasumura et al., 2012). Together the three alpha-NRXNs 1–3 are essential for survival and have a pivotal role in neurodevelopment and synaptic transmission where their roles partially overlap (Missler et al., 2003) and all have been implicated in ASD (Missler et al., 2003; Belloso et al., 2007; Wang et al., 2009; Sousa et al., 2010; Gauthier et al., 2011; Clarke et al., 2012; Vaags et al., 2012; Cristino et al., 2013; Jones et al., 2013; Kenny et al., 2013; Yadav et al., 2013). However, specific NTSC components do not associate with specific behavioral deficits in ASD. Moreover, many of the same NTSC gene families are found associated with other neuropsychiatric disorders outside the autism spectrum including Tourette syndrome (TS), Asperger syndrome, schizophrenia, and ID (Clarke et al., 2012). *Neuroligin 4X* (*NLGN4X*) is just one of the many NTSC single gene overlaps between TS, ASD, and ID. The first, a *NLGN4X* truncation mutation, was identified in a family comprising two affected brothers, one with autism and ID and the other with ASD–Asperger syndrome and normal intelligence (Jamain et al., 2003). Subsequently, a different *NLGN4X* truncating mutation was identified in a multigenerational pedigree with 13 affected males with either non-syndromic ID (10 individuals), ID with ASD, or ASD without ID (Laumonnier et al., 2004). In 2008, another familial *NLGN4X* truncating mutation was identified in two brothers with TS/motor tic, one with ASD and the other with attention deficit/hyperactivity disorder (ADHD) and a mother carrier with a learning disorder, anxiety, and depression (Lawson-Yuen et al., 2008). This latter NTSC association with TS and ADHD was just the first of many such associations which have emerged since between the NTSC and the divergent behavioral profiles of ASD and TS (Clarke et al., 2012).

Tourette syndrome (TS) is characterized by motor and vocal tics, with a pre-pubertal age of onset, a waxing and waning course, and improvement in symptoms in adulthood (Eapen and Crncec, 2009). Clinical and epidemiological studies point to a very high incidence of other childhood onset behavioral and developmental disorders including up to 60% with ADHD and up to 50% with obsessive–compulsive disorder (OCD). It has long been suggested that chronic tics and OCD within TS families are likely manifestations of the same underlying genetic etiology with gender-dependent differences in expression leading to male members of the family exhibiting more tic behaviors and the female members exhibiting OCD (Eapen et al., 1993). Furthermore, recent SNP association data suggests that OCD in the presence of TS/Chronic tics may have different underlying genetic susceptibility compared to OCD alone (Eapen et al., 1993).

In the fore mentioned affected families the different behavioral profiles appear to converge around the haploinsufficiency of *NLGN4X* as the common molecular deficit. The mutation, deletion, disruption and duplication of other NTSC components are also relatively common in ASD and consistent with dose effects (Sousa et al., 2010; Gilman et al., 2011; Sakai et al., 2011; Voineagu et al., 2011; O'Roak et al., 2012; Cristino et al., 2013). Association studies also show that many of the rare variants associated with ASD occur within NTSC genes including *NRXN1-3*, *NRXN4/CNTNAP2*, *NLGN1*, *NLGN3*, *NLGN4X*, *NLGN4Y*, *LRRTM1*, *LRRTM2*, *GRID1* (Sudhof, 2008; Sousa et al., 2010; Gilman et al., 2011; Sakai et al., 2011; Voineagu et al., 2011; Clarke et al., 2012; O'Roak et al., 2012; Cristino et al., 2013) and genes encoding NTSC interacting proteins like *SHANK1-3* (Cardno and Gottesman, 2000; O'Roak et al., 2011). Moreover, recent network analyses indicate synaptic transmission as the major protein hub within the ASD network and the only protein module with interactions with all other 12 major network modules including cell–cell adhesion (Cristino et al., 2013).

The recent identification of *GRID1* associating with ASD (Cristino et al., 2013) and *CBLN1* associating with autistic-like traits (Jones et al., 2013) extends the association between the NTSC and ASD and the molecular convergence between TS and ASD. GRID1 is an inter-synaptic ligand of the post-synaptic transmembrane protein CBLN2 found associated with TS (Clarke et al., 2012), that forms the tripartite NRXN–GRID1–CBLN2 trans-synaptic cell-adhesion complex (Matsuda and Yuzaki, 2011; Clarke et al., 2012). In fact all of the gene families encoding NTSC complexes, with the exception of the *GRIDs*, have been implicated in TS including the *NRXN*, *NLGN*, *LRRTM*, and *CBLN* gene families (Petek et al., 2001; Verkerk et al., 2003; Belloso et al., 2007; Lawson-Yuen et al., 2008; Sundaram et al., 2010; Patel et al., 2011; Clarke et al., 2012; Fernandez et al., 2012). Of the 11 novel TS gene disruptions, exonic deletions, and truncations reported to date that are either recurrent or familial, a total of 9 are associated with 1 of

the 20 gene families encoding the NTSC [$p = 5.5E-26$ (T-test)] (Petek et al., 2001; Verkerk et al., 2003; Belloso et al., 2007; Lawson-Yuen et al., 2008; Ercan-Sencicek et al., 2010; Sundaram et al., 2010; Patel et al., 2011; Clarke et al., 2012; Fernandez et al., 2012). As such, the NTSC emerges as a primary determinate for TS (Clarke et al., 2012) and thus by inference a primary determinate for that subset of ASDs with NTSC association. Moreover, as is the case with ASD the bulk of the NTSC mutations associated with TS to date are consistent with dose effects with no apparent correlation between any of the different receptors or ligands of the NTSC and specific behavioral deficits in or between ASD and TS. Rather, the stoichiometric balance between the various competitive NTSC ligands and receptors appears to play a protective gate-keeping role in behavioral control as outlined in the pathogenetic model (**Figure 1**) (Clarke et al., 2012).

The striking molecular convergence between TS and ASD at the NTSC may help explain epidemiological features shared between TS and ASD but not the behavioral divergence. TS and ASD are both conditions that begin during childhood (~1% of children affected) and both are more common in males than in females. The inheritance patterns of TS are also comparable with that of ASD. TS twin studies suggest a monozygotic to dizygotic concordance of up to 77% and family studies consistently demonstrate up to a 100-fold increase in the rates of TS in first-degree relatives comparable with the high heritability of the ASDs (O'Rourke et al., 2009). ASD is also over represented in TS, and clinically, symptoms such as obsessions, compulsive behaviors, involuntary movements (tics in TS and stereotypies in ASD), poor speech control, and echolalia are common in both conditions. Furthermore, the literature suggests that around 20–40% of individuals with ASD experience tics and over 50% of individuals with ID and ASD also exhibit tics (Kadesjo and Gillberg, 2000; O'Rourke et al., 2009). Such overlap in symptoms presumably stems from the interrelated neuronal circuitry involved in the final common pathways of behavioral expression (Eapen et al., 2013). However, the divergent behaviors seen in the two conditions with motor and vocal tics in TS, and impaired social interaction and communication and restricted interests seen in ASD presumably relate to secondary/auxillary molecular and/or environmental determinants impacting neuronal circuitry development/maintenance and/or transmission.

In addition to the prevalence of NTSC dose effects in ASD and TS, the competition for connections between NRXNs and their trans-synaptic ligands (**Figure 1**) further supports the requirement for NTSC-balance in behavioral control. This in turn provides insight into the behavioral role of molecules linked to the NTSC. For example, the SHANK proteins which function from the post-synaptic side of the NTSC are also commonly associated with ASD. The SHANK proteins mediate attachment of the intracellular PDZ-binding domains of NTSC receptor/ligand complexes, including NRXN–NLGN and NRXN–LRRTM (Clarke et al., 2012), to the local actin-based cytoskeleton within dendritic spines. Furthermore, in Purkinje cells, the post-synaptic clustering of SHANK2 with GRID2 appears dependent on the integrity of the tripartite NRXN–GRID2–CBLN1 trans-synaptic complex (Joo et al., 2011; Matsuda and Yuzaki, 2011; Jones et al., 2013). Another TS/ASD candidate gene of related interest to the SHANKs is *synapse-associated protein 97* (*SAP97*) which encodes a scaffold-like protein located on the post-synaptic side of the synapse. Linkage analysis of a large TS pedigree identified the strongest linkage marker (D3S1311) within *SAP97* (Verkerk et al., 2006) and a male individual with TS and ASD has been identified with duplication of the *SAP97* gene locus (unpublished data), whereas micro-deletion of 3q inclusive of *SAP97* is commonly associated with schizophrenia. SAP family proteins bind directly to NTSC complexes and to NMDA, AMP, and kainate receptors at the synapse (Rumbaugh et al., 2003) and membrane-diffusing AMPARs can be rapidly trapped at SAP90/PSD95 scaffolds assembled at nascent NTSC (NRXN–NLGN) adhesions (Mondin et al., 2011). Moreover, the TS candidate ZnT3 (Clarke et al., 2012) – a synaptic zinc transporter which controls concentrations of Zn^{2+} within post-synaptic vesicles – is of particular interest here given the concentration of Zn^{2+} ions within the post-synaptic density (PSD) is known to affect the recruitment of scaffolding proteins like SHANK2 and SHANK3 (Grabrucker et al., 2011).

NTSC RELATION TO NEUROLOGICAL PATHOLOGIES IN ASD AND TS

The neuronal cell-adhesion complexes of the NTSC promote synapse formation and/or maintenance bi-directionally in the glutamatergic and GABA-ergic nervous system. As such, NTSC-imbalance will translate as an imbalance in neuronal connectivity through changes in synapse patterning and transmission (Missler et al., 2003; Varoqueaux et al., 2006; Ko et al., 2009; Linhoff et al., 2009; Clarke et al., 2012). Loss of CBLN2, as reported in TS (Clarke et al., 2012), is associated with reduced mediation of inhibitory synaptogenesis (Yasumura et al., 2012). This however, appears in opposition with the reduced number of excitatory synapses associated with the downregulation of NLGN4X or the LRRTMs in TS (**Figure 1**) (Ko et al., 2009; Wright and Washbourne, 2011), albeit the recurrent loss/disruption of NRXN1 in TS and ASD infers loss of both excitatory and inhibitory synaptic connections. Together these findings further reinforce the importance of a balanced NTSC repertoire rather than "specific complexes" as the basis of NTSC related behavioral disorders.

Synaptic homeostasis depends on the balance between the strength of excitation, inhibition, and the intrinsic excitability of the neuronal circuitry. Evidence suggests that the balance between excitation and inhibition is tightly regulated with even small changes affecting neuronal firing (Atallah and Scanziani, 2009; Pouille et al., 2009). When this balance is perturbed, mechanisms come into play to restore synaptic homeostasis by modifying the balance between excitatory and inhibitory inputs or the application of intrinsic mechanisms to modify the balance of inward and outward voltage-dependent current (Gainey et al., 2009). Synapses are formed even when αNRXN 1 is deleted from the mouse genome, however, this compromises synaptic transmission (Missler et al., 2003). The pre-synaptic co-assembly of Ca^{2+} channels with the secretory apparatus is a prerequisite for the release of neurotransmitters like glutamate and this channel function is impaired in αNRXN1 knockout mice with consequent reductions in neurotransmitter release (Missler et al., 2003). The NTSC trans-synaptic connections NRXN–NLGN and NRXN–LRRTM are both

sensitive to extracellular Ca^{2+} concentrations which appear to trigger post-synaptic differentiation and control the balance of inhibitory GABA-ergic and excitatory glutamatergic inputs. Glutamate, the main excitatory neurotransmitter in the vertebrate brain, has a major role in cortico-striatal-thalamo-cortical circuits and several lines of evidence support the role of glutamate in TS including: the TS association of glutamate receptors that are localized in the cellular membranes of both neurons and glia; the recognized extensive interaction between glutamate and dopamine systems; results of familial genetic studies; and data from neurochemical analyses of post-mortem brain samples (Felling and Singer, 2011; Clarke et al., 2012). Interestingly, LRRTM1 null mice have altered distribution of the excitatory pre-synaptic vesicular glutamate transporter VGLUT1 (Ko et al., 2009; Linhoff et al., 2009). Furthermore, loss of excitatory synaptic connections results in a hypo-glutamatergic state that is consistent with a loss in the synaptic weight, which is an all important factor for the circuit strength required in language development (Matsuda and Yuzaki, 2011).

NEURAL CIRCUITRY AS A FUNCTION OF SYNAPTIC PRUNING AND BOUNDARY FORMATION

Synaptic pruning plays an important role during maturation of the brain by limiting neural circuitry, and neural circuitry within specific brain regions is implicated in behavioral control. As such the integrity of neural/brain boundaries may be a factor in neuropsychiatric disorders. In this respect, it is most interesting to note that both ASD and TS have been associated with leucine-rich repeat neuronal (LRRN) type I transmembrane protein genes. *LRRN3* is localized within the genomic region most commonly duplicated in ASD (Kroisel et al., 2001; Maestrini et al., 2010; Pagnamenta et al., 2010). *LRRN3* is also nested in an antisense orientation within the *IMMP2L* gene recurrently disrupted in TS and ASD (Clarke et al., 2012). Moreover, the nearest gene relation to *LRRN3*, *LRRN1*, has been duplicated in ASD (Davis et al., 2009). These associations suggest increased dose of LRRN1 and LRRN3 maybe pathogenic for ASD and TS. Little is known about the function of LRRN3, however, LRRN1 is known to have a key role in regional boundary formation within the brain (Chen et al., 2006; Tossell et al., 2011). Studies in the developing chick demonstrate that the midbrain–hindbrain boundary (MHB) is established through the down regulation of Lrrn1 by Fgf8 on the posterior side of the future boundary (Tossell et al., 2011), thereby creating a differential cellular affinity between the two compartments likely to involve an as yet unspecified extracellular binding partner for Lrrn1. Lrrn1 in turn regulates the expression of the *Lunatic Fringe* gene which modulates Notch signaling to complete MHB formation. Overexpression of Lrrn1 disrupts the MHB with mixing of cells between compartments (Tossell et al., 2011). For further insight into this association see (Clarke et al., 2012).

AUXILLARY MOLECULAR AND ENVIRONMENTAL DETERMINANTS SPECIFY BEHAVIORAL DEFICITS

Imbalance in the NTSC appears to be sufficient for but not definitive in specifying the nature of behavioral pathogenesis. Moreover, recent evidence suggests that numerous gene variants combine with environmental and physiological factors to specify behavioral deficits. For example, the sex-specific imprinting of *NRXN4/CNTNAP2*, *CTNNA3*, and *LRRTM1* is known to alter the expression of these genes and their parent-of-origin phenotypic inheritance patterns (Oudejans et al., 2004; Francks et al., 2007). Thus, a particular phenotypic co-morbidity may present based on the type and level of involvement of the different NTSC neurotransmitter pathways in combination with secondary determinates that mediate or modulate NTSC pathways during neurodevelopment whereas an early environmental insult could specify an alternate behavioral deficit/neural outcome (Herbert, 2010) including effects associated with prematurity, perinatal trauma, hypoxia, injury oxidative stress, inflammations, infections and autoimmunity, neural and psychosocial stressors, gender effects, etc. (Eapen, 2011). Gender-specific differences exist in the topographic segregation and functionality of GABA-A systems in the substantia nigra, moreover, circulating testosterone is essential for the development of the substantia nigra region in the neonatal period and to a lesser extent for final maturation in the peripubertal period (Veliskova and Moshe, 2001). In this regard, a role for testosterone has been suggested in the extreme male brain hypothesis in ASD (Baron-Cohen, 2002). Similar mechanisms may affect the TS genes leading to gender-dependent difference in phenotypic expression – with male members of TS families exhibiting more tic behaviors and female members more OCD (Eapen et al., 1993, 1997). Thus, an NTSC related imbalance that impacts development of different neuronal regions and circuitry maybe further specified by secondary genetic and/or environmental events (Eapen et al., 2014). The penetrance of the different co-morbidities may also be related to gender, gene dose effects, or the timing of events when different brain regions are being formed, thus resulting in different clinical phenotypes (Eapen et al., 2013).

CONCLUSION

The NTSC provides an invaluable window into the molecular basis of behavior. The role of NTSC-balance as a gate keeper of behavioral control provides a firm basis for more in depth molecular and clinical characterization and stratification of behavioral disorders. To this end, NTSC's common association with ASD and Tourette syndrome provides the ideal starting point for molecular and clinical comparisons between select ASD and TS families.

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Studying autism in rodent models: reconciling endophenotypes with comorbidities

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Autism spectrum disorder (ASD) patients commonly exhibit a variety of comorbid traits including seizures, anxiety, aggressive behavior, gastrointestinal problems, motor deficits, abnormal sensory processing, and sleep disturbances for which the cause is unknown. These features impact negatively on daily life and can exaggerate the effects of the core diagnostic traits (social communication deficits and repetitive behaviors). Studying endophenotypes relevant to both core and comorbid features of ASD in rodent models can provide insight into biological mechanisms underlying these disorders. Here we review the characterization of endophenotypes in a selection of environmental, genetic, and behavioral rodent models of ASD. In addition to exhibiting core ASD-like behaviors, each of these animal models display one or more endophenotypes relevant to comorbid features including altered sensory processing, seizure susceptibility, anxiety-like behavior, and disturbed motor functions, suggesting that these traits are indicators of altered biological pathways in ASD. However, the study of behaviors paralleling comorbid traits in animal models of ASD is an emerging field and further research is needed to assess altered gastrointestinal function, aggression, and disorders of sleep onset across models. Future studies should include investigation of these endophenotypes in order to advance our understanding of the etiology of this complex disorder.

Keywords: autism, epilepsy, sleep, motor deficits, aggression, sensory, gastrointestinal function, anxiety

Studying endophenotypes in rodent models of Autism spectrum disorder (ASD) can offer insights into the heterogeneity and underlying biological causes of this complex disorder. Patients with ASD demonstrate a high degree of variability in both the severity of core diagnostic symptoms (social communication deficits alongside repetitive behaviors) and in the nature and strength of a range of associated comorbidities. If comorbid traits associated with ASD are integral to the disorder we expect that many of these traits will present in animal models. However, despite the prevalence of comorbidities in patients, studies in animal models to date have largely focused on characterizing core behavioral traits. Here we review findings from salient rodent models of ASD identifying endophenotypes that parallel core ASD deficits in combination with one or more comorbid traits commonly reported in patients.

ASD: COMORBID TRAITS

Comorbid traits in ASD include seizures, heightened aggression, and anxiety disorders as well as gastrointestinal problems, altered sensory processing, motor deficits, and sleep disorders (**Table 1**). While treatment of these issues can significantly improve quality of life for patients and their families, the biological mechanisms underlying these symptoms and their co-expression are generally unknown in the context of ASD.

Current estimates for the prevalence of epilepsy in ASD patients range between 8 and 25% (Hara, 2007; Jeste, 2011; Sansa et al., 2011; Woolfenden et al., 2012). Recent meta-analysis data show that epilepsy is more common in ASD patients with an intellectual disability (21.5 vs. 8%; Woolfenden et al., 2012). When epilepsy and abnormal EEG data are compared within the general ASD population, 15% of ASD subjects have an epilepsy diagnosis whereas a larger proportion (24.6%) shows interictal epileptiform EEG abnormalities during sleep (Ekinci et al., 2010). Other reports reveal that as many as 25% of ASD patients have comorbid epilepsy, and that 45.5% show non-seizure-related EEG abnormalities (Parmeggiani et al., 2010). Furthermore, one third (34%) of patients with idiopathic ASD have treatment resistant epilepsy (Sansa et al., 2011).

Aggressive behavior and elevated anxiety are frequently reported in children and adolescents with ASD. Caregiver surveys suggest that as many as 68% of ASD patients show episodes of aggression toward them (Kanne and Mazurek, 2011). Pouw et al. (2013) found that aggression behaviors in ASD are most likely due to a relative impairment in the understanding of the emotions of others. It is also estimated that 40% of ASD patients have at least one anxiety disorder (van Steensel et al., 2011). Specific phobias, obsessive compulsive disorder, and social anxiety disorder are most frequently observed.

A significant proportion of patients with ASD also suffer from gastrointestinal problems (42–90%); with constipation, chronic diarrhea, abnormal stool patterns, and stomach cramps frequently reported (Parracho et al., 2005; Valicenti-McDermott et al., 2006; Ibrahim et al., 2009; Buie et al., 2010; Wang et al., 2011a). Alterations in gastrointestinal function in the context

Table 1 | Clinical comorbidities commonly associated with ASD.

Domain	Comorbid symptoms
Epilepsy	High prevalence of epilepsy (8–25%) and EEG abnormalities (46%) in ASD patients (Amiet et al., 2008; Parmeggiani et al., 2010; Jeste, 2011) High rate of treatment resistant epilepsy in idiopathic autism (34%) (Dudova et al., 2011; Sansa et al., 2011)
Heightened aggression	Approximately 70% of ASD patients exhibit aggression toward caregivers (Kanne and Mazurek, 2011) Reactive aggression correlates with impairments in emotional regulation in children with ASD but not in typically developing children (Pouw et al., 2013)
Anxiety	40% of ASD cases associated with at least one comorbid anxiety disorder (van Steensel et al., 2011)
Gastrointestinal disturbances	Up to 90% ASD patients have chronic GI problems, most commonly constipation, also abdominal pain, diarrhea, and bloating (Parracho et al., 2005; Ibrahim et al., 2009; Buie et al., 2010)
Sensory	Tactile: heightened sensitivity to vibration and thermal pain in palm and forearm (Blakemore et al., 2006; Cascio et al., 2008) Auditory: atypical change detection of auditory stimuli (Gomot et al., 2006; Kwakye et al., 2011) Visual: superior performance in detail oriented tasks, deficits in motion perception (Dakin and Frith, 2005; Latham et al., 2013; Robertson et al., 2013) Altered olfaction and taste in high-functioning ASD patients (Bennetto et al., 2007; Dudova et al., 2011)
Motor impairment	Delays in gross and fine motor domains (Jeste, 2011) Deficits in motor planning, coordination, and gait (Rinehart et al., 2006; Jeste, 2011)
Sleep	Sleep disturbances (quality, quantity, latency to sleep) found in 40–80% of children and adolescents with ASD (Allik et al., 2006; Malow et al., 2006; Jeste, 2011) Sleep onset problems and night waking common in 2- to 5-year-olds with ASD (Krakowiak et al., 2008)

Comorbid traits observed in patients with ASD are heterogeneous and include enhanced seizure susceptibility, heightened aggression, anxiety, gastrointestinal (GI) disturbances, altered sensory and motor function, and sleep disorders.

of ASD are thought to be linked to the effects of anxiety and thereby mediated via CNS function; however investigations into mechanisms involving the enteric nervous system have not been reported.

By far the most common changes associated with ASD are those related to sensory processing which are present in over 90% of individuals diagnosed with ASD (Leekam et al., 2007). Patients with Asperger Syndrome show significantly higher sensitivity to high frequency tactile stimuli compared to control subjects (Cascio et al., 2008). Abnormalities in tactile sensitivity, as well as hypersensitivity to hot and cold stimuli have also been reported in adults with ASD (Blakemore et al., 2006). Auditory processing deficits related to the discrimination of temporally separated tones (Kwakye et al., 2011) and impaired odor detection thresholds (Bennetto et al., 2007; Dudova et al., 2011) have been documented in patients with high-functioning autism as well as subtle impairments in identifying tastes (Bennetto et al., 2007). Interestingly, aberrant motion perception can occur alongside superior visual processing performance in detail oriented tasks, highlighting the potential complexity of sensory changes in ASD patients (reviewed in Dakin and Frith, 2005; also see Latham et al., 2013; Robertson et al., 2013).

Motor abnormalities occur in 60–80% of individuals with ASD and include hypotonia, apraxia, and subtle gait anomalies (see Geschwind, 2009 for review). Abnormal fine and gross motor function, as well as delayed motor learning, dyspraxia, and postural abnormalities are also commonly reported in ASD patients (reviewed in Jeste, 2011). Finally, difficulties initiating sleep, frequent night time waking, and insomnia are frequently reported in children with ASD (Allik et al., 2006; Malow et al., 2006; Krakowiak et al., 2008; Jeste, 2011).

The systematic analysis of traits in animal models corresponding to patient comorbidities can potentially provide insight into the underlying biological mechanisms of ASD. Such outcomes may lead to the design of new therapies and benefits to patients.

ANIMAL MODELS OF ASD

Over the last decade, a substantial number of rodent models of ASD have been generated (reviewed in Silverman et al., 2010a; Peca et al., 2011; Penagarikano et al., 2011; Wang et al., 2011b; Schmeisser et al., 2012; Won et al., 2012) and demonstrate face validity by replicating behavioral traits relevant to ASD. Well-characterized social and communication assessment paradigms and tests for the presence of repetitive behaviors exist for rodent models of ASD (Silverman et al., 2010a). In addition, a battery of tests is available to determine the presence of potential comorbidities including anxiety-like and aggressive behaviors, seizures, disrupted motor activity, sleep dysfunction, and sensory processing deficits (Crawley, 2007) as well as assays for gastrointestinal motility dysfunction (Roberts et al., 2007) in these models. Here we outline findings derived from investigations using these tests (**Table 2**) and highlight areas requiring further research (**Table 3**).

Animal models are discussed in three groups; (i) models with acquired behaviors resulting from environmental insult, (ii) models expressing a human genetic mutation associated with ASD,

Table 2 | Endophenotypes identified in rodent models relevant to comorbid features of ASD.

Domain	Model	Behavior
Seizure susceptibility	VPA	↑ Sensitivity to PTZ (Sobrian and Nandedkar, 1986) and electroshock-induced seizures (Kim et al., 2011)
	PPA	↑ Susceptibility to kindling with repeated intracerebroventricular infusions (MacFabe et al., 2007)
	Shank3B$^{-/-}$	Occasional handling-induced seizures (Peca et al., 2011)
	CNTNAP2	Handling-induced seizures common in adults (Penagarikano et al., 2011)
	FAST	↑ Sensitivity to kindling and chemoconvulsant-induced seizures (McIntyre et al., 1999; Xu et al., 2004; Gilby et al., 2005)
	EL	Handling-induced seizures (Todorova et al., 1999)
	BALB/c	↑ Audiogenic seizures (Morin et al., 1994; Banko et al., 1997)
	C58/J	↑ Sensitivity to PTZ-induced seizures (Nutt and Lister, 1988)
Aggression	Shank2$^{-/-}$	↑ Aggression in home cages although no change in resident-intruder test (Schmeisser et al., 2012)
	FAST	↑ (Reinhart et al., 2004)
	BALB/c	↑ (Brodkin, 2007; Velez et al., 2010)
Anxiety-like behavior	VPA	↑ (Mice) (Markram et al., 2008)
	Shank3B$^{-/-}$	↑ (Peca et al., 2011)
	Shank2$^{-/-}$	↑ (Schmeisser et al., 2012; Won et al., 2012)
	FAST	↑ Fear-potentiated startle (Anisman et al., 2000)
	BALB/c	↑ (Brodkin, 2007)
	BTBR	↑ Under some conditions (McFarlane et al., 2008; Pobbe et al., 2011)
Gastro-intestinal disturbances	BALB/c	Altered intestinal motility compared to C57BL/6 mice in response to serotonin antagonists (Neal et al., 2009)
Sensory	VPA	↓ PPI, ↑ tactile sensitivity (Schneider and Przewlocki, 2005), ↓ olfactory (Schneider and Przewlocki, 2005; Roullet et al., 2010) and pain (Markram et al., 2008) sensitivity
	PPA	↓ Sensorimotor function (increased tendency to slip/fall during beam task; Shultz et al., 2009)
	NL3^{R451C}	↓ Acoustic startle at high decibel levels (Chadman et al., 2008)
	Shank3B$^{-/-}$	↓ PPI (Peca et al., 2011)
	CNTNAP2	↑ Pain and olfactory sensitivity (Penagarikano et al., 2011)
	FAST	↓ Acoustic startle (Anisman et al., 2000)
	BTBR	↓ Thermal response (Silverman et al., 2010b)
Motor	NL3^{R451C}	↑ Latency to fall from rotarod (Chadman et al., 2008)
	Shank3^{e4-9}	Mild motor impairments (Wang et al., 2011b)
	CNTNAP2	Slight ↑ motor coordination (↑ latency to fall from rotarod Penagarikano et al., 2011)
	EL	Delays in visuomotor development (McFadyen-Leussis and Heinrichs, 2005)
Sleep	VPA	Abnormal circadian rhythms (Tsujino et al., 2007)

Endophenotypes relevant to enhanced seizure susceptibility, altered sensory function, and anxiety-like behavior were observed across environmental, monogenetic, and phenotype first models. However, each model was assessed for only a subset of the endophenotypes listed and further research is required to clarify full endophenotypic profiles. VPA, rodents administered valproate.

and (iii) naturally occurring rodent strains that demonstrate behavioral endophenotypes relevant to ASD.

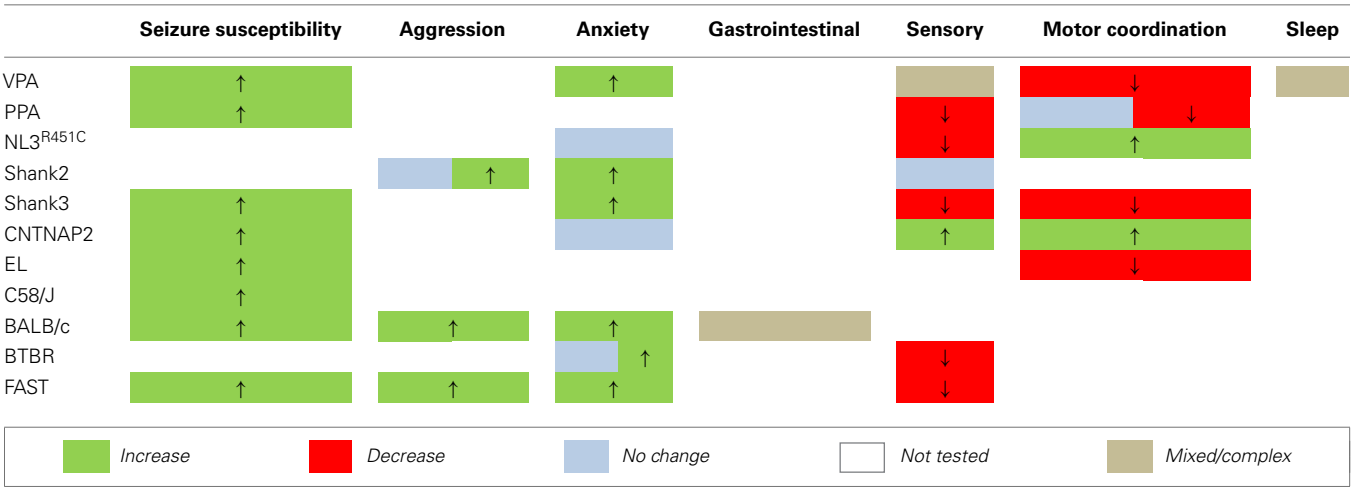
ENVIRONMENTAL MODELS

Autism spectrum disorder-like features exhibited by environmental rodent models are generally elicited in response to an overt insult or developmental challenge, such as exposure to toxins resulting in altered neurological development.

Valproate models

During pregnancy, maternal exposure to the first generation antiepileptic drug valproate has been shown to significantly increase the risk of ASD in children (Rasalam et al., 2005; Meador et al., 2006; Bromley et al., 2008). Valproate is a short-chain fatty acid and is thought to reduce neuronal excitability primarily by increasing concentrations of the inhibitory neurotransmitter GABA and modulating voltage-gated sodium channels (Chapman et al., 1982; Rogawski and Loscher, 2004). In both mice and rats, exposure to valproate during gestation via intraperitoneal injection or orally with food produces deficits in social interaction and repetitive behaviors (Schneider and Przewlocki, 2005; Wagner et al., 2006; Roullet et al., 2010; Kim et al., 2011). These animals also show reduced sensitivity to pain (Markram et al., 2008) and olfactory cues (Schneider and

Table 3 | An overview of endophenotypes assayed in rodent models of ASD.



Seizure susceptibility, sensory function, motor coordination, and anxiety-like behaviors are most commonly tested across models. Aggressive behavior, gastrointestinal function, and sleep cycles are generally understudied. Dual colored cells: formal aggression testing in Shank2$^{-/-}$ mice did not yield data suggesting abnormal aggressive behavior, however excessive aggression was observed in home cages; PPA rats had impaired sensorimotor abilities when tested using the beam task but showed no change in swim speed in other assays. VPA, rodents administered valproate.

Przewlocki, 2005; Roullet et al., 2010), increased tactile sensitivity (Schneider and Przewlocki, 2005), and diminished acoustic pre-pulse inhibition, a test commonly used to index abnormalities in sensorimotor gating (Schneider and Przewlocki, 2005; Markram et al., 2008; Gandal et al., 2010; Roullet et al., 2010). Valproate-exposed adult rats show increased levels of anxiety-like behaviors (Markram et al., 2008) and a reduced threshold for electroshock (Kim et al., 2011) and pentylenetetrazole (PTZ)-induced seizures (Sobrian and Nandedkar, 1986). These rats also show altered circadian rhythms characterized by frequent arousal during the light/sleep phase (Tsujino et al., 2007; **Tables 2** and **3**).

Propionic acid model

The gut microbiota have been suggested to play a role in the etiology of ASD (Mulle et al., 2013). Potential mechanisms contributing to ASD phenotypes are unknown, however excess toxin-producing bacteria have been identified in patients with ASD (Parracho et al., 2005) and increased levels of short-chain fatty acids (such as propionic acid; PPA) produced by enteric bacteria have been studied in rats (MacFabe et al., 2007). In rodent models, administration of the endogenous short-chain fatty acids butyric acid (Thomas et al., 2010), sodium acetate (Shultz et al., 2008, 2009), and PPA directly into the cerebral ventricles produces endophenotypes relevant to ASD (MacFabe et al., 2007, 2011; Shultz et al., 2008, 2009; Thomas et al., 2010). Acute intracerebral ventricular infusion of PPA in rats reduces sociability and learning and also produces sensorimotor impairments (Shultz et al., 2009). This paradigm also results in reduced cognitive flexibility during reversal learning (MacFabe et al., 2011). Furthermore, repeated intraventricular PPA infusion leads to increased susceptibility to kindling-induced seizures and stereotypic behavior (MacFabe et al., 2007, 2011; Shultz et al., 2009; **Tables 2** and **3**).

A small number of ASD patients (5%) show mitochondrial dysfunction along with altered levels of various metabolites suggestive of altered fatty acid processing (Frye et al., 2013). Further investigation to assess the effects of both PPA and valproate on gastrointestinal function (i.e., following oral administration) is needed (see **Table 3**), as the short-chain fatty acid receptor (GPR43) expressed by some mucosal enteroendocrine cells may play a role (Karaki et al., 2006). The effects of orally administered PPA in particular would be of interest and would serve to strengthen construct validity of this model.

GENETIC MODELS

Many gene mutations associated with ASD code for proteins involved in the formation and maintenance of synapses (Sudhof, 2008; Betancur et al., 2009; Bourgeron, 2009; Chakrabarti et al., 2009; Betancur, 2011; Geschwind, 2011). Here we review findings from monogenic mouse models expressing mutations in four genes modulating synaptic function; the neuroligin-3^{R451C} (NL3^{R451C}) mice (Tabuchi et al., 2007; Chadman et al., 2008) two models expressing specific mutations in the Shank3B/ProSAP2 gene [Shank3B knockout mice and Shank3B^{e4-9} partial knockout mice (Peca et al., 2011; Wang et al., 2011b)], as well as two SHANK2 knockout models (Schmeisser et al., 2012; Won et al., 2012) and the contactin associated protein-like 2/Neurexin IV (CNTNAP2/NRXN4; Penagarikano et al., 2011) knockout mouse model (**Table 2**). Electrophysiological studies in these mice report altered glutamatergic and GABAergic synaptic function (Tabuchi et al., 2007; Etherton et al., 2009, 2011; Peca et al., 2011; Wang et al., 2011b; Schmeisser et al., 2012; Won et al., 2012). Each of these models also expresses strong ASD behavioral endophenotypes suggesting a role for these genes in shaping core behaviors relevant to ASD diagnosis. However, it is not well established whether these animal models replicate comorbid traits observed in patients.

Neuroligin-3^{R451C} mice

Neuroligins are adhesion molecules which interact with a range of post-synaptic scaffolding proteins including Shank3 and CNTNAP2 and bind to members of the presynaptic neurexin family across the synaptic cleft (Sudhof, 2008; Krueger et al., 2012; Verpelli and Sala, 2012). Mutations in the neuroligin family of post-synaptic adhesion molecules were implicated in ASD after a spontaneous point mutation in the gene encoding NL3 was identified in two brothers with ASD; one with comorbid epilepsy (Jamain et al., 2003). Mice expressing the NL3^{R451C} mutation show a subtle reduction in pup distress calls (on post-natal day 8) and reduced acoustic startle (Chadman et al., 2008). Under some conditions and on some genetic backgrounds, NL3^{R451C} mice also show impaired social interaction (Tabuchi et al., 2007; Etherton et al., 2011). Delays in meeting developmental milestones (e.g., slower righting reflexes), which may appear as motor deficits early in development, have also been observed in these mice (Chadman et al., 2008). However, adult NL3^{R451C} mice showed better motor coordination in the accelerating rotarod test compared with wild type littermates (Chadman et al., 2008).

Shank3-related models

The Shank (SH3 and multiple ankyrin repeat domains) gene family (also known as Proline-rich synapse-associated proteins; ProSAPs) contains three members; Shank1-3 that code for post-synaptic scaffolding proteins involved in the recruitment of several receptors and proteins (including the neuroligins and neurexins) to the excitatory post-synaptic membrane (Irie et al., 1997; Meyer et al., 2004; Baron et al., 2006; Hayashi et al., 2009; Arons et al., 2012). Rare microdeletions within the 22q13 locus (containing Shank3) are associated with intellectual disability, speech delay, and ASD (Nesslinger et al., 1994; Bonaglia et al., 2006; Durand et al., 2007). Mutations in Shank2 are also associated with ASD (Berkel et al., 2010; Kumar, 2010). Two different genetic models in which Shank3 is altered; Shank3B$^{-/-}$ (Peca et al., 2011) and Shank3^{e4-9} (Wang et al., 2011b) in addition to two recently reported Shank2 knockout models (Schmeisser et al., 2012; Won et al., 2012) demonstrate core and comorbid traits relevant to ASD. A third model in which one full length copy of Shank3 is deleted shows core ASD endophenotypes; however the expression of secondary/comorbid features outlined here has not been investigated in these mice (Bozdagi et al., 2010). Shank3B$^{-/-}$ mice lacking the Shank3α and β isoforms show increased repetitive behavior (self-injurious grooming) and reduced interaction with a stranger mouse as well as occasional handling-induced seizures (Peca et al., 2011 and reviewed in Herbert, 2011). Shank3^{e4-9} mice (in which exons 4–9 are deleted) show core ASD-like deficits including social impairments, repetitive behaviors, and altered communication (i.e., less complex vocalization patterns), with learning deficits and mild motor abnormalities also evident (Wang et al., 2011b). In addition to a role as a structural protein in the central nervous system, Shank3 is present at enteric nervous system synapses (Huett et al., 2009). The enteric nervous system controls gastrointestinal motility and mucous secretion and therefore gene mutations leading to changes in synaptic function (including many ASD candidate genes) may also affect gastrointestinal function (Gershon and Ratcliffe, 2004). The Shank3 mouse models of ASD are therefore excellent candidates for investigating effects of ASD-associated gene mutations on gastrointestinal motility. Shank2 knockout mice demonstrate abnormal vocal and social behaviors, and increased grooming behaviors. Hyperactivity (e.g., repetitive jumping) and anxiety-like behaviors have also been reported in these mice (Schmeisser et al., 2012; Won et al., 2012). Schmeisser et al. (2012) detected no change in aggressive behaviors in Shank2 knockout mice using a resident-intruder assay. Despite this negative result, a high level of aggression between Shank2 knockout males was observed in home cages (Schmeisser et al., 2012).

CNTNAP2 mice

Genetic ablation of the contactin associated protein-like 2 (CNTNAP2) gene, a member of the neurexin transmembrane protein superfamily (also known as CASPR2 and Neurexin IV), results in ASD-like deficits in social interaction and stereotypic behaviors in mice (Penagarikano et al., 2011). In addition, CNTNAP2 knockout mice show hyperactivity, impaired nest building, and frequent handling-induced seizures after 6 months of age (Penagarikano et al., 2011). The CNTNAP2 gene has been associated with ASD and a recessive form of epilepsy (Strauss et al., 2006). These mice exhibit sensory endophenotypes including hyper-reactivity to thermal sensory stimuli and superior performance in the buried food test, an assay for olfactory function (Penagarikano et al., 2011). CNTNAP2 knockout mice also showed slightly improved motor coordination on the rotarod compared to wild type littermates. Perhaps surprisingly, the atypical antipsychotic risperidone (prescribed to treat aggression and irritability in some cases of ASD) reversed nest building deficits as well as locomotor hyperactivity in these mice (Penagarikano et al., 2011), demonstrating predictive validity in this model (**Table 2**).

Behavioral analyses in transgenic mouse models of ASD confirm that a range of proteins regulating synaptic function are likely to be integral to this disorder. Most studies involving genetic models have investigated one or two endophenotypes relevant to patient core and comorbid traits (**Tables 2** and **3**). However, to better understand the relationship between these traits a focus on assessing the more subtle secondary endophenotypes is required. Seizure susceptibility, gastrointestinal function, sleep cycles, and aggressive behaviors remain to be investigated in the majority of these genetic models of ASD (**Table 3**). Still, the presence of endophenotypes relevant to comorbid traits of ASD in each of these genetic models suggests that at least some of these traits may be associated with the core behavioral features of the disorder.

PHENOTYPE FIRST MODELS

Interplay between genomic and non-genomic influences (e.g., maternal effects) is almost certainly involved in the symptom heterogeneity associated with ASD. To further understand their relative degree of contribution, animal models in which clinically relevant endophenotypes occur "naturally" are of great interest. There are currently several rodent models developed via breeding processes alone that exhibit measurable endophenotypes relevant to the diagnostic criteria and comorbid traits associated with ASD. These animal models include the FAST/SLOW rats and the C58/J, BALB/c, BtBR T + tf/J (BTBR), and epileptic-like (EL) mice (**Tables 2** and **3**).

FAST/SLOW rats and EL mice

The FAST and SLOW rat strains were derived from parent populations of Long Evans Hooded and Wistar rats using selective breeding processes based on relative seizure susceptibility in the amygdala kindling model (Racine et al., 1999). This process ultimately produced a seizure-prone (FAST) and seizure-resistant (SLOW) strain. FAST rats have since proven highly seizure-prone in both the kindling model and in chemoconvulsant (e.g., pilocarpine, kainate) seizure-induction models (McIntyre et al., 1999; Xu et al., 2004; Gilby et al., 2007; Gilby and O'Brien, 2013). EL mice, like FAST rats, were also created via selective breeding based on relative seizure susceptibility and originated from the non-epileptic DDY mouse strain (Meidenbauer et al., 2011). EL mice typically exhibit handling-induced seizures by postnatal day 50–60 (Todorova et al., 1999). Remarkably, the breeding processes used to create heightened seizure sensitivity in both colonies simultaneously produced robust, comorbid ASD-like traits. Both FAST rats and EL mice exhibit significant social impairment (Reinhart et al., 2004, 2006; Gilby et al., 2007; Lim et al., 2007; Turner et al., 2007) and repetitive behaviors (e.g., overgrooming, self-injurious scratching, and/or myoclonic jumping; Gilby, 2008; Meidenbauer et al., 2011) alongside delays in social, physical, and visuomotor development (McFadyen-Leussis and Heinrichs, 2005), learning deficits, impulsivity, and hyperactivity in various testing paradigms (Anisman and McIntyre, 2002; McFadyen-Leussis and Heinrichs, 2005; Azarbar et al., 2010). FAST rats are also more aggressive than their comparison (SLOW) strain (Reinhart et al., 2004, 2006) and show reduced acoustic startle but enhanced fear conditioning (Anisman et al., 2000). Thus, FAST rats and EL mice offer a similar endophenotypic profile relevant to core and comorbid symptoms observed in ASD.

C58/J mice

C58/J mice naturally exhibit ASD-like traits including poor sociability (Moy et al., 2008; Ryan et al., 2010), relative learning deficits, hyperactivity (Moy et al., 2008), and stereotypic behaviors (i.e., jumping and flipping; Ryan et al., 2010). Interestingly, C58/J mice also demonstrate a reduced threshold for PTZ-induced seizures (Nutt and Lister, 1988). However, in contrast to the ASD-like developmental delays observed in FAST and EL animals, C58/J mice meet developmental milestones earlier than their comparison strain (C57BL/6J; Ryan et al., 2010).

BALB/c and BTBR mice

The BALB/c and BTBR mouse strains exhibit core ASD traits in the form of impaired social interaction and repetitive behaviors (i.e., overgrooming and/or excessive marble burying; Brodkin, 2007; Shoji and Kato, 2009; Pearson et al., 2011). BTBR mice also demonstrate increased social anxiety-like behavior (Pobbe et al., 2011) although anxiety responses to novel situations are inconsistent (McFarlane et al., 2008). BTBR mice are less reactive to thermal (hotplate) stimuli than the C57Bl/6J standard strain (Silverman et al., 2010b), suggesting subtle sensory changes exist in this model. In addition, several BALB/c substrains displaying distinct behavioral phenotypes offer particular strengths for comorbidity investigation. BALB/cJ mice exhibit altered gastrointestinal function (Neal et al., 2009) and are highly aggressive (Velez et al., 2010) while the epilepsy-prone (EP) BALB/c substrain is susceptible to audiogenic seizures (Morin et al., 1994; Banko et al., 1997). Notably, BTBR and BALB models have a high incidence of corpus callosal agenesis and severely reduced hippocampal commissural volumes (Wahlsten et al., 2003), which may be relevant to reports of reduced corpus callosal volumes in ASD patients (Anderson et al., 2011).

The characterization of ASD-relevant traits in these "natural" models is a relatively new initiative. Still, the documented commonalities thus far are striking; particularly the co-expression of repetitive behaviors and impaired social interaction together with heightened seizure sensitivity (**Table 3**). Finally, while we are aware that a few studies have investigated aggression and sensory processing in these rodent models, further testing using validated assays (Silverman et al., 2010a) should be applied to fully characterize the presence of core and comorbid features in these models.

SUMMARY

The primary aim of this review was to compare endophenotypic clustering within a selection of animal models of ASD. Here we focus on models expressing at least two core ASD endophenotypes with additional endophenotypes relevant to comorbid traits reported in ASD patients.

ENDOPHENOTYPING: A NEW APPROACH

We report that models generated via environmental insult, genetic manipulation, and selective breeding processes demonstrate a number of overlapping endophenotypes (**Tables 2** and **3**) relevant to both clinical comorbid (**Table 1**) and core traits of ASD. Detailed investigation into the more subtle endophenotypes associated with these models is a relatively novel approach. Indeed, many clinical traits highlighted here have yet to be investigated in these models or should be re-examined using consistent methodological approaches. Until then any ranking of the clinical relevance of the phenotypic profiles would be premature. Interestingly however, enhanced seizure susceptibility, altered sensory function, anxiety-like behaviors, and changes in motor coordination were the most frequently reported endophenotypes across models (**Table 3**). Although not routinely investigated, several of the models also showed atypical aggressive interactions (**Tables 2** and **3**). Despite evidence for disturbed sleep and abnormal gastrointestinal function in a significant number of ASD patients (see **Table 1**), to our knowledge, circadian rhythms and gastrointestinal function have only been investigated in two models; valproate-exposed rats and BALB/c mice, respectively. As discussed, gastrointestinal motility was insensitive to serotonin antagonists in BALB/c mice in comparison to a control strain (Neal et al., 2009) and valproate-treated rats showed increased arousal during sleep compared to untreated controls (Tsujino et al., 2007; **Tables 2** and **3**).

OVERLAPPING TRAITS

The presence of both core and comorbid endophenotypes in a range of animal models suggests that at least some of these traits may be interrelated and possibly integral to the etiology of ASD. Some endophenotypes are indeed co-expressed across different model constructs (for example, seizure susceptibility is consistently increased, as are anxiety-like behaviors in examples of environmental, genetic, as well as phenotype first models;

Table 3). Both environmental models (i.e., rodents administered the fatty acids valproate and PPA) and phenotype first models show heightened seizure susceptibility and anxiety-like behaviors together with sensory and motor deficits (**Table 3**). In contrast, genetic models show varied changes in sensory and motor domains (**Table 3**) for which the underlying mechanisms are unknown.

FUTURE DIRECTIONS: POTENTIAL MECHANISMS UNDERLYING ASD ENDOPHENOTYPES

Animal models are an important tool with which to tease apart the biological mechanisms underlying ASD. Given the diverse nature of ASD, it is unlikely that a single cause is responsible for this disorder and more recent research suggests some degree of interaction between the CNS and peripheral systems. Many gene mutations identified in patients with ASD affect synaptic function (Betancur et al., 2009; Bourgeron, 2009; Betancur, 2011). This supports an emerging hypothesis that ASD is primarily a disorder of neuronal communication (Grabrucker et al., 2011; Ebert and Greenberg, 2013) and we suggest that subtle changes in neural function could underlie many of the comorbid traits described here. For example, it is well established that gene mutations coding for ion channels that result in altered synaptic function in the CNS can cause seizures in patients (Helbig et al., 2008; Goldberg and Coulter, 2013). It is also important to acknowledge, however, that many neurotransmitters and receptors that regulate neuronal communication in the CNS are of functional importance in the periphery and may thereby contribute to common comorbid traits in patient subsets. For example, in the case of gastrointestinal issues, many of the synaptic genes associated with ASD including the Shanks, neurexins, and neuroligins are also expressed in the enteric nervous system (Huett et al., 2009; Raab et al., 2010; Zhang et al., 2013), which regulates gastrointestinal motility and secretion. It is, therefore, feasible that synaptic mutations may underlie gastrointestinal symptoms in at least a subset of patients with ASD (Gershon and Ratcliffe, 2004) in addition to altering neuronal communication in the CNS. Future research should explore potential neural mechanisms underlying endophenotypes, in particular, those that are currently understudied (such as gastrointestinal disorders and altered circadian rhythms) in animal models of ASD.

In summary, rigorous endophenotyping in animal models of ASD can assist in identifying the molecular mechanisms underlying these common comorbid traits. Such information may also contribute to the identification of putative patient subsets within this spectrum of disorders and the subsequent tailoring of potential therapies. However, in order to achieve these goals, a more consistent approach in the assessment and comparison of endophenotypes is needed.

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A "bottom-up" approach to aetiological research in autism spectrum disorders

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Autism spectrum disorders (ASD) are currently diagnosed in the presence of impairments in social interaction and communication, and a restricted range of activities and interests. However, there is considerable variability in the behaviors of different individuals with an ASD diagnosis. The heterogeneity spans the entire range of IQ and language abilities, as well as other behavioral, communicative, and social functions. While any psychiatric condition is likely to incorporate a degree of heterogeneity, the variability in the nature and severity of behaviors observed in ASD is thought to exceed that of other disorders. The current paper aims to provide a model for future research into ASD subgroups. In doing so, we examined whether two proposed risk factors – low birth weight (LBW), and *in utero* exposure to selective serotonin reuptake inhibitors (SSRIs) – are associated with greater behavioral homogeneity. Using data from the Western Australian Autism Biological Registry, this study found that LBW and maternal SSRI use during pregnancy were associated with greater sleep disturbances and a greater number of gastrointestinal complaints in children with ASD, respectively. The findings from this "proof of principle" paper provide support for this "bottom-up" approach as a feasible method for creating homogenous groups.

Keywords: autism spectrum disorders, heterogeneity, autism phenotype

INTRODUCTION

Autism spectrum disorders (ASD) are currently diagnosed in the presence of impairments in social interaction and communication, and a restricted range of activities and interests. However, there is considerable variability in the behaviors of different individuals with an ASD diagnosis. Traditionally, researchers have conceptualized ASD as a unitary disorder with a large spectrum, and have sought to discover a single aetiological factor that leads to disorder. However, the behavioral heterogeneity has been mirrored at the genetic level, for instance, many susceptibility loci have been identified, yet each has been found to account for a small amount of variance only (1–2%) (Weiss et al., 2008). A proposition that has gathered momentum over the last decade involves moving away from the traditional conceptualization of ASD as a unitary disorder toward conceptualizing a syndrome of multiple and separate disorders; in essence, re-examining "autism" as "the autisms" (Geschwind and Levitt, 2007; Whitehouse and Stanley, 2013).

Research in this area has traditionally adopted a "top-down approach" by constraining behavioral phenotypes in the hope that this will facilitate the identification of biological subtypes. For example, Buxbaum et al. (2001) reported linkage evidence for a susceptibility gene for Autistic Disorder on chromosome 2. In an analysis of 95 affected-relative pair families with Autistic Disorder they found a maximum multipoint heterogeneity LOD score (HLOD) of 1.96 and a maximum multipoint NPL score of 2.39 on chromosome 2q (at 186cM, for D2s364). When families were grouped according to delayed onset (at age >36 months) of phrase speech, linkage to chromosome 2 increased (HLOD = 2.99, NPL = 3.32). Shao et al. (2002) found further evidence for a susceptibility gene on chromosome 2. In an analysis of 82 sibling pairs with Autistic Disorder they found a HLOD of 0.53 at D2S116. When the analysis was restricted to a subset of 45 families with phrase speech delay (>36 months), linkage to chromosome 2q increased (HLOD = 2.12). Whilst this approach has received the most attention in aetiological research, generally speaking, it has underperformed, with only weak evidence that stratification based on IQ, age at first word, or verbal ability yield a more genetically homogenous population (Geschwind and Levitt, 2007).

A "bottom-up" approach to identify biological subtypes of ASD has not received the same level of research attention. This methodology focuses on known aetiological risk factors, and whether individuals exposed to these risk factors have a more homogenous phenotype. In this paper, we report on this bottom-up approach, focusing on aspects of the phenotype that are not part of the core defining features of the disorder. We know that comorbid medical conditions are highly prevalent in ASD (Bauman, 2010). Sleep problems are thought to affect 40–80% of children on the spectrum (Richdale, 1999) and estimates of gastrointestinal disorders in ASD range from 9 to 70% (Buie et al., 2010). The high prevalence of these comorbid conditions in children with ASD may suggest the presence of important genetic and/or biological markers, which if identified, can refine our ability to be more precise in categorizing clinical and genetic subtypes within the autism spectrum (Bauman, 2010). In this paper, we have adopted a bottom-up

approach by stratifying groups based on two previously identified risk factors, namely, maternal use of selective serotonin reuptake inhibitors (SSRIs) during pregnancy and low birth weight (LBW). The second part of our strategy involved examining the homogeneity within the groups based on medical complaints such as sleep problems and gastrointestinal complaints in addition to core features of ASD such as social behavior, language characteristics, and severity.

Selective serotonin reuptake inhibitors use during pregnancy has gained considerable attention over the last 2 years and is thought to be implicated in an increased risk of ASD diagnosis (Croen et al., 2011). Prevalence studies in the US estimate that up to 8% of mothers may be treated with SSRIs during pregnancy for conditions such as anxiety disorders or major depression (Alwan et al., 2011). SSRIs act primarily by blocking the serotonin transporter, thereby raising extracellular serotonin (5-HT) levels (Oberlander et al., 2009). These SSRIs readily cross the placental and blood-brain barriers to the fetus, with the potential to alter central 5-HT signaling (Oberlander et al., 2009). The neuroactive properties of SSRIs are thought to be a potential risk to fetal neurodevelopment, since 5-HT plays such a critical role in regulating diverse processes such as cell division, differentiation, migration, myelination, synaptogenesis, and dendritic pruning (Gaspar et al., 2003). A number of researchers have hypothesized that the increase in ASD diagnoses in recent years may be associated with a commensurate increase in maternal use of antidepressant medication during pregnancy (Croen et al., 2011). A recent population-based case-control study by Croen et al. (2011) reviewed record-based data describing the postnatal development of children exposed to SSRIs *in utero*. This study examined data for children born through a medical care program during the period of 1995–1999. Infants in the sample who were later diagnosed with ASD were considered cases. Children without an ASD diagnosis were randomly sampled from the remaining cohort at a ratio of five control children per one case child. Using this matched sample, Croen et al. (2011) investigated SSRI use throughout pregnancy and found that 70 women who took antidepressant medication the year before the birth of their child had twice the risk of having a child with ASD ($n = 20$ offspring with ASD, 28.57%) compared with 1735 women who did not take any antidepressant medication ($n = 278$ offspring with ASD, 16.02%).

Using a similar population-based nested case-control design, Rai et al. (2013) investigated the extent to which parental depression and maternal antidepressant use during pregnancy were associated with ASDs in offspring. For parental depression, record-based data was available for 4429 cases of ASD and 43277 age- and sex-matched controls, and for maternal antidepressant use, data existed for 1679 ASD cases and 16845 non-ASD controls. They found that a history of maternal but not paternal depression was associated with higher risk of autism in offspring. These associations were largely limited to children of mothers who reported using antidepressants at the first antenatal interview. Antidepressant use during pregnancy was reported by 1.3% of mothers of children with ASD and by 0.6% of control mothers, equating to an almost twofold increase in risk of ASD with use of antidepressants (Rai et al., 2013). Other studies that have examined the effect of maternal SSRI use during pregnancy have observed several atypical behavioral outcomes among offspring, including delay in meeting gross motor milestones (Pedersen et al., 2010), a wide range of feeding difficulties (Oberlander et al., 2006) and sleep disturbances (Zeskind and Stephens, 2004).

Low birth weight (<2500 g) has also been considered an environmental risk factor implicated in a range of psychiatric disorders including ASD, anxiety disorder, and depression (Indredavik et al., 2004; Gardener et al., 2011; Jaspers et al., 2012). Lampi et al. (2012) examined data from the case-control Finnish Prenatal Study of Autism and ASDs and found that children with very LBW (<1500 g) had a greater than threefold increased odds of autism compared with children with normal birth weight (NBW) (2500–3999 g). Interestingly, LBW did not significantly increase the odds of Asperger syndrome (Lampi et al., 2012). In addition to these associated psychiatric disorders, when compared to children with NBW, children with LBW have been found to show pervasive motor impairments, increased socio-emotional issues, increased risk of sleep-disordered breathing, and reductions in language ability (Paavonen et al., 2007; de Kieviet et al., 2009; Spittle et al., 2009; Barre et al., 2011; Scott et al., 2012). Despite evidence supporting the role of these environmental risk factors in the development of ASD, no single factor has been identified that poses a determinant risk for this disorder.

This paper will adopt a "bottom-up" approach to parsing ASD heterogeneity by investigating the behavioral phenotype associated with two possible environmental risk factors. The first study compared the behavioral and developmental phenotype of children with ASD whose mothers used SSRIs during pregnancy with the phenotype for a tightly matched group of children with ASD whose mothers did not use SSRIs during pregnancy. It was hypothesized that those children with ASD whose mothers used SSRIs during pregnancy would display early feeding and sleep disturbances compared to the control group of children with ASD. We also examined whether these children showed a distinguishable behavioral phenotype. Study 2 compared the phenotype of children with ASD born with LBW with a matched group of children with ASD born with NBW. It was hypothesized that those LBW children with ASD would display greater sleep disturbances (e.g., sleep-disordered breathing), language difficulties, and socio-emotional problems compared to the NBW group. This "proof of principle" study seeks to examine two potential risk factors within the context of a "bottom-up" research design. If the hypotheses are supported this paper may provide a blueprint for using the "bottom-up" approach as a feasible method for creating homogenous groups compared with the more costly "top-down" approach which requires large sample sizes.

STUDY 1

MATERIALS AND METHODS

Participants

Participants were part of the Western Australian Autism Biological Registry (WAABR), which is an ongoing study of children with a clinical diagnosis of an ASD and their families taking place at the Telethon Institute for Child Health Research in Perth, Western Australia (see Taylor et al., in press). Diagnosing ASD in Western Australia mandates assessment by a clinical team comprising a Pediatrician, Psychologist, and Speech-Language Pathologist under

DSM-IV guidelines (American Psychiatric Association, 1994). A diagnosis is only made when there is consensus amongst the team. The current study included nine participants from the WAABR whose mothers reported SSRI use during pregnancy (cases). Each of these participants was individually matched on gender and chronological age at assessment (within 15 months) with three further children with ASD ($n = 27$) whose mothers did not take an SSRI during pregnancy.

Measures and procedure

Prior to attending a face-to-face assessment, families were mailed and asked to complete a comprehensive case-history questionnaire relating to the mother's pregnancy and the ASD child's development. Mothers were asked to provide details of any history of psychological disorder such as major depression or anxiety. They were also asked to provide the name of any prescription or non-prescription medications, the dosage, and the amount they used during pregnancy. A series of questionnaires were also included in this package, including the Social Responsiveness Scale (SRS; Constantino and Gruber, 2002), Children's Sleep Habits Questionnaire (CSHQ; Owens et al., 2000), Children's Communication Checklist-2 (CCC-2; Bishop, 2003), and a gastrointestinal complaints questionnaire (Ibrahim et al., 2009).

The SRS is a 65-item questionnaire used to examine a range of social behaviors characteristic of ASD in children over the last 6 months. A total score can be calculated for the SRS as well as five subscale scores, namely, social communication, autism mannerisms, social motivation, social awareness, and social cognition. Parents respond using a four-point scale ranging from "not true" (1) to "almost always true" (4). A higher total score on this measure is indicative of greater social difficulties. The CSHQ is a 34-item parent-report instrument that was used to examine sleep behavior over a "typical week." Parents were asked to rate how often their child showed behaviors such as "struggle at bedtime" and "show fear at sleeping alone" using a one to three point scale corresponding to "rarely," "sometimes," or "usually," respectively. A total score and eight subscale scores (bedtime resistance, sleep onset delay, sleep duration, sleep anxiety, night wakings, parasomnias, sleep-disordered breathing, and daytime sleepiness) can be calculated for responses on the CSHQ. Higher total scores on the CSHQ indicate that the child has a greater number of sleep problems.

The CCC-2 is a parent-report questionnaire designed to assess the communication skills of children aged 4–16 years. The purposes of the CCC-2 are the identification of pragmatic language impairment, screening of receptive and expressive language skills, and assistance in screening for ASD. The CCC-2 consists of 70 items that are divided into 10 scales, each with 7 items. The first four scales focus on specific aspects of language and communications skills (content and form). The next four scales assess the pragmatic aspects of communication. The last two scales measure behaviors that are usually impaired in children with ASDs. The parent rates the frequency of the communication behavior described in each item from 0 (less than once a week or never) to 3 (several times a day or always). Interpretation is based on a General Communication Composite (GCC), with lower scores indicative of greater language and communication difficulties.

Parents also completed a brief questionnaire related to their child's history of gastrointestinal problems. This questionnaire was developed specifically for the WAABR case-history questionnaire based on the list of complaints in Ibrahim et al. (2009). After reviewing the literature related to gastrointestinal symptoms they identified five categories that have been reported to be common in patients with autism, namely, constipation, diarrhea, gastroesophageal reflux or vomiting, abdominal discomfort/irritability, or feeding issues (Ibrahim et al., 2009). If the parent reported their child had experienced any of the five gastrointestinal complaints for a period of at least a month, resulting in consultation with their doctor, they received a score of one for the indicated complaint(s). Any other reports received a score of zero. Using this scoring method these complaints were analyzed in two ways: (1) individually to see if the frequency of each complaint differed between the two groups and (2) as a summary measure of gastrointestinal complaints (score of one or more) versus no gastrointestinal complaints (score of zero).

Families were then invited to the Telethon Institute for Child Health Research for a face-to-face behavioral assessment. Clinical diagnoses of ASD were confirmed using the Autism Diagnostic Observation Schedule-Generic (ADOS-G; Lord et al., 2000). The present study used the child's age and ADOS module (reflective of their quantity of speech) to calibrate severity scores (0–10) for each participant according to the severity scale of Gotham et al. (2009). This enabled comparisons between the participants, irrespective of the module they completed.

Statistical analyses

Between-group differences in the quantitative scores of the SRS, CCC-2, CSHQ, and ADOS severity scale were investigated with independent-samples t-tests. Responses to the gastrointestinal complaints questionnaire were analyzed according to whether parents reported zero complaints or one or more complaints for their children using chi-square analyses with Fisher's exact test of significance.

RESULTS

The SSRI case ($n = 9$) and control ($n = 27$) groups did not significantly differ on gestational age [$F(1, 34) = 1.05$, $p > 0.05$] or maternal age at conception [$F(1, 34) = 3.45$, $p > 0.05$]. **Table 1** provides details of the maternal, pregnancy and offspring characteristics of the case group.

Independent-samples t-tests revealed that there were no significant differences between the two groups on any of the SRS, CCC-2, CSHQ, or ADOS severity scores (**Table 2**). However, analysis of responses to the gastrointestinal complaints questionnaire found that mothers who used SSRIs during pregnancy were more likely to have a child with ASD who experienced one or more gut problems ($n = 8$, 88.9%), compared to the control group ($n = 13$, 48.1%), $\chi^2(1)$, $p = 0.05$. To further investigate this association, chi-square analyses with Fisher's exact test were performed on the five individual complaints (**Table 3**). The individual complaints did not significantly differentiate between the groups. However, the percentage of constipation complaints was noticeably larger (though, not significantly) for cases compared to controls.

Table 1 | Study 1: maternal and offspring characteristics of the SSRI case group.

	Maternal			Offspring						
	SSRI taken during pregnancy	**Period of pregnancy SSRI taken**	**Psychiatric diagnosis**	**Gestational age at birth (weeks)**	**Age at assessment**	**ADOS module administered**	**ADOS severity score**	**CSHQ score**	**SRS score**	**Number of gut problems**
Case 1	Lexapro	Daily	Major depression	41	5, 6	2	1	42	146	2
Case 2	Lexapro	Daily	Major depression	40	4, 6	2	6	62	157	1
Case 3	Lovan	3 months	Major depression	36	5, 2	2	8	54	158	4
Case 4	Effexor	Daily	Major depression	38	10, 2	3	3	46	172	2
Case 5	Not specified	–	Major depression	38	4, 3	2	7	59	166	0
Case 6	Escitalopram	Daily	–	38	2, 9	1	4	77	207	1
Case 7	Fluoxetine	Daily	Anxiety disorder	40	8, 5	3	3	63	145	1
Case 8	Aropax	1 month	Anxiety disorder	39	3, 1	1	6	54	120	2
Case 9	Zoloft	Daily	Depression	38	3, 5	1	6	41	90	1

Table 2 | Study 1: descriptive statistics and independent-samples *t*-tests for CSHQ, SRS, and ADOS severity scores.

	SSRI cases		Controls		Statistic	*p*
	M	**SD**	***M***	**SD**		
CSHQ	55.56	11.36	51.58	11.96	$t(31) = 0.86$	0.40
Bedtime resistance	9.56	2.79	9.83	3.25		
Sleep onset delay	1.89	0.60	1.83	0.87		
Sleep duration	5.11	2.42	4.96	2.39		
Sleep anxiety	4.89	1.45	4.79	1.82		
Night wakings	6.00	1.50	5.04	2.03		
Parasomnias	11.33	2.78	9.58	3.36		
Sleep dis-breathing	4.11	1.83	4.08	1.56		
Daytime sleepiness	12.67	3.16	11.46	2.95		
SRS	151.22	32.84	145.11	25.86	$t(34) = 0.57$	0.57
Social awareness	16.56	1.81	16.44	2.94		
Social cognition	28.78	8.23	26.56	6.24		
Social communication	50.33	12.58	47.74	8.88		
Social motivation	25.56	5.36	24.93	5.95		
Autistic mannerisms	30.00	7.78	29.44	7.54		
ADOS severity	4.89	2.26	5.93	1.96	$t(34) = 1.32$	0.19
CCC-2						
GCC	30.75	6.99	36.15	16.53	$t(15) = 0.63$	0.54

Table 3 | Study 1: chi-square analyses using Fisher's exact test for both groups of children for the five gastrointestinal complaints.

Gut complaint	**SSRI cases *N* (%)**	**Control *N* (%)**	***p***
Constipation	4 (44.4)	4 (14.8)	0.09
Diarrhea	2 (22.2)	3 (11.1)	0.58
Gastro reflux	2 (22.2)	3 (11.1)	0.58
Abdominal	1 (11.1)	2 (7.4)	1.00
Feeding	5 (55.6)	8 (29.6)	0.24
One or more complaints	8 (88.9)	13 (48.1)	0.05

DISCUSSION

This is the first study to examine the relationship between SSRI exposure and ASD phenotype. There were no differences between the cases and individually matched control participants in scores on the SRS, CCC-2, CSHQ, or ADOS-G severity. However, children with ASD whose mothers took SSRIs during pregnancy were significantly more likely to experience gastrointestinal complaints during childhood. Further examination of the relationship between gastrointestinal complaints and *in utero* SSRI exposure revealed that no individual complaint could significantly differentiate the two groups. While this does not support Oberlander et al. (2009) who found evidence for feeding disturbances in typically developing infants exposed to SSRIs *in utero*, it is possible that the small sample size contributed to the null findings for the less-frequent individual complaints.

The current study was limited by the absence of a control group of children whose mothers had affective disorders but who did not take SSRIs during pregnancy, and therefore we are unable to parse out whether the differences in the frequency of gut problems is related to mood disturbances or SSRI use. Rai et al. (2013) reported an association between maternal depression and an increased risk of offspring ASD. Although they found that this association was largely confined to antidepressant use in a subsample of mothers, future studies could build on the findings presented here and in Rai et al. (2013) by comparing the phenotype for children with ASD whose mothers report untreated depression during pregnancy with a matched ASD control group of children. The hypothesized association between ASD and gastrointestinal pathology is the subject of increasing amounts of research. Despite the numerous parental reports of gastrointestinal complaints among their children with ASD, studies have failed to find a significant difference in the prevalence of these complaints between children with ASD and control groups of children (e.g., Ibrahim et al., 2009). The current findings suggest that SSRI exposure *in utero* may be one potential candidate accounting for variance in the gut phenotype in children diagnosed with ASD.

STUDY 2

MATERIALS AND METHODS

Participants

The study involved using data for 16 participants from WAABR whose birth weight was ≤2500 g (LBW). Each of these participants was individually matched on gender and chronological age at assessment (within 18 months) with two further control children with ASD ($n = 32$) whose birth weight was within the normal range (NBW; 2500–3999 g).

Measures and procedure

Within the case-history questionnaire, mothers were asked to report their child's birth weight. For the purposes of Study 2, data collected for each child using the SRS, CSHQ, ADOS severity, CCC-2, and gastrointestinal complaints questionnaire were analyzed.

Statistical analyses

Between-group differences in the quantitative scores of the SRS, the CSHQ, CCC-2, and ADOS severity scale were investigated with independent-samples t-tests. Responses to the gastrointestinal complaints questionnaire were analyzed using chi-square analyses with Fisher's exact test of significance.

RESULTS

The LBW ($n = 16$) and the NBW ($n = 32$) groups did not significantly differ on maternal age at conception [$F(1, 45) = 0.07$, $p > 0.05$]. Mean gestational age was significantly lower for the LBW group [$F(1, 43) = 28.53$, $p < 0.05$, $M = 34.25$ weeks, SD = 4.55 weeks] relative to the NBW group ($M = 39.07$ weeks, SD = 1.33 weeks, $p < 0.05$). **Table 4** provides details of the offspring characteristics of the case group. Independent-samples t-tests (see **Table 5**) revealed that LBW children with ASD had significantly higher scores relative to the NBW group on the CSHQ for Total Sleep Disturbance and two of the subscales, namely, Sleep-Disordered Breathing and Daytime Sleepiness. There were no significant differences between the two groups on the SRS, CCC-2, or ADOS severity scores (**Table 5**).

Similarly, children with LBW ($n = 16$, 81%) did not experience significantly greater gastrointestinal issues compared to the NBW group ($n = 32$, 53%), $\chi^2(1)$, $p = 0.07$. To further investigate this association, chi-square analyses with Fisher's exact test were performed on the five individual complaints (**Table 6**). The individual complaints did not significantly differentiate between the groups.

DISCUSSION

The second study examined the phenotype of children with ASD born with LBW relative to a group of children with ASD born with NBW. This study did not find any significant differences between the groups on the gastrointestinal complaints questionnaire, SRS, ADOS-G severity, or CCC-2. This is inconsistent with findings of greater socio-emotional issues and reduced language ability in LBW children compared to NBW children in the absence of an ASD diagnosis (Barre et al., 2011; Scott et al., 2012). The present study did find that children in the LBW group obtained higher mean scores on the CSHQ for total sleep disturbance, Daytime Sleepiness, and Sleep-Disordered Breathing relative to the NBW group. This supports the finding of sleep-disordered breathing in children with LBW without an ASD diagnosis (e.g., Paavonen et al., 2007). Interestingly, compared to norms from typically developing children ($M = 3.24$) and children with ASD ($M = 3.92$) (Hoffman et al., 2006), LBW children with ASD obtained larger mean scores for the Sleep-disordered Breathing subscale ($M = 4.31$).

Currently, there are no norms to describe performance of typically developing LBW children on the CSHQ. It would be interesting to compare sleep disturbance between LBW typically developing children and LBW children with ASD. Thus it may be useful to conduct a more comprehensive study of LBW and NBW children with and without ASD to look more closely at the significance of the present findings. Unsurprisingly, the LBW children

Table 4 | Study 2: offspring characteristics of the LBW case group.

	Birth weight	Gestational age at birth	Age at assessment	ADOS module	ADOS severity score	CSHQ score	SRS score	GCC	Number of gut problems
Case 1	600	24	11; 1	3	4	45	152	42	1
Case 2	895	27	7; 4	2	6	57	166	32	3
Case 3	985	29	5; 6	1	3	39	133	38	1
Case 4	1565	30	4; 7	1	6	42	172	–	0
Case 5	1640	37	5; 2	1	6	65	169	40	2
Case 6	1665	37	5; 2	2	4	56	143	49	1
Case 7	1725	35	14; 4	3	4	63	171	28	3
Case 8	1765	34	2; 8	1	7	47	107	–	1
Case 9	2097	40	13; 1	1	6	54	183	–	2
Case 10	2285	36	5; 2	2	8	54	158	–	4
Case 11	2300	37	5; 11	1	7	51	163	–	0
Case 12	2426	37	9; 7	1	8	69	200	-	1
Case 13	2450	32	4; 7	2	6	52	159	56	1
Case 14	2500	38	4; 3	2	7	59	166	39	0
Case 15	2125	37	11; 3	3	6	66	191	7	1
Case 16	2480	38	4; 6	2	5	66	156	–	2

Table 5 | Study 2: descriptive statistics and independent-samples *t*-tests for CSHQ, SRS, CCC-2, and ADOS severity score.

	LBW		NBW		Statistics	*p*
	M	SD	*M*	SD		
CSHQ	55.31	9.08	47.84	8.84	$t(45) =$ 2.72	0.01
Bedtime resistance	9.81	2.76	8.13	2.38		
Sleep onset delay	1.88	0.72	1.52	0.68		
Sleep duration	5.69	2.39	4.55	1.93		
Sleep anxiety	4.63	1.63	4.74	1.86		
Night wakings	5.19	1.87	4.19	1.66		
Parasomnias	10.44	2.71	9.97	2.56		
Sleep dis-breathing	4.31	1.49	3.29	0.82	$t(45) =$ 3.04	0.00
Daytime sleepiness	13.38	3.36	11.19	2.56	$t(45) =$ 2.48	0.02
SRS					$t(45) =$ 0.75	0.46
Social awareness	17.31	2.73	17.97	2.33		
Social cognition	30.25	3.64	28.77	5.81		
Social communication	54.00	8.25	51.13	8.58		
Social motivation	27.69	4.70	26.68	4.88		
Autistic mannerisms	32.56	7.16	31.68	7.36		
ADOS severity	5.81	1.47	6.56	1.98	$t(46) =$ 1.34	0.19
CCC-2						
GCC	36.78	13.92	28.88	13.67	$t(31) =$ 1.47	0.15

Table 6 | Study 2: chi-square analyses using Fisher's exact test for both groups of children for the five gastrointestinal complaints.

Gut complaint	LBW *N* (%)	NBW *N* (%)	*p*
Constipation	7 (43.8)	9 (28.1)	0.22
Diarrhea	2 (12.5)	3 (9.4)	0.55
Gastro reflux	5 (31.3)	8 (25)	0.45
Abdominal	0 (0)	4 (12.5)	0.19
Feeding	9 (56.3)	13 (40.6)	0.24
One or more complaints	8 (88.9)	13 (48.1)	0.05

had a significantly lower gestational age at birth than the NBW children, which raises the possibility that gestational age may be driving the findings and not birth weight. However, it is important to note that the study by Lampi et al. (2012), which informed our hypotheses, found that LBW was a better predictor of ASD diagnosis than was prematurity.

GENERAL DISCUSSION

This present study used a "bottom-up" approach to seek understanding of the heterogeneity of ASD by investigating the behavioral phenotype associated with two suspected environmental risk factors, namely, *in utero* SSRI exposure and LBW. It was hypothesized that children with ASD who were exposed to one of these environmental risk factors would present with a more homogenous phenotype relative to individually matched control groups of children with ASD. There was some preliminary support for this hypothesis. While the children in the LBW and SSRI-exposed groups were no different to their respective control groups in quantitative and qualitative measures of the core symptomatology of autism, there was evidence that the two groups were distinct in the level of their non-core symptomatology such as sleep and gastrointestinal complaints, respectively.

The numbers of children with ASD in the "aetiological risk" subgroups are small, and therefore we urge caution in drawing conclusions from these data. Rather, we seek to highlight a different method for understanding the heterogeneity in the ASD phenotype. We believe that the preliminary findings of increased levels of non-core symptoms of ASD among certain "aetiological risk" subgroups, provides evidence that this "bottom-up" methodology may assist ASD research. Studies including larger samples of children with ASD will build on the research presented here, and provide the opportunity to validate our preliminary findings.

Whilst the present study did not find any differences in core ASD symptoms between LBW and SSRI-exposed children with their respective control groups, we know that each child who is given an ASD diagnosis presents with the triad of core symptoms irrespective of their severity. It is unlikely that a single environmental factor could be attributed to "causing" one of these core impairments. Rather we may expect that the interplay between the environment and a child's genetic profile contributes to the variable expression of autistic-related traits (Ratajczak, 2011). Therefore, it seems reasonable that environmental factors may be related to the expression of non-core ASD symptoms among these children rather than to any variance in core symptomatology.

Recently, Whitehouse and Stanley (2013) reaffirmed an emerging view in the literature with regard to reconceptualizing autism in moving away from a unitary disorder with one cause, and toward an "umbrella" for a collection of behavioral disorders resulting from a range of causal pathways. In their paper they describe how research in cerebral palsy may be analogous to research on autism. Initially cerebral palsy was thought to be a unitary disorder caused by anoxia secondary to trauma occurring during labor and delivery. However, the heterogeneity in symptoms and severity amongst children with cerebral palsy led researchers to hypothesize that there may be many causal pathways. Many other causes were identified for cerebral palsy following this reconceptualization, such as complications of preterm birth, infections, and inflammation *in utero* (McIntyre et al., 2012). For diagnosis, cerebral palsy is now considered an umbrella term covering a wide range of syndromes that arise secondary to a number of brain lesions/anomalies occurring early in development (Badawi et al., 2008).

A key question facing the field is whether the long-held view that autism is a unitary disorder with a single causal pathway is correct, or whether autism may best be conceptualized as an umbrella term for a collection of behavioral disorders resulting from a range of causal pathways, analogous to cerebral palsy. Current evidence suggests that the latter may be a more accurate representation. Heterogeneity in the distal causes of autism is now well-established. It is estimated that between 10 and 15% of individuals with autism have a known genetic aetiology, but the loci and nature of these lesions vary, from known syndromes to observable cytogenetic

lesions and rare *de novo* mutations (e.g., copy number variations) (Abrahams and Geschwind, 2008). Among those with idiopathic autism, no single genetic risk variant has been found to occur in more than 1% of individuals (Abrahams and Geschwind, 2008). Similarly, environmental risk factors identified through epidemiological studies and examined in this study – *in utero* exposure to SSRIs (Croen et al., 2011) and LBW (Lampi et al., 2012) – differ considerably in the hypothesized biological paths to disorder, and as yet, no known environmental exposure is deterministic of autism.

Given that diagnosis is currently based on behavior, the question of whether autism is one or multiple disorders is ultimately a query over the proximal causes of these behaviors, and one perhaps best addressed in neuroscience. Neuroscientific studies may help determine whether (a) distal risk factors "fan in" on a common neurobiological substrate that has the capability of underpinning the considerable behavioral heterogeneity in autism (one disorder), or (b) the exact combination of distal risk factors determines the brain regions and functions that are affected, which in turn prescribe the behavioral profile of each individual (multiple disorders). A key research aim will be to investigate the correspondence (if any) between known distal (genetic and environmental) and proximal (neurobiological) risk factors for autistic behaviors, using increasingly sophisticated environmental monitoring, genetic sequencing, and neuroimaging techniques.

Using preliminary data in this study we have demonstrated how a "bottom-up" approach can be applied to current aetiological research. Grouping individuals using this method may facilitate the identification of subtypes of people with ASD. Elucidating the underlying nature of the disorder(s) is a crucial step toward achieving perhaps the "holy grail" of autism research: tailoring intervention to the biological and cognitive makeup of each individual (Whitehouse and Stanley, 2013).

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On the application of quantitative EEG for characterizing autistic brain: a systematic review

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Autism-Spectrum Disorders (ASD) are thought to be associated with abnormalities in neural connectivity at both the global and local levels. Quantitative electroencephalography (QEEG) is a non-invasive technique that allows a highly precise measurement of brain function and connectivity. This review encompasses the key findings of QEEG application in subjects with ASD, in order to assess the relevance of this approach in characterizing brain function and clustering phenotypes. QEEG studies evaluating both the spontaneous brain activity and brain signals under controlled experimental stimuli were examined. Despite conflicting results, literature analysis suggests that QEEG features are sensitive to modification in neuronal regulation dysfunction which characterize autistic brain. QEEG may therefore help in detecting regions of altered brain function and connectivity abnormalities, in linking behavior with brain activity, and subgrouping affected individuals within the wide heterogeneity of ASD. The use of advanced techniques for the increase of the specificity and of spatial localization could allow finding distinctive patterns of QEEG abnormalities in ASD subjects, paving the way for the development of tailored intervention strategies.

Keywords: autism-spectrum disorder, quantitative electroencephalography, coherence, asymmetry, non-linear techniques

INTRODUCTION

Autism-Spectrum Disorders (ASD) are neurodevelopmental conditions characterized by deficits in social communication and interactions and by the presence of repetitive patterns of behavior, interests, and activities (American Psychiatric Association, 2013). These features appear in early childhood, tend to persist life-long, and often lead to poor outcome in adulthood. Recent epidemiological studies estimate the prevalence of ASD to be 1 in 88 children in the USA (Centers for Disease Control and Prevention, 2012). Despite an extensive research, there is still much debate about the morphological, functional, and neuropsychological characteristics of the "autistic" brain (Schipul et al., 2011; Calderoni et al., 2012; Muratori et al., 2012; Narzisi et al., 2012), and thus the neural basis of altered behaviors in ASD remains largely unclear. Several neuroimaging and neurophysiological techniques have been used in order to understand the correlation between brain functionality and autistic behavior. Among them, Quantitative Electroencephalography (QEEG) is currently receiving great interest and it is increasingly used in studies on neurodevelopmental disorders, especially the ASD. It has been found relevant for evaluating heterogeneity of behavioral disorders, treatment responses, and outcomes amongst other issues (Sheikhani et al., 2009). The ease and simplicity of the EEG procedure and its millisecond resolution of brain activity coupled with standardized analysis protocols provides an opportunity for elaborate analysis of brain functions and dysfunctions. Emerging EEG analysis techniques that involve interesting applications of signal analysis protocols have given us new and exciting measures of brain function.

According to the American Academy of Neurology, QEEG is defined as "... The mathematical processing of digitally recorded EEG in order to highlight specific waveform components, transform the EEG into a format or domain that elucidates relevant information, or associate numerical results ..." (Nuwer, 1997). Therefore, QEEG applies computerized mathematical algorithms to transform raw EEG data into a number of frequency bands of interest. Five wide frequency bands are usually studied, typically defined as delta (1.5–3.5 Hz), theta (3.5–7.5 Hz), alpha (7.5–12.5 Hz), beta (12.5–30 Hz), and gamma (30–70 Hz) (Steriade et al., 1990). In addition, although not included in the standard classification of EEG bands, a further alpha-like rhythm, called mu rhythm, has been extensively studied in the research on ASD (Oberman et al., 2005, 2008; Barnier et al., 2007), since mu suppression during the observation of biological actions is thought to reflect mirror neuron system (MNS) functioning and, in its turn, MNS dysfunction has been proposed to explain the core social deficits observed in ASD (Williams et al., 2001). EEG recordings may be performed at rest, in both closed and opened eye conditions, or while subjects perform specific tasks. Normative or control data are usually needed, in order to give meaning to the functional information obtained.

In this article we carried out a systematic survey of existing research to present an integrated view on how QEEG may help

in characterizing autistic brain and present the future direction in the same domain. The paper is organized as follows: after briefly describing the fundamentals of QEEG in Section "QEEG Methods," we reviewed the recent works on application of QEEG in characterizing the autistic brain in Section "QEEG in Autism." Three particular directions have been surveyed in detail – QEEG during rest, during specific tasks, and how QEEG may be applied for classifying autistic subgroups. The paper is concluded and future research directions are discussed in Section "Discussion and Conclusion."

QEEG METHODS

For QEEG analysis raw EEG data are collected non-invasively via a set of electrodes typically following an international 10/10 or 10/20 electrode placement configuration on the scalp. The collected data is then transformed into frequency domain using computerized algorithms (i.e., Fourier Transform, Welch Method) and scalp map of different frequency bands is obtained (Dumermuth and Molinari, 1987).

Absolute and relative spectral power (SP) consists in transforming the EEG traces from time domain into frequency domain providing information about the harmonic content of the signal. The spatial analysis provides information about the distribution of electrical activity in the brain and the interconnectivity among cortical regions measured through coherence and symmetry analyses.

EEG power spectrums are regulated by anatomically complex homeostatic systems in the various frequency bands. Brainstem, thalamic, and cortical processes involving large neuronal populations mediate this regulation, using all the major neurotransmitters. The spectrum is quite stable in healthy individuals because it is regulated by homeostatic regulation of neurotransmitters and can be abnormal in some psychiatric disorders due to the dysfunction of this regulation (Hughes and John, 1999).

Coherence measures the degree of coupling between signals generated by specific neuronal assemblies, which are located in proximity of the recorded electrodes. Coherently oscillating neuronal assemblies exhibit electrical activity with common spectral properties. When a coherent oscillation occurs these neural groups can effectively communicate, because their communication windows for input and for output are open at the same times (Fries, 2005). The coherence pattern is flexible, changing according during specific cognitive or motor task and allowing the maintenance of our cognitive flexibility (Fries, 2005).

Brain asymmetry is due to hemispheric specialization, so that the global neural activity is not the same in the two hemispheres. However it has been shown that hemispheric differences in competence are not fixed and structure-dependent but are subject by dynamic processes. According to this view asymmetry can vary inter- and intra-individually according to arousal or other factors (Hugdahl, 1996).

Taken together, frequency and spatial information, and their modification over consecutive EEG epochs, provides a quantitative view of the dynamic evolution of connectivity between different brain areas, getting therefore cues on the functional organization of underlying neuronal networks in static and dynamic settings.

Before applying a quantitative analysis, a pre-processing step needs to be performed. First of all the signal is segmented in epochs of the same length and visually inspected, in order to reject those epochs with evident artifacts. Any remaining artifact is then removed by using high-pass, low-pass, and notch filters.

In most cases, frequencies below 0.5 Hz, due to movement artifacts, and higher than 60 Hz, afflicted by muscle artifacts, are filtered out, although this latter operation impairs the possibility to analyze gamma band. Notch filter allows removing artifacts caused by electrical power lines (50 or 60 Hz according to the country).

After preprocessing, spectral analysis can be applied to the signal. The Power Spectral Density (PSD) can be calculated by transforming the time domain signal to the frequency domain, using different techniques such as the Fast Fourier Transform (FFT) or the Welch method (Welch, 1967). From PSD the absolute power of the signal can be computed. However, since the absolute power measures may vary significantly in humans, it is more useful to calculate the power ratios among bands, which show less variability among subjects and are less affected by artifacts. Power ratios are expressed as a percentage, and are obtained by dividing the absolute power of a specific band by the total absolute power of the spectrum. Power ratio can be calculated also between only two bands (e.g., α/θ) or between band sets (e.g., $\alpha+\beta/\theta+\delta$).

Classical spectral analysis techniques, like the FFT, are very useful when analyzing stationary signals. Nevertheless, when dealing with non-stationary signals, as is the case of EEG, they show the big disadvantage of not preserving information on the temporal evolution/localization of the frequency components. This occurs because changes in frequency content, at a given time instant, cause changes to all the Fourier coefficients and therefore it is not possible to localize at which times these frequencies occur. In QEEG analysis, temporal information is important to detect and monitor changes in brain activity at different time-scales following a specific event. For this reason in some studies other techniques as the Short-Time Fourier Transform (STFT) have been used (Sheikhani et al., 2007). STFT can be interpreted as the Fourier transform of the signal observed through a sliding time window of finite duration. The STFT allows constructing the signal spectrogram, which is an image representation of the magnitude of Fourier coefficients within that time window and therefore describes the frequency contents of the signal in the neighborhood (bounded by the time window) of the selected time instant (Walter, 1963).

Spectral analysis is often associated with spatial analysis that allows characterizing relationships between activities of different brain areas. The spatial information may be mainly derived from QEEG data through symmetry and coherence analysis. The symmetry between the two hemispheres can be computed using the Brain Symmetry Index (BSI) (John et al., 1977; Van Putten et al., 2004). The BSI captures a particular asymmetry in SP between hemispheres, and is normalized between 0 (perfect symmetry) and 1 (maximal asymmetry). Asymmetry is defined by the difference on the EEG absolute power between homologous contralateral electrodes and it is calculated as:

$$\mathrm{BSI} = (\mathrm{LH} - \mathrm{RH}) \,/\, (\mathrm{LH} + \mathrm{RH})$$

where LH is the absolute power at one electrode in the left hemisphere and RH at its homologous electrode in the right hemisphere. On the other hand, the coherence function is a measure of the degree of association or coupling of frequency spectra

between two EEG signals simultaneously recorded from different scalp locations per frequency band. Mathematically coherence is defined as the squared cross-correlation between two waveforms within a specific frequency band that has been normalized for amplitude (Otnes and Enochson, 1972). It is assumed to be an index of functional coupling between different brain areas. In neuroscience the coherence measure is generally distinct from synchrony, which refers to signals oscillating at the same frequency with identical phases (Singer, 1999).

Finally, another interesting QEEG index, recently introduced by Pop-Jordanova and Pop-Jordanov (2005), is the "brain rate." This is calculated as the EEG spectrum weighted frequency with the following formula:

$$f_b \sum {}_i P_i = \sum {}_i f_i V_i / V \text{ with } V = \sum {}_i V_i$$

where the index corresponds to the frequency band ($i = 1$ for delta, $i = 2$, for theta, etc.), f_i is the mean frequency of the corresponding band and V_i is the mean amplitude of the electric potential associated to each band. The brain rate can thus be defined as an integral state attribute correlated to brain electric, mental, and metabolic activity (Pop-Jordanova and Pop-Jordanov, 2005).

The techniques described above are all linear methods and are the most commonly applied in the analysis of QEEG data. Given the non-linear nature of EEG, however, non-linear methods could be more suitable for the analysis of this signal. Although less conventional, a set of non-linear techniques have been sometimes used in QEEG analysis, allowing to obtain new information not detectable through linear methods, such as non-linear interactions and the complexity and stability of underlying brain sites. Some of these techniques, like higher-order statistical analysis, complexity analysis, and the phase synchronization analysis, have been applied to the study of QEEG signals in ASD.

Finally, the analysis of microstates represents another promising technique although not yet used in ASD research.

Among the higher-order statistical analysis techniques, the bispectral analysis is an advanced technique that quantifies quadratic non-linearities amongst the components of the EEG signal. In particular it measures the phase relationships between different frequency components and on that basis quantifies the degree of dependence amongst these components. Bispectrum is computed by the Fourier transform of the third order cumulant (a statistical measure of correlation). As the bi-spectrum depends not only on phase coupling but also on the power, it can be normalized in order to make it sensitive only to changes in phase coupling. This normalized bispectrum is then termed as bicoherence (for a detailed description of the mathematical bases of the bispectral analysis, see Sigl and Chamoun, 1994).

Another interesting property of EEG signal is complexity, which reflects random fluctuations over multiple time scales in the dynamics of neural networks, thus providing insights about neural connectivity. The most interesting methods employed for computing complexity of EEG signals are entropy and fractality.

Entropy is a physical measure related to the amount of disorder in a system, and it describes the irregularity or unpredictability characteristics of a signal.

Since regularity is not necessarily correlated with complexity, the quantification of complexity of EEG signals can be computed using the multiscale entropy (MSE), which measures the entropy across multiple time scales (Costa et al., 2002). This method is based on the principle that biological systems are modulated by multiple mechanisms, which interact over multiple temporal scales generating complex data. Another quantity that identifies the degree of complexity of a system is the fractal dimension. This is a non-integer number describing the self-similarity of a system: the whole can be fitted by parts of it by shifting and stretching (Mandelbrot, 1977).

The phase synchrony analysis may be useful when needed to analyze the phase relationships between EEG signals at different electrodes, independently of their amplitude (Lachaux et al., 1999). The basic idea of this technique is to generate an analytic signal from which a phase, and a phase difference between two signals, can be defined. On the basis of this phase difference a phase synchronization index can be computed, which will be zero if the signals under investigation are not synchronized and will be one for a constant phase difference.

Finally, the technique of functional microstates allows studying brief transactions occurring in the brain in the time range of milliseconds. Microstates are defined as time periods, of 80–120 ms, during which the potential distribution over the scalp shows stable topographical configuration after which a rapid transition to another stable configuration (another microstate) occurs (Lehmann et al., 1987). Microstates could be considered as the basic blocks of human information processing (Lehmann, 1990), reflecting the interactions between environmental information and the subject's previous knowledge and internal state. Microstates can only repeat several times within a period so that a cluster approach can be adopted to identify different classes of electrical states composing the EEG signal. Several statistical measures can then be extracted and related to the different experimental conditions and microstate class, such as mean microstate duration, mean number of microstates per second, or the percentage time covered by each state (Koenig et al., 2002) (for a detailed mathematical description of the non-linear analysis techniques described above, see Tong and Thakor, 2009).

Quantitative electroencephalography data are usually obtained using commercial or free software that are able to extract the most common features of EEG signals. The use of advanced techniques such as Independent Component Analysis (ICA), and neuroimaging techniques such as Low Resolution Electromagnetic Tomography (LORETA) (Pascual-Marqui et al., 1994) are used to map the actual sources of the cortical rhythms. These advanced techniques may, therefore, represent a promising approach to understand the dynamics and functions of the brain in a number of neurological diseases, including ASD.

QEEG IN AUTISM

Quantitative electroencephalography has been adopted in several studies for the assessment of ASD with the aim of finding out quantitative indices characterizing brain functions. An understanding of how this evolving technique can aid future research in ASD is very important.

Several studies highlight QEEG capacity to classify subjects with ASD from controls, or different subgroups of ASD. Moreover, QEEG has been also applied as a tool for therapeutic intervention through a neurofeedback approach, although its use in ASD is still poorly reported in literature. A description of neurofeedback methods and of its application in ASD, however, is beyond the aim of this article, and readers may refer to dedicated original researches and reviews (Pineda et al., 2008; Kouijzer et al., 2009, 2010; Coben et al., 2010).

In the following subsections, we will review the main applications of QEEG in three different scenarios: (1) closed or open-eyes rest condition; (2) subjects performing specific tasks; (3) subtyping of ASD through QEEG information. All the studies reviewed here are summarized in **Table 1**. This research provides evidence for the utility of QEEG in extracting objective measures that can characterize brain activity in different conditions.

QEEG DURING REST

Quantitative electroencephalography during rest conditions represents one of the most used applications of this technique in the field of autism research. Neural oscillations reflect the synchronous firing of large populations of neurons mediated by excitatory/inhibitory interactions and can be registered on the scalp. QEEG at rest may therefore inform, *in vivo*, on the balance between excitatory and inhibitory activities, which in turn could affect the cognitive and social functioning in ASD (Rubenstein and Merzenich, 2003; Bourgeron, 2009). The observation of spontaneous brain activity also allows characterizing different patterns in functional connectivity that might specify functionally relevant brain networks.

Finally, by studying power fluctuations of different bands it is possible to notice deviations from normal patterns that reflect the organization of the underlying system.

One of the first studies on QEEG in autism (Cantor et al., 1986) tried to examine if QEEG analysis could be used to differentiate low-functioning children with ASD from subjects with mental retardation without ASD, age-matched subjects with typical development and toddlers with typical development. Data were acquired during open-eyes rest condition and spectral as well as spatial analysis on the acquired data were performed.

Differences in PSD, coherence, and symmetry were found. In particular, children with ASD showed a significantly greater percentage delta and less alpha activity, higher degree of coherence between and within hemispheres, especially in delta and alpha band, and less amplitude asymmetry in every band. Interestingly, while ASD subjects significantly differentiate from age-matched controls and mentally retarded subjects, the pattern of activation was similar to the one obtained in typical toddlers, suggesting a maturational lag in cerebral functioning of subjects with ASD.

Chan et al. (2007) also studied PSD in children with ASD and in neurotypical controls by recording EEG in an open-eyes condition. In this study both low and high-functioning ASD children were included in the sample set. In consistence with the previous study, the ASD group showed significantly higher absolute delta and lower relative alpha. The authors attempted to localize the abnormalities in EEG signal, and found similar results at each channel suggesting that QEEG characteristics were not regionally specific, but were observed across all the cortex of children with ASD. This conclusion is also consistent with neuroimaging data indicating widespread brain abnormalities in ASD that include increased total intracranial volume (Courchesne et al., 2007), abnormal gray matter (Waiter et al., 2004; McAlonan et al., 2005), altered white matter (Barnea-Goraly et al., 2004; Billeci et al., 2012), and disrupted anatomical functioning (Belmonte and Yurgelun-Todd, 2003; Hubl et al., 2003).

Abnormalities in symmetry in ASD children were also found in the study of Stroganova et al. (2007). They acquired EEGs in open-eyes condition, in a large group of high-functioning children with ASD and in age-matched controls. PSD was calculated for delta, theta, and alpha frequency bands and BSI was computed. Atypical EEG asymmetry in children with ASD was found. In particular: (1) a broad-band leftward EEG asymmetry at the temporal and some adjacent regions that was absent in controls and (2) a symmetrically distributed mu rhythm in ASD across central sites of the left and right hemisphere, whereas in controls it was lateralized to the left. The first result could reflect structural asymmetries in the brain, although research on this issue is still inconclusive. For example, a decrease of deep white matter predominantly in the right hemisphere of ASD individuals has been highlighted in two recent studies (Boddaert et al., 2004; Waiter et al., 2005). However, further research is needed to adequately correlate structural and neurophysiologic data in ASD. Concerning the second result, the asymmetric distribution of mu is known to be linked to motor function and could mean that there is a greater down regulation of sensorimotor areas of the left hemisphere, involved in the control of the dominant right hand. On the contrary, the symmetrical distribution may be linked to a decreased control of motor function of the right hand.

Coben et al. (2008) demonstrated how the closed-eyes condition might cause changes in PSD and in coherence. In this study EEG was acquired on high-functioning children with ASD and controls and absolute and relative PSD for each band were computed. Moreover, intra-hemispheric and inter-hemispheric coherences were calculated. Opposite results in delta and beta band spectra with respect to the open-eyes condition were detected: in fact, a reduction in absolute and relative delta and an increase in absolute beta and relative theta distinguished ASD subjects from controls.

Also the coherence analysis revealed opposite results with respect to the open-eyes condition. In this study ASD subjects showed reduced intra-hemispheric as well as inter-hemispheric coherence in particular in the delta and theta band. Moreover they displayed lower inter-hemispheric coherences in the delta and theta bands in frontal regions, lower coherences in the delta, theta, and alpha bands in temporal regions and lower coherences in delta, theta, and beta bands in the central/parietal/occipital regions. The large amount of significant differences in coherence values in several brain regions, suggests altered connectivity in ASD (Belmonte et al., 2004).

Murias et al. (2007) have studied the eye-closed condition by using a high-density EEG system (124 electrodes) in adults with ASD. Indeed, a high-density approach, by employing more number of electrodes, allows increasing spatial resolution of the EEG potentials and improving signal source localization. A spectral analysis as well as a coherence analysis was performed. In

Table 1 | Summary of QEEG studies in autism.

Study	Design	Participants	Measures	Results in ASD
QEEG DURING REST				
Cantor et al. (1986)	10/20 System, open eyes	$n = 11$ ASD (age: 4–12 years, IQ = 37.45 ± 11.4) $n = 88$ TD (age: 5–15 years, IQ = 113.35 ± 9.5) $n = 18$ Intellectually disabled (age: 5–15 years, IQ = 71.1 ± 12.6) $n = 13$ TD toddlers (age: 16 months to 5 years, IQ = 121.0 ± 25.4)	PSD, coherence, symmetry	Higher percent delta and less alpha; higher coherence between and within, hemispheres; less asymmetry
Chan et al. (2007)	10/20 System, open eyes	$n = 66$ ASD (age: 5–18 years, TONI = 83.36 ± 21.61) $n = 90$ TD (age: 6–12 years, TONI = 111.42 ± 16.16)	PSD	Higher absolute delta and lower relative alpha; same results for all channel
Stroganova et al. (2007)	32 or 24 Electrode system, open eyes	$n = 40$ ASD (age: 3–8 years, IQ = 111 ± 8.29) $n = 40$ TD (age: 3–8 years)	PSD, asymmetry	Higher prefrontal delta; leftward asymmetry at the temporal regions; symmetric mu rhythm in ASD across central sites
Coben et al. (2008)	10/20 System, closed eyes	$n = 40$ ASD (age: 6–11 years, IQ = 93 ± 16.8) $n = 40$ TD (age: 6–11 years, IQ = 98 ± 15.4)	PSD, coherence	Less absolute (left frontal and posterior region) and relative delta (left frontal and vertex regions) and higher absolute beta (midline regions) and relative theta (right posterior regions); less delta and theta intrahemispheric coherence; less inter-hemispheric coherences in delta and theta in frontal regions in delta, theta, and alpha in temporal regions and in delta, theta, and beta bands central/parietal/occipital regions
Murias et al. (2007)	128 Channels, closed eyes	$n = 18$ ASD (age: 18–38 years, IQ = 107.33 ± 13.96) $n = 18$ TD (age: 18–38 years, IQ = 106.11 ± 13.56)	PSD, coherence	Higher relative theta in primarily frontal and prefrontal regions, less relative alpha in primarily frontal/prefrontal and occipital/parietal regions and higher relative beta in occipital/parietal regions; higher coherence in theta and less coherence in alpha
Pop-Jordanova et al. (2010)	10/20 System, open eyes and closed eyes	$n = 9$ ASD (age: 3–6 years) database of TD	PSD, brain rate	Higher delta/theta; higher beta in open eyes than in closed eyes; reduction of brain rate in all regions
Mathewson et al. (2012)	128 Channels, open eyes and closed eyes	$n = 15$ ASD (age: 19–52 years, IQ = 100.9 ± 18.6) $n = 18$ TD (age: 18–38 years, IQ = 107.1 ± 11.9)	PSD, coherence, correlation with AQ	Higher alpha in eye opens; less alpha suppression in 01; no difference in coherence; negative correlation between alpha and preferential attention to detail in posterior and frontal regions both in eye open and eye closed; negative correlation between attention to details and coherence in eye opened in the right centro-parietal region and in eye closed in the parieto-occipital regions; negative correlation between alpha coherence in eye-open and social functioning in the right fronto-central region; positive correlation between theta coherence in the left centro-parietal region in eye-closed and social functioning
Sheikhani et al. (2007)	10/20 System, closed eyes	$n = 10$ ASD (age: 9.3 ± 1.8 years) $n = 7$ TD (age: 9.2 ± 0.7 years)	PSD (STFT and STFT-BW) and bispectrum	No differences in STFT or bispectrum; significant differences in STFT-BW over Fp1, F3, F7, T3, T5, and O1

(Continued)

Table 1 | Continued

Study	Design	Participants	Measures	Results in ASD
QEEG DURING REST				
Ahmadlou et al. (2010)	10/20 System, closed eyes	$n = 9$ ASD (age: 6–13 years) $n = 6$ TD (age: 7–13 years)	Fractal dimension (HFD and KFD)	Significant difference in HFD in gamma, beta, and alpha and in KFD in gamma, beta, and delta
Thatcher et al. (2009)	10/20 System, open eyes	$n = 54$ ASD (age: 2.6–10.74 years) $n = 241$ TD (age: 2.2–11 years)	Phase synchronization analysis	Shorter phase shift durations in ASD in the alpha-1 frequency band (8–10 Hz); longer phase lock durations in ASD in the alpha-2 frequency band (10–12 Hz) and; differences in short and long inter-electrode pairs
Bosl et al. (2011)	64 Channels, open eyes	$n = 46$ HRA (age: 6–24 months) $n = 33$ TD (age: 6–24 months)	Multiscale Entropy (MSE) analysis	Reduced MSE in HRA subjects especially in 9–24 months range; discrimination between HRA and controls at 9 months with 80% of accuracy
Duffy and Als (2012)	32 Channels, open eyes	$n = 463$ ASD (age: 1–18 years) $n = 571$ TD (age: 1–18 years)	Coherence	High classification success between ASD and TD groups. decrease in short–distance coherence and increase in long-distance coherence in ASD group within a wide spectral range
QEEG DURING SPECIFIC TASKS				
Oberman et al. (2005)	10/20 System, tasks: (1) moving their own hand, (2) watching a video of a moving hand, (3) watching a video of two bouncing balls (non-biological motion), and (4) watching visual white noise	$n = 10$ ASD (age: 9–14 years, IQ > 80) $n = 10$ TD (age: 9–14 years)	PSD mu	Decreased mu only during the self-initiated hand movement
Orekhova et al. (2007)	10/20 System, sustained visual attention	$n = 40$ ASD (age: 3–8 years) $n = 40$ TD (age: 3–8 years)	PSD in high frequency bands	Higher power especially in gamma1 in midline, central, and parietal regions
Sheikhani et al. (2009)	10/20 System, tasks: (1) eye-closed condition, (2) eye-opened condition, (3–5) looking at three samples of Kanizsa shapes, (6) looking at mother's picture upright and (7) inverted, (8) looking at stranger's picture upright, and (9) inverted in frequency bands	$n = 15$ ASD (age: 6–11 years, IQ > 85) $n = 11$ TD (age: 6–11 years, IQ > 85)	PSD, spectrogram	Lower spectrogram criteria at Fp1, Fp2, and T6 in gamma and higher spectral power at FP1 and FP2 in open-eyes condition; difference in alpha at T3, F7, and C3 in looking at the inverted mother's picture; difference in alpha and beta at F7, F4, F8, C4, Pz. In looking at a stranger's picture inverted
Sheikhani et al. (2012)	128 Channels (reduced to 10/20), sustained visual attention	$n = 17$ ASD (age: 6–11 years, IQ > 85) $n = 11$ (age: 6–11 years, IQ > 85)	Spectrogram criteria	Lower spectrogram criteria in alpha, beta, and gamma especially in temporal and frontal regions in left hemisphere
Chan et al. (2011a)	Object recognition (OR) task	$n = 21$ ASD (age: 5–14 years, TONI = 101.86 ± 16.09) $n = 21$ TD (age: 5–14 years, TONI = 106 ± 14.59)	Coherence in theta	Elevated fronto-posterior coherences in left hemisphere; higher coherence in the left than in the right hemisphere; negative correlations between memory performance and the inter-hemispheric coherence

(Continued)

Table 1 | Continued

Study	Design	Participants	Measures	Results in ASD
QEEG DURING SPECIFIC TASKS				
Chan et al. (2011b)	10/20 System, Go/No-Go task	$n = 20$ ASD (age: 7–14 years) $n = 20$ TD (age: 7–14 years)	PSD in theta source localization with LORETA	In the "Go" condition theta decreased in the anterior cingulate cortex (ACC), in the "No-Go" condition in the ACC and in the precuneus
Lushchekina et al. (2012)	10/20 System, tasks: (1) eye-closed condition, (2) counting during eye closed	$n = 27$ ASD (age: 5–7 years) $n = 19$ TD (age: 5–7 years)	PSD, coherence	Higher gamma in baseline condition; right-sided predominance of spectral power in alpha both at rest and during counting
Catarino et al. (2011)	10/20 System, detection tasks: (1) faces, (2) chairs	$n = 15$ ASD (age: 23.79–42.34 years, IQ = 119 ± 13) $n = 15$ TD (age: 21.50–37.77 years, mean IQ = 119 ± 14)	MSE, PSD	Reduced MSE in ASD especially in parietal regions; higher MSE in response to faces in both groups; no differences in PSD
QEEG FOR THE IDENTIFICATION OF AUTISTIC SUBGROUPS				
Dawson et al. (1995)	10/20 System, open eyes	$n = 28$ ASD (age: 5–19 years, IQ = 119 ± 13) $n = 28$ Chronological-age-matched TD (age: 5–19 years) $n = 24$ Language-age-matched TD (age: 2–7 years)	PSD	Reduced delta and theta in the passive group in all brain regions and reduced alpha in the frontal regions
Sutton et al. (2005)	10/20 System, open eyes and closed eyes	$n = 23$ ASD (age: 9–14 years, IQ: 110.13 ± 21.21) $n = 20$ TD (age: 9–14 years IQ: 116.80 ± 11.69)	PSD	Higher alpha in anterior, central, and posterior cortical regions; more left-sided mid-frontal and central regions; subgroups with greater left-sided mid-frontal activity had greater social anxiety, greater general anxiety, greater social stress, and less satisfaction with interpersonal relations

ASD, autism-spectrum disorder; TD, typical developing; HRA, "high risk" of autism.

this experiment, ASD group showed an elevated relative theta and reduced relative alpha power primarily in the frontal and prefrontal regions. In addition, a reduced relative alpha and increased relative beta power was observed in the occipital/parietal regions, with bilateral central regions approaching significance. Significant differences in coherence analysis between the two groups were also observed in theta and alpha bands. The results of this study are in agreement with the theory of local overconnectivity and global under-connectivity in ASD (Courchesne and Pierce, 2005). In fact EEG oscillations in the theta range reflect locally dominant neocortical processes, whereas alpha oscillations represent more globally dominant phenomena that are more dependent on cortico-cortical and callosal fibers (Nunez, 2006).

In a more recent study (Pop-Jordanova et al., 2010) both the open-eyes and the closed-eyes conditions were investigated. In this study EEG data obtained on ASD children were compared to data belonging to neurotypical subjects contained in a database. The authors found an increase delta/theta power in ASD in both conditions. Moreover they noticed that in the open-eyes condition there was an increase in beta power with respect to the closed-eyes condition in both groups. These results were only partially in agreement with the previous studies. The authors also have introduced here a new index, namely the spectrum weighted frequency (brain rate), as an indicator of general mental arousal in these subjects. They found a reduction of brain rate in all regions in autistic children compared to the controls, indicating a lower general mental arousal in ASD.

Also in the most recent study by Mathewson et al. (2012) where QEEG technique was applied in the study of high-functioning adults with ASD, both the open-eyes and the closed-eyes condition were analyzed. The novelty of this study is that the features extracted by QEEG analysis related to power and frequency, were correlated to behavioral performances measured with the Autism-Spectrum Quotient (AQ; Baron-Cohen et al., 2001). In particular in this study the scores obtained with this instrument were clustered in two groups: preferential attention to detail and deficits in social interaction. EEG data were continuously recorded by means of a 128-channel system during a resting baseline condition

in which eyes-open and eyes-closed conditions alternated. The absolute PSD was calculated for each band separately at each single electrode, however, in order to reduce the computational complexity, power was averaged within four quadrants: left frontal, right frontal, left posterior, and right posterior. The main focus of the study was alpha band because it is thought to indirectly reflect the level of cortical excitability in the regions where it is found. It means that higher resting alpha power might denote cortical deactivation or inactivity (Rihs et al., 2007; Sauseng et al., 2009). Power analysis showed that in the eye-open condition the alpha power was higher in ASD group than in the controls in all regions, while in eye-closed condition no difference was found.

Regarding the other bands, beta, gamma, and theta powers were increased in ASD group. An analysis of the amount of alpha suppression in occipital regions was also performed. The results indicated that at O1 a significantly greater alpha suppression was present in control subjects than in ASD group. This difference in suppression is related to the fact that alpha suppression is associated with optimal neurological functioning that is impaired in ASD. In contradiction with the previous studies, no differences in coherence between the two groups were found.

While correlating the SP with the AQ score it was found that in ASD participants, alpha power was inversely correlated with preferential attention to detail in posterior and frontal regions both in eye-open and eye-closed condition, while no correlation was found in controls. From the coherence analysis, a positive correlation between alpha coherence in eye-closed condition and attention to details was found in the right centro-parietal region in controls, while in ASD group, attention to details was inversely correlated with coherence in eye-opened condition in the right centro-parietal region and in eye-closed condition in the parieto-occipital regions. Moreover in both groups alpha coherence in eye open was inversely correlated to social functioning in the right fronto-central region. For the other bands, increased theta coherence in the left centro-parietal region in eye-closed condition appeared to be related to poorer social functioning in ASD, while increased gamma coherence in the same region and condition appeared to be beneficial to social functioning in controls. The negative correlation between attention to detail and alpha power and coherence in parietal regions in ASD is consistent with the theory of altered parietal functioning in ASD. An explanation of this result could be that in ASD the mapping of attention focus may be exaggerated at the expense of attention prioritizing. Moreover the negative correlation between alpha coherence and attention to details is consistent with the fact that the brain is more receptive to incoming sensory information when neural activity is desynchronized than when it is engaged in performing a specific task.

In a few articles non-linear analysis techniques have been used to study particular properties of EEG signals. In the study of Sheikhani et al. (2007) the authors computed three types of transforms at each electrode for discriminating between ASD and controls: the STFT, the STFT-BW calculated considering the bandwidth of all the spectra as window, and the bispectrum. Data were acquired using a standard 10/20 system during eye-closed condition. The authors did not find any difference in STFT or bispectrum but they found significant differences in STFT-BW over Fp1, F3, F7, T3, T5, and O1. Since the sample of ASD and controls is very small in this study, it is difficult to draw any conclusions from the results. The bispectral analysis needs to be tested in a larger sample of subjects to see if it gives significant results and relevant information on brain activation not evidenced by the most common power spectral analysis.

In another study by Ahmadlou et al. (2010), the complexity of EEG signals in ASD and controls was investigated by using fractal dimension analysis. Data were acquired during eye-closed condition and a wavelet analysis approach aimed at decomposing the signal into the five standard EEG bands was performed. For each band and electrode the fractal dimension was computed using two methods: Higuchi's Fractal Dimension (HFD) (Higuchi, 1988) and Katz's Fractal Dimension (KFD) (Katz, 1988). Significant differences between ASD and controls in HFD were detected, especially in gamma, beta, and alpha and in KFD in gamma, beta, and delta. Differences were most significant for KFD, meaning that this method is a more effective tool for discriminating between ASD and controls. This study shows how fractal dimension, providing additional information about EEG signals, could be an important instrument for the identification of brain abnormalities in ASD.

Thatcher et al. (2009) applied phase analysis for the investigation of phase-reset mechanism in high-functioning children with ASD. Phase reset (PR) is defined as the succession of phase shifts (e.g., 30–80 ms) and phase locking (e.g., 100–800 ms) of clusters and sub-clusters of neurons. This process is regulated by GABA mediated thalamo-cortical circuits that is believed to be compromised in ASD (Orekhova et al., 2008). EEG data were acquired on ASD subjects and age-matched controls during eyes-open resting condition. Complex demodulation was used to compute phase differences between the signals from each pairs of electrodes. Each PR was composed by a phase shift of a finite duration (SD) and a phase locking of an extended duration (LD). SD is the interval of time from the onset of phase shift to its termination, defined by a peak in the first derivative and a peak in the second derivative or inflection on the declining side of the time series of first derivatives. LD was defined as the interval of time between the end of a significant phase shift and the beginning of a subsequent significant phase shift. Both in ASD and in controls SD, LD, and PR were computed for each pairs of electrodes in each EEG band. The comparison between the two groups showed that SD was significantly shorter in ASD than in the controls in particular in alpha-1 frequency band (8–10 Hz). On the contrary, LD was consistently longer in ASD especially in alpha-2 frequency band (10–12 Hz). The results of this study demonstrate an altered mechanism of neural synchronization in ASD, and suggest that increased phase lock periods, that represent the time in which clusters of neurons are synchronized, could reflect less cognitive flexibility and less availability of neural resources. However, higher statistically significant differences were found at short rather than long inter-electrode distances, suggesting that the defect in the mechanism of phase locking is particularly present in local neural.

In a recent study by Bosl et al. (2011) a non-linear analysis based on the calculation of MSE was performed. The analysis was applied to EEG signals acquired on a group of infants at "High Risk" because siblings of children with ASD. Data were obtained during resting state eyes open using a 64 channels EEG system. For

a more accurate comparison, the sample set was divided in subgroups according to age: 6, 9, 12, 18, and 24 months. The authors found a decrease of MSE in HRA over all EEG channels across all scales and ages. When considering the trajectories of mean MSE with age, it was shown that while the pattern of complexity was almost the same in the two groups in the age range 6–9 months, there was a strong decrease in EEG complexity in the HRA groups in the age range 9–12 months. Several classification approaches were then applied to distinguish between the groups of controls and HRA subjects according to the EEG complexity features. The machine learning approach represents an accurate classification method, especially in the first year of life of the children. As underlined by Griffin and Westbury (2011), in their commentary to the article of Bosl et al. (2011), these results should be viewed with caution because they identify an alteration of MSE in the whole population of HRA subjects without differentiating between the ones who will really develop autism and the ones who will have a typical development or other neuropsychiatric disorders. However, this study could be a promising starting point to carry out further investigation in order to highlight the differences in EEG complexity at the individual level, possibly helping to predict the risk of developing autistic traits.

Another interesting attempt in differentiating ASD and controls group based on a data-driven approach has been recently performed by Duffy and Als (2012). In this study, EEG data were recording on a large sample of ASD and TD subjects during awake state and coherence values were measured for each EEG frequency band.

In a first step, the dimensionality of the coherence data was reduced by applying a Principal Component Analysis (PCA) based on a Singular Value Decomposition (SVD) approach (SVD-based PCA). In the following step a classification algorithm, in particular the Discriminant Function Analysis (DFA), was applied to the variables selected by PCA. This technique allows producing a new canonical variable, the discriminant function, which is based on a weighted combination of the input variables and allows maximally separating the ASD and TD groups.

Given the wide age range of subjects, the classification analysis was applied also to subset of subjects with a narrower age span. The coherence factors given by DFA analysis allowed to accurately classifying ASD and TD across all three age spans. Moreover, it was observed that 70% of the factors were associated with reduced coherence for ASD subjects, in particular in the left temporal regions and frontal (short–distance connections). The decreased connectivity within these regions could be associated to language and communication problems. The other coherence factors showed an increased coherence in ASD subjects in particular in the long-distance connection. This finding could be explained as a compensatory mechanism of the autistic brain which establish atypical, spatially disparate, cortical networks to replace deficit function normally associated to more localized network. These results were quite stable across all broad spectral ranges.

The results of this study are very encouraging because it seems that the coherence factors could be used as a possible useful neurophysiological ASD-phenotype.

QEEG DURING SPECIFIC TASKS

The application of QEEG processing technique during cognitive tasks can give the possibility to view the dynamic changes which take place in the brain during these conditions, determining in this way which areas of the brain are engaged. Although in the past some researchers have considered that EEG signals acquired during task conditions are destabilized or otherwise corrupted (Thatcher, 1998), recent researches have challenged this conclusion. For example, McEvoy et al. (2000) have demonstrated greater stability of QEEG signals recorded during cognitive tasks, with respect to the resting condition and therefore paving the way for developing task-specific understandings of brain operation.

Oberman et al. (2005) have analyzed the mu (8–13 Hz) power, an index of neuron synchronization or desynchronization, over the sensorimotor cortex during imitation tasks. At rest, sensorimotor neurons spontaneously fire in synchrony, leading to large amplitude EEG oscillations and to elevated power in mu frequency band. Conversely, during action, these neurons fire asynchronously, and therefore the power in mu band decreases. In this investigation, EEG from high-functioning ASD and controls were recorded during observation of biological and non-biological motion. While controls showed a decreased mu power both in self-initiated hand movement and in observed biological motion conditions, the ASD group obtained the same effect only during the first task, suggesting mirror neuron dysfunction in autism.

In fact, sensorimotor neurons could be considered as belonging to the well-known mirror neurons system (Rizzolatti et al., 2001). Several studies have related the imitation deficit in subjects with autism to an impairment of this neural circuitry (Williams et al., 2001; Nishitani et al., 2004; Iacoboni and Dapretto, 2006). Nevertheless, the findings of some recent studies argue against a mirror system dysfunction in ASD (Dinstein et al., 2010; Fan et al., 2010).

Sheikhani et al. (2012) used the spectrogram method to analyze data acquired on a group of children with ASD and age-matched controls during sustained visual attention. The spectrogram criteria – defined as the average of all the frequency component values of spectrogram >70% of the maximum value for each frequency band – as well as the coherence between pairs of different electrodes were computed. The ASD group exhibited lower values of spectrogram criteria in alpha, beta, and gamma bands, whereas no significant difference was observed in the delta band. According to these results, EEG signal show the most significant differences in the temporal and in the frontal regions of the left brain hemisphere. These results agree with several studies showing an impairment of left hemisphere, in particular in temporal and frontal regions, in ASD (Rojas et al., 2002, 2005; Chandana et al., 2005). The authors also showed an increase in the degree of coherence in the ASD group, and suggested increased functional connectivity of temporal lobes with other regions in the gamma band frequency.

In another investigation of the same authors (Sheikhani et al., 2009), children with ASD and controls underwent EEG acquisition in nine different conditions: [(1) eye-closed condition, (2) eye-opened condition, (3–5) looking at three samples of Kanizsa shapes, (6) looking at mother's picture upright and (7) inverted, (8) looking at stranger's picture upright, and (9) inverted in

frequency bands]. Spectrogram and PSDs were calculated for each band at each condition. In the relaxed eye-opened condition, children with ASD obtained significant differences in gamma band with lower values of spectrogram criteria and higher values of SP. Spectrogram criteria were also significantly different in the alpha band when ASD and control children looked at the inverted mother's picture, and in alpha, beta, and gamma bands, when they looked at an inverted stranger's picture. Given that gamma band seems to play a role in the synchronization of cortical nets region, especially in recognition and perception, the authors suggested an abnormal functioning on these issues in ASD. Furthermore, since the alpha band is associated with the coordination of wider areas of the brain, and beta band plays a role in integrating neighboring areas, the abnormal spectrogram criteria found in this study might suggest a defect of coordination and integration in ASD.

In a recent study (Chan et al., 2011a), QEEG techniques were employed in order to examine the association between memory performance and fronto-posterior theta coherence in individuals with ASD. Several studies have found associations of theta-band amplitude with the performance of working memory tasks (Klimesch et al., 1994) and long-term memory encoding and retrieval (Larson et al., 1986). Moreover, basic research in animals and neuroimaging studies in humans have shown that during working memory tasks multiple brain areas are activated, in particular the prefrontal and postrolandic association cortices as well as the cingulate cortex and medial temporal areas (Fuster, 1995; Krause et al., 2000; Postle and D'Esposito, 2000). Given these findings, it has been suggested that connectivity abnormalities in these brain areas could be the neural bases of memory deficits in autism (Rippon et al., 2007). In the study by Chan et al. (2011a), EEG data were recorded during an object recognition task. ASD individuals showed elevated fronto-posterior long-range theta coherences, both intra-hemispheric (in the left hemisphere) and inter hemispheric (from left anterior to right posterior regions). Moreover, an opposite asymmetry pattern was observed: coherences in controls were higher in the right than in the left hemisphere, while in ASD children the pattern was opposite. A significant negative correlation between memory performance and inter-hemispheric long-range coherence was present in ASD subjects, whilst no significant correlations were found in controls. The abnormal pattern in ASD children could be explained with a hyper-functional connectivity in theta band with respect to controls that decrease the efficiency of memory processing.

In another study by the same research group (Chan et al., 2011b) the association between the performance of children with ASD in attention and inhibitory control and brain activity was investigated. The analysis was focused on relative PSD within theta band, which is related to attentional and inhibitory processing during a Go/No-Go task.

The authors found a decrease of theta activity in ASD children with respect to controls in the anterior regions for the "Go" and in anterior and centrotemporal regions for the "No-Go" condition. The application of LORETA software allowed a more accurate source localization ad it showed that in the "Go" condition theta decreased occurred in particular in the anterior cingulate cortex (ACC), while in the "No-Go" condition in the ACC and also in the precuneus. Significant correlations were found between theta power and scores obtained at the several tests performed showing an association between depressed brain activity, in particular in the ACC, and poorer performance in attention and inhibition.

Some authors have outlined the importance of studying the high EEG frequencies in order to characterize brain activity in ASD. The paper from Orekhova et al. (2007) aims at analyzing the differences between controls and ASD in high frequency EEG bands. EEG activity was recorded in young children with autism and age-matched controls during sustained visual attention. The mean PSD was calculated in three high frequency bands: beta (13.2–24 Hz), gamma 1 (24.4–44.0 Hz), and gamma 2 (56.0–70 Hz). An enhancement of spontaneous high frequency EEG oscillations in ASD was found, especially in gamma 1 band. The most involved brain areas were the midline, central, and parietal regions. Moreover, a significant positive correlation between the power spectrum value of gamma 1 and the degree of developmental delay in ASD group was detected. The excess of high frequencies in ASD agrees with the theory of an increase in ratio of excitation/inhibition in autism that leads to the formation of "noisy" and unstable cortical networks (Rubenstein and Merzenich, 2003).

In the most recent study (Lushchekina et al., 2012), the authors have tried to identify the neurophysiological components of cognitive abnormalities in ASD. EEG recordings, made in the standard 10/20 scheme were performed at baseline (rest with closed eyes), and during a cognitive task, consisting of counting, adding, and subtracting. SP and mean coherence were studied in the alpha, beta, and gamma ranges. Both typical and ASD subjects showed a marked frontal-occipital alpha gradient in baseline conditions. ASD individuals were characterized by right-sided predominance of PSD in the alpha range, both at rest and during cognitive tasks. In addition, in ASD the PSD of the gamma rhythm in baseline conditions was higher than that in the controls. During the cognitive task in ASD group the SP and mean coherence of fast rhythms did not change.

Another study (Catarino et al., 2011) analyzed EEG data acquired from adult ASD individuals and controls during a visual task (pictures of neutral faces versus pictures of chairs); a complexity analysis was performed using the MSE measure already described (Bosl et al., 2011). The task consisted in the detection of pictures of neutral faces and of chairs. Both a MSE investigation and a more traditional power spectral analysis were performed for each group and condition. While no differences were found in PSD, the authors demonstrated reduced entropy in ASD with respect to controls, especially at higher time scales, confirming that the decrease of MSE can be associated to impairments in brain function and connectivity.

QEEG FOR THE IDENTIFICATION OF AUTISTIC SUBGROUPS

Clinical observation as well as research data, suggest that ASD are a set of neurodevelopmental disorders with a considerable heterogeneity in the phenotypic presentation (Witwer and Lecavalier, 2008; Georgiades et al., 2013). Among the several methods used to stratify ASD subjects into more homogeneous subgroups, the QEEG may provide more objective and quantitative features characterizing different groups of affected individuals. However, despite the fact that QEEG approach seems very promising, only few studies have so far been directed to this end.

The first investigation on this topic (Dawson et al., 1995) showed how QEEG is not only useful in differentiating subjects with high-functioning ASD from controls, but also in distinguishing amongst subgroups which differ in degree and nature of social impairments. Twenty-eight children with autism were classified according to Wing and Gould (1979) classification system: "Aloof," "Passive," and "Active-but-odd." In particular, the authors studied the "passive" and the "active-but-odd" groups. EEG was acquired during sustained visual attention, and the PSD were calculated for each band. The passive group showed reduced EEG power in delta and theta bands in all brain regions and reduced alpha power in the frontal regions. Since the alpha activity is related to social engagement, a reduced alpha activity could reflect, in this "passive" group, a lack of active engagement in social information processing.

A subsequent study by Sutton et al. (2005), QEEG analysis was performed in order to correlate resting cortical brain activity with social-emotional abilities behaviors in high functioning children with autism (HFA).

Data were acquired on high-functioning ASD children and controls during eyes-opened and closed conditions. The analysis focused on the alpha band, due to its stronger relation with behavioral measures with respect to other frequency bands. Moreover an asymmetry index was calculated for homologous electrode pairs and was used for subgrouping autistic children. Three subgroups were obtained using computer-generated cut points: (1) the most extreme right mid-frontal asymmetry scores (RFA group), (2) the most extreme left medial frontal asymmetry scores (LFA group), and (3) the intermediate frontal asymmetry (IFA group A reduction of alpha power density in anterior, central, and posterior cortical regions of control individuals compared with HFA subjects was detected. Moreover, the HFA group showed a different asymmetry pattern compared to the control group. Finally, some brain-behavior relationship were found: in particular, the LFA group reported greater symptoms of anxiety and social stress, while the RFA group was characterized by a greater social impairment.

DISCUSSION AND CONCLUSION

This review focuses on key findings of quantitative EEG application in subjects with ASD. Despite conflicting results, literature analysis suggests that QEEG may help in detecting features of altered brain function, in linking behavior with brain activity and in defining more phenotypically homogeneous subgroups within the affected individuals.

Taken together, reviewed studies show that children with ASD present several differences in power spectra, coherence, and symmetry measures with respect to controls. This is true both when the signals are acquired in resting conditions – with either open or closed eyes – and when specific tasks are performed. However, QEEG features strongly depend on the diverse experimental settings (for example EEG recorded during observation of actions or during execution of actions) that may lead to different results. In addition, most parameters such as power spectra, coherence, and asymmetry, change with age and may vary according to behavioral, cognitive, and comorbid features of ASD subjects. The wide heterogeneity of the samples examined in the literature, particularly with regard to the cognitive level and age of subjects, and the different criteria used to diagnose ASD, makes it difficult to compare these studies and achieve unique general conclusions. In addition, drugs could influence the EEG activity (Muroka et al., 1992; Banoczi, 2005) with a potential impact on brain developmental process, especially in the frontal regions, which are the slowest in maturating. While some studies are more restrictive in defining the exclusion criteria of the sample, avoiding the enrollment of subjects taking medication, others do not define strict exclusion/inclusion criteria, or at least there are not clearly mentioned in the participant description. Thus, the variability in medication use across studies may be responsible for mixed findings in the literature reviewed. A possible role of immune dysregulation, toxicant exposures, and metabolic factors on the development of ASD abnormalities has been suggested (for a recent review, see Rossignol and Frye, 2012). However, the evaluation of these issues are often not specified or considered in the studies examined, potentially accounting for variation across studies and within subjects.

In open-eyes condition, the differences between ASD and TD are more pronounced. Studies performed in an eye-open rest condition present some replicated finding, i.e., the constant increase on delta power in ASD with respect to controls and the decrease in high frequency, especially alpha in childhood and adolescents (Cantor et al., 1986; Chan et al., 2007; Stroganova et al., 2007; Pop-Jordanova et al., 2010), but also some contradictory results. The power of alpha band in ASD, with respect to typical controls, was found to be reduced (Cantor et al., 1986; Chan et al., 2007), unchanged (Stroganova et al., 2007), or even increased (Mathewson et al., 2012) in different studies. A greater level of alpha amplitude reflects the inhibition of non-essential activity and consequently a better performance on the task (Klimesch et al., 2007) that could be explained by the *neural efficiency hypothesis* (Doppelmayr et al., 1998).

Also, the degree of asymmetry was found either broad-band decreased in ASD (Cantor et al., 1986) or leftward increased (Stroganova et al., 2007). This latter study also showed a symmetric mu rhythm, which was paradoxically asymmetric in healthy controls. The analysis of coherence between and within hemispheres in ASD subjects revealed an increased (Cantor et al., 1986) or reduced finding (Mathewson et al., 2012).

Several demographic and clinical differences characterize the samples involved in the above-mentioned studies, which may in part explain the conflicting results. In particular, the age range varies among the studies considered: young children (Cantor et al., 1986; Stroganova et al., 2007) children and adolescents (Chan et al., 2007), and adults (Mathewson et al., 2012) were respectively enrolled. Moreover, the cognitive level of ASD children displays a wide range of abilities: low functioning (Cantor et al., 1986), both high and low functioning (Stroganova et al., 2007), and only high-functioning (Chan et al., 2007; Mathewson et al., 2012) ASD subjects were evaluated.

Since QEEG indices are related to brain maturation and development (Clarke et al., 2001), the age represents a critical factor in the interpretation of results.

In typical development children low frequencies tend to decrease with age from childhood to adulthood while high frequencies increase (Gasser et al., 1988a). As regards coherence

it broad-band increases with age (Gasser et al., 1988b). Autistic children seems to have a late maturation as they show more slow-wave and less alpha activity, as well as greater coherence than the age-matched typical controls. As coherence increases with age in TD subjects, in adulthood coherence values became comparable between ASD and TD groups (Murias et al., 2007; Mathewson et al., 2012).

In addition, EEG activity is influenced by level of cognitive abilities: in fact, Thatcher et al. (2005) showed that absolute EEG power is positively correlated with full scale, verbal, and performance IQ, while coherence is negatively correlated with these scores.

The comorbidity between ASD and other psychiatric or neurological disorders is a common feature of ASD clinical manifestation (Simonoff et al., 2008). The presence of an additional non-ASD disorder represents a potential confounding factor in EEG research that frequently hasn't been taken into account in the interpretation of results. Thus, future exploration into the EEG presentations of subjects with comorbid psychopathology versus ASD singly may be of seminal importance for a better knowledge of the ASD biological underpinnings.

In closed-eyes condition, results are even more contradictory both in terms of power and coherence (Murias et al., 2007; Coben et al., 2008; Pop-Jordanova et al., 2010; Mathewson et al., 2012). Except for the study of Pop-Jordanova et al. (2010), which do not define the cognitive level, all these studies enrolled high-functioning individuals. In addition, two studies (Murias et al., 2007; Mathewson et al., 2012) have been performed on adults, whereas one investigation (Coben et al., 2008) on scholar children and another (Pop-Jordanova et al., 2010) on pre-scholar children. This suggests again that the different age range of the subjects participating to the different studies may at least in part influence the results. Moreover, the different findings of Murias et al. (2007) and Mathewson et al. (2012) on adult subjects (with respect to both alpha power and coherence) may in part be due to the different methodological approach. By considering all the 128 electrodes in computing power spectrum and coherence, both a decrease in power and coherence in alpha band (Murias et al., 2007), and no differences (Mathewson et al., 2012) were detected. Therefore, the analysis of neural networks with higher spatial resolution seems to allow a thinner characterization of brain activation and connectivity. Source localization using software like LORETA used in the study by Chan et al. (2011b) can also be useful to increase the spatial resolution of EEG.

In closed-eyes condition the increase in slow-way activity is more related to theta than delta. Moreover there is an increase in beta frequencies from childhood to adulthood, which is not observed in open-eyes condition (Murias et al., 2007; Coben et al., 2008). Increase of beta activity is associated with a strengthening of sensory feedback in static motor control when movement has to be resisted or voluntarily suppressed (Lalo et al., 2007; Zhang et al., 2008). In children with ASD the increased beta activity in closed-eyes condition may reflect the difficulty in motor and sensorial regulation that they present in this situation.

With regards to coherence, it seems that increases with age such as controls: in fact, it is decreased in childhood and adolescence and become equal or increased in adulthood.

Although the exact meaning of changes in SP and coherence in ASD children is not easy to understand, in resting state condition, both dysfunction of general state of arousal or of more specific systems of cognitive processing may explain these findings. However, by correlating brain activity findings with behavioral measures, Mathewson et al. (2012) showed that cognitive function and modulation might influence QEEG also at rest.

Acquiring data while children perform specific tasks allows having a better characterization of the link between behavior and brain activation, although the possibility to drive definitive conclusions is limited due to the small number of studies and of sample size. Differences between ASD subjects and controls during tasks mainly involve high frequencies, alpha, beta, and gamma, which have been found increased (Orekhova et al., 2007; Sheikhani et al., 2009; Lushchekina et al., 2012) in ASD population, regardless of the type of task. Moreover some authors also found an increase in coherence during tasks, in ASD with respect to control, supporting the hypothesis of an enhanced functional connection between cortical networks (over-connectivity) at the basis of the aberrant behaviors observed in autism.

In literature, moreover, QEEG was used for subtyping ASD subjects (Dawson et al., 1995; Sutton et al., 2005), suggesting that some QEEG parameters may correlate with different behavioral phenotype The identification of subgroups of subjects with different QEEG profiles could contribute to increase the homogeneity of ASD samples, with the aim to detect specific developmental time course, treatment responses, and possibly pathophysiological underpinnings.

In addition, due to the fact that brain activation and QEEG measures are strictly dependent on age, it is very important evaluating developmental processes in autism. QEEG, in fact, may show different developmental patterns in infants with high and low risk for ASD, and could be therefore used as a promising endophenotype for early diagnosis in at-risk children (Tierney et al., 2012). Non-linear techniques, such as entropy (Bosl et al., 2011) have also been used at this end. This technique, like also fractal dimension or phase coupling (Sheikhani et al., 2007; Thatcher et al., 2009; Ahmadlou et al., 2010; Bosl et al., 2011), is appealing not only in order to characterize autistic brain, but also to obtain potential biomarkers of the disorder, not otherwise detectable with common linear methods.

Overall, it is important to underline that QEEG activity components may also have some individual characteristics that differentiate each subject. The assessment of these features has a crucial importance for establishing a QEEG "baseline," which may be different for each person.

New advanced analysis methods such as entropy or cluster analysis could be useful to identify autistic subgroups with specific neurophysiological characteristics, providing in this way different brain endophenotypes, which may benefit from different intervention strategies. Thus, this sort of metrics on the brain's function could be used, in the future, to develop personalized treatments (for example by using connectivity-guided neurofeedback), and evaluate the effects of therapies through quantitative measures of brain activity. Also in this case, source localization is extremely important: in fact, variation of brain activity in a specific brain area can be a quick and objective indicator to monitor the effect of the treatment.

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Heterogeneity within autism spectrum disorders: what have we learned from neuroimaging studies?

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Autism spectrum disorders (ASD) display significant heterogeneity. Although most neuroimaging studies in ASD have been designed to identify commonalities among affected individuals, rather than differences, some studies have explored variation within ASD. There have been two general types of approaches used for this in the neuroimaging literature to date: comparison of subgroups within ASD, and analyses using dimensional measures to link clinical variation to brain differences. This review focuses on structural and functional magnetic resonance imaging studies that have used these approaches to begin to explore heterogeneity between individuals with ASD. Although this type of data is yet sparse, recognition is growing of the limitations of behaviorally defined categorical diagnoses for understanding neurobiology. Study designs that are more informative regarding the sources of heterogeneity in ASD have the potential to improve our understanding of the neurobiological processes underlying ASD.

Keywords: autism, psychiatry and developmental disabilities, intellectual disability, functional magnetic resonance imaging, structural magnetic resonance imaging

INTRODUCTION

The Autism Spectrum Disorders (ASD) are a group of lifelong neurodevelopmental syndromes which manifest in early childhood, defined by the presence of difficulties with social interactions and communication together with restricted, repetitive patterns of interests or behaviors (American Psychiatric Association, 2013). The variety of clinical presentations considered to fall within autism has gradually increased over the past 60 years. Leo Kanner first used the diagnosis in 1943 to describe a relatively homogenous group of individuals with deficits in all three domains but intact intellectual capacity (Kanner, 1968). Work by Lorna Wing and others then went on to place Kanner's subgroup within a broader spectrum that shared some level of deficit in these core domains but was otherwise highly heterogeneous, including allowing a wider spread of cognitive function (Wing, 1981).

In the previous version of the Diagnostic and Statistical Manual IV (American Psychiatric Association, 2000), this heterogeneity was captured primarily through the different categorical diagnoses within the Pervasive Developmental Disorders (PDD). The PDD category included Autism, Asperger's Syndrome, and Pervasive Developmental Disorder not otherwise specified (PDD-NOS), as well as two regressive neurodevelopmental disorders of early childhood frequently associated with autistic symptoms, Rett's syndrome and Childhood Disintegrative Disorder. As research progressed, the clinical and neurobiological validity of the categorical distinctions between Autism, Asperger's Syndrome, and PDD-NOS appeared increasingly doubtful, until they were dropped altogether in the recently released DSM-5 (American Psychiatric Association, 2013). Instead, a broader ASD diagnosis with dimensional specifiers of severity has been adopted, and a new diagnosis of Social Communication Disorder added for those individuals with problems in social communication but without the symptoms in the domain of restrictive and repetitive behaviors (RRIB) required for an ASD diagnosis.

An explicit goal of the recent reformulation of the diagnostic criteria for ASD was to refocus attention on the aspects of heterogeneity within ASD that were likely to be more meaningful than the previous categorical divisions, both clinically and in relation to underlying pathophysiology. This shift in conceptualization of ASD has occurred within a larger context of increasing dissatisfaction with existing diagnostic categories for a variety of psychiatric syndromes, particularly when seeking to link clinical symptoms to specific neurobiological processes (Insel et al., 2010; Lord and Jones, 2012; Uher and Rutter, 2012).

Heterogeneity of clinical presentation among individuals who meet criteria for a specific diagnosis is a particular impediment. One contributor to this heterogeneity is the checklist approach currently used to assign categorical diagnoses. While allowing a good level of reliability between diagnosticians, it has the unfortunate effect of allowing different individuals to meet the threshold for a particular syndrome without necessarily sharing many specific clinical features. A second contributor to heterogeneity of clinical presentations within a particular syndrome such as ASD is the extensive comorbidity between psychiatric diagnoses (Matson and Williams, 2013). It has been estimated that between 14 and 78% of children with ASD also meet criteria for Attention Deficit and Hyperactivity Disorder (ADHD) (Gadow et al., 2005; Gargaro et al., 2011), up to 42% for anxiety disorders (Simonoff et al., 2008; Matson and Williams, 2013), and between 25 and 70% have some level of intellectual disability (ID) (Fombonne, 2009). There are a wide variety of other symptoms not considered as "core" but which are nevertheless prominent in sizeable fractions

of individuals with ASD, such as poor attention, seizure disorders, poor sleep, and gastro-intestinal dysfunction (Silver and Rapin, 2012). How these symptoms relate to the pathophysiology of ASD is not clear, but their frequency is suggestive that they may represent at least in part pleiotropic expressions of common processes (Brock, 2011; Rommelse et al., 2011).

In addition to the clinical heterogeneity within ASD, it is associated with a wide variety of different risk factors, implying the potential for many different pathways to generate symptoms (Geschwind, 2009; Herbert, 2010). ASD has been associated with over 100 different genes affecting different aspects of neural development and function (Betancur, 2011), as well as a wide variety of environmental factors (Herbert, 2010). Attempting to understand the links of specific risk factors to autism is further complicated by the different ways risk factors can relate to clinical phenomena, described as *equifinality* and *multifinality* (Cicchetti and Rogosch, 1996). Equifinality refers to the observation that a single clinical syndrome may be associated with many different risk factors. Multifinality describes the converse situation, where a single risk factor can be associated with different clinical outcomes. Examples pertinent to ASD include Fragile X, where up to 45% of affected individuals have been estimated meet criteria for ASD (but 55% do not) (Gallagher and Hallahan, 2012), and 22q11.2d syndrome, which appears to increase risk for ASD in some individuals (Antshel et al., 2007), and schizophrenia in others (Murphy et al., 1999). Research on genetic risk factors for ASD in general has been notable for the lack of specificity to ASD (Betancur, 2011). Risk-genes instead appear to confer vulnerability for a variety of neurodevelopmental disorders (Sahoo et al., 2011; Rapoport et al., 2012), suggesting that understanding the role of genetic risk factors for ASD will require identifying the factors that direct an individual with a risk-gene down the path to one neurodevelopmental disorder versus another as much as identifying the genes themselves (Moreno-De-Luca et al., 2013).

Investigators have taken different approaches to the clinical and neurobiological heterogeneity of ASD. Geschwind and Levitt (2007) drew attention to the variety of associated genetic syndromes, endorsing the view that the field should be considering a multiplicity of "autisms" rather than a single condition. They went on to hypothesize that what these "autisms" shared was a common abnormality in brain connectivity that occurred early in development and most strongly affected the links between frontal and temporal/parietal cortices. A large body of work has accrued using multiple neuroimaging techniques across a variety of presentations and ages that has shown impaired functional and structural connectivity between brain regions (Amaral et al., 2008; Horder and Murphy, 2012; Just et al., 2012; Travers et al., 2012).

However, limitations to this formulation have also been recognized (Vissers et al., 2012). While the evidence of abnormalities in connectivity are convincing, it does not in itself explain either how the observed abnormalities in connectivity explain the heterogeneity of specific presentations, or how they differentiate autism from a variety of other disorders characterized by poor connectivity between similar brain regions, such as ADHD and schizophrenia. Pelphrey et al. (2011) proposed an alternative approach, in which heterogeneity was constrained by narrowing attention to the deficits in social communication felt to be the heart of ASD, and focusing on early failures of the neural systems relevant to these. While acknowledging that disruptions in these systems could arise from many different sources, congruent with the observations of multiple risk factors, they argued that what was relevant to ASD was the convergence of these factors on the development of specific aspects of the social brain network, such as the posterior superior temporal sulcus (Pinkham et al., 2008; Kaiser et al., 2010).

Concerns have been raised that this approach focuses too closely on the social communication aspect of ASD, and thus not only does not address the range of clinically relevant symptoms, but also stands to lose information potentially crucial for determining what types of pathophysiology are relevant to a particular individual. Brock (2011) have argued that a better method is to consider the heterogeneity along with the findings: for example, rather than just determining whether the amygdala is hyper- or hypo-activated, one should characterize what differentiates those with hyperactivity from those with hypoactivity.

This leads to the strategy of tackling heterogeneity by identifying more homogeneous subgroups, with the hypothesis that this will decrease noise due to variation and facilitate detection of meaningful group differences or brain-behavior relationships (Volkmar et al., 2009). Comparing putative subgroups against each other can also test whether a particular feature distinguishing the subgroups is related to brain differences, or conversely, if there is something that appears consistently found in individuals meeting diagnostic criteria despite other heterogeneous aspects of the presentation. The subgroups explored the most to date are the different disorders within the PDD category in DSM-IV (American Psychiatric Association, 2000), but other ways of dividing ASD have also been used, including divisions based on clinical features such as regression or level of ID, or subgroups defined by presence of a specific risk factor such as gender or a known genetic disorder.

Heterogeneity can also be addressed by using a dimensional approach to relate variation in neuroimaging measures to some other aspect of the presentation (Constantino, 2011; Lord and Jones, 2012; Uher and Rutter, 2012). Being able to determine whether some aspect of the brain is predictive of a clinical symptom can both support the relevance of the imaging data, and perhaps, depending on how much is known about the function of those brain regions, inform the larger questions as to what neural processes are responsible for the clinical presentation.

We first briefly discuss which findings appear to be the most consistent in neuroimaging studies of ASD. The second section will review studies that have looked at subgroups within ASD, and the third section work that has instead focused on dimensional approach, both of the features considered as core symptoms of ASD and of common co-occurring conditions.

WHAT IS COMMON WITHIN NEUROIMAGING FINDINGS IN ASD?

Most imaging studies of ASD done to date have been done taking the categorical diagnosis as their framework, asking the basic question of whether there are one or more brain differences corresponding to the clinical syndrome that can be recognized despite the various sources of heterogeneity. As is the case for other psychiatric disorders, clinical MRIs of individuals with autism do not typically show gross lesions or other abnormalities that can

be used to distinguish affected individuals. Instead, alterations in brain structure or function are most easily detectable as differences between groups, and, given the variability of the syndrome, preferably using large samples.

Meta-analysis provides one method of combining data to create larger databases in which to search for patterns. A meta-analysis by Stanfield et al. (2008) included 43 structural neuroimaging studies comprised of data from over 800 subjects between 3 and 30 years of age with ASD and similar number of matched controls. Although there was considerable heterogeneity of results across the studies they included, significant findings on meta-analysis included enlarged total brain volume, hemispheres, cerebellum, and caudate in ASD, and decreased volumes in other brain regions including midbrain regions, regions of the cerebellar vermis, and area of the corpus callosum. Gray and white matter (WM) volumes were not reported separately in this meta-analysis.

Another recent meta-analysis of differences in brain morphometry in ASD limited its scope to fully automated voxel-based morphometry (VBM) studies of gray matter volumes (Via et al., 2011). Twenty studies met inclusion criteria, including 496 adolescents or adults with ASD (a combination of Autism and Asperger's Syndrome) and 471 controls. Of the 17 studies that reported IQ, only one included subjects whose mean IQ was less than 70. This meta-analysis found no differences in global gray matter volume between ASD and controls; regional differences consisted of smaller gray matter volume in the ASD group in the amygdala-hippocampus complex and medial parietal regions. A linked meta-analysis by the same group of WM differences measured with VBM identified 13 eligible studies, including 246 patients with ASD, and 237 controls; while global WM volumes were not different, they did find evidence of increased volumes in regions relevant to language and social cognition (Radua et al., 2011).

Meta-analyses of functional magnetic resonance imaging (fMRI) studies have additional challenges due to the even greater variety of possible tasks and conditions to be compared. Philip et al. (2012) examined available fMRI studies in ASD across six different functional domains: visual processing, executive function and language tasks, basic social processing, and more complex challenges of social cognition. They performed their meta-analysis using the activation likelihood estimation (ALE) method, in which data regarding loci of activations are spatially normalized and then the overlap calculated across different experiments (Eickhoff et al., 2009). Ninety papers met inclusion criteria, with an average sample size of ASD participants of 12. Although the wide variety of tasks used made comparison challenging, there were regions of activation that were significantly different between the ASD subjects and controls in each of the functional domains examined. Notable findings included a tendency for decreased activation in ASD across several prefrontal and subcortical brain regions during tasks tapping executive function. Activation patterns in the superior temporal gyri were significantly different between ASD and controls across several domains, although the direction varied: ASD had decreased activation during tasks related to auditory and language processing, but increased during tasks of simple social processing, and mixed findings as demands became more complex. The authors also provided a qualitative review of available studies of functional connectivity that could not be included in an ALE analysis, finding multiple observations of decreased connectivity between areas of the cortex in ASD across a variety of resting or task based paradigms, and conversely some instead of increased connectivity, particularly between subcortical and cortical areas.

Robustness of findings in imaging studies has been limited by the small sample sizes and methodological variability of the studies available. However, technical advances in methods for data acquisition and automated processing are making multi-site large-scale imaging studies increasingly feasible. One such collaboration is the Autism Brain Imaging Data Exchange (ABIDE[1]), a consortium of investigators in which members contribute both published and unpublished resting-state fMRI data from ASD subjects and controls obtained using similar clinical and imaging protocols. The first paper from this consortium reported on measures of brain connectivity in a dataset collected across 17 sites. Neuroimaging data included in this analysis were limited to that collected from males (360 ASD, 403 controls) who had IQ within 2 standard deviations of the overall sample mean of 108 (Di Martino et al., 2013). The results helped to clarify conflicting results from earlier studies, confirming that both hyper- and hypo-connectivity are characteristic of brain activity in ASD. Hypo-connectivity was much more prominent, affecting to varying degrees all cortico-cortical connections tested, with particularly strong effects in unimodal association areas such as the fusiform and superior temporal gyri, paralimbic regions such as the insula and paracingulate cortex, and connections between hemispheres. Hyperconnectivity was more limited, affecting primarily subcortical nuclei and parietal cortex. The study represented a significant advance in reconciling previously inconsistent observations (Muller et al., 2011; Vissers et al., 2012), although the population studied was limited to the subset of individuals with ASD with normal range IQ, and did not have the younger subjects needed to answer questions around early developmental changes.

In summary, despite the clinical and etiological heterogeneity with ASD, the use of quantitative methods to look for common patterns across datasets collected thus far has detected evidence of some relatively consistent differences in both brain structure and function on a group level. However, a significant contributor to differences in findings among studies of ASD is age.

AGE EFFECTS

As suggested by the meta-analyses above, structural neuroimaging studies of adolescents and adults have had inconsistent findings, with some reporting enlarged volumes (Piven et al., 1995), but the majority normal or even reduced volumes (Garber et al., 1989), consistent with the meta-analyses of VBM data in older individuals by Via et al. (2011) discussed above (Radua et al., 2011). In contrast, studies of head circumference and brain volumes in children with autism have suggested that brain enlargement is a more consistent finding (Wolff, 2013). In the meta-analysis by Stanfield discussed above, age effects are described for the amygdala, such that the differences in amygdala volume correlated with age, becoming larger in younger subjects. While this analysis did not find similar age effects for total brain volume, unlike what had been reported in

[1] http://fcon_1000.projects.nitrc.org/indi/abide/

a previous meta-analysis (Redcay and Courchesne, 2005), there are increased effect sizes for larger total brain volumes in younger children. These and similar observations have led to the hypothesis that the trajectory of brain development in some individuals with ASD may show a two-step deviation: an early acceleration in brain growth, followed by a flattening of the growth trajectory or even relative loss in brain volume sometime during early adolescence (Aylward et al., 2002; Redcay and Courchesne, 2005).

It should be noted that while increased early brain volume is one of the most consistently reported observations, it has not been found by all studies (Raznahan et al., 2013a). A recent paper re-examining past reports of increased head circumference in ASD found evidence to suggest that much of this could actually due to bias from cohort effects within commonly used population databases such as the CDC. Those studies in which control data had been collected from the same community as the ASD group were less likely to find head circumference differences (Raznahan et al., 2013b). MRI comparisons of global gray and WM volumes in young children have also had mixed results. Studies that limited participants to those that met narrow DSM-IV criteria for Autism were less likely to find group differences, while larger brain volumes were more frequently observed when the patient group was extended to the broader autism spectrum. A recent study of brain volumes in toddlers with regressive versus non-regressive autism (Rogers, 2004; Hansen et al., 2008) of the same clinical severity also only found increased brain volumes in males with the regressive subtypes, while males with non-regressive autism were not different than controls. Brain volumes were not increased in females with either regressive or non-regressive autism, although sample size differences may have affected the ability to detect differences in the latter, as there were many more males in the study than females (114 males/22 females) (Nordahl et al., 2011). These findings, together with the observation that many genetic disorders associated with autism often result in microcephaly (Betancur, 2011), suggest that early increased brain growth is present in only a subset of individuals with ASD (Raznahan et al., 2013a).

There is as yet relatively little work attempting to assess developmental shifts in brain functional activity in ASD. In a recent meta-analysis, Dickstein et al. (2013) addressed developmental questions in fMRI results by using ALE methods to directly compare fMRI study results in children less than 18 years old with those obtained in adults. Tasks were split into those testing aspects of social function, such as theory of mind, face processing, and language, versus non-social capacities such as executive function and reward processing. Forty-two studies met inclusion criteria: 18 studies in children (including 262 ASD participants, average age 12.95 ± 1.74 years) and 24 in adults (288 participants, average age 30.55 ± 4.94 years), with similar numbers of age-matched controls. They reported that loci of both hyperactivation and hypoactivation were more pronounced in the younger subjects: in the group of social tasks, the convergence of hyperactivation was higher in the children in the left postcentral gyrus, and hypoactivation greater in the right parahippocampal gyrus/hippocampus and right superior temporal gyrus. In the non-social tasks, hyperactivation was greater in the ASD children in areas such as the right insula, right middle frontal gyrus, and left cingulate gyrus, while hypoactivation was more pronounced in the right middle frontal gyrus; there were no instances in either condition where convergences of hypo- or hyperactivation were greater in the adult ASD group. While the results need to be followed up in longitudinal analysis, they do suggest that development stage plays an important role in functional differences in ASD as well.

SUBGROUPS WITHIN ASD

SUBGROUPS DEFINED BY CLINICAL FEATURES

Autism and Asperger's syndrome

As discussed above, until the recent revision of DSM-5, ASD were divided into several categorical diagnoses. The question of whether Autism and Asperger's syndrome should be considered different disorders has been contentious, particularly when comparing individuals with Asperger's syndrome to those with Autism and normal cognitive function (Kozlowski et al., 2012; Planche and Lemonnier, 2012). Studies directly comparing the two have tended to report evidence of more severe brain abnormalities in Autism, with Asperger's syndrome being intermediate (Lotspeich et al., 2004; Schumann et al., 2004). The recent meta-analysis of VBM measures of gray matter volume described above (Via et al., 2011) did not find evidence of significant differences in gray matter volume between Autism and Asperger's syndrome, supporting the hypothesis that the conditions had similar neural substrates with differing levels of severity.

Intellectual disability

It is estimated that approximately of 60–80% of the total ASD population have mild to severe ID (Fombonne, 2003). How best to understand the relationship of ID to the core features of ASD is not clear – whether they should be considered as arising from the same fundamental process in individuals with both, whether ID should instead be treated as a co-occurring disorder, or whether ID may serve as an "unmasking" element that decreases an individual's ability to compensate for other factors that place them at risk for autistic behaviors (Skuse, 2007).

A handful of studies have compared ASD with and without co-occurring ID, often referred to as "low-functioning autism" (LFA), and "high-functioning autism" (HFA). A study of global brain volumes and amygdala and hippocampal volumes in a group of children and a group of adolescents, divided into the four subgroups of LFA and HFA, Asperger's syndrome and matched controls, did not find significant differences in patterns of neural abnormalities between LFA and HFA; both had significantly enlarged amygdala and hippocampal volumes in the younger children but not adolescents, and global brain volumes were the same as controls throughout (Schumann et al., 2004). A cross-sectional analysis of a subset of the same sample reported differences in cortical folding patterns between the four groups (Nordahl et al., 2007). Cortical morphometry and sulcal depths were modeled using a surface-based registration system (Van Essen et al., 2001). The LFA group had an area of deeper sulci than controls in the left frontal operculum and anterior insula; in the HFA group, sulcal depth was deeper in the left parietal operculum, approximately 12 mm distant from the area affected in the LFA group, and correlated with a similarly affected region in the right hemisphere. In the Asperger's syndrome group, this region was not affected, but there was evidence of greater sulcal depth bilaterally in the intraparietal sulcus.

Shape analysis showed an abnormal region in the pars opercularis portion of the left inferior frontal gyrus of the LFA group, which coincided with the sulcal depth abnormality. The sample was also divided into a younger (7.5–12.5 years) and older (12.75–18.5 years) in order to explore developmental effects: similarly to the study of amygdala size in this cohort, findings were more pronounced in the younger group, despite the smaller sample size, and no longer evident in the adolescent group.

Scanning children with intellectual disabilities is challenging, and relatively few studies have focused on LFA. Riva et al. (2011) compared brain volumes in a group of children with ASD aged 3–10 years, average IQ of approximately 52, against controls with normal IQ. They reported similar global brain volumes between the two groups, but a pattern of regional gray matter deficits in the autistic group. Other studies have used control groups matched on level of ID. Predescu et al. (2010) did not find differences between 15 children aged 2–8 with ASD and 10 age-matched children with developmental delay (DD) in global gray and WM volumes measured using VBM. A slightly larger study of 34 children aged 2–7 years with ASD and 13 controls matched for age and developmental level also did not find any significant differences in brain volumes, although there appeared to be a positive relationship between developmental stage in the developmentally delayed group that was absent in the ASD children (Zeegers et al., 2009). A study in older children with significant DD (27 ASD, 17 controls; both groups had chronologic age of approximately 14 years and developmental age of approximately 4.5 years) reported that the area of the corpus callosum was significantly smaller in the ASD group, although head circumference and cerebellar volumes were not different (Manes et al., 1999). A different approach was taken by Hrdlicka et al. (2005), who used cluster analysis to determine which traits grouped together across several domains, including brain volumes, autistic symptoms, IQ, facial dysmorphism, and comorbidities such as epilepsy. They found that level of ID differentiated between clusters, while ASD symptoms did not; more severe ID was associated with smaller volumes of the amygdala, hippocampus, and corpus callosum genu/splenium, along with higher frequency of epilepsy, facial dysmorphic features, and abnormal early psychomotor development. A significant limitation to the studies available thus far is their relatively small size. Compounding this is that the pathology underlying ID and DD is itself not well understood, and so control groups defined on basis of cognitive function are likely to introduce additional variation related to the causes of the cognitive impairment.

SUBGROUPS DEFINED BY RISK FACTORS

Environmental risk factors

Another strategy for parsing heterogeneity is subdividing based on the presence of a specific risk factor, either comparing individuals that have a specific risk factor who also have ASD features against those who do not, or comparing individuals with ASD and a specific risk factor against idiopathic ASD (iASD) and controls in order to see if the same patterns of differences is present regardless of the presence of the risk factor. Analyses of this type thus far have been carried out in primarily in regards to genetic risks. Although ASD has also been associated with a variety of environmental risk factors (Herbert, 2010), much less is yet known about how these might relate to brain differences. The strongest environmental risk identified thus far is severe early neglect, which has been associated with development of autistic behavioral features (Rutter et al., 2007), although studies of children raised in these conditions have also shown a capacity for significant improvements on exposure to a socially enriched environment that ASD generally does not. Some small neuroimaging studies have been done in these populations, which have documented decreased brain volumes and abnormal WM architecture, but the relationship of brain findings to autistic symptoms in these subjects is not known (Bos et al., 2011).

Prenatal exposure to maternal autoantibodies has been suggested as another environmental risk factor potentially playing a role in some individuals with ASD. The blood-brain barrier is permeable to maternal IgG during prenatal development, and maternal autoantibodies have been observed to react with fetal brain tissue, with reactivity to several specific antigens in the 37 and 73 kDa range of molecular weight linked to significantly increased risk for ASD in offspring (Braunschweig et al., 2013). Prenatal exposure of rhesus macaques to these autoantibodies from mothers of ASD children resulted in abnormal social function and a more rapid increase in brain volume in the males (although not females) during the first two years of life compared to controls (Bauman et al., 2013). These intriguing findings were followed up by a study of maternal autoantibodies and brain volume in male children with ASD and matched controls (Nordahl et al., 2013). In this study, 7.5% of the mothers of ASD children and none of the mothers of controls had the autoantibodies in the 37/73 kDa range; and the children of this subset had significantly larger brain volumes than the children with ASD who did not have exposure to these autoantibodies. Together, these findings support the hypothesis that there may be a subgroup of individuals with ASD in which pathophysiology is linked to a particular immune-mediated process during prenatal development. There have not been imaging studies yet related to other possible environmental factors such as prenatal maternal influenza.

Genetic risk factors

As extensively reviewed elsewhere, ASD is highly heritable (Ronald and Hoekstra, 2011), and has been linked to a large number of genetic risk factors (Betancur, 2011). The Y chromosome could be considered one of the strongest of these, conferring up to a 4:1 greater risk in males compared to females (Fombonne, 2009). Otherwise, the genes identified to date with the strongest impact on risk for ASD are those associated with known genetic neurodevelopmental disorders which include an increased likelihood of ASD (Fombonne, 2009). Examples include Fragile X syndrome (FXS) (Gallagher and Hallahan, 2012) and 22q11.2d, also known as velocardio-facial syndrome (VCFS) (Antshel et al., 2007). However, even in these disorders, ASD symptoms are only present in a subset of affected individuals, and each of these syndromes increases risk for a number of psychiatric disorders. Neuroimaging has begun to be used to try to determine whether having the ASD phenotype or not in individuals with the same genetic abnormality is reflected in variation in brain structure, and also whether brain differences associated with the ASD phenotype in a specific genetic condition are similar to those in "idiopathic" ASD. Such studies

within specific genetic disorders have the additional benefit of allowing comparison of individuals with ASD and ID within a relatively homogeneous cohort. There is an element of controversy regarding the relationship of ASD symptoms to genetic disorders such as FRX, with some arguing that despite appropriate scores on standardized assessments, a closer analysis of clinical features of individuals with genetic syndromes reveals that similarities are superficial, and symptom profiles are not the same as in individuals with iAUT (Moss and Howlin, 2009). An alternative is that the pattern appears atypical precisely because they represent a subgroup associated with a single major genetic risk factor, and so do not display the same characteristics described as averaged observations from a much more heterogeneous group.

Gender

Autism spectrum disorders is significantly more common in males than females (1 in 54 in boys and 1 in 252 in girls, up to 5 times more frequent in boys; Center for Disease Control and Prevention, 2012), although there has been longstanding debate whether the risk factors disproportionately affects males, or if instead other protective factors tend to make symptoms less noticeable in girls. It has also been uncertain whether the proportion of males and females may change based on the level of ID. Several early studies reported an interaction of gender with ID, such that the ratio of males to females is nearly equal in individuals with ID, and becomes more prominent in the groups with higher IQ (Wing, 1981; Lord et al., 1982; Volkmar et al., 1993). However, some more recent studies have not found a relationship of gender ratio and IQ (Carter et al., 2007; Hartley and Sikora, 2009; Mandy et al., 2012). The preponderance of males in recruitment samples has resulted in many imaging studies excluding females altogether, in order to reduce potential variance related to gender. Those that have included both males and females have generally had insufficient numbers of females to examine effects of gender on outcomes, and there have only been a few MRI studies that have addressed differences in gender directly.

Most studies thus far that have reported on gender effects have not found significant differences in which brain areas are affected, although the magnitude of effects in areas has tended to be larger in females (Bloss and Courchesne, 2007; Schumann et al., 2009, 2010; Calderoni et al., 2012). One study however found reduced cerebral GM and WM volumes and reduced temporal GM volumes in females versus males with ASD; in addition, cerebral, frontal, parietal, and occipital WM volumes were only correlated with age in girls but not in boys (Bloss and Courchesne, 2007). This was consistent with the gender effects observed in a meta-analysis of brain structural differences in ASD, which found that differences in the cerebellum were more likely to be observed when there were fewer males included in the study, suggesting that females may be contributing greater differences (Stanfield et al., 2008).

A notable recent study, the largest to date designed explicitly to examine gender differences, found that brain regions affected in ASD males had little overlap with those affected in ASD females (Lai et al., 2013a). In this study, VBM was used to compare gray and WM global and regional volumes in high-functioning adult males and females with ASD (age range 18–49; 30 males with ASD; 30 females with ASD; 30 male controls and 30 female controls). There were not significant interactions of gender and diagnosis for gray matter volumes. However, for WM, several regions had divergent findings for males and females. In the temporo-parieto-occipital region, females with ASD had larger WM volumes than female controls, while there was no difference in males; while WM in the internal capsule in the area around the basal ganglia was larger in the ASD males than male controls, but smaller in ASD females than female controls. In addition to the difference in direction of findings, there was little spatial overlap between affected regions in males and in females. These findings suggest a difference in neuroanatomical substrates of ASD for males and females, despite similar clinical characteristics. Although in general females who have been identified with ASD have tended to be characterized as clinically more severely affected than males (Dworzynski et al., 2012), suggested by some as due to ascertainment biases, this study as well as the others described above did not find gender differences in their samples in either ID or symptom severity (Bloss and Courchesne, 2007).

There has been one fMRI study to date that targeted gender differences, comprised of a verbal fluency task and a mental rotation task (Beacher et al., 2012). These tasks were chosen based on previous evidence of gender specific performance in health populations: females tend to perform better than males on measures of verbal fluency (Herlitz et al., 1997), and males better than females on tests of mental rotation (Crucian and Berenbaum, 1998; Astur et al., 2004; Parsons et al., 2004; Kozaki and Yasukouchi, 2009). The authors found evidence of interaction of group and gender. On the verbal fluency task, AS males, but not females, had greater activation in the left medial superior frontal gyrus than controls. On the mental rotation task, AS males had greater activation in the left precuneus, bilateral occipital gyri, and left inferior temporal gyrus than controls; the opposite was true for females, with control females having greater activation in the same regions. The authors speculated that differences could have been due to gender differences in cognitive styles.

Genetic syndrome

Fragile X Syndrome is an X-linked disorder, the most common inherited form of ID, caused by a trinucleotide repeat in the Fragile X mental retardation 1 (FMR1) gene (Gallagher and Hallahan, 2012). It is estimated that 25–47% of individuals with FXS have ASD (Gallagher and Hallahan, 2012), resulting in 2–6% of all ASD cases (Reddy, 2005; Hagerman et al., 2010). Kaufmann et al. (2003) were the first to directly compare children with FXS and ASD, finding hypoplasia of the posterosuperior vermis in both groups compared to controls. A study of 10 adults with FXS, 10 iASD, and 10 TD using VBM also reported decreased volume of the cerebellar vermis in FXS and iASD compared to TD; the FXS group had increased volumes of caudate and dorsolateral prefrontal cortex (PFC), and decreased volumes of left postcentral, middle temporal, and right fusiform gyrus (FG) compared to both ASD and TD (Wilson et al., 2009). Meguid et al. (2010) reported on a comparison of cortical thickness in 10 children with iAUT and 7 children with FXS + AUT; they found that that for the most part there were no significant differences in measures of cortical thickness, gyrification, or sulcal depth between the two groups, except that the iAUT had thinner cortex in the left medial frontal

and anterior cingulate cortices, which correlated with an index of social maturity.

A series of reports using different imaging analysis methods have come from work by Hazlett and colleagues regarding a longitudinal study of boys with FXS recruited between 2–4 years of age, including a group who had FXS with autism (FXS + AUT), FXS without autism (FXS-AUT), iASD, and controls (TD, a mix of typically developing and developmentally delayed children). In the baseline study using predefined regions of interest they found that the FXS group, both with and without autism, had enlarged caudate and decreased amygdala volumes, compared to both TD and iAUT. The most significant finding in the iAUT group was enlarged amygdala volume compared to either FXS or TD (Hazlett et al., 2009). At the next time point, when subjects were 5–6 years of age, additional differences were observed. Global volumes were similar between the FXS and iAUT groups, in both cases larger than the TD group, but frontal lobe gray and WM volumes were smaller in the FXS than in iAUT, and temporal WM and cerebellar volumes were larger (Hazlett et al., 2012). A longitudinal analysis of the same cohort using VBM found a complex pattern of differences between the groups. Interestingly, several regions important for social function such as medial PFC, orbitofrontal cortex, superior temporal sulcus, and temporoparietal region appeared to differ in opposite directions, being smaller in the FXS (including FXS + AUT) and larger in the iAUT. There was no significant difference in the overall severity of the autistic symptoms, which the authors interpreted as an example of different patterns of brain structural differences underlying similar symptoms (Hoeft et al., 2011).

Rett's syndrome (RS) is an X-linked genetic disorder commonly associated with autistic features that was previously included within the same PDD category as ASD. Most affected individuals have a mutation in the Methyl-CpG-binding Protein 2 (MECP2), a transcription regulator important in activity-dependent synaptic maturation (Amir et al., 1999). As evidence grows supporting the role of synaptic development as a potential convergent pathway for pathophysiology in ASD, there has been strong interest in the MECP2 mutation as a prototypic model (Neul, 2012). Imaging studies of RS have described decreases in both gray and WM volume affecting frontal, temporal, and parietal regions (Naidu et al., 2001). Decreased volumes are consistent with the characteristic microcephaly, and appear to be more pronounced in individuals with more severe clinical phenotypes (Carter et al., 2008a). Interestingly, a study of the milder preserved speech variant subtype of RS found that while 76% of a cohort of 17 intermediate and high-functioning participants met criteria for ASD, only 3 had microcephaly, 11 had normal head circumference, and 2 were even macrocephalic (Zappella et al., 2001). There have not been studies to date explicitly examining imaging findings in RS in relationship to the autistic phenotype.

Velo-cardio-facial syndrome is caused by a deletion in the 22q11.2 region, and associated with increased risk of ASD (Antshel et al., 2007), as well as schizophrenia and other psychiatric conditions, and DDs (Shprintzen, 2008). One study to date has compared brain structures in VCFS with ASD to those without ASD. This study found that those with an ASD diagnosis had larger right amygdala volume (Antshel et al., 2007), consistent with other reports of increased amygdala size in ASD.

Down's syndrome is the most common genetic cause of ID, and has been reported as being comorbid with ASD in a subset of between 1–11% (Lowenthal et al., 2007). Brain volumes in DS subjects are usually reported as smaller than controls. Findings have been mixed when comparing DS with or without autistic features. Studies that further subdivide DS by presence of autistic features have not found differences total brain volume or total cerebellar volume between those with (DS + AUT, ID + AUT) or without (DS-AUT, ID-AUT) autistic features (Kaufmann et al., 2003; Spencer et al., 2006; Carter et al., 2008b). Some evidence of differences however has been identified in cortical areas related to social functions such as the thalamus and left superior temporal sulcus (Spencer et al., 2006), and associations with motor or RRB in brainstem and cerebellar WM (Kaufmann et al., 2003; Carter et al., 2008b).

Other genetic risk factors

A large number of other genes not associated with specific genetic disorders have also been linked to increased risk for ASD. Most studies of the effects of these genes on brain structure in relation to ASD thus far have focused on demonstration of the effects of risk-genes on relevant aspects of brain structure or function within healthy subjects (e.g., contactin-associated protein-like 2 (CNTNAP2) (Scott-Van Zeeland et al., 2010a; Tan et al., 2010; Dennis et al., 2011; Whalley et al., 2011); homeobox A1 (HOXA1) (Canu et al., 2009; Raznahan et al., 2012); MET receptor tyrosine kinase (MET) (Hedrick et al., 2012), oxytocin receptor (OXT) (Inoue et al., 2010), and brain-derived neurotrophic factor (BDNF) (Raznahan et al., 2009). However, a few have sought to determine if risk alleles contribute to heterogeneity within ASD and may be useful for intra-diagnostic stratification; i.e., whether ASD subjects with a specific risk-gene allele have differences in brain structure or function from ASD subjects who do not have that particular allele.

Monoamine-oxidase A (MAOA) is an enzyme found in the brain that is a key regulator of serotonin, dopamine, and norepinephrine, all neurotransmitters that have been linked with ASD. VNTR, a polymorphism in the promoter region for the MAOA gene, has been demonstrated to affect the level of activity of this enzyme. A large body of work has linked the low activity (LA) allele to a variety of adverse effects on cognition and behavior, including decreased IQ and worse symptomatology within ASD (Cohen et al., 2003). A study of the relationship of MAOA and ASD compared brain volumes between individuals with the high (HA) versus LA alleles of VNTR, both within a sample of young males with ASD (18–35 months of age, 17 HA, 12 LA) and controls (7–18 years of age, 28 HA, 11 LA). While there was no effect of the allele type in the control subjects, within the ASD sample the LA allele was associated with larger volumes of both gray and WM (Davis et al., 2008).

MET is another candidate genetic risk factor with relatively robust support for a role in ASD. MET is a gene encoding a protein within the ERK/PI3 signaling pathway, and is closely regulated during the development of excitatory neurons during synapse formation in regions of the brain important for social cognition (Levitt and Campbell, 2009). Variations in MET have been linked to increased risk for ASD (Campbell et al., 2010), and in animal models have been associated with developmental

abnormalities consistent with ASD phenotypes (Judson et al., 2009). Alleles of rs158830, located within the promoter region of MET, have been of particular interest because of their effects on transcription and protein expression of MET. The presence of the "C" allele of rs158830 has been associated with more severe deficits in social function and communication (Campbell et al., 2010). Rudie et al. (2012) measured the impact of the rs158830 risk allele on structural and functional brain development in a population of 162 children and adolescents with ($n = 75$) and without ($n = 87$) ASD. Participants contributed to one or more of three separate neuroimaging experiments: an fMRI paradigm involving passive viewing emotional faces; a resting fMRI scan; and a diffusion tensor imaging scan. Group differences between ASD and controls were present for both functional imaging paradigms. Independent of diagnosis, the MET risk allele was also associated with abnormalities in brain activation in both paradigms, and with decreased fractional inositropy in several WM tracts in related regions. Notably, the effects of the risk allele were more pronounced in the ASD group than in the controls. Across all three testing conditions, the intermediate heterozygote (GC) within the ASD group were more similar to the high risk homozygote (CC); while within the control group the heterozygote condition was more similar to the low risk (GG) homozygote. The differences between the ASD participants with and without the risk allele supported the value of stratifying samples both by diagnosis and by specific genetic risk factors.

DIMENSIONAL APPROACHES TO HETEROGENEITY

Although most imaging studies in ASD have concentrated on group comparisons, there have been some which have sought instead to determine whether the variation observed in clinical characteristics could be linked to differences in brain structure and function. Some of these have concerned the different domains considered as part of the core criteria of ASD, while others have started to explore the impact of common co-existing features such as ID, anxiety symptoms, and problems with attention and impulsivity. As the vast majority of the imaging literature is based on DSM-IV criteria, this review will accordingly consider the domains of language, social communication, and repetitive/restricted interests and behaviors separately, rather than following the revised criteria in which language and social interaction are combined (American Psychiatric Association, 2013).

SOCIAL COGNITION

Impairment in social interactions is the defining feature of ASD. There has been significant progress in delineating the neural systems playing key roles in the enormously complex cognitive processes underlying routine social activity, highlighting brain structures such as the medial frontal and superior temporal cortex, insula, cingulate, and limbic regions (Blakemore, 2008). Neuroimaging studies comparing ASD with controls have demonstrated that these regions among those most consistently showing abnormalities, confirming their likely involvement in the clinical phenomena (Di Martino et al., 2009; Sugranyes et al., 2011).

However, as in other aspects of ASD, the severity of impairment of social function can vary widely. Fewer studies have attempted to determine if MRI measures of these neural systems predicts the clinical symptoms. The amygdala has been one of the regions that has received the most attention, due to its well-established role in relevant aspects of social cognition such as response to facial expressions and threat detection (Adolphs et al., 2005; Adolphs, 2010; Pessoa, 2010), and evidence of abnormal structure and function of the amygdala in ASD (Abell et al., 1999; Baron-Cohen et al., 2000; Howard et al., 2000; Sparks et al., 2002; Schumann et al., 2004, 2009; Hazlett et al., 2009; Mosconi et al., 2009; Groen et al., 2010; Stigler et al., 2011; Nordahl et al., 2012). Studies relating amygdala volume to the degree of social impairment have had mixed results. Two studies found social impairment correlated with decreases in amygdala volume (Nacewicz et al., 2006; Mosconi et al., 2009), one with larger amygdala volume (Schumann et al., 2009), and two others no relationship at all (Dziobek et al., 2006; Juranek et al., 2006). The two studies that found positive correlation assessed social ability by experimental procedures, such as eye-tracking, rating joint attention from camera recordings, or facial emotion recognition tasks (Nacewicz et al., 2006; Mosconi et al., 2009). The other three studies which found no correlation or negative correlation (Dziobek et al., 2006; Juranek et al., 2006; Schumann et al., 2009) instead assessed social ability through clinical interviews such as the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2000) or Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994), demonstrating the complexity of characterizing social ability and of relating clinical behaviors to results of cognitive tests. Other areas in which there has been some degree of correlation observed to measures of social function have included the medial PFC (Rojas et al., 2006; Schulte-Ruther et al., 2011), inferior frontal gyrus (Rojas et al., 2006), superior temporal sulcus (Pelphrey et al., 2005), FG (Greimel et al., 2010), temporal-parietal junction (TPJ) (Lombardo et al., 2011), and the anterior cingulate cortex (ACC) (Scott-Van Zeeland et al., 2010b).

LANGUAGE IMPAIRMENT

Delayed or atypical language development is a core feature of ASD (American Psychiatric Association, 2000). Language ability can vary enormously, from the 25% who never develop functional language to mild abnormalities in prosody (Mody et al., 2013). Neuroimaging studies of language function in autism have focused on language-associated brain regions such lateral inferior frontal cortex, including Broca's area, and temporoparietal cortex, which contains Wernicke's area (Shapleske et al., 1999; Dronkers et al., 2007). Language-associated areas are anatomically and functionally asymmetric, with predominance normally found in the hemisphere opposite the dominant hand. Although results are mixed, observations from multiple studies have suggested that asymmetry is often reduced or reversed in ASD (e.g., Herbert et al., 2002, 2005; Rojas et al., 2002, 2005; Just et al., 2004; McAlonan et al., 2005; Knaus et al., 2009; Anderson et al., 2010; Catarino et al., 2011).

Bigler et al. (2007) reported on gray matter volumes of the superior temporal gyrus in a group of 30 children with ASD compared to 39 controls matched on age and IQ; 13 of the controls had reading deficits. They found that superior temporal gyrus volume correlated with a standardized measure of language ability in the control group, but not the children with ASD. An early fMRI study reported that adults with ASD did not exhibit normal lateralization

of brain response to vocal stimuli (Gervais et al., 2004). A more recent fMRI study of brain activity of sleeping toddlers (40 ASD and 40 matched controls, aged 12–48 months) during the reading of a bedtime story found that the ASD group did not show normal left-lateralized responses; there was instead a right-lateralized temporal cortex response which was most pronounced in the children toward the older end of the age range studied, suggesting an increasingly deviant developmental trajectory of laterality. Interestingly, within the ASD group, greater right hemisphere activity correlated with less severe symptoms (Redcay and Courchesne, 2008; Eyler et al., 2012).

These findings are consistent with those from a study comparing ASD and specific language impairment (SLI), a disorder in which delayed language development is present despite preservation of other cognitive abilities, and for which there is evidence of shared genetic risks with ASD (Fisher et al., 2003). This study compared children aged 6–12 years with SLI and typical controls to individuals with ASD with and without language impairment (De Fosse et al., 2004). They found overall larger brain volumes in the children with ASD. However, while reversed asymmetry of language-associated regions was present in the two groups that had language impairment, it was not present in the ASD group with normal language ability, suggesting that the asymmetry was more related to the presence or absence of language impairment than the ASD diagnosis.

RESTRICTED AND REPETITIVE INTERESTS AND BEHAVIORS

The third core domain of ASD as defined in DSM-IV is the presence of restricted interests and RRIB, including both "higher order" RRIB, such as unusual preoccupations or patterns of interests and compulsive adherence to rituals or routines, and "lower order" RRIB, referring to stereotyped and repetitive motor mannerisms and preoccupation with parts of objects (Lord et al., 1994). Much of the work on RRIB has focused on the relationships between the frontal cortex and basal ganglia, taking as a model conditions with similar features such as obsessive-compulsive disorder (Scarone et al., 1992; Rosenberg et al., 1997) and Tourette syndrome (Peterson et al., 2003; Langen et al., 2012). Volumes of frontal regions have been correlated with RRB severity in ASD (Hardan et al., 2003; Rojas et al., 2006; Ecker et al., 2012), and in fMRI studies of tasks requiring cognitive inhibition, RRB severity has been shown to correlate with abnormal activation in areas including dorsolateral PFC, anterior cingulate, and the intraparietal cortex (Shafritz et al., 2008; Agam et al., 2010). Studies relating RRB symptoms to basal ganglia volumes have had mixed results. In a comparatively large sample of individuals with autism ($n = 99$; TD: $n = 89$), Langen et al. (2009) found that caudate volume was negatively correlated with insistence on sameness within higher order RRB. However, in Hardan et al. (2003), only scores on lower order RRB complex mannerisms were negatively correlated with caudate volume. Two other studies that included subjects across the autism spectrum and did not exclude ID found instead a positive correlation between caudate volume and severity of RRB (Hollander et al., 2005; Rojas et al., 2006).

SENSORY ABNORMALITIES

Abnormal sensory function, either hyper- or hyposensitivity, is extremely common in ASD. Described as an associated feature in DSM-IV-TR (American Psychiatric Association, 2000), in DSM-5 it was changed to be one of the diagnostic criteria in the restricted/RRIB category (American Psychiatric Association, 2013). Although key brain regions for sensory function such as the primary somatosensory cortex and insula are among those most consistently showing abnormalities in ASD, to date few neuroimaging studies have examined imaging correlates of abnormal sensory function in ASD directly. Cascio et al. (2012) used fMRI to compare responses to pleasant, neutral and unpleasant tactile stimuli between a sample of 13 adults with ASD and 14 matched controls. They found that the subjective descriptions of the sensations were on average similar between the groups, although there was more variability in the ASD responses, highlighting the heterogeneity within the group. Despite the similarity of the subjective reports, there were significant differences in brain activation: the ASD group had significantly less BOLD response to the pleasant and neutral stimuli, but some areas of increased activation during the unpleasant stimuli, including primary somatosensory cortex and insula. Increased activation in the insula correlated with social impairment scores, supporting the theory put forth by some that abnormal sensory function during early development may contribute to abnormal social development (Hilton et al., 2010). The thalamus has also been examined due to its central role in sensory processing. While thalamic volumes have not been found to relate to sensory function, a magnetic resonance spectroscopy study observed indications of a relationship between thalamic brain metabolites (*N*-acetyl aspartate and glutamate + glutamine) and sensory function (Hardan et al., 2008a,b). Another study reported that GM volume in the brainstem and oral sensitivity measures were associated in high-functioning ASD (Jou et al., 2009).

ANXIETY SYMPTOMS

Anxiety symptoms are also very common in ASD, estimated to affect over 40% (de Bruin et al., 2007; Eussen et al., 2012; Strang et al., 2012). A handful of studies have looked at how varying levels of anxiety affects neuroimaging findings in ASD, most of which have targeted structures in the limbic system. Higher levels of social anxiety (Corbett et al., 2009), and generalized anxiety/depression scores in children (Juranek et al., 2006) have each been correlated with decreased amygdala volume. fMRI studies have been more mixed. Kleinhans et al. (2010) found higher social anxiety scores correlated with reduced activation in the FG and greater activation in the amygdala in adults in response to angry and fearful faces. In contrast, another study in adolescents showed that brain activations were not associated with depression or anxiety scores (Weng et al., 2011).

POOR ATTENTION AND IMPULSIVITY

Symptoms such as inattention, hyperactivity, and impulsivity (American Psychiatric Association, 2000) are present in a majority of individuals with ASD (Schatz et al., 2002; Goldstein and Schwebach, 2004). The frequency of these types of symptoms in ASD was previously acknowledged through exclusion of a separate diagnosis of ADHD in the presence of PDD. Recognition that this exclusion unfortunately diminished the likelihood of recognition of co-occurring ADHD and associated symptoms resulted in its removal in DSM-5. Although work to date on ADHD and ASD has been

largely been done separately, making explicit comparison between the two conditions and difficult, data thus far has demonstrated that there is significant overlap in both affected brain regions and genetic risk factors in the two disorders (Gargaro et al., 2011). This has led to hypotheses that that the underlying biology for both may fall along a continuum, with increasingly prominent social impairments as one moves from ADHD to ASD, and suggestions that more research studies should include both groups to allow direct comparisons (Brieber et al., 2007; Rommelse et al., 2011).

SUMMARY OF FINDINGS

Most studies in autism have not had understanding heterogeneity as a goal, but instead have effectively treated it as noise in the search for brain differences that correspond to the categorical diagnosis. These efforts have had some success. Findings have implicated many regions with prominent roles in social cognition, such as the superior temporal sulcus, amygdala, and insula (Di Martino et al., 2009). Volumetric and functional differences appear to be more pronounced in younger individuals, with a tendency toward larger volumes earlier in life (Courchesne et al., 2011; Wolff, 2013). With maturation these differences decrease in magnitude, particularly for brain structure, such that by adolescence summative measures such as total brain volume are not significantly different than controls. Other measures of brain structure continue to show differences into adulthood, and newer multivariate techniques have been able to identify subtle and widespread cortical differences consistent with the ongoing differences in function and behavior (Ecker et al., 2010). Abnormalities in structural and functional measures of connectivity are a consistent finding.

Efforts to address the heterogeneity of autism in neuroimaging studies have chiefly taken two forms: the first, to subdivide ASD into more homogeneous subgroups, using a variety of criteria; the second, to take a dimensional approach to examining the relationship between neuroimaging data and clinical features. Far fewer studies have been done using these approaches, and sample sizes are often quite modest; negative findings in particular may reflect lack of statistical power, or that studies using more sensitive measures have not yet been performed.

Clinically defined categories have included subgroups such as Aspergers's versus narrowly defined Autism, ASD with and without significant language impairment, and low-functioning versus high-functioning autism. None of these comparisons have provided a strong case for a neurobiologically robust and distinct subtype, which is not to say variation along these clinical dimensions is not of ongoing interest. The relationship of ID to the pathophysiology of ASD has continued to be a challenging issue, complicated by the fact that brain imaging studies of individuals with significant ID are very difficult to carry out, and so samples including these subjects are often underpowered. This has created a situation where despite the predominance of ID in ASD, most imaging studies, particularly those with the sample sizes necessary for multivariate analyses, are carried out in ASD individuals with normal or near-normal IQ.

There have been a few specific risk factors identified with strong enough associations to ASD to look at affected individuals as specific subgroups. One of these is male gender, whose much higher rates compared to females imply the presence of risk factors unique to males. The few structural imaging studies explicitly designed for gender comparison have generally not found significant differences in the pattern of abnormalities, except for the likelihood of females to show more pronounced brain differences than males. Of other specific genetic risk factors, by far the most work has been done in FXS. Here, the pattern of imaging findings appeared to be driven by the FXS genotype, regardless of whether the individuals met criteria for ASD or not. Some of the brain differences associated with the presence of autistic features in the FXS and non-FXS groups affected similar regions but in opposite directions.

The other most frequently used approach in neuroimaging to heterogeneity with ASD has been through relating dimensional variation in clinical or cognitive measures to brain measures. Although these analyses have been generally intended less to describe heterogeneity than to strengthen the case for the likely relevance of the observed brain differences to the clinical symptoms, they can be informative about whether the variation observed clinically and in imaging measures are likely to be reflective of each other. Although reported findings have been mixed, many have been consistent with what would be expected for brain regions known to be associated with specific functions, as described above.

CONCLUSION

So, what has neuroimaging told us about heterogeneity in ASD? The main finding may be that neuroimaging provides no refuge from the multiplicity of presentations and candidate risk factors found in the clinic and the genetics laboratory. Studies to date have made progress in identifying patterns of brain abnormalities present in groups of people with ASD, but inconsistency between study results is still more the norm than the exception, and biomarkers robust enough to be meaningful on an individual level have yet to be identified. Adoption of multivariate methods and pattern identification methods based on techniques such as machine learning may improve results, as more reflective of the widespread and subtle morphologic differences that have become apparent with larger scale studies (Ecker et al., 2012), and are consistent with current hypotheses of ASD as being rooted in abnormalities of synaptic development (State and Sestan, 2012). The difficulty with this approach, however, as has been discussed extensively elsewhere (Hyman, 2010), is that it continues to center around a concept of autism, or even the broader range of ASD, as a discrete entity. Such a designation can be highly useful from the practical level of providing a diagnostic label and indications for intervention. However, as a constraint for inquiries into biology, it may more distort than illuminate. Autistic disorders exist not only as a spectrum within the realm of pathology, but also the severe end of a set of continuous traits which extend into the general population, and do not have clear boundaries with other disorders such as ADHD (Lai et al., 2013b). Abundant evidence from epidemiologic, genetic and twin studies supports the common nature of the risk factors affecting autistic traits within individuals meeting criteria for the disorder and in the general population (Robinson et al., 2011; Ronald and Hoekstra, 2011; Lundstrom et al., 2012). ASD might be better considered a name assigned to designate individuals whose expression of a particular set of continuously varying

traits has reached a certain threshold of severity (Uher and Rutter, 2012).

These issues have been much discussed of late, prompted by the most recent revision of DSM (Kendler, 2012; Lord and Jones, 2012). Although DSM-5 left the previous categorical system largely intact, acknowledging its clinical utility and the lack of sufficient evidence to support more substantive revision, for research purposes there has been increasing support for decreasing the emphasis on categorical diagnoses (Uher and Rutter, 2012). Proposed alternatives include transdiagnostic dimensional approaches such as the Research Domain Criteria (RDoCs) currently under development at the National Institutes of Mental Health (NIMH) in the U.S.[2] (Insel et al., 2010), which focus on simpler constructs (for example, response to social stimuli, or working memory) that may be more amenable to linking across multiple levels of neural, cognitive, and behavioral function regardless of which clinical syndrome a particular feature is occurring within.

Neuroimaging has revolutionized our understanding of neurodevelopmental disorders by affording observations of brain structure and function *in vivo*, including the tracing of developmental trajectories in children and adolescents from the very first months of life. It should be kept in mind that despite the rapid technical advances in the field, MRI techniques remain limited to a level of spatial and temporal resolution too coarse to visualize the synaptic or neuronal-level abnormalities that may be core features of disorders such as ASD. If this is the level from which heterogeneity arises, neuroimaging may ultimately not be the best tool for parsing these differences. However, in combination with more finely grained methods such as post-mortem tissue analysis and animal models, neuroimaging studies have the potential to provide a critical intermediate step between risk factors such as specific genes and the cognitive or behavioral features of interest. Such approaches, by focusing on the links between genetic, biological, and behavioral domains, allow us the opportunity to deconstruct our conceptions of ASD back to where they can be grounded in biology. Eventually, heterogeneity may no longer be considered as noise in neuroimaging studies of ASD, and instead take its place as a guide to pathophysiology (Brock, 2011; Georgiades et al., 2013).

[2]www.nimh.nih.gov/research-priorities/rdoc

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Converging pathways in autism spectrum disorders: interplay between synaptic dysfunction and immune responses

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Autism spectrum disorders (ASD) are highly heritable, yet genetically heterogeneous neurodevelopmental conditions. Recent genome-wide association and gene expression studies have provided evidence supporting the notion that the large number of genetic variants associated with ASD converge toward a core set of dysregulated biological processes. Here we review recent data demonstrating the involvement of synaptic dysfunction and abnormal immune responses in ASD, and discuss the functional interplay between the two phenomena.

Keywords: autism spectrum disorders, immune response, synapses, genomics, gene expression

INTRODUCTION

Autism spectrum disorders (ASD) are a spectrum of neurodevelopmental conditions characterized by language deficits, social impairments, and repetitive behaviors (Abrahams and Geschwind, 2008). Typically the disorder is diagnosed around 2–3 years of age and manifests with a regression in acquired language and behavioral skills. However, there are wide variations in the clinical presentation and disease progression. In addition to variable severity of the core symptomatology, ASD patients also present with a variable mix of co-morbid conditions: epilepsy, gastro-intestinal problems, intellectual disability, anxiety, and depression (Kim and Lord, 2013). Mirroring its clinical heterogeneity, ASD is also genetically very heterogeneous (State and Levitt, 2011). Based on the results of genome-wide association (GWAS) studies, candidate gene re-sequencing, and exome-sequencing studies, it is currently estimated that hundreds of genetic variants, including common and rare genetic variants, contribute to the disease (Murdoch and State, 2013). What are the molecular pathways that mediate the phenotypic expression of this myriad of genetic variants into a recognizable triad of symptoms? Here we review recent studies demonstrating a convergence of ASD genetic changes toward two main biological processes: synaptic function and immune responses, and discuss their functional interplay, with a focus on immune modulation of neuronal synapses (**Figure 1**).

FROM MANY GENES TO COMMON BIOLOGICAL PROCESSES

Results from genome-wide studies are beginning to confirm the long-held hypothesis that the wide variety of genetic variants associated with ASD ultimately converge on a core set of molecular pathways (Murdoch and State, 2013). It is worth noting that pathway enrichment analyses are inherently limited by our current knowledge of signaling pathways and molecular interactions, and thus the identification of distinct pathways by different studies might partially reflect yet uncharacterized complexity of molecular pathways.

Four recent studies undertook exome-sequencing in several hundreds of parent-child trios in order to identify *de novo* single nucleotide variants (SNVs) and copy number variants (CNVs) associated with the disease (Iossifov et al., 2012; Neale et al., 2012; O'Roak et al., 2012; Sanders et al., 2012). The study by O'Roak et al. found that the most disruptive *de novo* mutations converged onto a highly interconnected beta-catenin/chromatin remodeling protein network, which is involved in neuronal differentiation and synaptic formation (Ille and Sommer, 2005). Iossifov et al. found an enrichment of *de novo* variants in genes encoding proteins associated with the fragile X syndrome protein, FMRP, suggesting an involvement in synaptic plasticity. The study by Neale et al. demonstrated that genes carrying functional *de novo* variants were functionally related to each other and to synaptic genes previously implicated in ASD. Using a network-based analysis of genetic association data, Talkowski et al. (2012) showed that rare *de novo* CNVs occurring in ASD cases affect primarily genes related to synapse development, axon targeting, and neuron motility. Collectively, these studies highlighted the fact that genes containing pathogenic DNA sequence variants in ASD patients affected primarily genes involved in various aspects of synaptic function.

While genetic association studies identify genetic loci potentially implicated in the disease, they do not assess the functional consequences of the associated variants. On the other hand, transcriptome analyses comparing disease and control groups assess

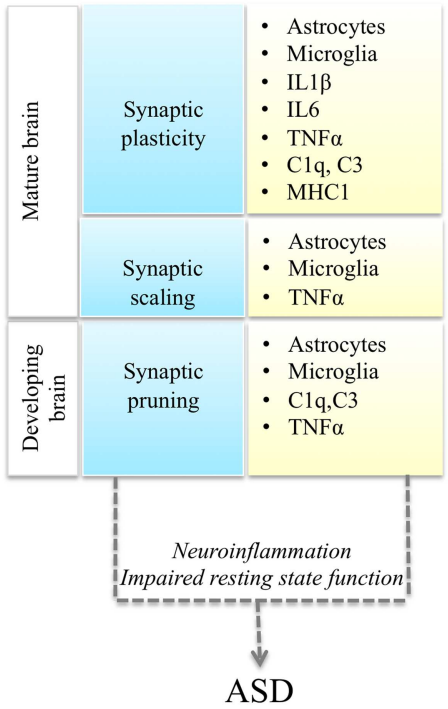

FIGURE 1 | Interactions between synaptic function and immune molecules and cells, and possible mechanisms leading to ASD. Multiple aspects of synaptic function (blue boxes) are regulated by astrocytes, microglia, and immune molecules (yellow boxes). Either immune activation during neuroinflammation or impaired resting state activity of immune cells in the brain could impair synaptic function and lead to ASD.

gene function (by quantifying mRNA output), but are liable to environmental variations in gene expression, and functional changes unrelated to the disease. Thus ideally, functional genomic studies should simultaneously assess DNA sequence variation and gene expression, in a disease-relevant tissue. However, the scarce availability of post-mortem brain tissue from ASD cases limits the sample size of such studies below appropriate statistical power. Attempting to address this problem, we performed a genome-wide assessment of gene expression in multiple brain regions (frontal cortex, temporal cortex, and cerebellum) from 19 autism cases and 17 unaffected controls, and integrated these results with previous ASD GWAS data (Wang et al., 2009). Using a network-based approach for the analysis of gene expression data we identified two modules of co-expressed genes dysregulated in a large subset of ASD cases (Voineagu et al., 2011). One of these modules was downregulated in ASD brain and was enriched for neuronal genes involved in synaptic function. A second module was upregulated in ASD brain and contained primarily genes functioning in immune and inflammatory responses. To integrate the gene expression results with previously published GWAS data, we performed a pathway enrichment analysis of ASD GWAS data using the two co-expression modules as pre-defined "pathways." We found that the neuronal genes downregulated in ASD, but not the immune/inflammatory genes, showed an enrichment for genetic association, as measured by a large ASD GWAS study (Wang et al., 2009). These results supported the heritability of synaptic gene dysfunction in ASD and suggested that the upregulation of immune and inflammatory genes is likely environmentally mediated or secondary to the synaptic dysfunction.

Although gene expression analyses of ASD brain are just beginning to emerge, several studies have evaluated gene expression in readily available peripheral tissues (blood and lymphoblast cell lines) from ASD patients (Hu et al., 2006, 2009; Gregg et al., 2008; Enstrom et al., 2009). A common result of these studies was the demonstration of increased expression of immune and inflammatory genes in ASD. Moreover, a comparison of gene expression studies of peripheral tissues in idiopathic autism and related neurodevelopmental disorders showed a convergence of gene expression abnormalities on genes involved in immune responses (Lintas et al., 2012). Interestingly, an analysis of genetic variants nominally associated with ASD found that these variants were enriched in brain expression quantitative trait loci (brain eQTLs), but not lymphoblast eQTLs (Davis et al., 2012). Thus gene expression studies collectively support the concept that (a) immune and inflammatory genes are upregulated in ASD, a phenomenon observed both in the brain and in peripheral tissues, and (b) neuronal synaptic genes are downregulated in ASD brains.

The involvement of synaptic dysfunction and immune responses in ASD had been demonstrated by multiple approaches (Betancur et al., 2009; Pizzarelli and Cherubini, 2011; Wright and Washbourne, 2011; Grabrucker, 2012; Onore et al., 2012; Zoghbi and Bear, 2012; Ebert and Greenberg, 2013), but it was not until large-scale genomic studies that these biological processes could be regarded as points of convergence of the heterogeneous genetic variants underlying ASD.

INTERPLAY BETWEEN BRAIN IMMUNE PROCESSES AND SYNAPTIC FUNCTION

Microglia, the main resident immune cells in the brain, have been long believed to be active only in response to immune insults, and to exist in a "resting state" in the normal brain. However, this view has dramatically changed over the last decade, and it is becoming increasingly clear that immune cells and molecules play an active role in the normal brain function. Microglia are believed to populate the CNS in the non-vascularized embryonic period and to originate from progenitors from the yolk sac. It has also been proposed that a second wave of microglia, originating from blood monocytes, may populate the CNS during the early postnatal period, a period particularly important for neurodevelopment (Davis and Carson, 2013). Microglia actively survey the brain parenchyma, constantly extending their processes to survey their microenvironment every few hours (Nimmerjahn et al., 2005). Importantly, microglia are required for synaptic pruning during postnatal neurodevelopment (Paolicelli et al., 2011). A recent study (Schafer et al., 2012) demonstrated that microglial synaptic pruning is developmentally regulated and depends on neuronal activity. This process was shown to be mediated by the complement receptor (CR3) pathway, and inhibiting CR3 signaling led to sustained deficits in synaptic connectivity.

Thus immune cells could affect neuronal synaptic function either as a result of their activation during immune responses, or due to a failure of their non-immune roles in the brain (**Figure 1**).

Recent evidence supports the potential involvement of both of these mechanisms in ASD pathogenesis.

Active neuroinflammation has been consistently demonstrated in ASD brains. Prominent activation of microglia (Vargas et al., 2005; Morgan et al., 2010), as well as increased levels of inflammatory cytokines and chemokines [interferon-gamma, IL-1β, IL-6, tumor necrosis factor (TNF)-α] have been documented in post-mortem brain tissue and cerebrospinal fluid from ASD patients (Onore et al., 2012). Recently, activated microglia have also been observed by positron emission tomography in ASD subjects in several brain regions (Suzuki et al., 2013). While it is not clear what is the cause of microglial activation in ASD brain, the cytokines produced by activated microglia have been demonstrated to affect neuronal synaptic function (Onore et al., 2012). TNF-α regulates neuronal cell proliferation and synaptic pruning (Cacci et al., 2005), and modulates synaptic scaling (i.e., the adjustment of synaptic strength for all synapses on a neuronal cell in response to prolonged changes in electrical activity) (Stellwagen and Malenka, 2006). IL-1β regulates long-term potentiation and alters synaptic plasticity (Schneider et al., 1998), while IL-6 has been implicated in behavioral changes associated with maternal immune activation (Patterson, 2009). Mounting evidence suggests that maternal immune activation, particularly during the first and second trimester of pregnancy, may be an important environmental factor in ASD (Onore et al., 2012). Rodent models of maternal immune activation exhibit ASD-like behavioral changes (Patterson, 2009), and the behavioral effects observed in offspring after maternal immune activation appear to be mediated by microglia and IL-6 (Hsiao and Patterson, 2011). In some mouse models increased levels of IL-6 have been sufficient to induce behavioral changes (Onore et al., 2012). Unlike peripheral macrophages, microglia are long-lived, and thus it has been hypothesized that they could maintain an "immunological memory" of an early immune insult, leading to long-term neuronal deficits (Davis and Carson, 2013).

One of the first studies to demonstrate a direct causal relationship between microglial function and a behavioral phenotype, was a mouse model of obsessive-compulsive disorder (Chen et al., 2010). *HOXB8* encodes a homeobox transcription factor expressed in the brain exclusively in bone-marrow-derived microglia. *HOXB8*-null mice exhibit excessive pathological grooming behavior similar to the obsessive-compulsive symptoms of trichotillomania. Chen et al. demonstrated that normal bone marrow transplant could rescue the excessive grooming and hair removal phenotype in the *HOXB8* mutant mouse, and that selective disruption of *HOXB8* in the hematopoietic lineage recapitulates pathological grooming. More recently, a role for non-immune functions of microglia has also been demonstrated in Rett syndrome, a pervasive developmental disorder, belonging to the wider group of ASD. Rett syndrome is caused by loss of function of the methyl-CpG binding protein 2 (*MECP2*) and is characterized by an initial period of normal development of about 5 months followed by deceleration of language development, psychomotor retardation, seizures and loss of social engagement skills (Chahrour and Zoghbi, 2007). It was initially believed that Rett syndrome is primarily due to loss of *MECP2* function in neurons. However several recent studies clearly demonstrated that *MECP2* loss in glial cells impairs neuronal function and contributes to the Rett syndrome symptomatology. *MECP2* deficiency in astrocytes leads to impaired BDNF regulation, cytokine production, and neuronal dendritic arborization (Maezawa et al., 2009). Moreover, *MECP2*-deficient astrocytes are unable to support normal dendritic ramification of wild-type neurons (Ballas et al., 2009). Remarkably, astrocyte-specific expression of *MECP2* in a *MECP2*-null mouse restored the normal neuronal dendritic morphology, improved locomotion, anxiety, and respiratory abnormalities (Lioy et al., 2011). A recent study by Derecki et al. (2012) demonstrated that not only astrocytes but also microglia contribute to the Rett syndrome phenotype. Using irradiation-mediated immune ablation in *MECP2*-null mice, followed by wild-type bone marrow transplantation, this study demonstrated that the wild-type microglia could arrest disease development. In addition, targeted expression of *MECP2* in myeloid cells ameliorated the phenotype in *MECP2*-null mice. These results implicated microglia as important players in the pathophysiology of Rett syndrome, and suggested a potential therapeutic benefit of bone marrow transplantation in Rett syndrome.

CONCLUSION AND FUTURE DIRECTIONS

Understanding the core biological processes underlying the clinical and genetic heterogeneity of ASD is as yet in incipient stages. Further advances in elucidating the molecular underpinnings of ASD are expected to result from (a) larger cohort sizes of GWAS and exome-sequencing studies, (b) increased availability of archived post-mortem brain tissue for transcriptome studies, and (c) integrative analyses of genomic, transcriptomic, and epigenomic data.

At the same time, understanding the role of immune cells in regulating synaptic function is also a newly developing field. As discussed above, accumulating evidence supports the notion that immune cells play important roles in normal brain function, outside of neuroinflammation. Of particular relevance to ASD is the role of microglia in synaptic pruning during postnatal brain development, a period that coincides with the onset of ASD symptoms. While it has been demonstrated that increased numbers of activated microglia are present in brain parenchyma of ASD patients (Vargas et al., 2005; Morgan et al., 2010; Suzuki et al., 2013), these studies have not captured the early postnatal development window. Future studies, facilitated by early ASD diagnosis, could shed further light on microglial activation occurs during postnatal brain development and on potential changes in the magnitude of this phenomenon across development and adult life in ASD. Notably, abnormal synaptic density, which could result from a deficit of synaptic pruning, is a feature of several ASD animal models [e.g., increased synaptic density in *Fmr1* KO mice, and decreased synaptic density in Rett syndrome mouse models (Delorme et al., 2013)], but it remains to be demonstrated whether it is also a feature of idiopathic ASD in human brain.

Since microglia and astrocytes have been shown to play a role in synaptic formation and maturation, and mutations in neuronal cell adhesion molecules have been associated with ASD, it is also tempting to speculate that ASD neurons might be particularly vulnerable to immune cell dysfunction in the brain.

Given the large amount of data supporting the role of immune responses in ASD and other neuropsychiatric disorders, advances in deciphering the functional interplay between immune cells and

neuronal synaptic function will likely provide vital insights into the mechanisms and potential therapy of neurodevelopmental disorders.

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Intellectual development in autism spectrum disorders: new insights from longitudinal studies

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The presence/absence of Intellectual Disability (ID) is considered to be the most critical factor affecting outcomes in individuals with Autism Spectrum Disorders (ASD). However, the question of the specific nature of ID in ASD has received little attention, with the current view being that ID is a comorbid condition (i.e., one that is unrelated in etiology and causality from the ASD itself). Recent advances in developmental neuroscience, highlighting the importance of early exposure to social experiences for cognitive development, support an alternative view; that ID in ASD might emerge as a consequence of severe social-communication deficits on the experience-dependent mechanisms underlying neurocognitive development. We tested this prediction in two independent samples of young children with ASD (*N*s = 23 and 60), finding that children with greater ASD severity at an initial assessment were more likely to present with poorer cognitive outcomes at a later assessment, irrespective of initial cognitive level. The results of this proof of principle study suggest that ASD symptom severity contributes to the extent to which the environmental input required to support "typical" brain development can be processed by the individual, so that the risk of developing ID increases as the number and severity of ASD social-communicative impairments increase.

Keywords: autism, intellectual disability, cognitive development, comorbidity, developmental cognitive neuroscience

INTRODUCTION

Intellectual Disability (ID) is characterized by significant limitations in intellectual functioning and in adaptive behavior as expressed in conceptual, social, and practical adaptive skills, with age of onset before age 18 years (Schalock et al., 2010). Approximately two thirds of individuals with Autism Spectrum Disorders (ASD) have co-occurring ID (Dykens and Lense, 2011), and the presence/absence of ID is considered to be the most critical factor affecting outcomes in this population (Howlin et al., 2004; Henninger and Taylor, 2013). However, the question of the nature of the association between ID and ASD has received little attention. One common view in the current conceptualization of ASD is that ID is a *comorbid* condition that occurs over and above ASD symptomatology in some individuals with ASD (Nordin and Gillberg, 1998; Cashin et al., 2009; Matson and Worley, 2013). The term "comorbidity" is used in medicine to denote clinical entities "unrelated in etiology or causality to the principal diagnosis" (e.g., cancer diagnosed after a stroke), and therefore conceptually distinct from complications or sequelae of the principal diagnosis (Greenfield, 1989; Iezzoni, 1994, p. 52; see also Lilienfeld et al., 1994). Other authors suggest that ID and ASD are related in terms of their etiology (i.e., that which causes ID also causes ASD) but they are not themselves causally related (e.g., Waterhouse, 2013). The perspective according to which ID is a distinct additional entity to ASD is reflected in many aspects of ASD research. For example, many studies report that participants with "comorbid ID" were excluded, to allow for the study of "pure autism"; that is, autism not confounded by ID.

The main argument supporting the idea of ID as a comorbid feature of ASD is the notion that a person can have either one without also having the other. While we do not disagree that such dissociation is possible, we argue that the presence of such a situation is not sufficient to demonstrate the independence of the two conditions. We will provide a number of counter arguments supporting an alternative view that severe ASD symptoms increase the risk of an individual also developing ID. We propose a theoretical model indicating the specific mechanisms through which the risk of having ID is related to the severity of ASD symptoms, and we provide novel data from two independent studies to support this model.

THEORETICAL ARGUMENTS FOR THE ASSOCIATION OF ID AND ASD

The notion of ID and ASD as independent clinical entities reflects a modular conceptualization of cognition, according to which one processing domain/module (in the case of ASD, the processing of social information) can be selectively disrupted without negative repercussions on the rest of cognitive system (that is, other domains will not be affected). According to this framework, "pure autism" is the exemplification of a "modular impairment" involving selective difficulties with social processing, while in the situation of "autism confounded by ID," additional (non-social) processing domains happen to be disrupted as well, albeit for a

different reason (i.e., the occurrence of a distinct clinical entity which is causally unrelated to ASD). However, an alternative to this account on the relationship between ID and ASD can be advanced within a developmental neuroscience framework (see Thomas and Karmiloff-Smith, 2002). Recent research emphasizes the experience-dependent nature of early brain development (e.g., Grossmann and Johnson, 2007; Kuhl, 2007; Makinodan et al., 2012), pointing to the crucial role of early exposure to social experience opportunities for cognitive development. For example, education and active engagement in a socially rich environment is associated with both structural and functional brain changes, whilst rearing in minimally stimulating environments (e.g., some orphanages) has a negative impact on brain functioning (Blakemore and Frith, 2005; Cicchetti and Cohen, 2006; Nelson, 2007).

Given the relevance of social input for normal brain development (Kuhl, 2007), it has been hypothesized that a decrease in the attentional and processing weight assigned to social information, in children with ASD, might preclude the usual social experiences that are necessary for "normal" cognitive development during early sensitive periods (Dawson, 2008; Klin et al., 2009; see also Hobson, 2004). This process would affect a number of different domains. For example, as noted by Whitehouse et al. (2007) language impairments in this population might be a secondary consequence of ASD symptoms. If infants with ASD do not have access to the appropriate input that supports the efficient organization and specialization of the brain in neurotypical development, this might ultimately result in the child also having an ID. A corollary of this model is that the more severe the ASD symptoms, the more the child would be "at risk" for developing an ID. Therefore, according to this view, ID is not a comorbid condition (i.e., an unrelated clinical entity), but a developmental *consequence* of the virtual "social deprivation" caused by the ASD symptoms. In this model, we do not imply that the presence of ASD necessarily results in ID in all children, regardless of symptom severity. Rather, we argue that ASD symptoms put the child "at risk" for ID, and that this risk will increase as the severity of ASD symptoms increase. One objection to this perspective is that the severity of ASD and ID are unrelated, so that ID can be equally present in children with mild or severe ASD symptoms. We turn to recent literature suggesting that this is not the case.

EMPIRICAL ARGUMENTS FOR THE ASSOCIATION OF ID AND ASD

If the notion that ID is a comorbid feature of ASD is correct, then measures of ASD severity and of cognitive abilities should be independent in the ASD population, so that a child could have mild ASD with severe ID, or severe ASD with mild ID. Empirical data, however, appear more consistent with our reasoning, showing that ID is more likely to be present in children with more severe ASD symptoms than in those with milder presentations. A review by Dykens and Lense (2011) using diagnostic categories from DSM-IV-TR (American Psychiatric Association, 2000) indicates that IQ levels vary substantially across diagnoses under the umbrella of the Pervasive Developmental Disorders (i.e., ASD), with more severe forms associated with lower cognitive scores. Furthermore, a recent longitudinal study by Gotham et al. (2012), involving a sample of 345 participants, documented that individuals with more severe autism symptoms had lower IQ, leading the authors to conclude that autism characteristics and cognitive functioning are not entirely independent features.

Another argument supporting our position derives from recent research on intervention in this population by Dawson et al. (2010). This study, focusing on the efficacy of the Early Start Denver Model – an intervention program specifically targeting ASD symptoms in very young children – found that children undergoing this program experience significant gains on measures of cognitive development and adaptive behavior. If ASD symptoms and ID (defined by low cognitive ability and adaptive functioning) are independent features, how is it that intervention targeting ASD symptoms results in gains in cognitive and adaptive functioning?

CURRENT AIMS AND HYPOTHESES

Based on the aforementioned arguments, we conducted a proof of principle study testing the hypothesis that severity of ASD in early childhood is associated with poorer development of cognitive abilities. We did this by collating secondary data across two independent samples of young children with ASD, each followed longitudinally. We predicted that (1) children with more severe ASD symptom presentation would be more likely to also demonstrate lower overall levels of cognitive ability, and (2) that these children would make slower gains in cognitive skills across time.

MATERIALS AND METHODS

PARTICIPANTS

Data for this study were available from two pre-existing, independent samples of young children with ASD; one comprising preschoolers with ASD diagnoses given by expert autism assessment teams in the community, and the other comprising toddlers with ASD prospectively identified and diagnosed from a low-risk, community-based sample following developmental surveillance. Ethics approval for each of these was provided via the La Trobe University Human Ethics Committee; HEC 10-084 and HEC06-94, respectively. We present data from each study as collected across two visits (hereafter, Time 1 and Time 2). Initial characterization data for each sample at Time 1 is presented in **Table 1**.

Table 1 | Sample characteristics at time 1; mean (SD) range.

	Preschoolers with ASD (community diagnosis)	Toddlers with ASD (developmental surveillance)
Chronological age (months)	40 (11) 22–60	25 (2) 23–33
ADOS-G[a] total algorithm	14.9 (4.6) 6–21	14.0 (4.1) 6–20
MSEL[b] Age-equivalence (months)		
Overall mental age	21.4 (12) 8–54.5	16.7 (3.4) 8–26
Subscales		
Visual reception	22.6 (8.9) 10–54	18.9 (3.2) 10–29
Fine motor	26.3 (10.7) 13–68	21.6 (3.7) 9–30
Receptive language	17.4 (10.7) 1–47	12.2 (5.1) 1–25
Expressive language	19.8 (11.5) 3–43	14.2 (4.1) 3–26

[a] *Autism diagnostic observation schedule – generic (Lord et al., 1999).*

[b] *Mullen scales of early learning (Mullen, 1995).*

Sample 1: preschoolers with community-based ASD diagnosis

Data for 23 children with ASD (1 female), aged 22–60 months at Time 1, were available from their enrollment and ongoing participation at a community early intervention center (see Vivanti et al., 2013). Children were accepted into the center on the basis of having an existing (or provisional) ASD diagnosis given by a community professional. This was confirmed at Time 1 via administration of the Autism Diagnostic Observation Schedule – Generic (ADOS-G; Lord et al., 1999) by an independent clinician with demonstrated research-reliability in the use of this measure. All participants were free from other medical conditions, and any visual, hearing, or motor impairment. Cognitive abilities for each child were assessed at entry to the early intervention center (Time 1) and again 1 year later (Time 2).

Sample 2: toddlers with ASD identified via developmental surveillance

Data for 60 toddlers with ASD (15 female), aged 23–33 months at Time 1, were drawn from a larger pool of participants identified within the Social Attention and Communication Study (SACS; Barbaro and Dissanayake, 2010; Barbaro et al., 2011). This longitudinal, community-based developmental surveillance study investigated the utility of a set of early markers in infants and toddlers for the prospectively identification of ASD at 12-, 18-, and 24 months of age. A sample of 110 children were identified at "at risk" for ASD on the SACS, and assessed at 24 months using the ADOS-G (Lord et al., 1999) and ADI-R (Lord et al., 1994), administered by a researcher with demonstrated reliability in using these measures. Of these, 89 toddlers were identified as having ASD at 24 months of age (Time 1), with 60 returning for a follow-up assessment 2 years later (Time 2; mean age 50 months, SD = 4.8). The current sample therefore comprises those 60 toddlers for whom an ASD diagnosis was initially given and for whom longitudinal cognitive ability data were available at follow-up.

MEASURES

Common measures of autism symptoms and cognitive ability were used to characterize children across the two samples. As already noted, the ADOS-G (Lord et al., 1999) was administered at Time 1 for all children (early intervention entry for the preschoolers, and initial diagnostic assessment visit for the toddlers). This standardized tool is considered the gold-standard observational measure for use in quantifying symptoms relevant to a diagnosis of ASD; that is, impairments in the areas of communication, reciprocal social interaction, and restricted/repetitive behaviors. While serving to inform diagnostic decisions around ASD, ADOS-G total algorithm scores (comprising communication and social interaction items only) can also be considered to index relative symptom severity, in so far as a range of scores is available beyond that considered to signal the "cut-off" for an ASD. Total algorithm scores for Module 1 (minimally verbal young children) plausibly range from 0 to 26, with a cut-off score of 7 used to identify an ASD (and with higher scores indexing greater symptom expression). As shown in **Table 1**, Time 1 ADOS scores varied substantially across the available range within each of our samples, showing clear individual differences on this measure.

Cognitive ability was assessed for all children at both Time 1 and Time 2 (following 1 year of early intervention for the preschoolers, and at a 2-year follow-up, post diagnosis, for the toddlers). This was achieved using the Mullen Scales of Early Learning (MSEL; Mullen, 1995), a standardized measure of ability across four domains important for cognitive functioning in early development; Visual Reception, Fine Motor, Receptive Language, and Expressive Language. The MSEL yields standardized *T*-Scores for each domain. However, as these can be of limited use for samples where children have ASD, as floor-level performance is often observed, we instead report Age-Equivalence (AE) scores here. These scores demonstrate good variability and can be meaningfully interpreted (i.e., a typically developing child should be expected to have an AE score in line with their own chronological age, and to make gains of 12 months' AE over a 1-year period). As shown in **Table 1**, Time 1 MSEL scores varied substantially, showing clear individual differences on this measure within each sample.

DESIGN AND ANALYTIC PROCEDURE

Associations between autism symptom presentation and cognitive ability were evaluated, separately within each sample, using Pearson Product Moment Correlation Coefficients. First, we examined concurrent associations among our indices of autism symptoms and cognitive ability at Time 1. Second, we computed a measure of gains in cognitive ability between Time 1 and 2 assessments (subtracting the former from the latter, for each child) and then examined the association of ASD symptoms at Time 1 with this measure of cognitive AE gains.

RESULTS AND DISCUSSION

SAMPLE 1: PRESCHOOLERS WITH COMMUNITY-BASED ASD DIAGNOSIS

For the sample of 23 preschoolers with ASD, a significant association, of large effect size, was evident between ASD symptoms and cognitive AE scores assessed concurrently at Time 1 (i.e., early intervention intake); $r = -0.49$, $p = 0.010$, $d = 0.9$. Longitudinally, a significant association, of larger effect size, was evident for this sample between Time 1 ASD symptoms and gains in cognitive AE made between Time 1 and Time 2 assessments; $r = -0.65$, $p = 0.001$, $d = 1.2$. Average cognitive AE scores increased from 21.4 months (SD = 12) to 30.4 months (SD = 18) over the 1-year period, representing an average growth of 9 months within this time. The results from the correlational analyses indicated that those preschoolers with more severe ASD symptoms also presented with lower concurrent cognitive ability. Moreover, they also had fewer gains in cognitive ability over the following year. **Figures 1** and **2** present individual data-points for these associations.

SAMPLE 2: TODDLERS WITH ASD IDENTIFIED VIA DEVELOPMENTAL SURVEILLANCE

Among the sample of 60 toddlers prospectively identified via developmental surveillance and meeting criteria for ASD at 2 years of age, a significant association, of medium effect size, was evident among concurrent ASD symptoms and cognitive AE assessed at Time 1 (i.e., initial diagnostic assessment); $r = -0.52$, $p < 0.001$, $d = 0.7$. A significant association, also of medium effect size, was

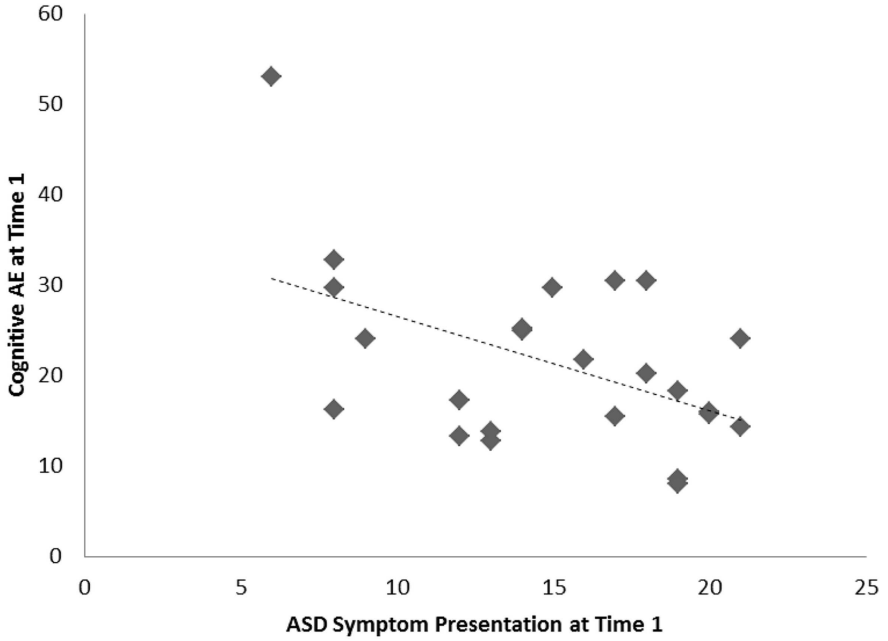

FIGURE 1 | Scatterplots for 23 preschoolers with community ASD diagnoses presenting associations among Time 1 ASD symptoms and concurrent cognitive age-equivalence scores.

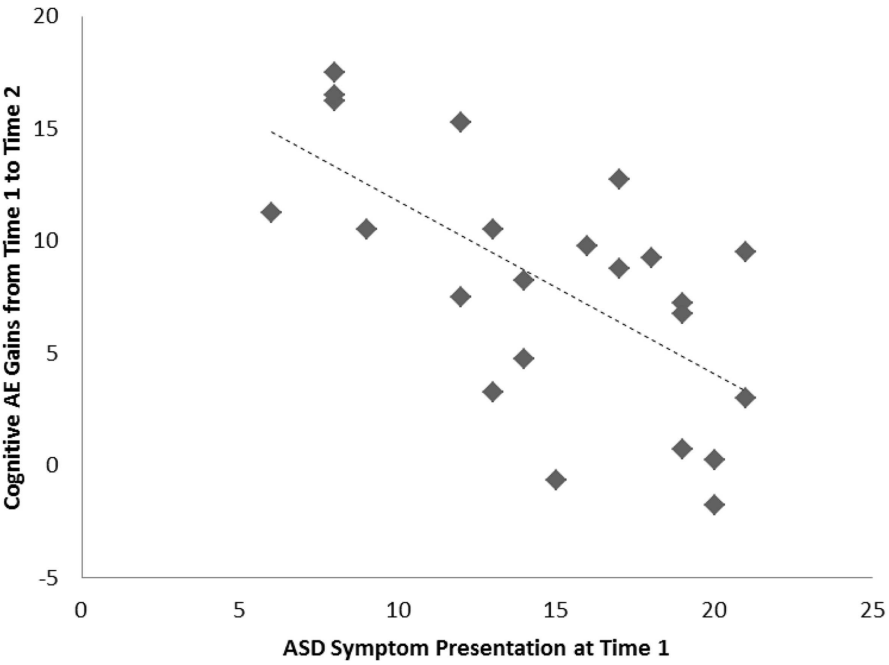

FIGURE 2 | Scatterplots for 23 preschoolers with community ASD diagnoses presenting associations among Time 1 ASD symptoms and gains in cognitive age-equivalence over a 1-year follow-up period.

also evident for this sample between ASD symptom presentation at Time 1 and gains in cognitive AE made between Time 1 and Time 2 assessments; $r = -0.32$, $p = 0.007$, $d = 0.7$. On average, cognitive AE scores increased from 16.7 months (SD = 3.4) to 37.5 months (SD = 11.5) over the 2-year period, representing an average growth of 20.8 months during this time (or around 10.4 months per year).

As with our findings for the preschoolers with ASD, and in keeping with our hypothesis, toddlers with more severe ASD symptoms showed lower cognitive ability at concurrent assessment and also made more limited cognitive gains across the following 2 years. These associations are shown in **Figures 3** and **4**, and a summary of our data across both samples is presented in **Table 2**.

CROSS-SAMPLE SIMILARITIES AND DIFFERENCES

Across these two independent samples of young children with ASD, similar magnitude of associations between Time 1 ASD symptom scores and concurrent cognitive AE are noted. However, effect sizes for the correlations between Time 1 ASD symptoms and longitudinal gains in cognitive AE were of quite different magnitude, albeit presenting a negative association for both groups. A somewhat stronger effect was observed here for our sample of preschoolers, followed across a 1-year period, with a more modest one apparent for our toddlers who were observed across a 2-year period. This variation in effect sizes may be due to sample differences. First, while our toddler sample included a greater number of individuals ($N = 60$) than our preschool sample ($N = 23$), the former comprised a relatively homogenous group, while the latter presented greater heterogeneity in terms of chronological age at each assessment and Time 1 cognitive AE scores (see standard deviation metrics in **Table 1**). Differences in the range of scores present within the groups may account, at least in part, for the differing magnitudes of effect size we observed. Variability in Time 1 ASD symptom presentation was relatively well balanced across the samples. However, by Time 2, cognitive AE scores were more varied in each of the groups. As such, this is unlikely to provide a full explanation for our finding.

The most likely potential contributing factor for the difference in effect size lies in the different intervals spanning Time 1 and Time 2 assessments for each group. In the case of the preschoolers, intake assessment to the community center was followed with an outcome evaluation 1 year later. For toddlers, by contrast, ASD diagnosis was made at a visit when children were ∼2 years of age (having been referred to the study across infancy and toddlerhood via developmental surveillance in the SACS) and the follow-up visit was scheduled ∼2 years later. This longer-interval, over which gains were evaluated, may explain the more modest effect size observed here between early ASD symptom presentation and change in cognitive AE ability.

GENERAL DISCUSSION

Autism Spectrum Disorder is defined in terms of limitations in social-communication and behavioral flexibility, and ID is defined in terms of limitations in intellectual functioning and in adaptive behavior as expressed in conceptual, social, and practical skills. Whilst the validity of ASD as a specific construct independent from ID and other factors (e.g., language) is well established, the majority of individuals with ASD also have co-occurring ID, posing the problem of the nature of this association. One possibility, which is widely accepted in the ASD field, is that ID represents a comorbid condition in ASD (i.e., one that is unrelated in etiology and causality from ASD). In this paper, we outlined a number of theoretical and empirical arguments indicating that this notion is questionable. Whilst there is clear evidence that mild ASD symptoms are compatible with normative or superior IQ, the evidence is less clear with regards to severe ASD symptoms, with recent data (including the novel data presented here) suggesting that the more "autism

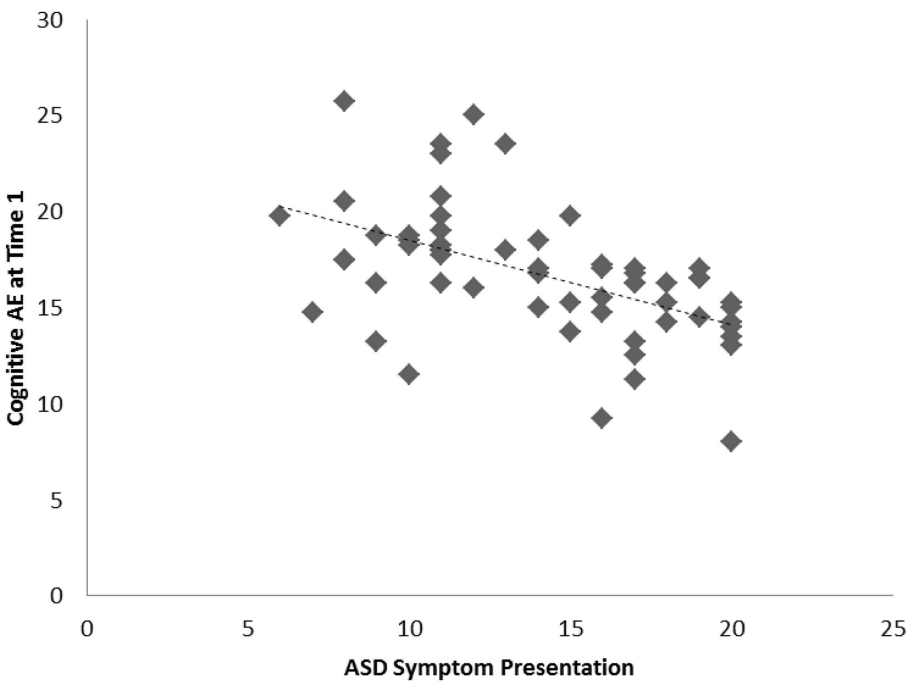

FIGURE 3 | Scatterplots for 60 toddlers with ASD identified via developmental surveillance presenting associations among Time 1 ASD symptoms and concurrent cognitive age-equivalence scores.

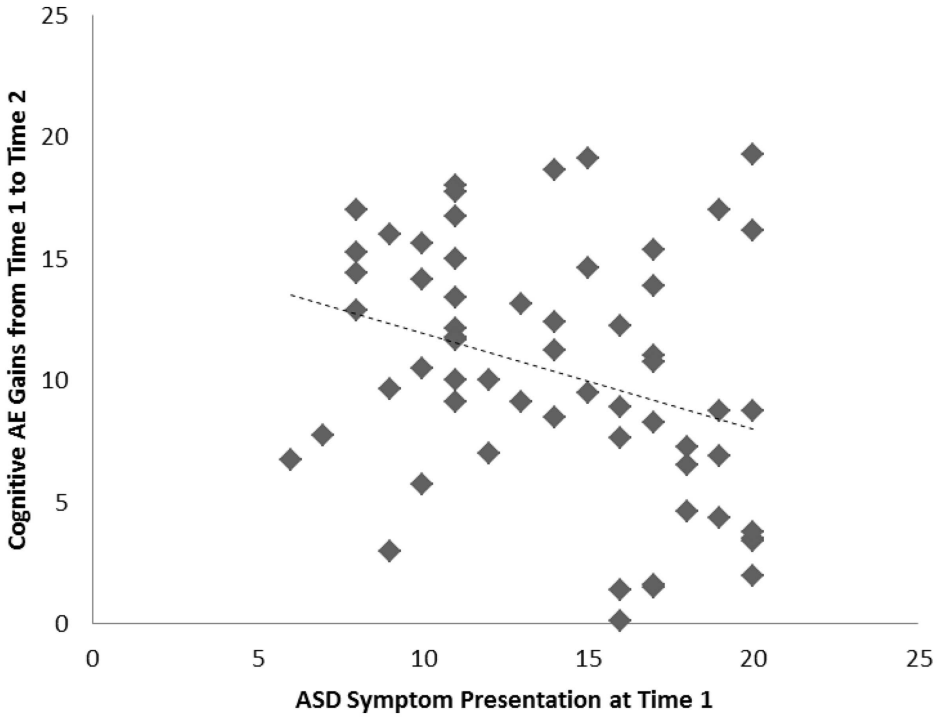

FIGURE 4 | Scatterplots for 60 toddlers with ASD identified via developmental surveillance presenting associations among Time 1 ASD symptoms and gains in cognitive age-equivalence over a 2-year follow-up period.

Table 2 | Summary of the strengths of association across the two samples.

	Preschoolers with ASD (community diagnosis)	Toddlers with ASD (developmental surveillance)
Concurrent associations		
Time 1 ADOS[a] and MSEL[b]	$r = -0.49$, $p = 0.010$, $d = 0.9$	$r = -0.52$, $p < 0.001$, $d = 0.7$
Longitudinal associations		
Time 1 ADOS[a] and Time 2 MSEL[b]	$r = -0.65$, $p = 0.001$, $d = 1.2$	$r = -0.32$, $p = 0.007$, $d = 0.7$
Interval between Time 1 and 2	1 year	2 years

[a] *Autism diagnostic observation schedule – generic (Lord et al., 1999).*

[b] *Mullen scales of early learning (Mullen, 1995).*

specific" symptoms a child has, the more at risk he or she is of poor cognitive outcome. Moreover, early behavioral intervention targeting ASD symptoms results in positive changes in IQ, again indicating some inter-dependence, rather than independence, of these two dimensions.

We argue that the presence of severe ASD symptoms is a risk factor for low IQ, in the same way that severe hypertension or severe obesity increases the risk of cardiovascular events (Flynn et al., 2011). Advances in developmental neuroscience, emphasizing the experience-dependent nature of early brain development (e.g., Grossmann and Johnson, 2007; Kuhl, 2007), allow us to explain this association of ID and ASD from a neurodevelopmental perspective. Based on this framework, we suggest that ASD symptom severity moderates the extent to which the environmental input required to support "typical" brain development can be processed by the individual, so that the risk of poor cognitive developmental outcomes increases as the number and severity of ASD social-communication impairments increase. That is, emerging social-communication deficits early in development might deprive the developing brain from receiving important environmental inputs, with downstream effects on global cognitive development. The result is lower IQ in those individuals with the most severe ASD symptoms.

According to this perspective, we argue that the practice of excluding children with ID in ASD research to study "pure autism unconfounded by ID" is ill considered, just as studying the risk of cardiovascular events in individuals who are slightly overweight, or who have mild presentation of hypertension, would not be informative on the most relevant aspects affecting the outcomes of individuals with those conditions. Rather, research should target those factors that place affected individuals at an increased risk of negative outcomes, by investigating the mechanisms underlying symptoms and their sequelae, and identifying prevention/remediation strategies to foster positive developmental outcomes.

LIMITATIONS AND FUTURE DIRECTIONS

There were several limitations of the current study. First, we do not have data on participants' adaptive behavior level, which form part

of the criteria required for the diagnosis of ID. Second, whilst all of the children involved in this study would likely have been enrolled in community early intervention programs, we have not collected details on the specific programs the children were involved in, nor on the amounts of intervention being received. As such, this potentially critical factor mediating the impact of ASD severity on cognition could not be taken into account in the current study. Future research will involve collecting data on adaptive behavior and participation in intervention in order to build on the results from the current study.

Whilst the aim of this proof of principle study was to test the notion that the severity of ASD symptoms has a negative influence on cognitive development, it is important to mention various other risk factors that are known to affect cognitive development in other groups of children. These include demographic factors, preterm birth, maternal age and education, and birth weight, among others (Zeanah, 2009). In order to disentangle the relative importance of ASD symptom severity from that of other potentially significant factors on cognitive development, more empirical research is needed that utilizes prospective research designs and includes larger sample sizes.

CONCLUSION

Intellectual Disability is known to result from a number of different risk factors. Here, we have argued that the presence of severe (but not mild) symptoms of ASD is one such risk factor, so that ID is unlikely to be a comorbid condition to ASD but, rather, one that is intimately linked to certain ASD presentations. Consistent with Ockham's razor (Popper, 1992), the presence of ID in the majority of the ASD population, in particular in those individuals who are more severely affected with ASD, can be more parsimoniously explained by positing a relationship between these two frequently co-occurring clinical entities than by claiming their independence. A developmental neuroscience framework provides a good explanatory model on the nature of such a relationship, indicating that it is plausible that children with very severe disabilities affecting social understanding and social learning are more vulnerable to poor cognitive outcomes (Coch et al., 2007). As the poor outcomes associated with the presence of ID in ASD result in large human and societal costs, it is important that future research systematically investigate the risk and protective factors associated with the development of ID in ASD. Indeed, excluding individuals with ID from research in ASD only renders more difficult the ultimate goal of fostering positive outcomes for individuals with ASD.

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