FRONTIERS IN BRAIN BASED THERAPEUTIC INTERVENTIONS AND BIOMARKER RESEARCH IN CHILD AND ADOLESCENT PSYCHIATRY

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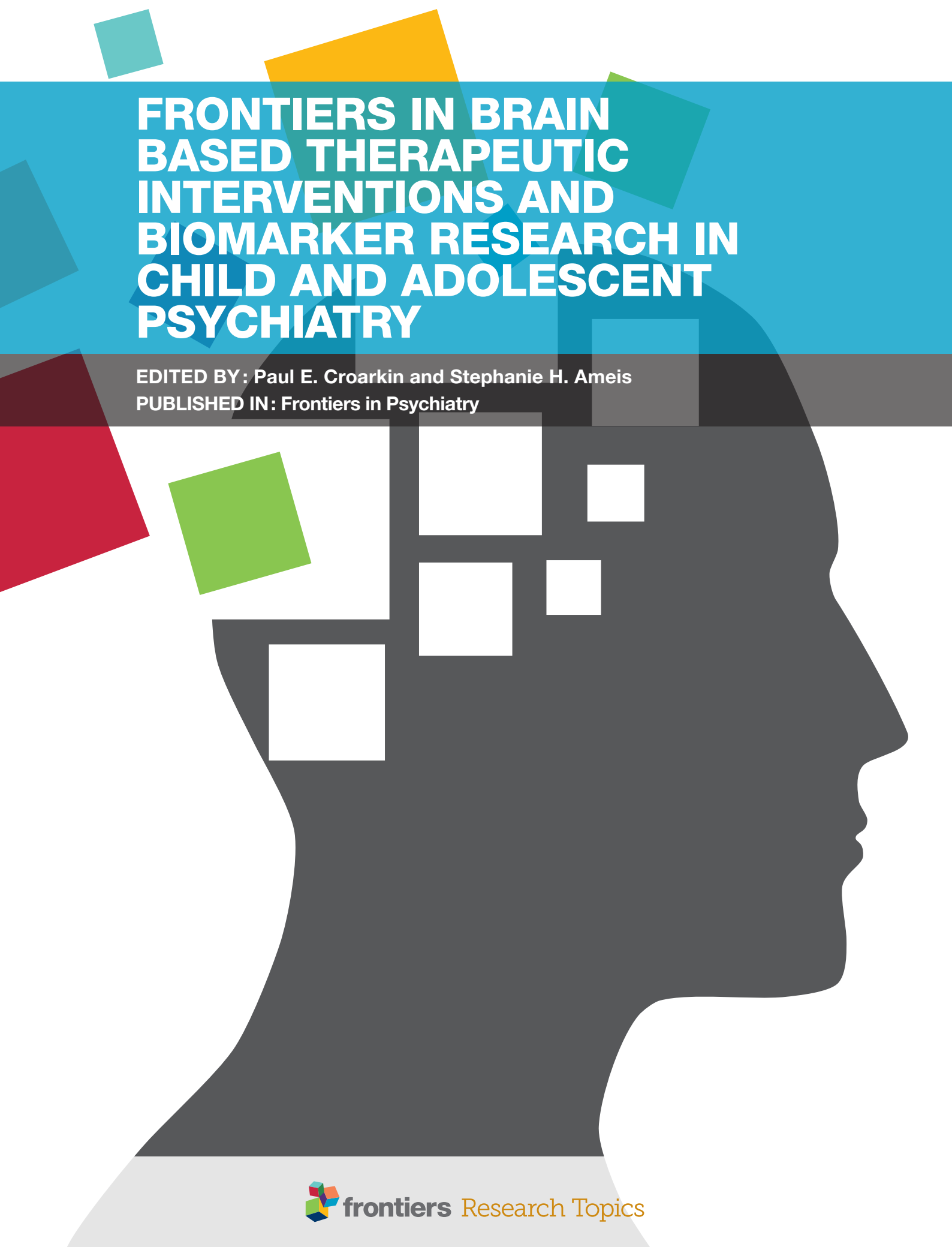

frontiers Research Topics

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FRONTIERS IN BRAIN BASED THERAPEUTIC INTERVENTIONS AND BIOMARKER RESEARCH IN CHILD AND ADOLESCENT PSYCHIATRY

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Developmental neuroscience research is on the cusp of unprecedented advances in the understanding of how variations in brain structure and function within neural circuits confer risk for symptoms of childhood psychiatric disorders. Novel dimensional approaches to illness classification, the availability of non-invasive, diverse and increasingly sophisticated methods to measure brain structure and function in humans in vivo, and advances in genetics, animal model and multimodal research now place brain-based biomarkers within reach in the field of psychiatry. These advances hold great promise for moving neuroscience research into the clinical realm. One exciting new area of translational research in child and adolescent psychiatry, is in the use of a variety of neuroscience research tools to track brain response to clinical intervention. Examples of this include: using longitudinal neuroimaging techniques to track changes in white matter microstructure following a training intervention for children with poor reading skills, or using functional imaging to compare brain activity before and after children with bipolar disorder begin taking psychotropic medication treatment. Brain stimulation is another cutting-edge research area where brain response to therapeutic intervention can be closely tracked with electroencephalography or other brain imaging modalities. Research using neuroscience tools to track brain response to clinical interventions is beginning to yield novel insights into the etiopathogenesis of psychiatric illness, and is providing preliminary feedback around how therapeutic interventions work in the brain to bring about symptom improvement. Using these novel approaches, neuroscience research may soon move into the clinical realm to target early pathophysiology, and tailor treatments to both individuals and specific neurodevelopmental trajectories, in an effort to alter the course of development and mitigate risk for a lifetime of morbidity and ineffective treatments. Excitement and progress in these areas must be tempered with safety and ethical considerations for these vulnerable populations. This research topic focuses on efforts to use neuroscience research tools to identify brain-based biomarkers of therapeutic response in child and adolescent psychiatry.

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EDITORIAL
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Editorial: Frontiers in Brain-Based Therapeutic Interventions and Biomarker Research in Child and Adolescent Psychiatry

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Keywords: adolescent, biomarker, child, child and adolescent psychiatry, neuroimaging, non-invasive brain stimulation, pediatric

The Editorial on the Research Topic

Frontiers in Brain-Based Therapeutic Interventions and Biomarker Research in Child and Adolescent Psychiatry

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Childhood psychiatric disorders present challenges given the heterogeneity of presentations, instability of phenotypes, and nascent understanding of neurodevelopment. Recent efforts, such as the National Institute of Mental Health Research Domain Criteria, aim to hone precision medicine approaches for psychiatric disorders (1). Elucidating the ontogeny of psychiatric illnesses and underlying neurobiology is a mandate for advancing modern clinical practice. Recent advances in neuroimaging, preclinical studies, genomics, and non-invasive brain stimulation may soon provide improved monitoring of development in health and disease. These tools also hold great promise for developing biological markers of illness that may be targeted through treatment innovation. This research topic surveys recent developmentally informed clinical neuroscience efforts focused on conditions that affect children and adolescents. Broadly, this includes studies focusing on neurodevelopmental disorders, eating disorders, mood disorders, and treatment innovations.

NEURODEVELOPMENTAL DISORDERS

Recent changes in descriptive diagnostic criteria, such as DSM-5 (2), aim to bridge basic science findings with clinical practice (3). Goldani et al. examine existing literature focused on putative biomarkers of autism spectrum disorder (ASD). Markers of mitochondrial function, oxidative stress, genetic clustering, and inflammation are promising approaches. However, at present, there is insufficient evidence to embed these markers into clinical practice (Goldani et al.). In other efforts to better understand neurobiology in ASD and related neurodevelopmental disorders, Baribeau and Anagnostou review neuroimaging correlates of ASD and schizophrenia. Volumetric changes, cortical thickness differences, and white matter changes in childhood onset schizophrenia (COS) appear to attenuate with age. Impaired local connectivity may also be coupled with amplified long-range connectivity in this condition. Neuroimaging findings in ASD collectively suggest an initial period of brain verdancy followed by dysmorphogenesis in adolescence. Furthermore in ASD, patterns of

local hyper-connectivity are coupled with impaired long-range neural communication (Baribeau and Anagnostou). Nagamitsu et al. present recent dimensional work with brain single-photon emission computed tomography (SPECT) in participants with attention deficit hyperactivity disorder (ADHD). Children with ADHD, and higher scores on the child behavior checklist-dysregulation profile had significantly increased ^{123}I-iomazenil biding in the posterior cingulate cortex. These data suggest that GABAergic inhibitory neurons are involved in the pathophysiology of ADHD (Nagamitsu et al.).

AFFECTIVE DISORDERS

Recent controversies surrounding the phenotyping of childhood mood disorders underscore the necessity of ongoing work focused on the neurobiological characterization of affective disorders during neurodevelopment (4). Henderson et al. examine white matter microorganization as assessed with diffusion tensor imaging (DTI) in adolescents with depression and healthy controls. Anhedonia and irritability were associated with unique neuroanatomical signatures. This promising early work suggests that prefrontal and limbic tracts are disrupted in depression and unique symptom presentations may have DTI signatures (Henderson et al.). Lee et al. examine brain function of healthy control participants, high-risk offspring, and youth with bipolar disorder by means of a meta-analytic approach. The authors postulated that greater activity in high-risk participants signifies potential compensatory mechanisms, whereas more widespread findings in bipolar patients signified chronic disease processes (Lee et al.). Finally, Walker et al. propose a compelling model for the mood disorder prodrome. These authors posit that early life stress, inflammation, and allosteric load are key contributors to disease burden, disease progression, and neuropathology (Walker et al.).

EATING DISORDERS

Eating disorders are severely impairing psychiatric illnesses, with high mortality rates, and profound neurobiologic underpinnings (5). Herein, McAdams and Krawczyk describe findings from a study of patients with anorexia, patients with bulimia, and healthy controls. Participants were exposed to social attribution, social identity, and physical identity fMRI tasks. Consistently throughout each region of interest, average activation levels for bulimic participants were greater than the group of patients with anorexia, but less than healthy participants. The authors concluded that patients with eating disorders could have a similar biological substrate in terms of social functioning, yet a distinctive functional characterization is a plausible pursuit for future work (McAdams and Krawczyk). Nagamitsu et al. also present intriguing work focused on developing SPECT biomarkers to guide the treatment of children with anorexia nervosa. In children with anorexia nervosa, decreased ^{123}I-iomazenil binding in the anterior cingulate and left parietal cortices was associated with a suboptimal response to treatment. Successful weight restoration was associated with increased relative binding of ^{123}I-iomazenil in the posterior cingulate and occipital cortices (Nagamitsu et al.).

TREATMENT INNOVATION IN CHILDREN AND YOUTH

Transcranial magnetic stimulation (TMS) is a powerful therapeutic and neurophysiological probe. Neurocognitive outcomes are key both in terms of safety and for intervention development, as they may serve as optimal clinical outcome measures in youth (6, 7). Wall et al. report on a study in which eighteen depressed adolescents received 30 sessions of 10-Hz rTMS, applied to the left dorsolateral prefrontal cortex. Participants demonstrated improvements in delayed verbal recall and memory. Furthermore, there were no decrements in other neurocognitive dimensions (Wall et al.). Desarkar et al. postulate that imbalances in excitatory and inhibitory neurotransmission could underlie aberrant neuroplasticity in ASD. At the receptor level, this may involve excessive NMDA and deficient GABA-mediated neurotransmission. Interventions with high frequency rTMS may have a role in stabilizing dysregulated neuroplasticity in ASD (Desarkar et al.).

In conclusion, the synthesis of neuroscience with child and adolescent psychiatry is yielding important discoveries and new directions for treatment innovation. However, we have yet to make the discoveries necessary to bring neuroscience research into the clinical realm through specific biomarker discovery that could pave the way for precision medicine where biomarkers are profiled in the clinic and individualized treatments are selected to optimize neurodevelopmental trajectories, mitigate the long-term effects of psychiatric illness, and maximize functioning for individuals. Novel research tools, innovative study designs that go beyond the case–control model, longitudinal research that identifies developmental trajectories within heterogeneous conditions, and large-scale studies with the power to detect small effects are likely the next frontier in research focused on advancing our understanding of neurobiological underpinnings and developing biologically informed treatments for children and youth with mental illness.

AUTHOR CONTRIBUTIONS

All authors listed have made substantial, direct, and intellectual contribution to the work and approved it for publication.

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Biomarkers in autism

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Autism spectrum disorders (ASDs) are complex, heterogeneous disorders caused by an interaction between genetic vulnerability and environmental factors. In an effort to better target the underlying roots of ASD for diagnosis and treatment, efforts to identify reliable biomarkers in genetics, neuroimaging, gene expression, and measures of the body's metabolism are growing. For this article, we review the published studies of potential biomarkers in autism and conclude that while there is increasing promise of finding biomarkers that can help us target treatment, there are none with enough evidence to support routine clinical use unless medical illness is suspected. Promising biomarkers include those for mitochondrial function, oxidative stress, and immune function. Genetic clusters are also suggesting the potential for useful biomarkers.

Keywords: biomarker, autism spectrum disorders, epigenetics, treatment targets, neuroimaging, genetics

INTRODUCTION

Several neurodevelopmental disorders have complex genetic and epigenetic features that lead to their phenotype and for some there is no single genetic marker for the diagnosis; therefore, the diagnosis is made phenotypically as in schizophrenia, ADHD, and autism spectrum disorder (ASD). While phenotypic characterization of neurodevelopmental disorders is an integral part of advances in clinical practice and research, a given phenotype may arise from a diverse set of biochemical processes (especially when the disorder is caused by numerous genetic and epigenetic factors). Therefore, the treatment of a "phenotypic diagnosis" with a specific drug or intervention might be extremely effective for one "phenotypically characterized" individual with a given set of genetic and/or epigenetic biomarkers, but completely ineffective for another with a different pattern of biomarkers. An important goal of ongoing research in ASD, therefore, is to more precisely identify the many different abnormal genetic and epigenetic processes that underlie the phenotype of the disorder. This might allow individuals with ASD to be characterized into subsets with certain biomarker profiles that would respond more favorably to specific treatments. It also has the potential to elucidate the abnormal physiology that leads to autism, which could improve the understanding of the disorder and lead to earlier diagnosis and more targeted treatments.

A significant challenge in identifying biomarkers in ASD is that biomarkers may reflect genetic and neurobiological changes or epigenetic (broadly defined, see below) processes that may be active only during particular periods of time and do not define the disorder, only the process that led to it. In addition, treatment research should ideally include biomarkers that are believed to predict improvements in clinical symptoms from clinical interventions (1) to know if an intervention is altering or targeting an active biomedical process that relates to response in the subject at that time. Indeed, the National Institute of Mental Health (NIMH) has changed how they fund clinical trials so that "trial proposals will need to identify a target or mediator; a positive result will require not only that an intervention ameliorated a symptom but also that it had a demonstrable effect on a target, such as a neural pathway implicated in the disorder or a key cognitive operation"(2).

Traditionally, research in psychiatry has been guided by DSM symptom based diagnoses and selection criteria for clinical trials were based on these symptom clusters. Biomarkers have not been reliable or valid markers of response to treatment in past trials, and this may be due to the wide variety of genetic and epigenetic processes that underlie the DSM-based diagnosis. Recently, progress in biomarker research has led to the commitment to the Research Domain Criteria project (RDoC) as a basis for future NIMH funding for biomarker based research (3, 4). The RDoC goal is to define basic dimensions of functioning to be studied across multiple units of analysis, from genes to neural circuits to behaviors, cutting across disorders as traditionally defined. The intent is to translate rapid progress in basic neurobiological and behavioral research to an improved integrative understanding of psychopathology and the development of new and/or optimally matched treatments for mental disorders (5).

In this article, we review the literature on biomarkers for ASD including genetic, epigenetic, brain based, and body metabolism biomarkers. This is a huge area and this review is not intended to be comprehensive. New potential biomarkers for ASD are being identified every day so the list needs to be updated frequently. We do extensively review the literature at the time of this writing, report on methodologically sounds studies, offer summary tables, and summarize what we know.

GENETIC BIOMARKERS

The literature supports a hereditary component in the susceptibility to ASDs, there are much higher concordance rates of ASDs in monozygotic twins (92%) than dizygotic twins (10%), and a recent estimate of the sibling recurrence risk ratio (λs) is 22 for autism. Despite being highly heritable, ASDs show heterogeneous clinical symptoms and genetic architecture, which have hindered the identification of common genetic susceptibility factors. Although

previous linkage studies, candidate gene association studies, and cytogenetic studies have implicated several chromosomal regions for the presence of autism susceptibility loci, they have not consistently identified and replicated common genetic variants that increase the risk of ASDs other than some clearly genetic disorders such as fragile X, tuberous sclerosis, and RASopathies whose phenotypes meet the ASD category description (5). As autism is not a single clinical entity, it can be viewed as a behavioral manifestation of tens or perhaps hundreds of genetic and genomic disorders (6). It has been estimated that there are over 500 distinct genetic loci that may be related to ASD (7) (**Figure 1**).

In addition, recent research has shown that there are many epigenetic mechanisms that could account for hereditary influences. A study by Hallmayer et al. (9) reports that the environment may actually account for more of the etiology of autism than genetics. Their study, the largest population-based twin study of autism that used contemporary standards for the autism diagnosis, found that heritability estimated at 38%, while shared environmental component was 58% (9). Heritability of ASD and autistic disorder is estimated to be approximately 50% (10).

Being one of the most familial psychiatric disorders, autism has garnered inquiries about possible genetic biomarkers (11); however, progress has been slow until recently with the introduction of genome-wide association studies (GWAS) and microarrays (12). Research into the microbiological underpinnings of ASDs suggests that it is not a monogenic disorder following Mendelian tendencies, with a few studied individuals and families as notable exceptions (11). In fact, the literature suggests that the risk of developing autism is derived by variations across many genes, none of which have been conclusively, definitively responsible for ASDs although some individuals with single gene disorders such as fragile X also meet the criteria for ASD.

Genome-wide association studies have identified, with replication, *de novo* variations that are strongly associated (with sufficient

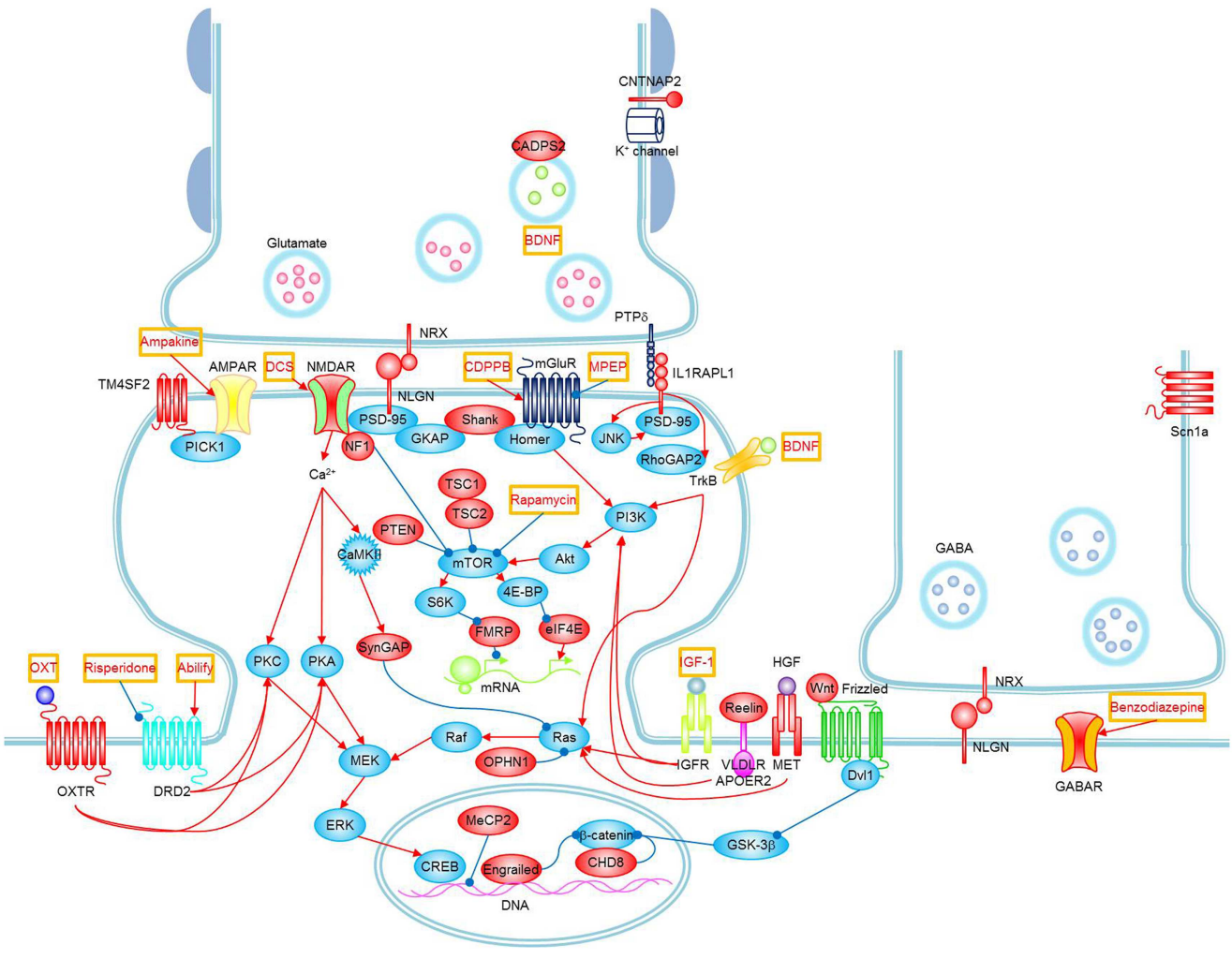

FIGURE 1 | Signaling pathways and possible treatments associated with ASD. Molecules whose mutations or polymorphisms are associated with ASD are indicated in red. Stimulations and inhibitions are indicated by red and blue arrows, respectively. Possible treatments and their target molecules are indicated by red texts in orange boxes. SynGAP1, which directly interacts with PSD-95, could not be placed next to PSD-95 for simplicity. Figure as originally published in Won et al. (8).

Table 1 | Genetic biomarkers in ASD (see text for references).

Neurexin 1 (NRXN1) deletion
7q11.23 duplication
15q11-13 duplication
16p11.2 duplication and deletion
SHANK 3
SHANK 2
SNC2A
CHD8
DYRKIA
POG2
GRIN2B
KATNAL2
CNTN4 deletion
CNTNAP2
5p14.1
CDH10
CDH9
MTHFR 677 >T
SEMA5A
TAS2R1
2q22.1
3p26.3
4q12
14q23
NLGN4

power) with ASDs (**Table 1**): deletions at the Neurexin 1 (NRXN1) locus, duplications at 7q11.23, duplications at 15q11-13, and deletions and duplications at 16p11.2. Earlier studies found rare, functional mutations in genes encoding for NRXN1, SHANK3, and SHANK2, all of which are proteins that affect the functioning of synapses and have been linked to other, known genetic disorders (12). In addition, whole exome sequencing verified by four reports have found genetic mutations associated with autism including SNC2A, CHD8, DYRKIA, POG2, GRIN2B, and KATNAL2 (13).

Studying particular genes in certain, recognized disorders with social deficits, such as fragile X syndrome and tuberous sclerosis, may shed light on the genetic underpinnings of ASDs. This strategy gives credence to the idea that ASD is the result of many variations among genes that converge to a similar phenotype. A prime example of implementation of such a strategy is with contactin 4 (CNTN4), and its association with social and intellectual disability in a recurrent deletion syndrome. Mutations in the respective genes are identified in idiopathic ASDs. Similarly, mutations in CNTNAP2 are linked to a variety of results, such as language delay, functional connectivity abnormalities, selective mutism, and anxiety. More importantly in the scope of ASDs, alterations in CNTNAP2 are noted in consanguineous pedigrees (12). Research shows an increased prevalence of ASDs in families that are consanguineous (11).

In a study published by Nature in 2009, Wang and colleagues completed a genetic analysis in a large number of ASD individuals and families, with a combined sample set of more than 10,000 subjects of European ancestry. They identified common genetic variants on 5p14.1 that are associated with susceptibility to ASDs and replicated these findings in separate analyses. The contribution of chromosome 5p14 to cell adhesion and its connection to autism susceptibility supports the conclusion that specific genes in this class help create the connectivity and structure of the brain that ultimately leads to ASD (14). Besides the potential role of the nearby CDH10 and CDH9 genes, pathway-based association analysis lend further support to neuronal cell-adhesion molecules in conferring susceptibility to ASDs, suggesting that specific genetic variants in this gene class may be involved in shaping the physical structure and functional connectivity of the brain that leads to the clinical manifestations of ASDs (14).

Among the common polymorphisms found to be associated with autism risk, the methylenetetrahydrofolate reductase (MTHFR) polymorphism is one of the most widely studied genetic correlations with autism. The MTHFR 677C > T polymorphism causes a reduction in enzyme activity, which results in higher production of 5-formyltetrahydrofolate (5-FTHF) necessary for DNA synthesis and repair along with lower 5-MTHF production. The MTHFR 677C > T polymorphism causes decline of normal enzyme activity to 35% (15). The MTHFR 677T-variant allele is correlated with a 2.79-fold increased risk for autism. However, this study also found that MTRR 66A and SHMT 1420T alleles demonstrated protective roles against autism risk (16). MTHFR also has a strong interaction with maternal folic acid intake before and during pregnancy, which is associated with autism risk. Children with high autism risk whose mothers carried MTHFR 677 TT allele and were reported taking prenatal vitamins had fewer diagnoses of autism than the children whose mothers with the same allele and did not take prenatal vitamins (17).

In several GWAS (14, 18–20), four genes have been associated with ASDs. These genes, cadherin (CDH9), cadherin 10 (CDH10), semaphorin 5A (SEMA5A), and taste receptor, type 2, member 1 (TAS2R1), are found on chromosome 5p14, which regulates axon growth and cell adhesion. While gene networks could not be established from the small number of genes, these findings do suggest that these genes and the dysregulation of synaptic connection may be a key feature in ASDs (21).

Griswold and coworkers found a significantly higher burden in the number and size of deletions carried by ASD individuals when compared with controls (22). Among the copy-number variations (CNVs) identified were several that overlapped with well-established autism-associated regions and candidate genes. They isolated four large, novel deletions on 2q22.1, 3p26.3, 4q12, and 14q23 that include new genes and regions linked to ASDs. Scattered findings related to NLGN4 and autism susceptibility occur across cultures. In the Chinese ASD cases, there were no significant findings regarding SNPs along NLGN4 gene and autism risk (23), yet in Greek ASD cases, nine nucleotide changes in NLGN4X are found to be associated with autism (24).

Copy-number variations has unveiled the overexpression of rare, *de novo* structural variations in the genome of simplex families (families which have one affected offspring) when compared to families with multiple affected offspring, and especially control families. Furthermore, these results have been replicated in later studies, bolstering the confidence in which discoveries can be made about genetic ties with common diseases and autism

(12); however, *de novo* CNVs have been found in only 5–10% of researched subjects, and thus, do not make up the majority of affected, researched individuals. Despite this finding, it seems as though large (>100 kb), multigenic *de novo* CNVs are the most indicative of ASD risk at this time.

The genetic component of a disorder can be transmitted or acquired through *de novo* ("new") mutations. A study based on a 343 family subset of the Simons Simplex collection did not find significantly greater numbers of *de novo* missense mutations in affected versus unaffected children, but gene-disrupting mutations (nonsense, splice site, and frame shifts) were twice as frequent (59 versus 28) (25). They found that the father is more frequently the parent of origin for *de novo* mutations than the mother (50/17) for single nucleotide variants (SNVs). Parental age also appears to play a role in mutation rate. A study published in *Nature* found that the rate of *de novo* SNVs increases with paternal age ($p = 0.008$) and that paternal and maternal ages are highly correlated ($p < 0.0001$) (26). Overall these data demonstrate that non-synonymous *de novo* SNVs, and particularly highly disruptive nonsense and splice-site *de novo* mutations, are associated with ASD.

Several companies are marketing genetic testing for autism based on clusters of genes with a strong clustering for ASD risk (27, 28). In the future, there may be biomarkers that can pinpoint for high risk for ASD diagnosis. For example, a mother who may be high risk for immune dysfunction leading to ASD in a second child once the first child has ASD (29) or the increase in the Akt-mTOR pathway, which can be seen in fragile X syndrome and in other ASD subtypes (30).

EPIGENETICS

Considerable symptom severity differences within ASD-concordant monozygotic twins, strongly implicates a role for non-genetic epigenetic factors (31). Epigenetics refers to the study of heritable changes in gene activity that are not caused by changes in the DNA sequence; it also can be used to describe the study of stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable. Epigenetic changes in ASD occur through methylation, histone modification (31), chromatin remodeling, transcriptional feedback loops, and RNA silencing (32). Processes in the gene × environment interaction that influence gene expression include metabolic processes such as oxidative stress, mitochondrial function, methylation, immune function, and inflammation that are byproducts of influences such as the mothers and fathers immune systems, environmental toxicants, and diet to name a few. This section will review these epigenetic influences associated with ASD.

Studies show that DNA methylation differences can occur in many loci including AFF2, AUTS2, GABRB3, NLGN3, NRXN1, SLC6A4, UBE3A (31), the oxytocin receptor (33), MeCP2 (a cause for most cases of Rett syndrome) in the frontal cortex (34), and changed chromatin structure in prefrontal cortex neurons at hundreds of loci (35). The severity of the autistic phenotype is related to DNA methylation at specific sites across the genome (31). Environmental and physiological influences are important factors accounting for interindividual DNA methylation differences, and these influences differ across the genome (36). The following sections describe markers for metabolic pathways and environmental influences that can effect epigenetic changes.

METABOLIC BIOMARKERS

There are no autism-defining, metabolic biomarkers, but examining the biomarkers of pathways associated with ASD can point to potentially treatable metabolic abnormalities and provide a baseline that can be tracked over time. Each child may have different metabolic pathologies related to SNPs, nutrient deficiencies, and toxic exposures. Examples of metabolic disorders that can lead to an autistic-like presentation include phenylketonuria (PKU) (37), disorders of purine metabolism (38), biotinidase deficiency (39), cerebral folate deficiency (40), creatine deficiency (41), and excess propionic acid (which is produced by *Clostridium*) (42, 43).

A recent review assessed the research on physiological abnormalities associated with ASD (44). The authors identified four main mechanisms that have been increasingly studied during the past decade: immunologic/inflammation, oxidative stress, environmental toxicants, and mitochondrial abnormalities. In addition, there is accumulating research on the lipid, GI systems, microglial activation, and the microbiome, and how these can also contribute to generating biomarkers associated with ASD (45, 46).

Pathways are interconnected with a defect in one likely leading to dysfunction in others. Many metabolic disorders can lead to endpoints such as impaired methylation, sulfuration, and detoxification pathways and nutritional deficiencies. Mitochondrial dysfunction, environmental risk factors, metabolic imbalances, and genetic susceptibility can all lead to oxidative stress (47), which in turn leads to inflammation, damaged cell membranes, autoimmunity (48), impaired methylation (49), cell death (48), and neurological deficits (50). The brain is highly vulnerable to oxidative stress (51), particularly in children (52) during the early part of development (47). As environmental events and metabolic imbalances affect oxidative stress and methylation, they also can affect the expression of genes.

Several studies have detected altered levels of a large collection of substances in body-based fluids from ASD subjects compared to controls (e.g., serum, whole-blood, and CSF) (53). These findings encompass either of two main disease-provoking mechanisms: a CNS disorder that is being detected peripherally [e.g., serotonin and its metabolites, sulfate (54), low platelet levels of gamma-aminobutyric acid (GABA) (55), low oxytocin (which affects social affiliation) (56), and low vitamin D levels (57, 58)] or a systemic abnormality that has repercussions in the brain (59).

Serotonin in the brain promotes prosocial behavior and correct assessment of emotional, social cues (60) and can contribute to immune abnormalities (61). Oxytocin can affect social affiliation and social communication deficits (62). Vitamin D has many effects including regulating serotonin synthesis, reducing maternal antibodies that attack the fetal brain, modulating oxytocin synthesis, lowering GI inflammation by lowering gut serotonin (58), DNA repair, anti-inflammatory actions, anti-autoimmune activities, antiseizure activity, increase in regulatory T cells, mitochondrial protection, stimulation of antioxidant pathway (63), and increasing glutathione (64).

OXIDATIVE STRESS MARKERS

Oxidative stress can be detected by studying antioxidant status, antioxidant enzymes, lipid peroxidation, and protein/DNA oxidation, all of which have been found to be elevated in children with autism (**Table 2**). Different subgroups of children with ASD have different redox abnormalities, which may arise from various sources (65). A recent meta-analysis from 29 studies of blood samples from subjects with ASD shows that reduced levels of glutathione, glutathione peroxidase, methionine, and cysteine along with increased levels of oxidized glutathione are statistically different in ASD (66). The level of antioxidants excreted in urine was found to be significantly lower than normal in autistic children. These findings correlated with the severity of the ASD (67).

Measurements of antioxidant status include measurement of *glutathione*, the primary antioxidant in the protection against oxidative stress, neuroinflammation, and mitochondrial damage (68, 69). Glutathione is instrumental in regulating detoxification pathways and modulates the production of precursors to advanced glycation end products (AGEs) (70). Measuring reduced glutathione, oxidized glutathione, or the ratio of reduced glutathione to oxidized glutathione helps determine the patient's oxidation status. In many patients with ASD, the ratio of reduced glutathione to oxidized glutathione is decreased, indicating a poor oxidation status (71).

The enzyme glutathione peroxidase has been used as a marker and is typically reduced. There are mixed results concerning the enzyme levels of *superoxide dismutase (SOD)* (72). Other markers for glutathione inadequacy include alpha hydroxybutyrate, pyroglutamate, and sulfate, which can be assessed in an organic acid test. Lipid peroxidation refers to the oxidative degradation of cell membranes. There is a significant correlation between the severity autism and urinary lipid peroxidation products (67), which are increased in patients with ASD.

Plasma F2t-Isoprostanes (F2-IsoPs) are the most sensitive indicator of redox dysfunction and are considered by some to be the gold standard measure of oxidative stress (73). They are increased in patients with ASD and are even higher when accompanied by gastrointestinal dysfunction (73). F2t-isoprostanes (F2-IsoPs) can be measured in the urine as well.

Urine 8-OHdG is biomarker for oxidative damage to DNA. It is commonly used although there are confounding factors and intra individual variations (74) and some researchers have reported that the increases in urine 8-OHdG in patients with ASD is not significant. The increases in urine 8-OHdG did not reach statistical significance (75).

Decreased levels of major antioxidant serum proteins *transferrin* (iron-binding protein) and *ceruloplasmin* (copper binding protein) have been observed in patients with ASD. The levels of reduction in these proteins correlate with loss of previously acquired language (47) although there are mixed reviews of the significance of this (66).

Plasma *3-chlortyrosine (3CT)*, a measure of reactive nitrogen species and myeloperoxidase activity, is an established biomarker of chronic inflammatory response. Plasma 3CT levels reportedly increased with age for those with ASD and mitochondrial dysfunction but not for those with ASD without mitochondrial dysfunction (65).

Table 2 | Oxidative stress biomarkers in ASD (see text for references).

Glutathione – reduced/oxidized
Methionine
Cysteine
Organic acid test – alpha hydroxybutyrate, pyroglutamate, and sulfate
Plasma F2t-isoprostanes (F2-IsoPs)
Urine8-OHdG
Transferrin
Ceruloplasmin
Plasma 3-chlortyrosine (3CT)
3-Nitrotyrosine (3NT)

Table 3 | Mitochondrial function biomarkers in ASD (see text for references).

Lactate
Pyruvate
Lactate/pyruvate ratio
Carnitine (free and total)
Alanine
Quantitative plasma amino acids
Ubiquinone
Ammonia
CD
AST/ALT
CO_2
Creatine kinase
Aspartate aminotransferase
Serum creatine kinase

3-Nitrotyrosine (3NT) is a plasma measure of chronic immune activation and is a biomarker of oxidative protein damage and neuron death. This measure correlates with several measures of cognitive function, development, and behavior for subjects with ASD and mitochondrial dysfunction but not for subjects with ASD without a mitochondrial dysfunction (65).

MITOCHONDRIAL DYSFUNCTION MARKERS

Mitochondrial dysfunction is marked by impaired energy production. Some children with ASD are reported to have a spectrum of mitochondrial dysfunction of differing severity (44) (**Table 3**). Mitochondrial dysfunction, most likely an early event in neurodegeneration (76), is one of the more common dysfunctions found in autism (77) and is more common than in typical controls (78). There is no reliable biomarker to identify all cases of mitochondrial dysfunction (79). It is possible that up to 80% of the mitochondrial dysfunction in patients with both ASD and a mitochondrial disorder are acquired rather than inherited (44).

Mitochondrial dysfunction can be a downstream consequence of many proposed factors including dysreactive immunity and altered calcium (Ca^{2+}) signaling (80), increased nitric oxide and peroxynitrite (68), propionyl CoA (81), malnutrition (82), vitamin B6 or iron deficiencies (83), toxic metals (83), elevated nitric acid (84, 85), oxidative stress (86), exposure to environmental toxicants, such as heavy metals (87–89), chemicals (90), polychlorinated

biphenyls (PCBs) (91), pesticides (92, 93), persistent organic pollutants (POPs) (94), and radiofrequency radiation (95). Other sources of mitochondrial distress include medications such as valproic acid (VPA), which inhibits oxidative phosphorylation (96) and neuroleptics (97, 98).

Markers of mitochondrial dysfunction include lactate, pyruvate and lactate-to-pyruvate ratio, carnitine (free and total), quantitative plasma amino acids, ubiquinone, ammonia, CD, AST, ALT, CO_2 glucose, and creatine kinase (CK) (44). Many studies of ASD report elevations in lactate and pyruvate, others report a decrease in carnitine, while others report abnormal alanine in ASD patients (44) or elevations in aspartate aminotransferase and serum CK (99). Increases in lactate are not specific and may only occur during illness, after exercise or struggling during a blood draw (100).

Rossignol and Frye (44) recommend a mitochondrial function screening algorithm. This includes fasting morning labs of lactate, pyruvate, carnitine (free and total), acyl carnitine panel, quantitative plasma amino acids, ubiquinone, ammonia, CK, AST/ALT, CO_2, and glucose (44). The interpretation of such a panel and the indications for specific treatments has not yet been established.

METHYLATION

The methylation pathway provides methyl groups for many functions, including the methylation of genes, which can result in the epigenetic changes of turning genes on and off (**Table 4**). This transfer occurs when *S*-adenosylmethionine (SAM) donates a methyl group and is transformed to *S*-adenosylhomocysteine (SAH). SAH can be transferred to homocysteine, which can either be re-methylated to methionine or be transferred by the sulfuration pathway to cysteine to create glutathione. With increased oxidative stress, SAH might be diverted away from the methylation pathway to the sulfuration pathway in order to make more glutathione. This will result in less methionine and less methylation ability.

Impaired methylation may reflect the effects of toxic exposure on sulfur metabolism. Oxidative stress initiated by environmental factors in genetically vulnerable individuals, can lead to impaired methylation and neurological deficits (49) both of which may contribute to the manifestation of autism (71).

A marker of methylation dysfunction is decreased SAM/SAH ratio in patients with ASD. Fasting plasma methionine decreases since through SAM it is the main methyl donor. Fasting plasma cysteine, a sulfur containing amino acid is the rate-limiting step in the production of glutathione and is significantly decreased. Plasma sulfate is decreased, which may impair detoxification pathways. Homocysteine is generally increased, but the studies are mixed (66). Vitamin B12 and folate are required for the methylation pathway. The MTHFR genetic SNP is reported to heavily influence the methylation pathway (66).

IMMUNE DYSREGULATION

Cytokine evaluation

Chronic inflammation and microglia cell activation is present in autopsied brains of people with ASD (101, 102) (**Table 5**). Factors that increase the risk of activating brain microglia include traumatic brain injury (TBI) (103) reactive oxygen species (104) and a dysfunctional blood brain barrier (105). The blood brain barrier can be compromised by oxidative stress (106), acutely stressful situations (107), elevated homocysteine (108), diabetes (109), and hyperglycemia (110). Cytokines can pass through a permeable blood brain barrier and start this process (111). Hence, cytokines can serve as a marker of the immune dysregulation, which can further complicate ASD.

Irregular cytokines profiles are found in ASD (112, 113) and elevations in plasma cytokines are reportedly correlated with regressive onset and severity of autistic and behavioral symptoms (113). Altered pro-inflammatory cytokines, complement proteins, chemokines, adhesion molecules, and growth factors are correlated with ASD. More specifically, altered TGF-beta, CCL2, and CCL5, IgM and IgG classes of immunoglobulin circulating levels are linked with a worsening of behavioral scores (114). An imbalance in Th1/Th2 has are found as well, which may play a role in the pathogenesis of autism (115).

Neopertin as a urine marker of immune dysfunction and activation. Neopterin is associated with increased production of reactive oxygen systems and can be considered as a measurement of the oxidative stress elicited by the immune system. Neopterin levels are found to be significantly higher in children with autism than in the comparison subjects (116).

Increased *S100B protein*, a calcium binding protein produced primarily by astrocytes, is a biomarker reflecting neurological/brain damage found elevated in ASD and correlated to autistic severity (117).

Table 4 | Methylation biomarkers in ASD (see text for references).

S-adenosylmethionine (SAM)/*S*-adenosylhomocysteine (SAH)
Homocysteine
MTHFR

Table 5 | Immune biomarkers in ASD (see text for references).

Subjects with ASD
TGF-beta
CCL 2
CCL 5
IGM
IgG
Th1/Th2
Neopterin
S110B protein
Anti ganglioside M1 antibodies
Antineronal antibodies
Serum anti-nuclear antibodies
BDNF
Mothers of subjects with ASD
IFN-Y
Il-4
Il-5
Il-6

AUTOIMMUNITY AND MATERNAL ANTIBODIES

Autoimmune autistic disorder is proposed as a major subset of autism (118), and autoimmunity may play a role in the pathogenesis of language and social developmental abnormalities in a subset of children with these disorders (119). There are many autoantibodies found in the nervous system of children with ASD who have a high level of brain antibodies (120, 121). These can be measured as biomarkers in this subset of ASD patients. The anti ganglioside M1 antibodies (122), antineuronal antibodies (123), and serum anti-nuclear antibodies (123, 124) correlate with the severity of autism. Other autoantibodies postulated to play a pathological role in autism include: anti neuron-axon filament protein (anti-NAFP) and glial fibrillary acidic protein (anti-GFAP) (125), antibodies to brain endothelial cells and nuclei (119), antibodies against myelin basic protein (126, 127), and anti myelin associated glycoprotein, an index for autoimmunity in the brain (128). BDNF antibodies were found higher in ASD (129), and low BDNF levels may be involved in the pathophysiology of ASD (130).

Antibodies in patients with autism are found to cells in the caudate nucleus (131), cerebellum (132, 133), hypothalamus and thalamus (121), the cingulate gyrus (134), and to cerebral folate receptors (135). Children with cerebellar autoantibodies had lower adaptive and cognitive function as well as increased aberrant behaviors compared to children without these antibodies (132).

MOTHER'S IMMUNE STATUS

Research studies indicate an association between viral or bacterial infections in expectant mothers and their ASD offspring (136, 137). Maternal antibodies cross the underdeveloped blood brain barrier of the fetus (138) leading to impaired fetal neurodevelopment and long-term neurodegeneration, neurobehavioral, and cognitive difficulties (139).

A maternal infection or immune response includes cytokines, which affect aspects of fetal neurogenesis, neuronal migration (140), synaptic plasticity, and stem cell fate (141). Elevated serum IFN-γ, IL-4, and IL-5 were more common in women who gave birth to a child subsequently diagnosed with ASD (142). Fetal IL-6 exposure, especially in late pregnancy, leads abnormalities of hippocampal structural and morphology, and decreased learning during adulthood (139).

Some of the antibodies that cross the fetal developing blood brain barrier recognize and attack the brain (138). The presence of fetal brain protein antibodies in ASD can result in an inappropriate approach to unfamiliar peers (143).

Braunschweig et al. developed a panel of clinically significant maternal autoantibody-related autoantibody biomarkers with over 99% specificity for autism risk (144). This panel is suggested to lead to an early diagnosis of maternal autoantibody-related autism, allow for interventions that limit fetal exposure to these antibodies and allow for early behavioral intervention.

DYSBIOSIS

When the gut becomes inflamed, it breaks down and becomes permeable, sometimes referred to as dysbiosis. Dysbiosis is reported to be an upstream contributing factor to autoimmune conditions and inflammation. Markers under consideration include circulating antibodies against tight junction proteins, LPS, actomyosin (145) calprotectin (146), and lactoferrin (147). Dysbiosis was found in 25.6% of patients with ASD (148). It is proposed to have a direct effect on the brain as it is a hypothesized source of inflammation (149–151) and autoimmunity (152, 153), possibly through molecular mimicry (154). Diet is one source of dysbiosis (155).

Table 6 | Other potential biomarkers in ASD.

Glutamate
GABA
BDNF
RBC fatty acids

AMINO ACIDS AND NEUROPEPTIDES

Platelet hyperserotonemia is considered one of the most consistent neuromodulator findings in patients with ASD (**Table 6**). As for other neuropeptides, a recent review reported approximately 15 components that are altered in ASD compared to controls (53). Among them, interesting research has been done on glutamate, GABA, BDNF, and dopamine and noradrenaline systems. A recent study reported a positive correlation between severity of clinical symptoms and plasma GABA levels in patients with ASD, supporting the idea of a disrupted GABAergic system (156). Additionally, a similar grouping of substances measured in the urine is suggested as a more convenient and less invasive way to draw information on these patients (41).

FATTY ACID ANALYSIS

Abnormal fatty acid metabolism may play a role in the pathogenesis of ASD and may suggest some metabolic or dietary abnormalities in the regressive form of autism (42, 157). There is evidence of a relationship between changes in brain lipid profiles and the occurrence of ASD-like behaviors using a rodent model of autism (42). Hyperactivity in patients was inversely related to the fluidity of the erythrocyte membrane and membrane polyunsaturated fatty acid (PUFA) levels (158). Imbalances of membrane fatty acid composition and PUFA loss can affect ion channels and opiate, adrenergic, insulin receptors (159) and the modulation of $(Na + K)$-ATPase activity (160). Analysis of red blood cell membrane fatty acids is a very sensitive indicator of tissue status and may reflect the brain fatty acid composition (161).

Seventeen percent of children with ASD manifest biomarkers of abnormal mitochondrial fatty acid metabolism, the majority of which are not accounted for by genetic mechanisms (162). Patients with ASD had reduced percentages of highly unsaturated fatty acids (163) and an increase in ω6/ω3 ratio (158).

ENVIRONMENTAL TOXICANTS

For environmental toxicant biomarkers, it is difficult to interpret abnormal levels in ASD. For instance, a high burden of aluminum, cadmium, lead, mercury, and arsenic was found in a subgroup of a sample of over 500 patients with ASD (164). Other studies have described decreased levels of some of these heavy metals in urine and in hair samples, which may imply that the body is not excreting the heavy metals adequately (41).

A systematic review of toxicant-related studies in ASD found that pesticides, phthalates, PCBs, solvents, toxic waste sites, air

pollutants, and heavy metals were implicated in ASD, with the strongest evidence found for air pollutants and pesticides (165).

BRAIN FOCUSED BIOMARKERS

MAGNETIC RESONANCE IMAGING

Like other areas in psychiatry, new approaches are being devised to tackle ASD in a "bottom-up paradigm" – that is, identifying genetic or biological alterations, which are associated with the clinical manifestations of symptoms. In neuroimaging, much progress has been made toward understanding the condition, but only very few observed biomarkers have sufficient evidence to suggest that they might hold diagnostic or treatment significance.

One of the best-replicated brain findings from subjects with ASD is an early-accelerated brain volume growth. The increase is usually around 10%, peaking between 2 and 4 years of age followed by a plateau (166). Head circumference (HC), an adequate proxy for brain size, is being investigated for diagnostic relevance for ASD (167). However, recent findings on HC in ASD show that there might be an unrelated growth in HC in both patients and controls. Thus, the abnormal overgrowth observed in older studies might be because of a biased Center for Disease Control (CDC) HC norm, which is commonly used as the control group (168).

Gray matter thickness and surface areas and white matter integrity are also being studied. A general trend demonstrating increased gray matter thickness in subjects with ASD compared to controls is observed with an age-dependent effect (166). Even though there are studies correlating symptom severity with altered thickness there are several limitations such as using a cross-sectional approach and a small number of subjects that hinder clinical application (169). Likewise, diffusion tensor imaging (DTI) studies on white matter connectivity are not yet conclusive across studies.

Early studies using functional magnetic resonance imaging (fMRI) focus on task specific cognitive networks (e.g., face recognition, theory of mind, imitation, language processing, and proxies for receptive behavior) (166). In these cognitive network studies, individuals with ASD and controls perform a task while the fMRI is monitored. More recently, researchers are investigating the connectivity between these network and resting-state methods where fMRI is obtained while a subject is at rest and not performing a task. These more recent studies reveal a pattern that suggests less activity in the brain areas that typically perform executive function tasks (such as organization or planning). This combination of activity patterns in ASD is often called a "high noise-information ratio," supporting an excitatory/inhibitory imbalance theory of ASD (170). Conversely, even though all these fMRI findings shed light on the pathophysiology of ASD, they also are not mature enough to translate into a reliable biomarker that can be used in clinical practice.

ELECTROENCEPHALOGRAPHY

Aligned with the notion that ASD is an abnormal connectivity disorder, studies using electroencephalography (EEG) have explored local changes in signal complexities in patients (171). Some studies were able to detect abnormalities as early as 6 months of age, suggesting an important tool for early detection and risk group assessment (172). However, despite findings like multi-scale entropy differences being proposed as an early diagnostic biomarker, EEG has not yet been established as a reliable tool for diagnosis or to document clinical changes (173).

NEUROCHEMISTRY

Neuroimaging techniques also are used to monitor *in vivo* concentration of substances in the brain, and include positron emission tomography (PET), single photon emission tomography (SPECT), and magnetic resonance spectroscopy (MRS). So far, the majority of studies report abnormalities in several of neurotransmitter networks and their respective metabolites (e.g., dopamine, GABA, serotonin, glutamate, and *N*-acetyl-aspartate), varying from synthesis, transport, and receptor activity in different regions of the brain in the glutamate–glutamine system, in particular, there appears to be either hyper (174) or hypoglutamatergic (175) states depending on the brain region, which could be interpreted as an excitatory increase relative to inhibition in key neural circuits (176). In addition, studies pointing toward GABA alterations also are accumulating, with findings of reduced levels of GABA in the frontal lobes of subjects with ASD. Using MRS (177), corroborated the histopathologic research on altered density and distribution of the GABA receptors (178).

BIOMEDICAL INTERVENTIONS

There are no published studies of interventions for ASD that use neuroimaging or genetic biomarkers in a prospective manner to guide treatment. Biomedical interventions based on body fluid/product biomarkers have been used in a small but growing numbers of well designed, published studies. Several recent reviews summarize these (179–181).

FUTURE RESEARCH DIRECTIONS

A common feature of all prior studies of these putative biomarkers is that most consist of small samples of patients, and therefore, do not grasp the heterogeneity that characterizes ASD. Also, since they mainly compare subjects with ASD to typically developing controls, it is uncertain whether these biomarker profiles are unique to ASD – they may be present in other neurodevelopmental disorders. A promising new method that is designed to increase specificity of biomarkers in ASD is the multiplex immunoassay, a method that analyzes sets of biomarkers to create a diagnostic profile (182, 183). Furthermore, advances in chromatographic and proteomic techniques are also contributing to the progress of the field, allowing easier assessment of several substances (184, 185).

Thus far, numerous studies examining a diverse set of potential biomarkers have found a large number of genetic, imaging, and metabolic tests that are abnormal in children with ASD compared to control subjects. For most of these measures, it is not yet clear if the abnormal biomarker is a contributing factor to the development of ASD or a result of another underlying abnormality (i.e., causal or merely associated). Not surprisingly, the conclusion is that more studies are needed to further explore these possible mechanisms individually. However, the future in the ASD research might involve a broader view of these biomarkers, which might hold more value in combination than in isolation. As a result of new technological advances, it is possible to use a machine learning technique that is trained to identify complex patterns of data

that can be applied to new individuals to make predictions (186). A recent study pooled regional white and gray matter volumes of whole-brain MRI scans in ASD subjects using this computer algorithm program, known as super vector machine. As a result, they could classify a new patient as having an ASD diagnosis or not with a high true positive rate (187). Although exemplified with neuroimaging, this approach could be generalized to other biomarkers (53, 188). In other words, individually insignificant biomarkers when analyzed together might generate a pattern of clinical relevance like diagnosis, severity staging, or response to treatment. These techniques might also be able to identify the most relevant or most predictive biomarkers among the many candidate biomarkers described above.

Although the maxim that "further studies are needed" still holds, ASDs may be witnessing the emergence of clinically relevant biomarkers in the near future.

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A comparison of neuroimaging findings in childhood onset schizophrenia and autism spectrum disorder: a review of the literature

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Background: Autism spectrum disorder (ASD) and childhood onset schizophrenia (COS) are pediatric neurodevelopmental disorders associated with significant morbidity. Both conditions are thought to share an underlying genetic architecture. A comparison of neuroimaging findings across ASD and COS with a focus on altered neurodevelopmental trajectories can shed light on potential clinical biomarkers and may highlight an underlying etiopathogenesis.

Methods: A comprehensive review of the medical literature was conducted to summarize neuroimaging data with respect to both conditions in terms of structural imaging (including volumetric analysis, cortical thickness and morphology, and region of interest studies), white matter analysis (include volumetric analysis and diffusion tensor imaging) and functional connectivity.

Results: In ASD, a pattern of early brain overgrowth in the first few years of life is followed by dysmaturation in adolescence. Functional analyses have suggested impaired long-range connectivity as well as increased local and/or subcortical connectivity in this condition. In COS, deficits in cerebral volume, cortical thickness, and white matter maturation seem most pronounced in childhood and adolescence, and may level off in adulthood. Deficits in local connectivity, with increased long-range connectivity have been proposed, in keeping with exaggerated cortical thinning.

Conclusion: The neuroimaging literature supports a neurodevelopmental origin of both ASD and COS and provides evidence for dynamic changes in both conditions that vary across space and time in the developing brain. Looking forward, imaging studies which capture the early post natal period, which are longitudinal and prospective, and which maximize the signal to noise ratio across heterogeneous conditions will be required to translate research findings into a clinical environment.

Keywords: autism spectrum disorder, childhood onset schizophrenia, neuroimaging, magnetic resonance imaging, child development, review

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder of increasing prevalence in the modern era. Presently, this condition is reported to affect 1 in 88 individuals (1). Manifested by social communication deficits and restricted or repetitive interests and behaviors, children with ASD present along a wide spectrum of clinical severity, from mild social difficulties to severe functional impairment. This condition typically presents in the first 3 years of life, manifested by a failure to gain, or a loss of, social communication milestones.

Childhood onset schizophrenia (COS), on the other hand, is a relatively rare disorder, affecting 1 in 10,000–30,000 children (2). The diagnostic criteria are the same as in adult onset schizophrenia, including the presence of positive and/or negative symptoms (3), but with onset occurring prior to the 13th birthday (4). Despite clinical heterogeneity, COS typically presents with psychotic symptoms after age seven, and is associated with a more severe course and poorer outcomes as compared to adult onset schizophrenia (2).

Although presently considered to separate clinical entities, prior to the twentieth century, catatonia, social withdrawal, bizarre behavior, and/or psychosis in children were considered undifferentiated conditions, labeled as "hereditary insanity," "dementia praecox," or "developmental idiocy" (5). With the onset of contemporary nosology, "autistic behavior and social withdrawal" were initially specified as features of "childhood schizophrenia" in the first and second editions of the Diagnostic and Statistical Manual of Mental Disorder (DSM-I and -II). Although formally defined as separate entities in DSM-III (6), at present the DSM-5 permits concurrent diagnosis of both conditions, should an

individual with ASD subsequently develop prominent delusions or hallucinations (3).

In the current review, a comparison between ASD and COS was chosen for several reasons. Firstly, children with co-occurring and overlapping symptoms complicate a diagnosis (2, 4). At times, a period of medication washout and inpatient observation is required to achieve a diagnostic consensus (7), further supporting a need for brain based biomarkers of disease state and treatment response. Indeed, over one quarter of patients diagnosed with COS display prodromal neurodevelopmental disturbances, meeting criteria for pervasive developmental disorder, or ASD (8, 9). Children diagnosed with ASD are more likely to report psychotic symptoms in adolescence and adulthood (10, 11), although the exact incidence of a subsequent diagnosis of schizophrenia varies by study, ranging from 0 to 7% (12–14). From a neuroimaging perspective, analysis of atypical brain "growth curves" may afford an opportunity for early identification and risk stratification; consistent with the present goal of moving toward biologically based diagnostic categories in neuropsychiatric disease.

Secondly, a growing body of literature supports a neurodevelopmental origin of both schizophrenia and autism, with a shared genetic architecture contributing to, or precipitating, the development of both conditions (15, 16). Some have hypothesized that ASD and schizophrenia are diametrically opposed with respect to underlying pathology (17). While adult onset schizophrenia and ASD have been compared in previous reviews [see Ref. (18)], a focus on COS specifically permits a more in-depth analysis of aberrant neurodevelopmental trajectories across comparable age ranges, which may provide insight into disease pathogenesis.

This review intends to translate several decades of neuroimaging research for a clinical audience, to highlight our current understanding of similarities and differences in the clinicopathogenesis of ASD and COS from a neuroimaging perspective. To our knowledge, this is the first focused review of neuroimaging findings in ASD and COS.

STRUCTURAL MRI STUDIES (VOLUMETRIC ANALYSIS, CORTICAL THICKNESS AND MORPHOLOGY, AND REGION OF INTEREST STUDIES)

VOLUMETRIC ANALYSIS

Structural magnetic resonance imaging (MRI) analysis for neuropsychiatric diseases began to emerge in the 1990s. Early trials employed manual delineation of gray and white matter to investigate specific regions of interest. With advancement in high resolution MRI technology and automated analysis, voxel-based morphometry (VBM) made it possible to quantify the specific gray matter content of each voxel (a volumetric pixel) in an image, allowing large data sets to be processed more efficiently (19). For statistical comparisons between case and control populations, images are "warped" onto a common template, and the degree of transposition of each voxel can be quantified. Inferences must be heeded with the consideration that the relative volumetric differences by region can vary by age, gender, whole brain volume, and by IQ, thus the degree to which these factors have been controlled for must be kept in mind.

Volumetric analysis in COS

Initial trials conducted by the National Institute of Mental Health (NIMH) on a cohort of children with COS, identified a pattern of reduced cerebral volumes and larger ventricles, consistent with findings in the adult onset schizophrenia population (20). With expansion and longitudinal analysis of this patient sample, investigators were able to localize and describe patterns of change in brain structure and volume over time. While typically developing children were found to have a small decrease in cortical gray matter (~2%) in the frontal and parietal regions throughout adolescence, children with COS displayed exaggerated gray matter losses (~8%), involving the frontal, parietal, and temporal lobes. Of note, baseline IQ varied significantly between case and control groups in this data set (70 vs. 124) (21).

Subsequent analysis on the same NIMH sample ($n = 60$ patients), suggested that this pattern took on a "back to front" trajectory, with losses originating in the parietal lobes and spreading anteriorly over time (22). This pattern persisted after controlling for IQ and medication administration (23). Despite significant differences at an early age, the rate of gray matter loss was shown to level off in early adulthood, implicating adolescent neurodevelopment as a key window in disease pathogenesis (22, 24). This data is consistent with hypotheses pertaining to exaggerated synaptic pruning as a feature of schizophrenia (25).

Later work by the same group demonstrated that the above-described pattern was specific for COS. Using VBM, 23 COS patients were compared to 38 age and gender matched healthy control subjects and 19 patients with other psychotic symptoms but not meeting criteria for COS, defined as "multidimensionally impaired" (MDI). MRI scans were conducted at study intake, and at 2.5 years follow up. The MDI group had equal exposure to neuroleptics at study intake, and had a similar degree of cognitive impairment. Total gray matter loss between the two time points demonstrated 5.1% loss for COS patients, 0.5% loss for MDI patients, and 1.5% loss for healthy control subjects. Thus, exaggerated gray matter loss during adolescence was considered to be a potential biomarker of COS (26).

There is very little literature looking at infants or toddlers who subsequently develop schizophrenia, given the methodological complexities of such a study. That being said, offspring of mothers with schizophrenia were found on average to have *larger* intracranial volumes, greater volumes of CSF, and greater gray matter volume on structural MRI in male neonates, compared to controls, although controlling for total intracranial volume resulted in all differences being non-significant (27).

Volumetric analysis in ASD

In ASD, earlier studies suggested a pattern of increased total brain volume, as well as increased ventricle size (28–30). Analyses across age ranges helped to further elucidate the chronology of this brain overgrowth picture. Indeed, exaggerated gray and white matter volumes seemed most pronounced in younger children, while older children with ASD had more typically appearing brains, when compared to their peers (31, 32) (see **Figure 1**). The hypothesis of brain overgrowth correlated with the measureable increase in rate of growth of head circumference during the first few years of life as well in this population (33, 34).

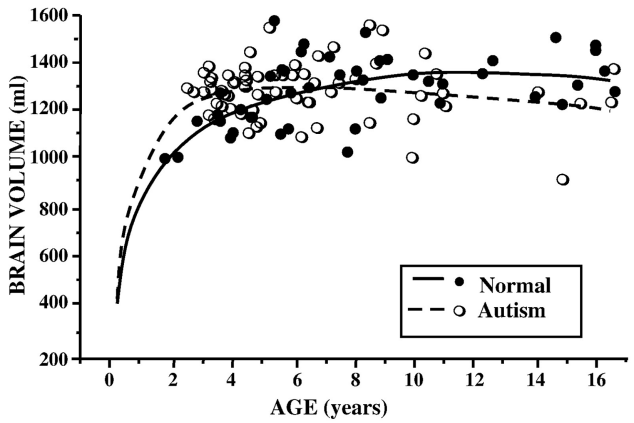

FIGURE 1 | Brain volume (milliliters) by age (years) in children with ASD and controls. Reproduced with permission from Courchesne et al. (37), adapted from Courchesne et al. (32).

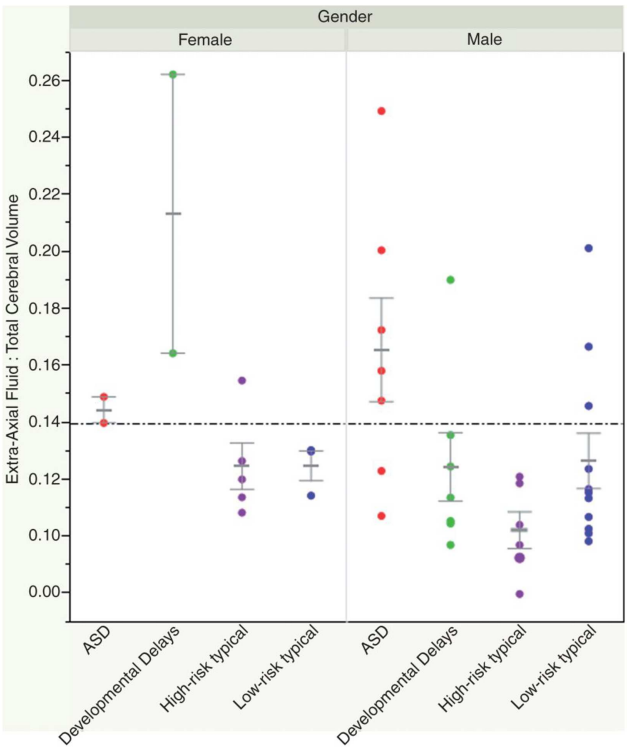

FIGURE 2 | Shen et al. (42) showed how an elevated ratio of fluid:brain volume (above 0.14) at 12–15 months of age was predictive of a subsequent diagnosis of ASD, with 78% sensitivity and 79% specificity in their sample. Reproduced with permission from Shen et al. (42).

In 2005, a meta-analysis of published data on brain volume, head circumference, and post-mortem brain weight in ASD, further described the effect of age, with most marked differences occurring in the first few years of life. In adulthood, however, brain sizes did not vary from controls (35). Subsequent longitudinal and cross-sectional data from hundreds of children and adults with ASD documented volume enlargement during preschool years, most prominently in the anterior regions, followed by possible growth arrest or exaggerated losses later in childhood (36–38). Using cross-sectional age-adjusted data, Schumann et al. (36), for example, showed that children with ASD had 10% greater white matter volume, 6% greater frontal gray matter volume, and 9% greater temporal gray matter volume at 2 years of age. Longitudinal data showed altered growth trajectories at follow up scans (36).

Volumetric differences did not hold true in all ASD studies however, for example, when structural MRI from children with ASD were compared to children with other developmental delays (39, 40). Similarly, a recent systematic review of published data on head circumference overgrowth in children with ASD suggests differences may be much more subtle than previously thought. The authors attribute exaggerated differences to biased normative data in the CDC head circumference growth curves, to the selection of control groups from non-local communities, as well as to a failure to control for head circumference confounders such as weight and ethnicity (41).

Recently, a small study looked at whether volumetric MRI might be predictive of a subsequent diagnosis of ASD, prior to the development of clinical symptoms. A group of 55 infants (33 of which were considered high risk given that they had a sibling with ASD) were scanned prospectively at three time points prior to 24 months of age. At 24 and 36 months, they underwent detailed developmental assessments, at which point 10 infants were identified as having a diagnosis of ASD, and 11 were noted to have other developmental delays. The authors found increased extra-axial fluid volume in infants who developed ASD, and quantified the difference through manual delineation of CSF compartments. They were able to show that a ratio of fluid:brain volume of >0.14 yielded 79% specificity and 78% sensitivity in 12–15 month old infants regarding a subsequent diagnosis of ASD (42) (see **Figure 2**). The finding remains to be replicated.

Summary and comparison. In summary, volumetric analyses in ASD describe early brain overgrowth in the first few years of life, a finding that is difficult to contrast to COS, given the methodological complexity of acquiring neuroimaging data in very young children or neonates who subsequently develop this condition. During childhood and adolescence, volumetric data suggests that individuals with ASD may have attenuated brain growth or exaggerated volume loss, since adults with ASD have comparable brain volumes to their typically developing peers. Some similarities emerge with the COS population, given findings of exaggerated gray matter loss during adolescent years.

CORTICAL THICKNESS AND MORPHOLOGY

With advancements in computational statistics, it became possible extract a more detailed analysis of the cortical gray matter with respect to surface morphology. Specifically, the transposition of cortical imaging data onto a common surface template allowed cortical gray matter volume to be further quantified in terms of cortical thickness, surface area, and gyrification. More recently, complex statistical approaches employing mathematical algorithms and machine-learning models have manipulated

neuroimaging data collected from both volumetric and cortical thickness measurements, in efforts to generate diagnostic classifiers of ASD/COS.

Cortical measurements are of interest for neurodevelopmental disorders as they are thought to represent distinct embryological processes under tight regulatory control (43). Cortical surface area, for example, reflects to the process of neural stem cell proliferation and migration early in embryologic development (44). Cortical thickness, on the other hand, reflects axon and dendrite remodeling, myelination, and synaptic pruning, in a dynamic process lasting from birth into adulthood (45).

Cortical thickness and morphology in COS

In the NIMH-COS sample (46), a combination of cross-sectional and longitudinal data from 70 patients compared to controls revealed diffuse decreases in mean cortical thickness in childhood (~7.5% smaller), which became localized specifically to the frontal and temporal lobes with increasing age. Statistical significance survived correction for covariates such as sex, socioeconomic status, and IQ. Accordingly, while individuals with COS displayed global gray matter and cortical thickness losses in childhood, with age these losses became similar to those observed in adult onset schizophrenia, with deficits localizing more anteriorly (see **Figure 3**).

Interestingly, in two separate samples, non-affected siblings of COS probands also demonstrated a pattern of decreased cortical thickness in the frontal, temporal and parietal lobes during childhood and adolescence, which then normalized in early adulthood, implicating some sort of compensatory mechanism despite underlying genetic risk (47, 48).

With hospitalization and medication management, symptom remission correlated with localized increases in cortical thickness measurable in specific subregions of the cortex (49), irrespective of choice of antipsychotic (50). Children who had other psychiatric conditions with comorbid psychotic symptoms but not meeting full criteria for COS demonstrated cortical deficits in prefrontal/temporal pattern as well, but deficits were smaller and less striking than in COS patients (51).

As mentioned in the introduction to this section, complex algorithms and mathematical protocols have been designed to identify and combine measurements that may be predictive of disease state. A multivariate machine-learning algorithm applied to cortical thickness data from the NIMH cohort was able to correctly classify 73.7% of patients with COS and controls. Through this method, 74 "important" regions were identified. Areas with the most predictive power clustered in frontal regions (primarily the superior and middle frontal gyris), and the left temporoparietal region (52). Given the rarity of COS in the general population, and the case-control study design, these results were not validated in a separate study population, precluding any calculation of positive or negative predictive value, and thus limiting any inferences regarding clinical utility.

Cortical thickness and morphology in ASD

There is significant heterogeneity in the literature with respect to cortical thickness and morphology in ASD, with at times seemingly contradictory results depending on the age, IQ, and clinical severity of the study population.

In a very young group of patients with ASD, cortical volume, and surface area (but not thickness) were found to be increased compared to controls at the age of 2 years. The rate of cortical growth between ages 2 and 5 years did not differ between groups, further implicating the prenatal and early postnatal periods as central to disease pathogenesis (53).

In slightly older age groups, many authors have observed evidence of exaggerated cortical thinning in ASD. For example, Hardan et al. (54) demonstrated that children with ASD ages 8–13 years had increased cortical thickness, particularly in the temporal lobe, as compared to aged matched controls. The small sample size ($n = 17$ cases), however, precluded co-variation for IQ, or analysis of age-related interactions (54). Longitudinal imaging 2-years later on seemingly the same cohort, showed that those with a diagnosis of ASD underwent exaggerated cortical thinning compared to controls, and that the degree of thinning correlated with the severity of symptoms. Differences, however, were mostly non-significant after controlling for multiple comparisons

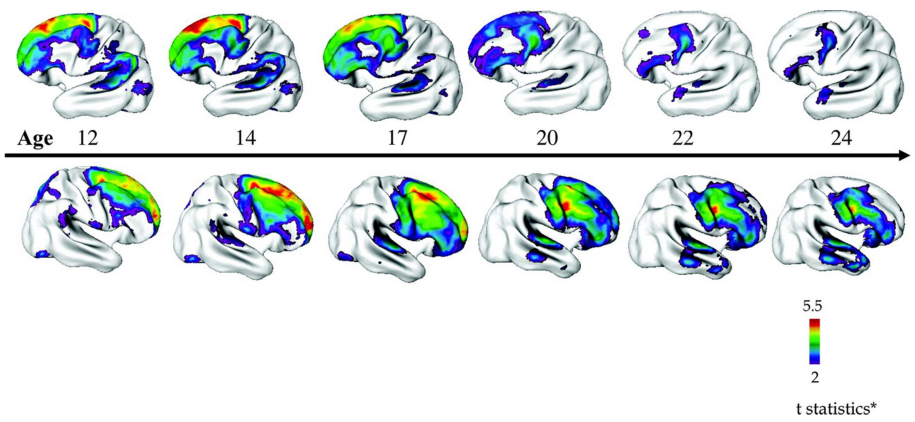

FIGURE 3 | Progressive loss of cortical thickness in a "front to back" pattern observed through longitudinal imaging of 70 children with COS compared to 72 control participants. Reproduced with permission from Gogtay (160), adapted from Greenstein et al. (46).

and variation in IQ (55). In a comparable age group (6–15 years). Mak-Fan et al. (56) showed a similar pattern of increased cortical thickness, surface area, and gray matter volume in children with ASD at earlier ages (6–10 years), that then underwent exaggerated losses compared to controls, such that by 12–13 years of age, controls surpassed patients on all three measures (56). Wallace et al. (57), on the other hand, found baseline *deficits* in cortical thickness for adolescents with ASD, but also observed exaggerated rates of cortical thinning during adolescence and early adulthood (57). In the same study population, no differences in overall surface area were noted, but more overall gyrification in the ASD group, particularly in the occipital and parietal regions was observed. Both groups showed a decline in gyrification overtime (58).

On the other hand, several authors have noted deficits in cortical thinning in ASD. Looking over a wide age range, Raznahan et al. (59) used cross-sectional MRI data from 76 patients with ASD (primarily Asperger's syndrome) and 51 controls from ages 10 to 60 years to study the effects of age on cortical thickness and surface area. While surface area was relatively stable and comparable between both groups, they found significant differences with respect to cortical thickness. Typically developing individuals had greater cortical thickness in adolescence, which thinned steadily overtime. Individuals with ASD had reduced cortical thickness early in life, which underwent relatively little cortical thinning overtime, such that by middle age, they had surpassed their typically developing peers (59). ASD associated deficits in expected age-related cortical thinning during adolescence and adulthood has been shown in several other studies as well, both diffusely and in specific subregions (60, 61).

Recently, Ecker et al. (62) sought to tease apart the relative contributions of cortical thickness and cortical surface area to overall differences in cortical volume in a group of adult males (mean age of 26 years) with ASD compared to controls. While total brain volume and mean cortical thickness measurements were not significantly different between the two groups, several regional clusters emerged with both increased and decreased cortical volumes. The authors found that these relative differences were accounted for by variability primarily in cortical surface area, and less so from cortical thickness. As well, differences in cortical thickness/surface area were largely non-overlapping, and were deemed to be spatially independent from each other (62).

As in COS, several groups have aimed to combine the predictive power of multiple measurements by applying mathematical algorithms to neuroimaging data. Ecker et al. (63), for example, included five parameters (cortical convexity, curvature, folding, thickness and surface area) in their support vector machine analytic approach. These combined measurements were able to correctly classify patients with ASD ($n = 20$) and controls ($n = 20$) with 80–90% specificity and sensitivity, with cortical thickness being the most predictive measurement. This approach also demonstrated proof of principle in separating patients with ASD from patients with ADHD, despite the small sample size, and lack of reproduction in a separate group of patients with ASD from which the algorithm was generated (63). Similarly, Jiao et al. (64) incorporated cortical thickness and volume data from children with ASD and controls (ages 7–13) into a machine-learning model with the aims of predicting presence or absence of ASD. One algorithm was able to predict diagnostic stratification with 87% accuracy based on cortical thickness measurements. The most predictive regions included both areas of decreased cortical thickness (in the left pars triangularis, orbital frontal gyrus, parahippocampalgyrus, and left frontal pole) and increased cortical thickness (left anterior cingulate and left precuneus) (64). Again, the case control design was not representative of true population prevalence, precluding calculation of positive predictive values.

Summary and comparison. In ASD, a small number of studies support a pattern of very early overgrowth in cortical surface area and volume (<2 years of age), which is immediately followed by cortical dysmaturation throughout childhood and adolescence, with evidence suggesting both exaggerated and impaired cortical thinning, depending on the study. Changes in cortical thickness and surface area seem to occur in non-overlapping regions. In COS on the other hand, cortical thickness is reduced diffusely in childhood, although data from very young patients (<8 years) are lacking. During adolescence, reductions in cortical thickness become more localized to frontal regions, although less has been written about the specific rates of cortical thinning in this patient group.

REGIONS OF INTEREST

Studies seeking out and investigating specific regions of interest in both COS and ASD have employed several different approaches. On the one hand, a general approach simultaneously comparing dozens of regions of interest or thousands of specific points in the absence of an *a priori* defined hypothesis has been used to survey for areas associated with the greatest differences between patient and control samples, and can help guide future areas of research. On the other hand, a predefined hypothesis regarding volumetric differences in a particular region allows optimization of statistical power, to more precisely elucidate candidate regions.

Regions of interest in COS

A meta-analysis of studies conducted in adult onset schizophrenia patients describes global deficits in volume, most consistently in the left superior temporal gyrus and the left medial temporal lobe (65). Looking specifically at COS, in the NIMH cohort, an automated and longitudinal analysis of over 40,000 points across the cortical surface found that the superior and middle frontal gyris showed the greatest overall reduction in cortical thickness compared to controls (46). In a different sample COS population from UCLA, specific analysis of the right posterior superior temporal gyrus (Wernicke's area, involved in verbal comprehension), found volume to be *increased* in this region (66). Investigations conducted by the same group on the anterior cingulate gyrus, a central and highly connected structure in the prefrontal cortex involved in many functions including error monitoring, yielded volume reductions (67).

Hypothesis driven approaches in the NIMH-COS cohort have been able to identify specific regional volume deficits as well. The insular cortex, for example, has been implicated in schizophrenia, given its role in distinguishing self from non-self, in visceral somatosensory interpretation, in processing of emotional experiences, and in salience. Patients with COS were found to have

smaller insular volumes, whereas COS-siblings and controls were not statistically different, suggesting reduced insular size as an indicator of disease state. Additionally, level of functioning and severity of symptoms correlated with insular volume (68).

The cerebellum, classically understood to be involved in motor coordination and planning, has been implicated in schizophrenia given its association with learning and cognition. In longitudinal data from the NIMH cohort, smaller overall and regional cerebellar volumes were detected in affected individuals, with siblings falling between patients and controls on various measures (69).

Regarding subcortical structures, enlargement of the caudate (70) has been shown. In the limbic system, increased amygdala volume (71), but volume loss in the hippocampus and fornix (72, 73) has also been found in COS.

Regions of interest in ASD

Brain regions proposed to play a role in social cognition, communication, and "theory of mind" have been a focus of investigation in ASD. The region of the temporoparietal junction in particular, is thought to be central to the integration of social information and empathy, as well as selective attention to salient stimuli (74). Thinning of several areas in the temporoparietal region, particularly on the left side, has been shown in children, adolescents, and adults with ASD (38, 57, 59, 61, 75).

The orbital frontal cortex, in the ventromedial prefrontal region, is thought to play a role in sensory processing, goal directed behavior, adaptive learning, and attachment formation (76). Patients with autism, despite increased overall cortical thickness in the frontal region, have been shown to have specific deficits in cortical thickness (38), volume, and surface area (62) in the orbital frontal cortex, which correlated with symptoms severity (62). Other frontal lobe structures showing reduced cortical thickness in ASD include the inferior and middle frontal gyri, and the prefrontal cortex, depending on the study (38, 64, 77).

The anterior cingulate is a highly connected part of the social brain network situated along the medial aspect of the frontal cortex. Its role in self-perception, social processing, error monitoring, and reward based learning has been described (78). Relative increases (60, 64) and decreases (62, 75, 77) in volume and thickness of the anterior cingulate have been shown in ASD. Given that different regions may grow at different rates in individuals with ASD vs. controls (60, 61), variation in the age and distribution of study populations may account for some inconsistencies.

Volume deficits in the insular cortex have been demonstrated in young adults with pervasive developmental disorders (79). In adults with ASD, those who had a history of psychotic symptoms also demonstrated reduced insular volumes, particularly on the right side, as well as reduced cerebellar volumes (80).

Looking at subcortical structures, the caudate has been shown to be enlarged in ASD, across whole brain volumetric meta-analyses (81–83), and in targeted ROI analysis, even after controlling for confounding medication administration (84). Volume loss in the putamen has been shown across whole brain meta-analyses in adults with ASD (81, 83, 85), but enlargement of the putamen has also been observed in younger populations (86). In the amygdala, volume losses emerge across whole brain meta-analytic approaches (83, 85, 87), but volume gains are noted in younger patient groups as well (88). From a functional perspective, enlargement of the caudate may be associated with repetitive or self-injurious behavior (89–92), while volume loss in the amygdala may pertain to impaired emotional perception and regulation (93).

Summary and comparison. Volume losses have been noted in some overlapping prefrontal regions in both ASD and COS, particularly along the middle frontal gyrus. The anterior cingulate is also implicated in both conditions, although bidirectional changes in volume have been noted in ASD, depending on age of study participants. The area of the temporal-parietal junction shows volume loss in ASD, and was an area strongly predictive of diagnosis in group of individuals with COS (discussed in see Cortical Thickness and Morphology in COS). The insula is implicated in patients with COS, and in those with ASD who have comorbid psychotic symptoms. Looking at deep structures, both conditions are associated with volume gains in the caudate, which may pertain to repetitive behaviors, or concomitant neuroleptic treatment.

STRUCTURAL WHITE MATTER ANALYSIS (VOLUMETRIC ANALYSIS AND DIFFUSION TENSOR IMAGING)

Magnetic resonance imaging analyses that incorporate diffusion measurements allow for further sub-characterization of white matter microstructure, above volumetric differences. The diffusion of water molecules is measurable with MRI technology, and the magnitude and direction of diffusion within each individual voxel can be modeled mathematically with vector algebra. Axial diffusivity (AD) is the measurement of diffusion occurring *parallel* to white matter fibers; increased AD occurs in diseases involving axonal degeneration, and is thought to reflect both the integrity and density of axon structures. Radial diffusivity (RD) on the other hand, is a measurement of diffusion occurring *perpendicular* to the white matter fibers; it is used as a measure of myelination, and is increased in demyelinating diseases. Mean diffusivity (MD) (also known as the apparent diffusion coefficient, ADC) is a measure of average diffusion in absence of a directional gradient (94).

A summary ellipsoid vector incorporating the overall spherical nature of the combined vectors is termed "fractional anisotropy" (FA). A perfectly "isotropic" solution (FA = 0), such as free water, contains molecules that diffuse freely in all directions, whereas an anisotropic solution (i.e., a white matter fiber bundle) would restrict diffusion in one direction resulting in an elongated ellipsoid and FA values closer to 1. In white matter tract analysis, increased FA is thought to be a sensitive but not specific measure of fiber myelination, the integrity of cell membranes as well as the diameter of the fibers (95). Typically developing individuals show age related increases in FA and decreases in MD throughout development, in keeping with increasing white matter maturation (96). As in gray matter analyses, DTI can be applied to the whole brain in a voxel-based approach, or alternatively, specific regions of interest can be investigated with this method. Along these lines, specific anatomic white matter tracts can be reconstructed and analyzed from DTI data, in a method known as tractography. DTI data can also be transposed onto a common FA template, in tract-based spatial statistics (TBSS) (97).

Magnetic resonance imaging data collected in the absence of diffusion measurements can still be utilized in studying white matter integrity and growth. Similar to gray matter analysis, simple volumetric studies on white matter structures have been employed. Alternatively, 3D mapping of volumetric changes in white matter tracts via tensor-based morphometry (TBM) has been validated as a method of studying white matter development over time. In brief, TBM applies initial and follow up scans to a standardized brain template to ensure precise anatomical alignment. Next, an elastic-deformation algorithm is used to calculate the specific degree of volume expansion in a set area, represented by an expansion factor called the "Jacobian determinant." Growth rates are calculated by comparing the Jacobian determinant measures across patient and control samples.

WHITE MATTER ANALYSIS IN COS

The corpus callosum is the largest white matter structure in the human brain, and is central for connectivity and relay of information between hemispheres. Deficits in the corpus callosum have been inconsistently demonstrated in adult onset schizophrenia populations (95). In a longitudinal analysis of children and young adults with COS, differences in the midsagittal area of the splenium of the corpus callosum emerged around age 22, with patients having significantly smaller structures (98). Later analysis looking at volumetric differences in subsections of the corpus callosum revealed no differences between NIMH-COS patients, their siblings and controls with respect to overall volume, and/or volume change over time (99).

Comparison of whole brain TBM data between 12 patients with COS and 12 age matched controls followed over a 5-year interval revealed aberrant white matter development between ages 13 and 19 years. Specifically, at baseline MRI, patients had a 15% deficit in white matter volume in the frontal regions. At follow up, control patients showed an average of 2.6% growth in white matter per year, while COS patient had only 0.4% white matter growth per year. The white matter deficits in the COS sample seemed to progress in a front to back pattern, opposite to previous findings regarding gray-matter deficits, but consistent with expected growth patterns in healthy adolescent brains (100). Unaffected siblings of children with COS showed delayed white matter growth at younger ages (<14 years) but not at older ages (14–18 years) as measured by TBM. Delayed white matter growth was most significant in the parietal regions for siblings, but normalized by age 18 (101).

There are relatively few DTI studies in specific COS populations. Clark et al. (102) found no significant differences in FA diffusely between 18 children and adolescents with COS, and 25 controls. Of note, five COS patients had a comorbid diagnosis of ASD, of which four were tested as having a linguistic impairment. Increased RD and AD was noted for patient vs. control groups in several white matter tracts (see **Table 1**). Increases in RD and AD in these regions were explained primarily by the presence of a linguistic impairment, and not the diagnosis COS, however (102).

There is a growing body of literature, however, on diffusion tensor imaging in adult onset schizophrenia and early-onset schizophrenia (EOS: defined as symptom onset prior to age 18 years). Findings investigating these patient groups are summarized in several reviews (103, 104). Given the paucity of literature applying DTI in COS, some conclusions may be extrapolated from the early-onset schizophrenia literature; therefore they will be discussed briefly.

In general, while results have varied, the corpus callosum, superior and inferior longitudinal fasciculus, cingulum, and the uncinate fasciculus have been suggested as areas most affected with respect to white matter integrity as measured by decreases in FA (103, 104). Some studies have attempted to correlate DTI findings with symptomatology. Ashtari et al. (105), for example, found decreased FA in the left inferior longitudinal fasciculus was more pronounced for EOS patients with a history of visual hallucinations (105). As in volumetric imaging, studies that incorporate

Table 1 | Summary of white matter findings in ASD and COS.

	COS vs. controls		ASD vs. controls	
	White matter volume in COS	**DTI in COS**	**Meta-analysis on white matter volume in ASD**	**Meta-analysis on DTI in ASD**
Study	(160); (99); (98)	(102)	(109)	(110)
Mean age of patient group	(160) 14.1–18.7; (99) 17.3; (98) 14.8	14.7	21.4	15.2
Whole brain white matter	↓ (160)	ND FA	ND	–
Corpus callosum	↓ (98); ND (99)	ND FA; ↑ RD/AD in LI	↓	↓ FA; ↑ MD
Superior longitudinal fasciculus	–	ND FA; ↑ RD/AD in LI (L)	–	↓ FA (L); ↑ MD
Arcuate fasciculus	–	ND FA	↑	–
Inferior longitudinal fasciulus	–	ND FA; ↑ RD/AD in LI (L)	–	ND FA
Inferior fronto-occipital fasciculus	–	ND FA; ↑ RD/AD in LI (L)	↑	ND FA
Cingulum	↓ (160)	ND FA	↓	ND FA
Uncinate fasciulus	–	ND FA	↑	↓ FA (L); ND MD

Note that for ASD, significant findings are reported from meta-analyses only. COS, childhood onset schizophrenia; ASD, autism spectrum disorder; DTI, diffusion tensor imaging; ND, no difference; FA, fractional anisotropy; RD, radial diffusivity; MD, mean diffusivity; AD, axial diffusivity; L, left side; R, right side; LI, COS patients with language impairment.

analyses for age effects provide evidence of dynamic white matter abnormalities as well, in EOS. For example, FA in the anterior cingulate region increased with age in the healthy control population, but decreased with age in the early onset psychosis population (106). Similarly, patients with EOS showed decreased FA in parietal regions, while patients with adult onset schizophrenia had findings localizing to the frontal, temporal, and cerebellar regions (107).

WHITE MATTER ANALYSIS IN ASD

Earlier volumetric analyses suggested a pattern of accelerated of white matter volume and growth in younger children, particularly in the frontal regions, but that adolescents with ASD had similar or reduced white matter volume compared to controls (108). Meta-analysis of 13 VBM studies on white matter volume found no differences globally in white matter volume, and no differences between child/adolescent groups and adults groups, although no studies included very young children (<6 years). Some regional differences emerged, however (109) (see **Table 1**).

With respect to diffusion tensor imaging, a recent systematic review and meta-analysis, combining DTI data from 14 studies, including both children and adults with ASD, summarized some areas of consensus and heterogeneity in the literature. In summary, decreased FA was most consistently demonstrated in the corpus callosum, left uncinate fasciculus, and left superior longitudinal fasciculus of individuals with ASD. Mean diffusivity was increased in the corpus callosum, and bilaterally in the superior longitudinal fasciculus (110). This meta-analysis included data from ROI and tractography studies only, however, excluding whole brain TBSS and voxel-based analyses. A recent literature review on DTI in ASD by Travers et al. (97), identified decreased FA, increased MD, and RD as the most common finding across methods, with the corpus callosum, cingulum, arcuate fasciculus, superior longitudinal, and uncinate fasciculus showing the greatest differences (97).

Most imaging studies in autism to date, as well as those included in the above-described meta-analyses, have been conducted in older children, adolescents, or adults. In these age groups, decreased FA and increased MD have been repeatedly documented in many white matter regions. The specific rate of change in white matter markers, as well as the effect of age on white matter maturation seems to vary by study, however. For example, Mak-Fan et al. (56) showed RD and MD measurements stayed stable between the ages 6 and 14 years in subjects with ASD, while control subjects showed expected decreases with age (111). Ameis et al. (112) found the between group differences in RD, AD, and MD, but not FA, which were more pronounced in childhood than in adolescence (112).

Few studies have been conducted in very young children, however, and less consistency emerges in the data from this age range. Contrary to literature in older populations, Weinstein et al. (113), reported that FA was *greater* for children ages 1.5–6 years with ASD compared to controls in the areas of the corpus callosum, superior longitudinal fasciculus, and cingulum. Differences in FA were attributable to decreased RD, while AD was the same between cases and controls (113). Similarly, Ben Bashat et al. (114), found evidence of accelerated white matter maturation marked by increased FA and reduced displacement values in a small sample of children with ASD ages 1.8–3.3 years, most prominently in frontal regions (114). Abdel Razek and colleagues (115), found ADC scores to be greater for preschool children with ASD in several regions, which correlated with severity of autistic symptoms as measured by the childhood autism rating scale (115). Walker et al. (116) on the other hand, found that 39 children between ages 2 and 8 years with ASD had decreased MD and FA compared to controls, accompanied by an attenuated rate of increase in FA, as well an accelerated rate of decreased MD compared to controls (116). Longitudinal data looking at high risk infants found evidence of higher FA at 6 months in children who were subsequently diagnosed with ASD, but that they had then had a slower rate of change such that by 24 months typically developing children had surpassed them in this measure (117).

For most studies, although differences have been statistically significant for certain regions, the magnitude of these differences has been quite small, on the range of 1–2%, thus limiting the predictive ability of any individual measurement. Lange et al. (118) generated a discriminant function that was able to distinguish between individuals with and without ASD with 94% sensitivity, 90% specificity, and 92% accuracy, by combining the predictive ability of DTI data points centered primarily around the superior temporal gyrus and the temporal stem. The sensitivity and specificity was reproduced in a replicate sample as well, however the case-control design was not reflective of true population prevalence, again precluding inferences regarding predictive ability in a real life clinical setting (118).

Emerging efforts have tried to correlate neuroimaing findings to functional and behavioral outcomes. For example, increased MD in the superior longitudinal fasciculus correlated with degree of language impairment in children and adolescents (119). Increase FA and decreased RD in the arcuate fasciculus correlated with greater language abilities in another group of children with ASD (120). Similarly, lower FA in the dorsal lateral prefrontal region was associated with increased social impairment in a group of children with ASD in Japan (121). Attempts to identify structural deficits in areas involved socio-emotional processing have yielded mixed results as well. Further focus on understanding the functional connectivity between distant regions is described in the next section.

Summary and comparison. White matter development in COS patients compared to controls appears marked by global deficits in white matter volume and decreased rates of white matter growth/integrity in adolescence, although the specific chronology, most affected regions and the relation to symptoms continues to be explored. In ASD, meta-analyses suggest no differences overall in white matter volume in adults, although early white matter volumetric overgrowth may occur in younger patient samples. Looking at specific white matter regions, volume losses have been noted in both ASD and COS in the corpus callosum and cingulum. In both conditions, decreased white matter integrity as measured though DTI has been observed in the superior longitudinal fasciculus, which may pertain to comorbid language impairments.

FUNCTIONAL CONNECTIVITY

While imaging of white matter tracts through techniques like DTI permits the quantification of structural connectivity between

regions, *functional* connectivity requires *in vivo* analysis of brain activation. Functional magnetic resonance imaging (fMRI) measures regional changes in blood oxygen level dependent (BOLD) signaling, given the subtle differences in magnetic field strength between oxygenated and deoxygenated blood. Brain activation patterns may be analyzed in subjects at rest (termed resting state) or during a specific cognitive or behavioral task performed in an MRI scanner. Data can be analyzed with respect to a specific region of interest (seed technique), where connections to and from an *a priori* defined region are studied. Alternatively, independent component analysis (ICA), or similar techniques, look at overall activation patterns across all regions, and can comment on patterns in functional networks (i.e., default mode network, salience network). Data from functional neuroimaging studies are often analyzed using graph theory. In this approach, the relationship between certain areas of central activation (termed "nodes") and the vectors of connectivity between nodes (termed "edges") are described using discrete mathematics (122). Short-range connectivity (i.e., within a specific lobe, or to a neighboring lobe) and long-range connectivity between remote regions can be quantified in this manner.

FUNCTIONAL CONNECTIVITY IN COS

Two separate analyses in the NIMH cohort of COS have suggested exaggerated long-range connectivity, and impaired short-range connectivity, in keeping with a hypothesis of exaggerated synaptic pruning. Resting state fMRI data was used to graph the connectivity between 100 regional nodes for 13 patients and 19 controls. Data showed that patients with COS had signals that were less clustered with more disrupted modularity marked by fewer edges between nodes of the same module. On the other hand, they showed greater global connectedness and greater global efficiency (123). Subsequent analyses with a slightly larger sample again found reduced connectivity at short distances and increased connectivity at long distances for patients with COS compared to controls on resting state fMRI. Relative to healthy controls, patients with COS had several regions in the frontal and parietal lobes that were "nodes" of over-connectedness with respect to long-range associations (124). White et al. (125) on the other hand, interpreted an opposite pattern from a study using a visual stimulus to analyze connectivity in the occipital lobe of children and adolescents with early onset schizophrenia (125). Similarly, structural connectivity analysis in neonates at high risk for schizophrenia found decreased global efficiency, increased local efficiency, and fewer nodes and edges overall compared to control infants (126).

FUNCTIONAL CONNECTIVITY IN ASD

In ASD on the other hand, there is an abundance of recent literature on functional connectivity. An emerging hypothesis suggests that frontoparietal under connectivity in ASD results in reduced "bandwidth" in long-range circuits [reviewed by Just et al. (127)]. Some propose that this coincides with local increases in connectivity within a specific lobe, resulting in a failure to integrate and regulate multiple sources of information (128). This hypothesis is consistent with structural white matter deficits in long-range association fibers, as well as structural patterns in gray matter showing increased local, but deficits in global modularity (129).

With respect to functional analyses, impaired synchronization, and under connectivity between large-scale networks has been shown in fMRI studies incorporating various task-based assessments, including those pertaining to language comprehension and auditory stimuli (130–132), executive functioning (133), visual spatial processing (134), and response to emotional cues (135, 136). Under connectivity has not been the only finding however, with many functional MRI studies showing evidence of increased connectivity or altered developmental trajectories with respect to integrated neural networks (137–139). For example, a recent meta-analysis of fMRI studies found greater activation in children with ASD in response to a social task in certain specific regions (i.e., in the left-precentral gyrus) but relative under activation compared to controls in other areas (superior temporal gyrus, parahippocampal gyrus, amygdala, and fusiform gyrus). In adults with ASD, activation was greater in the superior temporal gyrus, but less in the anterior cingulate during social processing (140).

The literature is also divided with respect to functional neuroimaging in resting state MRI, in the absence of any particular stimulus or task. Some have proposed that methodological issues may be contributing to observed inconsistencies (141). While hypoconnectivity seems most prevalent in the literature, [Ref. (142, 143); reviewed by Uddin et al. (144)], Uddin et al. (144) observed long-range hyperconnectivity via ICA across remote regions in 20 children ages 7–12 years with autism compared to controls. Hyperconnectivity was noted to involve the default mode network, frontotemporal, motor, visual, and salience networks. Hyperconnectivity of the salience network (which involves the anterior cingulate and insula) was most predictive of the diagnosis of ASD and was able to discriminate between cases and controls with 83% accuracy, a finding that was reproduced in a separate image dataset (145). Other resting state fMRI studies have also observed mixed patterns, which vary by region, network, and by age of the sample (146, 147).

The literature in very young patients with ASD is relatively sparse but seems to suggest altered developmental trajectories for affected children beginning at very young ages. A recent publication observed increased functional connectivity at 3 months, which disappeared by 12 months in high risk infants (148). Alternatively, Redcay and Courchesne (139) found increased connectivity between hemispheres in 2–3 year old children with ASD compared to chronological age matched controls, however the opposite pattern emerged when they were compared to mental age matched controls (139). Dinstein et al. (132) observed hypoconnectivity between hemispheres and in language regions in toddlers with ASD in response to auditory stimuli (132).

A recent review article by Uddin et al. (144) summarizes the literature to date with respect to resting state functional connectivity analyses. While intrinsic connectivity and seed-based analyses across 17 published studies suggest both hyper- and hypo-connectivity, Uddin and colleagues propose that the developmental age of the sample may be one explanatory factor with respect to variability in results. They describe a hypothesis in which increased functional connectivity in prepubescent children with ASD as compared to their peers is then met with altered maturational trajectories such that adults with ASD seem to have reduced connectivity compared to controls (144).

A recent publication put forth by a data sharing initiative entitled "autism brain imaging data exchange" (ABIDE) proposes to remedy disagreement in the literature through a large-scale international collaboration combining 1112 resting state fMRI scans. Analysis of 360 male subjects with ASD compared to controls found hypo connectivity in cortical networks but hyper connectivity in subcortical networks. They also identified localized differences in connectivity in certain regions, including the insula, cingulate, and thalamus. They did not perform specific analyses looking for age-associated differences, however, given that the majority of included participants were adolescents or adults (146).

Summary and comparison. There are only a handful of studies looking at functional connectivity in COS, but data from fMRI suggest a pattern of increased long-range connectivity, with disrupted short-range connectivity, in keeping with pathology of exaggerated synaptic pruning. In comparison, data from fMRI in ASD suggest to some extent an opposite pattern, with increased local but decreased global connectivity. fMRI data sharing between research centers reveal hyperconnectivity in subcortical networks, and hypoconnectivity in cortical networks in adult males with ASD. Smaller studies in younger age groups suggest important age effects regarding the connectivity hypothesis as well, with younger children with ASD seemingly showing more "over-connectedness" than adults.

DISCUSSION

This review compares and contrasts neuroimaging findings in ASD and COS. Overall, across volumetric, structural, and functional neuroimaging data, there arises evidence for a dynamic changes in both conditions. In ASD, a pattern of early brain overgrowth is seemingly met with dysmaturation in adolescence, although the literature in this regard is far from certain. Functional analyses have suggested impaired long-range connectivity as well as increased local and/or subcortical connectivity, which may also progress with age. In COS, global deficits in cerebral volume, cortical thickness, and white matter maturation seem most pronounced in childhood and adolescence, and may level off in early adulthood. Deficits in local connectivity, with increased long-range connectivity have been proposed, in keeping with exaggerated cortical pruning; however the opposite has also been shown. Symptom and neuroimaging overlap across conditions was illustrated via a meta-analysis of fMRI data in both schizophrenia and ASD, which identified shared deficits in regions involved in social cognition (149).

The significance of these findings is tempered, however, by heterogeneity in results across other pediatric onset neurodevelopmental disorders. In ADHD for example, longitudinal MRI analyses in children suggest overall reduced cortical thickness prior to the onset of puberty (158) with peak cortical thickness and onset of cortical thinning occurring at later ages (159). In the future, clinical neuroimaging must be able to identify not only the presence of aberrant neurodevelopment, but also be able to discern across overlapping conditions.

While there is heterogeneity in the literature in both conditions, findings regarding COS at times appear more consistent. It is important to note that, given the rarity of this condition, these findings emerge from relatively few research samples, and are derived primarily from data collected from the same population of individuals. In ASD on the other hand, there has been an international explosion of investigation at numerous institutions, across ages, IQ ranges, and diagnostic severity, which has resulted in at times seemingly contradictory results. A call for collaboration (150) has been met with a first international compilation of neuroimaging datasets, which has helped to clarify some discrepancies in the literature with respect to fMRI (146). Going forward, ongoing collaboration to facilitate large scale, prospective, longitudinal neuroimaging studies, will be necessary to separate signals from noise in these complex and heterogeneous diseases. A focus on genetic subtypes may help to unite synapse pathology with neuroimaging findings and network dysfunction, to permit some degree of hypothesis generation with respect to molecular pathogenesis.

In ASD, for example, a loss of inhibitory control leading to exaggerated growth, premature cortical thinning, and then early stabilization of cortical structures has led some to suggest that overall the developmental curve has been "shifted to the left" along the time axis in this condition, with respect to brain maturation (75, 151). Current genetic investigations suggest alterations in structural scaffolding at the excitatory synapse could be contributory in ASD (152). Single gene disorders associated with autism may shed light on underlying final common pathways (153). Fragile X syndrome (FXS), for example, is a genetic condition comorbid with ASD in 20–30% of cases (154). Individuals afflicted with this condition have dysfunction or absence of the fragile X mental retardation protein (FMRP). FMRP is now understood to play a critical role in regulation of protein synthesis at the excitatory synapse, and without it, exaggerated receptor cycling and dysfunctional neuroplasticity can results (153). A similar mechanism in idiopathic ASD would hypothetically results in a loss of inhibitory control on expected maturational changes, uncoupling the structural and temporal timeline of synaptic neurodevelopment.

In schizophrenia, exaggerated synaptic pruning has been a long held hypothesis with respect to an etiology (25), which is consistent with aspects of the neuroimaging literature in COS. On the other hand, a small study in high risk infants suggests enlarged cerebral volumes may exist early in life, implying that some type of early dysregulated growth may be at play in this condition as well, similar to the process occurring in ASD (27). Investigations in 22q11.2 deletion syndrome (DS), a genetic disorder associated with schizophrenia in 20–25% of cases (155), permits longitudinal and prospective analysis of children at high risk for schizophrenia. Interestingly, MRI data collected in children as young as 6 years old with 22q11.2 DS found early increases in cortical thickness and deficits in cortical thinning in preadolescence, which are then met with exaggerated cortical thinning during adolescent years. Patients who subsequently developed schizophrenia indeed had more exaggerated deficits in cortical thickness (156).

In studies recruiting adolescents, it is difficult to tease out the possible influence of confounders such as substance abuse on both clinical and radiologic findings. While comorbid substance abuse is common in adult onset schizophrenia populations (occurring in 50–80% cases), the rate of substance abuse in COS, while presumed

lower, has not been described (157). Ongoing study of clinical, environmental, and cultural confounding factors in both ASD and COS is needed.

Many investigators have sought to use neuroimaging protocols as predictors of diagnosis in case-control studies. The accuracy, sensitivity, and specificity of these analyses have on average ranged between 60 and 90%, and some groups have been able to reproduce high levels of diagnostic accuracy in separate patient samples. The clinical utility of these algorithms, however, remains uncertain in the absence of their application to populations reflecting realistic disease prevalence (i.e., positive predictive values are low or not reported). The development of clinically useful, cost-effective wide scale diagnostic tests for neurodevelopment conditions remains a common goal, and several groups have initiated prospective trials on high risk patient populations which may perhaps yield some hopeful results in the next decade.

AUTHORS CONTRIBUTION

Danielle A. Baribeau authored the manuscript. Evdokia Anagnostou developed the research topic, provided guidance, editing, and supervision.

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Upregulated GABA inhibitory function in ADHD children with child behavior checklist–dysregulation profile: 123I-iomazenil SPECT study

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The child behavior checklist–dysregulation profile (CBCL–DP) refers to a pattern of elevated scores on the attention problems, aggression, and anxiety/depression subscales of the child behavior checklist. The aim of the present study was to investigate the potential role of GABA inhibitory neurons in children with attention deficit/hyperactivity disorder (ADHD) and dysregulation assessed with a dimensional measure. Brain single photon emission computed tomography (SPECT) was performed in 35 children with ADHD using 123I-iomazenil, which binds with high affinity to benzodiazepine receptors. Iomazenil binding activities were assessed with respect to the presence or absence of a threshold CBCL–DP (a score $\geq$210 for the sum of the three subscales: Attention Problems, Aggression, and Anxiety/Depression). We then attempted to identify which CBCL–DP subscale explained the most variance with respect to SPECT data, using "age," "sex," and "history of maltreatment" as covariates. Significantly higher iomazenil binding activity was seen in the posterior cingulate cortex (PCC) of ADHD children with a significant CBCL–DP. The Anxiety/Depression subscale on the CBCL had significant effects on higher iomazenil binding activity in the left superior frontal, middle frontal, and temporal regions, as well as in the PCC. The present brain SPECT findings suggest that GABAergic inhibitory neurons may play an important role in the neurobiology of the CBCL–DP, in children with ADHD.

Keywords: CBCL-dysregulation profile, iomazenil, GABA, ADHD

Introduction

Severe behavioral and affective dysregulation with symptoms, such as hyperactivity, aggression, irritability, mood instability, and anxiety, contribute to significant academic and psychosocial impairment in children. Some of these symptoms are consistent with attention deficit hyperactivity disorder (ADHD). ADHD is the most frequent neuropsychiatric disorder in children and often presents with co-occurring disruptive behavior disorders, anxiety disorders, and bipolar disorder. Hyperactivity, irritability, and impulsivity place children at risk of maltreatment as a result of

strained parent–child interactions (1, 2). The insecure parent–child relationship further exacerbates the behavioral and affective dysregulation observed in children.

The child behavior checklist-dysregulation profile (CBCL–DP) refers to a pattern of elevated scores on the Attention Problems, Aggression, and Anxiety/Depression subscales of the child behavior checklist (CBCL) (3). The CBCL–DP was originally proposed as a means of identifying youth with bipolar disorder (4). However, recent studies suggest that the results of the CBCL–DP are not simply an early manifestation of a single disease process, but rather that the CBCL–DP can be used as a developmental risk marker for a persisting deficit in self-regulation of affect and behavior (5, 6). The CBCL–DP may be best interpreted as an indicator of symptom severity and functional impairment (7, 8). Children with ADHD who had a threshold level CBCL–DP score ($\geq$210) showed higher rates of comorbidity disorders, including oppositional defiant disorder (ODD), conduct disorder (CD), anxiety disorder, bipolar disorder, and depression (9). The CBCL–DP is also associated with mood, anxiety, disruptive behavior disorders, and substance use in adulthood (3).

The underlying neurobiological defects or aberrant neuronal activity leading to the dysregulation profile in children with ADHD are elusive. Reducing serotoninergic function in children with ADHD and a significant CBCL–DP resulted in slower cognitive performance compared to children with ADHD who did not have the CBCL–DP, indicating that serotoninergic function could play a decisive role in the etiology of the CBCL–DP (10). In addition, the CBCL subscale of "Aggression" was found to be the main discriminator of ADHD children with CBCL–DP versus those without CBCL–DP with respect to serotoninergic dysfunction. Conversely, prior translational work with magnetic resonance spectroscopy and transcanial magnetic stimulation paradigms suggest that GABAergic neurochemistry and neurotransmission are dysregulated in children with ADHD (11). Ongoing work also suggests that defects in the GABAergic system in adults increase an individual's vulnerability to severe psychiatric illnesses due to aberrant regulation of serotoninergic and/or dopaminergic neurons (12, 13). Previous biochemical and pharmacological studies indicate that deficits in GABA receptor function, induced by intravenous infusion of iomazenil followed by a serotoninergic agonist, predispose healthy volunteers to increased anxiety and dissociative disturbances, suggesting that deficits in the GABAergic system may contribute to the pathophysiology of serotonin-induced psychosis (12).

123I-iomazenil is a radioactive ligand for central-type benzodiazepine receptors that forms a complex with GABA(A) receptors. Thus, 123I-iomazenil single photon emission computed tomography (SPECT) can indirectly index GABA receptor function. 123I-iomazenil is a frequently used radionuclide tracer for presurgical evaluation of patients with refractory partial epilepsy (14, 15). Moreover, recent neuroimaging studies have explored the role of GABAergic inhibitory function in psychiatric disorders such as schizophrenia, anxiety disorders, and developmental disorders (16–20). To our knowledge, there is no previous work which characterizes GABA receptor functioning with 123I-iomazenil SPECT among children with ADHD. The working hypothesis of the present study was that behavioral and affective symptoms in children with ADHD, reflected in CBCL–DP scores, would correlate with changes in cortical GABAergic neuronal activity. To confirm this hypothesis, brain SPECT was performed using 123I-iomazenil in ADHD children with or without CBCL–DP. Further, we tried to identify which of the three significant scales in the CBCL–DP explains the most variance with respect to SPECT data using "age," "sex," and "history of maltreatment" as covariates.

Materials and Methods

Ethics Statement

The design of the study and procedures for obtaining informed consent were approved by the Medical Ethics Committee of Kurume University School of Medicine (#10081). Informed consent was obtained from each child and his/her parents prior to their participation in the study.

Participants

Thirty-five children with ADHD (23 boys, 12 girls) enrolled in the study. Participants were recruited after visits from the Department of Pediatrics, Kurume University, for the management of externalizing symptoms (e.g., difficulty maintaining attention, restlessness, hyperactivity, and aggressive behavior) or internalizing symptoms (e.g., anxiety, dissociation, and depressive symptoms). A diagnosis of ADHD was made using the *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (Dsm-Iv-Tr)* (21). Children who had anxious or depressive symptoms, but did not have ADHD symptoms were excluded in this study. Of the 35 child participants with ADHD, 15 had the combined type, 11 had hyperactive-impulsive type, and 9 had inattentive type. Seventeen children (7 male, 10 females) had experienced an obvious maltreatment, such as physical ($n = 9$), psychological ($n = 6$), or sexual abuse ($n = 1$), or sexual assault ($n = 1$) during preschool. In 11 of these instances, a child-welfare consultation center had previously supported the families in hopes of preventing maltreatment. Two children stayed in a child residential care institution. However, none of the participants met the diagnostic criteria for posttraumatic stress disorder (PTSD) on assessment. Seven of the 35 subjects (20%) had comorbid disorders, such as ODD ($n = 2$), CD ($n = 1$), anxiety disorder ($n = 2$), and depression ($n = 2$). The mean age of the children at the time of their hospital visit was 10.4 years. All participants were medication naïve prior to enrollment.

Child Behavior Checklist

Behavioral and psychiatric assessments of the children included the CBCL, ADHD rating scale (hyperactivity/impulsive and inattention scores, as well as total score) (22, 23), the Child Depression Inventory (CDI), the Child Dissociative Checklist (CDC), and the Wechsler Intelligence Scale for Children (WISC-III). The CBCL was used to evaluate children's emotional and behavioral functioning, competencies, and social problems, with specific items evaluating internalizing and externalizing symptoms, as well as attention and thought problems. Items evaluating internalizing symptoms focus on withdrawal, somatic complaints, and anxiety/depression. Items evaluating externalizing symptoms focus on delinquent or aggressive behavior. The CBCL–DP refers to

a pattern of elevated scores on the Attention Problems, Aggression, and Anxiety/Depression subscales of the CBCL. A threshold CBCL–DP was defined as a score ≥210 for the sum of three subscales. (4) Physicians rated the participants using the ADHD rating scale, CDI, and CDC, and the parents rated their children using the CBCL.

Iomazenil Single Photon Emission Computed Tomography and Analysis of Regions of Interest

All 35 children underwent iomazenil SPECT imaging of the brain. Briefly, children were injected intravenously with a bolus of 95–117 MBq 123I-iomazenil (Nihon Medi-Physics, Tokyo, Japan), which binds with high affinity to benzodiazepine receptors. The SPECT scan was performed 3 h after injection of the tracer, without any sedation, using a large field-of-view dual-detector camera and a computer system equipped with a low-energy, high-resolution, parallel-hole collimator. The dual detector camera rotated over 180° in a circular orbit and in 32 steps of 40 s each to cover 360° in about 22 min. Brain magnetic resonance imaging (MRI) was performed using a superconducting magnet operating at 1.5 T. For coregistered SPECT and MRI analysis, a method of image integration was applied using Fusion Viewer software (Nihon Medi-Physics) with a registration algorithm based on maximum mutual information (**Figure 1**). Subsequently, the cortical and subcortical regions of interest (ROIs) in the acquired SPECT data were defined. Using elliptical templates, the ROIs were placed over the following regions: the superior frontal, middle frontal, parietal, temporal, and occipital regions in each hemisphere; the midbrain; and the anterior and posterior cingulate cortex (ACC and PCC, respectively; **Figure 1**). Each relative iomazenil binding activity in ROIs was expressed as a ratio of that in the occipital cortex. As 123I-iomazenil affinity in the occipital region was maximum and stable in brain cortex, the occipital region was used as a reference (24).

Data Analysis

The differences of each CBCL subscale, ADHD-RS, CDI score, CDC score, and Intelligence scale between ADHD children with/without CBCL–DP were compared by student's *t*-test. We first analyzed correlations between the relative iomazenil binding activity expressed as a ratio in each ROI and psychometric profiles after controlling for the effects of age, sex, and history of maltreatment. Further, we compared iomazenil binding activity with respect to the presence or absence of a threshold CBCL–DP score in these children and tried to identify which of the three CBCL–DP subscales explained the most variance with respect to the SPECT data. Associations between each of the CBCL–DP subscales and iomazenil binding activity in each brain area were evaluated using liner regression models, with "age," "sex," and "history of maltreatment" as covariates.

Results

Behavioral and psychiatric assessments of the participants were shown in **Table 1**. Of the 35 participants, 15 had a threshold CBCL–DP score (i.e., a score ≥210) and 20 had CBCL–DP scores <210. The group with threshold CBCL–DP scores had a lower ratio of male to female participants and more instances of maltreatment. Each ADHD rating scale, all subscales of CBCL with the exception of somatic problems, and CDC score were significantly higher in ADHD children with threshold CBCL–DP scores. Four participants with threshold level CBCL–DP scores had comorbidity disorders, including depression ($n = 2$), ODD ($n = 1$), and CD ($n = 1$). Three participants without threshold CBCL–DP scores had comorbidity disorders, including anxiety disorder ($n = 2$) and ODD ($n = 1$). There was a difference in the CBCL–DP scores between participants with/without comorbidity disorders; however, this difference did not reach statistical significance ($n = 28$, 199 ± 20, and $n = 7$, 209 ± 12, respectively, $p = 0.10$).

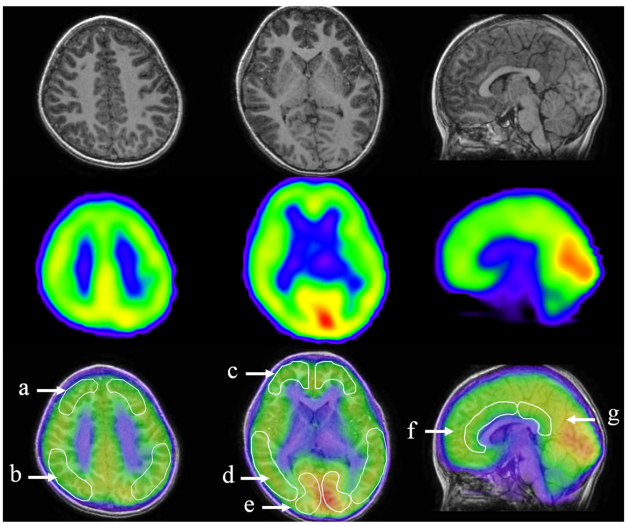

FIGURE 1 | Designated regions of interest (ROIs) in fusion images of 123I-iomazenil SPECT and MRI. The top panel shows brain MRI (transverse and sagittal T_1 sequences), the middle panel shows corresponding results of 123I-iomazenil SPECT, and the bottom panel shows fusion imaging. Outlined regions in the bottom panel indicate designated ROIs, namely (a) the superior frontal, (b) parietal, (c) middle frontal, (d) temporal, (e) occipital regions, (f) anterior, and (g) posterior cingulate gyrus.

Analyses of all participants ($n = 35$) revealed correlations between iomazenil binding activity in several brain regions and some part of the CBCL profile, after controlling for the effects of age, sex, and a history of maltreatment (**Table 2**). In both ACC and PCC, iomazenil binding activity had a statistically significant positive correlation with scores on the Anxiety/Depressed (**Table 2**; **Figure 2**), Internalizing, and Withdrawal Problems subscales of the CBCL (**Table 2**). In addition, significant positive correlations were noted for iomazenil binding activity in the ACC and Thought Problems on the CBCL, as well as for iomazenil binding activity in the PCC and Attention problems and Social Problems on the CBCL (**Table 2**). These significant correlations were not seen for other combinations in other brain regions, except for iomazenil binding activity in the midbrain and Thought Problems on the CBCL, and iomazenil binding activity in the right temporal region and Social Problems on the CBCL. There were no significant correlations between iomazenil binding activities in any brain region and any of the ADHD rating scales, CDI score, and CDC score

TABLE 1 | Behavioral and psychiatric assessments of participants.

	ADHD children without significant CBCL–DP	ADHD children with significant CBCL–DP
Number of participants	20	15
Male:Female	17±3	6 ± 9
Mean age (years)	10.7 ± 1.9	10.0 ± 1.8
Experience of maltreatment (%)	40	80
ADHD-RS		
Total score	23.5 ± 9.2	30.6 ± 9.0*
Inattention score	14.8 ± 5.7	17.9 ± 4.9*
Impulsivity/hyperactivity score	9.2 ± 5.6	12.6 ± 6.0*
CBCL score		
Internalizing score	64.4 ± 9.7	80.8 ± 8.2**
Externalizing score	62.7 ± 8.5	71.1 ± 4.8**
Aggressive behaviors	63.7 ± 9.0	79.2 ± 7.2**
Anxious/depressed	58.1 ± 4.9	66.4 ± 4.5**
Attention problem	66.2 ± 5.4	73.7 ± 5.5**
Delinquent behavior	62.7 ± 8.5	71.1 ± 4.8**
Withdrawn	61.5 ± 5.6	66.3 ± 5.6**
Somatic problems	55.5 ± 15.2	58.4 ± 9.5
Social problems	62.0 ± 8.8	66.9 ± 8.1*
Thought problems	59.9 ± 9.7	67.8 ± 9.9*
CDI score	14.3 ± 9.6	15.6 ± 6.3
CDC score	6.7 ± 3.8	13.3 ± 4.7**
WISC-III	90.0 ± 15.6	87.3 ± 13.7

CBCL–DP, child behavior checklist–dysregulation profile; ADHD-RS, attention deficit hyperactivity disorder rating scale; CDI, child depression inventor; CDC, children dissociative checklist; WISC, Wechsler intelligence scale for children.
*Significant difference from children without significant CBCL–DP (*indicates $p < 0.05$, **indicates $p < 0.001$).*

(data not shown). Iomazenil binding activity in the PCC was significantly higher in ADHD children with a threshold CBCL–DP score than in ADHD children with scores <210 after controlling for the effects of age, sex, and a history of maltreatment (**Table 3**, F-value = 4.36, $p < 0.05$). Of the three CBCL–DP subscales, the Anxiety/Depression subscale had significant effects on higher iomazenil binding activity in the left superior frontal, middle frontal, and temporal regions, as well as in the PCC (**Table 4**).

Discussion

This is the first neuroimaging study showing that behavioral and affective symptoms in children with ADHD, reflected in CBCL–DP scores, are correlated with changes in cortical GABAergic neuronal activity. Overall, increased iomazenil activity in the ACC and PCC was associated with higher scores on many of the CBCL subscales. In ADHD children with a significant CBCL–DP, iomazenil activity was upregulated in the PCC. Of the three CBCL–DP subscales, the Anxiety/Depression subscale had a significant effect on iomazenil binding activity in many brain regions. These results suggest that behavioral and affective dysregulation in ADHD children may be characterized by changes of GABAergic neural activity. In this section, we discuss the role of the cingulate cortex in GABA function, the association between CBCL–DP scores and GABA function, and age-dependent differences in GABA function.

The cingulate cortex is one of the largest parts of the limbic lobe and the prefronto-limbic circuitry. The ACC plays key roles in emotion, motivation, and motor functions, whereas the PCC is involved in emotion, facial recognition, and memory functions (25–27). In the present study, we found higher iomazenil binding activity in ACC and PCC that was associated with higher scores on many of the CBCL subscales in ADHD children with and without CBCL–DP. Similar findings have been reported in healthy adults. For example, Kim et al. (28) found a positive correlation in healthy subjects between high GABA concentrations in the ACC and a high harm avoidance temperament, characterized by worrying about potential problems, fearful of uncertainties, and being shy in unfamiliar environments. Moreover, increased activity in the PCC has been observed in emotional disorders, including obsessive–compulsive disorder, major depression, and social phobia (29, 30). Because the cingulate cortex has been suggested to have an important role in modulating human fear and anxiety by modulating the activity of other limbic structures, including the amygdala (31), the increased GABAergic function in the cingulate cortex of ADHD children in the present study may have inhibited excessive excitation of the limbic system, which contributes to the development of behavioral and affective dysregulation.

We found that the Anxiety/Depression subscale of the CBCL–DP explains the most variance with respect to SPECT data in various brain regions using "age," "sex," and "history of maltreatment" as covariates. The Aggression and Attention Problem subscales of the CBCL–DP had no significant effects on SPECT data in various brain regions. These findings strongly support previous converging lines of evidence regarding the association between GABAergic activation and increased anxiety (32). Conversely, several biochemical and genetic studies have provided evidence of a significant role of serotoninergic function in aggressive behavior. For example, an inverse correlation has been reported between downregulated platelet or CSF 5-hydroxyindolecetic acid (5-HIAA), a major metabolite of serotonin, and levels of aggression and impulsivity (33, 34). Furthermore, Haberstick et al. (35) reported an association between certain promoter polymorphisms in the serotonin transporter (5HTTLPR) and greater aggressive behavior in middle childhood, suggesting that differences in serotonergic functioning may be a contributing factor to different levels of aggressive behavior. In terms of the biological mechanism underlying attention function, an important role for dopaminergic neurons has been proposed. Several neuroimaging studies have shown aberrant dopamine transporter (DAT) levels in the nucleus accumbens, caudate, and midbrain, as well as a positive relationship between DAT levels in the putamen and inattention scores in ADHD patients (36–38). Together, these findings suggest that changes in several neurotransmitter systems, including serotoninergic, dopaminergic, and GABAergic neurons, are likely to be involved in constructing the clinical manifestations of the CBCL–DP.

Significant positive correlations between GABAergic inhibitory function and the Anxiety/Depression subscale were also seen in our study. Although previous neuroimaging studies have reported those correlations in adulthood with psychiatric disorders (17, 28), the present study is the first report of the correlation in childhood with psychiatric disorders. Despite the positive correlation in childhood, previous neuroimaging studies using iomazenil SPECT have revealed negative correlations between

TABLE 2 | Partial correlation coefficients between CBCL profiles and the iomazenil binding activites in each brain region.

	Superior frontal		Parietal		Middle frontal		Temporal		Mid brain	ACC	PCC
	R	L	R	L	R	L	R	L			
CBCL profiles											
Total problems	0.341	0.237	0.192	0.267	0.301	0.229	0.284	0.191	0.178	0.385	0.245
Internalizing problems	0.199	0.160	0.026	0.068	0.110	0.188	0.136	0.332	0.274	0.477*	0.536**
Externalizing problems	0.158	0.086	0.057	0.090	0.148	0.027	0.127	−0.072	0.032	0.078	−0.041
Aggressive behaviors[a]	0.119	0.052	0.036	0.077	0.113	−0.042	0.088	−0.111	−0.040	0.017	−0.026
Anxious/depressed[a]	0.309	0.284	0.120	0.129	0.264	0.335	0.246	0.369	0.355	0.536**	0.524**
Attention problem[a]	0.265	0.120	0.234	0.157	0.228	0.166	0.248	0.160	0.212	0.378	0.483**
Delinquent behavior	0.122	0.091	0.068	0.102	0.133	0.087	0.175	0.108	0.159	0.077	0.008
Withdrawn	0.278	0.105	0.163	0.006	0.192	0.057	0.338	0.214	−0.012	0.425*	0.509**
Somatic problems	−0.131	0.045	−0.162	0.035	−0.133	0.074	−0.246	0.080	0.216	−0.050	0.205
Social problems	0.304	0.112	0.325	0.217	0.264	0.108	0.406*	0.204	0.129	0.383	0.440*
Thought problems	0.322	0.221	0.269	0.207	0.307	0.320	0.310	0.239	0.546**	0.432*	0.360

CBCL, child behavior checklist; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; R, right; L, left.
**Indicates $p < 0.05$; **indicates $p < 0.01$.*
[a]*Indicates subscale which comprises CBCL–DP (dysregulation profile).*

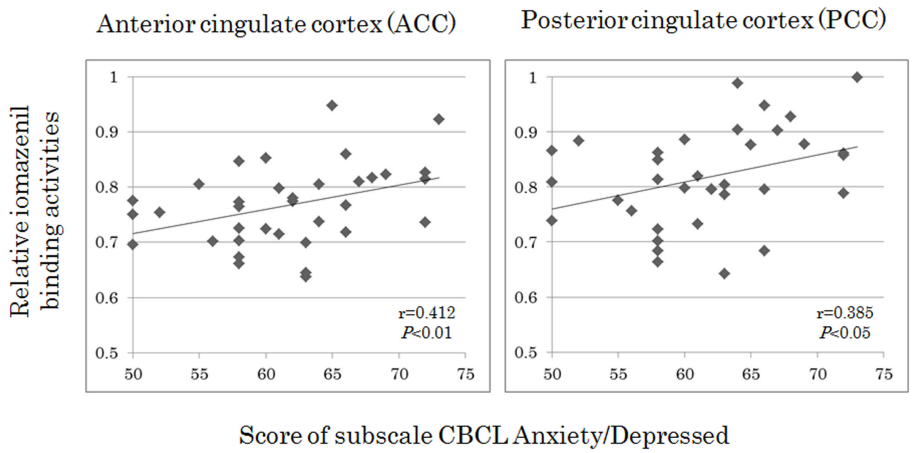

FIGURE 2 | Correlations between iomazenil binding activity in the anterior and posterior cingulate cortices and score of subscale CBCL anxiety/depressed in all subjects. The higher relative iomazenil binding activities in ACC and PCC are significantly associated with the higher scores of "Anxiety/Depressed" subscale in the CBCL. CBCL, child behavior checklist.

TABLE 3 | Effect of the significant CBCL–DP (score ≥210) on iomazenil binding activities in each brain region.

Brain area	Effect	*F*-Test	*p*-Value
Right superior frontal	Significant CBCL–DP	0.88	0.35
Left superior frontal	Significant CBCL–DP	0.18	0.67
Right parietal	Significant CBCL–DP	2.07	0.16
Left parietal	Significant CBCL–DP	0.37	0.55
Right middle frontal	Significant CBCL–DP	1.57	0.22
Left middle frontal	Significant CBCL–DP	0.44	0.51
Right temporal	Significant CBCL–DP	1.26	0.27
Left temporal	Significant CBCL–DP	0.05	0.82
Midbrain	Significant CBCL–DP	0.2	0.66
Anterior cingulate cortex	Significant CBCL–DP	0.99	0.33
Posterior cingulate cortex	Significant CBCL–DP	4.36	<0.05

CBCL–DP, child behavior checklist–dysregulation profile.

GABA–benzodiazepine receptor binding activity and the severity of anxiety symptoms in adults with panic or traumatic disorders (17–19). It is well known that there are considerable changes in the number of GABA receptors and in subunit expression during brain development (39). Specifically, the greatest number of GABA receptors is found in the youngest children, with numbers decreasing exponentially with age, and there are age-related increases in α1-subunit-containing GABA receptors (40). These age-related changes in GABA receptors may affect outcomes when assessing increases and/or decreases in overall iomazenil binding activity in children.

The present study has several limitations that require consideration in future studies. For example, in the present study, SPECT exhibited poor resolution around some limbic regions, such as the amygdala and hippocampus, which are important for emotion processing. In these small regions, the obtained radioactivity might differ from the true activity because of partial volume effect (PVE). The PVE can be defined as the underestimation of binding per unit brain volume in small objects or regions because of the blurring of the radioactivity (spill-out and spill-in) between regions. These regions need to be resolved using MR imaging-based correction for PVE (41, 42). Brain imaging data from normal healthy children are not available

TABLE 4 | Effect of the CBCL–DP subscale on iomazenil binding activities in each brain region.

Brain area	Effect	*F*-Test	*p*-Value
Right superior frontal	Aggressive behavior	0.03	0.8708
	Anxious/depressed	2.79	0.1058
	Attention problem	0.12	0.7268
Left superior frontal	Aggressive behavior	0.01	0.9374
	Anxious/depressed	4.28	0.0479*
	Attention problem	1.6	0.2157
Right parietal	Aggressive behavior	0	0.9675
	Anxious/depressed	0.13	0.7249
	Attention problem	0.39	0.5367
Left parietal	Aggressive behavior	0.33	0.5703
	Anxious/depressed	0.67	0.4214
	Attention problem	0.02	0.8843
Right middle frontal	Aggressive behavior	0	0.9771
	Anxious/depressed	1.83	0.1871
	Attention problem	0.06	0.8060
Left middle frontal	Aggressive behavior	0.5	0.4873
	Anxious/depressed	5.1	0.0319*
	Attention problem	0.92	0.3457
Right temporal	Aggressive behavior	0.03	0.8709
	Anxious/depressed	1.46	0.2363
	Attention problem	0.02	0.8888
Left temporal	Aggressive behavior	0.12	0.7354
	Anxious/depressed	7.55	0.0104*
	Attention problem	1.91	0.1777
Mid brain	Aggressive behavior	0.66	0.4231
	Anxious/depressed	1.98	0.1699
	Attention problem	0.21	0.6482
Anterior cingulate cortex	Aggressive behavior	0.97	0.3331
	Anxious/depressed	3.94	0.0570
	Attention problem	1.06	0.3118
Posterior cingulate cortex	Aggressive behavior	0.99	0.3277
	Anxious/depressed	5.62	0.0248*
	Attention problem	1.26	0.2714

CBCL–DP, child behavior checklist–dysregulation profile.
**Indicates significant effects on higher iomazenil binding activity.*

because of ethical concerns with SPECT studies of this population. Therefore, we focused our research questions on the correlation between GABAergic inhibitory function in specific brain regions and psychometric profiles. It is possible that, in addition to the population of people with ADHD, our result is generalizable to the normal population. Furthermore, we selected iomazenil activity in the occipital regions as a reference, meaning that we could not evaluate inhibitory function in occipital regions. We did not clarify how putative dopaminergic or serotoninergic changes are involved in other subscales, such as the Aggression and Attention Problem subscales, of the CBCL–DP. It is possible that investigations incorporating the simultaneous assessment of benzodiazepine receptor binding activity and homovanillic acid (HVA) and 5HIAA in the urine (principal metabolites of dopamine and serotonin, respectively) could provide new insights into the underlying neurobiological defects or aberrant neuronal activity leading to the dysregulation profile in children.

In conclusion, the present 123I-iomazenil brain SPECT study provides evidence that changes in GABAergic inhibitory neuronal activity correlate with some elements of function measured by the CBCL–DP. Brain SPECT may be useful for the evaluation of the possible pathogenesis of neuropsychiatric symptoms observed in children.

Author Contributions

SN participated in the design of this study and compiled the manuscript. SN, YY, and HC saw the patients and obtained informed consent and their agreement to participate in the study. Diagnosis of comorbidity disorders was made by HC. SN and HC summarized participant behavioral and psychiatric assessments, including CBCL–DP data. Three radiologists (HT, HK, and MI) were in charge of radioactive measurements and calculations of iomazenil activity using ROIs. TK, a statistician, conducted the statistical analyses. PC and TM supervised the preparation of the manuscript.

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A preliminary study of white matter in adolescent depression: relationships with illness severity, anhedonia, and irritability

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Major depressive disorder (MDD) during adolescence is a common and disabling psychiatric condition; yet, little is known about its neurobiological underpinning. Evidence indicates that MDD in adults involves alterations in white and gray matter; however, sparse research has focused on adolescent MDD. Similarly, little research has accounted for the wide variability of symptom severity among depressed teens. Here, we aimed to investigate white matter (WM) microstructure between 17 adolescents with MDD and 16 matched healthy controls (HC) using diffusion tensor imaging. We further assessed within the MDD group relationships between WM integrity and depression severity, as well as anhedonia and irritability – two core symptoms of adolescent MDD. As expected, adolescents with MDD manifested decreased WM integrity compared to HC in the anterior cingulum and anterior corona radiata. Within the MDD group, greater depression severity was correlated with reduced WM integrity in the genu of corpus callosum, anterior thalamic radiation, anterior cingulum, and sagittal stratum. However, anhedonia and irritability were associated with alterations in distinct WM tracts. Specifically, anhedonia was associated with disturbances in tracts related to reward processing, including the anterior limb of the internal capsule and projection fibers to the orbitofrontal cortex. Irritability was associated with decreased integrity in the sagittal stratum, anterior corona radiata, and tracts leading to prefrontal and temporal cortices. Overall, these preliminary findings provide further support for the hypotheses that there is a disconnect between prefrontal and limbic emotional regions in depression, and that specific clinical symptoms involve distinct alterations in WM tracts.

Keywords: depression, adolescent, white matter, diffusion tensor imaging, anhedonia, irritability

INTRODUCTION

Major depressive disorder (MDD) in adolescence is a prevalent and disabling psychiatric illness associated with serious consequences including academic failure, social withdrawal, substance abuse, and most critically, suicide (1–5). Converging evidence derived from neuroimaging studies suggests that adolescent MDD entails morphological, functional, and neurochemical alterations (6–10). Importantly, since adolescence represents a critical period of rapid neuroplasticity [e.g., increased myelination, synaptic pruning; (11–13)], white matter (WM) alterations can contribute to the neurobiology of adolescent MDD. Indeed, in our prior investigation of gamma-Aminobutyric acid in the anterior cingulate cortex (ACC), we incidentally found reduced WM volume in adolescents with MDD compared to healthy controls [HC; (9)]. However, there has been sparse research focusing on WM alterations in this age group.

Diffusion tensor imaging (DTI) enables the non-invasive examination of *in vivo* structural connectivity by providing measures of WM microstructure and integrity based on the extent of water diffusion (14). Several DTI measures are typically quantified, with fractional anisotropy (FA) being the most commonly used to reflect WM integrity. Higher FA values suggest greater diffusion in the direction of the axon, and thus greater WM integrity. Other measures, including mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD), can also be determined to investigate different aspects of WM microstructure.

To date, most DTI research in MDD has investigated adults and consistently reported decreased FA in tracts connected to the prefrontal cortex (PFC) or tracts connecting the two hemispheres within the PFC (15, 16). Only one DTI study was carried out in adolescents with depression, demonstrating a similar pattern to adult MDD of reduced FA in tracts connected to the subgenual ACC and the PFC [i.e., uncinate fasciculus, inferior-fronto-occipital fasciculus, anterior cingulum, superior longitudinal fasciculus; (17)]. However, results may have been impacted by the concurrent use of psychotropic medication and past substance abuse in some subjects. Relatedly, medication-naïve adolescents with a familial risk for unipolar depression also demonstrated reduced FA compared to HC in similar tracts (18).

In this study, we aimed to expand on prior work by examining WM integrity in psychotropic medication-free adolescents with MDD compared to HC. Given the inherent heterogeneity

of adolescent MDD, we further sought to identify specific WM alterations in relation to MDD severity as well as anhedonia and irritability – two core symptoms of adolescent MDD. Due to our desire to explore a range in depression severity, we included patients with mild to moderate severity. Based on others' and our prior findings (8, 15–17), we hypothesized that adolescents with MDD would manifest less restricted diffusion (i.e., decreased WM integrity) compared to HC in tracts connecting frontal, striatal, and limbic regions. We also predicted that similar tracts would be associated with depression severity. For anhedonia, we expected reduced WM integrity in tracts that have been implicated in reward-related processing in the ventral striatum [i.e., subgenual cingulate, forceps minor, inferior-fronto-occipital fasciculus, anterior thalamic radiation (ATR), anterior limb of the internal capsule; (19)], and that these would be distinct from those associated with irritability. Finally, only one study has examined irritability in a clinical population [i.e., Huntington's disease; (20)] and found involvement of the amygdala. As such, we predicted the impairment of tracts connecting to the amygdala (e.g., uncinate fasciculus, inferior-fronto-occipital fasciculus, inferior longitudinal fasciculus).

MATERIALS AND METHODS

PARTICIPANTS

The sample population partially overlapped with that used for our previously published resting state functional magnetic resonance imaging research (8), and was comprised of 17 adolescents with MDD (ages 13–20 years, $M = 16.8$, $SD = 2.2$, 8 female) and 16 HC (ages 13–19 years, $M = 16.4$, $SD = 1.4$, 10 female) group matched for age, and all were right-handed. Depressed adolescents were recruited through the New York University (NYU) Child Study Center, the Bellevue Hospital Center Department of Psychiatry, and local advertisements in the NY metropolitan area. HC were recruited from the greater NY metropolitan area through local advertisements and from the families of NYU staff. The study was approved by the NYU School of Medicine and the Icahn School of Medicine at Mount Sinai institutional review boards. Prior to enrollment, study procedures were explained to the subjects and parents. Written informed consent was provided by participants age 18 and older; those under age 18 provided signed assent and a parent/guardian provided signed informed consent.

Inclusion and exclusion criteria

All subjects were ≤20 years old and did not present with any significant medical or neurological disorders. Other exclusionary criteria consisted of an IQ < 80, MRI contraindications as assessed by a standard screening form, a positive urine toxicology test or a positive urine pregnancy test in females.

All MDD subjects met the DSM-IV-TR diagnosis of MDD with a current episode ≥8 weeks duration, and raw severity score ≥40 (i.e., T score ≥ 63) during the initial evaluation on the Children's Depression Rating Scale-Revised (CDRS-R), and were psychotropic medication-free for at least 7 half-lives of the medication. To explore a wider range of depression severity we included patients presenting with mild to severe depression on the date of the scan. Exclusionary criteria for the MDD group included current or past DSM-IV-TR diagnoses of bipolar disorder, schizophrenia, pervasive developmental disorder, panic disorder, obsessive-compulsive disorder, conduct disorder, Tourette's disorder, or a substance-related disorder in the past 12 months. Current diagnoses of post-traumatic stress disorder or an eating disorder were also exclusionary. In addition, acute suicidality requiring immediate inpatient admission was exclusionary. HC subjects did not meet the criteria for any major current or past DSM-IV-TR diagnoses and had never received psychotropic medication.

CLINICAL ASSESSMENTS

All subjects were assessed by a board-certified child and adolescent psychiatrist or a clinical psychologist at the NYU Child Study Center. Clinical diagnoses were established using the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version [KSADS-PL; (21)], a semi-structured interview performed with both the subjects and their parents. Depression severity was assessed by the CDRS-R and the Beck Depression Inventory, second edition [BDI-II; (22)]. Additionally, suicidality and anxiety were assessed using the Beck Scale for Suicidal Ideation [BSSI; (23)] and the Multidimensional Anxiety Scale for Children [MASC; (24)], respectively. The Kaufman Brief Intelligence Test (25) or the Wechsler Abbreviated Scale of Intelligence (26) were used to estimate IQ. Urine toxicology and pregnancy tests were administered on the day of the scan.

Anhedonia

Our approach to quantifying anhedonia allows for clinician- and self-rated assessments to contribute equally to the anhedonia score (range 1–13). As in our previous studies (8, 9, 27), the score for each subject was computed by summing the responses to three items associated with anhedonia from the clinician-rated CDRS-R (item 2: "difficulty having fun;" scale of 1–7) and the self-rated BDI-II (items 4: "loss of pleasure" and 12: "loss of interest;" scales of 0–3).

Irritability

Our approach again combined both clinician- and self-rated assessments to contribute to the irritability score (range 1–10). The score for each subject was computed by summing the responses to the items associated with irritability from the CDRS-R (item 8: "irritability;" scale of 1–7) and the BDI-II (item 17: "irritability;" scale of 0–3).

DATA ACQUISITION AND ANALYSIS

Diffusion data were acquired on a Siemens Allegra 3.0 T scanner at the NYU Center for Brain Imaging using a single-channel head coil. Diffusion-weighted echo-planar images (EPI) were acquired along 12 diffusion gradient directions for acquisition of 35 slices through the whole brain (TR = 6000 ms, TE = 82 ms, flip angle = 90°, b value = 1000 s/mm^2, FOV = 192 mm, 128 × 128 matrix, slice thickness = 2.5 mm, with four averages). High-resolution T1-weighted anatomical images were acquired using a magnetization-prepared gradient echo sequence (TR = 2530 ms; TE = 3.25 ms; TI = 1100 ms; flip angle = 7°; 128 slices; FOV = 256 mm; acquisition voxel size = 1.3 mm × 1 mm × 1.3 mm).

All preprocessing was performed using FMRIB's Software Library (FSL; Oxford, UK): FMRIB's Diffusion Toolbox (FDT). Preprocessing began with eddy current correction to correct for gradient-coil distortions and small head motions, using affine registration to a b0 reference volume. A diffusion tensor model was fitted to each voxel along the principal λ_1, λ_2, λ_3 directions to generate FA, MD, RD, and AD. We then implemented FSL's tract-based spatial statistics pipeline [TBSS; (28)], in which a non-linear registration aligned each subject to the FMRIB58_FA template in 1 mm × 1 mm × 1 mm standard space, and then warped each subject into standard Montreal Neurological Institute space (MNI152). A WM "skeleton" was then generated representing a single line running down the centers of all of the common WM fibers by using an FA cut-off of 0.2, and relevant diffusivity measures (i.e., FA, MD, RD, AD) were projected onto the skeleton. Group statistical analysis was then conducted only on voxels within the WM skeleton mask, therefore restricting the voxel-wise analysis only to voxels with high confidence of lying within equivalent major WM pathways in each individual.

In order to assess differences in FA, MD, RD, and AD between the MDD and HC groups, we used FSL's Randomise with 5000 permutations to perform voxel-wise independent samples *t*-tests using voxel-based thresholding while controlling for age and sex. Group comparisons did not withstand stringent correction for multiple comparisons using family-wise error correction (FWE; implemented by Randomise) or FDR correction (implemented by FSL's FDR program). As such, we performed a second, more exploratory analysis in which we accepted clusters of at least 10 contiguous voxels at $p < 0.001$.

To investigate relationships with depression severity, anhedonia, and irritability, Randomise was again used to perform a series of one-sample *t*-tests using the score for each category, respectively, while controlling for age and gender. Due to the low variability in scores in the HC group, as well as the nature of the study topic, the analysis was limited to the MDD group whose scores were normally distributed. Once again, tests did not withstand correction for multiple comparisons and we used the more exploratory approach of accepting clusters of at least 10 contiguous voxels at $p < 0.001$. We used FSL's cluster program to extract all clusters across the brain, and anatomical localization of each cluster was determined using the FSLView atlas toolbox and the relevant gray matter (Harvard-Oxford Cortical/Subcortical) and WM (Johns Hopkins University WM tractography) atlases. Brain imaging results were prepared for display using FSL's tbss_fill script, which displays results superimposed upon the WM skeleton from the group TBSS analysis.

RESULTS

PARTICIPANTS

Demographic and clinical characteristics are summarized in **Table 1**. One subject with MDD had been treated with Lexapro and Ambien for 7 months, but was medication-free for approximately 14 months prior to scanning. A second subject with MDD had a brief trial with Prozac which was self-discontinued prior to participation in this study. All other subjects were psychotropic medication-naïve. Fifteen subjects with MDD had experienced only one episode of depression, with length of episode ranging from 4 to 48 months, and two patients reported having two distinct episodes. Two Shapiro–Wilk tests revealed that anhedonia and irritability were both normally distributed within the MDD group, $ps = 0.81$, 0.48, respectively. Depression severity (excluding the anhedonia- or irritability-related items) was significantly correlated with both severity of anhedonia ($r = 0.66$, $p < 0.005$) and irritability ($r = 0.66$, $p < 0.005$) within our MDD sample. Anhedonia and irritability were not correlated ($r = 0.37$, $p = 0.15$).

Table 1 | Demographic and clinical characteristics of adolescents with major depressive disorder (MDD) and healthy controls.

Characteristic	MDD subjects (*N* = 17)	Healthy controls (*N* = 16)
Age (range)	16.8 ± 2.2 (13–20)	16.4 ± 1.4 (13–19)
Gender (female/male)	8/9 (47/53%)	10/6 (63/38%)[a]
Ethnicity (Caucasian/African American/Hispanic/Asian/other)	9/2/5/0/1 (53/12/29/0/6%)	6/5/1/1/3 (38/31/6/6/19%)
ILLNESS HISTORY		
Current episode duration in months (range)	24.4 ± 16.0 (7–72)	0
Number of MDD episodes	1 (*n* = 15), 2 (*n* = 2)	0 (*n* = 16)
History of suicide attempts (range)	0.2 ± 0.5 (0–2)	0
Medication-naïve/medication-free	15/2 (88/12%)	16/0 (100/0%)
CDRS-R (range)[b]	45.7 ± 9.7 (29–64)	19.4 ± 2.7 (17–27)
Anhedonia (range)	5.76 ± 2.28 (2–10)	1.44 ± 0.73 (1–3)
Irritability (range)	4.71 ± 1.40 (2–7)	1.18 ± 0.83 (0–3)
BDI-II (range)[c]	19.2 ± 10.4 (1–37)	1.9 ± 2.6 (0–10)
BSSI (range)[d]	2.0 ± 4.2 (0–14)	0.1 ± 0.3 (0–1)
MASC (range)[e]	44.2 ± 21.3 (11–75)	33.5 ± 12.4 (9–55)
CURRENT COMORBIDITY		
ADHD[f]	1 (6%)	0
Any anxiety disorder	8 (47%)	0
GAD[g]	7 (41%)	0

[a] *Respective percentages (may not add up to 100% due to rounding).*

[b] *Children's depression rating scale – revised.*

[c] *Beck depression inventory, 2nd ed.*

[d] *Beck scale for suicidal ideation.*

[e] *Multidimensional anxiety scale for children.*

[f] *Attention deficit hyperactivity disorder.*

[g] *Generalized anxiety disorder.*

WHOLE-BRAIN GROUP COMPARISON

No voxel-wise group comparisons for FA, MD, RD, or AD withstood correction for multiple comparisons. An exploratory analysis using a threshold of $p < 0.001$, uncorrected with clusters exceeding 10 contiguous voxels, revealed 4 significant clusters (**Table 2**, **Figure 1**). Compared with HC, the MDD group had lower FA in the anterior cingulum, and lower AD in the anterior corona radiata (ACR). However, the MDD group also had greater FA and lower RD in the posterior cingulum compared to HC.

Table 2 | Voxel-wise group comparison results.

Region	Tract	COG coordinates			Cluster size	Values mean (SD) HC/MDD
		x	*y*	*z*		
FA: MDD > HC						
L cerebral WM (hippocampus)	Posterior cingulum	−20	−39	−5	16	0.45 (0.06) 0.57 (0.08)
FA: HC > MDD						
R cerebral WM (precuneus)	Anterior cingulum	18	−51	34	10	0.50 (0.05) 0.43 (0.04)
MD: MDD > HC						
None						
MD: HC > MDD						
None						
RD: MDD > HC						
None						
RD: HC > MDD						
L cerebral WM (hippocampus)	Posterior cingulum	−20	−39	−5	16	0.0006 (7E−05) 0.0005 (8E−05)
AD: MDD > HC						
None						
AD: HC > MDD						
L cerebral WM (putamen)	ACR	−23	22	−5	10	0.0013 (9E−05) 0.0012 (8E−05)

Units for AD and RD = mm²/s. Coordinates in MNI space. Threshold p < 0.001, uncorrected, k > 10. COG, center of gravity; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity; AD, axial diffusivity; WM, white matter; L, left; R, right; ACR, anterior corona radiata.

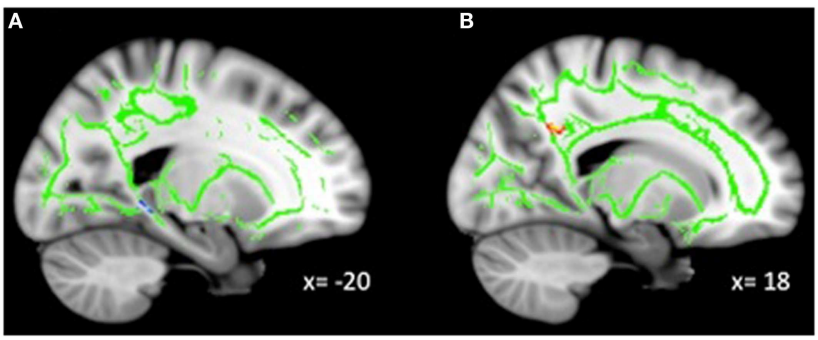

FIGURE 1 | (A) Increased WM integrity in the MDD group vs. HC in the posterior cingulum near the hippocampus; **(B)** decreased WM integrity in the MDD group vs. HC in the anterior cingulum near the precuneus.

WHOLE-BRAIN CORRELATIONS WITH DEPRESSION SEVERITY IN MDD

Exploratory analyses revealed a total of 16 uncorrected clusters, with 5 overlapping clusters between the 4 diffusivity measures (**Table 3**, **Figure 2**). As depression severity increased, FA decreased in the genu of the corpus callosum, the sagittal stratum, the ATR, and the anterior cingulum. Additionally, a positive correlation between MD and depression severity was found in the same sagittal stratum cluster as the FA analysis, as well as in clusters in the ATR and corticospinal tract. Similarly, illness severity was positively correlated with RD in the same sagittal stratum cluster as the FA and MD analyses, the same genu of the corpus callosum and ATR clusters as the FA analysis, the same ATR cluster as the MD analysis, and a cluster in the superior longitudinal fasciculus (SLF). Finally, increased illness severity was associated with increased AD in the same corticospinal cluster as the MD analysis as well as in clusters in the inferior-fronto-occipital fasciculus (IFOF), the SLF, and fibers projecting to the orbitofrontal cortex (OFC).

WHOLE-BRAIN CORRELATIONS WITH ANHEDONIA IN MDD

Analyses revealed a total of 14 uncorrected clusters, with 2 overlapping clusters between FA and RD (**Table 4**, **Figure 3**). As anhedonia increased, FA increased in a cluster in the posterior cingulum near

Table 3 | Voxel-wise correlations with depression severity (CDRS-R).

Region	Tract	COG			Cluster size
		x	*y*	*z*	
FA: POSITIVE RELATIONSHIP					
None					
FA: NEGATIVE RELATIONSHIP					
L cerebral WM (PHG)	Sagittal stratum (ILF + IFOF)	−42	−35	−9	29
L cerebral WM (pallidum)	ATR	−10	−3	−3	20
L cerebral WM (ACC)	Genu of corpus callosum	−2	30	4	14
L cerebral WM (precuneus)	Anterior cingulum	−7	−72	39	11
MD: POSITIVE RELATIONSHIP					
L cerebral WM (PHG)	Sagittal stratum (ILF + IFOF)	−41	−33	−13	20
R cerebral WM (pallidum)	ATR	14	−3	3	11
R cerebral WM (postcentral gyrus)	Corticospinal	22	−40	47	10
MD: NEGATIVE RELATIONSHIP					
None					
RD: POSITIVE RELATIONSHIP					
L cerebral WM (PHG)	Sagittal stratum (ILF + IFOF)	−42	−35	−10	45
L cerebral WM (pallidum)	ATR	−10	−3	−4	17
R cerebral WM (supramarginal gyrus)	SLF	30	−39	37	13
L cerebral WM (ACC)	Genu of corpus callosum	−2	29	4	14
R cerebral WM (pallidum)	ATR	14	−2	3	10
RD: NEGATIVE RELATIONSHIP					
None					
AD: POSITIVE RELATIONSHIP					
R cerebral WM (postcentral gyrus)	Corticospinal	22	−33	44	25
R cerebral WM (precuneus)	IFOF	23	−55	30	17
L cerebral WM (MFG)	SLF	−31	21	25	10
R cerebral WM (OFC)	Frontal projection fibers	−24	19	−18	10
AD: NEGATIVE RELATIONSHIP					
None					

Coordinates in MNI space. Threshold $p < 0.001$, uncorrected, $k > 10$. COG, center of gravity; L, left; R, right; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity; AD, axial diffusivity; WM, white matter; PHG, parahippocampal gyrus; ACC, anterior cingulate cortex; MFG, medial frontal gyrus; OFC, orbitofrontal gyrus; ILF; inferior longitudinal fasciculus; IFOF, inferior-fronto-occipital fasciculus; ATR, anterior thalamic radiation; SLF, superior longitudinal fasciculus.

the hippocampus – similar to a cluster from the group comparison analysis – and decreased in the anterior limb of the internal capsule, OFC projection fibers, and the posterior cingulum near the precuneus. Furthermore, anhedonia was positively correlated with MD in OFC projection fibers, the external capsule, and the sagittal stratum. Additionally, increased anhedonia severity was associated with greater RD in the same posterior limb of the internal capsule and posterior cingulum clusters as the FA analysis, the same OFC projection fibers as the MD analysis, and clusters in the ATR and corticospinal tract. Finally, there were positive correlations between anhedonia and AD in the corticospinal tract and projection fibers into the occipital cortex.

WHOLE-BRAIN CORRELATIONS WITH IRRITABILITY IN MDD

Analyses revealed a total of 14 uncorrected clusters, with 2 overlapping clusters (**Table 5**, **Figure 4**). As irritability increased, FA decreased in clusters in the sagittal stratum and IFOF, while MD increased in the same sagittal stratum cluster as well as in clusters in the ACR, SLF, and IFOF. For RD, positive correlations with irritability were evident in the same sagittal stratum cluster, the anterior limb of the internal capsule, and the SLF. Positive correlations were also found between AD and irritability in the same ACR cluster as the MD analysis as well as in the IFOF, the corticospinal tract, and the SLF.

DISCUSSION

Consistent with our hypotheses, analyses revealed reduced WM integrity (i.e., decreased FA, and increased MD, RD, AD) in the MDD group compared to HC as well as in the more severely depressed, anhedonic, and irritable patients. Furthermore, despite significant correlations between the two dimensional measures and depression severity, we found distinct WM alterations for both anhedonia and irritability that differed from those for depression severity. Reduced integrity was found in fronto-striatal and thalamic tracts, the corpus callosum, and tracts connected to the inferior temporal (IT) cortex. Additionally, reduced integrity in the sagittal stratum was consistently found in our analyses to be correlated with increasing depression severity, anhedonia, and

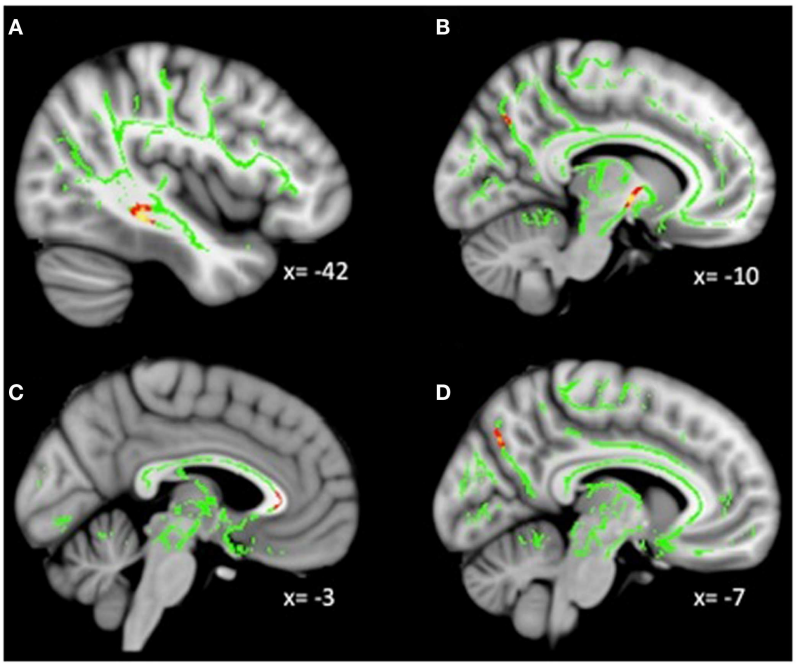

FIGURE 2 | Decreased WM integrity as depression severity increased in the (A) sagittal stratum near the PHG; (B) ATR near the pallidum; (C) genu of the corpus callosum and anterior cingulate; (D) anterior cingulate near the precuneus. Key: PHG, parahippocampal gyrus; ATR, anterior thalamic radiation.

irritability. Unexpectedly, analyses also revealed a cluster in the posterior cingulum near the hippocampus which demonstrated more anisotropic diffusion, both as anhedonia increased and in the MDD group vs. HC. Finally, it is interesting to note that in many of our analyses there was overlap in the clusters demonstrating a relationship with FA and RD, potentially suggesting that structural issues related to RD are driving the observed relationships with FA in this and other studies.

GROUP DIFFERENCES IN WM INTEGRITY

Group differences were observed in both the posterior and anterior cingulum. Specifically, depressed adolescents demonstrated decreased WM integrity in the anterior cingulum near the precuneus, and increased integrity in the posterior cingulum near the hippocampus, compared to HC. The cingulum connects the cingulate and entorhinal cortices and is broadly involved in attention, memory, and emotions (29, 30). The anterior portion of the cingulate has been implicated in emotional processing and depression (31). Altered functioning, connectivity, and diffusion around the precuneus are frequently reported in MDD (32, 33). Given the role of the precuneus in self-related processes, and that self-processing is typically altered in depression (34), this potentially suggests that reduced WM integrity contributes to altered functioning in this region early in the course of the disease.

The MDD group also demonstrated more coherent diffusion in the posterior cingulum near the hippocampus. The posterior cingulate is involved in cognitive functions including attention and memory (35). Functional hyperactivity in the hippocampus (36–38), as well as decreased hippocampal volume (39), are consistent findings in adult MDD. Given the role of the hippocampus in learning and memory (40), but also in the regulation of motivation and emotion (41, 42), this region is critical to carrying out normal behaviors that may be altered in depression. Furthermore, greater WM integrity in tracts leading to the hippocampus would be consistent with the literature demonstrating hyperactivity of this region in non-medicated MDD patients. Overall, the categorical comparison between depressed adolescents and HC revealed differences in an important tract connecting prefrontal and limbic regions.

DEPRESSION SEVERITY AND WM INTEGRITY

Our use of an approach that accounts for a range of depression severity in our sample revealed a pattern of reduced WM integrity as depression severity increased. Specifically, we found reduced integrity in the genu of the corpus callosum, a region that connects prefrontal and orbitofrontal cortices. Many studies have documented altered diffusivity in the genu (16) as well as reduced volume (6, 43–45). Given that the prefrontal and orbitofrontal cortices are involved in critical processes, including decision-making, attention, reward processing, and the evaluation and regulation of emotion (46–48), an interruption in communication between these areas has implications for depression and mood disorders.

Additionally, we found decreased integrity in the sagittal stratum with increasing severity, not only in this analysis, but also in the dimensional analyses within the MDD population. The sagittal stratum is a complex fiber bundle connecting the occipital cortex to the rest of the brain, and includes fibers from many major tracts including the ILF and IFOF (49). The ILF and IFOF both connect the occipital cortex to temporal limbic structures (i.e., amygdala, hippocampus) and the PFC, although the IFOF connects directly

Table 4 | Voxel-wise correlations with anhedonia.

Region	Tract	COG			Cluster size
		x	*y*	*z*	
FA: POSITIVE RELATIONSHIP					
R cerebral WM (hippocampus)	Posterior cingulum	25	−31	−14	13
FA: NEGATIVE RELATIONSHIP					
R cerebral WM (thalamus)	Anterior limb IC (ATR)	14	−2	4	14
R cerebral WM (OFC)	IFOF	34	31	−6	12
L cerebral WM (precuneus)	Posterior cingulum	18	−58	42	10
MD: POSITIVE RELATIONSHIP					
R cerebral WM (OFC)	Projection fibers	17	20	−18	14
L cerebral WM (putamen)	External capsule (IFOF)	−32	−22	2	11
L cerebral WM (fusiform gyrus)	Sagittal stratum (ILF + IFOF)	−40	−35	−14	11
MD: NEGATIVE RELATIONSHIP					
None					
RD: POSITIVE RELATIONSHIP					
L cerebral WM (pallidum)	ATR	−10	−2	−3	24
R cerebral WM (OFC)	Projection fibers	17	20	−17	14
R cerebral WM (precuneus)	IFOF	18	−58	44	11
R cerebral WM (postcentral gyrus)	Corticospinal	18	−39	57	10
R cerebral WM (thalamus)	Anterior limb IC (ATR)	14	−2	4	10
RD: NEGATIVE RELATIONSHIP					
None					
AD: POSITIVE RELATIONSHIP					
R cerebral WM (postcentral gyrus)	Corticospinal	22	−37	46	34
L cerebral WM (occipital)	Projection fibers	−15	−65	50	12
AD: NEGATIVE RELATIONSHIP					
None					

Coordinates in MNI space. Threshold $p < 0.001$, uncorrected, $k > 10$. COG, center of gravity; L, left; R, right; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity; AD, axial diffusivity; WM, white matter; OFC, orbitofrontal gyrus; IC, internal capsule; ATR, anterior thalamic radiation; IFOF, inferior-fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus.

to the OFC and the ILF does so indirectly through the uncinate fasciculus (50). Therefore, both tracts are involved in connecting visual information with areas involved in emotional memories, judgments, and behaviors. A meta-analysis of diffusion studies of patients with MDD found WM alterations in both the ILF and IFOF (16). Additionally, alterations in WM have been found in the IFOF for depressed adolescents (17), adolescents with a familial risk for depression (18), and adults with MDD (51).

We also observed reduced integrity with increasing severity in bilateral clusters in the ATR near the pallidum. The ATR connects thalamic nuclei with the PFC through the anterior limb of the internal capsule. Reduced WM integrity has been reported in the ATR in several studies of depressed adults (16). Furthermore, given the role of the thalamus in motivation and goal pursuit (52, 53), altered connectivity within this circuit could contribute to the motivational deficits associated with depression.

Additionally, increased illness severity was associated with reduced integrity in the corticospinal tract near the postcentral gyrus. The corticospinal tract transmits motor impulses from the motor and premotor cortices to the spinal cord. Although this was an unexpected finding, motor disturbances and retardation are a relevant clinical symptom of depression (54). In this way, altered diffusivity may be related to the observed slowing and impairment of motor functions. Finally, we again found decreased integrity with increasing severity in the previously described anterior cingulum near the precuneus, which is consistent with the findings from our group analysis. Overall, our analysis with varied levels of depression severity was more robust than the categorical comparison and revealed a more extensive network of reduced WM integrity.

ANHEDONIA AND WM INTEGRITY

The dimensional analysis with anhedonia revealed an association between increased anhedonia and reduced integrity in the anterior limb of the internal capsule near the thalamus, a tract implicated in reward processing (19). The anterior limb of the internal capsule connects the thalamus with cingulate and prefrontal cortices, which are heavily involved in motivation, decision-making, and evaluating the saliency of emotional and rewarding stimuli. Additionally, increased anhedonia was correlated with reduced integrity in tracts (i.e., IFOF, projection fibers) connected to the posterior lateral OFC (BA 47), an area involved in many functions including emotional and reward processing, complex learning, and the inhibition of responses (46, 47, 55). Depressed patients

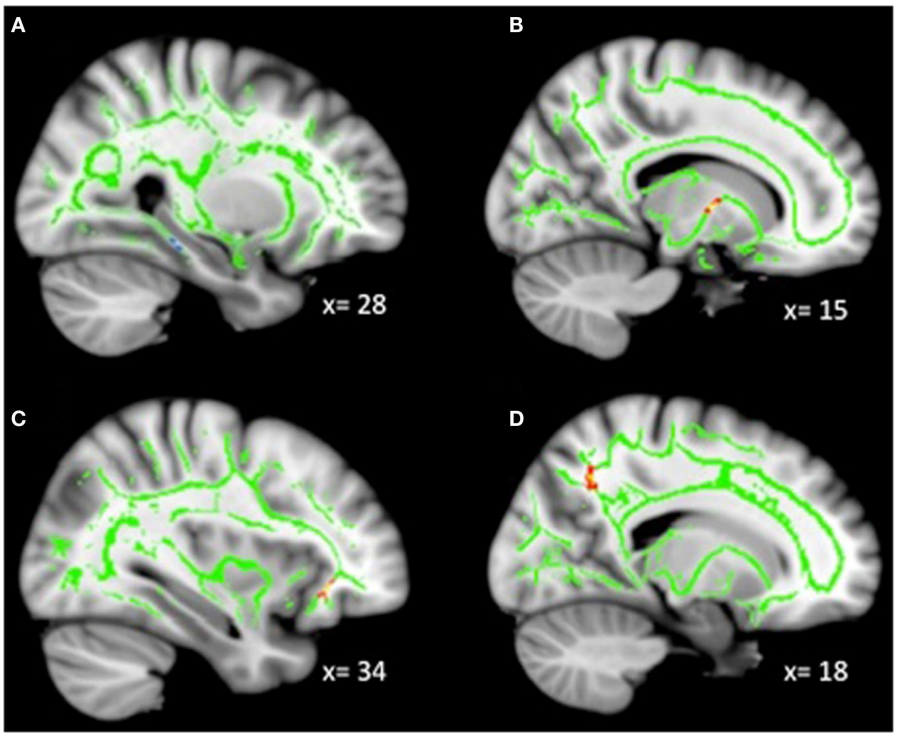

FIGURE 3 | (A) Increased WM integrity as anhedonia increased in the posterior cingulum near the hippocampus; decreased WM integrity as anhedonia increased in the **(B)** anterior limb of the internal capsule; **(C)** IFOF near the OFC; **(D)** posterior cingulum near the precuneus. Key: IFOF, inferior-fronto-occipital fasciculus; OFC, orbitofrontal cortex.

have demonstrated reduced gray matter volume in the posterior lateral OFC as well as altered functional responses to emotional stimuli, reward processing, and reversal learning (56).

We also found reduced integrity in the external capsule as anhedonia increased. The external capsule contains cholinergic fibers projecting from the basal forebrain to the cerebral cortex. Reduced integrity in the external capsule has been found previously in adult MDD (57, 58). Furthermore, we once again found reduced integrity in the previously discussed sagittal stratum and posterior cingulum near the precuneus with greater symptom severity. Finally, the analysis revealed increased integrity with increased anhedonia in the posterior cingulum near the hippocampus, in an area fairly close to the cluster that showed increased integrity in the MDD group in our categorical comparison. In this way, it is possible that anhedonic symptoms are related to the group differences we observed. Given the previously discussed role of the hippocampus and limbic system in the regulation of motivation and emotion, the relationship between hippocampal functioning and anhedonia represents an important area for future research.

IRRITABILITY AND WM INTEGRITY

As predicted, we found decreased integrity as irritability increased in a tract near the amygdala (i.e., sagittal stratum including the ILF and IFOF). However, increased irritability was correlated with decreased integrity in tracts primarily connecting to prefrontal and occipital cortices. We also found clusters in the previously discussed IFOF, although one was in the lingual gyrus while the other was in the middle frontal gyrus. The lingual gyrus has been implicated in processing emotional faces (59), which is typically altered in MDD (60). Altered cerebral blood flow and resting state connectivity have been demonstrated in the lingual gyrus in adults with MDD (61, 62). Additionally, decreased integrity in WM has previously been found around the middle frontal gyrus (63), an area broadly involved in a variety of higher-level cognitive processes (64) which are often compromised in MDD.

Reduced integrity related to elevated irritability was also found in the ACR, which connects the striatum to the ACC. Reduced integrity has previously been demonstrated in the ACR in pediatric bipolar patients (65), and dysfunctional activity in the ACC is typically considered a hallmark of depression (37, 42, 66–70). Furthermore, altered intrinsic functional connectivity (i.e., resting state) between the striatum and ACC has been documented for depressed adolescents (8). Finally, a cluster in the SLF in the IT gyrus was found. The SLF is a major bidirectional association tract connecting large parts of the frontal cortex with the parietal, temporal, and occipital lobes. Less restricted diffusion in the SLF has been previously demonstrated for depressed adolescents (17), adolescents with a genetic risk for depression (18), and adults with MDD (71).

MEASURES OF WM INTEGRITY

Although a complete discussion of RD and AD goes beyond the scope of this paper, it is interesting to note that for both the categorical and dimensional analyses we found overlap in

Table 5 | Voxel-wise correlations with irritability.

Region	Tract	COG			Cluster size
		x	*y*	*z*	
FA: POSITIVE RELATIONSHIP					
None					
FA: NEGATIVE RELATIONSHIP					
L cerebral WM (IT)	Sagittal stratum (ILF + IFOF)	−43	−33	−9	51
L cerebral WM (MFG)	IFOF	−31	33	12	13
MD: POSITIVE RELATIONSHIP					
L cerebral WM (IT)	Sagittal stratum (ILF + IFOF)	−42	−34	−9	82
L cerebral WM (putamen)	ACR (ATR)	−22	17	17	19
L cerebral WM (IT)	SLF	−47	−39	−4	13
L cerebral WM (lingual gyrus)	IFOF	−14	−86	−6	11
MD: NEGATIVE RELATIONSHIP					
None					
RD: POSITIVE RELATIONSHIP					
L cerebral WM (IT)	Sagittal stratum (ILF + IFOF)	−45	−34	−8	99
R cerebral WM (supramarginal gyrus)	SLF	34	−43	33	15
R cerebral WM (supramarginal gyrus)	SLF	38	−43	26	12
R cerebral WM (putamen)	Anterior limb IC (ATR)	14	7	6	12
RD: NEGATIVE RELATIONSHIP					
None					
AD: POSITIVE RELATIONSHIP					
R cerebral WM (postcentral gyrus)	Corticospinal	22	−30	46	22
R cerebral WM (precuneus)	IFOF	23	−55	32	18
L cerebral WM (MT)	SLF	−49	−46	−1	16
L cerebral WM (putamen)	ACR	−22	18	18	10
AD: NEGATIVE RELATIONSHIP					
None					

Coordinates in MNI space. Threshold $p < 0.001$, uncorrected, $k > 10$. COG, center of gravity; L, left; R, right; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity; AD, axial diffusivity; WM, white matter; IT, inferior temporal gyrus; MFG, medial frontal gyrus; MT, middle temporal gyrus; ILF, inferior longitudinal fasciculus; IFOF, inferior-fronto-occipital fasciculus; ACR, anterior corona radiata; ATR, anterior thalamic radiation; SLF, superior longitudinal fasciculus; IC, internal capsule.

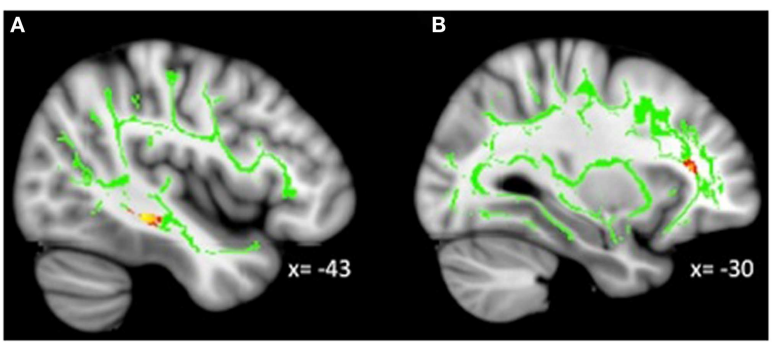

FIGURE 4 | Decreased WM integrity as irritability increased in the (A) sagittal stratum in the IT; (B) IFOF near the MFG. Key: IT, inferior temporal cortex; IFOF, inferior-fronto-occipital fasciculus; MFG, medial frontal gyrus.

clusters with reduced FA and increased RD, but no overlapping relationships with AD. Increased RD may be caused by disturbances in myelin, whereas decreased AD has been suggested to reflect disrupted axonal integrity (72–74). As such, our findings and those from previous research may reflect that alterations in FA for MDD are being driven more by issues of myelination than axonal integrity. However, further research is needed to replicate and expand upon a possible mechanism.

LIMITATIONS AND FUTURE DIRECTIONS

Although our findings are consistent with other clinical studies investigating altered WM in depressed adolescents, it should be noted that very liberal thresholds were used for the analyses and the inability to correct for multiple comparisons is an issue of concern. Although our statistical methodology and sample size were comparable to those of other studies of clinical populations using DTI (17, 63, 75), it is possible that the sample sizes used in many clinical studies are not large enough to produce adequate statistical power. In this way, it is difficult to adequately balance the concerns of committing a Type I error by not correcting while also avoiding a Type II error due to small sample sizes and reduced statistical power. Therefore, our findings are considered preliminary. Furthermore, the inclusion of patients with milder symptomatology may have weakened our ability to detect group differences.

Although small sample sizes may be a possible explanation for the relatively weak results in our and other clinical studies of adolescent depression, another possibility is that the adolescent brain is still malleable and the alterations in WM structure may not fully take hold until adulthood (11). Therefore, it is even more pressing to understand a neuroimmunological model of depression and the factors that may contribute to changes in WM before chronicity begins to take effect. For example, given past findings that depressed adolescents exhibit higher levels of circulating inflammatory cytokines (76), one possible explanation for the observed reduction in FA in adult MDD is that it may reflect effects of chronic low grade inflammation. Additionally, given our previous research on fronto-striatal functional connectivity in MDD, future studies should investigate altered WM microstructure using a targeted tractography approach. Finally, further research is needed to investigate this hypothesis and other models of the systemic consequences of depression. To this end, a better understanding of what FA, MD, AD, and RD illustrate in an adolescent population, as well as the factors that contribute to these diffusivity measures, is needed in the field.

CONCLUSION

Our investigation of altered WM microstructure in medication-free adolescents with MDD revealed a general pattern of impaired WM integrity in the depressed adolescents, and as depression severity, anhedonia, and irritability increased. Our findings are consistent with an overall hypothesis that depression, even in adolescence, involves a disconnection of prefrontal, striatal, and limbic emotional areas (16). Although this represents a good step toward understanding depression during this critical period, more research is needed to understand the factors that ultimately contribute to altered WM microstructure in order to develop potential interventions.

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Meta-analyses of developing brain function in high-risk and emerged bipolar disorder

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Objectives: Identifying early markers of brain function among those at high risk (HR) for pediatric bipolar disorder (PBD) could serve as a screening measure when children and adolescents present with subsyndromal clinical symptoms prior to the conversion to bipolar disorder. Studies on the offspring of patients with bipolar disorder who are genetically at HR have each been limited in establishing a biomarker, while an analytic review in summarizing the findings offers an improvised opportunity toward that goal.

Methods: An activation likelihood estimation (ALE) meta-analysis of mixed cognitive and emotional activities using the GingerALE software from the BrainMap Project was completed. The meta-analysis of all fMRI studies contained a total of 29 reports and included PBD, HR, and typically developing (TD) groups.

Results: The HR group showed significantly greater activation relative to the TD group in the right DLPFC–insular–parietal–cerebellar regions. Similarly, the HR group exhibited greater activity in the right DLPFC and insula as well as the left cerebellum compared to patients with PBD. Patients with PBD, relative to TD, showed greater activation in regions of the right amygdala, parahippocampal gyrus, medial PFC, left ventral striatum, and cerebellum and lower activation in the right VLPFC and the DLPFC.

Conclusion: The HR population showed increased activity, presumably indicating greater compensatory deployment, in relation to both the TD and the PBD, in the key cognition and emotion-processing regions, such as the DLPFC, insula, and parietal cortex. In contrast, patients with PBD, relative to HR and TD, showed decreased activity, which could indicate a decreased effort in multiple PFC regions in addition to widespread subcortical abnormalities, which are suggestive of a more entrenched disease process.

Keywords: pediatric bipolar disorder, high risk, meta-analysis, GingerALE, dorsolateral prefrontal cortex, amygdala

INTRODUCTION

The relationship between pediatric and adult bipolar disorder has been the subject of controversy. It is not clear whether pediatric bipolar disorder (PBD) is the pediatric form of the typical adult bipolar disorder or an entity of its own, as bipolar disorder usually manifests differently in childhood than in adulthood. Some studies in adults have reported that a portion of adults with bipolar I disorder experienced childhood or adolescent onset, and some of them began showing symptoms even before 12 years of age (1, 2). Identifying early markers of brain function among those at high risk (HR) for PBD could serve as a screening measure when children and adolescents present with subsyndromal clinical symptoms prior to the conversion to bipolar disorder (pediatric or adult form). These biomarkers can also aid as a stand-alone bio-signature for the identification of risk even prior to the emergence of any clinical symptoms and could allow an opportunity to prevent the onset of full-blown illness (3). One way to begin identifying the biomarkers is to examine the brain function in the genetically HR offspring of patients with bipolar disorder. While some studies of HR have been published (4–11), due to their small sample sizes and corrections for multiple comparisons, the findings remain inconclusive.

To offer robust and reliable findings, we used a recently developed activation likelihood estimation (ALE) technique. This method assumes that the peak co-ordinates reported by each study represent the activation maps from which they are derived and uses the reported co-ordinates in voxel-wise analysis to assess the consistency of activation in any given set of studies (12–14). By performing the quantitative voxel-wise meta-analysis of already published results from the HR population and comparing them with those from the converted PBD and typically developing (TD) youth, we can provide objective, unbiased, and statistically based quantified evidence.

Ideally, a separate meta-analysis would be conducted for each individual domain, such as emotion processing or attention, as they relate to bipolar disorder diathesis. However, given the infancy of the current literature regarding HR patients, this is not practical, as no individual construct has included a sufficient number of studies to date. Instead, it is more feasible to study the commonalities probed across multiple domains in a systematic and

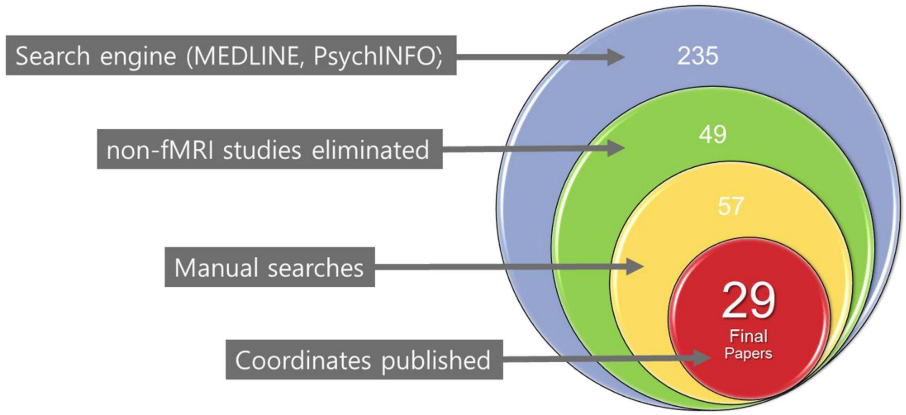

FIGURE 1 | Flow chart of the literature search for included studies.

statistically driven fashion. There is a certain advantage to combining all the studies that include multi-domain probes. First, the brain does not work in isolation across individual domains; therefore, it is necessary to examine the brain's function as a whole while it is engaged in affective, cognitive, and motor control tasks (15). Furthermore, pooling several pilot studies produces an exploratory power of how the brain functions in a larger sample, eventually offering the possibility of correlating the results with the clinical manifestations of domains and disorders presenting with combined affective, cognitive, and motoric symptoms (16). This approach is a segue into future studies that can explore the interface of multiple domain functions in individual studies.

We consider emotional systems and circuits, in illness or wellness, to be closely linked to cognitive and motor control circuits of attention, working memory, and response inhibition (17). These systems interface at three tiers as shown in animal (18) and human studies of PBD (19): (1) at the prefrontal level between the ventrolateral prefrontal cortex [VLPFC; inferior frontal gyrus; Brodmann areas (BAs) 45, 47] and the dorsolateral prefrontal cortex (DLPFC; middle frontal gyrus; BAs 9, 9, 46), (2) at the intermediary cortex in the anterior cingulate cortex (ACC), such as between the dorsal (BA 32) and pregenual ACC (BA 24), and (3) at the subcortical level between the amygdala and striatum (19). While we could not determine which probe or domain dysfunction would contribute to activity in any given co-ordinate in this meta-analysis, we developed our hypotheses based on knowledge derived from the emerging literature. Emotion-processing tasks probing the affective systems entered into our meta-analysis would contribute to the increased prefrontal activity at the interface of VLPFC and DLPFC in HR and the decreased activity in PBD relative to TD (19). Increased subcortical amygdala activity would be a specific marker of PBD (20) relative to HR and TD. Based on our knowledge of attention and working memory task response, the DLPFC will manifest with increased activity in HR (6) and decreased activity in PBD (21), relative to TD. Impaired subcortical striatal activity would be a more entrenched specific marker of PBD's cognitive and motor dysfunction (20, 22, 23) relative to the HR and TD groups.

MATERIALS AND METHODS

SEARCH STRATEGY

We identified primary studies through a comprehensive literature search of the MEDLINE (using both free-text and MeSH search) and PsychINFO databases using the following keywords: pediatric or child or adolescent, plus bipolar disorder or high-risk or at risk, and plus functional magnetic resonance imaging or fMRI. In addition, manual searches were conducted via reference sections of review articles and individual studies to check for any missing studies that were not identified using computerized searches. There were no language restrictions; in fact, all the included manuscripts were written in English. Only fMRI studies were chosen for review. An initial list of studies was produced that included any report of fMRI studies of PBD and HR offspring published in print or online by December 31, 2013. The selection process for the final list of primary studies for the planned meta-analyses in this study was very specific. The first-level literature search yielded 235 unique published articles with 49 studies meeting the initial inclusion criteria. A further manual search leads to eight other studies. After a second-level review of these 57 studies, only 29 contained the co-ordinates essential for inclusion in our meta-analysis (**Figure 1**). Any ambiguity in inclusion was resolved through a consensus decision by the authors of this manuscript. Study data (e.g., co-ordinates, participant numbers, and imaging spaces) were entered and crosschecked by participating authors.

SELECTION CRITERIA

"High risk" in this project refers to adolescents who have a biological parent diagnosed with BD. We selected studies with participants whose mean age was less than 19 years. Every study that we included had participants between the ages of 7 and 18 except for the study performed by Thermenos et al. (11), where the ages ranged up to 24. All reports included in the meta-analysis satisfied the following criteria: (1) a healthy comparison group is included, (2) the studies conducted whole-brain analyses, (3) all studies provided standard Talairach or Montreal Neurological Institute (MNI) spatial co-ordinates for the key findings, (4)

patient participants had been diagnosed with bipolar disorder, and (5) there were at least five members in each of the participant groups. We included only those studies that reported activation foci as 3D co-ordinates in stereotactic space, examined active task constructs, and presented results for groups of participants.

Excluded manuscripts consisted of the following: (1) reviews or meta-analyses, (2) those with subject overlap, and (3) other MRI modalities (e.g., structural imaging, spectroscopy, diffusion tensor imaging, and functional connectivity studies).

ACTIVATION LIKELIHOOD ESTIMATION METHODS AND PAIRWISE ALE META-ANALYSIS

GingerALE software version (version 2.3.1) from the BrainMap project was used to conduct ALE meta-analysis of eligible studies (13, 14, 43). Meta-analyses were performed using the revised ALE software (i.e., GingerALE 2.3). The key modification in the revised ALE software included the change from fixed-effects (convergence between foci) to random-effects inference (convergence between studies but not individual foci reported for the same study), as well as greater meta-analytic weighting for primary studies that involved more participants. In line with our goal of gaining insight on the whole brain's function through tasks that probe combined domains, we performed exploratory analyses using all eligible data in the HR offspring, BD patient, and TD groups in the pediatric age group. Conversely, we did not separate the analyses by the type of the task or the brain domain probed. This method also helped to harness sample size and power. Activation co-ordinates reported in the MNI space were converted to Talairach co-ordinates using the Lancaster transform (icbm2tal) in GingerALE. Our meta-analysis was conducted in Talairach space. Co-ordinates originally presented as MNI space were transformed into Talairach space using Lancaster transformation. For uniformity, Talairach co-ordinates expressed by the previous Brett transformation (44) were converted into MNI space and re-transformed into Talairach space. The meta-analysis was performed using pairwise ALE meta-analysis.

Pairwise ALE meta-analyses included the following comparisons at first: greater activation in PBD versus HR, in HR versus PBD, in PBD versus TD, in TD versus PBD, in HR versus TD, and in TD versus HR. However, two pairwise ALE meta-analyses (greater activation in PBD versus HR and greater activation in TD versus HR) were not performed due to the lack of available data. The input co-ordinates were weighted to form estimates of activation likelihood for each intracerebral voxel. The activation likelihood of each voxel in standard space was then combined to form a statistic map of the ALE score at each voxel. Statistical significance of the ALE scores was determined by a permutation test controlling the false discovery rate (FDR) at $p < 0.05$ (45). The statistic maps were thresholded by default at this critical value, and a recommended minimum cluster size was suggested from the cluster statistics. By using this minimum cluster size for the supra-threshold voxels, we can obtain the thresholded ALE image. Pairwise ALE analyses results were reported at $p = 0.05$ and were whole-brain corrected. A Talairach Daemon was used for anatomical locations for significant clusters.

RESULTS

The meta-analysis of all fMRI reports included 29 studies (PBD, HR, and TD). There was no overlap in patients who completed the same task across the selected studies. The primary studies included in the meta-analysis are listed in **Table 1**. Findings are summarized in **Table 2** and **Figure 2**.

HR AND TD: RECOGNIZING HIGH-RISK PARTICIPANTS

Participants in the HR group showed significantly greater activation in the right DLPFC, insula, inferior parietal lobule, and left cerebellum relative to TD. No other group differences were found. In case of greater activation in the TD group relative to HR, the analysis was not performed due to the lack of a large enough sample size and of experiments showing significant results.

PBD AND HR: RECOGNIZING THE EMERGENCE OF THE DISORDER

The HR group showed significant greater activation of the right DLPFC, insula, and left cerebellum than PBD. No other group differences were identified. In case of greater activation in the PBD group relative to HR, the analysis was also not performed due to a small sample size and few experiments showing significant results.

PBD AND TD: RECOGNIZING THE ILLNESS FROM WELLNESS

Patients with PBD demonstrated greater activation in the subcortical regions of the right amygdala, the parahippocampal gyrus, the subgenual ACC, and the medial PFC, and in the left ventral striatum, VLPFC, and cerebellum relative to TD. The TD group showed greater activation in the right VLPFC, DLPFC, superior frontal gyrus, dorsal ACC, and striatum than patients with PBD.

DISCUSSION

We found the recently published developmental meta-analysis of bipolar disorder performed by Wegbreit et al. The researchers compared different age groups with bipolar disorder (youths and adults). PBD youths showed increased activation in the amygdala, the inferior frontal gyrus, and precuneus compared to bipolar disorder adults during tasks using emotional stimuli. These findings revealed that these structures are underdeveloped and work less efficiently when compared with those of adults (46). However, our meta-analysis was conducted using the comparison between participants of the same age (participants' mean age is less than 19 years). The central findings of the meta-analyses of brain function among the PBD, HR, and TD groups, during the performance of mixed cognitive and emotional activities, illustrated a coherent pattern of group differences in line with our *a priori* hypothesis. The HR group showed a significantly greater activation in the *right DLPFC–insular–parietal–cerebellar regions* relative to TD, and this may be a bio-signature – an earlier sign of potential PBD development. At the junction of the DLPFC and VLPFC regions, where prefrontal systems interface in voluntary modulation of cognition, emotion, and motor control, brain function was amplified in the HR group (6, 7). Large future studies of symptomatic HR population (47) and genetic HR population must be compared both at a symptomatic and brain functional level to look at the definitive predictability of symptoms and the correlation of brain activity patterns.

Table 1 | Primary fMRI studies of participants with pediatric bipolar disorder (PBD), those at high risk (HR) for PBD, and typically developing (TD): children included in meta-analysis.

Primary study	Sample size	Age (mean ± standard deviation, years)	Medication status	Task
Cerullo et al. (24)	PBD (11, female = 7), TD (13, female = 6)	Age range: 11–18, PBD: 14.2 ± 1.5, TD: 14.5 ± 1.9	All bipolar participants had been off atypical anti-psychotics for at least 72 h and had undetectable levels of mood stabilizers.	Continuous performance task with a response inhibition component
Chang et al. (25)	PBD (12, all male), TD (10, all male)	Age range: 9–18, PBD: 14.7 ± 3.0, TD: 14.4 ± 3.2	All PBD participants except one were taking medication at the time of the fMRI.	Two-back visuospatial working memory task and an affective task showing emotionally valenced pictures
Deveney et al.[a] (5)	PBD (19, female = 12), HR (13, female = 7), TD (21, female = 8)	PBD: 14.76 ± 2.9, HR: 13.46 ± 1.8, TD: 13.78 ± 2.0	10 of 19 PBD participants were medicated.	Stop signal task
Deveney et al. (26)	PBD (32, female = 17), TD (21, female = 8)	Age range: 8–18, PBD: 14.5 ± 2.5, TD: 13.8 ± 2.0	17 of 32 PBD youths were medicated.	Stop signal task
Dickstein et al. (27)	PBD (16, female = 7), TD (16, female = 7)	Age range: 7–18, PBD: 14.1 ± 2.5, TD: 13.9 ± 2.4	13 of 16 PBD youths were medicated.	Probabilistic reversal task
Dickstein et al. (28)	PBD (23, female = 14), TD (22, female = 12)	PBD: 14.2 ± 3.1, TD: 14.7 ± 2.3	18 of 23 PBD youths were medicated.	Encoding task and subsequent memory task
Diler et al. (29)	PBD (10, female = 8), TD (10, female = 8)	Age range: 12–17, PBD: 15.6 ± 0.9, TD: 15.6 ± 1.2	7 of 10 youths were medicated.	Emotional face gender-labeling task
Diler et al. (30)	PBD (10, female = 8), TD (10, female = 8)	Age range: 12–17, PBD: 15.6 ± 0.9, TD: 15.6 ± 1.2	All PBD youths were medicated.	Go/no go block design cognitive control task
Garrett et al. (20)	PBD (20, female = 6), TD (21, female = 8)	Age range: 9–17, PBD: 15.63 ± 2.10, TD: 15.35 ± 2.68	Exact total percentage of medicated participants was not shown.	Emotional (happy, sad, and neutral) facial expression
Kim et al.[a] (6)	PBD (28, female = 16), HR (13, female = 7), TD (21, female = 8)	Age range: 8–17, PBD: 14.37 ± 2.63, HR: 13.90 ± 2.02, TD: 13.73 ± 1.96	18 of 28 PBD youths were medicated.	The change task
Kim et al. (31)	PBD (18, female = 8), TD (15, female = 10)	Age range: 9–18, PBD: 14.29 ± 2.54, TD: 14.98 ± 2.03	15 of 18 PBD youths were medicated.	Emotional face gender-labeling task
Ladouceur et al.[a] (7)	HR (16, female = 7), TD (15, female = 11)	Age range: 8–17, HR: 14.2 ± 2.3, TD: 13.8 ± 2.7	All participants were unmedicated.	Emotional face N-Back task
Leibenluft et al. (32)	PBD (26, female = 14), TD (17, female = 8)	PBD: 13.6 ± 2.6, TD: 14.6 ± 1.8	13 of 26 PBD youths were medicated.	Stop signal task
Mourao-Miranda et al.[a] (8)	HR (16, female = 9), TD (16, female = 9)	Age range: 12–17, HR: 14.8 ± 1.8, TD: 15.3 ± 1.2	All participants were unmedicated.	Emotional face gender-labeling task
Nelson et al. (33)	PBD (25, female = 13), TD (17, female = 8)	Age range: 8–17, PBD: 13.4 ± 2.5, TD: 14.6 ± 1.8	13 of 25 PBD youths were medicated.	The change task
Olsavsky et al.[a] (9)	PBD (32, female = 15), HR (13, female = 6), TD (56, female = 30)	Age range: 8–18, PBD: 14.7 ± 2.7, HR: 14.0 ± 2.4, TD: 14.0 ± 2.6	24 of 32 PBD and 1 of HR youths were medicated.	Emotional face presentation

(Continued)

Table 1 | Continued

Primary study	Sample size	Age (mean ± standard deviation, years)	Medication status	Task
Passarotti et al. (34)	PBD (15, female = 8), TD (15, female = 8)	Age range: 10–18, PBD: 13.20 ± 2.65, TD: 14.13 ± 3.16	8 of 15 PBD youths had been medicated in the past. Patients were drug-free for at least 7 days before testing.	Stop signal task
Passarotti et al. (22)	PBD (23, female = 13), TD (19, female = 10)	Age range: 10–18, PBD: 13.55 ± 2.48, TD: 13.53 ± 3.16	All participants were medication free or had a washout period of at least 4–7 days before scanning.	Emotional face N-Back task
Passarotti et al. (35)	PBD (17, female = 11), TD (14, female = 7)	Age range: 10–18, PBD: 14.27 ± 1.98, TD: 14.14 ± 2.42	All participants were medication free or had a washout period.	Emotional valence Stroop task
Passarotti et al. (36)	PBD (17, female = 12), TD (13, female = 7)	Age range: 10–18, PBD: 14.29 ± 2.05, TD: 14.38 ± 3.57	All patients were medication free for at least 7 days prior to scanning.	Emotional face N-Back task
Pavuluri et al. (37)	PBD (10, female = 4), TD (10, female = 5)	Age range: 12–18, PBD: 14.9 ± 1.85, TD: 14.3 ± 2.36	All participants were unmedicated.	Emotional face presentation
Pavuluri et al. (21)	PBD (10, female = 5), TD (10, female = 5)	Age range: 12–18, PBD: 15.2 ± 2.0, TD: 14.3 ± 2.1	All participants were unmedicated.	Incidental and directed emotion-processing task
Pavuluri et al. (38)	PBD (13, female = 3), TD (13, female = 9)	Age range: 10–18, PBD: 14.4 ± 2.2, TD: 14.4 ± 2.8	All patients were medication free for at least 4–7 days prior to scanning.	Response inhibition task
Pavuluri et al. (39)	PBD (17, female = 11), TD (14, female = 7)	Age range: 12–18, PBD: 14.3 ± 1.1, TD: 14.1 ± 2.4	All participants were unmedicated.	Pediatric affective color matching task
Rich et al. (23)	PBD (22, female = 12), TD (21, female = 10)	Ager range: 9–17, PBD: 14.2 ± 3.1, TD: 14.5 ± 2.5	18 of 22 PBD youths were medicated.	Emotional face presentation
Singh et al. (40)	PBD (24, female = 11), TD (24, female = 15)	Age range: 13–18, PBD: 15.7 ± 1.7, TD: 15.0 ± 1.4	20 of 24 PBD participants had a history of medication exposure.	Monetary incentive delay task, affective priming task
Singh et al. (41)	PBD (26, female = 7), TD (22, female = 9)	Age range: 9–18, PBD: 15.4 ± 2.37, TD: 14.3 ± 2.33	History of medication exposure: valproic acid (13), lithium (8), antidepressants (16), atypical anti-psychotics (6), psychostimulants (14), or more than one medication (16).	Go/no go block design cognitive control task
Thermenos et al.[a] (11)	HR (10, female = 5), TD (10, female = 5)	Age range: 13–24, HR: 18.4 ± 4.2, TD: 17.1 ± 1.4	All participants were unmedicated.	2-back working memory task and 0-back control task
Weathers et al. (42)	PBD (16, female = 8), TD (21, female = 9)	PBD: 14.65 ± 2.19, TD: 13.79 ± 1.97	9 of 16 PBD youths were medicated.	Stop signal task

[a] *Studies including HR groups.*

Some studies were missing age range information and showed only the mean age. Accordingly, that information could not be included within the table. Specific medications were heterogeneous when reported and at times went unreported. Hence, we were only able to comment on participants' medicated/unmedicated status. Similarly, the mood and affect of participants were also largely unreported and, therefore, could not be included in the table.

A repeated and important observation of hemodynamics of the fMRI studies is the increased activity in the brain that reflects increased effort (48). If one construes TD as the reference point of normative activity, then the HR group showed increased effort to get the same work done by deploying the right DLPFC–insular–parietal regions relative to TD, while in PBD, these same regions went offline relative to TD. This finding is akin to the analogy of "stretching an elastic band" with increased DLPFC activity (requiring a greater effort than TD) in the HR group, whereas those with PBD who had a more severe illness had reached a breaking point with decreased right VLPFC and DLPFC activity (with no effort to spare relative to TD). We could not explain the increased left VLPFC activity in PBD relative to TD. While such a finding is not unexpected in a meta-analytic study, it was largely based upon the

Table 2 | Activation likelihood estimation (ALE) meta-analysis findings for fMRI studies comparing pediatric bipolar disorder (PBD) patients, participants with a high risk (HR) for PBD, and typically developing (TD) children.

Pairwise analysis	Side	Brain region	BA	Talairach			Cluster size (mm^3)	Extreme value
				X	*Y*	*Z*		
HR youths > TD youths (11 experiments)	L	Cerebellum, culmen		−8	−50	−26	1472	0.022
				−14	−36	−22		0.014
				−2	−54	−10	952	0.021
	R	Dorsolateral prefrontal cortex	9	46	8	22	1048	0.020
	R	Insular cortex	13	38	18	6	472	0.014
	R	Parietal lobe, inferior parietal lobule	40	32	−46	42	464	0.014
HR youths > BD youths (6 experiments)	R	Dorsolateral prefrontal cortex	9	46	8	22	1056	0.020
	L	Cerebellum		−8	−50	−26	944	0.022
	R	Insular cortex	13	38	18	6	496	0.014
BD > TD (43 experiments)	R	Amygdala, limbic lobe, parahippocampal gyrus,		26	−2	−12	1120	0.0221
	R	Frontal lobe, medial prefrontal cortex	10	4	62	14	872	0.030
				12	40	10	568	0.023
	L	Ventral striatum		−16	−12	28	640	0.024
	R	Somatosensory association cortex	7	42	−58	48	576	0.020
				2	−64	56	392	0.019
	L	Cerebellum		−16	−36	−24	560	0.022
	L	Lentiform nucleus, putamen, lateral globus pallidus		−22	6	−4	464	0.018
				−12	4	−6	368	0.017
				−16	−4	−8		0.013
	L	Ventrolateral prefrontal cortex	47	−30	20	−8	336	0.017
	R	Subgenual cingulate cortex	25	2	0	−4	256	0.016
TD > PBD (21 experiments)	R	Dorsal cingulate cortex	32	2	36	12	1576	0.017
	R	Dorsal striatum		10	10	6	696	0.014
	R	Ventrolateral prefrontal cortex	47	38	24	−4	336	0.011
	R	Dorsolateral prefrontal cortex	8	32	24	38	224	0.013
	R	Superior frontal gyrus	10	24	48	2	216	0.011

R: right, L: left.

participants of only one study (21). However, it can be explained by bilateral disturbances in the VLPFC in PBD, albeit with the common and prominent right-sided abnormality than the left (32, 37). In the end, while one can postulate with explanations consistent with repeatedly published findings, definitive interpretations are not possible in understanding the nature of abnormal hemodynamic activity. For example, decreased (5) or increased (6) activation of the striatum with failed trials cannot easily differentiate HR from PBD based on any individual study. It could be mediated by the severity of illness in case of PBD, subsyndromal symptoms in HR, type of task, or hemodynamic relationship between the striatum and the PFC control regions.

With regard to recognizing the fully formed illness, typically noted underactivity of the higher cortical regions of emotion modulation (i.e., the interfacing dyad of the right VLPFC and DLPFC in the prefrontal regions) and overactivity of the subcortical amygdala consistently reported in BD Type I participants relative to TD adolescents (19) has also emerged as a significant finding in the current meta-analyses. The VLPFC is believed to serve the dual function of emotion (49) and motor (50) control via top–down regulation of the amygdala (51) and striatum (52), respectively. The DLPFC also serves a dual function, but it is predominantly through diverse cognitive functions involving executive control, response selection, problem solving, and emotion (53), and by being closely connected to the medial PFC, VLPFC, and the subcortical regions directly (54) as well as indirectly (52). The cognitive and emotion control regions in the PFC are not able to moderate the overactive subcortical regions, a consistent finding that was further underscored in our meta-analysis. In addition to the top–down *affect modulation circuitry* problems, increased activity is lateralized to the left side in the evaluative medial PFC, pregenual ACC, and the striatal loop (55); furthermore, all these regions are known to be closely connected to the amygdala (56). This subcortical and medial PFC loop is the *affective evaluation circuit* that is overactive in PBD. These findings could explain the excessive reactivity to negative emotions reported in PBD (21, 57) and are also in line with the concept suggested for bipolar disorder in general, including adult patients. Phillips and Swartz conceptualized bipolar disorder as multiple dysfunctions in prefrontal hippocampal–amygdala, emotion processing,

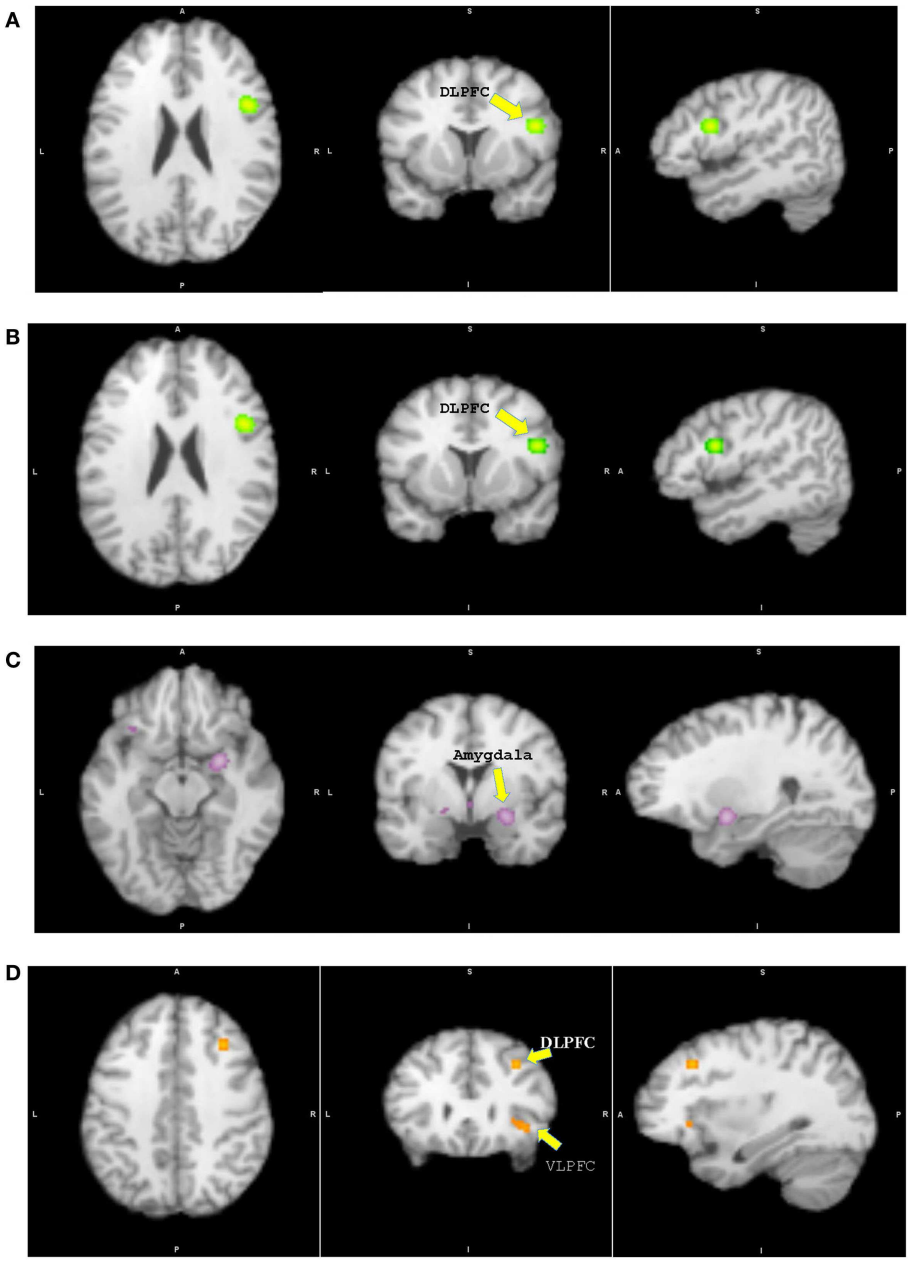

FIGURE 2 | Results from pairwise activation likelihood estimation (ALE) analysis. **(A)** High-risk youth > typically developing youth. **(B)** High-risk youth > youth with bipolar disorder. **(C)** Youth with bipolar disorder > typically developing youth. **(D)** Typically developing youth > youth with bipolar disorder. **(A)** DLPFC: dorsolateral prefrontal cortex, $x = 46$, $y = 8$, $z = 22$, cluster size = 1048 mm^3, extreme value = 0.020; **(B)** DLPFC: $x = 46$, $y = 8$, $z = 22$, cluster size = 1056 mm^3, extreme value = 0.020; **(C)** Amygdala: $x = 26$, $y = -2$, $z = -12$, cluster size = 1120 mm^3, extreme value = 0.022; **(D)** DLPFC: $x = 32$, $y = 24$, $z = 38$, cluster size = 224 mm^3, extreme value = 0.013; VLPFC: ventrolateral prefrontal cortex.

and emotion-regulation circuits, together with an "overactive," left-sided ventral striatal-ventrolateral, and orbitofrontal cortical reward-processing circuit (58). These results attest to the fact that, in relative terms of group comparison from fMRI studies, cognitive DLPFC and the corresponding dorsal circuitry hub that includes the parietal region and the insula are more involved in the HR population, while the wider multiple cortico (VLPFC, DLPFC, and medial PFC) and subcortical (limbic and basal ganglia) regions are implicated in PBD.

Published structural and fMRI studies of HR have not been conclusive and are limited to a comparison with the TD at times (7, 11). Singh et al. (59) reported that 8- to 12-year-old children with a familial risk for mania did not exhibit any statistically significant volumetric differences in the PFC, thalamus, striatum, or amygdala

compared with the TD group. However, they concluded that longitudinal studies will be needed to examine whether structural changes over time may be associated with a HR for BD (59). Bechdolf et al. (60) reported volume reduction in emotion-processing regions (i.e., the insula and amygdala) in HR, relative to TD, that corresponded to the functional abnormality involving increased amygdala activity in HR (9). While we found abnormal function in the insula in HR in this meta-analysis, three-way comparison did not reveal increased amygdala activity in HR. Existing studies consistently reported smaller amygdala and hippocampus (61), larger basal ganglia (62), and reduced PFC gray matter (63) in PBD. Hemodynamic (64) and resting state connectivity (65) findings in PBD relative to TD also point to frontolimbic and frontostriatal functional disturbance in PBD. Such uniformity in multi-modal imaging findings attests to the high reliability in establishing a significant pattern of brain dysfunction specific to PBD.

Limitations of this study include fewer and unequal numbers of participants in the HR group and the inclusion of studies that employed variable tasks used to probe multiple domains. However, due to the broad array of daily functions that draws from the active involvement of multiple and highly integrated networks, and the dual engagement of VLPFC, DLPFC, ACC, and the striatum in both cognitive and emotional tasks, this study was a reasonable first attempt to examine the entire brain's level of functionality from the existing data.

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Stress, inflammation, and cellular vulnerability during early stages of affective disorders: biomarker strategies and opportunities for prevention and intervention

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The mood disorder prodrome is conceptualized as a symptomatic, but not yet clinically diagnosable stage of an affective disorder. Although a growing area, more focused research is needed in the pediatric population to better characterize psychopathological symptoms and biological markers that can reliably identify this very early stage in the evolution of mood disorder pathology. Such information will facilitate early prevention and intervention, which has the potential to affect a person's disease course. This review focuses on the prodromal characteristics, risk factors, and neurobiological mechanisms of mood disorders. In particular, we consider the influence of early-life stress, inflammation, and allostatic load in mediating neural mechanisms of neuroprogression. These inherently modifiable factors have known neuroadaptive and neurodegenerative implications, and consequently may provide useful biomarker targets. Identification of these factors early in the course of the disease will accordingly allow for the introduction of early interventions which augment an individual's capacity for psychological resilience through maintenance of synaptic integrity and cellular resilience. A targeted and complementary approach to boosting both psychological and physiological resilience simultaneously during the prodromal stage of mood disorder pathology has the greatest promise for optimizing the neurodevelopmental potential of those individuals at risk of disabling mood disorders.

Keywords: prodrome, depression, bipolar, biomarker, stress, inflammation, cellular resilience, plasticity

INTRODUCTION

There is increasing appreciation for the need to both identify and treat mood disorders during their earliest stages (1). Although some dispute remains, maladaptive changes in mood and behavior first become evident during the prodromal period (2). However, the low specificity of these changes makes the prodromal stage difficult to definitively characterize prior to disease onset (3). Observable changes in mood and general physiologic functioning can include increases in sadness, anhedonia, irritability, anger, and anxiety, together with alterations in sleep and energy (4). Correlating these symptoms with prodromal biomarkers offers an exciting juncture whereby targeted interventions could be opportunistically employed to prevent neurodegenerative changes from accruing as the disease progresses (5). The potential to intervene during the prodromal stage of psychiatric illness through the detection and remediation of novel biomarkers has perhaps been best studied in schizophrenia, wherein most individuals experience a lengthy prodromal period prior to the full emergence of diagnosable psychotic symptoms (6). As an exemplar, low levels of nervonic acid appear to be a risk factor for conversion from high-risk to frank psychosis (7), and this risk of conversion may be reduced by targeted omega-3 fatty acid supplementation (8). Encouraging results from this work have renewed interest in the early detection of affective disorders, particularly bipolar disorder, with the hope that earlier and more targeted interventions might slow disease progression (3, 9–12). This can significantly impact neuroprogression and subsequent disease course for the individual (13). This concept of "neuroprogression" refers to the cumulative restructuring of the central nervous system which in turn mediates the development and persistence of psychiatric illness (14, 15). This process results from disturbances in inflammatory mediators, neurotrophins, oxidative stress, and energy regulation (14, 15).

BIOMARKER STRATEGIES FOR PRODROMAL MOOD DISORDERS

STRESS AND ALLOSTATIC LOAD

Stress sensitization and early detection

Stress is one of the best-studied mediators by which genetic vulnerabilities are translated into mood disorder pathology through the process of neuroprogression (16–18). Numerous studies have

demonstrated that both depression and bipolar disorder are more prevalent in individuals who have experienced adverse early-life events. This is partly because such experiences prime future physiologic and neural responses to stress, elicit a state of chronic inflammation (19), alter cellular mediators of plasticity and energy metabolism, and increase cellular "wear and tear" (20–22). Early-life stress (2) can be particularly deleterious because of its potential to influence the programing of the hypothalamic–pituitary–adrenal (HPA) axis (23) to induce persistent sensitization of neuroendocrine, autonomic, oxidative, and immune responses to stress. Over time these sensitized systems cumulatively contribute to the cellular and synaptic alterations underlying neuroprogression (21, 24–26). Specific examples include changes in reactivity of inflammatory cytokines [e.g., interleukin 6 (IL-6)] (25), alterations in markers for lipid peroxidation [e.g., 8-iso-prostaglandin F (2α)], oxidative damage to DNA (8-hydroxy-2′-deoxyguanosine) and RNA (8-hydroxyguanosine) (24), as well as altered cortisol, adrenocorticotropic hormone, and corticotrophin releasing factor responses (26). Identification of the state of physiologic and cellular resilience or sensitivity to stress may provide an important indicator of the level of neuroprogression and stress-mediated disease pathology for affective disorders, potentially prior to the initial manifestation of the mood episode (22).

One mechanism whereby HPA axis sensitization is likely to occur is through epigenetic regulation of stress response processes (21, 27). Evidence shows that exposure to various forms of stress result in multiple epigenetic changes in limbic regions as well as the HPA axis (21, 27). Interestingly, a recent study by Klendel and colleagues (18) found that only individuals who exhibited allele-specific DNA demethylation in functional glucocorticoid response elements of FK506 binding protein 5 (*FKBP5*), were prone to developing persistent cortisol dysregulation (18, 21). Further, this association was found to be dependent on an interaction effect with trauma in early life, suggesting that key developmental stages are directly related to stability of the observed effects across time (18). In another study, significant interactions between peripheral *FKBP5* mRNA expression and disease progression were reported, suggesting that polymorphisms in the gene directly impact the extent of neuroendocrine dysregulation, and corresponding neuroprogression (28). The *FKBP5* risk allele and corresponding levels of mRNA expression may represent useful biomarkers. These markers could be employed to identify individuals in the prodromal stages of stress-sensitive psychiatric disorders, such as major depression or bipolar disorder. Such detection would facilitate early intervention and could improve resilience and alleviate allostatic load in the prodromal individual.

Early-life stress and accumulation of allostatic load

Accumulation of allostatic load is a key mechanism through which early-life stress is thought to result in psychopathology (29). This is mediated via a series of enduring adaptive changes across a range of systems primed both to respond rapidly to challenge, as well as to restore homeostatic equilibrium (30). Adaptive allostatic mechanisms may fail when chronically challenged or when regulatory systems falter. This leads to a state of allostatic overload, which is thought to considerably impact the clinical course of mood disorders (31–33). Without sufficient opportunity for recovery, the brain and body are repeatedly exposed to molecular mediators of stress that can increase the level of cellular "wear and tear" (33). These mediators, which include metabolic factors, inflammatory cytokines, neurotrophins, and oxidative species, collectively impact an individual's mental and physical resilience as outlined below [for more detailed reviews see Ref. (6, 34, 35)]. Both physiological (i.e., immune and/or metabolic) and psychological (i.e., bullying) stressors contribute significantly to allostatic load, and thus need to be considered together when assessing both risk and relative staging of mood disorder pathology (6, 34).

Enhancing an individual's capacity to buffer the physiologic toll that accumulates through allostatic overload should be considered an important early intervention strategy. As allostatic load accumulates and attempts to maintain cellular homeostasis fail, cell danger signals are propagated and pro-apoptotic cell signaling pathways become increasingly engaged (36–39). This may play a role in medical comorbidities such as heart disease (40), as well as interfere with the therapeutic mechanisms of antidepressants and mood stabilizers to impair treatment efficacy (41–43). Internal stressors that activate the HPA axis and associated allostatic systems can limit an individual's capacity for allostasis even prior to the onset of external stressors (36). For example, an endogenous load can build through the expression of homocysteine or inflammatory cytokines, limiting the capacity of adaptive responses in the face of subsequent stressors. Interventions that counter this load and reduce levels of proinflammatory mediators or interfere with their neuromodulatory actions could limit neuroprogression in both bipolar and unipolar depression, as well as enhance capacity for antidepressant efficacy (44–46).

INFLAMMATORY PROFILE

Stress during earlier life is not only associated with disruption of the HPA axis, but may also serve to sensitize proinflammatory responses to future insults (47–49). Inflammatory mechanisms are increasingly appreciated for their critical role in mood disorder pathophysiology, in particular via their regulation of neuronal excitability, synaptic transmission, synaptic plasticity and neuronal survival (41, 50, 51). Of specific interest are proinflammatory mediators, such as cytokines [i.e., interleukin 1, IL-6, and tumor necrosis factor alpha (TNF-α)] and C-reactive protein (CRP). CRP is often used as a biomarker for inflammation in studies due to its relationship with proinflammatory cytokines and role in the immune response. As demonstrated by Slopen and colleagues (49), individuals at ages 10 and 15 who reported adverse life events at critical stages between the ages of 1.5 and 8 years were found to have significantly increased levels of CRP and IL-6. These heightened concentrations were correlated with immune activation and depressive-like symptoms. Notably, increased CRP levels have been used previously to predict depression severity and recurrence rates in males (48, 52).

There is a growing literature supporting the use of inflammatory biomarkers as predictors of ensuing mood disorder pathology (22). Research to date has been focused on investigating the relationship between inflammatory cytokines and affective disorders in adults; however, their specific role in early onset/adolescent psychopathology is less well explored (53). Cytokines are thought to influence neurodevelopment during key

stages, such as adolescence, interacting with biological systems including those of stress hormones and gonadal hormones (53). As such, perturbation of inflammatory balance in adolescents may significantly contribute to neuroprogression and development of psychiatric illness (19, 53, 54). For example, elevated serum levels of TNF-α, IL-6, and interleukin-10 (IL-10) have been reported during the early stages of bipolar disorder (55), and CRP appears to be a biomarker of *de novo* depression risk (56).

As the mood disorder pathology progresses, an increasing number of proinflammatory cytokines are observed, including elevated levels of interferon gamma (IFN-γ) (22, 54, 55). Notably, increases in IFN-γ are associated with dysregulation of the tryptophan metabolite pathway via direct role in indoleamine 2,3-dioxygenase (IDO) activation. Activation of IDO is commonly found in later stages of mood disorders, and is a biomarker of depression-like behavior mediated by neural inflammation in animal models (48). Proinflammatory cytokines activate IDO, resulting in depletion of serotonin and augmentation of quinolinic acid (QUIN) metabolism over kynurenic acid (KYNA). Tryptophan metabolites (kynurenine, KYNA, 3-hydroxykynurenine, and QUIN) act as neuromodulators to influence behavioral, neuroendocrine, and neurochemical aspects of depression (57–60). Consequently, this accumulation of QUIN facilitates neurodegeneration over neuroprotection, impacting mood disorder neuroprogression and resultant disability (61).

It is noteworthy to mention several other findings regarding altered inflammation in youth with psychiatric pathology. Increased mRNA and protein expression levels of IL-1β, IL-6, and TNF-α were reported in the anterior prefrontal cortex of adolescent suicide victims compared with normal control subjects (62). Elevated levels of inflammatory cytokines (among others: TNF-α, IL-1β, IL-6, and IFN-γ) were also observed in the serum of pediatric patients who experienced first-episode psychosis, in addition to increased leukocyte counts and evidence of blood–brain barrier damage (63). Quantification of inflammatory biomarkers (e.g., TNF-α, IL-6, IL-10, or CRP) may thus prove useful for detecting individuals at risk for developing a mood disorder. A recent study by Byrne and colleagues (64) suggests that levels of peripheral cytokines (e.g., IFN-γ) and CRP in salivary samples may correlate with serum samples in young people. Salivary assay may prove to be a simpler, less invasive method of estimating peripheral levels of inflammatory markers in adolescents (64). This provides one avenue whereby prodromal individuals could potentially be identified and their disease onset delayed.

DIMINISHED SYNAPTIC INTEGRITY

Homeostatic control of synaptic connections within key mood-related circuits plays a critical role in the etiology of mood disorders (65). Stress and inflammation as discussed in previous sections are implicated in disruption of synaptic signaling and integrity during the early stages of mood disorder pathogenesis. This is mediated in part through the inhibition of neurotrophin function, of which brain derived neurotrophic factor (BDNF) is the most thoroughly characterized. BDNF plays an important role in neuronal development, survival, and function, including activity-dependent synaptic plasticity (66). Synaptic plasticity is characterized by various processes, including synaptic remodeling, synaptogenesis, long-term potentiation, and long-term depression, all of which critically mediate the flow of electrochemical information throughout the central nervous system (67, 68). Stress, allostatic load, inflammation, antidepressants, and mood stabilizers exert major effects on signaling pathways that regulate cellular plasticity, suggesting these are critical neurobiological mediators of mood dysfunction and therapeutic intervention (69–72).

Glycogen synthase kinase-3 (GSK-3), part of the signaling cascade regulated by BDNF, plays an important role in synaptic homeostasis through regulation of synaptic deconsolidation (pruning) and glutamate receptor cycling (73). Increased GSK-3-mediated synaptic deconsolidation has been suggested to be an important factor contributing to reduced spine density in mood disorders (74). Additionally, levels of activated GSK-3 are increased in postmortem brain tissue from individuals with unipolar and bipolar depression (74). In addition to BDNF, GSK-3 is deactivated by signals originating from numerous signaling pathways demonstrated to be dysregulated in mood disorders (e.g., Wnt and PI3K pathways), and is either the direct or downstream target of many mood stabilizer and antidepressant medications (75). GSK-3 activity is modulated by serotonin and dopamine, and is a critical node at the intersection of multiple neurotransmitter and cell signaling cascades (68). As a result, GSK-3 modulates not only synaptic plasticity but also apoptotic mechanisms and, in turn, plays a critical role in mediating cellular resilience (75). For this reason, GSK-3 has received much attention for its potential to be targeted as an early intervention strategy during the prodrome period.

IDENTIFYING IMPAIRED CELLULAR RESILIENCE

Stress, allostatic overload, and neuroinflammation function together to impair synaptic plasticity and cellular resilience. Disrupted plasticity along with increased cellular vulnerability contributes significantly to the pathophysiology of mood disorders and directly to the neuroprogressive nature of the disease course (3, 76). Some of the key mechanisms of disease progression affecting cellular resilience include: oxidative stress, decreased neurotrophic factor expression, reduced neurogenesis, impaired regulation of calcium, altered endoplasmic reticulum and mitochondrial function, together with dysregulated energy metabolism and insulin signaling. Each of these mechanisms are mediated by allostatic overload and neuroinflammation [for detailed reviews see Ref. (3, 36, 76–78)]. Together, these processes demonstrate that in addition to synaptic integrity, maintenance of cellular homeostasis is critical for facilitating cellular resilience and attenuating mood disorder pathogenesis (79), which is also likely to enhance the capacity for treatment response during later stages of the disorder (80).

Cellular vulnerability and resilience are mediated by apoptotic and anti-apoptotic intracellular signaling cascades, respectively. Apoptosis is important for the regulation of developmental processes and prevention of cancerous growths. Excessive apoptosis in neuronal systems, however, leads to neurodegeneration and certain cell populations are at increased risk of stress-mediated apoptotic cell death (80). Apoptosis is a tightly regulated and energy-dependent process, which coordinates programed cell death in response to different stimuli (81). This can occur through stimulation of death receptor proteins

[i.e., tumor necrosis factor (TNF) receptor] by cytokines of the TNF superfamily or in response to mitochondrial degradation. These stimuli result in activation of executioner caspases that function to coordinate cellular process necessary for apoptosis, including cessation of cell repair processes and cell cycle progression, cytoskeletal and nuclear disassembly, and flagging the cell for phagocytosis (82). Distinct classes of antidepressants and mood stabilizers have been demonstrated to facilitate cellular resilience to prevent progression of pro-apoptotic processes, and novel treatments are currently being developed to target these specific mechanisms (83). Biomarkers that characterize the level of neuronal vulnerability relative to resilience may prove useful as biomarkers of prodromal mood disorder pathology. This has been demonstrated for later stages of bipolar disorder (84), however more studies are needed to determine the utility of such cell danger biomarkers during the mood disorder prodrome (22).

OPPORTUNITIES FOR PREVENTION AND INTERVENTION

IDENTIFYING VULNERABILITIES AND BUILDING RESILIENCE AT THE CELLULAR LEVEL

Identification of individuals at risk of developing a mood disorder, or those in the prodromal stage, provides a potential opportunity to target these mechanisms for neuroprotective interventions that enhance cellular resilience, maintain synaptic plasticity and boost psychological resilience (**Figure 1**) (85). One of the longest held notions of brain plasticity is that certain critical periods or windows exist in development, during which circuitry is consolidated for lifetime functionality. Recently, there is a rising consensus that developmentally induced plasticity can, to an extent, be reversed by "re-opening" those windows of plasticity (86). Hyman and Nestler (87) have underscored the importance of shifting the brain into an "adaptive state" to necessitate the antidepressant response. Their theory of "initiation and adaption" is exemplified by psychotropic drugs wherein primary molecular targets that initiate alterations in brain function activate homeostatic mechanisms that return the system to an adaptive and treatment responsive state (87). Plasticity and cellular resilience are thus necessary for the efficacy of antidepressants and mood stabilizing treatments. McGorry and colleagues (6, 88) and others (89) have demonstrated this concept with pre-psychotic interventions, and repeatedly emphasized the need to take advantage of the "windows of opportunity" present within the prodromal stages of psychiatric disease (6, 88, 89). During this stage, the course of the disease remains theoretically plastic and amenable to intervention (90). Previous literature indicates that once risk or prodromal symptoms of mood disorders are identified, there is some (91), but not unequivocal (92) evidence that early intervention in adolescents can significantly reduce mood-related symptoms and incidence of fully diagnosable psychiatric disorders such as depression (93–95). Neuroprotective pharmacotherapies together with appropriate psychotherapy may reduce the risk of neuropsychiatric disease progression in young people which, together with allostatic load reducing behavioral interventions, may significantly slow the trajectory of the disease course into adulthood (6, 36, 96). Such interventions may include reducing lifestyle mediators of allostatic load (19, 97).

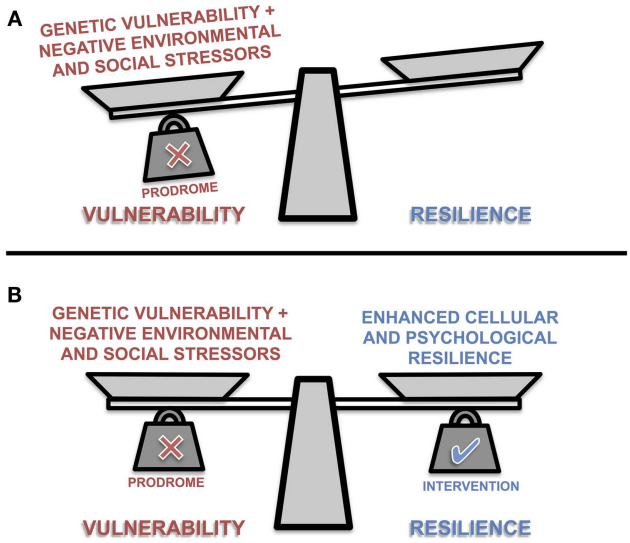

FIGURE 1 | A representation of the conceptual balance between vulnerability and resilience in prodromal individuals. The scale's balance beam teeters between vulnerability and resilience as scale pans are loaded with different positive and negative biological, psychological and social factors. The presence or absence of these factors influence the ability of the individual to cope with stressors, and maintain allostasis. **(A)** Prodromal individuals are somewhat predisposed to vulnerability; but with intervention **(B)** an individual may adopt more adaptive environmental coping strategies, support mechanisms, general healthy lifestyle choices, and/or receive pharmacological interventions that collectively enhance physiological and psychological resilience.

COGNITIVE AND BEHAVIORAL INTERVENTIONS TO BUFFER STRESS AND BUILD RESILIENCE

Individuals provided with effective social and emotional support to help cope with stressors that are adverse and potentially taxing will be much better placed to limit associated biological costs and maintain allostasis (98). The absence of emotional or social support and the implementation of maladaptive coping strategies can enhance the toxic effects of stress and contribute to allostatic overload (98). Exposure to regular and controllable stressors over the course of childhood and adolescence is essential for the development of effective coping strategies. Through such exposure, an individual can develop a repertoire of these coping strategies. Mathew and Nanoo (99) found that adaptive coping strategies (e.g., employing self-control, accepting responsibilities, problem solving, seeking social support, or positive reappraisal) are protective for suicide risk in adolescents. Conversely, maladaptive coping strategies, such as confrontation, distancing, and escape-avoidance were reported to be significant risk factors associated with adolescent suicide attempts (99). These findings provide evidence to support the notion that coping strategies can act as protective factors against both the development and progression of mood disorders. Importantly, educating children and adolescents in protective coping skills may be a promising intervention that could be implemented as early as elementary school. In recent years, patterns of threat perception such as optimism have attracted much attention in relation to later mood,

coping, and immune change in response to stress (100, 101). Moreover, it has been found to be protective against the development of depressive symptoms in later life (102). Its potential role in buffering against the negative emotional consequence of adverse events has led to a view of optimism as an index of resilience (103). Optimists may also choose lifestyles that promote physical as well as mental health, thereby reducing other aspects of allostatic load.

Healthy lifestyle, similar to optimism, provides a solid foundation for adaptation, and increases available resources for buffering the neurodegenerative effects of stress. Specifically, previous literature highlights the importance of healthy diet, adequate sleep, avoidance of smoking, and sufficient exercise (104). A population-based study reported higher emotional well-being among physically active youths, independent of social class and health status (105). Across a 2-year period, Motl and colleagues (106) found changes in physical activity were inversely related to a change in depressive symptoms. Levels of physical activity in childhood can modulate the risk of adult depression (107). Exercise modulates many of the core biomarkers of neuroprogression, including inflammation, oxidative stress, and neurotrophins (108). Poor eating habits and sleep have been linked to the manifestation of toxic stress and unhealthy growth in pediatrics by disrupting the architecture of the plastic, adaptive brain (109). There is now extensive evidence that poor diet quality is a risk for adolescent depression (110), and new data suggests that maternal diet influences the mental health of offspring (111). Similarly, smoking increases the risk of mood and anxiety disorders, and appears to influence similar biological pathways (112, 113). Parents and care givers of younger children need to be informed of the potential impact that a healthy lifestyle can have in mitigating mood-related symptoms and problematic behaviors. Low-risk interventions such as those aforementioned are critical for enhancing both psychological and biological resilience to stress. When such perspectives and lifestyle health behaviors are consolidated early in childhood and adolescence, the cumulative effect may be meaningful (103).

CONCLUSION

Early intervention offers the possibility of altering the trajectory of mood disorder pathology. In so doing, we may curtail the progressive nature of the illness, both through neuroprotection and maintenance of peripheral health. Prevention and intervention treatments should go beyond stabilizing mood to include various and complementary strategies for reducing allostatic load, perhaps through psychoeducation and lifestyle-related interventions, including effective stress management. The combination of these techniques with specific pharmacotherapies may significantly improve functional outcomes by both reducing cellular insults and enhancing resilience. In so doing, this optimizes the capacity for maintenance of synaptic integrity and cellular resilience, which must be aggressively targeted as a therapeutic strategy during the prodromal stage of mood disorder pathology (90). This neuroprotective approach not only slows neuroprogression associated with the disease, but lays a foundation for more treatment-responsive outcomes during later stages.

AUTHOR CONTRIBUTIONS

Adam J. Walker, Yesul Kim, J. Blair Price, Rajas P. Kale, Jane A. McGillivray, Michael Berk, and Susannah J. Tye each made contributions to the writing of this manuscript.

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Neural responses during social and self-knowledge tasks in bulimia nervosa

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Self-evaluation closely dependent upon body shape and weight is one of the defining criteria for bulimia nervosa (BN). We studied 53 adult women, 17 with BN, 18 with a recent history of anorexia nervosa (AN), and 18 healthy comparison women, using three different fMRI tasks that required thinking about self-knowledge and social interactions: the Social Identity task, the Physical Identity task, and the Social Attribution task. Previously, we identified regions of interest (ROI) in the same tasks using whole-brain voxel-wise comparisons of the healthy comparison women and women with a recent history of AN. Here, we report on the neural activations in those ROIs in subjects with BN. In the Social Attribution task, we examined activity in the right temporoparietal junction (RTPJ), an area frequently associated with mentalization. In the Social Identity task, we examined activity in the precuneus (PreC) and dorsal anterior cingulate (dACC). In the Physical Identity task, we examined activity in a ventral region of the dACC. Interestingly, in all tested regions, the average activation in subjects with bulimia was more than the average activation levels seen in the subjects with a history of anorexia but less than that seen in healthy subjects. In three regions, the RTPJ, the PreC, and the dACC, group responses in the subjects with bulimia were significantly different from healthy subjects but not subjects with anorexia. The neural activations of people with BN performing fMRI tasks engaging social processing are more similar to people with AN than healthy people. This suggests biological measures of social processes may be helpful in characterizing individuals with eating disorders.

Keywords: mentalization, identity, theory of mind, eating disorders, anorexia, bulimia, neuroimaging, social behavior

INTRODUCTION

Bulimia nervosa (BN) is an eating disorder characterized by frequent binge-eating followed by purging behaviors in concert with a self-esteem that is overly associated with body shape and weight (1). The symptoms of many eating disorder patients change during their lives (2, 3). For example, a patient may develop restricting behaviors with weight loss in high school, begin binging and purging behaviors at a low weight, continue binge-purge behaviors at a healthy weight throughout college, and then cease the purging behaviors but have occasional binge-eating problems. Such a patient would have met criteria for anorexia nervosa (AN), restricting subtype initially, then AN, binge-purge subtype, then BN, and finally binge-eating disorder. This diagnostic instability makes clinical treatment as well as research into eating disorders challenging (4, 5). A better understanding of biological and cognitive similarities and differences that contribute to eating disorders may improve clinical treatment. Currently, treatment of BN leads to sustained recovery in only about half of the patients (6, 7). Through the use of fMRI, we examined neural activations related to social processes in BN.

A specific set of neural regions is modulated in response to tasks that require thinking about people in healthy subjects (8, 9); this provides a framework to assess differences related to psychiatric illnesses. Severe impairments in social interaction are one of the diagnostic criteria for autistic spectrum disorders (1), but problems in social cognition have been reported in many psychiatric illnesses (10–14). Decreased social cognition has been reported in a variety of behavioral tasks in adults with AN (15–17). In BN, recent studies have concluded that there was little evidence of social cognition differences in psychological tasks (18, 19), although far fewer studies of social cognition have been completed in BN than in AN.

Because both BN and AN include in their diagnostic criteria an association between appearance and self-esteem (1), these experiments focused on neural pathways related to thinking about oneself. Self-esteem is a term used to describe one's overall sense of one's own value as a person, and is generally considered a fairly stable psychological characteristic (20). Although the diagnostic criteria in eating disorders connect self-esteem specifically to physical appearance, similarly unrealistic social expectations are reported and observed in eating disorder patients (21, 22). Self-knowledge, as used in MRI tasks, relates to the ability to evaluate oneself, and is expected to be a process that involves self-esteem as well as other criteria. For example, an individual whose self-esteem is highly related to appearance might be very good at her work, and correctly describe herself as a competent employe, but maintain an overall low self-esteem because of perceived inadequacies of appearance. Furthermore, low self-esteem has been related to

prognosis in AN (23, 24) as well as onset of bulimic symptoms (25). Negative beliefs about one's self, unrelated to physical appearance, have been observed in eating disorders (26, 27), and neural differences in the processing of these negative self-beliefs have been seen in BN (28). These data show not only that psychological similarities in self-esteem are present in AN and BN, but also that self-esteem is an important factor in assessing the prognosis and severity of eating disorders.

In healthy people, midline cortical structures, including the cingulate (Cing), dorsal anterior cingulate (dACC), and precuneus (PreC), have been specifically associated with thinking about oneself, using a variety of self-knowledge, appraisal, and viewing tasks (29). Most commonly, these areas show activation during neuroimaging tasks that ask healthy subjects to reflect upon whether specific characteristics describe oneself (30). Performance of this type of task is likely to acutely stimulate similar cognitive processes as those that generate one's longer-term sense of self-esteem. We recently reported differences in brain activations in AN and CN based on differences in self-knowledge using two neuroimaging tasks that required self-evaluations, one using social adjectives and the other physical descriptors (31). In that study, we identified regions in the dACC, PreC, and Cing with different activations in subjects with AN compared to the healthy controls. Here, we consider the responses of subjects with BN in the same self-evaluative tasks.

In addition to self-evaluative tasks, we included a more general social processing neuroimaging task that robustly engages additional regions in the social processing network associated with considering other people (32). This task, the Social Attribution Task, strongly activates the right temporoparietal junction (RTPJ), a region that has been closely associated with theory of mind (TOM), and mentalization [for reviews, see (9, 33)], as well as the fusiform gyrus, a region closely associated with facial processing (34, 35). Furthermore, differences in fMRI activations in both adult participants with AN (36) as well as adolescent participants with AN (37) have been examined using this task. In this manuscript, we describe the neural activations of subjects with BN during the Social Attribution Task.

METHODS AND MATERIALS

ETHICS STATEMENT

This study was approved by the institutional review boards at both the University of Texas Southwestern Medical Center and The University of Texas at Dallas. Additionally, the study adhered to the guidelines as set out in the Declaration of Helsinki. Written informed consent was required from all participants, and subjects were reimbursed for time spent participating.

PARTICIPANTS

A total of 53 female participants, between 18 and 42 years of age, were recruited for this study from the general public, from treatment providers, and support groups in the Dallas–Fort Worth area. Subjects volunteered to spend 2 h in clinical assessments and completing questionnaires and 1.5 h completing behavioral tasks in the MRI scanner, and were compensated for their time financially. The participant groups consisted of 18 healthy controls (CN), 18 individuals with a recent history of anorexia but were currently in the process of recovering from AN, and 17 individuals recovering from BN. All AN and BN participants had met full DSM-IV criteria for either AN or BN within the previous 2 years. The AN subjects were required to be maintaining a minimum BMI of 17.5 with no weight loss for the 3 months preceding the MRI scans. This was based primarily on a detailed eating disorder symptom and weight history obtained at the initial screening interview, only after which was it divulged that low or unstable weight was an exclusion factor for MRI scans. One of the AN and one of the CN participants did not complete the Social Attribution task and one of the BN participants did not complete the Social and Physical Identity neuroimaging task; these subjects were excluded in the analyses involving those tasks. Another BN participant was excluded from the neuroimaging analyses of the Social and Physical Identity tasks due to excessive movement. Eleven of the AN subjects had the restricting subtype and seven had the binge-purge subtype of AN. All subjects were recruited and scanned between 2009 and 2012. Because of difficulty recruiting BN subjects, data collection of AN and CN subjects finished nearly 1 year before the last four BN subjects were obtained. Therefore, the AN and CN data were analyzed and published in two earlier papers, one describing results obtained from the Social Attribution task and the other results from the two Identity tasks (31, 36).

Subjects provided written informed consent to participate in this study at an initial appointment. All subjects were then interviewed using the Structured Clinical Interview for DSM-IV disorders (SCID-RV). Participants were also screened for MRI compatibility. Some of the subjects had a history of recurrent MDD (1, CN; 7, AN; 9 BN) but none had met symptom criteria for an MDE for at least 3 months prior to the neuroimaging studies. No participants had a current or past diagnosis of any psychotic disorders or bipolar disorder based on the SCID-RV; no participants were currently taking mood-stabilizers, antipsychotics, or benzodiazepines. Participants on antidepressants whose dosage had not changed for at least 3 months prior to their MRI scans were included (1 CN; 8 AN; 7 BN).

Participants also completed the Quick Inventory of Depression, Self-Report (QIDS-SR), a self-report questionnaire consisting of 16 items to assess current symptoms of depression (38), and the Eating Attitudes Test-26 (EAT-26), a self-report questionnaire consisting of 26 items that relate to current eating behaviors (39). Subjects also completed the Self-Liking and Self-Competence Self-Esteem Questionnaire (SLCS), a 16 item self-report questionnaire that provides two measures of self-esteem (40), and the Social Problem-Solving Inventory (SPSI-R), a 26 item self-report questionnaire (41).

NEUROIMAGING TASKS

Three fMRI tasks were employed, the Social Attribution Task, the Social Identity task, and the Physical Identity task. Most subjects (35 of 53) preferred to complete the tasks in two scanner sessions, the first session consisting of the Social Attribution Task, and the second both the Identity Tasks. If all tasks were completed in 1 day, the Identity tasks were run before the Social Attribution task, and the total scan time was 80 min. When completed on separate days, the first day lasted about 30 min and the second session was about 50 min.

The Social Attribution Task presented short videos of moving shapes (32, 36). Briefly, subjects were asked to view the shapes in two conditions: the visuospatial or Bumper condition, preceded by the question "Bumper cars: Same weight?" and the social attribution or People condition, preceded by the question "People: All friends?". Each animation consisted of a moving display of three white shapes (circle, triangle, and square) and a white box with one side that opened as if hinged on a black background. Although the same shapes were presented in both conditions, the movements of the shapes in the two tasks differed. During the visuospatial task, the shapes moved around the box for the duration of the animation periodically bumping into one another. During the social task, the shapes moved in ways that suggested social behavior was occurring among the shapes (e.g., playing, fighting, avoiding etc.). We recorded responses to the weight and friendship questions about the animations to determine accuracy and maintenance of concentration.

The Identity tasks consisted of the presentation of written appraisal statements projected onto a screen within the MRI scanner (31). For both the Social Identity and the Physical Identity task, three different types of appraisals were shown: self (evaluation of an attribute about one's own identity based on one's own opinion), Friend (evaluation of an attribute about a close female friend), and Reflected (evaluation of an attribute about one's self from one's friend's perspective). Each statement was presented above a scale reading 1 "Strongly Disagree," 2 "Slightly Disagree," 3, "Slightly Agree," and 4 "Strongly Agree." Subjects were asked to read each statement and select a rating via a hand-held button. The Friend and Reflected statements were personalized to contain the name of a specific female friend of each subject. Each task was conducted separately, with all runs of the Social task preceding any runs of the Physical task. In the Social task, the statements were presented in a format ending with a socially descriptive adjective (ex. Self Statement "I believe I am nice," Friend statement "I believe my friend is mean," Reflected statement, "My friend believes I am responsible"). For the Physical task, the statements were presented in the format ending with a physical body part and a descriptor (ex. Self statement "I believe my arms are toned," Friend statement "I believe my friend's eyes are bloodshot," Reflected statement "My friend believes my stomach is flabby"). In all cases my friend was replaced with the name of a close female friend of the subject.

MRI ACQUISITION AND ANALYSIS

All images were acquired with a 3T Philips MRI scanner. High resolution MP-RAGE 3D T1-weighted images were acquired for anatomical localization with the following imaging parameters: repetition time (TR) = 2100 ms, echo time (TE) = 3.7 ms; slice thickness of 1 mm with no gap, a 12°flip angle, and 1 mm^3 voxels. For both fMRI tasks, each slice was acquired with a 22.0 cm^2 field of view, a matrix size of 64×64, and a voxel size of 3.4 mm × 3.4 mm × 3 mm using a one-shot gradient T2*-weighted echoplanar (EPI) image sequence sensitive to blood oxygen level-dependent (BOLD) contrast. Head motion was limited using foam head-padding.

For the Social Attribution task, images were acquired during four runs, each lasting 128 s and presenting four 17-s videos, two in each condition (People or Bumper). These sequences were acquired using a TR of 1.5 s, an TE of 25 ms, and a flip angle of 60°, and volumes were composed of 33 tilted axial slices (3 mm thick, 1 mm slice gap) designed to maximize whole-brain coverage while minimizing signal dropout in the ventral anterior brain regions. For the Identity tasks, images were acquired during eight runs (four for Social and four for Physical), each lasting 360 s, and presenting 12 statements of each condition (Self, Friend, and Reflected). These sequences were acquired using a TR of 2 s, an TE of 35 ms, and a flip angle of 0°, and volumes were composed of 36 axial slices (4 mm thick, no gap).

Prior to statistical analyses, preprocessing for all tasks consisted of spatial realignment to the first volume of acquisition, normalization to the MNI standard template, and spatial smoothing with a 6 mm 3D Gaussian kernel. fMRI task data were analyzed using Statistical Parametric Mapping software (SPM5, Wellcome Department of Imaging Neuroscience London)[1] run in MATLAB 7.4[2], and viewed with xjview[3].

The fMRI data were analyzed separately for each of the three tasks. For the Social Attribution task, the data were analyzed using a general linear model to create contrast images with a block design (blocks: People and Bumper); the Identity tasks were analyzed separately using an event-related design, in which each type of event (events: Self, Friend, and Reflected) corresponded to the BOLD signal during the 4 s presentation of each statement. With both techniques, the general linear model was used to create contrast images with activation of each condition assessed using a multiple regression analysis set as boxcar functions. Each regressor was convolved with a canonical hemodynamic response function (HRF) provided in SPM5 and entered into the modified general linear model of SPM5. Parameter estimates (e.g., beta values) were extracted from this GLM analysis for the regressors. Resulting single-subject one-sample *t*-test contrast images were created for each participant for each of the three tasks. These contrast images were combined for group map analyses.

REGIONS OF INTEREST

Previously we identified four regions showing group differences with whole-brain voxel-wide comparisons of the AN and CN group maps using the contrasts of conditions in the three tasks (31, 36). These were the *a priori* regions of interest (ROI) for this study focusing on BN. In the Social Attribution Task, the whole-brain voxel-wide comparisons of the AN and CN groups led to identification of a 94 voxel region in the RTPJ (MNI 52, −64, 20) that showed more modulation in the People condition than the Bumper condition in the CN subjects compared to the AN subjects. In the Social Identity Task, the whole-brain voxel-wide comparisons of the Reflected–Self contrast for the AN and CN groups led to identification of a 379 voxel region in the dACC (MNI 6, 26, 36) with the opposite modulation in the CN subjects compared to the AN subjects. In the Social Identity Task, the whole-brain voxel-wide comparisons of the Self–Friend contrast for the AN and CN groups led to identification of a 43 voxel region in the PreC (MNI −8, −48, 46) with more modulation in the Self

[1] www.fil.ion.ucl.ac.uk/spm
[2] http://www.mathworks.com
[3] http://www.alivelearn.net/xjview8/

condition than the Friend condition in the CN subjects compared to the AN subjects. In the Physical Identity Task, the whole-brain voxel-wide comparisons of the Self–Friend contrast for the AN and CN groups led to identification of a 61 voxel region in a ventral region of the dACC adjacent to the corpus callosum (cc-dACC, MNI −6, 20, 24) showing more modulation in the CN subjects than the AN subjects. In addition to the ROIs defined by group differences in these tasks, we also examined activations in medial prefrontal cortex (MPFC; vmPFC) and dorsolateral prefrontal cortex (DLPFC) based on prior reports of differences in these areas with similar tasks in eating disorder subjects. We created 5 mm spherical ROIs centered on the published coordinates for MPFC [10, 64, 18, (37)], vmPFC [−12, 44, −12, (42)], and DLPFC [−48, 6, 38 (28)]. For all ROI analyses, we extracted the percent signal change occurring within each of these regions for each subject using the MarsBar toolbox[4] and transferred this data to (SPSS, Inc., Chicago). In SPSS, we first conducted a three-group ANOVA to identify whether differences were present across the three subject groups for each ROI, and conducted follow-up analyses of significant results using between-group *t*-tests.

RESULTS

PSYCHOLOGICAL SCALES AND DEMOGRAPHIC DATA

The three groups were not significantly different in age or years of education. The AN group had a significantly lower body mass index than either the CN and BN groups (**Table 1**). The BN and AN groups both scored higher than the CN group on measures of depression and eating behaviors but were not significantly different from each other. The AN and BN subjects also reported lower levels of both self-liking and self-competence compared to the CN subjects. On the SPSI-R, the AN and BN groups had lower overall scores on social problem solving as well as lower levels of positive problem orientation and higher levels of negative problem orientation than the CN groups. The AN subjects also showed higher levels of avoidance than the CN subjects, whereas the BN subjects had lower levels of rational-problem solving than the CN subjects. However, there were no significant differences in the AN and BN groups in comparisons for any of the SPSI-R subscales.

SOCIAL ATTRIBUTION TASK

The Social Attribution Task required subjects to respond to a question about each video. There were no differences in the accuracy of the subjects in response to either the Bumper visuospatial-weight question [mean percent correct, CN 59%, AN 64%, BN 69%, $F(50) = 2.17$, $p = 0.13$], or the People social-friendship question [mean percent correct, CN 77%, AN 81%, and BN 84%, $F(50) = 1.67$, $p = 0.20$]. There were also no differences in reaction times for subjects in either task [Bumper, mean reaction time in seconds, CN 1.22, AN 1.30, BN 1.35, $F(50) = 0.28$, $p = 0.76$; People, mean reaction time in seconds, CN 1.30, AN 1.35, BN 1.40, $F(50) = 0.15$, $p = 0.86$].

The People–Bumper contrast of the Social Attribution Task resulted in significant clusters of activation in the middle temporal gyri, and temporoparietal junctions (TPJ) in all three groups (**Table 2**). Additionally, the CN subjects had bilateral activations in inferior frontal gyri, the fusiform gyri, the medial frontal gyrus, and the PreC. The AN and BN subjects also had activations in the right inferior frontal gyrus, but not the left inferior frontal gyrus. The AN subjects also showed modulation of the medial frontal gyrus like the CN subjects, and also activated a region in the ventral anterior Cing. The BN subjects did not modulate MPFC, like the CN and AN groups, but did modulate the PreC and the fusiform gyri, like the CN subjects but differing from the AN subjects.

In **Figure 1**, we show the percent signal change occurring in the ROI for this contrast, the RTPJ, in the CN, AN, and BN groups during the Social Attribution task [means People–Bumper, CN 0.35,

[4]sourceforge.net/projects/marsbar

Table 1 | Sociodemographic and symptom scale values for the participants.

	Healthy control ($n = 18$)	Anorexia nervosa ($n = 18$)	Bulimia nervosa ($n = 17$)	Between group comparisons[c]
Average age (years)	24.5 (18–39)[a]	26.1 (18–40)	28.1 (19–42)	No differences
Mean years of education	15.8 (14–20)	14.9 (12–19)	15.8 (13–18)	No differences
Current body mass index[b]	23.2 (18–35)	19.6 (18–23)	22.1 (19–27)	1 > 2, $p = 0.003$; 3 > 2, p=0.001
Quick inventory of depression	3.7 (0–9)	8.5 (2–17)	6.8 (1–16)	2 > 1, $p < 0.001$; 3 > 1, $p = 0.012$
Eating attitudes test	4.3 (0–15)	27.4 (1–61)	18.8 (2–51)	2 > 1, $p < 0.001$; 3 > 1, $p = 0.001$
Self liking from SLSC[d]	30.7 (19–40)	17.2 (8–29)	20.4 (8–32)	1 > 2, $p < 0.001$; 1 > 3, $p = 0.02$
Self competence from SLSC[d]	30.2 (24–40)	24.7 (18–37)	27 (17–38)	1 > 2, $p < 0.001$; 1 > 3, $p = 0.04$
Social problem solving inventory	15.3 (12–18)	12.1 (6–18)	11.6 (6–14)	1 > 2, $p < 0.001$; 1 > 3, $p < 0.001$
Positive problem orientation	13.7 (7–18)	10.4 (3–19)	10.3 (3–15)	1 > 2, $p = 0.02$; 1 > 3, $p = 0.02$
Negative problem orientation	3.9 (0–8)	10.8 (3–16)	9.2 (1–20)	2 > 1, $p < 0.001$; 3 > 1, $p = 0.001$
Rational problem solving	13.2 (6–18)	10.8 (0–16)	8.3 (3–17)	1 > 3, $p = 0.007$
Avoidance style	3 (0–14)	6.6 (0–17)	5.5 (0–13)	2 > 1, $p = 0.028$
Impulsivity/careless style	3.4 (0–10)	3.6 (0–15)	5.9 (0–16)	No differences

[a]*All entries under the subject groups contain the mean (range).*

[b]*Mean and range for AN subjects exclude one higher weight outlier.*

[c]*Statistical values obtained using a three group ANOVA for each metric; p values provided for significant differences (<0.05).*

[d]*SLSC: self-Liking and self-competence scale.*

Table 2 | Clusters in the CN, AN, and BN group maps during the People–Bumper contrast of the social attribution task.

Group	Region	Volume	Cluster *P*	Peak *T*	MNI *x, y, z*
CN	Temporal	4366	0	11.66	60, −8, −16
CN	Inferior frontal	769	0	10.9	54, 28, 8
CN	Inferior frontal	313	0	10.67	−52, 22, −4
CN	Temporoparietal	2273	0	9.07	−60, −44, 12
CN	Temporal	918	0	8.64	−54, 0, −20
CN	Cerebellum	281	0	7.47	−10, −80, −46
CN	Fusiform	764	0	7.39	44, −34, −20
CN	Precuneus	609	0	6.68	8, −46, 36
CN	Prefrontal	344	0	6.13	4, 56, 14
CN	Caudate	81	0.036	5.77	−6, 2, 4
CN	Precentral	151	0.001	5.58	44, 6, 42
CN	Medial frontal	86	0.027	4.95	−6, 46, 40
AN	Temporoparietal	531	0	9.71	56, −42, 10
AN	Inferior frontal	194	0.003	9.22	50, 30, 10
AN	Temporal	204	0.003	9.21	−56, −10, −16
AN	Temporal	402	0	8.18	54, −2, −20
AN	Medial frontal	244	0.001	6.6	6, 54, 14
AN	Cingulate	137	0.02	5.89	−2.48, −10
AN	Temporoparietal	111	0.048	5	46, 10, −34
BN	Temporoparietal	2152	0	12.34	50, −58, 2
BN	Temporal	667	0	8.65	54, −10, −14
BN	Fusiform	255	0	7.97	34, −34, −18
BN	Temporal pole	645	0	7.77	−42, 6, −36
BN	Precuneus	638	0	7.5	14, −60, 22
BN	Temporoparietal	1312	0	7.23	−48, −58, 6
BN	Inferior frontal	224	0	6.93	50, 28, 4
BN	Fusiform	282	0	6.12	−36, −50, −18

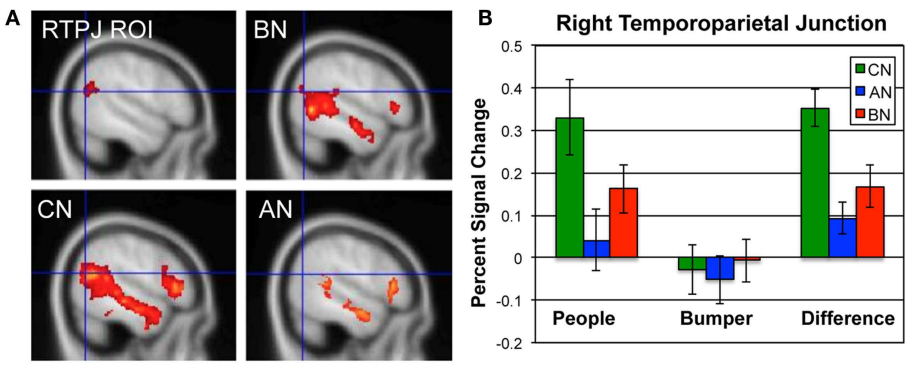

FIGURE 1 | Neural activations in the RTPJ during the social attribution task. **(A)** The RTPJ ROI is shown in the upper left, and the group maps for the People–Bumper contrast are all shown at sagittal coordinate $x = 52$ for the BN (upper right panel), CN (lower left panel), and AN (lower right panel) groups. **(B)** The percent signal change in the RTPJ ROI for the People condition, Bumper condition, and the difference in modulation for each group (green, CN; blue, AN; red, BN).

AN 0.09, BN 0.17, $F(50) = 9.7$, $p < 0.001$]. The BN group showed significantly less modulation of this region than the CN group [$t(33) = 2.7$, $p = 0.01$; Cohen's $d = -0.85$; effect size $= -0.39$] and no difference compared to the AN group [$t(33) = -1.2$, $p = 0.23$]. Similar to the AN group, the differences in activation are primarily the result of less activation of this region during the People condition. We also examined percent signal change by subject group in a MPFC ROI previously described in a similar task as related to outcomes in adolescent AN (37). Although the BN subjects had less activation in this ROIs than the other groups, it was not statistically different from either of the other groups [means, CN 0.30, AN 0.22, BN 0.17, $F(50) = 2.1$, $p = 0.13$].

SOCIAL IDENTITY TASK

The Social Identity Task required subjects to read and respond to social adjectives presented in three different conditions (Self, Friend, and Reflected) in the scanner. For each statement, we obtained a response on a four point scale and a reaction time. There were no significant differences across the three groups in any condition for either average response [mean response, Self: CN 2.53, AN 2.44, BN, 2.49, $F(51) = 0.178$, $p = 0.84$; Friend: CN 2.49, AN 2.46, BN 2.46, $F(51) = 1.54$, $p = 0.22$; Reflected: CN 2.53, AN 2.38, BN 2.41, $F(51) = 0.75$, $p = 0.48$] or the reaction times [mean reaction times in seconds, Self: CN 2.07, AN 2.18, BN 2.26, $F(51) = 0.35$, $p = 0.70$; Friend: CN 2.03, AN 1.97, BN 2.10, $F(51) = 1.97$, $p = 0.15$; Reflected: CN 2.17, AN 2.15, BN 2.16, $F(51) = 0.42$, $p = 0.66$].

The Social Identity Task activates regions associated with self-knowledge and personal mentalization. In the personal mentalization contrast (Social Reflected–Self), subjects were asked to imagine what a close friend thinks about their social characteristics in contrast to their own belief about themselves. This contrast differs somewhat from the mentalization processes activated in the Social Attribution task because the mentalization is now attributed to a known individual. The largest clusters of activation occurred in the PreC in all subject groups (**Table 3**). For both the CN and BN groups, this cluster also included a portion of the posterior Cing, but the AN group had a smaller PreC cluster and an additional cluster in the posterior Cing. The BN and CN groups also had other activation clusters including some consistent with activations seen in the impersonal mentalization task (CN subjects, cluster in left medial temporal gyrus; BN subjects, bilateral clusters in the TPJs). In **Figure 2**, the BN group showed a lower degree of modulation of the ROI from this task contrast, the dACC, than the CN group [means, Reflected–Self, CN 0.076, BN −0.011, $t(31) = 2.2$, $p = 0.03$, Cohen's $d = -0.79$, effect size = −0.37], and no difference from the AN group [means, Reflected–Self, AN −0.091, BN −0.01, $t(31) = -2.0$, $p = 0.06$].

The self-knowledge comparison (Social Self–Friend) led to very different activation patterns in the AN and BN subjects compared to the CN subjects (**Table 4**). Notably, the CN subjects only activated clusters in the occipital lobes, whereas the AN and BN subjects had many clusters with the largest in occipital, parietal, and frontal cortex. In **Figure 2**, the BN group also showed significantly less modulation of the ROI from this task contrast, the PreC, than the CN group [means, Self–Friend, CN 0.083, BN −0.001, $t(31) = 2.7$, $p = 0.01$, Cohen's $d = -0.94$, effect size = −0.43], and no difference from the AN group [means, Social Self–Friend, AN −0.039, BN −0.001, $t(31) = -0.9$, $p = 0.36$]. We also examined percent signal change in the vmPFC and DLPFC but found no differences in either region across the three groups [vmPFC, means CN 0.02, AN 0.02, BN 0.18, $F(50) = 1.75$, $p = 0.18$; DLPFC, means CN 0.08, AN 0.07, BN 0.14, $F(50) = 1.18$, $p = 0.31$].

PHYSICAL IDENTITY TASK

The Physical Identity Task required subjects to read and respond to physical descriptive phrases presented in three different conditions (Self, Friend, and Reflected) in the scanner. For each statement, we obtained a response related to agreeing or disagreeing with the description using a four point scale and a reaction time. There were no significant differences across the three groups in any condition for either average response [mean response, Self: CN 2.39, AN 2.40, BN, 2.36, $F(51) = 0.178$, $p = 0.84$; Friend: CN 2.36, AN 2.40, BN 2.31, $F(51) = 1.54$, $p = 0.22$; Reflected: CN 2.31, AN 2.31, BN 2.24, $F(51) = 0.75$, $p = 0.48$) or the reaction times [mean reaction times in milliseconds, Self: CN 2339, AN 2294, BN 2367, $F(51) = 0.35$, $p = 0.70$; Friend: CN 2392, AN 2267, BN 2440, $F(51) = 1.97$, $p = 0.15$; Reflected: CN 2493, AN 2414, BN 2492, $F(51) = 0.42$, $p = 0.66$].

In the Physical Identity self-knowledge contrast (Physical Self–Friend), very different activation patterns were present in the three groups (**Table 5**). The CN subjects had several clusters in the anterior and middle Cing; the AN subjects had clusters in the inferior frontal gyri; and the BN subjects showed no activation clusters at all. In **Figure 2**, the BN group showed no differences in the modulation of the ROI for this task contrast, the cc-dACC, with either the CN group [means Physical Self–Friend, CN 0.12, BN 0.04, $t(31) = 1.8$, $p = 0.09$] or the AN group [means, Physical Self–Friend, AN −0.014, BN 0.04, $t(31) = -1.3$]. We also examined percent signal change in vmPFC and DLPFC but found no differences in either region across the three groups [vmPFC, means CN 0.06, AN 0.04, BN −0.04, $F(50) = 0.44$, $p = 0.65$; DLPFC, means CN 0.06, AN 0.00, BN 0.05, $F(50) = 1.35$, $p = 0.27$].

Table 3 | Clusters in the CN, AN, and BN group maps during the Reflected–Self contrast of the social identity task.

Group	Region	Volume	Cluster *P*	Peak *T*	MNI *x, y, z*
CN	Lingual gyrus	151	0.001	8.15	18, −82, −6
CN	Posterior cingulate	1008	0	6.74	8, −24, 28
CN	Middle temporal	135	0.002	6.36	−52, −16, −12
CN	Cuneus	79	0.035	5.83	16, −76, 10
CN	Superior frontal	140	0.002	4.84	−2, 8, 56
AN	Precuneus	137	0.002	6.02	−8, −64, 34
AN	Posterior cingulate	85	0.024	5.44	4, −30, 24
BN	Precuneus	2180	0	12.76	12, −70, 34
BN	Temporoparietal	202	0	8.63	48, −42, 16
BN	Temporoparietal	361	0	6.88	−48, −58, 20
BN	Cingulate	108	0.003	6.31	18, −36, 40

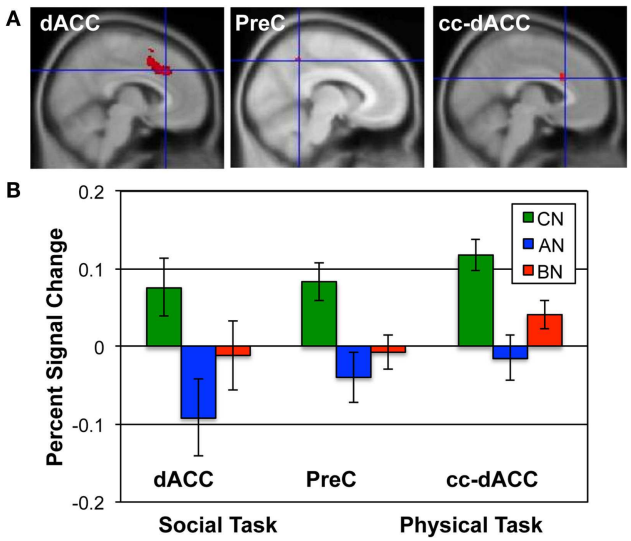

FIGURE 2 | ROIs and the percent signal change in each group for the Social and Physical Identity contrasts. **(A)** The upper row shows the extent of each ROI in red; from left to right, the dACC (MNI $x = 6$; Social Identity Reflected–Self); the precuneus ($x = -8$; Social Identity Self–Friend); and the cc-dACC ($x = -6$; Physical Identity Self–Friend). **(B)** The bar graphs shows the average percent signal modulation within each ROI for the CN (green), AN (blue), and BN (red) groups. Percent signal change was computed for the contrast and task that defined each ROI; the Social Identity task for both the dACC and PreC; the Physical Identity task for the cc-dACC.

DISCUSSION

Neuroimaging work in the last decade has shown that neural regions involved in self-knowledge are often also activated in social cognitive processing, so the same brain regions that enable understanding one's own self may also be involved in understanding others (8, 9, 30). One diagnostic criterion for both AN and BN is related to self-knowledge: body shape or weight having undue influence on self-esteem (1). Additionally, problems related to understanding self and others have long been observed in AN (14, 43, 44). Recently, neural evidence of differences in social processing has been reported in AN subjects (36, 37). Here, we assessed whether BN subjects showed more similarities to AN or CN subjects in their neural activations in response to fMRI tasks requiring social processing.

First, it is worth observing that the psychiatric and demographic data for the subjects with AN and BN were only significantly different from each other in that the AN subjects had a lower body mass index. On all other scales, including measures of self-esteem and social behavior, the two subject groups did not differ from one another. There were two differences in comparisons with the CN group on subscales of the SPSI-R: AN subjects had a higher avoidance style and BN subjects showed less rational-problem solving than the CN subjects, but there were no significant differences on these measures in the direct comparisons of the AN and BN subjects. These results are consistent with studies of clinical and personality characteristics in the literature that have examined both AN and BN subjects: few differences are identified, supporting a theory that similar psychological processes underlie both disorders (45–49). Many self-report and clinical measures of psychiatric symptoms depend both upon a subject's willingness to admit to their symptoms and concerns as well as their ability to recognize and report on their actual symptoms (50). In eating disorders, minimization and denial of symptoms are frequently observed, making psychological and cognitive assessments challenging (51, 52). Neuroimaging data is less likely to be affected by these problems. This study suggests that neural data may provide increased sensitivity for the detection of altered brain function in eating disorders.

Few studies have examined social cognition in BN (19). Interestingly, nearly all of these studies have examined social cognition using facial stimuli, either in a recognition of feelings portrayed by faces (16, 18), an identification of emotions in faces (53, 54), or through an emotional facial Stroop task (48). Amongst these tasks, only the emotional Stroop task, showed strong differences in direct comparisons of BN and CN subjects. Akin to these studies, the neural data from the Social Attribution task showed more similar activation clusters in the BN and CN group maps than in the AN and CN group maps. Notably, both the CN and BN subjects showed significant activations bilaterally in the fusiform face areas in the People–Bumper contrast but the AN group did not have activation in this region. In concert with the numerous behavioral observations of differences related to facial emotion processing in AN (15–17, 55), our neuroimaging data suggest that the neural regions that subserve the processing of facial expressions may be intact in BN but not in the AN (**Figure 1**; **Table 1**).

However, the BN subjects did show less modulation than the CN subjects within the RTPJ, the ROI previously identified as showing differences in the task activations using the whole-brain comparisons of the AN and CN groups. This area has been most consistently associated with TOM across a wide variety of imaging tasks that include imagining human movement, interpreting stories, and viewing complex videos (32, 56–59). Our demonstration of reduced modulation of this region in the BN group suggests that there are similarities in the neural processing of TOM in both types of eating disorders. This finding further highlights the fact that neuroimaging markers for cognitive processes may be more sensitive to measuring certain aspects of processing, as the behavioral studies have not detected mentalization differences in BN subjects.

Bydlowski (60) reported reduced TOM in BN subjects using the Levels of Emotional Awareness Scale (LEAS). This is a TOM task that involves answering questions about one's own emotions and another person's emotional state based on responses to short vignettes, rather than viewing faces or videos. In PET imaging studies, LEAS scores has been positively correlated with emotional arousal in the dACC in healthy people (61–63) but negatively correlated in post-traumatic stress disorder patients (63). Interestingly, the Social Identity mentalization contrast showed an opposite pattern of modulation in the dACC in the AN and BN groups compared to the CN group. In concert with our data, these results suggest that neural differences in the dACC related to social and emotional processing are present in both AN and BN. Interestingly, activations of the dACC are more commonly observed in tasks with personal relevance (30), a condition present in our Identity task mentalization condition but not the Social Attribution task.

Table 4 | Clusters in the CN, AN, and BN group maps during the Self–Friend contrast of the social identity task.

Group	Region	Volume	Cluster *P*	Peak *T*	MNI *x, y, z*
CN	Lingual	915	0	8.7	−4, −94, −4
CN	Lingual	454	0	6.83	8, −82, −4
CN	Occipital	115	0.007	6.13	32, −86, 12
CN	Occipital	290	0	6.11	−24, −68, −18
AN	Occipital	4399	0	8.86	24, −92, −2
AN	Superior parietal	1358	0	8.8	−22, −64, 48
AN	Caudate	95	0.009	7.35	16, 14, −2
AN	Middle frontal	1293	0	7.27	−54, 18, 30
AN	Superior frontal	923	0	7.23	6, 8, 58
AN	Inferior frontal	560	0	6.46	−44, 40, 2
AN	Superior temporal	166	0	6.14	48, 16, −10
AN	Precuneus	539	0	5.97	18, −70, 50
AN	Middle frontal	75	0.03	5.79	36, 32, 28
AN	Inferior parietal	75	0.03	5.71	38, −50, 42
AN	Cerebellum	74	0.032	5.64	10, −76, −44
AN	Inferior frontal	137	0.001	5.04	36, 2, 22
BN	Occipital	839	0	9.3	−12, −92, 14
BN	Parietal	1087	0	8.48	−52, −22, 38
BN	Occipital	979	0	8.13	14, −86, −2
BN	Cingulate	991	0	8.02	4, 8, 64
BN	Precuneus	185	0	7.17	22, −56, 36
BN	Fusiform	216	0	6.46	−36, −62, −18
BN	Precentral	128	0.001	6.46	54, 2, 38
BN	Inferior frontal	482	0	6.33	−56, 12, 22
BN	Precentral	169	0	6.1	48, −16, 42

Table 5 | Clusters in the CN, AN, and BN group maps during the Self–Friend contrast of the physical identity task.

Group	Region	Volume	Cluster *P*	Peak *T*	MNI *x, y, z*
CN	Anterior cingulate	614	0	7.64	−6, 22, 24
CN	Anterior cingulate	104	0.011	6.23	2, 42, 12
CN	Cingulate gyrus	184	0	5.14	6, −8, 34
AN	Inferior frontal	80	0.022	4.84	40, 16, −16
AN	Medial frontal	70	0.04	4.49	−2, 36, 38
AN	Inferior frontal	70	0.04	4.45	−50, 20, −6
BN	No regions				

One of the most intriguing findings relates to the differences seen in both eating disorder patients and healthy people with a mentalization process that is personal (*my friend thinks*. . .) compared to the impersonal task (*People: All friends?*). Very different neural regions are engaged in these two tasks, demonstrating that tasks that separate personal and impersonal mentalization may be important for examination of psychopathology related to social processing. Our neural data implies that the consideration of one's own self may fundamentally alter social cognitive processing. Recognition of the specific neurocognitive demands of both imaging and behavioral tasks may be essential in detecting psychological and biological differences in eating disorders. Further research with more complicated behavioral and neuroimaging tasks that assess personal and impersonal mentalization are warranted in BN.

Stein and Corte (64) described identity as the stable yet evolving set of memory structures relating to one's own experiences, and dissociable into different dimensions, which they referred to as self-schemas. They proposed that in eating disorders, the self-schemas related to emotional and physical understanding of one's own self-state are impaired (64–66). Limitations in assessment of their own emotions are seen in the elevated levels of alexithymia reported in both AN and BN (46, 60, 67–69). Problems in self-esteem are also present and often precede the development of both AN and BN (20–22, 70, 71). The presence of negative self-beliefs unrelated to shape and weight has been proposed as a core component of eating disorders (26, 27).

In Social Identity self-knowledge contrast (Social Self–Friend), the group maps of the AN and BN subjects were very different from the CN subjects. The CN subjects only activated areas in

the occipital and lingual cortex, whereas the AN and BN subjects showed significant activations not only in those areas, but also in frontal, parietal, and temporal regions (**Table 4**). Additionally, we observed reduced activation of the dorsal PreC in AN and BN subjects, the area previously identified with greater modulation in the CN subjects than the AN. Consistent with our findings, reduced modulation of the PreC has been reported in two other imaging tasks in BN. Ashworth and colleagues (72) examined cognitive processing related to social emotional appraisals by asking subjects to remember and match negative facial expressions, and Pringle and colleagues (28) asked subjects to consider whether negative eating and depression words were self-relevant or not. Together with our data, these studies support an idea that PreC activity in response to self-evaluation may be altered in eating disorders. The PreC has connections with temporal, limbic, and parietal regions, suggesting that it serves to integrate current physical and emotional status with prior experiences (73). The reduced modulation of the PreC in the eating disorder subjects observed here implies a reduced connection between physiological state and personal experience, supporting an idea that the psychological processes that mediate identity formation are disrupted in eating disorders.

In the physical self-knowledge contrast, subjects were asked to think about their own physical appearance. This task strongly activated a ventral region of the dACC, immediately adjacent to the corpus callosum in the CN subjects, with little modulation in the AN subjects, and no clusters identified in the BN subjects. In this comparison, BN subjects were not significantly different from either the AN or the CN subjects. The variance of the BN group was nearly twice that of either the AN or CN group for this ROI, supporting an idea that some subjects with BN may have problems activating this cortical area and others may not. For the other ROIs, the variance of the BN group was similar to that of the AN and CN groups. Interestingly, Marsh and colleagues have focused on the neural circuits involved in self-regulation in BN, and also reported differences in the activation of this area of the dACC in BN (74, 75). From a cognitive perspective, the differences in the cc-dACC suggests that some subjects with BN, but not all, think about their current physical or physiological state differently than healthy people. This neural difference may correspond to less information about physiological needs being available in the minds of eating disorder patients, making it easier for these individuals to develop feeding behaviors that are removed from nutritional needs. Future studies may want to focus on whether the activation of the cc-dACC can be related to psychological measures of body shape perception and interoceptive awareness.

Recently, Schulte-Ruther and colleagues (37) used an fMRI task similar to the Social Attribution task to examine whether longitudinal changes in social cognitive regions were associated with weight recovery in adolescents being treated for AN. They observed reduced activation of temporal and medial frontal regions both before and after weight recovery in AN subjects compared to CN subjects. They also reported that stronger modulation of one social cognition region, the MPFC at the start of treatment, was predictive of outcome. We also examined responses in this MPFC ROI, but found no differences in the three subject groups. One major difference in the studies is that all our subjects were at a stable weight when scanned, whereas the earlier study had observed changes in this region related to outcomes following treatment. Nevertheless, the observation that MPFC may be relevant to recovery is particularly exciting when considered in the context of a study by Somerville and colleagues (76) in which healthy people with low self-esteem showed modulation of responses in their vACC and MPFC in response to social feedback whereas people with high self-esteem showed no changes in this region in response to feedback. Low self-esteem has previously been shown to be predictive of the development of eating disorders (20, 22, 25) as well as an indicator of outcome and severity (77, 78). Neural responses within the ACC and MPFC to social feedback may provide a biological mechanism that connects social cognitive responses and self-esteem with eating pathology; understanding how biological factors impact specific patients may lead to improved outcomes by providing more individualized treatments in the future.

Additionally, two earlier studies have identified frontal cortical regions associated with eating disorders and processing verbal stimuli. Pringle and colleagues (28) found differences in dorsolateral PFC in response to negative emotional words considered in the context of oneself, and Miyake et al. (42) has reported differences in vmPFC associated with responses to selecting a negative body image words compared to selecting the most neutral of a random word sets. Although we did not observe differences across our subject groups in these same regions in either the Social Identity task or the Physical Identity task, this is likely related to differences in task design. Our subjects performed Self-appraisals, Friend-appraisals, and Reflected-appraisals, using the same sets of adjectives. As such, neither of the studies showing effects in frontal regions had a comparison situation involving the same stimuli words referenced to a different person. This suggests that the areas we have identified in the dACC, cc-dACC, and PreC may be specific for altered cognitions related to one's own self in eating disorder subjects, whereas frontal activation differences may relate to the cognitions evoked by physical and emotional descriptive terms.

There are a number of limitations to these studies. First, the sample groups were small and as such the study may be underpowered to identify both differences and similarities that are present. Furthermore, the BN group showed more variability in their neural activations than the AN and CN groups, and that variability may warrant collecting a larger group to identify specific differences. Additionally, a larger study could explore the relationships between neural activity and clinical symptoms for both AN and BN. Potentially, neural data may provide an additional tool to assess the severity and symptoms present in a specific patient with an ED.

Another limitation of any single-time point psychiatric study is that the presence of neural differences does not determine whether these differences are a cause or an effect of the disorder. In eating disorders, medical issues are likely to alter brain function. Purging behaviors alter electrolytes, a critical factor for neuronal signaling (79). Restriction leads to nutritional deficiencies and hormonal changes, additional factors that alter brain function (80). Neurodevelopment is critically dependent on myelination (81, 82), and that process may be impacted with the presence of an eating disorder in adolescence and young adulthood. The size of the anterior Cing, a brain region fundamental in self-processing, decreases with the severity of starvation in AN (83). Our studies show differences in neural activations in response to social tasks in patients currently or recently with eating disorders, including both AN and BN.

Differences in neural activations in psychiatric populations may be a result of a variety of processes. They can emerge because of pre-existing biological differences that lead to the disorder, because of the effects of having the disorder such as electrolyte changes, or may merely be a reflection of current psychological differences related to processing stimuli relevant to the disorder. Most commonly, differences are viewed as a biological predisposition to the illness but it is impossible to determine if these differences existed before the eating disorder and if they will still be present following recovery from the eating disorder. Our data show that there are differences in the neural activity that underlies social processing in people with BN. Clinically, this implies that social processing pathways, including TOM and self-knowledge, are engaged differently during an eating disorder. This reinforces choosing treatment models with a focus on issues related to social interaction and function in addition to disordered eating behaviors (84–86). Neural evidence of social processing differences in eating disorders may be important in helping patients accept treatments that appear indirectly related to alteration of eating patterns.

In summary, these experiments examined the neural modulations in response to fMRI tasks focusing on self-knowledge and social cognition in BN. We observed modulation in the BN group that was consistently intermediate between the AN and CN groups. In three ROIs, all of which were activated in CN during MRI tasks involving social evaluation, the BN subjects were significantly different from the CN subjects but not from the AN subjects. This suggests that neural processes that mediate social thinking are similar in AN and BN. Recently, Lavender and colleagues (87) examined outcomes for BN using a group therapy focusing on emotional and social mind training, and found recovery rates similar to more established cognitive behavior therapy. That study, in concert with our neural data, suggest that further exploration of social processing interventions may lead to improved outcomes in BN. One interesting observation in that pilot treatment study was that subjects in the emotional and social training group were more likely to attend sessions, suggesting that this type of treatment may help to engage patients in treatment. The fourth region, cc-dACC, was identified in a contrast of the Physical Identity task. In this area, the BN subjects were not significantly different from either the AN or the CN subjects. This suggests that cognitions surrounding physical appearance may be altered less in BN than in AN, or less consistently altered amongst patients with BN. Overall, our studies demonstrate similarities in neural processing in BN and AN, and suggest that there may be shared biological mechanisms related to processing social concepts that differ systematically from neural modulations seen in healthy CN subjects.

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Altered SPECT ^{123}I-iomazenil Binding in the Cingulate Cortex of Children with Anorexia Nervosa

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Several lines of evidence suggest that anxiety plays a key role in the development and maintenance of anorexia nervosa (AN) in children. The purpose of this study was to examine cortical GABA(A)-benzodiazepine receptor binding before and after treatment in children beginning intensive AN treatment. Brain single-photon emission computed tomography (SPECT) measurements using ^{123}I-iomazenil, which binds to GABA(A)-benzodiazepine receptors, was performed in 26 participants with AN who were enrolled in a multimodal treatment program. Sixteen of the 26 participants underwent a repeat SPECT scan immediately before discharge at conclusion of the intensive treatment program. Eating behavior and mood disturbances were assessed using Eating Attitudes Test with 26 items (EAT-26) and the short form of the Profile of Mood States (POMS). Clinical outcome scores were evaluated after a 1-year period. We examined association between relative iomazenil-binding activity in cortical regions of interest and psychometric profiles and determined which psychometric profiles show interaction effects with brain regions. Further, we determined if binding activity could predict clinical outcome and treatment changes. Higher EAT-26 scores were significantly associated with lower iomazenil-binding activity in the anterior and posterior cingulate cortex. Higher POMS subscale scores were significantly associated with lower iomazenil-binding activity in the left frontal, parietal cortex, and posterior cingulate cortex (PCC). "Depression–Dejection" and "Confusion" POMS subscale scores, and total POMS score showed interaction effects with brain regions in iomazenil-binding activity. Decreased binding in the anterior cingulate cortex and left parietal cortex was associated with poor clinical outcomes. Relative binding increases throughout the PCC and occipital gyrus were observed after weight gain in children with AN. These findings suggest that cortical GABAergic receptor binding is altered in children with AN. This may be a state-related change, which could be used to monitor and guide the treatment of eating disorders.

Keywords: anorexia nervosa, cingulate cortex, GABA, children, iomazenil SPECT

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INTRODUCTION

Anorexia nervosa (AN) typically presents in females during adolescence. It is a serious psychiatric illness conferring substantial morbidity and mortality, which manifests as disturbances in eating habits, excessive preoccupation with weight, restricted caloric intake, and body image distortion (1). Although some research regarding the outcome of childhood AN is encouraging in terms of mortality and recovery from AN (2), long-term comorbid psychiatric disorders, such as anxiety disorders and affective disorders, represent unfavorable prognostic factors (3). Anxiety is present in the majority of children with AN prior to abnormal eating or body image distortions (4). Anxiety in children with AN is also associated with decreased body mass index (BMI) (5, 6). Moreover, trait anxiety scales in children show significant positive correlations with eating disorder psychopathology such as "drive for thinness," "body dissatisfaction," and "perfectionism" (5).

Several lines of evidence implicate gamma-aminobutyric acid (GABA)ergic neurotransmission in the pathophysiology of anxiety (7). Recently, a large-scale candidate gene study found that allele frequency differences in the GABA receptor SNP, *GABRG1*, are related to levels of trait anxiety in AN and bulimia nervosa (8). Furthermore, elevated GABA(A) receptor levels in the amygdala were reported in activity-based anorexia (ABA), an animal model of the behavioral phenotype of AN (9). Upregulated GABA(A) receptor function may be associated with anxiety in ABA animals. Several neuroimaging studies have shown negative correlations between GABA-benzodiazepine receptor binding activity and severity of anxiety symptoms in adults with panic or traumatic disorders (10, 11). However, to date, no study has examined GABA(A) receptor binding or function in AN. It is possible that GABAergic neurons may play an important role in both premorbid anxiety of AN and the pathogenesis of childhood AN.

Single-photon emission computed tomography (SPECT) is a nuclear medicine tomographic modality employing gamma rays, and in which, injected radionuclides are attached to ligands selective for specific receptors of interest. ^{123}I-iomazenil is a radioactive ligand for central-type benzodiazepine receptors, which form a complex with GABA(A) receptors. Thus, ^{123}I-iomazenil SPECT measures GABA(A) receptor binding and indirectly assays GABA(A) receptor function. ^{123}I-iomazenil is a frequently used radionuclide tracer for presurgical evaluation of patients with refractory partial epilepsy (12). Moreover, recent neuroimaging studies have explored the role of GABAergic inhibitory function in psychiatric disorders, such as schizophrenia, Alzheimer's disease, and developmental disorders, as well as anxiety disorders including panic and traumatic stress disorders (10, 11, 13–17). In these reports, significant correlations between GABAergic function and dimensional scales measuring anxiety, panic, negative cognitions, and psychiatric status were found.

The aims of this study were to (1) determine if GABA(A) receptor binding is associated with AN symptoms and anxiety in children initiating clinical treatment for AN; (2) determine which brain regions are involved; (3) determine if measures of GABA(A) receptor binding can predict a participant's clinical outcome; and (4) determine if these measures change with successful treatment. We hypothesized that lower cortical iomazenil-binding activity is associated with greater baseline symptom severity and poor clinical outcome in children with AN.

MATERIALS AND METHODS

Participants

The study complies with the Declaration of Helsinki and informed consent was obtained from participants and parents or legal guardians prior to enrollment in the imaging study. The procedures for assent, informed consent, and study design were approved by the Medical Ethical Committee of Kurume University School of Medicine. Twenty-six female participants were recruited who fulfilled the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) criteria for AN, and had been admitted to the Department of Pediatrics, Kurume University School of Medicine between 2007 and 2012 for clinical treatment in an eating disorders program. All were restricted-type AN. The flow diagram for the study is shown in **Figure 1**. The eating disorder treatment program has a multimodal approach, which includes parenteral nutrition, psychotherapy, and behavioral intervention. On initiation of treatment, participants and families have extensive psychoeducation focused on major physical risk factors associated with restricted body weight and therapeutic goals for hospitalization. Individual behavior therapy with reward reinforcement is used to facilitate recovery. Although oral feeding was sufficient for the majority of participants, parenteral nutrition was implemented for select participants with severe AN. Behavioral therapy was combined with nutritional counseling and individual psychotherapy to target difficult emotions and family relational

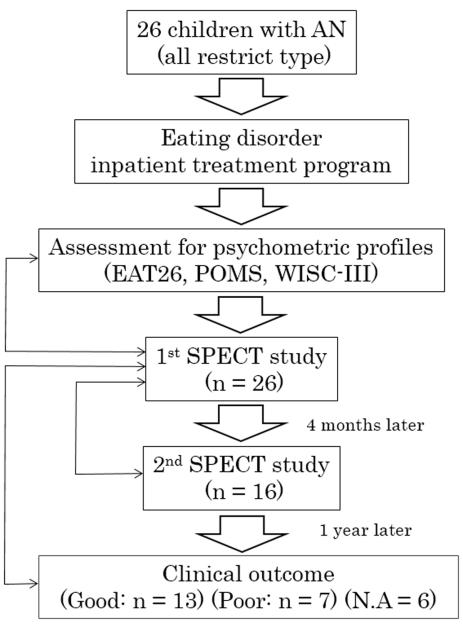

FIGURE 1 | Flow diagram of the study. AN, anorexia nervosa; EAT26, Eating Attitude Test with 26 items; POMS, Profile of Mood States; WISC-III, Wechsler Intelligence Scale for Children; SPECT, single-photon emission computed tomography; N.A., not available. Bidirectional thin arrows indicate statistical comparisons or correlation analyses between objects.

stress. As part of the treatment, participants completed the Eating Attitude Test (EAT-26), a standardized, self-report measure of eating disorder symptoms, which is widely used for screening and measurement of symptoms and characteristics of eating disorders (18). Participants rated their mood using the short form of the Profile of Mood States (POMS), a validated measure that consists of 30 items describing six moods: "Tension–Anxiety," "Depression–Dejection," "Anger–Hostility," "Vigor," "Fatigue', and "Confusion" (19). High Vigor scores reflect a good mood or emotion, and low scores in the other subscales reflect a good mood or emotion. Total mood disturbance (TMD) was obtained by subtraction of the Vigor score from the sum of Tension–Anxiety, Depression–Dejection, Anger–Hostility, Fatigue, and Confusion scores. Each original POMS score was converted to a *T*-score (20). We selected the POMS for measurement of anxiety, as we have neither a Japanese version of State-Trait Anxiety Inventory for Children (STAIC) nor other validated Japanese psychometric scales for anxiety. Upon enrollment in the study, a diagnosis of AN and comorbidities was confirmed in all participants by semi-structured interviews using the Mini-International Neuropsychiatric Interview (MINI) (21), which were performed by two psychiatrists (Michiko Matsuoka and Hiromi Chiba). All participants underwent cognitive assessment using the Wechsler Intelligence Scale for Children (WISC-III). Psychometric profiles were performed before treatment. Participants were medication naïve and did not receive pharmacological treatment during the course of the study. Twenty-three participants had secondary amenorrhea and three had not yet reached menarche. In all participants, brain magnetic resonance imaging (MRI) examination was performed on admission to identify any structural abnormalities, e.g., regional brain atrophy. Participants with severe, co-occurring medical illnesses (such as superior mesenteric artery syndrome) were excluded. Ethical concern regarding the use of ionizing radiation in healthy children precluded the enrollment of a control group for this study.

Clinical Outcome Measures

Follow-up clinical assessments were performed 1 year after hospital discharge by one pediatrician (Shinichiro Nagamitsu) and two psychiatrists (Michiko Matsuoka and Hiromi Chiba). A structured approach was used to define clinical outcome *a priori*. Clinical outcome score was based on eight items, as defined in prior work (22). This included weight change, menstrual status, abnormal eating behavior, body image, binge eating or purging behaviors, insight, school attendance, and quality of family relationship. Improved or impaired answers were scored "0" and "1," respectively. For items of weight change and school attendance, improved and unimproved ratings were scored "0" and "2," respectively. The middle score "1" indicates "unchanged condition." The outcome was considered good with total scores less than "4" and poor with total scores of "4" or over. The clinical outcome raters (Shinichiro Nagamitsu, Michiko Matsuoka, and Hiromi Chiba) were blinded regarding SPECT data.

Iomazenil SPECT

All 26 children underwent brain imaging using SPECT. The first ^{123}I-iomazenil SPECT examination was performed before treatment and the second one immediately before discharge (16 of 26 participants). Mean duration between the first and second SPECT examinations was approximately 4 months. Briefly, participants were injected intravenously with a bolus of 95–117 MBq ^{123}I-iomazenil (Nihon Medi-Physics Co., Tokyo, Japan), which binds with high affinity to the GABA(A)-benzodiazepine receptor. The SPECT scan was performed 3 h after injection of the tracer without any sedation, using a large field-of-view dual-detector camera and a computer system equipped with a low-energy, high-resolution, and parallel-hole collimator. The dual detector camera rotated over 180° in a circular orbit and in 32 steps of 40 s each to cover 360° in approximately 22 min.

Image and Statistical Analyses

Images of ^{123}I-iomazenil scintigraphs were analyzed by three-dimensional stereotactic surface projections (3D-SSP) using iSSP3 software (Nihon Medi-Physics Co.). Stereotactic anatomical standardization was performed as described previously (23). Briefly, rotational correction of the SPECT data set and three-dimensional centering were performed, followed by realignment to the anterior commissure–posterior commissure line. Differences in individual brain size were accounted for by linear scaling and regional anatomical differences minimized using a non-linear warping technique (24). Consequently, each brain was anatomically standardized to match a standard atlas brain. Brain MRI was performed using a superconducting magnet operating at 1.5 T. For coregistered SPECT and MRI analysis, a method of image integration was applied using Fusion Viewer software (Nihon Medi-Physics Co.) with a registration algorithm based on maximum mutual information (**Figure 2**). Subsequently, cortical and subcortical regions of interest (ROIs) in the acquired SPECT

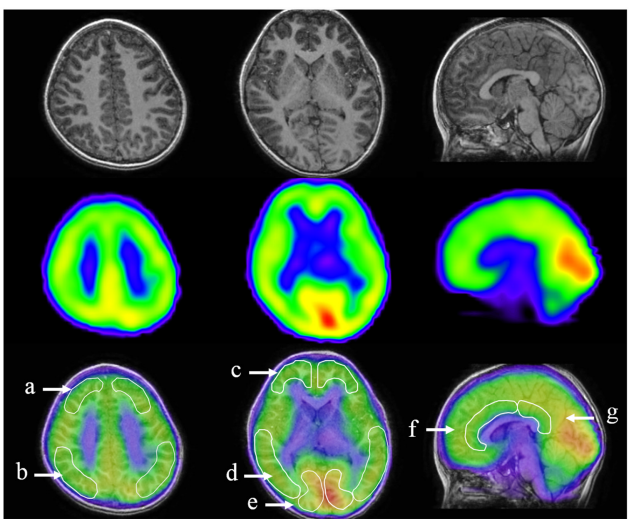

FIGURE 2 | Designated regions of interest (ROIs) in fusion images of ^{123}I-iomazenil SPECT and MRI. The top panel shows brain MRI (transverse and sagittal T_1 sequences), the middle panel the corresponding results of ^{123}I-iomazenil SPECT, and the bottom panel fusion imaging. Outlined regions in the bottom panel indicate designated ROIs, namely, **(a)** the superior frontal, **(b)** parietal, **(c)** frontal, **(d)** middle temporal, and **(e)** occipital regions; **(f)** anterior and **(g)** posterior cingulate gyrus.

data were defined. Using elliptical templates, ROIs were drawn manually for the major cortical and subcortical brain regions in a representative subject. To eliminate the disadvantage of lower reliability with manual operations, the same elliptical templates were used to define ROIs in other subjects. ROIs were placed over the following regions: superior frontal, middle frontal, parietal, middle temporal, and occipital regions; the cerebellum in each hemisphere; and the anterior and posterior cingulate cortex (ACC and PCC, respectively; **Figure 2**). Two neuroradiologists (Hitoshi Tanigawa and Masatoshi Ishibashi), blinded to clinical symptoms, independently drew ROIs. Each relative iomazenil-binding activity in ROIs was expressed as a ratio of that in the cerebellum, as patients with AN have no cerebellar symptoms. Spearman's correlation was used to determine correlations between relative iomazenil binding in each region on baseline SPECT scan and age, BMI-standard deviation score (BMI-SDS), EAT-26, and POMS subscale score. To test for possible differential relationships between POMS and iomazenil-binding activity in brain regions, the brain regions were classified into three groups: center region, left hemisphere region, and right hemisphere region. ROIs were grouped accordingly: ACC and PCC as the center region; left of superior frontal, parietal, frontal, temporal, and occipital as the left hemisphere region; and right of superior frontal, parietal, frontal, temporal, and occipital as the right hemisphere region. POMS subscales were separately analyzed using the mixed-effect model (SAS 9.3 PROC MIXED). Regions, POMS subscale, and their interactions were treated as fixed effects in the model, while the intercept was treated as a random effect, therefore accounting for correlations among iomazenil-binding activities. When the interaction between all three regions and POMS subscale was significant, ROIs were analyzed within the region and POMS using mixed model regression to determine significances between ROIs. The Mann–Whitney *U* test was used to compare between participants with good and poor outcomes.

NeurologicalStatisticalImageAnalysissoftware(NEUROSTAT, Stat_1tZ), which can perform a paired *t* test between two corresponding groups using cross-sectional images, was adopted to examine changes in iomazenil binding between the first and second SPECT. Statistical significance was set at *Z*-score >2, a level commonly used to discriminate abnormalities. The regions identified were transformed into three-dimensional anatomical data and Talairach coordinates to show brain landmarks.

RESULTS

Participants' Characteristics

The mean and SD of age before treatment was 14.1 (1.3) years of age (range, 10.5–15.6 years of age) (**Table 1**). Mean (SD) BMI before and after treatment were 13.7 (2.0) and 15.7 (0.9), respectively. Mean (SD) BMI-SDS before and after treatment were −3.7 (1.9) and −2.3 (1.1), respectively. Mean (SD) EAT-26 score before therapy was 22.4 (12.0), which was higher compared with the reference control value (25). Five participants had co-occurring psychiatric disorders including two with autism spectrum disorders, one with learning disability, and two with selective mutism. Mean (SD) scores for each of the POMS subscales were 46.1 (10.3) for Tension–Anxiety, 53.1 (11.8) for Depression–Dejection, 50.3 (11.9) for Anger–Hostility, 43.4 (11.8) for Vigor, 47.4 (11.4) for Fatigue, and 52.1 (15.5) for Confusion. Mean (SD) score for TMD of POMS was 205.6 (59.4). The subscale scores of Tension–Anxiety and Depression–Dejection were higher than normal ranges (26), but the differences did not reach significance. Mean (SD) IQ was 105 (13). None of the participants showed regional brain atrophy on brain MRI examination. Mean duration of hospitalization was approximately 4 months. Mean (SD) age on the second SPECT examination was 14.8 (1.1). The range of duration between the first and second SPECT was from 86 to 250 days (mean, 128 days).

TABLE 1 | Clinical characteristics and POMS scores for subjects with relative iomazenil-binding activity in each brain region.

	Before treatment (1st SPCET)		After treatment (2nd SPCET)
	All subjects	Subjects having 2nd SPECT	
N	26	16	16
Age	14.1 ± 1.3	14.4 ± 1.1	14.8 ± 1.1
BMI	13.7 ± 2.0	13.3 ± 1.6	15.7 ± 0.9
BMI-SDS	−3.7 ± 1.9	−4.1 ± 1.9	−2.3 ± 1.1
EAT-26	22.4 ± 12.0		
Menstrual cycle			
Not experienced	2		
Secondary amenorrhea	22		
POMS			
Tension–Anxiety	46.1 ± 10.3		
Depression–Dejection	53.1 ± 11.8		
Anger–Hostility	50.3 ± 11.9		
Vigor	43.4 ± 11.8		
Fatigue	47.4 ± 11.4		
Confusion	52.1 ± 15.5		
Total score	205.6 ± 59.4		
WISC-III	105 ± 13		
Relative iomazenil-binding activities			
R superior frontal	1.41 ± 0.11	1.40 ± 0.11	1.45 ± 0.12
L superior frontal	1.44 ± 0.15	1.45 ± 0.13	1.48 ± 0.16
R parietal	1.46 ± 0.14	1.48 ± 0.14	1.50 ± 0.14
L parietal	1.51 ± 0.16	1.55 ± 0.15	1.53 ± 0.13
R middle frontal	1.41 ± 0.11	1.41 ± 0.11	1.46 ± 0.10
L middle frontal	1.45 ± 0.14	1.47 ± 0.14	1.52 ± 0.12
R middle temporal	1.46 ± 0.12	1.46 ± 0.12	1.54 ± 0.12*
L middle temporal	1.44 ± 0.14	1.47 ± 0.14	1.54 ± 0.11*,†
R occipital	1.74 ± 0.14	1.78 ± 0.14	1.88 ± 0.26*
L occipital	1.77 ± 0.20	1.79 ± 0.19	1.88 ± 0.21‡
Anterior cingulate	1.44 ± 0.13	1.47 ± 0.12	1.59 ± 0.24*,†
Posterior cingulate	1.65 ± 0.17	1.69 ± 0.16	1.80 ± 0.33‡

SPECT, single-photon emission computed tomography; BMI, body mass index; EAT-26, Eating Attitude Test with 26 items; POMS, Profile of Mood States; WISC-III, Wechsler Intelligence Scale for Children; R, right; L, left.
**Significant difference compared with all subjects (P < 0.05).*
†Significant difference compared with subjects at second SPECT (P < 0.05).
‡Trend toward significance in all subjects (P < 0.1).

Participants' Outcome

Clinical outcome scores 1 year after treatment were examined in 20 out of 26 participants. Three participants did not complete the

treatment. It was not possible to examine three other participants, as two were transferred to locked psychiatric units and one was transferred to a local hospital. After the evaluations, 13 participants were classified as having a good outcome and 7 with a poor outcome.

Baseline Correlations between ^{123}I-iomazenil Binding and Clinical Measures

Relative iomazenil-binding activities in each brain region are summarized in **Table 1**. There were significant associations between some clinical measures and relative iomazenil-binding activity in several brain regions. Higher EAT-26 scores were significantly associated with lower iomazenil-binding activity in the ACC (**Table 2**). Higher "Tension–Anxiety" score at the beginning of therapy was significantly associated with lower iomazenil-binding activity in the left superior frontal, parietal, middle frontal cortex, and PCC (**Table 2**). Higher "Anger–Hostility," "Confusion," and "Total" scores at the beginning of therapy were significantly associated with lower iomazenil-binding activity in the same regions and left occipital cortex (**Table 2**). Furthermore, "Depression–Dejection" and "Fatigue" scores were also significantly associated with lower iomazenil-binding activity in the PCC (**Table 2**). There were no associations between BMI-SDS and iomazenil-binding activity in any brain region. However, there were significant positive correlations between age and iomazenil-binding activity in the left and right middle frontal, left parietal, and PCC ($r = 0.415$, 0.392, 0.430, and 0.454, respectively, $P < 0.05$) (data not shown).

Interactions between POMS Subscales and Brain Regions in Iomazenil-Binding Activity

The mixed-effect model detected significant interaction effects between three main brain regions and POMS total scale and subscales of "Depression–Dejection" and "Confusion" (**Table 3**). In the three main brain regions, significant differences were identified between the left and right hemisphere regions on POMS total score and subscales of "Depression–Dejection" and "Confusion" (**Table 4**). Furthermore, significant differences between left and right hemisphere regions were identified in the superior frontal region on POMS total score ($t = -2.63$, $P = 0.0094$), superior frontal and occipital regions on the subscale of "Confusion" ($t = -3.16$, $P = 0.0018$; $t = -3.49$, $P = 0.0062$, respectively), and superior frontal region on the subscale of "Depression–Dejection" ($t = -2.75$, $P = 0.0065$). This finding remained significant after Bonferroni correction for multiple comparisons ($P = 0.01$).

Comparison of ^{123}I-iomazenil-Binding Activity Before and After Treatment

Relative iomazenil-binding activity after treatment was significantly increased in the ACC, right occipital, and bilateral middle temporal gyrus (**Table 1**). Comparisons of adjusted iomazenil-binding activity before and after weight gain were examined in the same 16 participants using NEUROSTAT. There were significant increases in iomazenil-binding activity after treatment in the ACC, PCC, frontal gyrus, occipital gyrus, and hippocampus (**Figure 3**). By contrast, there was a significant decrease in iomazenil-binding activity after treatment in the bilateral inferior temporal cortex (data not shown). The Talairach coordinates of sites with Z-scores >3.0 are listed with the associated brain regions in **Table 5**.

Association between ^{123}I-iomazenil-Binding Activity in the ACC and Clinical Outcome

There was a significant baseline difference in iomazenil-binding activity between participants with good and poor clinical outcome scores in the ACC (1.48 ± 0.09 vs. 1.32 ± 0.12, respectively, $P < 0.05$) (**Figure 4**) and left parietal gyrus (1.57 ± 0.12 vs. 1.40 ± 0.15, respectively, $P < 0.05$). Relative baseline iomazenil-binding activity in the ACC and left parietal gyrus in participants with a poor clinical outcome were significantly lower than those with a good clinical outcome.

DISCUSSION

To our knowledge, this is the first investigation on SPECT ^{123}I-iomazenil brain imaging in children with AN. Using SPECT

TABLE 2 | Correlation coefficients between POMS subscale scores, EAT-26, and iomazenil-binding activity in each brain region from the first SPECT.

	TA	D	AH	V	F	C	Total	EAT-26
R superior frontal	0.036	0.290	−0.034	−0.014	0.161	0.097	0.116	−0.203
L superior frontal	−0.473*	−0.428*	−0.494*	0.155	−0.332	−0.646**	−0.530*	−0.338
R parietal	0.022	0.312	−0.037	−0.057	0.019	−0.001	0.073	−0.100
L parietal	−0.413*	−0.250	−0.414*	−0.029	−0.314	−0.604**	−0.417*	−0.338
R frontal	−0.213	0.057	−0.179	−0.073	0.054	−0.094	−0.061	−0.431
L frontal	−0.498*	−0.320	−0.468*	0.100	−0.288	−0.598**	−0.476*	−0.390
R temporal	−0.095	0.226	−0.241	−0.144	0.015	−0.009	0.009	−0.151
L temporal	−0.210	−0.130	−0.367	−0.040	−0.101	−0.374	−0.245	−0.141
R occipital	−0.175	0.003	−0.356	−0.111	−0.162	−0.222	−0.168	−0.109
L occipital	−0.0355	−0.378	−0.510*	0.027	−0.288	−0.589**	−0.454*	−0.097
Anterior cingulate	−0.043	−0.008	−0.034	−0.277	−0.016	−0.156	−0.005	−0.606**
Posterior cingulate	−0.553*	−0497*	−0.476*	0.262	−0.440*	−0.641**	−0.594*	−0.312

POMS, Profile of Mood States; TA, Tension–Anxiety; D, Depression–Dejection; AH, Anger–Hostility; V, Vigor; F, Fatigue; C, Confusion; EAT-26, Eating Attitude Test with 26 items.
**P < 0.05 and **P < 0.01 indicate significant correlations.*

^{123}I-iomazenil brain imaging, we found association between cortical GABAergic receptor binding and clinical manifestations of childhood AN. Higher EAT-26 and mood disturbance scores were significantly associated with lower GABAergic inhibitory binding in various brain regions. Poor clinical outcome was also associated with lower GABAergic receptor binding in the ACC and left parietal region. GABAergic receptor binding was mainly activated in the ACC, PCC, frontal gyrus, and occipital gyrus after treatment.

TABLE 3 | Interaction effects between POMS subscales and three main regions (center, left, and right hemispheres).

POMS subscales	df (between regions)	df (within regions)	*F*	*P*-value
POMS total	2	194	3.52	<0.05*
Tension–Anxiety	2	194	2.07	0.13
Depression–Dejection	2	194	4.09	<0.05*
Anger–Hostility	2	194	2.16	0.12
Vigor	2	194	0.22	0.8
Fatigue	2	193	1.49	0.23
Confusion	2	194	5.65	<0.01*

POMS, Profile of Mood States.
**Indicates significance.*

TABLE 4 | Interaction between three regions and POMS subscales.

	POMS total		Confusion		Depression–dejection	
	t	*P*-value	*t*	*P*-value	*t*	*P*-value
Center–left	0.88	0.38	−1.22	0.22	−0.5	0.62
Center–right	−1.12	0.27	−1.32	0.19	−1.61	0.11
Left–right	2.65	0.0087*	3.36	<0.001*	2.79	0.0057*

**indicates significance.*

We found significant correlation between reduced GABAergic receptor binding in various brain regions and mood disturbances, as assessed using POMS subscales. Mixed model regression showed significant effects for the interactions between brain regions and POMS total scale and subscales of "Depression–Dejection" and "Confusion." Furthermore, the effect of these POMS profiles showed significantly different binding activities between the left and right hemispheres, especially in the superior frontal region. These results indicate that GABAergic neuronal activity correlates to mood disturbances in children with AN. The potential involvement of GABAergic neurotransmission in the pathophysiology of AN was recently investigated by genetic allele frequency analysis of *GABRG1* in AN patients. This study showed significant correlation between specific allele frequency in this GABA receptor SNP and levels of trait anxiety in the patients (8). Further, in an animal model of AN, elevated GABA(A) receptor expression in the amygdala was associated with increased anxiety (9). It remains unclear whether GABA(A) receptor function is associated with the underlying pathophysiology of childhood AN or a result of long-term starvation. However, a relative strength of our present findings is that at repeat SPECT scan, the participants were not completely weight-restored [mean BMI 15.7 (0.9 SD)], suggesting that changes in brain ^{123}I-iomazenil binding may be related to clinical improvement rather than mere weight restoration. Nonetheless, this does suggest that GABAergic receptor-mediated inhibitory function may be associated with mood disturbances in children with AN.

We also found a significant negative correlation between ^{123}I-iomazenil-binding activity in the ACC and abnormal eating attitude described by EAT-26 score, with a greater decrease in activity in the ACC of AN children with poor clinical outcomes,

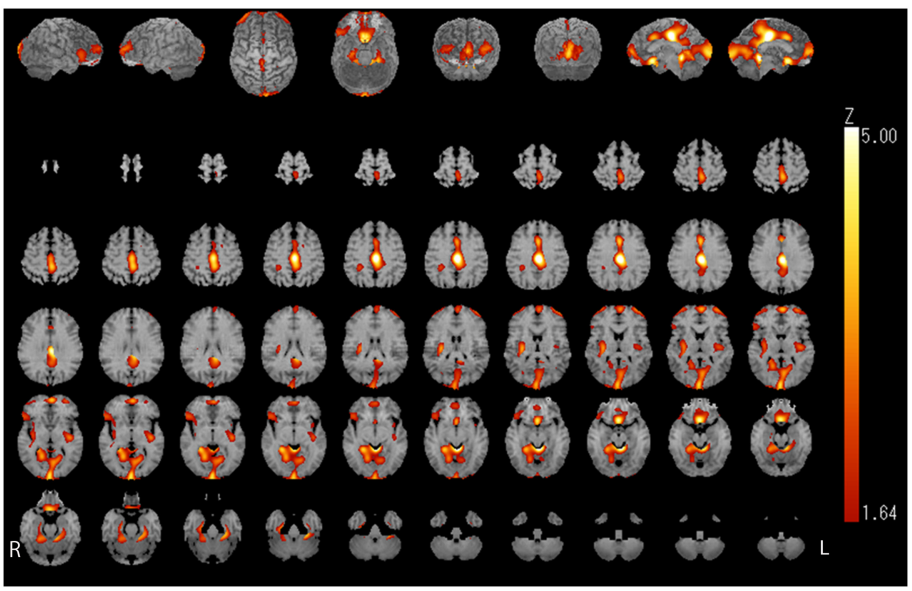

FIGURE 3 | Image analysis (1tZ) of increased iomazenil-binding changes before and after weight gain in the brain of children with AN. Significant increases in iomazenil-binding activity before and after weight gain are shown in the anterior and posterior cingulate cortex, occipital cortex, frontal cortex, and hippocampus, as indicated by the bright orange color.

TABLE 5 | Brain regions and Talairach coordinates showing significantly increased and decreased iomazenil binding in children with anorexia nervosa before and after weight gain.

Regions	Talairach coordinates				*P*-value
	X	*Y*	*Z*	*Z* score	
Increased regions					
Right anterior cingulate gyrus	3	−19	36	6.33	<0.00001
Right occipital gyrus	17	−103	−4	5.43	<0.00001
Left medial frontal gyrus	−1	19	−14	4.89	<0.00001
Left occipital gyrus	−1	−94	2	4.31	<0.00001
Right posterior cingulate gyrus	6	−40	22	3.99	<0.00005
Right parahippocampal gyrus	26	−31	−25	3.84	<0.0001
Right medial frontal gyrus	3	29	4	3.84	<0.0001
Decreased regions					
Right inferior temporal gyrus	55	−24	−9	4.28	<0.00001
Left inferior temporal gyrus	−53	−19	−14	3.05	<0.01

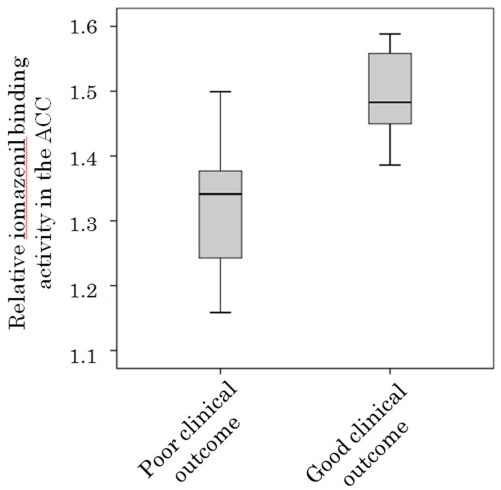

FIGURE 4 | Relative iomazenil-binding activity in the anterior cingulate gyrus in participants with different clinical outcomes. Relative iomazenil-binding activity in the anterior cingulate gyrus at initiation of treatment in participants with good clinical outcomes was significantly higher than those with poor clinical outcomes.

compared with those with good clinical outcomes. Furthermore, binding activity in the ACC was significantly increased after treatment. As the present clinical outcome score was composed of current BMI and presence of menstruation, as well as changes of eating behavior and social interaction, GABAergic functional activity in the ACC may be related to biological vulnerability of recovery from AN symptoms. Converging lines of evidence suggest correlations between morphological or functional neural changes and differential clinical outcomes in AN. For example, McCormick et al. (27) reported that although the dorsal ACC gray matter volume is significantly reduced in patients with AN compared with normal controls, greater normalization of the right dorsal ACC volume following weight restoration prospectively predicted sustained remission at 1 year post-hospitalization. Functional MRI studies have shown that increased activation in the dorsal ACC and prefrontal cortex in response to food stimuli differentiates recovered AN patients from chronically ill AN patients (28). Further, subcallosal cingulate deep brain stimulation has recently been applied as a treatment strategy for treatment-refractory AN and associated with improvement in mood, anxiety, affective regulation, and increased BMI (29). Taken together, our findings contribute to emerging evidence that variations in functional activities of the ACC may be predictors of outcomes of AN.

Similar to the ACC, neural activities in the PCC may play important roles in the pathophysiology of AN. The PCC is functionally coupled with other brain regions as a default mode network and involved in self-related aspects of cognitive processing such as self-reference and self-reflection (30). Functional brain imaging suggests that dysfunction in resting-state functional connectivity in regions involved in self-referential processing might be associated with development of AN (31). Further, several lines of evidence show that less activation in the PCC is associated with altered inhibitory processing, which might represent a behavioral characteristic and impairment of emotional processing in AN (32, 33). In the present study, several higher mood disturbance scores were significantly associated with lower GABAergic inhibitory binding, mainly in the PCC. Similarly, the PCC was one of the brain regions in which iomazenil-binding activity increased after treatment. In a previous neuroimaging study in children with AN, increased cerebral blood flow (CBF) was observed in the parietal cortex and PCC after inpatient treatment (34). Taken together, increased GABAergic inhibitory function in the PCC after weight gain in our study might indicate improved self-referential processing and cognitive control, which were missing during their starvation period.

We found evidence of increased ^{123}I-iomazenil-binding activity in the occipital cortex after treatment in children with AN. In general, iomazenil-binding activity is strongest in the occipital cortex, indicating that GABA receptors are densely distributed in this area. GABA is involved in interocular suppression in the visual cortex and plays a central role in determining visual cortex selectivity (35). As the brain has a limited capacity, attention allows relevant incoming information to be selectively enhanced while suppressing irrelevant information, the processing for which may be modulated by GABAergic inhibitory function (36, 37). A recent MR spectroscopy study revealed negative correlation between the amount of occipital GABA and cognitive failure in healthy patients, indicating that the inhibitory capacity in sensory areas affects their ability to ignore information that is irrelevant to current behavioral goals (37). In AN patients, cognitive deficits in impaired visuospatial ability, impaired complex visual memory, and impaired selective attention have been reported (38, 39). As GABA plays an important part in stimulus processing and suppression in sensory areas, increased GABAergic inhibitory activation observed in the occipital cortex in AN participants in our study may reflect enhanced suppression of visual information processing, possibly resulting in the improvement of cognitive deficits.

The present study has several limitations that require consideration in future studies. First, brain imaging data from normal healthy children are not available because ethics approval was not feasible for SPECT studies in healthy control children. Therefore,

we focused our research on the correlation between GABAergic inhibitory function in brain regions and psychometric profiles, and changes in these functions before and after treatment in AN participants. Thus, we could not determine if the basic GABAergic inhibitory function is upregulated or downregulated, compared to healthy controls. Second, it is possible that changes in iomazenil binding after treatment might be associated with confounding effects, secondary to starvation and restored body weight. However, as the average period for repeat SPECT was short (4 months), weight restoration was not complete. Furthermore, our previous neuroimaging report regarding CBF changes after treatment in AN patients showed no global increase in CBF changes except specific brain regions (bilateral parietal lobe and PCC) (34). Consequently, confounding effects are unlikely to be a cause of our SPECT findings. Third, SPECT exhibits poor resolution around some limbic regions, such as the amygdala and hippocampus, which are important for emotion processing. In these small regions, the obtained radioactivity might differ from the true activity because of a partial volume effect (PVE). The PVE can be defined as underestimation of binding per unit brain volume in small objects or regions because of blurring of radioactivity (spill-out and spill-in) between regions (40, 41). These regions need to be resolved using MR imaging-based correction for PVE. Fourth, we did not examine the participants' psychometric profiles at the end of hospitalization. Although the majority of participants obtained proper eating habits, attended school, and improved parental relationships by the discharge period, these socio-emotional behaviors were not evaluated using the psychometric profile. To confirm our understanding of altered ^{123}I-iomazenil-binding activity due to therapeutic intervention, improvement of socio-emotional difficulties should be consecutively measured.

In conclusion, GABAergic inhibitory receptor function in the brain may play an important role in manifesting clinical symptoms of childhood AN. Lower GABAergic ^{123}I-iomazenil binding in specific brain regions at initiation of treatment is associated with clinical severity of mood disturbances and abnormal eating attitude in this sample of participants. Decreased binding in the ACC and left parietal cortex were associated with poor clinical outcomes. Conversely, increased changes in GABAergic receptor binding in the ACC, PCC, and occipital gyrus might be important for the recovery process of childhood AN. Although GABAergic function in the cingulate cortex might be evaluated as a potential predictor of clinical outcome, it will be important to determine the association between function and long-term prognosis. Further research focused on GABAergic receptor-mediated function among participants with eating disorders is warranted.

AUTHOR CONTRIBUTIONS

SN participated in the design of this study and compiled the manuscript. SN, MM, HC, SO, and YY saw the patients and obtained informed consent and their agreement to participate in the study. Three radiologists (HT, HK, and MI) were in charge of radioactive measurements and calculations of iomazenil activity using ROIs. RS and TK, statistician, conducted the statistical analyses. PC and TM supervised the preparation of the manuscript.

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Assessing and stabilizing aberrant neuroplasticity in autism spectrum disorder: the potential role of transcranial magnetic stimulation

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Exciting developments have taken place in the neuroscience research in autism spectrum disorder (ASD), and results from these studies indicate that brain in ASD is associated with aberrant neuroplasticity. Transcranial magnetic stimulation (TMS) has rapidly evolved to become a widely used, safe, and non-invasive neuroscientific tool to investigate a variety of neurophysiological processes, including neuroplasticity. The diagnostic and therapeutic potential of TMS in ASD is beginning to be realized. In this article, we briefly reviewed evidence of aberrant neuroplasticity in ASD, suggested future directions in assessing neuroplasticity using repetitive TMS (rTMS), and discussed the potential of rTMS in rectifying aberrant neuroplasticity in ASD.

Keywords: autism spectrum disorder, transcranial magnetic stimulation, neuroplasticity, EEG, treatment

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by persistent deficits in social communication and interaction and stereotyped behaviors, interests, and activities [*Diagnostic and Statistical Manual of Mental Disorders*, 5th Edition (DSM-5)] (1). The most recent US Centers for Disease Control and Prevention data estimate that ASD now affects 1 in 68 children (2). These data establish ASD as the most common neurodevelopmental disorder. Thus, the social, clinical, and economic burden of ASD is tremendous.

Since the turn of the century, significant advancements have been made in ASD research, and a range of macro- and micro-structural, neurochemical, functional, anatomic, and genetic abnormalities have been proposed [see reviews by Rubenstein and Merzenich (3), Parellada et al. (4), Chen et al. (5), Ameis and Catani (6)]; however, despite gaining important leads, the exact etiology of ASD is still unknown and successful treatment remains elusive. Thus, there is an urgent need to explore novel and effective investigational and mechanism-driven treatment paradigms for ASD.

One mechanism that has recently received a large amount of support suggesting its role in the pathophysiology of ASD is aberrant neuroplasticity (7, 8) In fact, several lines of evidence from genetic (9–13) to animal model (7, 14), neuroimaging (15, 16), and brain stimulation (17, 18) research have all begun to implicate aberrant neuroplasticity in ASD. One neuroscientific tool that has become a widely used, safe, and non-invasive way to probe aberrant neuroplasticity is transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). Perhaps a fair example of this is the use of

TMS/rTMS in the study of Parkinson's disease [see review by Shukla and Vaillancourt (19)], depression (20), and schizophrenia (21). The diagnostic and therapeutic potential of rTMS in ASD is beginning to be realized. In this article, we will briefly review evidence of aberrant neuroplasticity in ASD, suggest future directions in assessing neuroplasticity using rTMS, and discuss the potential of rTMS in rectifying aberrant neuroplasticity in ASD.

Aberrant Neuroplasticity in ASD

Before describing the evidence in favor of aberrant neuroplasticity in ASD, it may be worthwhile briefly revisiting neuroplasticity first. Neuroplasticity refers to neuron's ability to reorganize and alter their anatomical and functional connectivity in response to the environmental input. Long-term potentiation (LTP), which involves a net increase in synaptic efficacy, and long-term depression (LTD), which indicates a net decrease in synaptic efficacy, are the two prototypes of neuroplasticity (22).

In a simplistic model, LTP is mediated by glutamate via *N*-methyl-D-aspartate (NMDA) receptors (23). The basic process of LTP generation involves the removal of the Mg^{2+} block of the post-synaptic NMDA receptors by a strong wave of depolarization in the dendritic spine, leading to a rapid inflow of Ca^{2+} that activates several kinases, eventually leading to the generation of LTP. Similarly, LTD too perhaps is dependent on NMDA receptors. The mechanism of LTD generation, however, requires milder activation of post-synaptic NMDA receptors, which leads to an intermediate intracellular Ca^{2+} elevation (23). One key regulator of LTP and LTD is gamma-aminobutyric acid (GABA) released by the inhibitory interneurons (24). At the synaptic level, the fine balance between excitation (mediated by glutamate) and inhibition (mediated by GABA) could be crucial for optimal level of neuroplasticity (25).

Evidence from the Structural Neuroimaging Studies in ASD

Most of the symptoms of ASD develop in the first few years of life when synaptic development and maturation are occurring at a rapid rate, and one of the most consistent morphological findings that emerged from the structural neuroimaging studies in ASD is early brain overgrowth (15) [also see review by Courchesne et al. (16)]. Such atypical brain enlargement appears to be most pronounced between 2 and 5 years of age (16), and it preferentially affects the frontal and temporal cortices (5). Furthermore, recent evidence indicates that atypical cortical development in ASD subjects persists beyond toddlerhood. In particular, evidence of cortical thinning has been observed among adolescents and young adults (26). These observations led to the hypothesis that ASD is associated with a significant disruption of the typical synaptic maturation and plasticity (5).

Evidence from the Genetic Studies in ASD

Of all the proposed neurobiological theories of ASD, the potential contribution of genetic factors is backed by a large body of evidence [see review by Chen et al. (5)]. It is important to note that many ASD-associated genes reported by genome-wide association studies encode proteins related to synaptic formation, transmission, and neuroplasticity, and results from recent genetic studies involving ASD clients have consistently linked mutations involving several genes supporting synaptic maturation and neuroplasticity. The examples of such mutations involve genes critically involved in (a) synaptic maturation, e.g., neuroligin 3 and 4 (10), c3orf58, NHE9, and PCDH10 (13); (b) neuronal migration, e.g., CNTNAP2 (12); and (c) dendritic development, e.g., SHANK3 (12).

Evidence from Animal Models of ASD

Further evidence of aberrant neuroplasticity in ASD is shown by animal models. Perhaps one of the best known among these models is the valproic acid (VPA) rat model of autism. This model predicts that brain in ASD is likely to be hyperplastic. It has been found that, following a Hebbian Pairing Stimulation protocol, the amount of post-synaptic LTP measured in the neocortex and the amygdala doubled in VPA-treated rats compared with controls (14). However, other animal models utilizing genetically modified mice showed that ASD brain could be characterized by both impairment and enhancement of neuroplasticity. For example, Shank3(G/G) mice (27) and mice with MECP2 mutations (model of Rett's syndrome) (28) were shown to have cellular hypoplasticity, but mice with neuroligin-3 mutation were associated with hyperplasticity (29). Such divergent outcomes with regard to the direction of neuroplasticity in these animal experiments could be due to the nature of the genetic modifications used and their impact on the brain substrates of neuroplasticity. Nevertheless, a key insight emerging from these animal models is that if the brain becomes too much or too less plastic (i.e., hyper or hypo), cognition and behavior will be affected. It has been suggested that an optimum level of plasticity is necessary for optimal performance (30), and this process essentially involves keeping excitability within a normal physiological range (31).

Excitation/Inhibition Imbalance in ASD

Perhaps one of the widely cited neurobiological models in ASD over the past decade is the increased excitation/inhibition ratio in ASD brain (3). It has been suggested that the excitation–inhibition imbalance could be the key determinant of neuroplasticity abnormalities in neurodevelopmental disorders such as ASD (32), and a deficit in the inhibitory neurotransmission has been implicated in the etiopathogenesis of ASD [see review by Baroncelli et al. (25)]. It is believed that such deficits could develop during neuronal maturation (25). At the synaptic level, abnormally increased NMDA-mediated state of excitation, and/or abnormally reduced GABA-mediated inhibition, may lead to abnormally increased neuronal excitability and neuroplasticity. In fact, studies involving subjects with ASD have shown that excitatory glutamate receptors (NMDA and metabotropic glutamate receptor 5) are overexpressed, whereas inhibitory gamma aminobutyric acid A ($GABA_A$) and B ($GABA_B$) receptors are underexpressed in the ASD brain (25, 33). Additionally, post-mortem studies of minicolumnar morphometry in subjects with ASD also demonstrate a significant reduction of the peripheral neuropil space, which is the site of GABA-ergic lateral inhibition in the brain (34).

Transcranial magnetic stimulation has also been used to investigate excitation–inhibition imbalance in ASD. Specifically,

paired-pulse TMS paradigms, involving the "pairing" of a "conditioning stimulus" with a "test stimulus" at different interstimulus intervals, have been used to assess cortical inhibition (CI) and facilitation. CI is the neurophysiological process in which inhibitory GABA-ergic interneurons selectively attenuate the activity of pyramidal neurons in the cortex. It has been suggested that CI is key to the regulation of neuroplasticity, and the therapeutic effects of rTMS could be mediated by the induction of local changes in CI (35). Emerging evidence indicates that post-synaptic $GABA_B$ receptor-mediated CI is crucial for the regulation of neuroplasticity. $GABA_B$ regulates neuroplasticity in two ways: (a) they contribute to the regulation of inhibition by mediating long-lasting inhibitory post-synaptic potentials (IPSPs) and (b) they reduce $GABA_A$ receptor-mediated inhibition through presynaptic auto-inhibition of inhibitory interneurons (36). Using paired-pulse TMS paradigms, studies have found evidence for excitation–inhibition imbalance in a subgroup of individuals with ASD (37, 38). Other studies have shown no abnormality in CI (18, 39) or a heterogeneous response to this paradigm (40). The heterogeneity in these findings reflects the known heterogeneity of ASD at both the behavioral and the physiological level.

rTMS in the Assessment of Neuroplasticity in ASD

Repetitive TMS, which involves repetitive delivery of pulses (>1 Hz), is used to modulate cortical activity for investigative and therapeutic purposes [see review by Kobayashi and Pascual-Leone (41)]. rTMS has been increasingly used to study neuroplasticity in humans. The basic premise is that rTMS can modulate activity in the targeted brain region for a duration that can outlast the effects of stimulation itself (30). It is believed that rTMS induces such lasting changes in the brain through altering neuroplasticity mechanisms (42). So far, two rTMS paradigms – theta-burst stimulation (TBS) (17) and paired associative stimulation (PAS) (18) – have been used to assess neuroplasticity in ASD.

Theta-Burst Stimulation

Theta-burst stimulation involves the delivery of a burst of three pulses at 50 Hz (i.e., 20 ms between stimulus) repeated at intervals of 200 ms (i.e., 5 Hz, hence called theta-burst) (43). TBS comprises two well-established patterned stimulation protocols – continuous TBS (also known as cTBS) and intermittent TBS or iTBS. cTBS paradigm involves the delivery of continuous uninterrupted TBS for 40 s. In the iTBS paradigm, a 2-s train of TBS is repeated every 10 s for a total of 190 s. However, the total number of pulses delivered may vary from one study to another. In the original study, Huang et al. (43) used 600 pulses. iTBS produces sustained enhancement, whereas cTBS is associated with lasting suppression of cortical activity, indexed by potentiation and suppression of motor-evoked potential (MEP) following single-pulse TMS in the contralateral thumb muscle, respectively (43). It is believed that such lasting changes induced by iTBS and cTBS reflect LTP- and LTD-like mechanisms in the brain (43), and in previous experiments, they have been found to be mediated by NMDA receptor (44) and GABA receptor pathways (45), respectively.

Paired Associative Stimulation

Paired associative stimulation is another well-established rTMS paradigm that has been associated with the induction of LTP-like neuroplasticity (PAS-LTP). It has been shown that PAS-LTP is mediated by NMDA receptors (46). The PAS protocol involves the repetitive delivery of two paired (180 pairs at 0.1 Hz for 30 min) stimulations: (1) an electrical peripheral nerve stimulation of the right median nerve, and 25 ms later, a (2) TMS pulse delivered to the contralateral motor cortex (M1) (hence PAS-25). PAS-25 results in LTP-like neuroplasticity that manifests as the potentiation of MEP in the thumb muscle following single-pulse TMS (46).

Safety of rTMS in ASD

Available limited data indicate that rTMS, when applied within established safety guidelines, is well tolerated and safe in both adult and pediatric ASD populations (47, 48). There is no current evidence of increased risk of seizure (48).

rTMS Studies Assessing Neuroplasticity in ASD

Asperger's disorder (AD), which was a subtype of the DSM-IV Pervasive Developmental Disorder, has now been subsumed under ASD in DSM-5 (1). A more direct evidence of aberrant neuroplasticity in AD subjects has been shown by recent rTMS studies using TBS and PAS paradigms. All these studies, however, have assessed neuroplasticity in the motor cortex (M1). One group found greater and long-lasting modulation of neuroplasticity (reflective of aberrant hyperplasticity) following both forms of TBS (cTBS and iTBS) in a small cohort (40) and, subsequently, in a relatively bigger sample of adults with AD (17). Another group, examining LTP-like neuroplasticity in a mixed cohort of adolescents and adults with AD using PAS, obtained similar results, i.e., aberrant neuroplasticity (18); however, the direction of aberrant neuroplasticity was different. In this study, it was found that, compared to typically developing subjects, PAS-induced LTP-like plasticity was significantly deficient (reflective of aberrant hypoplasticity) in the AD group.

Assessing Neuroplasticity in ASD Subjects Using rTMS: Future Considerations

At present, research assessing neuroplasticity using rTMS in ASD population is at an early stage. Studies so far have only tested high-functioning ASD subjects at the motor cortex (M1). Furthermore, findings obtained in the adult population may not be generalized to the pediatric population. For example, Oberman et al. (47) found a "paradoxical facilitatory effect" to cTBS in more than one-third of their sample consisting of children and adolescents. Therefore, to what extent current findings can be generalized is certainly not very clear at present. The potential factors that need to be considered by future research are heterogeneity in the ASD population, potential impact of the presence/absence of comorbidities including intellectual disabilities, medication use, developmental age, site of stimulation, stimulation parameters (e.g., TBS versus PAS), etc.

The other important point for consideration is that all existing studies utilizing rTMS have assessed neuroplasticity at the motor

cortex (M1) of ASD brain. In the future, studies need to look at neuroplasticity in other potential areas of interest in the ASD brain. Information regarding which sites to choose for assessing neuroplasticity in ASD brain may come from existing rTMS intervention studies. So far, studies that used rTMS for therapeutic purposes to improve either symptoms or physiological and cognitive indices have focused on four areas of ASD brain – the dorsolateral prefrontal cortex (DLPFC), medial prefrontal cortex (mPFC), supplementary motor area, and right pars triangularis and pars opercularis [for a review see Oberman et al. (49)]. The DLPFC was chosen due to its extensive network connection with other specialized distributed and local networks in brain (34). Dorsomedial PFC (dmPFC) is another key area for stimulation since it is believed to be uniquely linked with the mentalizing ability (50). A recent trial of deep rTMS delivered bilaterally to the dmPFC significantly improved social relatedness in ASD subjects (51). Therefore, both DLPFC and mPFC could be potential sites of interest for studying neuroplasticity in ASD. Other brain areas related to mentalizing, such as the temporoparietal junction (TPJ) (52), and facial processing, such as superior temporal sulcus (53), could be potential sites for stimulation as well.

Establishing a stimulation paradigm to reliably assess neuroplasticity from these key areas of brain is challenging; however, the combination of TMS with electroencephalography (TMS–EEG) offers researchers an exciting opportunity to gather a more direct measure of neuroplasticity from these areas of brain. Previously, our group established that TMS–EEG can be a reliable method to measure neuroplasticity from M1 and also DLPFC (54). More recently, using a pioneering technique that combines PAS with EEG – "PAS–EEG," our group assessed and successfully demonstrated PAS-induced potentiation of cortical evoked activity, which is reflective of LTP-like neuroplasticity, in DLPFC (55). A similar TMS–EEG approach may be useful for studying neuroplasticity in other key areas of brain. For example, TBS can be combined with EEG to investigate neuroplasticity measures.

In the future, TMS–EEG can also be combined with various social–cognitive tasks and functional neuroimaging to better elucidate the brain–behavior relationship in ASD. Ultimately, TMS–EEG will be combined with genetic research to better elucidate the link between underlying genetic factors (i.e., polymorphisms) and aberration in neuroplasticity captured more directly by TMS–EEG cortical readout. Results from a few early exploratory studies assessing the impact of single-nucleotide polymorphisms, e.g., brain-derived neurotrophic factor valine-to-methionine substitution at codon 66 (Val66Met) genotype (56), on TMS-induced plasticity measures have so far been encouraging.

Can rTMS be Used as a Therapeutic Tool to Rectify Aberrant Neuroplasticity in ASD?

Repetitive TMS affords researchers to design specific stimulation protocols that can modulate neuroplasticity, and such neuroplasticity-based brain stimulation interventions look promising. Recently, in a randomized double-blind sham-controlled study, our group demonstrated that application of 1,500 pulses/session of high-frequency (20 Hz) rTMS to DLPFC can "normalize" working memory deficits in schizophrenia (57). One possible mechanism of such improvement is enhancement of neuroplasticity in the DLPFC. There is a need to explore similar approach to treat aberrant neuroplasticity in ASD.

What rTMS Stimulation Protocol to Choose for Stabilizing Aberrant Neuroplasticity in ASD?

Since aberrant neuroplasticity has been linked with the pathogenesis of ASD (7, 8), there is an urgent need to explore treatment paradigms that can stabilize aberrant neuroplasticity and thus potentially facilitate optimal social and cognitive performance and improve restricted and repetitive behaviors in ASD. In this regard, we would like to propose the potential role of extended dosing (i.e., 6,000 pulses) of high-frequency (i.e., 20 Hz) rTMS (58).

In healthy adults, rTMS applied on M1 has been shown to enhance GABA-mediated inhibitory neurotransmission indexed by lengthening of the cortical silent period (CSP), a CI measure reflective of $GABA_B$-mediated inhibitory neurotransmission, with increased stimulation frequency. Our group found that the enhancement was maximal at 20 Hz (31). This finding breaks with convention that high-frequency stimulation results in excitation, whereas low-frequency stimulation results in inhibition, as 20-Hz rTMS, but not 1-Hz rTMS, resulted in a CSP prolongation (31, 58). One explanation is that 20-Hz rTMS may exert its inhibitory effect by selectively affecting networks involving fast-spiking inhibitory interneurons that mainly oscillate at higher (i.e., 30–70 Hz) frequencies (58). A recent study by our group investigating differing durations or doses of rTMS on CI in M1 in healthy subjects found that even a single session of extended dosing (6,000 pulses) with high-frequency (20 Hz) pulses led to significant lengthening of the $GABA_B$-mediated CSP compared with other paradigms (58). This effect was not seen with active or sham 1- or 20-Hz rTMS at 1,200 pulses or 3,600 pulses.

It has been suggested that, depending on the direction and magnitude of inhibition, $GABA_B$ receptor-mediated neurotransmission may attenuate neuroplasticity. In fact, baclofen, a $GABA_B$ agonist, significantly attenuated LTP-like neuroplasticity in M1 induced by PAS (59). Since extended dosing (i.e., 6,000 pulses) of such specific high-frequency (20 Hz) rTMS protocol (58) appears to maximally enhance $GABA_B$-mediated inhibitory neurotransmission, one approach would be to assess if such protocols are able to stabilize aberrant hyperplasticity seen in ASD. This line of approach is also consistent with the excitation–inhibition imbalance in ASD, i.e., a general deficit in GABA-ergic inhibition, an increased excitation/inhibition ratio (3), and an evidence of reduced expression of $GABA_B$ receptors (33). In the future, proof-of-principle studies are needed to test this assumption. Because of its simplicity and reliability, such experiments may begin at M1 to see if the delivery of 6,000 pulses at 20 Hz can stabilize aberrant neuroplasticity in ASD subjects. If successful, further pilot studies will be required to assess whether rectifying aberrant neuroplasticity translates into actual clinical improvement or not. These pilot studies may potentially stimulate key areas of ASD brain discussed above, i.e., DLPFC, TPJ, and dmPFC, and determine key stimulation parameters, duration of sessions, etc.

Conclusion

In summary, existing genetic and animal studies of ASD and evidence emerging from human rTMS studies have consistently indicated aberrant neuroplasticity in ASD brain. However, at this point, there are many unanswered questions regarding the exact etiopathological connection between aberrant neuroplasticity in the brain and development of autistic symptoms. Nevertheless, existing evidence still indicates that aberrant neuroplasticity could play a critical role in the pathogenesis of ASD. Therefore, it can be postulated that it may be possible to attain optimal social and cognitive performance in ASD by stabilizing aberrant neuroplasticity. In this context, we discussed a novel mechanism-driven approach toward achieving such goal using rTMS. If successful, this information will not only help us better understand the brain mechanisms involved in ASD but also stimulate trials testing mechanism-driven novel brain stimulation treatment paradigms for ASD.

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Neurocognitive effects of repetitive transcranial magnetic stimulation in adolescents with major depressive disorder

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Objectives: It is estimated that 30–40% of adolescents with major depressive disorder (MDD) do not receive full benefit from current antidepressant therapies. Repetitive transcranial magnetic stimulation (rTMS) is a novel therapy approved by the US Food and Drug Administration to treat adults with MDD. Research suggests rTMS is not associated with adverse neurocognitive effects in adult populations; however, there is no documentation of its neurocognitive effects in adolescents. This is a secondary *post hoc* analysis of neurocognitive outcome in adolescents who were treated with open-label rTMS in two separate studies.

Methods: Eighteen patients (mean age, 16.2 ± 1.1 years; 11 females, 7 males) with MDD who failed to adequately respond to at least one antidepressant agent were enrolled in the study. Fourteen patients completed all 30 rTMS treatments (5 days/week, 120% of motor threshold, 10 Hz, 3,000 stimulations per session) applied to the left dorsolateral prefrontal cortex. Depression was rated using the Children's Depression Rating Scale-Revised. Neurocognitive evaluation was performed at baseline and after completion of 30 rTMS treatments with the Children's Auditory Verbal Learning Test (CAVLT) and Delis–Kaplan Executive Function System Trail Making Test.

Results: Over the course of 30 rTMS treatments, adolescents showed a substantial decrease in depression severity. Commensurate with improvement in depressive symptoms was a statistically significant improvement in memory and delayed verbal recall. Other learning and memory indices and executive function remained intact. Neither participants nor their family members reported clinically meaningful changes in neurocognitive function.

Conclusion: These preliminary findings suggest rTMS does not adversely impact neurocognitive functioning in adolescents and may provide subtle enhancement of verbal memory as measured by the CAVLT. Further controlled investigations with larger sample sizes and rigorous trial designs are warranted to confirm and extend these findings.

Keywords: adolescents, depression, neurocognition, memory, learning, TMS

INTRODUCTION

Repetitive transcranial magnetic stimulation (rTMS) is a novel treatment approach for medication-resistant patients with major depressive disorder (MDD). Repetitive TMS has been approved by the US Food and Drug Administration for the treatment of adults with MDD who fail to achieve satisfactory improvement from one prior adequate antidepressant treatment trial. Although several sham-controlled studies have indicated that rTMS is efficacious in adults with MDD (1–4), there have been few studies in adolescents. We recently reported results of an open-label pilot study that found rTMS to be a potentially effective adjunctive therapy for adolescents with treatment-resistant MDD (5). Adolescents showed statistically significant improvement in the Children's Depression Rating Scale-Revised (CDRS-R) from baseline through the rTMS treatment series (30 sessions) and at 6-month follow-up. A second, recently completed replication trial in 10 adolescents revealed similar findings for clinical improvement in treatment completers (submitted). In both studies, assessments of cognitive functioning were performed at baseline and treatment completion.

A number of studies have indicated that rTMS treatment for MDD is not associated with adverse effects on neuropsychological functions such as attention, learning, and memory (2, 3, 6–9), but these investigations have only included adults. Not only have studies not shown any deterioration in neuropsychological functioning from rTMS, but several investigations have shown an improvement in neurocognitive function in adult patients

with MDD. For example, in a sham-controlled study by Avery et al. (1), adults with MDD showed considerably improved performance on measures of attention, learning and memory, and cognitive flexibility following 10 sessions of 10 Hz rTMS applied to the left dorsolateral prefrontal cortex (L-DLPFC). Furthermore, two sham-controlled studies reported improvement in verbal memory performance as a result of multiple sessions of 10 Hz rTMS treatment to the L-DLPFC in adults with MDD (10, 11). Also, following multiple sessions of 10 Hz rTMS to the L-DLPFC in depressed adults, Fitzgerald et al. (12) found that there was significant improvement in neuropsychological function, including autobiographical memory; and Martis et al. (13) reported improvement in working memory and executive function. Recently, Luber and Lisanby described a review of over 60 TMS studies that reported "significant improvements in speed and accuracy in a variety of tasks involving perceptual, motor, and executive processing" (14).

In the present study we evaluated the neurocognitive effects of rTMS when used as an adjunctive treatment for adolescents with treatment-resistant MDD. We hypothesized that adolescents would demonstrate no difference in measures of memory, executive functioning, or auditory and visual learning tasks following a robust course of left-sided, high-frequency rTMS.

MATERIALS AND METHODS

PARTICIPANTS

Participants were diagnostically assessed by a board-certified child and adolescent psychiatrists (Paul E. Croarkin and Christopher A. Wall). This included a comprehensive clinical evaluation and standardized diagnostic interview that utilized the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children – Present and Lifetime Version (K-SADS-PL) (15). At the time of enrollment, all participants were receiving active antidepressant treatment for an MDD episode according to the *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition, Text Revision (*DSM-IV-TR*) (16). Clinically significant depressive symptoms were defined by CDRS-R (17) total score of at least 40 (t score >63). Participants included those with treatment failure/non-response to at least one adequate antidepressant trial [i.e., treated with stable selective serotonin reuptake inhibitor (SSRI) dose regimen for at least 6 weeks as defined by a score of ≥ 3 on the Antidepressant Treatment History Form] (18). All participants continued treatment with a stable dose of their prestudy antidepressant during the rTMS course. Participants in psychotherapy were ineligible if they had changed therapists, type of psychotherapy, or providers in the 4 weeks prior to rTMS initiation. Participants were allowed to continue previous sleep aids such as melatonin, trazodone, or diphenhydramine during treatment. Stimulants, antipsychotics, mood stabilizers, and tricyclic antidepressants were not permitted during the active treatment phase.

Patients with comorbid secondary diagnoses of dysthymia, attention-deficit/hyperactivity disorder, or anxiety disorders were eligible for enrollment. However, patients with schizophrenia, schizoaffective disorder, bipolar spectrum disorders, substance abuse or dependence, somatoform disorders, dissociative disorders, post-traumatic stress disorder, obsessive-compulsive disorder, eating disorders, mental retardation, or pervasive developmental disorder/autism spectrum disorders were excluded from participation. Medical exclusions included preexisting seizure disorders or active neurologic conditions (e.g., brain tumor, dyskinesias, or paralysis). The screening process included a urine toxicology screen for drugs of abuse and a urine pregnancy test. All participants and treaters wore earplugs during the sessions to minimize the risk of auditory threshold changes.

STUDY OVERVIEW

Both trials were prospective, open, multicenter pilot trial of active rTMS in adolescents with MDD confirmed by the K-SADS-PL. Both studies received institutional review board approval and were performed under United States Food and Drug Administration Investigational Device Exemptions: trial #1 – G060269 and trial #2 – G110091. All patients provided written informed assent, and parents provided written informed consent per institutional review board-approved guidelines. Recruitment, outcomes, and potential adverse effects were monitored by a Data and Safety Monitoring Board comprised of clinicians with no direct involvement in the study.

rTMS PROCEDURES

Identification of the treatment site and stimulus dosing were based on previously defined techniques and guidelines noted in adult rTMS trials (4, 19). In both trials, the motor cortex was identified, using the rTMS machinery via a single pulse administered every 3–5 s, at the location that produced a localized contraction of the contralateral abductor pollicis brevis muscle. Once this site was defined, the resting motor threshold (MT) was determined using a computer-assisted maximum likelihood threshold-hunting algorithm (MT Assist, Neuronetics Inc., Malvern, PA, USA). Repeat MT determinations occurred at least once every 10 treatments to assess for possible changes that could produce safety issues due to changes in cortical excitability.

In trial #1, the L-DLPFC treatment location was determined by moving the treatment coil 5 cm anterior to the MT location along a left superior oblique plane (20). In trial #2, the L-DLPFC treatment location was determined via an MRI-based neurolocalization technique. The identified treatment site was then marked and spatial coordinates were recorded with a mechanical coil positioning system to ensure reproducibility of the coil placement.

In both trials, a total of 30 treatments were administered across a range of 6–8 weeks. This range was chosen for potential variation in patient schedules related to school and family events. Thus, each patient was offered a total of 40 treatment opportunities in which to complete 30 treatments. Each treatment was titrated to 120% of calculated MT, at a frequency of 10 Hz, with stimulus train duration of 4 s and an inter-train interval of 26 s, for a total of 3,000 stimulations per treatment session. In trial #1, rTMS was delivered using the Neuronetics Model 2100 Therapy System; in trial #2 treatments were delivered using the NeuroStar System (Neuronetics, Inc., Malvern, PA, USA).

NEUROCOGNITIVE ASSESSMENTS

Neurocognitive testing was administered by trained psychometrists at baseline and upon completion of the active rTMS treatments. Testing was typically performed in the afternoon hours, although not universally due to scheduling accommodations related to subject school obligations, family work hours, and clinician/psychometrist availability. A doctorate-level child psychologist (Leslie A. Sim) analyzed the results. The neurocognitive battery was tailored to assess a variety of neurocognitive domains including psychomotor speed, simple attention, learning, memory, and executive function. Specifically, the battery included the Children's Auditory Verbal Learning Test-2 (CAVLT-2) (21) and the Delis–Kaplan Executive Function System (D-KEFS) (22) Trail Making Test.

The CAVLT-2 (21) is a measure designed to quantify a child's (ages 6.6–17.11) verbal learning and memory abilities. The measure is comprised of a 16-item word list that is administered across five trials, and the participant is asked to recall the words after each trial. A different set of words is then presented and the participant is asked to immediately recall the items from the new list (Interference Trial). Following the interference list, the participant is instructed to recall as many items as possible from the original list (immediate recall). Following a 15 min delay, the participant is asked to recall the original list for a final time (delayed recall). Finally, the participant is presented with a 32-item word list and asked to recognize the 16 words from the original list (Recognition Trial). The CAVLT-2 yields multiple indices of learning and memory, including immediate memory span, level of learning, immediate recall, delayed recall, recognition accuracy, and total intrusions.

The D-KEFS Trail Making Test is used to assess components of cognitive flexibility on a visual-motor sequencing task (23). It consists of five conditions: (a) Visual Scanning, (b) Number Sequencing, (c) Letter Sequencing, (d) Motor Speed, and (e) Number-Letter Switching. The D-KEFS Trail Making Test was selected to assess aspects of executive function, particularly cognitive flexibility, as it is influenced by mood and anxiety states. Importantly, the D-KEFS has been normed for the age group of children in this trial and provides standardized scores (24). Based on adequate reliability and validity of these tests along with appropriate developmental normative data, these neuropsychological measures (CAVLT-2 and D-KEFS TMT) were thought to be developmentally appropriate tools to assess subtle changes in cognitive function of adolescents receiving rTMS treatments.

Safety and participant comfort were assessed and recorded before and after each study visit with prompted opportunities to report adverse events.

STATISTICAL ANALYSIS

The primary aim of this study was to assess whether adjunctive rTMS is a safe and feasible treatment approach in adolescents. This question was evaluated by neurocognitive assessments that occurred at baseline and immediately following treatment number 30. Within patient changes from baseline to treatment completion were examined with comparative statistics.

Neurocognitive measurements obtained at baseline and immediately following treatment were summarized using mean and standard deviation ($\pm$SD). The paired t-test was used to assess whether scores changed significantly from baseline to end of treatment. For these analyses, two-tailed P-values of ≤ 0.05 were considered statistically significant. In addition to the primary analyses which included all enrolled subjects, a subset analysis was performed that was restricted to subjects who completed treatment. All analyses were performed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Of the 18 adolescents enrolled in both trials, 14 adolescents (5 males and 9 females; ages 13.9–17.8 years; mean age, 16.3 ± 1.1 years) completed the entire rTMS treatment course (clinicaltrials.gov Identifier: NCT00587639). Fourteen out of 14 adolescents who completed the entire treatment course also completed neurocognitive testing at baseline and treatment completion. Subjectively, no reportable changes in memory, cognitive functioning, or attention were noted by any of the participating adolescents or their families. Objectively, subtle but statistically significant improvement was observed in immediate memory and delayed recall as measured by the CAVLT when measured in all study participants (**Table 1**; **Figure 1**) and participants who completed all 30 rTMS sessions of the treatment protocol (**Table 2**; **Figure 2**). All other CAVLT indices of learning and memory (interference, immediate recall, or level of learning) remained stable over the course of treatment (**Table 1**).

No significant changes were noted on the D-KEFS Trail Making Test indices from baseline to treatment completion for either the all participant group (**Table 3**; **Figure 3**) or the treatment completers only group (**Table 4**; **Figure 4**).

DISCUSSION

To our knowledge, this is the first report on neurocognitive outcomes within a clinical trial of rTMS in depressed adolescents. The neurocognitive safety findings of this case series of high-frequency rTMS in treatment-resistant depressed adolescents are consistent with previously reported findings in clinical trials of rTMS in adults with psychiatric illness. Previous adult trials have frequently included cognitive assessments that demonstrated rTMS to have no adverse effects on cognitive functions (2, 3, 6–9, 25). Interestingly, a number of these clinical trials in adults have shown time-limited improvements in various aspects of cognitive function, mainly in attention, concentration, working memory, and processing speed (10–13). Similarly, modest improvements in attention, and verbal and learning and memory were observed in this cohort of adolescents.

LIMITATIONS

Clearly, these findings must be interpreted with caution due to the small total number of participants and the lack of a control group. However, if there was a distinct pattern of clinically and psychometrically meaningful adverse cognitive effects – as could

Table 1 | Children's Auditory Verbal Learning Task (CAVLT) results (all participants).

CAVLT subscales		*N*	Score Mean	Change from baseline Mean (SD)	Change (95% CI)	*P*-value	Cohen's *d*
Immediate Memory Scale	BL	18	98.6	–	–	–	–
	PT	18	113.1	14.5 (12.0)	(8.5, 20.5)	<0.0001	0.81
Level of learning	BL	18	98.4	–	–	–	–
	PT	18	106.2	7.7 (17.7)	(−1.1, 16.5)	0.0814	0.37
Interference	BL	18	104.4	–	–	–	–
	PT	18	109.2	4.7 (13.1)	(−1.8, 11.2)	0.1434	0.30
Immediate recall	BL	18	98.5	–	–	–	–
	PT	18	100.2	1.7 (11.9)	(−4.2, 7.6)	0.5596	0.08
Delayed recall	BL	18	94.7	–	–		
	PT	18	102.3	7.6 (13.8)	(0.7, 14.5)	0.0319	0.33

BL, baseline; PT, post-treatment.

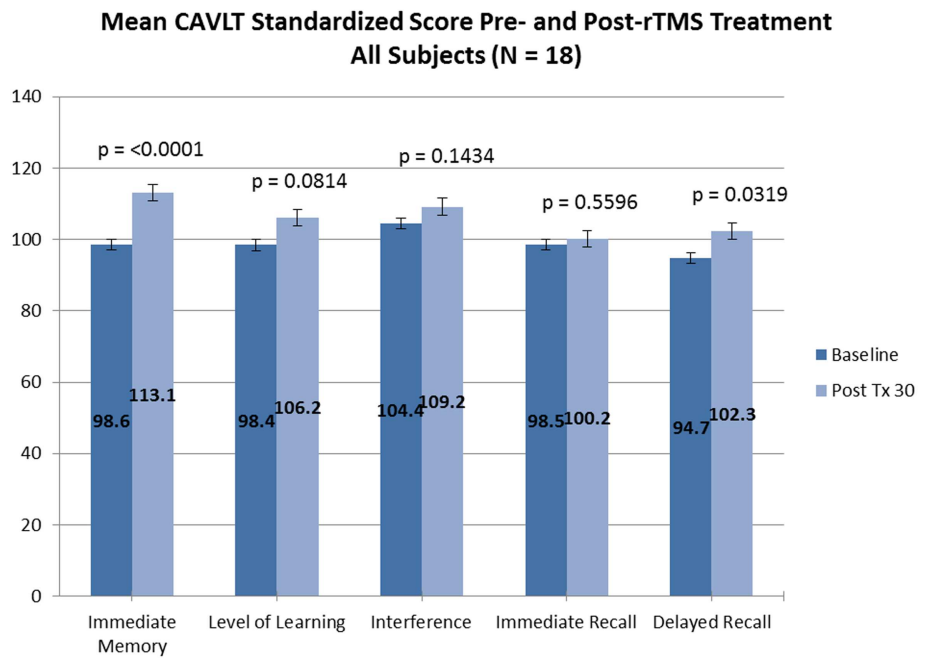

FIGURE 1 | CAVLT results (all participants).

be found in a robust course of electroconvulsive therapy – we would expect to see these findings even in this small group of participants. It is reassuring to note that none of the participants or their family members described any impairments (or marked improvements) in learning, memory, or other untoward cognitive effects.

CONCLUSION

Collectively, the findings of this study combined with our prior clinical findings suggest that rTMS may be a safe, feasible, and potentially efficacious adjunctive therapy for adolescents with MDD given the lack of negative changes in cognitive functioning and reduced overall side-effect burden (4, 5). Future studies of rTMS in adolescents will need to monitor for cognitive changes, which would benefit from the use of a comprehensive, standardized, and validated neurocognitive battery that will be sensitive to cognitive changes, particularly in those cognitive domains essential for continued academic maturation and instrumental activities of daily living. Such a neurocognitive battery should assess domains of intellectual ability, processing speed, attention,

Table 2 | Children's Auditory Verbal Learning Task (CAVLT) results (treatment completers).

CAVLT subscales		*N*	Score Mean	Change from baseline Mean (SD)	Change (95% CI)	*P*-value	Cohen's *d*
Immediate Memory Scale	BL	14	95.6	–	–	–	–
	PT	14	109.4	13.8 (12.6)	(6.5, 21.1)	0.0013	0.77
Level of learning	BL	14	99.4	–	–	–	–
	PT	14	105.1	5.7 (18.8)	(−5.2, 16.6)	0.2764	0.25
Interference	BL	14	101.1	–	–	–	–
	PT	14	106.1	5.0 (13.2)	(−2.6, 12.6)	0.1786	0.31
Immediate recall	BL	14	97.3	–	–	–	–
	PT	14	99.9	2.6 (13.3)	(−5.1, 10.3)	0.4707	0.11
Delayed recall	BL	14	94.9	–	–		
	PT	14	100.9	6.0 (14.1)	(−2.1, 14.1)	0.1353	0.24

BL, baseline; PT, post-treatment.

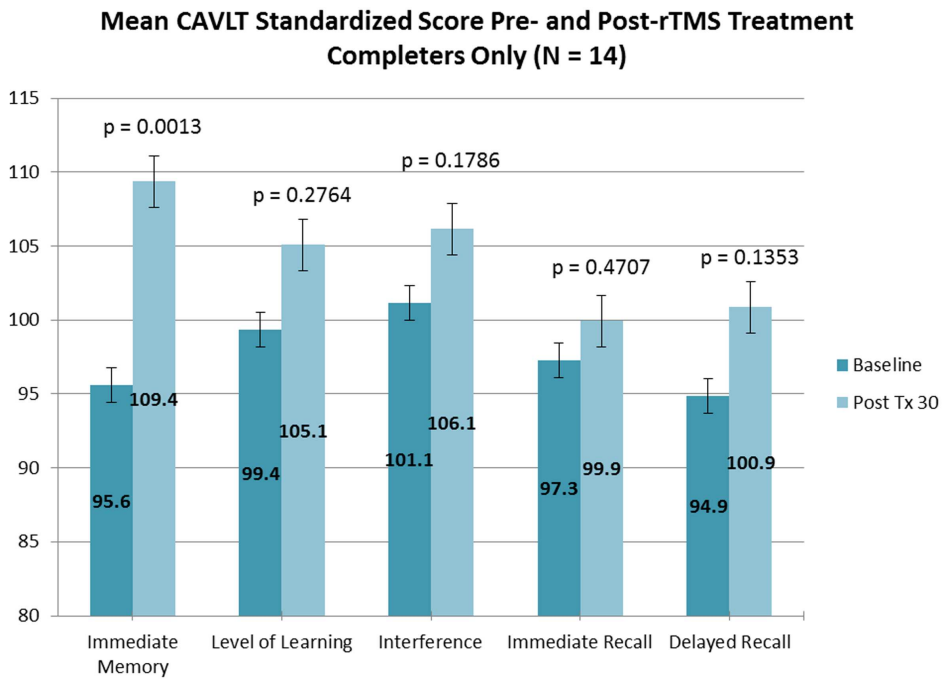

FIGURE 2 | CAVLT results (completers only).

Table 3 | Delis–Kaplan Executive Function System (D-KEFS) Trail Making Test results (all participants).

D-KEFS subscales		*N*	Score Mean	Change from baseline Mean (SD)	Change (95% CI)	*P*-value	Cohen's *d*
Number sequencing	BL	18	10.1	–	–	–	–
	PT	18	11.1	1.1 (3.2)	(−0.5, 2.7)	0.1735	0.38
Letter sequencing	BL	18	10.3	–	–	–	–
	PT	18	11.3	1.0 (2.9)	(−0.4, 2.4)	0.1604	0.42
Composite score	BL	18	10.7	–	–	–	–
	PT	18	11.8	1.1 (2.9)	(−0.4, 2.6)	0.1259	0.43

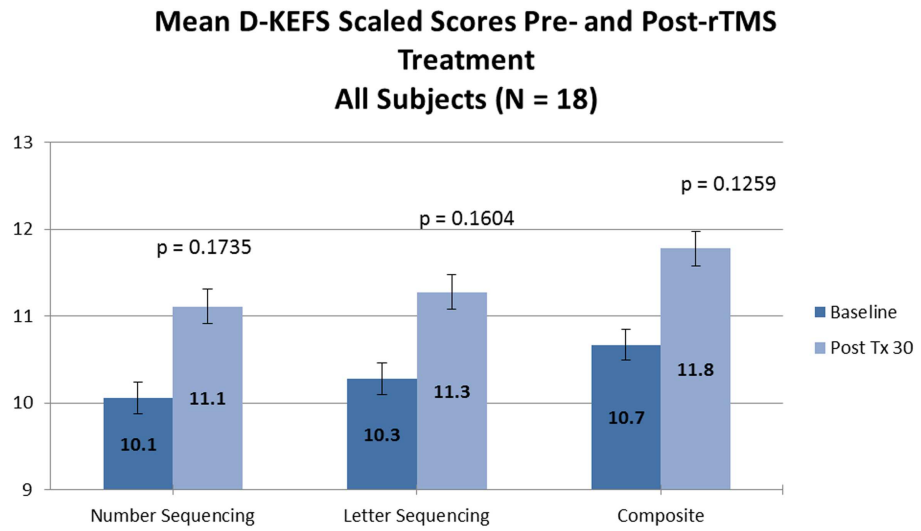

FIGURE 3 | D-KEFS results (all participants).

Table 4 | Delis–Kaplan Executive Function System (D-KEFS) Trail Making Test results (treatment completers).

D-KEFS subscales		*N*	Score Mean	Change from baseline Mean (SD)	Change (95% CI)	*P*-value	Cohen's *d*
Number Sequencing	BL	14	10.3	–	–	–	–
	PT	14	11.1	0.9 (3.6)	(−1.2, 3.0)	0.3854	0.28
Letter sequencing	BL	14	10.9	–	–	–	–
	PT	14	11.6	0.7 (2.7)	(−0.9, 2.3)	0.3405	0.31
Composite score	BL	14	11.2	–	–	–	–
	PT	14	12.0	0.8 (3.1)	(−1.0, 2.6)	0.3629	0.30

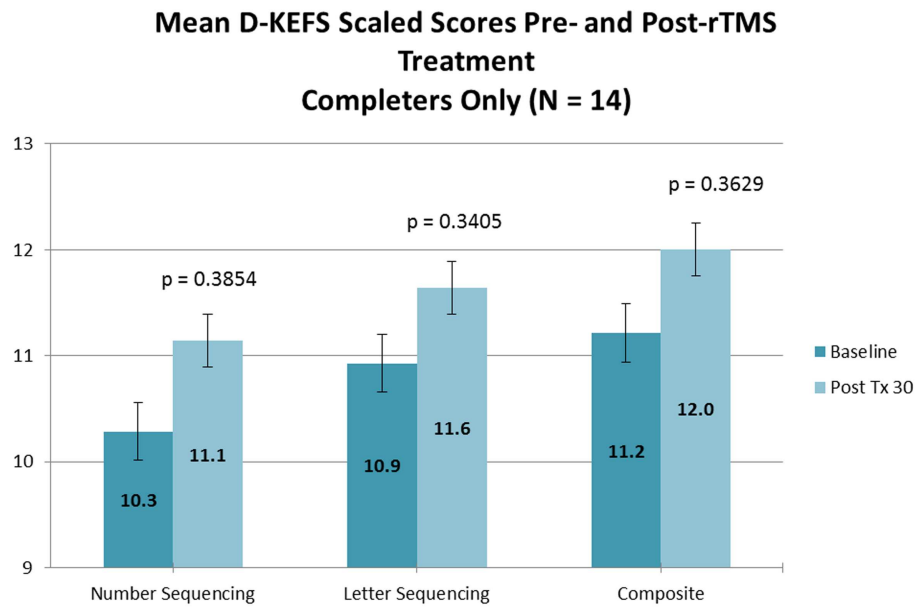

FIGURE 4 | D-KEFS results (completers only).

learning and memory, working memory, and executive function. Indeed, Semkovska and McLoughlin recommended the development of standardized and validated tools to measure the ability to recall autobiographical events in patients with depression (26). Such a measure would be of particular value in the adolescent population.

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