ORIGINAL RESEARCH

Abnormal Degree Centrality in Children with Low-Function Autism Spectrum Disorders: A Sleeping-State Functional Magnetic Resonance Imaging Study

Shoujun Xu[1,]*, Meng Li[2,]*, Chunlan Yang[3], Xiangling Fang[4], Miaoting Ye[4], Yunfan Wu[2], Binrang Yang[4], Wenxian Huang[4], Peng Li[1], Xiaofen Ma[2], Shishun Fu[2], Yi Yin[2], Junzhang Tian[2], Yungen Gan[1], Guihua Jiang[2]

[1]Department of Radiology, Shenzhen Children's Hospital, Shenzhen, People's Republic of China; [2]Department of Medical Imaging, Guangdong Second Provincial General Hospital, Guangzhou, People's Republic of China; [3]Department of Hematology and Oncology, Shenzhen Children's Hospital, Shenzhen, People's Republic of China; [4]Department of Department of Children Healthcare, Shenzhen Children's Hospital, Shenzhen, People's Republic of China

*These authors have contributed equally to this work

Correspondence: Guihua Jiang; Yungen Gan, Email jiangguh@gd2h.org.cn; gdszgyg-1@126.com

Purpose: This study used the graph-theory approach, degree centrality (DC) to analyze whole-brain functional networks at the voxel level in children with ASD, and investigated whether DC changes were correlated with any clinical variables in ASD children.

Methods: The current study included 86 children with ASD and 54 matched healthy subjects Aged 2–5.5 years. Next, chloral hydrate induced sleeping-state functional magnetic resonance imaging (ss-fMRI) datasets were acquired from these ASD and healthy subjects. For a given voxel, the DC was calculated by calculating the number of functional connections with significantly positive correlations at the individual level. Group differences were tested using two-sample t-tests ($p < 0.01$, AlphaSim corrected). Finally, relationships between abnormal DCs and clinical variables were investigated via Pearson's correlation analysis.

Results: Children with ASD exhibited low DC values in the right middle frontal gyrus (MFG) ($p < 0.01$, AlphaSim corrected). Furthermore, significantly negative correlations were established between the decreased average DC values within the right MFG in ASD children and the total ABC scores, as well as with two ABC subscales measuring highly relevant impairments in ASD (ie, stereotypes and object-use behaviors and difficulties in language).

Conclusion: Taken together, the results of our ss-fMRI study suggest that abnormal DC may represent an important contribution to elucidation of the neuropathophysiological mechanisms of preschoolers with ASD.

Keywords: autism spectrum disorders, ASD, functional magnetic resonance imaging, fMRI, Degree centrality, DC, whole-brain functional networks, Pearson's correlation analysis

Introduction

Autism spectrum disorders (ASDs) are early-onset neurodevelopmental disorders that are characterized by social-communication deficits, restricted interests, and stereotyped behaviors.[1] Diagnosis of ASDs is mainly based on a history of an impairment in expressive behaviors and cognitive development.[2] The prevalence of ASD has increased significantly over time, and currently affects nearly 1–2% of children worldwide.[3] The surge in the incidence of ASD has led to considerable impairment in major life areas,[1,4] as well as a declining quality of life,[4,5] both of which may last for a life time.[6] Therefore, identification of ASD etiology, accurate diagnosis, and effective interventions as early in life as possible are crucial for ASD patients and society overall.[7] However, the neurobiological bases underlying the symptoms and etiologies of ASD in children have remained unclear.

With the rapid development of neuroimaging technologies, many new methods have emerged for the detection of early brain changes in many neuropsychological diseases, including ASD. Positron-emission tomography (PET) and single-photon emission computed tomography (SPECT) have demonstrated cerebral hypoperfusion or insufficient blood flow in many areas of the brain,[8,9] as well as neurotransmitter and glucose-metabolism disorders in ASD patients.[8,10–12] Furthermore, remarkable advances in magnetic resonance imaging (MRI) technology in recent decades have greatly enriched our understanding of neuropathological differences in ASD. Magnetic resonance spectroscopy (MRS) studies have found that patients with ASD have an excitation/inhibition imbalance.[11,13–16] Previous diffusion-tensor imaging (DTI) studies have indicated alterations not only in the local microstructure of white-matter (WM) integrity[17–20] but also in interregional fiber tractography[21,22] in ASD patients compared with these parameters in control subjects. Voxel-based morphometry (VBM) and arterial spin-labeling (ASL) MR perfusion-imaging studies have also been used in investigating ASD and have also revealed that alterations of brain structure[23–25] and brain metabolism[26] existed in some areas of the cerebral cortex in ASD patients. Evidence from previous functional MRI (fMRI) studies have shown that ASD-induced alterations of brain functional activity are not only limited to regional changes[27–29] but also reflected in the level/degree of functional integration within brain neural-network-related regions.[7,30–32] For example, using regional homogeneity (ReHo) method, Dajani and Uddin studied the differences among groups in three age cohorts and found that local connections were disrupted during the development of ASD, and the most significant differences occurred in childhood.[27] In addition, a study of 119 children and adolescents interms of their dynamic functional connections (DFC) showed that network segregation in ASD individuals was significantly reduced within the default-mode network (DMN) during two transient states that were mainly concentrated in sensory/motor networks and higher-order cognitive networks.[31] Furthemore, Lee et al studied the fMRI during natural sleep and found greater dissimilarity between typically developing children and those with ASD in females than in males.[33] However, few studies have explored intrinsic dysconnectivity patterns within whole-brain functional networks at the voxel level in ASD children, which may represent an important contribution to elucidation of the neuropathophysiological mechanisms of children with ASD.

Degree Centrality (DC) is one class of graph-based network measures to assess the centrality or functional importance of individual elements in the brain by taking into account their relationships within the entire complex functional connectome at the voxel level, which has received extensive attention in recent years.[34,35] DC has also been widely used in studying various diseases, such as chronic users of codeine-containing cough syrups,[36] acute pontine infarcts and acute striato-capsular infarcts,[37] chronic migraine,[38] and type-2 diabetes mellitus.[39] Furthermore, using the DC metric in graph theory, previous studies have found abnormal DC values in patients with ASD.[40–44] For example, decreased DC in Broca's area in both children aged less than 12 years and adolescents aged 12–19 years with ASD have been reported by studying language networks in three age-related groups.[41] Additionally, Di Martino et al found increased DCs in bilateral temporolimbic areas in children with ASD aged 7.1–13.9 years.[40] However, many previous studies used DC have focused on adults or older children aged more than 5-years old with ASD, whereas less attention has been paid to children between aged 2.0–5.5 years.

In this study, in order to better understand intrinsic dysconnectivity patterns of whole-brain functional networks of children with ASD and their relationships with clinical features, we measured DC values in young children aged 2.0–5.5 years with ASD. In addition, we correlated changes in DCs with clinical variables in children with ASD. We hypothesized that (1) the ASD group would demonstrate abnormal DCs in some brain areas; and (2) the abnormal DCs in some brain areas might be related to alterations in social communication, interests, and behaviors in children with ASD.

Materials and Methods

Participants

The current study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). This prospective study was approved by the Ethics Committee of Shenzhen Children's Hospital. Written informed consent was obtained from each subject's parents before the investigation. A total of 140 subjects aged 2.0–5.5 years were recruited from November 2016 to April 2018 at Shenzhen Children's Hospital, (China), including 86 children with Low-Function ASD (developmental quotient (DQ) < 70) and 54 healthy subjects. All of the children fulfilled the

DSM-V criteria for the clinical interview diagnosis of ASD, and completed the Childhood Autism Rating Scale (CARS) and the Autism Behavior Checklist (ABC).[45] Each of the children with ASD was made a clinical diagnosis by Psychiatrists. All of the children with ASD were scanned upon diagnosis and before treatment. The exclusion criteria for children with ASD included seizure, intellectual disability, other developmental disability, brain trauma, hematological-system diseases, schizophrenia, any major medical illnesses, neurological diseases, a personal or family history of psychiatric disorders, routine MRI examination showing abnormalities, and any history of substance abuse. The inclusion criteria for the control subjects included no histories of neurological or psychiatric disorders.

Clinical Assessment Scales

CARS, ABC, and the Developmental Scale for Children Aged 0–6 years were used to evaluate the children with ASD before treatment, and these clinical assessment scales have been widely used in previous studies.[7,46,47] CARS scores were assessed by trained psychologists and consisted of 15 items on the scale, in which each item was rated from 1 to 4, and the maximum score was 60. A total score of less than 30 was classified as non-autism, a total score of 30–36 was considered mild-to-moderate autism, and a total score of 36 indicated severe autism. This scale is suitable for children over two years old. The ABC was completed by each child's parents. The ABC contains 57 items regarding the atypical behaviors of autistic individuals, which are related to five domains (sensory; relating; stereotypes and object use; language; and self-help and social). To determine the overall ABC scores, all of the items were summed. A total score of more than 30 and less than 67 indicated suspicious ASD symptoms, whereas a total score of 67 or more indicated ASD symptoms. This ABC scale is applicable to individuals aged 8 months to 28 years. The DQ of children with ASD was evaluated by the Developmental Scale for Children Aged 0–6 years (DQ < 70 as a low score); it consists of the following five items on the scale: gross motor; fine motor; ability to adapt; language; and social skills.

Data Acquisition

Before scanning, 0.5 mL/kg (maximum dose, 10 mL) of 0.5% chloral hydrate was given orally or via an enema to induce the maintenance of sleep. The subjects continued to sleep undisturbed during scanning.

All of the MRI datasets were obtained using a 3.0 T Siemens Skyra scanner in the Department of Radiology at Shenzhen Children's Hospital. Subjects' heads were fixed with foam pads to prevent head movement, and their ears were blocked with earplugs in order to prevent sleep disruption during scanning. Then, T2-weighted gradient echo-plane imaging was used to collect fMRI images. The parameters of the echo plane imaging sequence were as follows: repetition time (TR)/echo time (TE) = 2000 ms/30 ms, slice thickness/gap = 3.6 mm/0.72 mm, field of view (FOV) = 230 mm × 230 mm, matrix = 64 × 64, flip angle = 90°, and axial slices = 35. Interleaved scanning began in the plus direction with odd slices. Thirty-five axonal slices covering the whole brain were located approximately along the anterior commissure-posterior commissure (AC-PC) line, and 240 volumes were acquired in approximately 8 min. After the MRI scanning, the images of each participant were examined to ensure that the images met the requirements of the experiment. Meanwhile, sagittal structural images of T1-weighted sequences covering the whole brain (176 sagittal slices) were acquired using a 3D magnetization-prepared rapid acquisition gradient echo (MPRAGE). The corresponding acquisition parameters were as follows: TR/TE = 2300ms/2.26 ms, TI = 900 ms, flip angle = 8°, acquisition matrix = 256 × 256, FOV = 256 mm × 256 mm, and slice thickness/inter-slice gap = 1.0 mm/0.5 mm. In addition, T2-weighted images and T2-FLAIR images were obtained to exclude asymptomatic lesions.

Data Processing

Data were preprocessed using the Data Processing Assistant for Resting-State Functional MR Imaging toolbox (http://www.restfmri.net.sci-hub.cc/forum/DPARSF).[48] Before pretreatment, the first 10 volumes of each subject were discarded to keep the rest of the fMRI signals stable. The remaining 230 fMRI images for each subject were then subjected to slice-time correction for intra-volume acquisition time delay and realignment of intra-volume geometric displacements caused by head movement. In addition, the mean framewise displacement (FD) was calculated as a measure of the microhead motion for each subject to ensure that there were no significant differences between the two groups. Furthermore, individual's mean FD was retained as covariates for subsequent data processing to further reduce impacts of in-scanner

motion. Next, the T1 image for each subject was co-registered with the functional images of the same subject. This co-registered T1 image was then segmented and normalized to the standard MNI space. The corresponding deformation parameters were then used to normalize the functional images to the MNI space. White matter, cerebral spinal fluid, head motion parameters and global signals were entered as nuisance regressors. The normalized functional images were processed with spatial smoothing using a Gaussian Kernel of 6-mm full width at half maximum (FWHM) and removal of linear trends. Finally, the fMRI dataset was filtered using typical temporal bandpass (0.01–0.08 Hz) to reduce high-frequency respiratory and cardiac noise as well as low-frequency drift.

Degree-Centrality (DC) Calculations

According to previous studies,[34,35] DC refers to the number of direct connections to a given voxel in graphs based on voxels. DC has been widely used to describe the node characteristics of large-scale intrinsic connectivity networks in the brain. Specifically, the pre-processed fMRI data were used to perform voxel-based whole-brain correlation analysis. The time course of each voxel in each brain was related to every other voxel time course in the GM mask. We obtained an n × n matrix of Pearson's correlation coefficients between any pair of voxels, where n is the voxel number in the whole-brain mask. Next, the Pearson's correlation data were converted into a normally distributed Fisher z-score, and a whole brain functional network was constructed by setting each relevant threshold to $r > 0.25$.[49] The threshold is the default setting in calculating the DC map, and was selected to eliminate counting voxels with low temporal correlation due to signal noise. Buckner et al pointed out that different threshold choices did not qualitatively change the results.[49] The present study only considered the positive Pearson's correlation coefficients. For a given voxel, the DC was calculated by calculating the number of FCs with significant positive correlations at the individual level.[35,49]

Statistical Analysis

The difference in age between children with ASD and healthy subjects was assessed by a two-sample two-tailed *t*-test using SPSS statistical software (version 13.0). Sex differences between the two groups were determined by a two-tailed Pearson's chi-square test. In order to determine intergroup DC differences from the zDC map, a two-sample *t*-test was conducted between the two groups by using REST software. Meanwhile, the age and gender were included as covariates to reduce the influence on the results. Multiple comparisons were corrected by a Monte Carlo simulation using the AlphaSim program (http://afni.nimh.nih.gov).[50] We adopted a method of combining the height threshold ($p < 0.001$)[51] and the extent threshold ($p < 0.01$) in REST software to implement the AlphaSim program and obtained the corrected $p < 0.01$. Voxel-based analysis showed that the average DC values of all voxels in significantly different regions were extracted independently by time series extracted in REST and were then entered into SPSS. Then, a Pearson's correlation analysis was conducted to clarify the relationship of abnormal DC values in significantly different regions with clinical characteristics of children with ASD, including the total scores of ABC, DQ, and CARS as well as the subscales scores of ABC and DQ. Significance levels were set at $p < 0.05$ (two-tailed).

Results
Demographics and Clinical Characteristics

Details of the demographic data and corresponding clinical characteristics of the participants in this study are presented in Table 1. No significant differences were observed in terms of age ($t = -1.797$, $p = 0.076$) or sex distribution ($\chi2 = 0.814$, $p = 0.055$) between children with ASD and healthy subjects.

DC Analysis

A two-sample *t*-test revealed significantly decreased DC values within the right middle frontal gyrus (MFG) of children with ASD compared with those in healthy subjects ($p < 0.01$, AlphaSim corrected) (Table 2 and Figure 1, and the color bar in Figure 1 indicated the t value).

Table 1 Demographic and Clinical Characteristics of Children with ASD and of Healthy Subjects

Characteristic	ASD (n=86)	Healthy Subjects (n =54)	p-value	Chi-Square/t value
Sex (M/F)	76/10	47/7	0.055[a]	0.814
Age (years)	3.16±1.19	3.62±1.66	0.076[b]	−1.797
CARS total score	34.20±2.05	-	-	-
ABC total score	70.52±9.51	-	-	-
DQ total score	53.44±7.90	-	-	-
ABC SUB-SCALE				
Sensory	10.37±4.12	-	-	-
Relating	18.86±5.55	-	-	-
Stereotypes and object use	12.31±3.63	-	-	-
Language	14.90±3.01	-		
Self-help and social	14.08±2.72	-	-	-
DQ SUB-SCALE		-	-	-
Gross motor	70.64±12.29			
Fine motor	50.81±8.45	-	-	-
Ability to adapt	62.70±11.69	-	-	-
Language	46.93±12.07	-	-	-
Social skills	53.95±9.53	-	-	-

Notes: Values are means ± SDs. M, Male; F, Female. [a]The p-value was obtained using a chi-square test. [b]The p-value was obtained using a two-sample t-test.

Table 2 Brain Regions Showing Differences in Degree Centrality Between Children with ASD and Healthy Subjects

Brain Regions	MNI Coordinates			Voxels	T value
	x	y	z		
Right middle frontal gyrus	27	24	45	114	−4.0423

Note: The negative T value indicates the degree of decline in the ASD group.

Correlation results

Notably, the mean DC values within the right MFG observed in children with ASD showed significantly negative correlations with the total scores of ABC, and also with two ABC subscales measuring highly relevant impairments in ASD (ABC total scores: r =- 0.236, p = 0.029; stereotypes and object use: r =- 0.364, p = 0.001; language: r =- 0.320, p = 0.003.Figure 2). No other significant correlation was found between the mean DC value of the right MFG and any of the other clinical variables (CARS, DQ or any other subscale scores of the ABC and DQ) in children with ASD.

Discussion

In this study, we used DC analysis to describe the intrinsic dysconnectivity patterns of whole-brain functional networks in children with ASD. Significantly decreased DC values within the right MFG were found in children with ASD compared with those in healthy subjects. Furthermore, significantly negative correlations were established between the decreased average DC values within the right MFG in children with ASD and the total scores of ABC, as well as with two ABC subscales measuring highly relevant impairments in ASD (ie, stereotypes and object use behaviors, as well as difficulties in language).

Importantly, we found that children with ASD showed decreased DC values in the right MFG, which reflects decreased functional connections between this brain region and other brain networks and also reflects decreased centrality or importance of a specific voxel in the brain networks. The MFG has been proposed to be the focal point of the dorsal and ventral attention networks[52,53] and is mainly responsible for coordinating different messages.[7,52] The
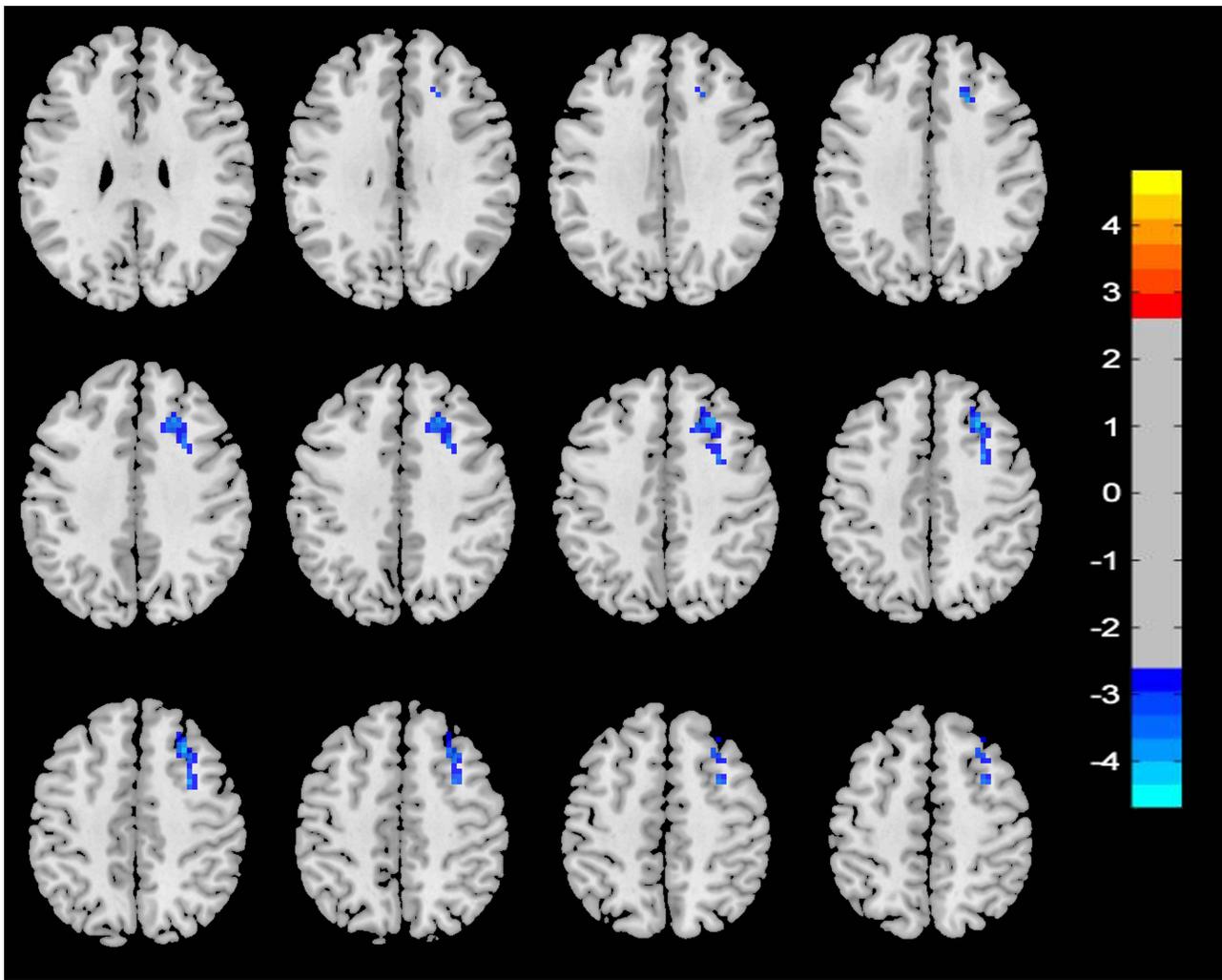
Figure 1 Brain regions showing decreased degree centrality within the right middle frontal gyrus in the autism spectrum disorder (ASD) group compared with that in healthy subjects. Regions showing decreased degree centrality in the axial map from Z = +30 to Z = +52 mm (every 2 mm) at the given threshold. The color bar indicated the t value.
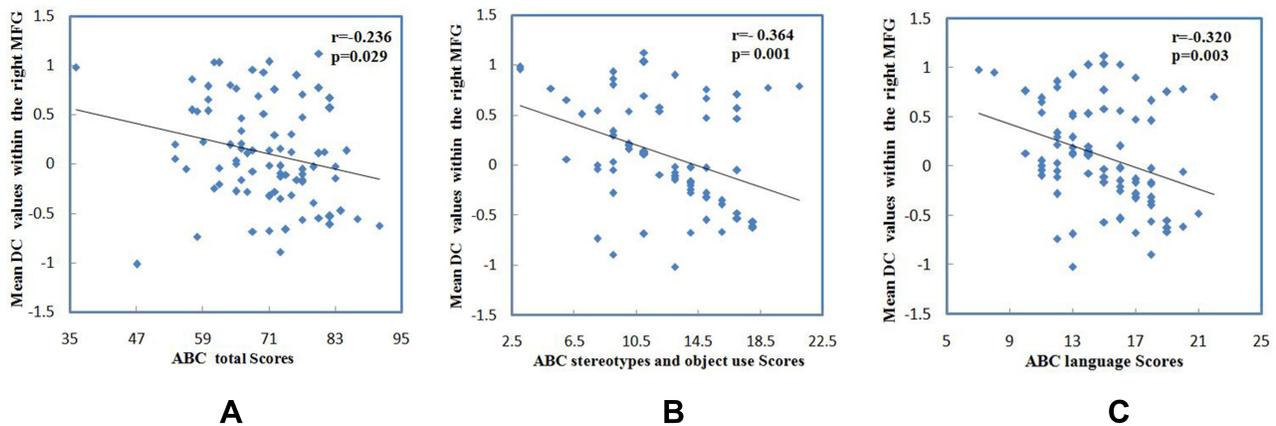

Figure 2 Significant negative correlations between the mean degree-centrality values within the right middle frontal gyrus and (**A**) the total scores of the Childhood Autism Rating Scale in children (ABC), and also with two ABC subscales measuring highly relevant impairments in children with ASD, namely, (**B**) stereotypes and object use behaviors, as well as (**C**) difficulties in language.

decreased direct connectivity of this brain region within the brain networks of children with ASD might indicate less efficient transmission of neural information in the brains of children with ASD compared with that in healthy subjects. Because of this decreased ability to integrate and process information, children with ASD might be unable to communicate normally in public places, which are consistent with previous studies.[7,54]

The MFG plays a key role in a host of high-level executive functions, such as self-control, rule learning and emotional regulation.[53,55–57] Perlman et al found that inadequate activation of the MFG in response to frustration was associated with irritability and emotional lability in non-autistic children.[58] Some other previous studies[57,59–61] have also suggested that the MFG plays a critical role in motor, emotional and cognitive self-regulation by guiding attention and initiating goal-directed behaviors in typical individuals. Therefore, we speculated that the abnormal DC values within the MFG that we found in the present study may contribute to the impairment of emotional and cognitive self-regulation, as well as stereotypical ASD behavior, in children with ASD. Moreover, the MFG is a key region involved in the formation of dorsolateral prefrontal cortical (dlPFC) circuits.[62,63] Specific dlPFC circuits have been shown to support self-regulation and habit learning,[62] which are associated with goal-oriented planning.[64,65] Additionally, dlPFC circuits play an important role in social motivation and their disruption has been postulated to represent the neural basis for social-interaction and communicative deficits in ASD.[66] Some previous studies[53,67,68] have found that increased GM volumes and numbers of neurons indicate structural abnormalities in dlPFC circuits in children with ASD. Luna et al found that the absence of spatial working memory in ASD was due to abnormalities in dlPFC circuits.[55] Some other previous studies[63,66] have also reported that interactions between the right MFG and the striatum are involved in the restricted interests and repetitive behaviors of ASD. Therefore, to some extent, impairments in social interaction and communication, as well as the limited interests and repetitive behaviors, of children with ASD may be explained by the functional variations in dlPFC circuits. However, the abnormal development of the right MFG, which is an important part of the dlPFC, may contribute to abnormal social characteristics, such as a lack of self-control and emotional modulation. Beyond all that, the MFG participates in forming default mode network (DMN), which is involved in the monitoring of internal processes, including internal and external cognition, self-monitoring, and autobiographical as well,[69] and plays an important role in expressing emotions and social processes.[70] The DMN is very important for the social understanding of others', however, the DMN dysfunction is an important component of social impairments in ASD.[71] Wang et al also found that the intensity of the FC between networks in the DMN was associated with the severity of autistic characteristics.[72] Our present finding of decreased DC values in the right MFG of children with ASD may help further elucidate the mechanisms underlying ASD and the role of the right MFG in this process.

Remarkably, the brain is strongly lateralized in adults and older children, such that the right hemisphere has been reported to be larger than the left hemisphere,[53,73] and the right hemisphere has been shown to be predominantly involved in emotional processing and working-memory-related patterns.[74] Barbey et al demonstrated that the right dlPFC is essential for manipulating information in a wider context of reasoning.[56] A previous spectroscopic study[75] suggested that the right prefrontal cortex is a major contributor to psychopathology in young children with ASD at the level of functional network structure. Consistent with previous studies, our present results suggest a right lateralization of the brain in children with ASD.

In the current study, we found that the average DC values within the right MFG in children with ASD were negatively correlated with ABC total scores, which may indicate that lower DC values within the right MFG of children with ASD are indicatives of more severe behavioral symptoms. Consistent with our findings, Qian et al suggested that the dysfunction of the right MFG was closely related to the diversity and severity of symptoms experienced in children with ASD.[53] In addition, a mounting body of evidence has suggested that several behavioral symptoms in ASD are the result of "network disorders", which are associated with high-level cognitive tasks, such as working memory, mental flexibility and language.[76] Grecucci et al also found a broad structural network that they called the "Autism-specific Structural Network" (ASN), which was different between adults with ASD and healthy controls.[77] Particularly, previous studies[52,53] have indicated that the right MFG is a critical part of different types of brain networks. Our present findings may provide a better understanding of the neural basis of ASD, which may aid in identifying targets for further ASD research and related therapeutic interventions.

In this study, we found that the average DC values within the right MFG in children with ASD were negatively correlated with ABC stereotypes and object-use behavioral scores, which may indicate that lower DC values within the right MFG are indicative of more dysfunctional stereotypes and object-use behaviors in children with ASD. Similar to our results, Grecucci et al suggested that the loading coefficient of the ASN, which contains the MFG, was significantly correlated with Autism Diagnostic Observation Schedule (ADOS) stereotypic behavioral scores.[77] In addition, Powell et al reported that there was a significantly positive correlation between the severity of motor stereotypy and the degree of ASD symptomology.[78] Previous studies[79–81] have also suggested that motor-stereotypy behaviors exhibited by children with ASD may be due to defects in executive functioning (EF), which is responsible for planning, inhibiting aggressive or inappropriate behavioral responses, and performing appropriate responses. Additionally, the frontal lobes are essential for intact EF capacities.[81] In particular, the MFG plays a crucial role in a host of high-level executive functions, such as self-control and set-shifting.[53,56,57] Taken together, our present findings may provide an explanation for the dysfunctional stereotypes and object-use behaviors in children with ASD.

In addition, in our study, the average DC values within the right MFG in were negatively correlated with ABC language scores, which may indicate that lower DC values within the right MFG are predictive of more serious communicative dysfunction in children with ASD. Language deficits mainly refer to delays in speech onset and literal language interpretation, as well as poor recognition of social and emotional cues in language,[82] all of which are present in children with ASD. Previous fMRI studies have shown that ASD impairments are often attributed to changes in connections between different brain regions[83] or abnormal activation in some brain regions.[84,85] Chen et al suggested that normally developing boys tend to perform high-level semantic feature retrieval and selection when making semantic judgments, while boys with ASD tend to perform low-level visual processing during these processes.[86] Consistent with this finding, Murdaugh et al also reported that in addition to recruitment of typical language networks, children with ASD were inclined to recruit additional visual processing in language-comprehension tasks to aid in language comprehension.[87] Specifically, visuospatial skills are related to the specialization of the right hemisphere,[88] and the right MFG is an important part of visual networks, which are associated with atypical visual information processing in ASD.[7,85] Therefore, our present results suggest that decreased DC values within the right MFG may be related to communicative dysfunction in children with ASD.

Our study had some limitations. First, all of the children with ASD were completed the CARS and the ABC for diagnosising of ASD, but there was no implementation of ADOS or Autism Diagnostic Interview-Revised (ADI-R) which was regarded as "gold standard" by many countries to confirm the diagnosis of ASD. Second, DC can only identify brain regions showing abnormal functional connectivities and cannot provide detailed information about the functional connections. Therefore, future studies should trace the brain sites to which these abnormal functional connectivities are linked. Third, our study was cross-sectional, and all of the subjects were preschoolers. Therefore, the interpretation of our findings may not be appropriate for other development stages. Longitudinal datasets with large sample sizes are warranted for a more comprehensive description of the precise nature of the underlying neural-circuits contributing to ASD, and ideally such studies would be sensitive to subtle changes over time within neural circuits in ASD over the human lifespan. Fourth, our subjects were in a state of sedation induced by chloral hydrate in our study. However, previous studies[89,90] on the effects of the sleep-induced states of chloral hydrate on the brain have been controversial. For example, Wei et al found that sedation-induced by chloral hydrate reduced functional interaction within the brain.[89] However, Doria et al compared sedated infants and deep-sleeping infants found no significant differences.[90] Therefore, we speculate that our results may only be applicable for children who are sleeping-state.

Conclusions

In the current study, we used chloral hydrate induced sleeping-state fMRI-derived DC values to investigate abnormal intrinsic connectivity patterns within whole-brain functional networks in children with ASD. Our results revealed significantly decreased DC values within the right MFG in children with ASD. Moreover, significant negative correlations were established between the decreased average DC values within the right MFG and the total scores of ABC, and also with two ABC subscales measuring highly relevant impairments in ASD (ie, stereotypes and object-use behaviors, as well as difficulties in language). Taken together, our findings suggest a novel approach to explore the neural correlates

of deficits in social interactions and communication, as well as the limited interests and repetitive behaviors, of preschoolers with ASD. Specifically, our findings suggest that abnormal DC values may represent an important contribution to elucidating the neuropathophysiological mechanisms of preschoolers with ASD.

Disclosure

The authors report no conflicts of interest in this work.

References

1. American Psychiatric Association [APA]. *The Diagnostic and Statistical Manual of Mental Disorders: DSM 5*. Washington, DC: American Psychiatric Association; 2013.
2. Bent CA, Barbaro J, Dissanayake C. Change in autism diagnoses prior to and following the introduction of DSM-5. *J Autism Dev Disord*. 2017;47 (1):163–171. doi:10.1007/s10803-016-2942-y
3. Lukito S, Jones CRG, Pickles A, et al. Specificity of executive function and theory of mind performance in relation to attention-deficit/hyperactivity symptoms in autism spectrum disorders. *Mol Autism*. 2017;8:60. doi:10.1186/s13229-017-0177-1
4. Bölte S, Girdler S, Marschik PB. The contribution of environmental Bucknerexposure to the etiology of autism spectrum disorder. *Cell Mol Life Sci*. 2019;76(7):1275–1297. doi:10.1007/s00018-018-2988-4
5. Jonsson U, Alaie I, Löfgren WA, et al. Annual research review: quality of life and childhood mental and behavioural disorders - a critical review of the research. *J Child Psychol Psychiatry*. 2017;58(4):439–469. doi:10.1111/jcpp.12645
6. Borràs-Ferrís L, Pérez-Ramírez Ú, Moratal D. Link-level functional connectivity neuroalterations in autism spectrum disorder: a developmental resting-state fMRI study. *Diagnostics*. 2019;9(1):32. doi:10.3390/diagnostics9010032
7. Xu S, Li M, Yang C, et al. Altered functional connectivity in children with low-function autism spectrum disorders. *Front Neurosci*. 2019;13:806. doi:10.3389/fnins.2019.00806
8. Zürcher NR, Bhanot A, McDougle CJ, Hooker JM. A systematic review of molecular imaging (PET and SPECT) in autism spectrum disorder: current state and future research opportunities. *Neurosci Biobehav Rev*. 2015;52:56–73. doi:10.1016/j.neubiorev.2015.02.002
9. Bjørklund G, Kern JK, Urbina MA, et al. Cerebral hypoperfusion in autism spectrum disorder. *Acta Neurobiol Exp*. 2018;78(1):21–29. doi:10.21307/ane-2018-005
10. Anil KBN, Malhotra S, Bhattacharya A, Grover S, Batra YK. Regional cerebral glucose metabolism and its association with phenotype and cognitive functioning in patients with autism. *Indian J Psychol Med*. 2017;39(3):262–270. doi:10.4103/0253-7176.207344
11. Hwang BJ, Mohamed MA, Brašić JR. Molecular imaging of autism spectrum disorder. *Int Rev Psychiatry*. 2017;29(6):530–554. doi:10.1080/09540261.2017.1397606
12. Żarnowska I, Chrapko B, Gwizda G, Nocuń A, Mitosek-Szewczyk K, Gasior M. Therapeutic use of carbohydrate-restricted diets in an autistic child; a case report of clinical and 18FDG PET findings. *Metab Brain Dis*. 2018;33(4):1187–1192. doi:10.1007/s11011-018-0219-1
13. Masuda F, Nakajima S, Miyazaki T, et al. Motor cortex excitability and inhibitory imbalance in autism spectrum disorder assessed with transcranial magnetic stimulation: a systematic review. *Transl Psychiatry*. 2019;9(1):110. doi:10.1038/s41398-019-0444-3
14. O'Neill J, Bansal R, Goh S, Rodie M, Sawardekar S, Peterson BS. Parsing the heterogeneity of brain metabolic disturbances in autism spectrum disorder. *Biol Psychiatry*. 2020;87(2):174–184. doi:10.1016/j.biopsych.2019.06.010
15. Port RG, Oberman LM, Roberts TP. Revisiting the excitation/inhibition imbalance hypothesis of ASD through a clinical lens. *Br J Radiol*. 2019;92 (1101):20180944. doi:10.1259/bjr.20180944
16. Pretzsch CM, Freyberg J, Voinescu B, et al. Effects of cannabidiol on brain excitation and inhibition systems; a randomised placebo-controlled single dose trial during magnetic resonance spectroscopy in adults with and without autism spectrum disorder. *Neuropsychopharmacology*. 2019;44 (8):1398–1405. doi:10.1038/s41386-019-0333-8
17. Goddard MN, van Rijn S, Rombouts SA, Swaab H. White matter microstructure in a genetically defined group at increased risk of autism symptoms, and a comparison with idiopathic autism: an exploratory study. *Brain Imaging Behav*. 2016;10(4):1280–1288. doi:10.1007/s11682-015-9496-z
18. Lu C, Qi Z, Harris A, et al. Shared neuroanatomical substrates of impaired phonological working memory across reading disability and autism. *Biol Psychiatry Cogn Neurosci Neuroimaging*. 2016;1(2):169–177. doi:10.1016/j.bpsc.2015.11.001
19. Ouyang M, Cheng H, Mishra V, et al. Atypical age-dependent effects of autism on white matter microstructure in children of 2–7 years. *Hum Brain Mapp*. 2016;37(2):819–832. doi:10.1002/hbm.23073

20. Li Y, Fang H, Zheng W, et al. A fiber tractography study of social-emotional related fiber tracts in children and adolescents with autism spectrum disorder. *Neurosci Bull*. 2017;33(6):722–730. doi:10.1007/s12264-017-0155-9

21. Wilkinson M, Wang R, van der Kouwe A, Takahashi E. White and gray matter fiber pathways in autism spectrum disorder revealed by ex vivo diffusion MR tractography. *Brain Behav*. 2016;6(7):e00483. doi:10.1002/brb3.483

22. Zhang F, Savadjiev P, Cai W, et al. Whole brain white matter connectivity analysis using machine learning: an application to autism. *Neuroimage*. 2018;172:826–837. doi:10.1016/j.neuroimage.2017.10.029

23. Carlisi CO, Norman LJ, Lukito SS, Radua J, Mataix-Cols D, Rubia K. Comparative multimodal meta-analysis of structural and functional brain abnormalities in autism spectrum disorder and obsessive-compulsive disorder. *Biol Psychiatry*. 2017;82(2):83–102. doi:10.1016/j.biopsych.2016.10.006

24. Ni HC, Lin HY, Tseng WI, et al. Neural correlates of impaired self-regulation in male youths with autism spectrum disorder: a voxel-based morphometry study. *Prog Neuropsychopharmacol Biol Psychiatry*. 2018;82:233–241. doi:10.1016/j.pnpbp.2017.11.008

25. Pappaianni E, Siugzdaite R, Vettori S, Venuti P, Job R, Grecucci A. Three shades of grey: detecting brain abnormalities in children with autism using source-, voxel- and surface-based morphometry. *Eur J Neurosci*. 2018;47(6):690–700. doi:10.1111/ejn.13704

26. Jann K, Hernandez LM, Beck-Pancer D, et al. Altered resting perfusion and functional connectivity of default mode network in youth with autism spectrum disorder. *Brain Behav*. 2015;5(9):e00358. doi:10.1002/brb3.358

27. Dajani DR, Uddin LQ. Local brain connectivity across development in autism spectrum disorder: a cross-sectional investigation. *Autism Res*. 2016;9(1):43–54. doi:10.1002/aur.1494

28. Li G, Rossbach K, Jiang W, Du Y. Resting-state brain activity in Chinese boys with low functioning autism spectrum disorder. *Ann Gen Psychiatry*. 2018;17(1):47. doi:10.1186/s12991-018-0217-z

29. Ciarrusta J, O'Muircheartaigh J, Dimitrova R, et al. Social brain functional maturation in newborn infants with and without a family history of autism spectrum disorder. *JAMA Netw Open*. 2019;2(4):e191868. doi:10.1001/jamanetworkopen.2019.1868

30. Funakoshi Y, Harada M, Otsuka H, Mori K, Ito H, Iwanaga T. Default mode network abnormalities in children with autism spectrum disorder detected by resting-state functional magnetic resonance imaging. *J Med Invest*. 2016;63(3–4):204–208. doi:10.2152/jmi.63.204

31. Mash LE, Linke AC, Olson LA, Fishman I, Liu TT, Müller RA. Transient states of network connectivity are atypical in autism: a dynamic functional connectivity study. *Hum Brain Mapp*. 2019;40(8):2377–2389. doi:10.1002/hbm.24529

32. Reiter MA, Mash LE, Linke AC, Fong CH, Fishman I, Müller RA. Distinct patterns of atypical functional connectivity in lower-functioning autism. *Biol Psychiatry Cogn Neurosci Neuroimaging*. 2019;4(3):251–259. doi:10.1016/j.bpsc.2018.08.009

33. Lee JK, Amaral DG, Solomon M, Rogers SJ, Ozonoff S, Nordahl CW. Sex differences in the amygdala resting-state connectome of children with autism spectrum disorder. *Biol Psychiatry Cogn Neurosci Neuroimaging*. 2020;5(3):320–329. doi:10.1016/j.bpsc.2019.08.004

34. Wang JH, Zuo XN, Gohel S, Milham MP, Biswal BB, He Y. Graph theoretical analysis of functional brain networks: test-retest evaluation on short- and long-term resting-state functional MRI data. *PLoS One*. 2011;6(7):e21976. doi:10.1371/journal.pone.0021976

35. Zuo XN, Ehmke R, Mennes M, et al. Network centrality in the human functional connectome. *Cereb Cortex*. 2012;22(8):1862–1875. doi:10.1093/cercor/bhr269

36. Hua K, Wang T, Li C, et al. Abnormal degree centrality in chronic users of codeine-containing cough syrups: a resting-state functional magnetic resonance imaging study. *Neuroimage Clin*. 2018;19:775–781. doi:10.1016/j.nicl.2018.06.003

37. Liu G, Tan S, Peng K, et al. Network change in the ipsilesional cerebellum is correlated with motor recovery following unilateral pontine infarction. *Eur J Neurol*. 2019;26(10):1266–1273. doi:10.1111/ene.13974

38. Lee MJ, Park BY, Cho S, Kim ST, Park H, Chung CS. Increased connectivity of pain matrix in chronic migraine: a resting-state functional MRI study. *J Headache Pain*. 2019;20(1):29. doi:10.1186/s10194-019-0986-z

39. Hu B, Yan LF, Sun Q, et al. Disturbed neurovascular coupling in type 2 diabetes mellitus patients: evidence from a comprehensive fMRI analysis. *Neuroimage Clin*. 2019;22:101802. doi:10.1016/j.nicl.2019.101802

40. Di Martino A, Zuo XN, Kelly C, et al. Shared and distinct intrinsic functional network centrality in autism and attention-deficit/hyperactivity disorder. *Biol Psychiatry*. 2013;74(8):623–632. doi:10.1016/j.biopsych.2013.02.011

41. Lee Y, Park BY, James O, Kim SG, Park H. Autism spectrum disorder related functional connectivity changes in the language network in children, adolescents and adults. *Front Hum Neurosci*. 2017;11:418. doi:10.3389/fnhum.2017.00418

42. Sato JR, Calebe Vidal M, de Siqueira Santos S, Brauer Massirer K, Fujita A. Complex network measures in autism spectrum disorders. *IEEE ACM Trans Comput Biol Bioinform*. 2018;15(2):581–587. doi:10.1109/TCBB.2015.2476787

43. Wan B, Wang Z, Jung M, et al. Effects of the co-occurrence of anxiety and attention-deficit/hyperactivity disorder on intrinsic functional network centrality among children with autism spectrum disorder. *Autism Res*. 2019;12(7):1057–1068. doi:10.1002/aur.2120

44. Sadria M, Karimi S, Layton AT. Network centrality analysis of eye-gaze data in autism spectrum disorder. *Comput Biol Med*. 2019;111:103332. doi:10.1016/j.compbiomed.2019.103332

45. Krug DA, Arick J, Almond P. Behavior checklist for identifying severely handicapped individuals with high levels of autistic behavior. *J Child Psychol Psychiatry*. 1980;21(3):221–229. doi:10.1111/j.1469-7610.1980.tb01797.x

46. Liu J, Liu X, Xiong XQ, et al. Effect of vitamin A supplementation on gut microbiota in children with autism spectrum disorders - a pilot study. *BMC Microbiol*. 2017;17(1):204. doi:10.1186/s12866-017-1096-1

47. Liu GH, Huang LS, Qian QF, Wang YX, Ge P. [Curative effect of progressively integrated sandplay therapy on core symptoms and sleep management in preschool children with mild-to-moderate autism spectrum disorder]. *Zhongguo Dang Dai Er Ke Za Zhi* [Chinese journal of contemporary pediatrics]. 2019;21(8):743–748. Chinese.

48. Yan CG, Zang YF. DPARSF: a MATLAB toolbox for "pipeline" data analysis of resting-state fMRI. *Front Syst Neurosci*. 2010;4:13. doi:10.3389/fnsys.2010.00013

49. Buckner RL, Sepulcre J, Talukdar T, et al. Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. *J Neurosci*. 2009;29(6):1860–1873. doi:10.1523/JNEUROSCI.5062-08.2009

50. Ledberg A, Akerman S, Roland PE. Estimation of the probabilities of 3D clusters in functional brain images. *Neuroimage*. 1998;8(2):113–128. doi:10.1006/nimg.1998.0336

51. Eklund A, Nichols TE, Knutsson H. Cluster failure: why fMRI inferences for spatial extent have inflated false-positive rates. *Proc Natl Acad Sci U S A*. 2016;113(28):7900–7905. doi:10.1073/pnas.1602413113

52. Japee S, Holiday K, Satyshur MD, Mukai I, Ungerleider LG. A role of right middle frontal gyrus in reorienting of attention: a case study. *Front Syst Neurosci*. 2015;9:23. doi:10.3389/fnsys.2015.00023

53. Qian L, Wang Y, Chu K, et al. Alterations in hub organization in the white matter structural network in toddlers with autism spectrum disorder: a 2-year follow-up study. *Autism Res*. 2018;11(9):1218–1228. doi:10.1002/aur.1983

54. Mohd Roffeei SH, Abdullah N, Basar SK. Seeking social support on Facebook for children with Autism Spectrum Disorders (ASDs). *Int J Med Inform*. 2015;84(5):375–385. doi:10.1016/j.ijmedinf.2015.01.015

55. Luna B, Minshew NJ, Garver KE, et al. Neocortical system abnormalities in autism: an fMRI study of spatial working memory. *Neurology*. 2002;59 (6):834–840. doi:10.1212/WNL.59.6.834

56. Barbey AK, Koenigs M, Grafman J. Dorsolateral prefrontal contributions to human working memory. *Cortex*. 2013;49(5):1195–1205. doi:10.1016/j.cortex.2012.05.022

57. Bennett RH, Somandepalli K, Roy AK, Di Martino A. The neural correlates of emotional lability in children with autism spectrum disorder. *Brain Connect*. 2017;7(5):281–288. doi:10.1089/brain.2016.0472

58. Perlman SB, Jones BM, Wakschlag LS, Axelson D, Birmaher B, Phillips ML. Neural substrates of child irritability in typically developing and psychiatric populations. *Dev Cogn Neurosci*. 2015;14:71–80. doi:10.1016/j.dcn.2015.07.003

59. Sylvester CY, Wager TD, Lacey SC, et al. Switching attention and resolving interference: fMRI measures of executive functions. *Neuropsychologia*. 2003;41(3):357–370. doi:10.1016/S0028-3932(02)00167-7

60. Grecucci A, Giorgetta C, Bonini N, Sanfey AG. Reappraising social emotions: the role of inferior frontal gyrus, temporo-parietal junction and insula in interpersonal emotion regulation. *Front Hum Neurosci*. 2013;7:523. doi:10.3389/fnhum.2013.00523

61. Depue BE, Orr JM, Smolker HR, Naaz F, Banich MT. The organization of right prefrontal networks reveals common mechanisms of inhibitory regulation across cognitive, emotional, and motor processes. *Cereb Cortex*. 2016;26(4):1634–1646. doi:10.1093/cercor/bhu324

62. Berner LA, Marsh R. Frontostriatal circuits and the development of bulimia nervosa. *Front Behav Neurosci*. 2014;8:395. doi:10.3389/fnbeh.2014.00395

63. Akkermans S, Rheinheimer N, Bruchhage M, et al. Frontostriatal functional connectivity correlates with repetitive behaviour across autism spectrum disorder and obsessive-compulsive disorder. *Psychol Med*. 2019;49(13):2247–2255. doi:10.1017/S0033291718003136

64. Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci*. 1986;9:357–381. doi:10.1146/annurev.ne.09.030186.002041

65. Vaghi MM, Vértes PE, Kitzbichler MG, et al. Specific frontostriatal circuits for impaired cognitive flexibility and goal-directed planning in obsessive-compulsive disorder: evidence from resting-state functional connectivity. *Biol Psychiatry*. 2017;81(8):708–717. doi:10.1016/j.biopsych.2016.08.009

66. Delmonte S, Gallagher L, O'Hanlon E, McGrath J, Balsters JH. Functional and structural connectivity of frontostriatal circuitry in Autism Spectrum Disorder. *Front Hum Neurosci*. 2013;7:430. doi:10.3389/fnhum.2013.00430

67. Courchesne E, Mouton PR, Calhoun ME, et al. Neuron number and size in prefrontal cortex of children with autism. *JAMA*. 2011;306 (18):2001–2010. doi:10.1001/jama.2011.1638

68. Kaushik G, Zarbalis KS. Prenatal neurogenesis in autism spectrum disorders. *Front Chem*. 2016;4:12. doi:10.3389/fchem.2016.00012

69. Zhou C, Yu M, Tang X, et al. Convergent and divergent altered patterns of default mode network in deficit and non-deficit schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*. 2019;89:427–434. doi:10.1016/j.pnpbp.2018.10.012

70. Wang W, Liu J, Shi S, et al. Altered resting-state functional activity in patients with autism spectrum disorder: a quantitative meta-analysis. *Front Neurol*. 2018;9:556. doi:10.3389/fneur.2018.00556

71. Padmanabhan A, Lynch CJ, Schaer M, Menon V. The default mode network in autism. *Biol Psychiatry Cogn Neurosci Neuroimaging*. 2017;2 (6):476–486. doi:10.1016/j.bpsc.2017.04.004

72. Wang K, Xu M, Ji Y, et al. Altered social cognition and connectivity of default mode networks in the co-occurrence of autistic spectrum disorder and attention deficit hyperactivity disorder. *Aust N Z J Psychiatry*. 2019;53(8):760–771. doi:10.1177/0004867419836031

73. Herbert MR, Ziegler DA, Deutsch CK, et al. Brain asymmetries in autism and developmental language disorder: a nested whole-brain analysis. *Brain*. 2005;128(Pt 1):213–226. doi:10.1093/brain/awh330

74. Yang LC, Ren P, Ma YY. Anodal transcranial direct-current stimulation over the right dorsolateral prefrontal cortex influences emotional face perception. *Neurosci Bull*. 2018;34(5):842–848. doi:10.1007/s12264-018-0242-6

75. Li Y, Yu D. Weak network efficiency in young children with Autism Spectrum Disorder: evidence from a functional near-infrared spectroscopy study. *Brain Cogn*. 2016;108:47–55. doi:10.1016/j.bandc.2016.07.006

76. Urbain C, Vogan VM, Ye AX, Pang EW, Doesburg SM, Taylor MJ. Desynchronization of fronto-temporal networks during working memory processing in autism. *Hum Brain Mapp*. 2016;37(1):153–164. doi:10.1002/hbm.23021

77. Grecucci A, Rubicondo D, Siugzdaite R, Surian L, Job R. Uncovering the social deficits in the autistic Brain. A source-based morphometric study . *Front Neurosci*. 2016;10:388.

78. Powell JL, Pringle L, Greig M. Investigation of the association between motor stereotypy behavior with fundamental movement skills, adaptive functioning, and autistic spectrum disorder symptomology in children with intellectual disabilities. *J Child Neurol*. 2017;32(2):222–229. doi:10.1177/0883073816678551

79. Hill EL. Executive dysfunction in autism. *Trends Cogn Sci*. 2004;8(1):26–32. doi:10.1016/j.tics.2003.11.003

80. Lopez BR, Lincoln AJ, Ozonoff S, Lai Z. Examining the relationship between executive functions and restricted, repetitive symptoms of Autistic Disorder. *J Autism Dev Disord*. 2005;35(4):445–460. doi:10.1007/s10803-005-5035-x

81. Shafritz KM, Dichter GS, Baranek GT, Belger A. The neural circuitry mediating shifts in behavioral response and cognitive set in autism. *Biol Psychiatry*. 2008;63(10):974–980. doi:10.1016/j.biopsych.2007.06.028

82. Anderson JS, Lange N, Froehlich A, et al. Decreased left posterior insular activity during auditory language in autism. *AJNR Am J Neuroradiol*. 2010;31(1):131–139. doi:10.3174/ajnr.A1789

83. Sharda M, Khundrakpam BS, Evans AC, Singh NC. Disruption of structural covariance networks for language in autism is modulated by verbal ability. *Brain Struct Funct*. 2016;221(2):1017–1032. doi:10.1007/s00429-014-0953-z

84. Boddaert N, Belin P, Chabane N, et al. Perception of complex sounds: abnormal pattern of cortical activation in autism. *Am J Psychiatry*. 2003;160 (11):2057–2060. doi:10.1176/appi.ajp.160.11.2057

85. Itahashi T, Yamada T, Watanabe H, et al. Alterations of local spontaneous brain activity and connectivity in adults with high-functioning autism spectrum disorder. *Mol Autism*. 2015;6(1):30. doi:10.1186/s13229-015-0026-z

86. Chen PJ, Gau SS, Lee SH, Chou TL. Differences in age-dependent neural correlates of semantic processing between youths with autism spectrum disorder and typically developing youths. *Autism Res*. 2016;9(12):1263–1273. doi:10.1002/aur.1616

87. Murdaugh DL, Deshpande HD, Kana RK. The impact of reading intervention on brain responses underlying language in children with autism. *Autism Res*. 2016;9(1):141–154. doi:10.1002/aur.1503

88. Floris DL, Lai MC, Auer T, et al. Atypically rightward cerebral asymmetry in male adults with autism stratifies individuals with and without language delay. *Hum Brain Mapp*. 2016;37(1):230–253. doi:10.1002/hbm.23023

89. Wei Z, Alcauter S, Jin K, Peng ZW, Gao W. Graph theoretical analysis of sedation's effect on whole brain functional system in school-aged children. *Brain Connect*. 2013;3(2):177–189. doi:10.1089/brain.2012.0125

90. Doria V, Beckmann CF, Arichi T, et al. Emergence of resting state networks in the preterm human brain. *Proc Natl Acad Sci U S A*. 2010;107 (46):20015–20020. doi:10.1073/pnas.1007921107