FOXP2 down expression is associated with executive dysfunctions and electrophysiological abnormalities of brain in Autism spectrum disorder; a neuroimaging genetic study

**Abstract**

**Background and aims:** Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by language impairment, and challenges with social interaction, communication, and repetitive behaviors. Although genetics are a primary cause of ASD, the exact genes and molecular mechanisms involved in its pathogenesis are not completely clear. The FOXP2 gene encodes a transcription factor that is known for its major role in language development and severe speech problems. The present study aimed to evaluate the role of FOXP2 in ASD etiology, executive functions, and brain activities.

**Methods:** In the present study, we recruited 450 children with ASD and 490 neurotypical control children. Three domains of executive functions (working memory, response inhibition, and vigilance) were assessed. In addition, five-
minute eyes closed electroencephalography was obtained from some of the children with ASD and neurotypical children. DNA sequence and expression level of FOXP2 in blood samples of children with ASD and the control group were evaluated by using sequencing and Real-time PCR, respectively.

**Results:** The results showed no mutations but a significant down expression of FOXP2 genes in children with ASD vs. neurotypical children. Several cognitive and executive function deficiencies were detected in children with ASD. Low alpha and gamma bands in the frontal lobe and high theta bands in the occipital lobe were revealed in children with ASD. We also found several correlations between FOXP2 expression levels and clinical assessments.

**Conclusions:** Our finding revealed the down expression of FOXP2, which could be considered as a biomarker for ASD as well as cognitive and executive dysfunction. Based on brain mapping data, FOXP2 may be related to the theta wave abnormality of children with ASD. FOXP2 may be considered a target of novel treatment to improve memory and executive functions.

**Implications:** Our findings highlight the role of FOXP2 mRNA level in ASD etiology, executive functions, and brain wave frequencies.

**Keywords**
ASD, executive function, electroencephalography, FOXP2, sequencing, gene expression

**Introduction**

Autism spectrum disorder (ASD) is a complex neuropsychiatric disorder with onset in early childhood characterized by social and communication deficits, language impairment, and ritualistic/repetitive behavior. The prevalence of ASD has been estimated at 1 in every 54 children in the United States and is more prevalent among boys compared to girls (Knopf, 2020). ASD has shown heterogeneity in its etiology, clinical phenotype expression, and severity in cognitive dysfunction (Rutter & Schopler, 2012). Different degrees of cognitive impairment, particularly executive function disabilities, are often found among children with ASD (Nydén, 2000; Rutter & Schopler, 2012; Wallace et al., 2016). These executive functions (EFs) include high-order cognitive abilities such as working memory, inhibitory control, cognitive flexibility, planning, reasoning, and problem-solving (Pennington & Ozonoff, 1996; Spear, 2010).

ASD is highly heritable (approximately 80%), and several common genetic variants contribute substantially to ASD susceptibility (Bai et al., 2019). Several genes in different chromosome regions are related to neurodevelopment, excitatory and inhibitory neuron functions, synaptogenesis, and the immune system. In addition, various biological processes are involved in ASD etiology and symptom severity (Ansel et al., 2017; Yang & Gill, 2007).

Executive dysfunction, including abnormalities in sustained attention, response inhibition, and error monitoring has a major impact on quality of life and is prevalent in a wide range of neurodegenerative and neurodevelopmental disorders. While most of the disorders with major executive function deficits are highly heritable, it seems that clarification of the genetic architecture of executive function in both clinical and non-clinical populations may lead to more advanced and precise therapy in a wide range of psychiatric disorders. Dopamine, serotonin, and noradrenaline are considered neuromodulators of executive functions and associations between dopamine pathway genes such as monoamine oxidase A, D2, and D4 dopamine receptors, and dopamine transporter gene (DAT1) and sustained attention, performance monitoring, and error processing and inhibitory control have been reported (Barnes et al., 2011).

Regions both within and outside of the frontal lobes are implicated as important in cognitive control and executive functional interactions. Several links between schizophrenia genetic risk factors and characteristic executive function deficits have been reported (Eisenberg & Berman, 2010). A genome-wide association study of executive function in children with ADHD identified one significant variant, rs852004, an intergenic variant between ESR1 and CCDC170 genes, related to executive dysfunctions. Genetic and functional magnetic resonance imaging (fMRI) analyses revealed that the presence of this variant may lead to a specific executive function pattern, including inhibiting and monitoring components and activity of inhibitory control-related brain regions (Sun et al., 2018). Further studies with a neuroimaging-genetic approach may provide new insights into the genetic basis of executive function in children with ASD.

Transcription factors expression alteration in children with ASD, as well as other psychiatric disorders, is responsible for the dysregulation of the immune system, cell signaling, bioenergetics system, and synaptogenesis. Although it has been reported that the restoration of transcription factor signaling may have great therapeutic potential in the treatment of children with ASD, the role of many transcription factors in ASD is still unclear (Ahmad et al., 2017).

While more than 1000 genes have been related to ASD risk, the pathogenicity and specific contribution of many of these genes are not clear. More than 1600 transcription
factor exist in the human genome that are members of the proteome and the gene expression regulation system as well. Attention to the issue of transcription factors regulation is mounting in inheritable disorders with genetic complexity, due to a better understanding of the etiology of the disorder and potential targeted treatments (Ayhan & Konopka, 2019).

Overlaps have been reported between transcription factors related to ASD and other neurodevelopmental disorders, such as common regulatory pathways, including chromatin remodeling, transcription, and alternative splicing. However, the differences between the neurobiology of ASD and other neurodevelopmental disorders could be due to the specific role of several transcription factor families such as the FOXP family (Bowers & Konopka, 2012).

Forkhead box protein P2 is a human protein encoded by the FOXP2 gene located on human chromosome 7. FOXP2 is a member of the forkhead box family of transcription factors and proteins, which are involved in regulating several gene expressions through binding to DNA (Becker et al., 2018). FOXP2 is expressed in several brain regions, including the basal ganglia and inferior frontal cortex. These regions are essential for brain maturation, speech and language development, memory formation, and cognitive functions (Énard et al., 2002; Spiteri et al., 2007). Genetic variants and translocation of FOXP2 are well-known as the genetic causes of severe speech disorders. Mutations in FOXP2 have been found to be associated with attention deficit hyperactivity disorder (ADHD) but no association has been discovered between FOXP2 mutations and ASD (Demontis et al., 2019; Gauthier et al., 2003).

The rate of language problems in ADHD is considerable, ranging from 30% to 50%. While different types of language impairments are heritable, two genes, FOXP2 and TOSPEAK have been reported concerning language abnormalities. There are different lines of evidence that indicate the association of FOXP2 with ADHD onset (Helland et al., 2016; Lewis et al., 2012). At least two Single nucleotide polymorphisms in FOXP2, rs12533005 and rs12533005, were found associated with ADHD (Ribasés et al., 2012). Also, 3-D modeling revealed a structural link between FOXP2 and ADHD in adults based on the presence of tag SNPs of FOXP2 and ADHD presence (Savoy et al., 2020).

Several sequence variants and even one tag SNP were directly involved in the genetic background of ADHD. Previous genome-wide association studies (GWAS), deep-sequencing of the FOXP2 genomic region, and also structure-function 3-D modeling, point to the role of FOXP2 in ADHD as well as several other neurodevelopment-related phenotypes, especially in self-regulation and inhibitory control. Determination of a strong structural link between FOXP2 and ADHD in children and also in adults may lead to promising insights into the FOXP2-related pathophysiology riddle. These insights are not just about ADHD, but also in all disorders with shared molecular pathways and similar developmental symptoms including ASD. Focus on genes that are regulating and regulated by FOXP2 may gain a better understanding of the complex functions and epigenetic modifications of the gene as well as sequence variants in FOXP2 (Meyer et al., 2022; Savoy et al., 2020).

In the present study, we have assessed associations between DNA mutations and expression level of FOXP2 gene, EF abilities, and electroencephalography markers of individuals with high functioning ASD in a large sample. We aimed to better understand the role of FOXP2 in the etiology, EF abnormalities, and brain functions of children with ASD.

Material and methods

Subject recruitment

The study comprised 450 children with ASD (260 boys (58%), 190 girls (42%)) with a mean age of 8.2 ± 1.3, and the control group included 490 neurotypical control children (245 boys (50%), 245 girls (50%)) with a mean age of 9.1 ± 3.1. Two independent senior psychiatrists diagnosed children with ASD by unstructured or semi-structured behavioral observations in children and interview them, their parents, and teachers based on DSM-5 criteria. In addition, Autism Diagnostic Observation Schedule-2 (ADOS-2) raw score was used to evaluate the exceeding threshold and the

| Table 1. Basic demographic data of each group. |
|-----------------------------------------------|
| Variable | ASD | control children |
|----------|-----|------------------|
| Sex      | 260 male, 190 | 245 male, 245 |
|          | female | female |
| Age      | 8.2 ± 1.3 | 9.1 ± 3.1 |
| Body mass index | 19 ± 2.3 | 20 ± 3.6 |
| Race     | 85% Caucasian/ | 83% Caucasian/ |
|          | 15% other | 17% other |
| Education (who didn’t go to School) | 17 | 11 |

ASD = Autism spectrum disorder.

| Table 2. Non-significant p-value in the basic demographic situation between groups. |
|-----------------------------------------------|
| Variable | ASD vs. control children |
|----------|--------------------------|
| Sex      | p = 0.55 |
| Age      | p = 0.49 |
| Body mass index | p = 0.77 |
| Race     | p = 0.62 |
| Socioeconomic situation | p = 0.58 |
| Familial situation | p = 0.49 |
| Education | p = 0.84 |

ASD = Autism spectrum disorder.
severities of symptoms in children with ASD. As all the recruited children with ASD were high functioning, the language function compatible module three was used for all individuals with ASD (Centers for Disease Control and Prevention, 2014). High functioning children with ASD are determined based on IQ scores (Wechsler Abbreviated Intelligence Scale, 1999) greater than 70 and clinical observation regarding pragmatic and language skills. Children with IQ scores lower than seventy were excluded from the study. Children with ASD were early diagnosed (mostly at age 4) and did not take any anti-psychotic or anti-depressant at the time of participation. We have recruited children with ASD from the outpatient psychiatric clinics of 18 provinces out of the 31 provinces of Iran. Neurotypical children were recruited from individuals who received regular medical checkups before school enrollment in local medical centers with no history of any psychological or somatic problems in reputations. It should be noticed that in Iran, after regular checkups, and further psychiatric assessments, if children get diagnosed with ASD, they will enroll in ASD special elementary schools. We matched subjects of the two groups with each other, based on gender, age, race, socioeconomic situation, and familial situation. Demographic and clinical data and statistical analysis of the two groups are presented in Table 1 and Table 2 respectively. We excluded individuals with combined ASD and ADHD or any other psychiatric or behavioral disorders from the study. None of the subjects or their parents had a current or a history of a severe medical condition, neurological disorder, history of head trauma with loss of consciousness, any psycho-stimulant or opioid drug abuse, or alcohol or nicotine dependence. Parents were provided information about the study’s purpose and informed consent before signing the written consent form. Children were verbally told about the study. The study was approved by the ethical committee of Islamic Azad University and performed based on Helsinki declaration obligations.

**Blood sampling and real-time PCR**

Blood (5 ml) was collected from the cubital vein without a tourniquet between 10.00 and 11.00 AM. Total RNA was extracted from peripheral blood samples immediately after samplings according to the standard protocols using a Genomic DNA Purification kit (GeneJET™ RNA Purification Kit #K0732, Thermo scientific – Fermentas, Latvia). Total RNA was treated with DNase for the removal of contaminating genomic DNA using DNase Treatment & Removal Reagents (DNase I, RNase-free (#EN0521) Fermentas, Latvia), according to the protocol. The quality of RNA was evaluated by Agarose gel electrophoresis and UV-spectroscopy. The cDNA was synthesized using a Transcription First Strand cDNA Synthesis Kit (RevertAid Premium First Strand cDNA Synthesis Kit #K1652, Thermo scientific -Fermentas, Latvia). Primers were designed by the oligo7 software and blasted on the NCBI website. The phosphoglycerate kinase 1 (PGK1) gene was used for normalization as an endogenous reference gene. Agarose gel electrophoresis was used to verify the predicted size of PCR amplicons of genes. Standard curves for each gene were prepared using serial dilutions (1: 4) of pooled cDNA from total RNA extracted from blood samples of 10 control subjects. In each experiment, the R² value of the standard curve was more than 0.99 and no-template control assays have resulted in no detectable signal. Quantitative RT-PCR was performed using SYBR green (Thermo Scientific Maxima SYBR Green/ROX qPCR Master Mix (2X) #K0221, Thermo scientific - Fermentas, Latvia). CFX96 Touch Real-Time PCR Detection System (BIO-RAD, California, United States) used for triplicate method Quantitative Real-time PCR. The Pfafle formula was used to calculate the ratio. Procedures of Real-time PCR and analysis of expression data were conducted based on the previous studies (Haghifard et al., 2018). Primer sequences are presented in Table 3.

| Genes primer        | primer sequence                        |
|---------------------|----------------------------------------|
| PGK1 forward primer | 5’GTGCCAATAGGAAACGGAGAG3’              |
| PGK1 reverse primer | 5’TGCCAAGGGAGATGCGAA3’                 |
| FOXP2 forward primer| 5’TGCACTAAACATGGAGGGGC3’               |
| FOXP2 reverse primer| 5’TTTGGAAGTGTTGGAGGAGGT3’              |

**DNA extraction**

Genomic DNA was extracted from peripheral blood samples according to the standard protocols using the Genomic DNA Purification Kit (Thermo Fisher Scientific #K0512). The quality and integrity of extracted DNA were evaluated by agarose gel electrophoresis and UV-spectroscopy.

**PCR amplification and DNA sequencing**

The entire length of the FOXP2 gene, including coding and non-coding regions, was amplified by PCR. In addition, DNA cycle sequencing on an automated sequencers ABI 3700 was conducted as described in previous studies (D’Gama et al., 2015).

**Sequence data and statistical analysis**

All the sequenced data were compared between individuals in groups by an optimized version of Phred software to ABI 3700, Phred version (0.020425.c). Hardy-Weinberg equilibrium (HWE) was tested using exact significance as implemented in STATA 12.1. Minor allele frequencies were measured using STATA 12.1. The normality of residuals was checked graphically with STATA 12.1.
Executive function assessments

Cambridge neuropsychological examinations. Four neuropsychological tests were obtained to test the response inhibition, vigilance, and working memory. Deficiencies in these essential executive functions had been reported in children with ASD.

1. Integrated Visual and Auditory (IVA) Continuous Performance Test (CPT)

The integrated visual and auditory (IVA) and continuous performance test (CPT) are used for the assessment of neurodevelopmental psychiatric disorders such as ASD. The IVA associates vigilance and impulsivity in a balanced design by visual and auditory modalities. The visual response control quotient (VRCQ) and auditory response control quotient (ARCQ) are the primary dependent variables of IVA (Corbett et al., 2009; Sandford & Turner, 2000).

2. IVA Visual Attention Quotient (VAQ) and Auditory Attention Quotient (AAQ)

VAQ and AAQ were used as primary dependent variables in this measure for the evaluation of vigilance. Tests were obtained based on previous study methods (Corbett et al., 2009; Sandford & Turner, 2000). The VRCQ, ARCQ, VAQ and AAQ are all part of the IVA-CPT test.

3. Spatial Span (SSP) of CANTABexpedio

Cambridge neuropsychological test automated battery (CANTABexpedio) examines cognitive domains such as attention, executive function, memory, processing speed, and visuo-spatial ability. Spatial Span (SSP) considers both forward and reverse spatial memory span (Corbett et al., 2009).

4. Spatial Working Memory (SWM)

Spatial Working Memory (SWM) analyzes the ability to maintain spatial information and manipulate the presented items in working memory. This test uses boxes and colors and is suitable for children. Method of performing test had followed based on previous researches. Total spatial working memory between search errors (SWM Btwn Error) and strategy scores (SWM Strategy) was calculated (Corbett et al., 2009).

Electroencephalography assessment. From all participants, 127 children with ASD (88 boys (69%), 39 girls (31%)) and 100 neurotypical control children (70 boys (70%), 30 girls (30%)) participated in electroencephalography examinations. Electroencephalography tests had recorded by custom-designed electrode caps with a 64-channel BioSemi Active-Two system (BioSemi B.V.) for ten minutes of the eyes-closed resting state and data were processed by the BrainVision Analyzer package, version 2.0 (Brain Products GmbH, Munich, Germany). All participants had normal hearing and normal or corrected-to-normal vision. Seven subjects with noisy EEG (muscle activity and exorbitance eye blink) were excluded. The algorithm works based on amplitude and frequency with acceptable z-scores between −1.96 and +1.96 (95% accuracy). After automatic artifact rejection, the average of the signal remaining was three minutes and nine seconds. The remaining signals were visually inspected for artifacts. Finally, 60 to 70 artifact-free signal segments with a length of three seconds were selected from the whole of the signals. The test-retest and split-half tests for all EEG channels remained over 0.9. Methods and data analysis process was conducted based on previous studies (Vettori et al., 2020).

Statistical analysis

Descriptive data are expressed as mean ± SD (range), and the level of statistical significance was set at P < 0.05. Compliance with the normal distribution of continuous variables was assessed via the Kolmogorov-Smirnov test. The Chi-square test was used for the detection of group differences in allele frequency in the DNA sequencing analysis. Statistical differences in all variables were calculated by one-way ANOVA followed by an independent Student’s t-test for multiple group comparisons. Pearson correlation test was used to determine the relationship between variables. Multiple-comparison analysis correction was evaluated by the Bonferroni correction test. Statistical analysis was conducted using SPSS software version 24.

Results

Results of FOXP2 sequencing

No variants associated with the onset of the disorder or cognitive assessments have been found in children with ASD compared with neurotypical control children.

Gene expression results

Significant down-regulation of FOXP2 was detected in children with ASD compared to the control group (p = .002, ratio (2^−ΔΔct) = 0. 56). No significant correlation was found between gene expression results and age, age of onset, duration of illness, and intelligence quality score.

Neuropsychological results and correlations with gene expression

1. IVA tests’ results

Significant deficiency and lower performance in children with ASD compared to the control group, determined in
both VRCQ (P = .0004) and ARCQ (P = .0002). In addition, significantly lower performances in the ASD group compared to the control group in VAQ (P = .002) and AAQ (P = .001). Also, down expression in FOXP2 was significantly correlated with the lower performance of VRCQ. Analysis of correlation between FOXP2 expression level and IVA tests in all subjects as the combined group showed a significant correlation between mRNA level reduction and the lower performance of all IVA tests.

2. Working memory tests results

Differences in SSP (P = .006), SWM Btwn Errors (P = .003), and SWM strategy (P = .008) were significant between children with ASD in comparison with the control group, which reveals the significant deficiency in working memory of children with ASD. Significant correlation between the down expression of FOXP2 and the increase of SWM Btwn Errors and SWM strategy score detected in children with ASD. In all subjects as a combined group, there was a significant correlation between the down expressions of FOXP2 with the increase in SWM strategy score. Clinical data and neuropsychological test results have presented in Table 4.

**EEG results**

Children with ASD showed a significant decrease in alpha (p = .0003) and gamma (p = .0005) band frequencies at the resting state in the frontal lobe compared to the neurotypical control children. In addition, the theta wave frequency in the occipital lobe was significantly higher (p = .0004) in children with ASD compared with neurotypical control children. Lower frequency of frontal lobe alpha (P = .002, r = .51) and high frequency of theta band in the occipital lobe (P = 0.002, r = -.55) showed significant correlations with mRNA level of FOXP2.

**Symptoms’ severity results**

The ADOS-2 test has been used to confirmation of the diagnosis and evaluation of the severity of symptoms in children with ASD. Then, the correlations between severity of symptoms with gene expression and executive functions have been assessed. As the children with ASD were high function, age-compatible module three of ADOS-2 has been used for all children with ASD. A significant correlation was found between the down expression of FOXP2 and the higher raw score of ADOS-2 (r = −0.52, P = .009). In addition, a significant correlation was determined between the lower score of VRCQ and the higher score of ADOS-2 (r = −0.58, P = .006).

**Sex effect analysis in ASD group**

Effects of gender on the severity of symptoms, executive functions, EEG factors, and FOXP2 expression have been assessed in children with ASD. Findings showed a significant decrease in SWM strategy score in girls with ASD compared with boys with ASD (P = .003).

**Discussion**

In the present study, we have assessed the blood expression level and DNA variants of FOXP2 in a large sample size of children with ASD along with neuropsychological and neuroimaging examinations. Although no mutation has been found associated with ASD onset or cognitive deficits of children with ASD, significant down-regulation of mRNA level was detected in peripheral blood of children with ASD, which was strongly related to cognitive dysfunctions and abnormalities in the brain map revealed by EEG.

Results indicated the role of FOXP2 as a transcription factor in the molecular process of cognitive functions, memory, and attention, as well as neural activity in the brain, but the abnormalities are not the same as FOXP2 abnormalities in language disorders (Gauthier et al., 2003). In all FOXP2-related speech and language disorders, childhood apraxia of speech (CAS), a disorder of speech motor programming or planning that affects the production, sequencing, timing, and stress of sounds, syllables, and words are present while most of these children show no Autistic features (Adegbola et al., 2015). On the other hand, in FOXP2-plus-related disorders, oral motor deficits, global developmental delay, and ASD spectrum disorder are common (Morgan, Fisher, Scheffer, & Hildebrand, 2017). Although the FOXP2 mutations and chromosomal translocation are associated with language impairments, it seems that epigenetic changes, as well as DNA sequence variants, are responsible for FOXP2 deficiency in children with ASD. Methylation analysis of a CpG island showed a higher degree of methylation of the first exon of the FOXP2 gene in the left parahippocampus gyrus.
individuals with schizophrenia, which leads to a low expression level of the gene (Tolosa et al., 2010). While down expression of FOXP2 in psychiatric disorders such as schizophrenia, has been reported in several studies (Sanjuán et al., 2021) present findings, indicate the same expression pattern in children with ASD. In addition, results showed FOXP2 down expression is correlated with the severity of symptoms in children with ASD, which may support the upstream effect of FOXP2 as a transcription factor on different pathways related to ASD.

Results in the neuropsychological battery of EF measures, response inhibition, vigilance, and working memory confirmed previous reports about EF deficit in children with ASD (Corbett et al., 2009). Genetic bases of cognition and executive functions are not clarified, but associations between deficiency of EF measures in children with ASD and expression levels of neuronal growth factors such as Neuregulin 1 were reported (Abbasy et al., 2018). FOXP2 regulates several genes and pathways involved in the neural development of the frontal lobe, striatal compartments, cerebral, and cerebellar structures, synaptogenesis and neural flexibility. All these functions are related to cognitive and executive functions and language acquisition (Newbury et al., 2002).

FOXP2 has a key role in synaptic plasticity, dopamine, and NMDA receptors that may lead to the tuning of corticostratal systems, a capacity potentially contributing to learning and speech and language acquisition in the human brain (Co et al., 2020; Schreiweis et al., 2019). It seems that FOXP2 dysfunction may cause deficiencies in a wide range of executive function domains, including response inhibition, vigilance, and working memory, as well as language and speech-related domains.

We detected significantly decreased resting alpha power in children with ASD that may signify atypical arousal levels and/or an excitatory/inhibitory imbalance. Both low alpha power and imbalance in excitatory/inhibitory neurons were detected in previous studies on children with ASD (Keehn et al., 2017). Also, Gamma-band abnormalities detected in the present study have been reported in several studies as one of the endophenotypes of ASD (Rojas & Wilson, 2014). Gamma-band activity is associated with perceptual and cognitive functions. Interestingly, gamma-band deficiency detected in normal first-degree relatives of children with ASD may support evidence for the genetic roots of gamma-band and cognitive abnormalities. The novel EEG findings of our study are the abnormality of the theta wave in the occipital lobe as a known marker for anxiety and stress (Hamid et al., 2010). Genetic bases of anxiety and stress have been studied before, but the role of stress on EF, especially in children with ASD is a new area of study. Reduction of stress and anxiety may help the improvement of EF in several disorders, including ASD (Azadmarzabadi et al., 2018).

We primarily suggest the involvement of FOXP2 in the etiology of autism spectrum disorders. In addition, deficiencies in EF and abnormalities in brain activities such as alpha, gamma, and theta frequency bands may relate to the FOXP2 expression level. The FOXP2 is a transcription factor that affects the expression regulation of several molecular pathways and is well-known in the language developments process.

Sample size restrictions and lack of language-related assessments are the main limitations of the present study. Gene expression study of all language-related genes, advanced neuroimaging data analysis, source localization Brian signals, and psychological tests that measure language impairments must consider in future studies.

Conclusion

Neuroimaging assessments along with gene expression and genotyping examinations could provide reliable evidence about the role of FOXP2 and their potential downstream genes in ASD etiology and EF functions in these children. Neuroimaging genetic approach with the evaluation of associations between genetic and neuroimaging data can shed light on the molecular mechanisms of neural activity in the central nervous system. Neuroimaging genetic approach mainly focuses on sequencing data, but our research combined sequencing and expression level analysis. This approach can be called neuroimaging epigenetic approach, which may lead to developing a pharmacological strategy for treating ASDs or at least improvement in EF and speech and language skills and potential surrogate markers for the effectiveness of other treatments.

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