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Association of estrogen receptor alpha polymorphisms with symptoms of autism among Chinese Han children

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Abstract

OBJECTIVES: Autism has a significant sex difference. This implies that the sex hormones might have effect on autism. Estrogens play an important role in early nervous system development and sex differentiation through estrogen receptors in brain. Thus, we tested the hypothesis that estrogen receptor alpha (ESR1) gene affects the pathogenesis of autism and related symptoms.

METHODS: Genotypes of rs11155819 and rs2234693 were determined in boys with autism and normal boys from Chinese Han population. A case–control study was performed to explore the association between polymorphisms in ESR1 gene and autism susceptibility. Assessment tool was used to evaluate the neuropsychological developmental level of autistic children. Finally, we analyzed the association of these single nucleotide polymorphisms (SNPs) with specific symptoms.

RESULTS: The results showed no significant differences between cases and controls in the distribution of genotypes and allele frequencies of the two SNPs. However, rs11155819 TT genotype showed a lower neuropsychological development level among autistic children, especially in the aspects of fine motor and adaptation ability (p=0.028; p=0.042).

CONCLUSION: Polymorphisms of ESR1 are relevant to autism symptoms in Chinese Han children.

ORIGINAL ARTICLE

Abbreviations

ESR1/ERα	- estrogen receptor alpha
SNPs	- single nucleotide polymorphisms
ASD	- autism spectrum disorder
CDD	- childhood disintegrative disorder
PDD-NOS	- pervasive developmental disorder not otherwise specified
AS	- Asperger syndrome
EMB	- extreme male brain
fT	- fetal testosterone
ESR2/ERβ	- estrogen receptor beta
DSM-IV	- Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
CARS	- Children Autism Rating Scale
ABC	- Autism Behavior Checklist
DQ	- Development Quotient
PCR	- polymerase chain reaction
AQ	- Autism Spectrum Quotient

INTRODUCTION

Autism spectrum disorder (ASD) is a group of complex neurodevelopment disorders, including autism, childhood disintegrative disorder (CDD), pervasive developmental disorder-not otherwise specified (PDD-NOS) and Asperger syndrome (AS) (APA. 2013), with an increasing incidence at 14.7 per 1,000 (Christensen *et al.* 2016). In addition to abnormalities of social interaction, communication and repetitive behaviors, other ASD symptoms are prevalent: motor impairment, sleep disorders, epilepsy, emotional problems, and adaptation difficulty (Hill *et al.* 2004; Jeste 2011).

ASD also has a dramatic sex difference, with a ratios of 3:1 (male:female) for autism and 11:1 for AS (Gillberg *et al.* 2006). That implies some potential relationship between sex hormones and ASD etiology. Baron-Cohen and his colleagues examined sex differences by 'extreme male brain' (EMB) theory, which hypothesizes that ASD represents an extreme of the typical male profile (Baron-Cohen 2002). One possible cause of EMB is fetal testosterone (fT). Previous studies have demonstrated that early exposure to testosterone affects brain sex differences in behavior, cognition, and brain structure (Arnold & Breedlove 1985; Simerly 2002). Human males experience three surges in testosterone from gestation to puberty; the first surge plays a strong role in brain masculinisation, during second trimester of pregnancy. Thus, scientists detected fT in the amniotic fluid from healthy pregnant women. After birth, the children were followed up to measure their cognition and behavior. These data indicated that fT level is related to autistic traits in children at early age (Auyeung *et al.* 2012). For example, fT is inversely associated with frequency of eye contact, size of vocabulary development, quality of social relationships, and the capacity to identify and understand other people. In contrast, fT is positively associated with narrow interests, performance on attention to detail, and the capacity to analyze or construct rule-based systems (Auyeung *et al.* 2009; Knickmeyer *et al.* 2005; Lutchmaya *et al.* 2004). Consequently, high levels of fT might be a risk factor for the development of ASD and autistic traits.

Many physiological actions of testosterone were achieved by its aromatization form, estrogen (Hofer *et al.* 2013). With aromatase enzyme, testosterone changes to estrogen, which binds to estrogen receptors of two types, estrogen receptor alpha (ESR1, alias of ERα in mouse) and estrogen receptor beta (ESR2, alias of ERβ in mouse) (Dahlman-Wright *et al.* 2006). These binding activates their function on target tissues in a wide range of brain areas, such as hypothalamus, hippocampus and amygdale (Ostlund *et al.* 2003). In animal models, this process plays a critical role in brain sexual differentiation during neonatal development (Beyer 1999; Patchev *et al.* 2004). Gene knockout mouse models have indicated that *ERα* is involved in masculinization, while *ERβ* affects defeminisation (Kudwa *et al.* 2006). Although these pathways engendering brain sexual differentiation by estrogen have not been supported by clinical human evidence, whereas androgen has been described as having direct effects, these variations of estrogen function might lead to alteration in the homeostasis of the aromatized steroid process and dysfunction of brain sexual differentiation.

The association between *ESR1/ESR2* and ASD has been little acknowledged. A study of Caucasian population reported that polymorphisms in *ESR1/ESR2* are related with susceptibility of AS as well as autistic traits of the general population (Chakrabarti *et al.* 2009). A twins study from Sweden also indicated that *ESR1* variants involved in the autistic traits, such as language and social interaction impairments (Zettergren *et al.* 2013). In addition, the mRNA and protein expression of *ESR2* in the middle frontal gyrus of autistic patients were both decreased, compared with normal people (Crider *et al.* 2014).

The range of autistic symptoms encompasses intellectual, social interaction, communication, behaviors, and sensory and motor activity. Thus, it is interesting to determine which specific symptoms are modulated by *ESR1*. In this study, we focused on autism patients (1) to detect if genetic variants in *ESR1* are related to Chinese Han children with autism from a case–control study; and (2) to explore possible association between variants in *ESR1* and severity of specific symptoms based on the assessment tool, Neuropsychological development examination table for children aged 0–6 years old.

MATERIALS AND METHODS

Ethics statement

The Ethics Committee of the Harbin Medical University approved the study. [HMUIRB20130019]. All parents or guardians provided written informed consent for participation in this research. The experimental protocol was conducted in accordance with the Declaration of Helsinki.

Participants

We recruited 125 boys (mean age = 4.38±0.99) with autism from the Children Development and Behavior Research Center, Harbin Medical University, Heilongjiang Province, China, during January 2010 to March 2013. Participants were diagnosed by more than two experienced psychiatrists according to standard criteria of Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (APA. 1994), which characterized autism by impaired social relationships and communication, language deficits or disorders, and restricted, repetitive, or stereotyped behaviors. Furthermore, they were assessed using Children Autism Rating Scale (CARS) and the Autism Behavior Checklist (ABC) (Krug *et al.* 1980). We only selected autism cases and ensured their CARS score ≥30 and ABC score ≥67. Participants were excluded if they were other disorders in the ASD group, or any other neurological condition suspected to be associated with autism (Liang *et al.* 2014).

116 normal development boys were recruited from kindergartens around Harbin (mean age = 4.45±0.88). None had below-average intelligence quotient, or had a neurological or psychiatric disorder. Also, they did not have any diseases or take any medicines. We matched the cases and controls based on their sex, age, ancestral geographical origin (three generations from the same province), life standard, and the parents' education level. All normal developments were examined clinically in the same way.

All participants were ethnically Chinese Han, with no parent of ethnicity other than Chinese Han.

Development Quotient (DQ) analysis

The neuropsychological development examination table for children aged 0–6 years old was produced by the Capital Institute of Pediatrics, Beijing, based on Gesell Development Schedules. This table is used widely for early screening of slowly developed and intellectually disabled children in China (Liu 2012). It specifically examines gross motor function, fine motor function, adaptation ability, language, and social behavior. The DQ in different ages was calculated according to the original score of these five items, the higher scores associated with more advanced development levels. For this study, we used the DQ value to assess the development level and severity of symptoms among children with autism.

Genotyping of SNPs

We chose two SNPs in *ESR1* gene, rs11155819 and rs2234693. Genomic DNA was extracted from whole blood according to the manufacturer's protocol (TaqMan Sample-to-SNP; Applied Biosystems, Foster City, CA, USA). The SNPs were genotyped applying TaqMan technology. Reference sequences were obtained from the GenBank database (RRID:nif-0000-02873) and GenBank Accession number (NG_008493.1). Real-time polymerase chain reaction (PCR) was performed in a 5 μl volume containing 1 μl DNA, 2.5 μl TaqMan GTX press Master Mix, 0.25 μl TaqMan Genotyping assay mix, and 1.25 μl MilliQ water. Real-time PCR reaction was run on a system (ABI Prism 7900HT; Applied Biosystems, Foster City, CA, USA) with the following conditions: initial denaturation at 95 °C (20 s), followed by 40 cycles at 95 °C (3 s), at 60 °C (20 s).

Statistical analysis

Hardy–Weinberg equilibrium was evaluated by chi-square test for each group. In case–control study, genotype distributions and allele frequencies were calculated by Pearson chi-square test or Fisher's exact test. Since the frequency of rs11155819 CC genotype was low in both groups (Table 1), we used a C allele carrier group and a TT homozygote group for this SNP in the next analysis. T-test was used to ascertain whether rs11155819 genotypes were associated with autistic symptoms, while One-way ANOVAs test was done for rs2234693 to analyze the same items by using SPSS software version 19.0.

Tab. 1. Allele and Genotype Frequencies of rs11155819 in Participants.

Test	Genotype/Allele	Cases number(%)	Controls number(%)	*p*-value
Additive model	CC	3 (2.4)	2 (1.7)	0.627
	CT	37 (29.6)	41 (35.3)	
	TT	85 (68.0)	73 (62.9)	
Dominant model	CC+CT	40 (32.0)	43 (37.1)	0.419
	TT	85 (68.0)	73 (62.9)	
Recessive model	CC	3 (2.4)	2 (1.7)	1.000
	TT+CT	122 (97.6)	114 (98.3)	
Allele association	C	43 (17.2)	45 (19.4)	0.533
	T	207 (82.8)	187 (80.6)	

Tab. 2. Allele and Genotype Frequencies of rs2234693 in Participants.

Test	Genotype/Allele	Cases number(%)	Controls number(%)	*p*-value
Additive model	CC	17 (13.6)	15 (12.9)	0.952
	CT	57 (45.6)	55 (47.4)	
	TT	51 (40.8)	46 (39.7)	
Dominant model	CC+CT	74 (59.2)	70 (60.3)	0.896
	TT	51 (40.8)	46 (39.7)	
Recessive model	CC	17 (13.6)	15 (12.9)	1.000
	TT+CT	108 (86.4)	101 (87.1)	
Allele association	C	91 (36.4)	85 (36.6)	0.957
	T	159 (63.6)	147 (63.4)	

RESULTS

Susceptibility to autism

Genotype distributions for cases and controls were in accordance with the Hardy–Weinberg equilibrium ($p>0.05$). No significant difference of genotype or allele frequencies was detected between cases and controls in rs11155819 and rs2234693 (Tables 1 and 2, $p>0.05$). These results suggest that genetic variants in *ESR1* do not contribute to susceptibility to autism in a Chinese Han population.

Severity to autism

For the rs11155819, C allele carrier group had higher DQ value and five sub-scores than those of TT genotype group. Especially, the differences of fine motor and adaptation ability between two genotypes were significant. The value of fine motor in TT genotype group (48.35±1.89) was lower than that of C allele group (56.36±3.41) (Figure 1, $p=0.028$). In addition, the TT genotype showed a lower level of adaptation ability at 50.71±1.89, which was 57.68±2.92 of C allele carrier group (Figure 1, $p=0.042$). These data suggest that autism patient who is TT genotype in rs11155819 tends to get a lower development level of neuropsychology.

For the rs2234693, TT genotype showed lower DQ value and five sub-scores compared with CC and CT genotypes. However, the differences between these genotypes were not significant ($p>0.05$), which indicates that rs2234693 polymorphisms do not affect neuropsychological development of autism.

DISCUSSION

In present study, no association was found between *ESR1* polymorphisms and susceptibility to autism in a Chinese Han population. However, association analyses of the severity of each symptom using the DQ score show that individuals with TT genotype in rs11155819 have a lower level of development of fine motor and adaptation ability. These results suggest that polymorphisms in *ESR1* are associated with the severity of autistic symptoms. This report is the first describing a study investigating the association between *ESR1* polymorphisms and autism in a Chinese Han children.

A previous study of Caucasian people revealed that carriers with CC genotype in rs11155819 among normal population had a significantly higher autistic traits score, which mean CC allele is associated with autistic traits (Chakrabarti *et al.* 2009). However, the results in the current study did not support that finding. A possible explanation is the different ethnic population and clinical status as samples: Caucasian normal population or Chinese autism. Another explanation might be using different assessment tools. The Caucasian study assessed autistic traits by Autism Spectrum Quotient (AQ), which was designed for AS and high-functioning autism, especially social functions (Baron-Cohen *et al.* 2001). By contrast, we used DQ, which is designed to assess autism, neuropsychological development of normal children, including mental retardation and motor disability. These differences suggest that CC genotype contributes to social disability, while TT genotype, as shown in the current study, contributes to motor disability. Previous studies proved that estrogen benefits motor skills. For example, women with higher levels of estrogen associated with faster movement (Jennings *et al.* 1998), while males show greater age-related decline in motor function than females do (Lacreuse *et al.* 2005). Thus, SNP rs11155819 might influence brain functions related to motor control by regulating expression of the *ESR1* gene. To understand our findings, further investigations of the relevance of this SNP for estrogen metabolism during development are needed.

The rs2234693 is an important SNP for regulating physiological traits. Women with TT genotype were more likely to have late-life depression (Ryan *et al.* 2011). In addition, elder women with T allele had an increased risk of developing cognitive impairment (Yaffe *et al.* 2002). On the other hand, the C allele was more frequent in African American schizophrenia (Weickert *et al.* 2008). Adolescent boys who carry CC genotype were

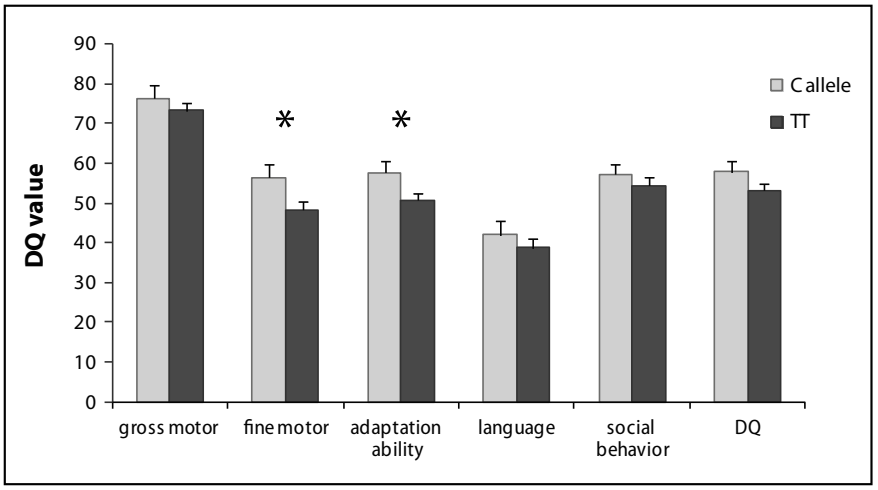

Fig. 1. Association between rs11155819 polymorphisms and DQ scores. TT genotype reflected lower developmental level in fine motor and adaptation ability (*p<0.05). The means and standard errors were presented.

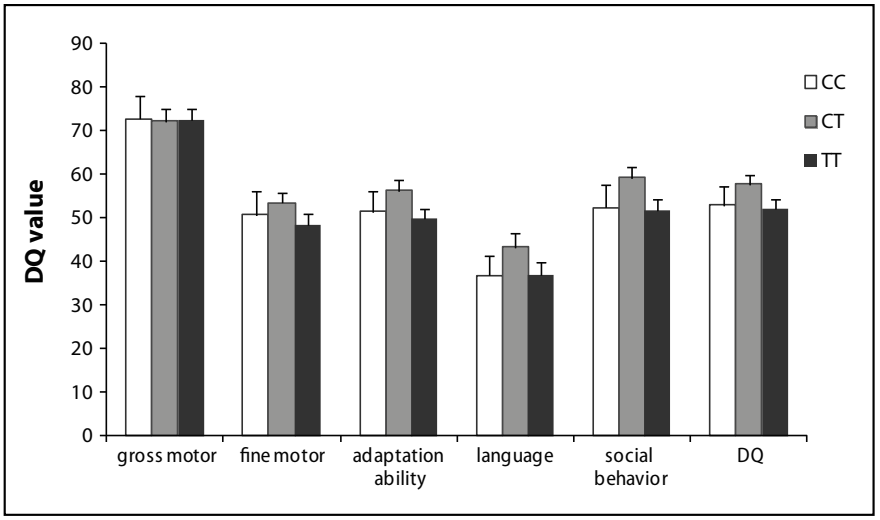

Fig. 2. Association between rs2234693 polymorphisms and DQ scores. No significant differences were found between rs2234693 genotypes and specific symptoms. The means and standard errors were presented.

apt to daily hassles and depressive symptoms, while the C allele related to higher level of anger expression in girls (Vermeersch *et al.* 2013). In this study, we found that there was no relationship between rs2234693 genotypes and abnormalities of autism. These controversial results might be due to differences in diseases, ethnic differences, and methods.

The main limitation of this study was limited sample size of participants, which might contribute to a false-negative observation. Thus, replication of our findings in large population-based studies is needed. Additionally, we have used DQ to evaluate autistic severity. It is reliable to carry out the gold standard, the Autism Diagnostic Observation Schedule and the Autism Diagnostic Interview-Revised, to confirm our findings. Furthermore, the cases in our study were completely autism. We need target the remainder of developmental disorders in the ASD group.

In conclusion, our findings indicated that *ESR1* polymorphisms affect the severity of specific symptoms in a Chinese Han population, such as fine motor and adaptation ability. To date, the mechanisms by which *ESR1* affects both susceptibility and symptoms of autism are not well understood. New investigations are requested to elucidate this association.

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