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Research paper

Chromatin remodeling gene *EZH2* involved in the genetic etiology of autism in Chinese Han population

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HIGHLIGHTS

- We performed a family based association study between EZH2 and autism in Chinese Han population.
- SNP rs6464926 was associated with autism significantly even after Bonferroni correction.
- Haplotype G-T constructed from rs740949 and rs6464926 was a risk haplotype for autism.

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ABSTRACT

Autism spectrum disorder (ASD) is a group of severe neurodevelopmental disorders. Epigenetic factors play a critical role in the etiology of ASD. Enhancer of zest homolog 2 (*EZH2*), which encodes a histone methyltransferase, plays an important role in the process of chromatin remodeling during neurodevelopment. Further, *EZH2* is located in chromosome 7q35–36, which is one of the linkage regions for autism. However, the genetic relationship between autism and *EZH2* remains unclear. To investigate the association between *EZH2* and autism in Chinese Han population, we performed a family-based association study between autism and three tagged single nucleotide polymorphisms (SNPs) that covered 95.4% of the whole region of *EZH2*. In the discovery cohort of 239 trios, two SNPs (rs740949 and rs6464926) showed a significant association with autism. To decrease false positive results, we expanded the sample size to 427 trios. A SNP (rs6464926) was significantly associated with autism even after Bonferroni correction ($p = 0.008$). Haplotype G-T (rs740949 and rs6464926) was a risk factor for autism ($Z = 2.655$, $p = 0.008$, Global $p = 0.024$). *In silico* function prediction for SNPs indicated that these two SNPs might be regulatory SNPs. Expression pattern of *EZH2* showed that it is highly expressed in human embryonic brains. In conclusion, our findings demonstrate that *EZH2* might contribute to the genetic etiology of autism in Chinese Han population.

1. Introduction

Autism spectrum disorder (ASD) is a group of severe neurodevelopmental disorders characterized by impaired social interactions and communication, and restricted repetitive interests or behaviors [1]. The heritability of ASD has been estimated to be approximately 90% [2]. Common genetic variations and *de novo* mutations as well as chromosomal abnormalities may contribute to autism. However, the exact etiology of autism remains unclear.

Epigenetic factors play a critical role in the causes of ASD [3,4]. For example, genetic variants of the chromatin regulation gene methyl CpG binding protein 2 (*MECP2*) are responsible for the cause of most patients of Rett syndrome which has characteristics similar to those of ASD. In addition, *MECP2* expression level significantly decreased in the brain samples of autistic patients compared to those of age-matched healthy controls [5]. Additional evidence comes from mutations in H3K9 methyltransferase complex genes such as *EHMT1*, *EHMT2*, and *WIZ* in Japanese autistic patients, suggesting the potential role of histone methyltransferase in the

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etiology of autism [6]. A whole exome-sequencing study of sporadic ASD reported that 39% of the most disruptive *de novo* mutations map to a highly interconnected β-catenin/chromatin remodeling protein network ranked significantly for autism candidate genes. These genes include three members of chromodomain helicase DNA binding protein (CHD) family (*CHD3*, *CHD7*, and *CHD8*) [7], which encode DNA helicases that function as transcription repressors by remodeling the chromatin structure. Another study also detected a *de novo* mutation in *CHD8* in ASD [8]. Chromatin remodeling genes appear to play a role in neurodevelopmental disorders including autism [9]. For example, an animal research indicated that knockdown of *Ankrd11*, which is a potential chromatin regulator, contributed to cortical abnormalities, ASD-like repetitive behavior and impaired social interaction in mice [10]. All of the evidence above reveals the role of variants of chromatin remodelers in autism, indicating the importance of genes that affect the epigenetic regulation of brain development and cognitive function in humans [11,12].

Enhancer of zest homolog 2 (*EZH2*), which encodes a member of the polycomb-group (PcG) family, is a component of polycomb repressive complex 2 and an important chromatin remodeler. *EZH2* is localized in chromosome 7q35–36. Autistic susceptibility genes such as Engrailed homeobox 2 (*EN2*) [13], Forkhead box P2 (*FOXP2*) [14], and Contactin associated protein-like 2 (*CNTNAP2*) [15,16] are localized in this region. *CNTNAP2*, which is located at a distance approximately 400 Kb from *EZH2*, was repeatedly reported to be associated with autism in genome-wide association studies as well as candidate gene studies. Further, missense mutations in *EZH2* were identified in patients affected with Weaver syndrome which shares some clinical features with autism such as intellectual disability and brain structure abnormalities [17].

EZH2 catalyzes the trimethylation of lysine residue 27 on histone H3 (H3K27me3), which results in the silencing of particular genes and ultimately impacts the expressed phenotype [18]. During developmental stages, a group of genes regulated by polycomb-group complexes contain both active (H3K4me3) and repressive (H3K27me3) marks. The altered balance might contribute to neurodevelopmental disorders including autism.

Ezh2 regulates the balance between self-renewal and differentiation of neural progenitor cells in the cerebral cortex of mice [19]. Conditional knockout of *Ezh2* in mouse cortical neural progenitor cells resulted in disrupted cortical development [19]. Moreover, Ezh2 regulated adult hippocampal neurogenesis in mice. Conditional knockout of *Ezh2* in mouse adult neural stem cells showed impaired spatial learning and memory, contextual fear memory, and pattern separation [20]. Besides, Ezh2 regulates dendritic arborization, which is one of the key determinants of precise circuits for information processing in neurons [21].

Considering the biological function of *EZH2* in regulating chromatin structure and its location in the chromosome, we hypothesized that *EZH2* might contribute to the genetic risks for autism. To investigate whether *EZH2* polymorphisms were associated with autism, we performed a family-based association study in Chinese Han population. Our results demonstrate that one SNP and two haplotypes in *EZH2* are associated with autism.

2. Methods

2.1. Ethics statement

This research was approved by the Ethics Committee of the Institute of Mental Health, The Sixth Hospital, Peking University. All patients and the legal guardians for children provided written informed consent to participate in this study.

2.2. Subjects

In this study, we recruited 427 Chinese Han family trios (including singleton autistic patients and their unaffected biological parents). All subjects were unrelated Chinese Han nationality born. They all have Chinese Han ancestry (All subjects' parents and grandparents are Chinese Han descent). Initially, we recruited 239 trios as a discovery cohort. Of the 239 autistic patients, 226 were male and 13 were female. The mean age of the children at the time of assessment was 7.5 years. Then we expanded sample size to 427 trios by recruiting additional 188 trios (median age of autistic children was 6.0 years).

The evaluation and diagnosis of autism were conducted by two senior psychiatrists, according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for autism. In addition, Childhood Autism Rating Scale (CARS) and Autism Behavior Checklist (ABC) were used to assess the clinical features of autistic children. Those who had CARS scores $\geq$35 and ABC scores $\geq$53 were included in this study. Children with Asperger's syndrome, Rett syndrome, fragile X syndrome, tuberous sclerosis, phenylketonuria, and chromosomal abnormalities were excluded from this study.

2.3. SNP selection and genotyping

Tagged SNPs in *EZH2* were targeted for this study. To identify tagged SNPs, the genotype data of Han Chinese in Beijing (CHB) were downloaded from HapMap phase II and phase III datasets (http://www.hapmap.org). SNPs with minor allele frequency (MAF) >0.05 and *p*-value of Hardy–Weinberg Equilibrium (HWE) >0.05 were selected. Pair-wise tagging in the Tagger module implemented in Haploview v4.1 program was used to select tagged SNPs that could capture >80% of the markers with $r^2 > 0.8$. Therefore, 38 SNPs were included for tagged SNPs detection. As a result, three tagged SNPs (rs740949, rs6464926, and rs10488070) were selected in our study. These three SNPs represented 37 SNPs and covered 95.4% of the genome of *EZH2*.

Genomic DNA was extracted from blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). All three selected SNPs were genotyped using iPLEX Assay on the Sequenom MassARRAY platform (Agena Bioscience, San Diego, USA) with standard protocols, which used the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry primer extension assay. Moreover, the efficiency and flexibility of the Sequenom MassARRAY system had been increased through single base primer extension with mass-modified terminators [22,23]. Quality control was performed by excluding individual SNPs or samples with genotype call rates less than 95% and SNP assays with poor-quality spectra/cluster plots. To confirm the genotype results by the Sequenom genotyping platform, all three SNPs were re-genotyped in 10% of the whole samples.

2.4. Statistical analyses

Mendelian errors were detected by the family-based association test (FBAT) program version 2.0.4 (http://www.biostat.harvard.edu/fbat/default.html). The genotypes of families with Mendelian errors were reset to zero. The HWE for genotype frequency distributions was tested using the Chi-square goodness-of-fit test.

The FBAT software was used to perform association test for SNPs under an additive model. This software uses generalized score statistics to perform a variety of transmission disequilibrium tests (TDT), including SNP association and haplotype association analyses. Bonferroni correction was used to reduce the chances of obtaining false-positive results (type I errors) when multiple statistical tests were performed. Haploview 4.1