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Association analysis of two synapse-related gene mutations with autism spectrum disorder in a Chinese population

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ABSTRACT

Background: Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder with a strong genetic basis. Recently, synaptic abnormality has been proved to have a strong association with the etiology of ASD. *PSD95* and *DLGAP2* are the members of postsynaptic scaffolding proteins that play crucial roles in synaptic plasticity and function. This study evaluated the association of the genetic variants in *PSD95* and *DLGAP2* with ASD.

Methods: We performed a case-control study in a Chinese population with samples of 529 cases and 1923 controls. We extracted genomic DNA from oral swabs and determined the SNP genotypes by using a PCR-RFLP assay.

Results: We sequenced five SNPs (rs7005715, rs2301963 and rs2906569 in *DLGAP2*; rs2521985 and rs2017365 in *PSD95*). Genetic analysis suggested the GA genotype and GG genotype of rs7005715 were significantly associated with increased risk of ASD (respectively: OR = 1.357, 95%CI = 1.103–1.669, $P = 0.016$; OR = 1.860, 95%CI = 1.359–2.551, $P < 0.001$). The dominant model (OR = 1.444, 95%CI = 1.186–1.758, $P < 0.001$) and recessive model (OR = 1.597, 95%CI = 1.187–2.149, $P = 0.011$) also showed the same trend. We did not detect any significant association between other SNPs and ASD.

Conclusions: The genetic variant of rs7005715 in *DLGAP2* increased susceptibility to the risk of ASD in a Chinese Han population.

1. Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by lifelong impairment in social communication and interaction, and the presence of stereotyped behaviors and restricted interests (Chan, Ho, Tsang, Lee, & Chung, 2007). Evidence of epidemiology showed that the prevalence of ASD was 27.5 per 10,000 in a middle-sized city among children aged 18–36 months in China, and it was 14.6 per 1000 in children aged 8 years in the USA (Christensen et al., 2016; Huang et al., 2014). A growing body of work has indicated that children with ASD would have pervasive deficits in various skills, especially in interpersonal interactions, language development, and intellectual disability (D'Mello, Moore, Crocetti, Mostofsky, & Stoodley, 2016; Kao, Kramer,

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Liljenquist, & Coster, 2015; Soto, Giserman Kiss, & Carter, 2016). Owing to the absence of effective treatments, ASD and its related consequences have become important public health concerns.

The mechanism of ASD pathogenesis is complex, involving both genetic and environmental factors (Masi, DeMayo, Glozier, & Guastella, 2017). Twin and family studies have stressed the importance of genetic predisposition to the etiology of ASD (Willfors et al., 2017; Gai et al., 2012). Synapses, across which neurons communicate, are associated with almost all brain function, such as sensory perception, movement coordination, and mental function. Genetic studies suggested that abnormality of synaptic homeostasis could be involved in the development of ASD (Bourgeron, 2009, 2015). The *postsynaptic density proteins 95 (PSD95)* and *discs, large (Drosophila) homolog associated protein 2 (DLGAP2)* are the members of postsynaptic scaffolding proteins that play crucial roles in synaptic plasticity and function (de Bartolomeis & Tomasetti, 2012; Winkler et al., in press). In recent years, whole-exome sequencing (WES) studies, whole-genome sequencing studies and genome-wide LncRNA studies explored the genes related to ASD, and discovered many candidate ASD risk genes, including synapse-related genes (Al-Mubarak et al., 2017; De Rubeis et al., 2014; Jiang et al., 2013; Tang, Yu, & Yang, 2017). Previous studies detected that rare missense mutations in synapse-related genes might increase the risk of ASD (Chen, Yu, Fu, & Li, 2014). For example, the G464R mutation in *Synapsin2* significantly impacted human Syn II ortholog protein function (Corradi et al., 2014), and the missense mutation in *SHANK3*, a leucine to proline at amino acid position 68, would be predicted to disrupt an alpha-helical domain (Gauthier et al., 2009). Furthermore, a recent study found genetic variant of rs13331 in the *PSD95* gene may increase susceptibility to ASD (Wang et al., 2016). An exon resequencing study showed some common and rare genetic variants of *DLGAP2* might contribute to the pathogenesis of ASD (Chien et al., 2013). Evidence suggested that the *Dlg4* mutant mice displayed abnormal behavioral plasticity (Vickers et al., 2006), and the *Dlgap2*$^{-/-}$ mice appeared to have elevated aggressive behaviors and enhanced social dominance (Jiang-Xie et al., 2014). With more and more research focusing on the linkage between genes and ASD, *PSD95* and *DLGAP2* have already proved to be ASD risk genes.

In recent years, some studies have found that variants in synaptic gene may contribute to ASD risk, for example the C allele of the rs9616915 of *SHANK3* decreased the risk of ASD (Shao et al., 2014). However, there has been little research focusing on the association of ASD with *PSD95* and *DLGAP2* in the Chinese population. From previous genetic studies, we believe it is worthwhile to evaluate the association of ASD with *PSD95* and *DLGAP2*. Given our hypothesis that the variants might contribute to ASD risk, we sequenced the SNPs of the *PSD95* gene and *DLGAP2* gene in 529 ASD patients and 1923 healthy controls to ascertain the association in a Chinese population.

2. Methods

2.1. Subjects

Our study included 529 ASD patients and 1923 healthy controls. The ASD patients were recruited from the Maternal and Child Care Service Centre in Zhuhai city, Shenzhen city and Luohu district in China, the Wuhan Mental Health Center in China and the Special Children's Education Agency in Guangzhou, Suzhou and Wuhan in China between July 2010 and July 2016. The ASD patients were diagnosed based on the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV), and subjects with fragile X, deletion, etc. were excluded. The healthy controls were selected from a Genome-wide association study (GWAS) in a Chinese population (Wang et al., 2018), who participated in health check-up programs in Tongji hospital in Wuhan during July 2010 to July 2013, and they were matched with ASD patients for gender. This case-control study was approved by the Ethics Committee of Tongji Medical College of Huazhong University of Science and Technology, China.

2.2. Identification of candidate SNPs and genotyping

The procedure for screening candidate SNPs that might be functional in the *DLGAP2* gene and *PSD95* gene was as follows. First, we extracted the SNPs having possible functional effects of protein coding, splicing regulation, transcriptional regulation or post-translation from the F-SNP database (http://compbio.cs.queensu.ca/F-SNP). Second, these SNPs whose minor allele frequency (MAF) of Han Chinese in Beijing (CHB) was more than 5% were filtered from theHapMap database (http://hapmap.ncbi.nlm.nih.gov/). Third, we assessed the linkage disequilibrium (LD) among these SNPs using SNAP Pairwise (http://www.broadinstitute.org/mpg/snap/ldsearchpw.php); if the SNPs were in strong LD with each other ($r^2 \geq 0.80$), we considered reserving only one for further analysis. As a result, there were five SNPs that could be used for further selection, including rs7005715, rs2301963 and rs2906569 in *DLGAP2,* and rs2521985 and rs2017365 in *PSD95*.

Genomic DNA was extracted from oral swab samples using TIANamp Swab DNA Kit DP080714 (Tiangen, Beijing, China) according to the manufacturer's instructions. DNA concentration and optical density were tested by a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Genotyping was performed at the BIO MIAOBIOLOGICAL Corporation (Beijing, China) with the Sequenom Mass ARRAY platform (San Diego, CA), according to the manufacturer's protocol. The Mass ARRAY Assay Designer software (v3.1) was used to design PCR primers and termination mixes for multiplexed assays. The mass of extended primer was determined using a MALDI-TOF mass spectrometer and we analyzed the resulting genotype spectra using Mass ARRAY Type4.0 software.

2.3. Statistical analysis

SPSS software v23.0 was used for all statistical analyses and the probability level considered significant was $P < 0.05$. The