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Voltage-gated calcium channel activity and complex related genes and schizophrenia: A systematic investigation based on Han Chinese population

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ABSTRACT

Schizophrenia (SCZ) is a devastating mental disorder affecting approximately 1% of the worldwide population. Early studies have indicated that genetics plays an important role in the onset and development of SCZ. Accumulating evidence supports that SCZ is linked to abnormalities of synapse transmission and synaptic plasticity. Voltage-gated calcium channel (VGCC) subunits are critical for mediating intracellular Ca^{2+} influx and therefore are responsible for changing neuronal excitability and synaptic plasticity. To systematically investigate the role of calcium signaling genes in SCZ susceptibility, we conducted a case-control study that included 2518 SCZ patients and 7521 healthy controls with Chinese Han ancestry. Thirty-seven VGCC genes, including 363 tag single nucleotide polymorphisms (SNPs), were examined. Our study replicated the following previously identified susceptible loci: *CACNA1C*, *CACNB2*, *OPRM1*, *GRM7* and *PDE4B*. In addition, several novel loci including *CACNA2D1*, *PDE4D, NALCN*, and *CACNA2D3* were also identified to be associated with SCZ in our Han Chinese sample. Combined with GTEx eQTL data, we have shown that *CASQ2*, *ITGAV*, and *TMC2* can be also added into the prioritization list of SCZ susceptible genes. Two-way interaction analyses identified widespread gene-by-gene interactions among VGCC activity and complex-related genes for the susceptibility of SCZ. Further sequencing based studies are still needed to unravel potential contributions of schizophrenia risk from rare or low frequency variants of these candidate genes.

1. Introduction

Schizophrenia (SCZ) is a devastating mental disorder that affects approximately 1% of the worldwide population. Early family, adoption, and twin studies have confirmed that heritability of SCZ is as high as 80% (O'Donovan et al., 2008). So far, through linkage analyses, candidate gene association studies, and genome-wide association studies (GWAS), many susceptibility genes have been identified including *CACNA1C* (He et al., 2014; Ripke et al., 2013; Schizophrenia Psychiatric Genome-Wide Association Study, 2011; Schizophrenia Working Group of the Psychiatric Genomics, 2014), *CACNB2*(Cross-Disorder Group of the Psychiatric Genomics, 2013; Wang et al., 2010), *CACNG5* (Curtis et al., 2011; Guan et al., 2016) and *CACNA1I* (Andrade et al., 2016; Irish Schizophrenia Genomics and the Wellcome Trust Case Control, 2012), which all encode voltage-gated calcium channel (VGCC) subunits. These findings provide a possibility that VGCCs are involved in the pathogenesis of SCZ. However, the underlying molecular mechanism remains unknown.

Accumulating evidence supports that SCZ is linked to abnormalities of synapse transmission and synaptic plasticity (Stephan et al., 2009); therefore, genes that affect neurotransmitter release and synaptic plasticity may contribute to the risk for SCZ. VGCCs are widely distributed in all parts of the brain. They are critical for mediating intracellular Ca^{2+} influx, which results in transmitter release from pre-

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synaptic endings, changing neuronal excitability and synaptic plasticity (Berger and Bartsch, 2014). Through studies of partial or conditional CACNA1C knockout mice, a defect in protein synthesis-dependent, N-methyl-D-aspartate receptor (NMDAR)-independent LTP in the CA1 region was found, which was paralleled by a deficit in spatial learning (Berger and Bartsch, 2014). These findings demonstrated that VGCCs serve a critical function in synaptic plasticity and learning memory (Moosmang et al., 2005). Data from studies in human genetics have strongly suggested a central role of VGCCs in SCZ. Giegling et al. presented converging genetic evidence for the contribution of genes potentially related to alterations in intracellular calcium-homeostasis to the risk of SCZ (Giegling et al., 2010). Recently, the Cross-Disorder Group of the Psychiatric Genomics found that two L-type voltage-gated calcium channel subunits, *CACNA1C* and *CACNB2,* showed significant association with psychiatric disorders (Cross-Disorder Group of the Psychiatric Genomics, 2013). These two genes code for the α1 and β2 subunits of VGCCs. β subunits regulate trafficking of the respective channel to the plasma membrane and are involved in the modulation of gating properties (Buraei and Yang, 2013; Chen et al., 2004). Moreover, a GWAS (Ripke et al., 2013) indicated that the single nucleotide polymorphism (SNP) rs1006737 in the *CACNA1C* gene is a susceptibility marker for SCZ in individuals of European ancestry (Ripke et al., 2013) (Schizophrenia Psychiatric Genome-Wide Association Study, 2011; Schizophrenia Working Group of the Psychiatric Genomics, 2014). This SNP leads to increased $Ca_V1.2$ α1 subunit mRNA expression (Yoshimizu et al., 2015). Functional magnetic resonance imaging (MRI) studies have also found that healthy individuals carrying the *CACNA1C* risk variant show compromised function of the anterior cingulate cortex, prefrontal cortex and hippocampus (Paulus et al., 2014; Wang et al., 2011). More significantly, combining GWAS with genome-wide expression data from human post-mortem brain samples of SCZ patients and controls has shown a significant enrichment of calcium channel activity genes (Hertzberg et al., 2015). Such studies may answer the question if calcium signaling serves as a key factor in the pathogenesis of SCZ. Activating Ca^{2+} channels triggers biological signal transduction and regulates a variety of downstream gene expression. Nevertheless, the effects of risk variants in VGCCs in SCZ are still unclear.

Our group has published a few consecutive replication studies on GWAS of schizophrenia focusing on *PED4* (Guan et al., 2012) and *CACNG* related genes based on Chinese Han populations (Guan et al., 2016). To investigate the role of calcium signaling genes in susceptibility of SCZ, we conducted a case-control study to evaluate the association of VGCC genes of complex and activity pathways with SCZ in Han Chinese population. Integration of data from different populations potentially increased the biological and statistical reliability of the results, and significantly reduced the false positive rate resulting from each of the separate analyses. Although some VGCC genes have been reported as susceptible loci for SCZ in some populations, it is still necessary to systematically explore the potential association of VGCC genes and SCZ.

2. Methods

2.1. Subjects

A total of 10,039 individuals, including 2518 patients with SCZ (1307 females and 1211 males; range of age was 18–51 years old; mean age 36.9 ± 9.3) and 7521 healthy controls (3903 females and 3618 males; range of age was 18–51 years old; mean age 37.8 ± 9.6) were recruited for this study. All SCZ patients were recruited from the inpatient and outpatient clinical services of a psychiatric unit at Xi'an Mental Health Center, and all unrelated healthy controls were recruited locally. All subjects were from local Han ethnic groups. Based on medical records and self-reports regarding their own and their paternal grandparents' place of birth, we excluded anyone not born in Shaanxi Province or those whose families within three generations were not born in Shaanxi Province, to rule out potential population stratification. All patients were assessed by the Structured Clinical Interview for DSM-IV (Diagnostic and statistical manual of mental disorders, 4th revision) Axis I disorders (SCID), which was administered by two experienced psychiatrists. Diagnostic assessments were supplemented with clinical information obtained by a review of medical records and interviews with family informants. Subjects with substance-induced psychotic disorders, learning disabilities, head injuries, and other symptomatic psychoses were excluded from the present study. All unrelated controls were enrolled from local volunteers based on selection criteria of frequency-matched age (± 5 years) and gender of the patients. Trained psychiatrists interviewed the control subjects by using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders-Nonpatient Edition (SCID-NP), and we excluded individuals with a personal family history of mental illness and with current or past evidence of psychoses from the study. This study was performed in accordance with the ethical guidelines of the Declaration of Helsinki (version 2002) and was approved by the Xi'an Jiaotong University Health Science Center Ethics Committee. All of the participants completed written informed consent forms.

2.2. SNP selection and genotyping

Gene sets for VGCC activity and complex were extracted using KOBAS (http://kobas.cbi.pku.edu.cn/index.php) and were summarized in Supplemental Table S1. A total of 45 genes were obtained. In this present paper, we reviewed 37 VGCC genes from genetic databases and utilized a custom set of 363 tag SNPs, whose heterozygosity (> 0.2), pair-wise tagging r^2 (> 0.8) and minor allele frequency (MAF) (> 0.05) were validated (www.ensembl.org and www.ncbi.nlm.nih.gov/SNP) based on gene coverage, functional prediction, and prior reports (Supplemental Table S2). Eight genes, *CACNG1-8*, were investigated in our previous work (Guan et al., 2016). One thing we need to note is that there is no consensus on definition of VGCC activity and complex gene sets and different gene sets might be obtained based on different criteria. For example, *NALCN* and *PDE* genes may regulate calcium channels, but are also associated with many more important functions. In addition, the *CACNA2D* genes have functions in the synapse that are critical for synaptogenesis and that are not necessarily linked with VGCC (Geisler et al., 2015).

Peripheral blood samples were collected from the vein of all subjects. Genomic DNA samples were extracted from peripheral blood leukocytes according to the standard protocol of the DNA Isolation Kit for Mammalian Blood (Tiangen Biotech Co., Ltd, Beijing, China). SNP genotyping was conducted for all of the SNPs using the Sequenom MassARRAY matrix-assisted laser desorption ionization-time of flight mass spectrometry platform, following the manufacturer's protocols (Sequenom, San Diego, CA, USA). To ensure the accuracy of genotyping, case and control samples were blinded for quality control during the genotyping process. Additionally, we chose randomly 5% of the samples from our study subjects, and the results were 100% concordant.

2.3. Statistical analyses

We calculated MAF and tested Hardy-Weinberg Equilibrium (HWE) for the 363 selected SNPs. The results and basic information are summarized in Supplemental Table S2. All of our selected SNPs had *P* values greater than 0.05 for testing of HWE. We investigated the potential genetic effects of our selected SNPs by fitting logistic models. Age and gender were included in each logistic model as covariates to eliminate any potential confounding effects. Three genetic coding modes including additive, dominant and recessive, were utilized. In addition to the single marker based analyses described above, we also performed haplotype based analyses. Haplotype analyses are supposed to be more powerful compared to single marker based methods, thus we expected