Research Paper

Calcium ion channels CACNA1C polymorphism and Schizophrenia in the Northern Han Chinese population

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ABSTRACT

Common psychiatric disorders are highly hereditary, indicating that genetic factors play an important role in their pathogenesis. The CACNA1C gene, located 12p13.3, which codes for subunit alpha-1C of the Cav1.2 voltage-dependent L-type calcium channel, was consistently found to be the shared risk gene for several kinds of mental disorder. There was a research reported that rs1006737 in intron of the CACNA1C gene being a risk for both schizophrenia and major depressive disorder in the Han population, but another research did not find a significant association between rs1006737 and schizophrenia in the Han Chinese population. To further investigate whether CACNA1C is a susceptibility gene for schizophrenia in the Han Chinese population, a case-control study of 1230 patients with schizophrenia and 1120 healthy controls was conducted. A tag single nucleotide polymorphism (SNP) rs1006737 in the CACNA1C gene were genotyped in all samples. It was observed that rs1006737 was associated with schizophrenia (P_all = 0.015, P_genotype = 0.011, odds ratio (OR) = 1.405, 95% CI 1.067-1.851). It was observed that the frequency of (AA+AG) in patients was obviously different when compared to the control (P=0.007). Our findings further support CACNA1C being a risk gene for schizophrenia in the Han Chinese population.

Key words: Calcium ion channels, schizophrenia, CACNA1C, Chinese, Han population, control, rs1006737, DSM-IV criteria, SNP, allele, genotype.

INTRODUCTION

Schizophrenia is a disease with high disability rate, high relapse rate and high mortality. It is a great burden to the society and the family. The etiology of schizophrenia is complex and genetic factors play a very important role in the development of the disease.

The CACNA1C gene, located 12p13.3, which codes for subunit alpha-1C of the Cav1.2 voltage-dependent L-type calcium channel, was consistently found to be the shared risk gene for several kinds of mental disorder (Green et al., 2010; Liu et al., 2011; Nyegaard et al., 2010; Ripke et al., 2011; Shi et al., 2011; Sklar et al., 2011). Cav1.2 couples transient increase of membrane permeability for calcium-causing cell-membrane depolarization leading to activated intracellular gene transcription and plays an important role in dendritic development, neuronal survival, synaptic plasticity, memory formation, learning and behavior (Moosmang et al., 2005; Shibasaki et al., 2010; White et al., 2008).

The CACNA1C gene is widely expressed in the cardiovascular system and entire nervous system, especially hippocampus and thalamus of the brain (Narayanan et al., 2010; Strueissnig et al., 2006). Genome-wide association studies (GWASs) detected the single nucleotide polymorphism (SNP) rs1006737 in intron of the CACNA1C gene as a shared risk factor for schizophrenia, bipolar disorder and major depressive disorder in the White population (Liu et al., 2011). Stephan Ripke conducted a multi-stage GWAS for schizophrenia beginning other research suggested that rs1006737 of CACNA1C association with schizophrenia with a Swedish national sample followed...
by meta-analysis prior to schizophrenia GWAS and finally by replication of SNPs in 168 genomic regions in independent samples, they found genome-wide significant support for CACNA1C (Ca1.2, \( P = 5.2 \times 10^{-12} \) at the intronic SNP rs1006737) (Stephan et al., 2013).

The positive association between CACNAIC rs1006737 and schizophrenia was found in the Han population (Kuanjun et al., 2014), whereas, other research did not found this association (Zhang et al., 2012). Here, to further investigate whether the rs1006737 in intron of the CACNAIC is associated with schizophrenia in the Northern Han Chinese population, we genotyped rs1006737 in 1230 patients with schizophrenia and 1120 healthy controls.

**MATERIALS AND METHODS**

**Participants**

Drawn from a population of Han descent, our study sample set consists of 1230 people with schizophrenia (739 males and 491 females), and 1120 healthy controls (633 males and 487 females). All of the participants in our study were unrelated, living in Shanxi, China and of Shanxi origin. Patients were in-patients with no impulse wounding risk and were interviewed by two independent psychiatrists and diagnosed strictly according to DSM-IV criteria. Exclusion criteria included the presence of other mood or neurodevelopmental disorders, epilepsy, or mental retardation. For the selection of controls, all participants gave informed consent, the details of which had been reviewed and approved by the local ethical committee of Shanxi, Rongjun Psychiatric Corelle Hospital. Controls were randomly selected from the general population in Shanxi (including normal volunteers, unrelated relatives of patients, interns and hospital staff).

The mean age of individuals in the schizophrenia group was 47.3 years (s.d. = 12). All of the participants with schizophrenia had paranoid schizophrenia and no lifetime history of an episode of mania or depression. The mean age of individuals in the healthy controls was 45.1 years (s.d. = 10). All controls were randomly selected from the general public of the Han Chinese population. Volunteers who replied to a written invitation completed an investigation of their medical history, with supplementary questions about psychosis and other major complex diseases. Before collecting their blood, a face-to-face inquiry was conducted that included a physical examination (height, weight and blood pressure).

**SNP selection**

The TagSNP rs 1006737 was selected based upon the HapMap database (http://hapmap.ncbi.nlm.gov) from Han Chinese in Beijing (CHB) data after configuring the criteria MAF > 0.05 and \( r^2 > 0.8 \).

**SNP genotyping**

The genotype of SNP was analyzed by the Shanghai Biowing Applied Biotechnology Co., Ltd. (www.biowing.com.cn) using the Ligase Detection Reaction-Polymerase Chain Reaction (LDR-PCR) method (O’Donovan et al., 2008; Shi et al., 2011). Genomic DNA extracted from clinical samples was first subjected to multiplex RCR to obtain a PCR product including SNPs. This PCR product and LDR probes were then subjected to mul-tiplex LDR reaction, with a DNA sequencer to detect the products. To test the validity of this procedure, approximately 10% of the samples was randomly selected and retested using the same process. Results in the retested 10% were consistent with those obtained in the larger sample.

**Statistical analysis**

Our statistical analyses was performed using PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/), including association studies, Hardy-Weinberg equilibrium (HWE) tests and the calculation of genotype and allele frequencies in schizophrenic patients and healthy controls. The statistics about the different patterns of the gene using SPSS-17.0 software and chi square test was used.

**RESULTS**

In this study, 1230 schizophrenia patients and 1120 unrelated normal controls was analyzed. HWE tests indicated that the allelic frequency distribution of CACNAIC polymorphisms does not deviate significantly from the Hardy-Weinberg equilibrium (\( P = 0.3618 \) for rs1006737). The total genotyping rate in all individuals is 99.05%. It was found that rs1006737 was significantly associated with schizophrenia (rs1006737: \( \text{P} = 0.015 \), \( \text{P} = 0.011 \), odds ratio (OR) = 1.405, 95% CI 1.067-1.851). (The detailed summary is shown in Table 1).

We compare different models of rs1006737 in the CACNA1C, we found that the frequency of (AA+AG) in patient was obviously different to control; the result shows that A allele of rs1006737 may be a risk site (Table 2).

**DISCUSSION**

Schizophrenia Psychiatric Genome-Wide Association Consortium (PGC) highlighted 81 single nucleotide polymorphisms (SNPs) where association with
schizophrenia, one of the genes was CACNA1C (Marian et al., 2013). CACNA1C is a gene that codes for the α1C subunit of the Cav1.2 voltage-dependent L-type calcium channel (LTCC).

The LTCC family consists of four distinct members referred to as Ca_{α1.1}–Ca_{α1.4} (Catterall, 2011). Ca_{α1.2} is the primary LTCC expressed in the mammalian brain. A functional Ca_{α1.2} channel consists of three sub-units: transmembrane α_{1C} (CACNA1C) and α_{δ} (encoded by CACNA2D1, 2 or 3) as well as, intracellular β (encoded by CACNB1-4 genes) and calmodulin (Dolphin AC, 2009). Using conditional knock-out mice model and pharmacological method, the results suggest that Ca_{α1.2} is a related gene sub-type in the amygdala pathway, not Ca_{α1.3} (Langwieser et al., 2010). Significant association between CACNA1C and schizophrenia was detected, Li et al. (2015) suggests that CACNA1C might play a role in the genetic etiology of autism in Chinese Han population.

In our research, we found that rs1006737 was significantly associated with schizophrenia (rs1006737: P = 0.015, P = 0.011, odds ratio (OR) = 1.405, 95% CI 1.067–1.851). Past research suggested that the CACNA1C rs1006737 risk variant is associated with heritable neuropsychiatric disorders (Green et al., 2013; Wray et al., 2012). Subsequent studies have demonstrated that this SNP is also associated with unipolar major depressive disorder and schizophrenia (Casamassima et al., 2010; Nyegaard et al., 2010). Ivorra et al. (2014) performed a genotyping study in schizophrenia using 86 previously associated SNPs identified by GWAS of schizophrenia, bipolar disorder (BPD) and autistic spectrum disorder (ASD) patients and found SNP rs1006737 in the CACNA1C gene was close to the significant threshold. In the research of Kuanjun et al. (2014), rs1006737 was found to be associated with schizophrenia in Han Chinese population. Another research also found that rs1006737, rs4765905 and rs882194 in CACNA1C showed significant associations with schizophrenia in the Han Chinese population (corrected global p<0.005) (Guan et al., 2014). Our conclusion is consistent with these results. It should also be noted that Zhang et al. (2012) did not find a significant association between rs1006737 and schizophrenia in the Han Chinese population. We believe that the results may not be consistent with the study of region of human, geographical and other factors.

We also found that the frequency of (AA+AG) in schizophrenia was obviously different to control; it shows that A allele of rs1006737 may be a risk site. Yan et al. (2014) used Meta analysis method and found that the single nucleotide polymorphism (SNP) rs1006737 in intron of the CACNA1C type A gene has clear connection with schizophrenia, and more susceptibility with the Caucasian and Asian population. Hiroaki et al. (2012) investigated the association between rs1006737 within CACNA1C and neurocognitive functions in schizophrenia patients and in healthy subjects; the result A-allele carriers demonstrated significantly worse logical memory performance than the G-allele homozygotes suggesting that rs1006737 may be associated with schizophrenia through its detrimental effect on endophenotypic traits. All of those researches prompt A allele of rs1006737 may be a risk site.

The CACNA1C gene is widely expressed in the cardiovascular system and the entire nervous system, especially hippocampus and thalamus of brain. Zhang et al. (2012) tested the association between the SNP rs1006737 and spatial working memory as measured by an N-back task and dot pattern expectancy (DPE) task, among SCZ patients and healthy controls, the clinical risk allele was associated with impaired working memory. Another study demonstrate that rs1006737 is associated with significant functional alterations in human iNs, and may direct future efforts at developing novel therapeutics for the treatment.

### Table 1. Association study of rs1006737 in the CACNA1C gene between case and control.

<table>
<thead>
<tr>
<th>Variable</th>
<th>A</th>
<th>G</th>
<th>A/A</th>
<th>A/G</th>
<th>G/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>133(0.055)</td>
<td>2293(0.945)</td>
<td>2(0.002)</td>
<td>129(0.106)</td>
<td>1082(0.892)</td>
</tr>
<tr>
<td>Control</td>
<td>88(0.040)</td>
<td>2132(0.960)</td>
<td>4(0.004)</td>
<td>80(0.072)</td>
<td>1026(0.924)</td>
</tr>
<tr>
<td>X^2</td>
<td>5.898</td>
<td>9.093</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.015</td>
<td>0.011</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: P <0.05 is meaningful.

### Table 2. Association study of rs1006737 in the CACNA1C under different models.

<table>
<thead>
<tr>
<th>SNP(A1:A2)/test model</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>freq &amp;</td>
</tr>
<tr>
<td>Dominant(AA+AG)/GG</td>
<td>131/1082</td>
<td>0.108</td>
</tr>
<tr>
<td>Recessive(GG+AG)/AA</td>
<td>1211/2</td>
<td>0.998</td>
</tr>
</tbody>
</table>

The major allele frequency for allelic and trend model, "DD+Dd" frequency for dominant model, "D" indicates major allele, "d" indicates minor allele.
of psychiatric disease (Yoshimizu et al., 2015).

At present, many studies have reported that rs1006737 are involved in the function and structure of the brain; variation occurs in healthy people without mental illness and gene mutation can increase the incidence of mental illness. Krug (year) suggested that carrying the risk allele can reduce the level of language, fluency, attention is not focused, personality changes, a high degree of injury avoidance, anxiety, paranoia, panic reaction (Rousso et al., 2011). Some researchers reported CACNA1C genotype carriers can increase brain grey matter volume and stem volume (Kempton et al., 2009; Franke et al., 2010).

The effect of the genotype on brain function or volume changes occurred in which stages is unclear temporarily, need further study.

A few limitations to the present study should be acknowledged. The present sample size is considered insufficient to detect a significant difference, although our data may contribute to future meta-analytic studies.

Our results further support that CACNA1C being a risk gene for schizophrenia in northern Han Chinese population, but how it influence the development of the disease is also a mystery. In the next study, we will further explore the pathogenesis of schizophrenia.

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REFERENCES


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