Association of the CACNA2D2 gene with schizophrenia in Chinese Han population

Yingli Fu 1,2, Na Zhou 3, Wei Bai 1, Yaoyao Sun 1, Xin Chen 1, Yueying Wang 1, Mingyuan Zhang 1, Changgui Kou 1, Yaqin Yu 1, Qiong Yu 1

1 Department of Epidemiology and Statistics, School of Public Health, Jilin University, Changchun, Jilin, China
2 Division of Clinical Research, First Hospital of Jilin University, Changchun, Jilin, China
3 Department of Pharmacy, School and Hospital of Stomatology, Jilin University, Changchun, Jilin, China

Corresponding Author: Qiong Yu
Email address: yuqiong@jlu.edu.cn

Background. Schizophrenia is a severely complex psychiatric disorder in which ~80% can be explained by genetic factors. Single nucleotide polymorphisms (SNPs) in calcium channel genes are potential genetic risk factors for a spectrum of psychiatric disorders including schizophrenia. This study evaluated the association between SNPs in the voltage-gated calcium channel auxiliary subunit alpha2delta 2 gene (CACNA2D2) and schizophrenia in the Han Chinese population of Northeast China.

Methods. A total of 761 schizophrenia patients and 775 healthy controls were involved in this case-control study. Three SNPs (rs3806706, rs45536634, and rs12496815) of CACNA2D2 were genotyped by the MALDI-TOF-MS technology. Genotype distribution and allele frequency differences between cases and controls were tested by Chi-square ($\chi^2$) in males and females respectively using software SPSS24.0. Linkage disequilibrium and haplotype analyses were conducted using Haploview4.2. The false discovery rate (FDR) correction was utilized to control for Type I error by R3.2.3.

Results. There was a significant difference in allele frequencies ($\chi^2 = 9.545, P_{adj}=0.006$) and genotype distributions ($\chi^2=9.275, P_{adj}=0.006$) of rs45536634 between female schizophrenia patients and female healthy controls after adjusting for multiple comparisons. Minor allele A (OR=1.871, 95%CI=1.251-2.798) and genotype GA+AA (OR=1.931, 95%CI=1.259-2.963) were associated with an increased risk of schizophrenia. Subjects with haplotype AG consisting of rs45536634 and rs12496815 alleles had a higher risk of schizophrenia (OR=1.91, 95%CI=1.26-2.90) compared those with other haplotypes.

Conclusions. This study provides evidence that CACNA2D2 polymorphisms may influence the susceptibility to schizophrenia in Han Chinese women.
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\textsuperscript{1}Department of Epidemiology and Statistics, School of Public Health, Jilin University, Changchun, Jilin, China

\textsuperscript{2}Division of Clinical Research, First Hospital of Jilin University, Changchun, Jilin 130021, China

\textsuperscript{3}Depart of Pharmacy, School and Hospital of Stomatology, Jilin University, Changchun, Jilin, China

Corresponding Author:
Qiong Yu\textsuperscript{1}
1163 Xinmin Street, Changchun, Jilin, 130021, China.

Email address: yuqiong@jlu.edu.cn
Abstract

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Background

Schizophrenia (SCZ) is a severely debilitating psychiatric disorder characterized by positive and negative symptoms as well as cognitive dysfunction (Allen et al. 2008; Koike et al. 2014). The lifetime risk of schizophrenia is approximately 1% across the world (Mayilyan et al. 2008), and the lifetime prevalence of adults in China was 0.6% (Huang et al. 2019).

Schizophrenia has a marked impact on the life quality and accounts for approximately 2.8% of the global burden of diseases reported by the World Health Organization (WHO) in 2001, and the prevalence of schizophrenia disability was 0.41% in China (Liu et al. 2015).

Genetic and environmental factors may combine to increase schizophrenia risk (Plomin et al. 1994). Genetic factors explain ~80% of the risk for schizophrenia, and the risk of the schizophrenia decrease as the parental relationship recedes (Zhu et al. 2009). Despite vigorous genome-wide association studies have been conducted to elucidate the common genetic variations associated with the susceptibility to schizophrenia (Børglum et al. 2014; Riley et al. 2009), the etiology of schizophrenia remains obscure, suggesting that additional studies are required to discover the “missing heritability”.

The voltage-gated calcium channel auxiliary subunit alpha2delta 2 gene (CACNA2D2), located in 3p21.31 and highly expressed in the brain, encodes a calcium channel protein. Voltage-gated calcium channels are widely distributed throughout the brain and mediate the intracellular Ca2+ influx of synaptic action potentials (Guan et al. 2016). In recent years, the gene encoding voltage-gated calcium channel subunit attracts wide attention in the field of schizophrenia pathogenesis. SNPs in calcium channel genes have been identified as genetic risk factors for a spectrum of psychiatric disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium2013; Cross-Disorder Group of the Psychiatric Genomics Consortium 2014). For example, several studies showed that SNPs of CACNA1C were significantly
associated with schizophrenia, and the finding has been confirmed in different populations 
(Gasso et al. 2016; Zhang et al. 2017; Zhu & Li 2019). Although there are no reported 
studies on the relationship between CACNA2D2 and schizophrenia, CACNA2D2 may cause 
other psychiatric disorders (Berridge 2013) and severe neurological diseases (Strupp et al. 
2005). In this study, we conducted a genetic association study to examine the association 
between SNPs of CACNA2D2 and schizophrenia by a case-control study.

Materials and Methods

Study Population

A total of 761 patients with schizophrenia from the Mental Hospital of Changchun and 
775 healthy controls from the physical examination center of the First Hospital of Jilin 
University were recruited. All participants were Han Chinese. The patients were diagnosed 
according to the criteria of the International Statistical Classification of Disease and Related 
Health Problems, Tenth Revision (ICD-10) independently by at least two experienced 
psychiatrists. Healthy controls matched the patients by gender and age. All controls had no 
personal or family history of mental illness. This study was performed under protocols 
approved by the Ethics Committee of Jilin University, China (2014-05-01). All subjects 
signed written informed consent before participating in this study, and all experiments were 
performed in accordance with relevant guidelines and regulations.

DNA Extraction and SNP selection

Peripheral blood of 5mL was collected from each subject, and the genomic DNA was 
extracted from blood samples using a commercial DNA extraction kit (Kangwei Biotech 
Company, Beijing, China). SNPs located in the promoter and 3’ untranslated region (UTR) 
of CACNA2D2 were searched in NCBI-SNP (https://www.ncbi.nlm.nih.gov/snp/) and 
Ensembl (https://asia.ensembl.org/) databases. We predicted the function of these SNPs in 
SNPinfo (http://snpinfo.niehs.nih.gov/) and searched for minor allele frequency (MAF) each 
SNP in 1000 Genomes. Finally, we chose three SNPs (MAF > 0.05) located in promoter or 
3’UTR regions (rs3806706 in the promoter region and rs45536634 and rs12496815 in the 3’
UTR) of CACNA2D2 and predicted to be located in transcription factor binding sites (TFBS). SNP genotyping was performed using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). SNP genotyping reactions were performed in a 384-well Spectro-CHIP using a Mass Array nanodispenser (Sequenom Inc.). The primers for genotyping were designed by AssayDesigner3.1 and were listed in Table 1.

**Statistical Analysis**

Pearson’s Chi-square ($\chi^2$) test and Student’s t-test were used to test the distribution of sex and age between case and control groups, respectively. The distributions of allele and genotype were analyzed using $\chi^2$ tests. The odds ratio (OR) was used to estimate the relative risk of schizophrenia associated with genotypes with minor alleles. The Type I error due to multiple testing was corrected by the false discovery rate (FDR) method. All the above analyses were performed using Software SPSS 24.0 (IBM SPSS, IBM Corp, Armonk, NY, USA), and R version 3.2.3 was used for FDR corrections. The Hardy-Weinberg equilibrium (HWE) test was conducted in the case and control group separately by Goodness of fit $\chi^2$ test using online software SNPStats (https://www.snpstats.net/snpstats/start.htm). The linkage disequilibrium (LD) between SNPs was estimated in both females and males separately using Haploview 4.2 (Barrett et al. 2005), and the haplotype analysis was further performed using Haploview. The statistical power for each SNP was calculated according to the MAF of each SNP (rs45536634: 0.073, rs3806706: 0.378, and rs12496815: 0.388). The prevalence of schizophrenia (1%) was estimated by Quanto 1.2.4 (Gauderman 2002). The OR was set from 1.4 to 2.0. All tests were two-sided and $P$-adj-value less than 0.05 was considered to be statistically significant.

**Results**

**Demographic Characteristics**

A total of 1,536 subjects were included in this study, comprised of 761 schizophrenia patients (58.2% males, mean age= 34.61±12.02 years) and 775 healthy controls (56.2% males, mean age= 34.74±11.41 years). There was no significant difference either in sex ($\chi^2$
The distribution of alleles and genotypes in males and females

The detection rate of rs45536634, rs12496815 and rs3806706 were 97%, 92% and 98%, respectively. Genotype and allele frequencies of females were depicted in Table 3. A significant difference ($P_{adj}=0.012$) was observed in allele frequencies of rs45536634 between female schizophrenia patients and female healthy controls. Subjects who carried minor allele A had a 1.9 times higher risk of schizophrenia than those homozygous for the major G allele. Similarly, a significant difference ($P_{adj}=0.006$) was observed in the genotype distribution in females, and subjects with the minor allele (GA+AA) has an increased risk of schizophrenia when compared those with genotype GG ($OR=1.931$, 95%CI=1.259-2.963). These associations were found only in females, but not in males. In the male group, there was a difference between cases and controls in the genotype distribution of rs3806706 ($P=0.02$); however, after adjusting for multiple testing, the difference was not significant ($P_{adj}=0.12$) (Table 4).

LD and Haplotype Analysis

As shown in Figure 1, the LD analysis of rs45536634 and rs12496815 in CACNA2D2 showed that the D’ values were equal to 1 in both female and male groups. According to the results of LD analysis, haplotype association analyses of rs45536634-rs12496815 were conducted in females and males respectively, and the results are shown in Table 5. Three common haplotypes were estimated to have a frequency>1%, and haplotype AG was significantly associated with schizophrenia ($OR=1.91$, $P_{adj}=0.0096$) in females.

Statistical power

The statistical power for rs45536634, rs12496815, and rs3806706 were 0.675-0.999, 0.872-0.999, and 0.878-0.999, respectively, if the OR varied from 1.4 to 2.0.

Discussion
The association between variants of a number of genes and schizophrenia has been reported in previous studies. To the best of our knowledge, this is the first report of a significant association between rs45536634 of *CACNA2D2* and schizophrenia in females of the Northeast Han Chinese population.

It is known that Ca2+ ion represents one of the most important second messengers in the brain and plays an essential role in neuronal development, synaptic transmission and plasticity, besides regulating various metabolic pathways (Striessnig et al. 2006). Notably, as demonstrated by several studies, the Ca2+ homeostasis disorder is associated with many pathological mechanisms, especially those related to neurodegenerative disorders, such as schizophrenia, Alzheimer’s disease, and bipolar disorder (Berridge 2013; Sulzer & Surmeier 2013). *CACNA2D2* encodes the Alpha 2 delta 2 subunit of voltage-gated calcium channel (Tedeschi et al. 2016) which is a key signaling element, allowing changes in membrane potential to control a large number of Ca2+ dependent neurotransmitter release and neuronal plasticity in electrically excitable cells (Striessnig et al. 2006). A study conducted by Villela et al. showed that the copy number change in *CACNA2D2* was a risk factor for Alzheimer’s Disease (Villela et al. 2016).

Moreover, *CACNA1C*, a gene in the same family as *CACNA2D2*, has been repeatedly confirmed as one of the susceptibility genes for schizophrenia in various populations (Guan et al. 2014; He et al. 2014). In addition, Zhang et al. conducted a review research on calcium channel genes associated with schizophrenia in the Han Chinese population and found that *CACNA1C, CACNB2, CACNA2D1* and *CACNA2D3* were related to schizophrenia (Zhang et al. 2018).

The sex-specific molecular phenotype of schizophrenia was observed in previous studies. A study conducted by Oumaima et al. indicated that minor alleles of SNPs in genes *LTA* and *TNFA* were over-represented in male schizophrenia patients but not in female schizophrenia patients (Inoubli et al. 2018). Jemli et al. researched the association between the functional polymorphism of *IFNGR2* with schizophrenia and found the *IFNGR2* Q64R
polymorphism was associated with schizophrenia in males (Jemli et al. 2017). The study conducted by Yang Guang et. al showed that the genotypes and allele distributions of rs3087494 in PLA2G12A were significantly associated with schizophrenia in males, but not in females (Yang et al. 2016). Another study focused on sex-specific molecular phenotypes found that eight genes showed a differential expression in female and male schizophrenia patients (Ramsey et al. 2013).

For a better understanding of the association between schizophrenia and CACNA2D2, a more in-depth investigation — haplotype analysis was carried out to determine whether the combination of specific alleles was associated with the schizophrenia risk. The AG haplotype, consisted of rs44536634 and rs12496815 alleles, was correlated with an increased risk of schizophrenia in Han Chinese women. The haplotype analysis not only confirmed the association between rs44536634 and schizophrenia, but also supported that rs44536634 allele A was associated with an increased risk of schizophrenia in females. Furthermore, our research provided an evidence to support the distinct molecular phenotypes of schizophrenia patients with different gender, as reported in previous studies.(Ben Nejma et al. 2013; Jemli et al. 2017; Ramsey et al. 2013).

In this study, several limitations should be considered. Firstly, our study was performed at a single center and only three SNPs were analyzed, it ignored SNPs in other genes that may be associated with schizophrenia. Secondly, the study is limited to interpreting the causal relationship between genetic risk factors and schizophrenia as this is a cross-sectional study. Furthermore, the representative of this study was limited to the adults of Northeast China because the samples were collected from Jilin Province. Finally, we lost some demographic covariates of the controls when analyzing the association between CACNA2D2 SNP polymorphism and schizophrenia patients due to the difficulty of demographic characteristics collection. Large-scale examination with more demographic characteristics is warranted to further examine the association between CACNA2D2 and schizophrenia.

Conclusion
The sample size of the present study was sufficient for detecting the effect of 
CACNA2D2 variants on schizophrenia. This study demonstrated that CACNA2D2 
polymorphisms might influence the susceptibility to schizophrenia in Han Chinese women 
from Northeast China. The findings support the hypothesis that CACNA2D2 may represent a 
novel susceptibility gene for schizophrenia in females. Functional genomics studies should 
be performed in future to validate the function of schizophrenia-associated CACNA2D2 
variants.

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Table 1 (on next page)

Primers for polymerase chain reaction
Table 1. Primers for polymerase chain reaction

| SNP    | Primer sequence(5'-3')                      |
|--------|---------------------------------------------|
| rs12496815 | F: ACCTGGGATGGTGGTTTTGGCACCAGTGTGCT        |
|        | R: ACCTGGGATGTGGCACCCAAATCACATCTC          |
| rs3806706  | F: ACCTGGGATGTGGCTCAACAGCTGTCCCTT          |
|        | R: ACCTGGGATGTCCAGCAACAGGTAAGAG           |
| rs45536634 | F: ACCTGGGATGCAATGTATGTCAAGGCTGT          |
|        | R: ACCTGGGATGGAGTCACTTTAGTGCTCTG          |
Table 2 (on next page)

Test of HWE for case and control groups

Ho: observed heterozygosity; He: expected heterozygosity.
Table 2. Test of HWE for case and control groups

| Gene      | SNP          | case       |          | control      |          |
|-----------|--------------|------------|----------|--------------|----------|
|           | H0           | He         | $\chi^2$ | $\chi^2$     | $P$      |
|           | H0           | He         |          | H0           | He       | $\chi^2$ | $P$      |
| CACNA2D2  | rs45536634   | 0.172      | 0.171    | 0.024        | 0.9      |
|           | rs12496815   | 0.469      | 0.497    | 2.126        | 0.1      |
|           | rs3806706    | 0.458      | 0.437    | 1.66         | 0.2      |

$H_o$: observed heterozygosity; $H_e$: expected heterozygosity.
Table 3 (on next page)

Genotype and allele distributions of CACNA2D2 SNPs in female

* \( P_{adj} \) represent \( P \) corrected by FDR

\* \( P_{adj} < 0.05 \)
Table 3. Genotype and allele distributions of *CACNA2D2* SNPs in female.

| SNPs       | Genotype | Case | Control | $\chi^2$ | $P$  | $P_{adj}$ | OR(95%CI) |
|------------|----------|------|---------|---------|------|-----------|-----------|
| rs3806706  | GG       | 140  | 158     | 0.032   | 0.858| 0.858     | 1         |
|            | GC+CC    | 165  | 181     |         |      |           | 1.184(0.689-2.035) |
|            | Allele   |      |         |         |      |           |           |
|            | G        | 417  | 460     | 0.039   | 0.843| 0.858     | 1         |
|            | C        | 193  | 218     |         |      |           | 0.977(0.772-1.235) |
| rs45536634 | GG       | 227  | 297     | 9.275   | 0.002| 0.006*    | 1         |
|            | GA+AA    | 62   | 42      | 9.545   | 0.002| 0.006*    | 1.931(1.259-2.963)* |
|            | Allele   |      |         |         |      |           |           |
|            | G        | 513  | 635     |         |      | 1         | 1.871(1.251-2.798)* |
|            | A        | 65   | 43      |         |      |           | 1         |
| rs12496815 | GG       | 62   | 69      | 1.421   | 0.233| 0.395     | 1         |
|            | GA+AA    | 190  | 268     |         |      |           | 0.789(0.534-1.165) |
|            | Allele   |      |         |         |      |           |           |
|            | G        | 247  | 308     | 1.268   | 0.263| 0.395     | 1         |
|            | A        | 257  | 366     |         |      |           | 0.876(0.695-1.103) |

$P_{adj}$ represent $P$ corrected by FDR

* $P_{adj} < 0.05$
Table 4 (on next page)

Genotype and allele distributions of CACNA2D2 SNPs in male

$P_{adj}$ represent $P$ corrected by FDR
Table 4. Genotype and allele distributions of CACNA2D2 SNPs in male.

| SNPs      | Genotype | Case | Control | $\chi^2$ | $P$   | $P_{adj}$ | OR (95% CI)          |
|-----------|----------|------|---------|----------|-------|-----------|----------------------|
| rs3806706 | GG       | 190  | 225     | 5.373    | 0.02  | 0.12      | 1                    |
|           | GC+CC    | 241  | 208     |          |       |           | 1.372 (1.050-1.793)  |
|           | Allele   |      |         |          |       |           |                      |
|           | G        | 580  | 617     | 3.185    | 0.074 | 0.172     | 1.205 (0.982-1.478)  |
|           | C        | 282  | 249     |          |       |           |                      |
| rs45536634| GG       | 361  | 369     | 0.127    | 0.721 | 0.865     | 1                    |
|           | GA+AA    | 67   | 64      | 0.00025  | 0.987 | 0.987     | 1.070 (0.738-1.552)  |
|           | Allele   |      |         |          |       |           |                      |
|           | G        | 786  | 795     | 0.987    | 0.987 |           | 1.997 (0.707-1.407)  |
|           | A        | 70   | 71      |          |       |           |                      |
| rs12496815| GG       | 85   | 105     | 0.66     | 0.416 | 0.624     | 1                    |
|           | GA+AA    | 303  | 327     | 1.145    | 0.826 | 1.586     | 1.145 (0.826-1.586)  |
|           | Allele   |      |         |          |       |           |                      |
|           | G        | 347  | 423     | 2.953    | 0.086 | 0.172     | 1.186 (0.976-1.440)  |
|           | A        | 429  | 441     |          |       |           |                      |

$P_{adj}$ represent $P$ corrected by FDR
Table 5 (on next page)

Association between haplotypes and schizophrenia by sex

\*P_{adj} < 0.05
Table 5. Association between haplotypes and schizophrenia by sex

| Haplotype         | Male(freq) | Control | Case | OR(95%CI)   | P   | P_adj |
|-------------------|------------|---------|------|-------------|-----|-------|
|                   |            |         |      |             |     |       |
| rs45536634-rs12496815 |            |         |      |             |     |       |
| GA                | 0.510      | 0.551   | 1    | —           | —   | —     |
|                   | 5          | 8       |      |             |     |       |
|                   | 0.407      | 0.366   | 1.03 | 0.84        | 0.08| 0.28  |
|                   | 5          | 6       |      |             |     |       |
|                   | 0.082      | 0.081   | 1.31 | 0.93        | 0.66| 0.7   |
|                   | 6          | 6       |      |             |     |       |
|                   | 0          | 0       | —    | —           | —   | —     |
| GG                | 0.082      | 0.081   | 1.31 | 0.93        | 0.66| 0.7   |
|                   | 6          | 6       |      |             |     |       |
| AG                | 0          | 0       | —    | —           | —   | —     |
| AA                | 0          | 0       | —    | —           | —   | —     |
| rs124996815-rs3806706 |            |         |      |             |     |       |
| GG                | 0.446      | 0.393   | 1    | —           | —   | —     |
|                   | 2          | 3       |      |             |     |       |
|                   | 0.265      | 0.279   | 1.51 | 1.18        | 0.18| 0.3   |
|                   | 9          | 6       |      |             |     |       |
|                   | 0.243      | 0.275   | 1.61 | 1.27        | 0.04| 0.23  |
|                   | 9          | 2       |      |             |     |       |
| AC                | 0.243      | 0.275   | 1.61 | 1.27        | 0.04| 0.23  |
|                   | 9          | 2       |      |             |     |       |
|     | 0.044 | 0.051 | 1.33 (0.77 - 2.29) | 0.31 | 0.388 | 0.0473 | 0.069 | 1.43 (0.77 - 2.62) | 0.26 | 0.65 |
|-----|-------|-------|-------------------|------|-------|--------|-------|-------------------|-----|------|
| GC  |       |       |                   |      |       |        |       |                   |     |      |

rs124996815
-
rs3806706-
rs45536634

|     | 0.371 | 0.314 | 1                  | —    | —     | 0.3492 | 0.329 | 7                  | 1   | —    | —     |
|-----|-------|-------|-------------------|------|-------|--------|-------|-------------------|-----|-------|--------|
| GGG |       |       |                   |      |       |        |       |                   |     |       |        |

|     | 0.266 | 0.279 | 1.23 (0.95 - 1.59) | 0.12 | 0.28  | 0.2683 | 0.259 | 8                  | 1.04 (0.76 - 1.43) | 0.79 | 0.87 |
|-----|-------|-------|-------------------|------|-------|--------|-------|-------------------|-------------------|-----|------|
| AGG |       |       |                   |      |       |        |       |                   |                  |     |      |

|     | 0.243 | 0.273 | 1.31 (1.03 - 1.69) | 0.03 | 0.23  | 0.2741 | 0.249 | 6                  | 0.97 (0.72 - 1.31) | 0.85 | 0.87 |
|-----|-------|-------|-------------------|------|-------|--------|-------|-------------------|-------------------|-----|------|
| ACG |       |       |                   |      |       |        |       |                   |                  |     |      |

|     | 0.074 | 0.078 | 1.23 (0.83 - 1.81) | 0.3  | 0.288 | 0.061  | 0.094 | 1                  | 1.67 (1.02 - 2.72) | 0.042 | 0.167 |
|-----|-------|-------|-------------------|------|-------|--------|-------|-------------------|-------------------|-----|------|
| GGA |       |       |                   |      |       |        |       |                   |                  |     |      |

|     | 0.036 | 0.050 | 1.58 (0.87 - 2.87) | 0.14 | 0.28  | 0.045  | 0.048 | 2                  | 1.18 (0.60 - 2.34) | 0.63 | 0.87 |
|-----|-------|-------|-------------------|------|-------|--------|-------|-------------------|-------------------|-----|------|
| GCG |       |       |                   |      |       |        |       |                   |                  |     |      |

|     | 0.007 | 0.003 | 0.72 (0.13 - 3.85) | 0.7  | 0.7   | 0.0024 | 0.018 | 6                  | 8.05 (1.01 - 64.35) | 0.05 | 0.167 |
|-----|-------|-------|-------------------|------|-------|--------|-------|-------------------|-------------------|-----|------|
| rare|       |       |                   |      |       |        |       |                   |                  |     |      |

*P<0.05
Fig. 1 Linkage disequilibrium (LD) of SNPs within CACNA2D2 in female (A) and male (B), D’ values were used to estimate the LD between pairwise SNPs.