

# Association of the CACNA2D2 gene with schizophrenia in Chinese Han population

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**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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# Abstract

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**Keywords:** Schizophrenia; *CACNA2D2*; SNPs; haplotype

## 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 Ca<sup>2+</sup> 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 Consortium 2013; 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*-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 ( $\chi^2$

=0.681,  $P=0.409$ ) or age ( $t=0.221$ ,  $P=0.825$ ) between patients and healthy controls. The results of the HWE test were shown in Table 2. All SNPs were in accordance with the HWE in both cases and controls ( $P>0.05$ ).

### 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 ( $P_{\text{adj}}=0.012$ ) 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 ( $P_{\text{adj}}=0.006$ ) 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 ( $P_{\text{adj}}=0.12$ ) (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 ( $OR=1.91$ ,  $P_{\text{adj}}=0.0096$ ) 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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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

1

Table 1. Primers for polymerase chain reaction

SNP	Primer sequence(5'-3')
rs12496815	F: ACGTTGGATGTGGTTTTGGCACCAGTGCGT
	R: ACGTTGGATGTGGCACCCAAATCACATCTC
rs3806706	F: ACGTTGGATGTGAGCTCAACAGCTGCCTTC
	R: ACGTTGGATGGTCCAGCAAACAGGTAAGAG
rs45536634	F: ACGTTGGATGCAATGTATGTCAAGGGCCTG
	R: ACGTTGGATGGAGTCCCAGTTAGTGCTCTG

2

# **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			
		H <sub>0</sub>	He	$\chi^2$	P	H <sub>0</sub>	He	$\chi^2$	P
<i>CACNA2D2</i>	rs45536634	0.172	0.171	0.024	0.9	0.127	0.137	2.42	0.12
	rs12496815	0.469	0.497	2.126	0.1	0.498	0.499	0.002	0.968
	rs3806706	0.458	0.437	1.66	0.2	0.403	0.422	1.583	0.208

Ho: observed heterozygosity; He: 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	$\chi^2$	<i>P</i>	<i>P<sub>adj</sub></i>	<i>OR(95%CI)</i>
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				1.931(1.259-2.963)*
	Allele			9.545	0.002	0.006*	
	G	513	635				1
	A	65	43				1.871(1.251-2.798)*
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<sub>adj</sub>* represent *P* corrected by FDR

\**P<sub>adj</sub>*<0.05

# **Table 4**(on next page)

Genotype and allele distributions of *CACNA2D2* SNPs in male

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

1 Table 4. Genotype and allele distributions of *CACNA2D2* SNPs in male.

SNPs	Genotype	Case	Control	$\chi^2$	<i>P</i>	<i>P<sub>adj</sub></i>	<i>OR(95%CI)</i>
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
	C	282	249				1.205(0.982-1.478)
rs45536634	GG	361	369	0.127	0.721	0.865	1
	GA+AA	67	64				1.070(0.738-1.552)
	Allele			0.00025	0.987	0.987	
	G	786	795				1
	A	70	71				1.997(0.707-1.407)
rs12496815	GG	85	105	0.66	0.416	0.624	1
	GA+AA	303	327				1.145(0.826-1.586)
	Allele						
	G	347	423	2.953	0.086	0.172	1
	A	429	441				1.186(0.976-1.440)

2 *P<sub>adj</sub>* 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(frequency)					Female(frequency)				
	Contro l	Case	OR(95%CI)	P	P <sub>adj</sub>	Control	Case	OR(95%CI)	P	P <sub>adj</sub>
rs45536634- rs12496815										
GA	0.510 5	0.551 8	1	—	—	0.5424	0.506 9	1	—	—
GG	0.407 5	0.366 6	0.84 (0.68 - 1.03)	0.08 6	0.28	0.3942	0.380 7	1.02 (0.80 - 1.31)	0.87	0.87
AG	0.082	0.081 6	0.93 (0.66 - 1.31)	0.66	0.7	0.0634	0.112 5	1.91 (1.26 - 2.90)	0.002 6	0.026 *
AA	0	0		—	—	0	0		—	—
rs124996815- rs3806706										
GG	0.446 2	0.393 3	1	—	—	0.4098	0.419 6	1	—	—
AG	0.265 9	0.279 6	1.18 (0.92 - 1.51)	0.18	0.3	0.2686	0.264	0.96 (0.71 - 1.29)	0.77	0.87
AC	0.243 9	0.275 2	1.27 (1.00 - 1.61)	0.04 6	0.23	0.2742	0.247 3	0.88 (0.66 - 1.16)	0.37	0.74

GC	0.044	0.051 9	1.33 (0.77 - 2.29)	0.31	0.388	0.0473	0.069 1	1.43 (0.77 - 2.62)	0.26	0.65
rs124996815										
-										
rs3806706-										
rs45536634										
GCG	0.371 2	0.314 8	1	—	—	0.3492	0.329 7	1	—	—
AGG	0.266	0.279 8	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
ACG	0.243 9	0.273 5	1.31 (1.03 - 1.69)	0.03 1	0.23	0.2741	0.249 6	0.97 (0.72 - 1.31)	0.85	0.87
GGA	0.074 9	0.078 2	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
GCG	0.036 9	0.050 2	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
rare	0.007 1	0.003 5	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

\* $P_{adj} < 0.05$

# Figure 1

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

