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Association of *NKAPL*, *TSPAN18*, and *MPC2* gene variants with schizophrenia based on new data and a meta-analysis in Han Chinese

Li Z, Shen T, Xin R, Liang B, Jiang J, Ling W, Wei B, Su L. Association of *NKAPL*, *TSPAN18*, and *MPC2* gene variants with schizophrenia based on new data and a meta-analysis in Han Chinese.

Background: Schizophrenia (SZ) is suggested to be a complex polygenetic disorder with high heritability. Genome-wide association studies have found that the rs1635, rs11038167, and rs10489202 polymorphisms are associated with SZ in Han Chinese. However, results of validation studies are inconsistent. This study aimed to test the association between the *NKAPL* rs1635, *TSPAN18* rs11038167, and *MPC2* rs10489202 polymorphisms and SZ in a Chinese population.

Methods: This study contained 700 unrelated SZ patients (300 Zhuang and 400 Han) and 700 gender- and age-matched controls (300 Zhuang and 400 Han). The polymorphisms in *TSPAN18* (rs11038167), *NKAPL* (rs1635), and *MPC2* (rs10489202) were genotyped using the Sequenom MassARRAY method. Statistical analyses were performed with PLINK program and SPSS l6.0 for Windows. STATA11.1 was used for meta-analysis.

Results: No statistically significant difference was found in different allele and genotype frequencies of rs1635, rs11038167, and rs10489202 between SZ cases and controls of Zhuang and Han ethnicities and the total samples (all $p > 0.05$). Further meta-analysis suggested that single-nucleotide polymorphism rs10489202 was significantly associated with SZ in a Han Chinese population ($p_{OR} = 0.002$).

Conclusions: Our case–control study failed to validate the significant association of *NKAPL* rs1635, *TSPAN18* rs11038167, and *MPC2* rs10489202 polymorphisms with SZ susceptibility in the southern Zhuang or Han Chinese population. However, meta-analysis showed a significant association between *MPC2* variant rs10489202 and SZ susceptibility in Han Chinese.

Zhen Li[1,2,†], Tingting Shen[3,†], Ran Xin[4,†], Baoyun Liang[3], Juan Jiang[1], Weijun Ling[1], Bo Wei[1], Li Su[1,5]

[1]School of Public Health, Guangxi Medical University, Nanning, Guangxi, China; [2]Education Department, Guangxi Zhuang Autonomous Region, Nanning, Guangxi, China; [3]First Affiliated Hospital, Guangxi University of Chinese Medicine, Nanning, Guangxi, China; [4]Chinese Center for Disease Control and Prevention, Nanning, Guangxi, China; and [5]Guangxi Colleges and Universities Key Laboratory of Prevention and Control of Highly Prevalent Diseases, Nanning, Guangxi, China

[†]First co-author.

Keywords: association studies; *MPC2*; *NKAPL*; schizophrenia; *TSPAN18*

Bo Wei, School of Public Health of Guangxi Medical University, 22 Shuangyong Road, Nanning, Guangxi 530021, China.
Tel: +867 715 358 847;
Fax: +867 715 350 823;
E-mail: weibogx@163.com
Li Su, School of Public Health of Guangxi Medical University, 22 Shuangyong Road, Nanning, Guangxi 530021, China; Guangxi Colleges and Universities Key Laboratory of Prevention and Control of Highly Prevalent Diseases, Nanning, Guangxi 530021, China.
Tel: +861 387 889 0526;
Fax: +867 715 350 823;
E-mail: suli2018@hotmail.com

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Significant outcomes

- There was no statistically significant difference in different allele and genotype frequencies of rs1635, rs11038167, rs10489202 between schizophrenia (SZ) cases and controls in Zhuang ethnic population.
- No significant difference was observed between *NKAPL* rs1635, *TSPAN18* rs11038167, *MPC2* rs10489202 polymorphism and the susceptibility to SZ in Han ethnic population.
- Further meta-analysis suggested that single-nucleotide polymorphism (SNP) rs10489202 was significantly associated with SZ in the Han Chinese population.

Limitations

- The limited sample size may not have been powerful enough to identify the tiny influence of some SNPs with susceptibility to SZ. Therefore, additional studies with larger sample sizes should be conducted.
- The recruited SZ patients of the study may have different levels of heterogeneity, which may confuse the association result. So, it could be feasible to narrow down the phenotype to a more homogeneous subgroup, enabling an accurate distinction of underlying genetic subtypes.
- We only investigated one SNP in each gene, which failed to cover all the genetic variants of *NKAPL*, *TSPAN18*, and *MPC2* gene. Consequently, additional studies should take the combined effects of more polymorphisms and the potential gene–gene interactions into consideration.

Introduction

Schizophrenia (SZ) is a severe neuropsychiatric disorder characterised by a series of psychotic symptoms, such as delusions, hallucinations, and cognitive impairments (1), and epidemiological data have shown that the lifetime prevalence of SZ is up to about 1% worldwide (2). SZ, as a complex disease, is influenced by both genetic and environmental factors (3), whereas heritable factors of SZ account for 80% (4). Thus, genetic research of SZ is popular worldwide. However, the definite genetic mechanism remains unclear.

Numerous genome-wide association studies (GWASs) in SZ have been conducted, and many susceptibility loci have been identified. However, these GWAS-identified genetic associations still require further validation. In 2011, a GWAS (768 cases and 1733 controls) found that two SNPs, rs1635 and rs11038167, have a significant association with SZ in a Han Chinese population, and the follow-up independent Chinese sample of 4027 cases and 5603 controls also successfully replicated this result (5). Another GWAS of Han Chinese descent (3750 cases and 6468 controls) in 2011 (6) reported that the association between *MPC2* variant rs10489202 and SZ reaches genome-wide statistical significance, thereby validating the significant association with an additional sample of 4383 cases and 4539 controls. SNP rs1635 is located in the *NKAPL* gene, which encodes for nuclear factor-κB-activating protein-like, whereas *NKAPL* is located at 6p21-p22.1, which belongs to the extended major histocompatibility complex (MHC) region. Accumulating evidence from GWASs (7–9), pathway analysis studies (10), and expression studies (11,12) demonstrated the significant role of MHC in SZ. The rs11038167 polymorphism is located at the *TSPAN18* gene and it encodes tetraspanin 18, a member of a superfamily of tetraspanins that are involved in signalling, antigen presentation, and diverse cellular processes (13). *TSPAN18* was proposed as a susceptibility locus containing the rs11038167, rs11038172, and rs835784 polymorphisms for SZ in the GWAS by Yue et al. (5) of Han Chinese, and a similar study (14) also revealed that *TSPAN18* rs835784 is significantly associated with SZ in a Chinese population. Another SNP rs10489202 of the *MPC2* gene was found on chromosome 1q24.2, which encodes mitochondrial pyruvate carrier (MPC) 2 protein. Previous studies (15–17) have shown that the MPC involved in some metabolic pathways is closely related to psychiatric disorders. Thus, these studies suggested that the *NKAPL*, *TSPAN18*, and *MPC2* genes may be associated with susceptibility to SZ.

Since the 2011 GWASs (5,6), subsequent validation research was carried out in succession in different regions of China. In 2012, Ma et al. (18) genotyped nine GWAS-identified risk loci in an independent case–control study of Han Chinese from Hunan Province, and they failed to replicate the associations of the SNP rs1635, rs11038167, and rs10489202 with SZ. In 2013, a replication study by Yuan et al. (14) indicated no significant association in both the allele and genotype frequencies of rs11038167 in *TSPAN18* with SZ among a Han Chinese population in Jiangsu. Another replication study (19) for *MPC2* did not support the previous GWAS findings, and results showed that rs10489202 is not associated with SZ in Han Chinese. In 2014, Chen et al. (20) used a sample of Han Chinese subjects from Taiwan, and validated the association of rs1635 in the *NKAPL* gene with SZ, which supported the view that *NKAPL* is a susceptible gene for SZ. In 2015, a recent study by Zhang et al. (21) reported that *TSPAN18* variant rs11038167 is not significantly associated with SZ risk in northwestern Han Chinese subjects in Shanxi Province. Another study by Wang et al. (22) detected a positive association of the *NKAPL* rs1635 polymorphism with SZ in Han Chinese from Jiangsu Province, but their meta-analysis failed to validate a significant association. Notably, these replication studies used samples from different provinces in China, which implied that differences in the genetic background of individuals in different regions may lead to conflicting results.

Hence, this study aimed to examine whether the three GWAS-identified positive SNPs (rs1635, rs11038167, and rs10489202) can influence susceptibility to SZ in a

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Chinese population. China is a united multi-ethnic state, where the Han is a major ethnic group, and the Zhuang nationality is the largest minority. First, we conducted an independent case–control study including subjects from Zhuang and Han Chinese populations in Guangxi. In addition, a meta-analysis of the SNPs combining our case–control study with previous replication studies was performed to accurately assess the relationship between these SNPs and SZ in the Chinese population.

Methods

Case–control study

Participants. The case group consisted of 700 SZ patients from the two ethnic groups of Mainland China: 400 Han subjects and 192 Zhuang subjects from Guangxi Brain Hospital, and 108 Zhuang subjects from a public health project funded by the government in Liujiang County, Guangxi. The diagnosis was confirmed by two independent, trained psychiatrists according to ICD-10 (The International Classification of Disease, tenth revision). All patients are of one ethnic origin and born in Guangxi within three generations based on their own reports or reports by their first-degree relatives. Exclusion criteria included mental disorders caused by various organic diseases of the nervous system or other system disorders; substance-induced psychotic disorders; mood or neurodevelopmental disorders or mental retardation; and stroke, epilepsy, and other neurological diseases or other serious physical illnesses. Healthy controls were selected by well-trained investigators using a simple non-structured interview; these controls included 154 Zhuang healthy volunteers and 400 Han healthy individuals recruited from two comprehensive hospitals in the same region, as well as 146 healthy Zhuang volunteers in rural communities of Liujiang County. The ICD-10 was used to assess the subjects to exclude individuals with psychiatric conditions from the control group. This group was free of past or present major psychiatric or neurological disorders; in addition, the control subjects had no family history of mental illnesses in first-degree relatives.

All participants were unrelated Han or Zhuang Chinese born and living in Guangxi, and all of their biological grandparents were of Han or Zhuang Chinese ancestry. All the research subjects or their legal guardians signed informed consents.

Genotyping assays. Blood samples were collected using K2EDTA tubes (Weihai Hongyu Medical Instrument Co. Ltd, Shangdong, China) from all participants. In 7 days, genomic DNA was extracted from peripheral blood leucocytes using a commercial kit (Tiangen Biotech, Beijing, China). DNA samples were then stored at −80°C for genotype analysis. Primers were designed using Sequenom Assay Designer 3.1 software (Sequenom, San Diego, CA, USA), and the primer sequences are shown in Table 1. The polymorphisms in *TSPAN18* (rs11038167), *NKAPL* (rs1635), and *MPC2* (rs10489202) were genotyped using the Sequenom MassARRAY method. The design and synthesis of primers for allele identification were completed by Bomiao Technologies Co. (Beijing, China). Moreover, a 5% random sample was repeatedly tested, and the concordance rate was 100%.

Table 1. Primers of the rs1635, rs11038167, and rs10489202 polymorphisms

SNP_ID	Primer	Sequence
rs1635	forward primer	5′-ACGTTGGATGCGAGTTGGAATCTGAACTGC-3′
	reverse primer	5′-ACGTTGGATGTCCCTCAGCTAGATTCTGAC-3′
rs11038167	forward primer	5′-ACGTTGGATGCTCAGATAATTTCTCTGTGGG-3′
	reverse primer	5′-ACGTTGGATGAGGCTCAGAGAAACTAAGTG-3′
rs10489202	forward primer	5′-ACGTTGGATGCTCTCAATAACTGTCAGTTC-3′
	reverse primer	5′-ACGTTGGATGTGGTGTGCCTCCAACTTGTC-3′

SNP = single nucleotide polymorphism.

Statistical analysis. The PLINK program (http://pngu.mgh.harvard.edu/~purcell/plink/) was applied to evaluate the genetic association between SNP genotypes and SZ susceptibility. χ^2 goodness of fit test was used to assess the genotypic distributions among the control groups of each SNP for Hardy–Weinberg equilibrium (HWE). The correlation between each SNP and SZ was performed using unconditional logistic regression. After adjusting for age and gender, we obtained odds ratios (ORs) and 95% confidence intervals (95% CIs) to evaluate the strength of genotypes or alleles in different genetic models. Three genetic models were used, namely, Additive (AA vs. Aa vs. aa), Dominant (AA + Aa vs. aa), and Recessive (AA vs. Aa + aa), in which A is the mutant genotype, and a is the wild type. Power analysis was undertaken by Quanto software (http://hydra.usc.edu/gxe). SPSS version 16.0 was used to complete the statistical analysis of general characteristics of subjects. The independent *t*-test was employed to compare ages between SZ patients and normal controls of the two ethnic groups, and Pearson's χ^2-test was used to compare the categorical variable gender.

Meta-analysis

Searches were done in PubMed, Embase, Chinese National Knowledge Infrastructure database, Chinese Wanfang, and Chongqing VIP database, using a

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combined retrieval strategy that includes the following key terms: 'schizophrenia', 'rs1635', 'rs11038167', 'rs10489202', 'NKAPL', 'TSPAN18', 'MPC2', 'association study', 'case-control study' and 'China'. In addition, some potential studies which were closely related to the research subject were identified by examining reference lists. The available articles cover all English and Chinese publications from their commencement to 16 September 2015. The identified studies were required to comply with the following inclusion criteria: (1) case–control studies that examined the association of *NKAPL* rs1635 or *TSPAN18* rs11038167 or *MPC2* rs10489202 polymorphisms with SZ susceptibility in a Chinese population; and (2) studies provided with data on 'ORs and 95% CIs', or 'genotype and allele frequencies'. Studies were unsuitable if they complied with any one of characteristics below, namely, exclusion criteria: (1) the articles were not original research, for instance, reviews, commentaries, editorials, conference papers, and so on; (2) the duplicate publications and incomplete articles. Data extraction was in line with the inclusion criteria, including name of first author, publication year, country, ethnicity, number of cases and controls, ORs, 95% CIs, genotype, and allele frequencies. STATA software (version 11.1) was used for meta-analysis. The associations between SNPs and suspectibility to SZ in all samples were tested by pooled ORs and 95% CIs. Here, the pooled ORs were calculated based on the original data including ORs and 95% CIs from each included study. Heterogeneity across the included studies was assessed using the Q-test and I^2 statistics. If I^2 was <50% and $p > 0.10$, no significant heterogeneity existed, and the fixed-effect model (Mantel–Haenszel method) was used to merge the data; otherwise, the random-effect model (The DeSimonian and Laird method) was used. All of the tests were two-tailed, with statistical significance of $p < 0.05$.

Results

General characteristics of participants

The Zhuang ethnic population comprised 300 SZ cases (with 207 males and 93 females aged 33.68 ± 11.99 years) and 300 controls (with 198 males and 102 females aged 32.37 ± 12.27 years). The Han ethnic sample included 400 patients (67.2% males, 32.29 ± 11.56 of mean age) and 400 controls (62.0% males, 33.09 ± 11.17 of mean age). In the total sample group, 700 SZ patients (68% males) and 700 controls (63.7% males) were included, with an average of 32.89 ± 11.76 and 32.78 ± 11.65 years, respectively. No significant differences in age or gender distributions were observed between the case and control subjects in the Zhuang, Han, and total sample population (all $p > 0.05$).

Power analysis

The power test was calculated under the following parameters: the prevalence of SZ was 1%; the ORs were set as 1.3; the sample size of the Zhuang, Han, and total group was 300, 400, and 700, respectively; the allele frequencies of rs1635, rs11038167, and rs10489202 polymorphisms were 0.340, 0.375, and 0.128 in Zhuang controls, 0.364, 0.345, and 0.144 in Han controls, and 0.354, 0.358, and 0.137 in the total controls, respectively. According to the above parameters, the statistical power of rs1635, rs11038167, and rs10489202 polymorphisms was 59.05%, 60.51%, and 35.56% among Zhuang subjects, 72.54%, 71.72%, and 48.57% among Han subjects, and 92.05%, 92.15%, and 70.38% among the total subjects, respectively.

Association between the *NKAPL* rs1635, *TSPAN18* rs11038167, and *MPC2* rs10489202 polymorphisms and SZ susceptibility.

The genotype distributions of the SNPs rs1635, rs11038167, and rs10489202 in the healthy controls did not show any significant deviations from HWE in each group (Table 2). No significant difference in the genotypic and allelic frequency distribution of the three SNPs was observed between SZ patients and controls (Table 2). As presented in Table 3, we found no significant association between the *NKAPL* rs1635, *TSPAN18* rs11038167, and *MPC2* rs10489202 polymorphisms and SZ susceptibility in different genetic models in the Zhuang, Han, and total sample groups, respectively.

Meta-analysis

Nine studies (5, 6, 14, 18–22) (including our study) were finally included in this meta-analysis. This meta-analysis comprised five data sets containing 8070 SZ cases and 10 237 controls for the *NKAPL* rs1635 polymorphism, five data sets (7685 cases and 10 295 controls) for the *TSPAN18* rs11038167 polymorphism, and seven data sets (10 602 cases and 13 472 controls) for the *MPC2* rs10489202 polymorphism (Table 4). Results of the present meta-analysis indicated that the SNPs rs1635 and rs11038167 were not significantly associated with SZ susceptibility (both $p_{OR} > 0.05$), whereas the rs10489202 polymorphism showed a significant association with SZ susceptibility in the Han Chinese population ($p_{OR} = 0.002$), and the corresponding pooled OR and 95% CI are shown in Table 5. A random-effect model was applied to calculate the association between rs1635, rs11038167, rs10489202 polymorphisms and SZ risk

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Table 2. Genotype and allele distribution of single-nucleotide polymorphisms (SNPs) and Hardy–Weinberg equilibrium (HWE) test

		Genotype (A1A1/A1A2/A2A2)					Allele (A1/A2)			
SNP_ID	Samples	Case	Control	χ^2	p	p_{HWE}	Case	Control	χ^2	p
rs1635	Zhuang	23/136/137	36/132/132	2.990	0.224	0.797	182/410	204/396	1.443	0.230
	Han	63/177/158	53/185/162	1.084	0.582	1.000	303/493	291/509	0.488	0.485
	Total	86/313/295	89/317/294	0.053	0.974	0.804	485/903	495/905	0.053	0.819
rs11038167	Zhuang	43/143/110	44/136/119	0.526	0.769	0.622	229/363	224/374	0.189	0.664
	Han	43/183/170	42/189/165	0.183	0.913	0.316	269/523	273/519	0.045	0.833
	Total	86/326/280	86/325/284	0.023	0.989	0.680	498/886	497/893	0.016	0.901
rs10489202	Zhuang	7/78/210	3/71/226	NA	NA	0.441	92/498	77/523	1.860	0.173
	Han	14/98/285	10/95/295	0.874	0.646	0.540	126/668	115/685	0.693	0.405
	Total	21/176/495	13/166/521	2.794	0.247	1.000	218/1166	192/1208	2.300	0.129

A1, minor allele; A2, major allele; P_{HWE}, HWE for control subjects.

Table 3. Association between single-nucleotide polymorphisms (SNPs) and SZ in Zhuang and Han groups

		Additive model		Dominant model		Recessive model		Allelic model	
SNP_ID	Samples	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
rs1635	Zhuang	0.86 (0.67–1.10)	0.224	0.91 (0.66–1.26)	0.575	0.62 (0.36–1.07)	0.086	0.86 (0.68–1.10)	0.230
	Han	1.07 (0.88–1.31)	0.491	1.03 (0.78–1.37)	0.817	1.23 (0.83–1.83)	0.302	1.08 (0.88–1.32)	0.485
	Total	0.98 (0.84–1.14)	0.819	0.98 (0.79–1.21)	0.848	0.97 (0.71–1.33)	0.856	0.98 (0.84–1.15)	0.819
rs11038167	Zhuang	1.05 (0.83–1.33)	0.665	1.12 (0.80–1.56)	0.509	0.99 (0.63–1.55)	0.948	1.05 (0.83–1.33)	0.664
	Han	0.98 (0.79–1.21)	0.829	0.95 (0.72–1.26)	0.719	1.03 (0.65–1.61)	0.909	0.98 (0.79–1.20)	0.832
	Total	1.01 (0.86–1.18)	0.900	1.02 (0.82–1.26)	0.879	1.01 (0.73–1.38)	0.976	1.01 (0.86–1.18)	0.901
rs10489202	Zhuang	1.26 (0.91–1.76)	0.168	1.24 (0.86–1.78)	0.253	2.41 (0.62–9.40)	0.206	1.26 (0.91–1.74)	0.173
	Han	1.12 (0.85–1.46)	0.418	1.10 (0.81–1.51)	0.534	1.43 (0.63–3.25)	0.399	1.12 (0.85–1.48)	0.405
	Total	1.17 (0.95–1.44)	0.134	1.16 (0.91–1.47)	0.224	1.65 (0.82–3.33)	0.159	1.18 (0.95–1.45)	0.129

SZ, schizophrenia; OR, odds ratio; CI, confidence interval.

Table 4. Genotype distribution of the studied single-nucleotide polymorphisms (SNPs) in the meta-analysis

		rs1635 (T/G)		rs11038167 (A/C)		rs10489202 (T/G)	
References	Ethnicity	Case	Control	Case	Control	Case	Control
Yue et al. (5)	Chinese Han	1303/3470	2348/4854	2185/2588	2883/4319	–	–
Shi et al. (6)	Chinese Han	–	–	–	–	–	–
Ma et al. (18)	Chinese Han	331/645	360/683	373/603	414/629	151/825	148/895
Yuan et al. (14)	Chinese Han			877/1307	806/1234		
Jin et al. (19)	Chinese Han					324/1834	331/1703
Chen et al. (20)	Chinese Han	388/642	273/639				
Zhang et al. (21)	Chinese Han			193/250	274/354		
Wang et al. (22)	Chinese Han	897/1915	789/1483				
This study (2015)	Chinese Han	303/493	291/509	269/523	273/519	126/668	115/685

with significant heterogeneity ($p_{\text{Heterogeneity}} = 0.000$, $I^2 = 93.80\%$; $p_{\text{Heterogeneity}} = 0.000$, $I^2 = 89.00\%$; $p_{\text{Heterogeneity}} = 0.048$, $I^2 = 52.80\%$, respectively), showed in Table 5 and supplementary Fig. 1a–c. No publication bias of the meta-analysis upon the rs11038167 and rs10489202 polymorphisms were observed in the funnel plot by Egger's test (both $p > 0.05$, supplementary Fig. 2b–c), whereas a publication bias of the meta-analysis on the SNP rs1635 was found in the funnel plot by Egger's test ($p = 0.018$, supplementary Fig. 2a).

Table 5. Meta-analysis of single-nucleotide polymorphisms (SNPs) and the susceptibility of schizophrenia

SNP_ID	Number of cases/controls	OR (95% CI)	$p_{\text{Heterogeneity}}$	I^2 (%)	p_{OR}
rs1635	8070/10 237	1.05 (0.84–1.31)	0.000	93.80	0.681
rs11038167	7685/10 295	1.05 (0.89–1.23)	0.000	89.00	0.566
rs10489202	10 602/13 472	1.15 (1.06–1.26)	0.048	52.80	**0.002**

OR, odds ratio; CI, confidence interval.
Bold value showed a significant correlation, which was used for emphasis.

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Discussion

In our study, the results suggested that the GWAS-identified SNPs rs1635, rs11038167, and rs10489202 may not be associated with SZ susceptibility in Zhuang and Han individuals of China. But, our meta-analysis showed that the SNP rs10489202 was significantly associated with SZ in Han Chinese. This study was conducted for the first time to examine the association of the SNPs rs1635, rs11038167, and rs10489202 with the susceptibility of SZ in the Zhuang Chinese population, and further evaluate the association of the three SNPs with SZ susceptibility in the Han Chinese population.

In 2011, a GWAS (5) reported that the association between the *NKAPL* rs1635 polymorphism, *TSPAN18* rs11038167 polymorphism, and SZ reached the level of genome-wide significance in a Han Chinese population. In the same year, another GWAS (6) also identified the *MPC2* rs10489202 polymorphism as potential susceptibility loci for SZ in Han Chinese. Several replicated studies were conducted in different areas in China, including Hunan (18), Jiangsu (14,19,22), Taiwan (20), and Shanxi province (21). In addition, our case–control study showed no significant association between the SNPs rs1635, rs11038167, and rs10489202 and SZ risk in both Zhuang and Han groups in Guangxi, whereas our meta-analysis validate the association between rs10489202 and SZ in Han Chinese. Thus, these subsequent validation studies presented inconsistent association results compared with previous GWAS studies. One possible reason for this inconsistency is that the limited sample size may not provide sufficient power. Our sample size was smaller than the two GWASs, with a sample size (case/control) of 4773/7207 (5) and 8133/11 007 (6), respectively. Another important reason may be the genetic heterogeneity of these variants to SZ risk in different regions of China. Notably, these studies used samples from different geographic areas of China; the subjects of the 2011 GWAS (5) were recruited from northern China (including Beijing, Tianjin, Hebei, and Shandong Provinces), whereas the study subjects of Zhang et al. (21) were from northwestern China (Shanxi Province). Subjects from Ma et al. (18), Yuan et al. (14), and Wang et al. (22), as well as our present study, were enroled from southern China (Hunan, Jiangsu, and Guangxi Provinces). Meanwhile, another GWAS (6) collected samples from northern, central, and southern Han Chinese, whereas subjects of the replicated study (19) were only from Jiangsu Province (eastern China), and our subjects in the present study were from Guangxi Province (southern China). Previous studies by Chen et al. (23) and Xu et al. (24) showed a great genetic difference among the Han Chinese population, which implied that the population structure and geographic variation might generate conflicting association results.

Although our case–control study has failed to find the susceptibility of SZ, further meta-analysis of this study suggested that the SNP rs10489202 was significantly associated with SZ risk in Han Chinese. Rs10489202 polymorphism is located in intron 1 of *MPC2* gene on chromosome 1q24.2. *MPC2* gene encodes the MPC-2 protein that is the main component of MPC proteins (25). The main role of MPC proteins is to transport pyruvate by acting as a facilitative carrier. Pyruvate is a critical metabolite linking cytoplasmic and mitochondrial metabolism, which participates in the process of oxidative metabolism, glycolysis, amino acid catabolism, and anabolism (26). Currently, research showed that defects in mitochondrial function are related to some progressive neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and it is considered to be linked with perturbations in MPC activity (27–29). Though the mechanism of MPC gene disruption in neurons is currently not clear, mitochondrial pyruvate transport is believed to maintain neuronal function of the brain (30). Therefore, further studies are warranted to explore the role of *MPC2* gene variant in the occurrence of SZ.

Several possible limitations of this study should be noted. First, the limited sample size may not have been powerful enough to identify the minimal influence of some SNPs with susceptibility to SZ. Second, the recruited SZ patients of the study may have different levels of heterogeneity, which may confuse the results. SZ is recognised as a highly heterogeneous disease, and it possesses diverse clinical presentations and may have different risk genotypes. Thus, narrowing down the phenotype to a more homogeneous subgroup is feasible; for example, by using neuropsychological test scores, electrophysiological assessments, neuroimaging, or defined symptom subtypes with some symptom scales, an accurate distinction of underlying genetic subtypes may be created. Third, we only investigated one SNP in each gene, which failed to cover all the genetic variants of the *NKAPL*, *TSPAN18*, and *MPC2* genes. An association study by Zhang et al.(31) reported that the SNPs rs12214383 and rs12000 on the *NKAPL* gene are significantly associated with SZ in a Han Chinese population. Consequently, additional studies on the association of the three genes with SZ should consider the combined effects of more polymorphisms and the potential gene–gene interactions.

In conclusion, our case–control study suggested that the *NKAPL* rs1635, *TSPAN18* rs11038167, and *MPC2* rs10489202 polymorphisms might not be associated with susceptibility to SZ in the Zhuang or

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Han populations, whereas our meta-analysis validate a significant association between *MPC2* rs10489202 polymorphism and SZ susceptibility. Further studies with larger samples should include more ethnicities to further confirm the association of the *NKAPL*, *TSPAN18*, and *MPC2* genes with the risk of SZ.

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Conflicts of Interest

None.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Supplementary material

For supplementary material/s referred to in this article, please visit http://dx.doi.org/doi:10.1017/neu.2016.36

References

1. Owen MJ, Craddock N, O'Donovan MC. Schizophrenia: genes at last? Trends Genet 2005;**21**:518–525.
2. Saha S, Chant D, Welham J, McGrath J. A systematic review of the prevalence of schizophrenia. PLoS Med 2005;**2**:e141.
3. Jia P, Wang L, Meltzer HY, Zhao Z. Common variants conferring risk of schizophrenia: a pathway analysis of GWAS data. Schizophr Res 2010;**122**:38–42.
4. Sullivan PF, Daly MJ, O'Donovan M. Genetic architectures of psychiatric disorders: the emerging picture and its implications. Nat Rev Genet 2012;**13**:537–551.
5. Yue WH, Wang HF, Sun LD et al. Genome-wide association study identifies a susceptibility locus for schizophrenia in Han Chinese at 11p11.2. Nat Genet 2011;**43**:1228–1231.
6. Shi Y, Li Z, Xu Q et al. Common variants on 8p12 and 1q24.2 confer risk of schizophrenia. Nat Genet 2011;**43**:1224–1227.
7. Purcell SM, Wray NR, Stone JL et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 2009;**460**:748–752.
8. Shi J, Levinson DF, Duan J et al. Common variants on chromosome 6p22.1 are associated with schizophrenia. Nature 2009;**460**:753–757.
9. Stefansson H, Ophoff RA, Steinberg S et al. Common variants conferring risk of schizophrenia. Nature 2009;**460**:744–747.
10. Jia P, Wang L, Fanous AH, Chen X, Kendler KS, Zhao Z. A bias-reducing pathway enrichment analysis of genome-wide association data confirmed association of the MHC region with schizophrenia. J Med Genet 2012;**49**:96–103.
11. Mexal S, Frank M, Berger R et al. Differential modulation of gene expression in the NMDA postsynaptic density of schizophrenic and control smokers. Brain Res Mol Brain Res 2005;**139**:317–332.
12. Harrison PJ. The hippocampus in schizophrenia: a review of the neuropathological evidence and its pathophysiological implications. Psychopharmacology (Berl) 2004;**174**:151–162.
13. Berditchevski F, Odintsova E. Tetraspanins as regulators of protein trafficking. Traffic 2007;**8**:89–96.
14. Yuan J, Jin C, Qin HD et al. Replication study confirms link between TSPAN18 mutation and schizophrenia in Han Chinese. PLoS One 2013;**8**:e58785.
15. Halestrap AP, Scott RD, Thomas AP. Mitochondrial pyruvate transport and its hormonal regulation. Int J Biochem 1980;**11**:97–105.
16. Khaitovich P, Lockstone HE, Wayland MT et al. Metabolic changes in schizophrenia and human brain evolution. Genome Biol 2008;**9**:R124.
17. Olsen L, Hansen T, Jakobsen KD et al. The estrogen hypothesis of schizophrenia implicates glucose metabolism: association study in three independent samples. BMC Med Genet 2008;**9**:39.
18. Ma L, Tang J, Wang D et al. Evaluating risk loci for schizophrenia distilled from genome-wide association studies in Han Chinese from Central China. Mol Psychiatry 2013;**18**:638–639.
19. Jin C, Zhang Y, Wang J et al. Lack of association between MPC2 variants and schizophrenia in a replication study of Han Chinese. Neurosci Lett 2013;**552**:120–123.
20. Chen SF, Chao YL, Shen YC, Chen CH, Weng CF. Resequencing and association study of the NFKB activating protein-like gene (NKAPL) in schizophrenia. Schizophr Res 2014;**157**:169–174.
21. Zhang B, Li DX, Lu N, Fan QR, Li WH, Feng ZF. Lack of association between the TSPAN18 gene and schizophrenia based on new data from Han Chinese and a meta-analysis. Int J Mol Sci 2015;**16**:11864–11872.
22. Wang Z, Yang B, Liu Y et al. Further evidence supporting the association of NKAPL with schizophrenia. Neurosci Lett 2015;**605**:49–52.
23. Chen J, Zheng H, Bei JX et al. Genetic structure of the Han Chinese population revealed by genome-wide SNP variation. Am J Hum Genet 2009;**85**:775–785.

24. Xu S, Yin X, Li S et al. Genomic dissection of population substructure of Han Chinese and its implication in association studies. Am J Hum Genet 2009;**85**: 762–774.
25. Vigueira PA, McCommis KS, Schweitzer GG et al. Mitochondrial pyruvate carrier 2 hypomorphism in mice leads to defects in glucose-stimulated insulin secretion. Cell Rep 2014;**7**:2042–2053.
26. Gray LR, Rauckhorst AJ, Taylor EB. A method for multiplexed measurement of mitochondrial pyruvate carrier activity. J Biol Chem 2016;**291**:7409–7417.
27. Shetty PK, Galeffi F, Turner DA. Cellular links between neuronal activity and energy homeostasis. Front Pharmacol 2012;**3**:43.
28. Parnetti L, Gaiti A, Polidori MC et al. Increased cerebrospinal fluid pyruvate levels in Alzheimer's disease. Neurosci Lett 1995;**199**:231–233.
28. Ahmed SS, Santosh W, Kumar S, Christlet HT. Metabolic profiling of Parkinson's disease: evidence of biomarker from gene expression analysis and rapid neural network detection. J Biomed Sci 2009;**16**:63.
30. McCommis KS, Finck BN. Mitochondrial pyruvate transport: a historical perspective and future research directions. Biochem J 2015;**466**:443–454.
31. Zhang Y, Lu T, Yan H et al. Replication of association between schizophrenia and chromosome 6p21-6p22.1 polymorphisms in Chinese Han population. PLoS One 2013;**8**:e56732.

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