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Genetic variation in the 3'-untranslated region of *PAK1* influences schizophrenia susceptibility

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Abstract. The present study aimed to investigate the association of two polymorphisms (rs2844337 and rs11237200) in the P21 protein (cell division control protein 42/Rac)-activated kinase 1 gene with susceptibility to schizophrenia (SCZ) in Chinese Zhuang and Chinese Han populations. A total of 700 patients with SCZ and 700 healthy controls were recruited. Rs2844337 and rs11237200 polymorphisms were genotyped using Sequenom technology. A total of 591 patients completed the Positive and Negative Syndrome Scale (PANSS) assessment. Data were statistically analyzed using PLINK version 1.07 and SPSS version 17.0. In the Chinese Han population, the genotypic (P=0.038) and allelic (P=0.033) frequencies of the 3'-untranslated region (UTR) genetic variation of rs2844337 in patients were significantly decreased compared to that in controls; these frequencies were significantly associated with SCZ susceptibility in the additive model (P_{adj}=0.032) and in the recessive model (P_{adj}=0.031). Moreover, the TG haplotype constructed by rs2844337 and rs11237200 polymorphisms remained significantly associated with SCZ risk following adjustment for gender and age and applying a Bonferroni correction in the Chinese Han population (P_{adj}=0.003, P_{BC}=0.009). The adjacent 5'-UTR genetic variation of rs11237200 was significantly associated with the total score (P_{adj}=0.006), positive scale score (P_{adj}=0.014) and general psychopathology scale scores (P_{adj}=0.009) in the recessive model of the Chinese Han population. However, these polymorphisms were not significantly associated with SCZ susceptibility or the PANSS scores in the Chinese Zhuang population. In conclusion, variations in the *PAK1* gene influenced the susceptibility and severity of the clinical symptoms of SCZ in the Chinese Han population investigated in the present study.

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Introduction

Schizophrenia (SCZ) is characterized by cognitive impairment and disorganized behavior, which may result in debilitation and require costly maintenance. The global prevalence of SCZ varies; the median incidence and the median lifetime morbid risk of SCZ are 15.2/100,000 and 7.2/1,000 individuals, respectively (1,2). In China, a systematic review has demonstrated that the lifetime prevalence of SCZ is 5.44/1,000 individuals (3). In addition, SCZ induces a financial and social burden worldwide (4). Furthermore, the genetic contribution to SCZ is ~81% (5). Genetic studies on SCZ have predominantly focused on the identification of candidate genes and the corresponding genetic loci (6-9); >1,000 genetic association studies on SCZ risk have also been conducted (http://www.schizophreniaforum.org/). However, studies on candidate genes have yet to clearly elucidate the genetic basis of SCZ (10).

The P21 protein [cell division control protein (Cdc) 42/Rac]-activated kinase 1 (*PAK1*) gene is located in the 11q13-q14 chromosome, and encodes PAK proteins. These proteins are critical effectors that link the Rho family of GTPases to cytoskeleton reorganization and nuclear signaling, and also function as targets of small GTP binding proteins (Cdc42 and Rac). Rubio *et al* (11) previously revealed that damage on the Cdc42/PAK1 and Duo/Rac-1/PAK1 pathways in the anterior cingulate cortex may enhance myosin light chain phosphorylation, which is responsible for the cytoskeletal dysfunction and dendritic spine loss associated with SCZ. A study on *Caenorhabditis elegans* has demonstrated that disrupted-in-SCZ 1 (DISC1) leads to the abnormal branching formation of schizophrenic motor neurons by activating the UNC-73-RAC-PAK signaling pathway (12). Datta *et al* (13) found that mRNA levels of *PAK1* are significantly downregulated in the schizophrenic deep layer of three pyramidal cells. This evidence indicates that the *PAK1* gene may be involved in the molecular pathological mechanism of SCZ. However, studies have yet to describe the genetic association of the *PAK1* genetic variation and SCZ.

A total of 179 single nucleotide polymorphisms (SNPs) are present in the *PAK1* gene. In the rs2844337 polymorphism, a G allele is inserted in the 3'-untranslated region (UTR) in the 77322663 position of the *PAK1* gene. Genetic

variation in 3'-UTRs of eukaryotic genes may affect gene expression through the binding of these genes to specific microRNAs (miRNAs or miRs), potentially resulting in serious pathology (14). Caputo *et al* (15) found that two 3'-UTR functional variants (rs11030100 and rs11030099) of the brain-derived neurotrophic factor gene (*BDNF*) influence SCZ susceptibility by altering miRNA-BDNF binding. In human-induced pluripotent stem cells, a rare functional SNP (rs1130354) in the 3'-UTR of the dopamine receptor D2 gene (*DRD2*) disrupts the targeting of miR-326 to the mRNA of DRD2; as a result, dopamine signaling in patients with SCZ is dysregulated (16). The G allele of the rs11122396 polymorphism, which is located in the 3'-UTR of the *DISC1* gene, may be functionally related to the schizophrenic phenotypes of miR-135b-5p; however, this G allele may no longer regulate the mRNA level of *DISC1* (17). Furthermore, rs11237200 is located near the 5'-UTR in the 77474957 position of the *PAK1* gene. The ribosomal binding site near the 5'-UTR may be implicated in the inhibition of translation and may accelerate degradation by binding to small RNAs (18). In a meta-analysis, schizophrenic expression quantitative trait loci were suggested to be located near the 5'-UTR (19). Thus, the genetic variation in rs2844337 (3'-UTR) and rs11237200 (5'-UTR) of the *PAK1* gene may be involved in SCZ.

Fifty-six ethnic groups are found in China. Among these groups, Han is the largest, followed by Zhuang which ranks second, with a population of 52.82 million (20). More than 90% of the Zhuang population inhabits the Guangxi Zhuang Autonomous Region in South China, which is located near the border of Vietnam. At present, studies on the genetic association of SCZ have predominantly focused on the Han population in China, and few studies have investigated SCZ affecting individuals in other ethnic groups. The authors of the present study have recently published two studies that focus on the association between genetic variations and SCZ susceptibility in the Chinese Zhuang population (21,22). These studies revealed that the rs12807809 polymorphism of the neurogranin gene influences the severity of the clinical symptoms of SCZ in the Chinese Han population, but not in the Chinese Zhuang population (21). Considering ethnic differences, a case-control analysis was performed in the present study to investigate the potential genetic association between SCZ and two *PAK1* polymorphisms (rs2844337 and rs11237200) in Chinese Han and Chinese Zhuang populations. Moreover, SCZ predominantly manifests heterogeneous clinical symptoms; genetic variations are possibly related to different clinical symptoms. Therefore, the association between the *PAK1* polymorphisms and the clinical symptoms of SCZ were assessed, on the basis of the Positive and Negative Syndrome Scale (PANSS) (23).

Materials and methods

Inclusion and exclusion criteria. In the present study, the Chinese Han or Chinese Zhuang patients were of the same ethnic origin for at least three generations. Patients with SCZ were diagnosed independently by two senior psychiatrists on the basis of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, criteria (24); the final diagnoses made by these psychiatrists were consistent. Patients who satisfied the following conditions were excluded: Mental disorders caused by organic brain syndrome; mental retardation; nervous system disease; history of severe brain injury; uncooperative patients with severe excitement or impulse; and pregnant or breast-feeding women. Healthy controls were age- and gender-matched with the patients with SCZ. Healthy controls who met the following criteria were excluded: Family history of mental disease; severe head trauma or birth injury; or febrile convulsion in childhood or infant stage.

Subjects. A total of 1,400 unrelated individuals were recruited from the Guangxi Zhuang Autonomous Region between April 2010 and May 2012. The case group (400 Chinese Han and 300 Chinese Zhuang) was recruited from Guangxi Encephalopathy Hospital (Liuzhou, China) and Community Management Patients of Guangxi Liujiang County (Zhuang-inhabited region). The healthy controls (400 Chinese Han and 300 Chinese Zhuang) included individuals who visited Guangxi Liuzhou People's Hospital (Liuzhou, China) and The First Affiliated Hospital of Guangxi University of Chinese Medicine (Nanning, China) for a regular checkup and healthy volunteers living in Liujiang County. All participants provided informed written consent after the protocols were clearly understood or explained by legal guardians. All protocols in the present study were approved by the Ethics Committee of Guangxi Medical University (Nanning, China).

PANSS symptom assessment. PANSS (23) was used to assess the symptoms of patients with SCZ. The following scale scores were calculated: PANSS total score and three subscale scores (positive, negative and general psychopathology scale scores). Symptoms were assessed by two senior psychiatrists on the day that the patients were admitted to hospital.

Genomic DNA extraction. A total of 5 ml peripheral blood was drawn from each subject and collected in vacutainer tubes containing the anticoagulant ethylenediaminetetraacetic acid; the blood specimen was shaken and stored at 4˚C. Genomic DNA was isolated from leukocytes in seven days using a TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). Extracted DNA was divided into three tubes and stored at -80˚C.

Genotyping. Genotyping was performed using a MassARRAY microarray platform on an iPLEX GOLD technology system (Sequenom, Inc., San Diego, CA, USA). The primers of rs2844337 and rs11237200 polymorphisms were designed using Sequenom Assay Designer 3.1 (Sequenom, Inc.). Primer sequences are listed in Table I. In the present study, primer design, synthesis and genotypic tests were performed by Bomiao Biological Co., Ltd., (Beijing, China). Approximately 5% of the DNA samples were selected randomly and retested to assess the quality of genotypic data; the concordance rate was 100%.

Statistical analysis. SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA) was used for evaluating the frequencies of allele and genotype, and the comparison of age and gender. Linkage disequilibrium (LD) analyses were performed with Haploview version 4.2 software (Whitehead Institute for Biomedical Research, Cambridge, MA, USA). The following

Table I. Primer sequences of *PAK1* gene polymorphisms rs2844337and rs11237200.

SNPs	Forward primer (5'-3')	Reverse primer (5'-3')
rs2844337	ACGTTGGATGTGTGCTTCTAGCAGCTGTTC	ACGTTGGATGCATAGCCGAGAGCATTTCAC
rs11237200	ACGTTGGATGAAGAGTCCGGAATCAGGTTG	ACGTTGGATGGGCTTAGAAATGTCAAGGTC

PAK1, P21 protein (Cdc42/Rac)-activated kinase 1; SNP, single nucleotide polymorphism.

Table II. Distribution of genotype and allele frequency and HWE test for rs2844337 and rs11237200.

		Genotype (A1A1/A1A2/A2A2)					Allele (A1/A2)			
SNPs	Ethnicity	Case	Control	χ^2	P-value	P_{HWE}	Case	Control	χ^2	P-value
rs2844337	Han	2/64/235	10/74/220	6.538	**0.038**	0.269	68/534	94/514	4.525	**0.033**
(G/T)	Zhuang	6/61/161	7/61/159	0.087	0.957	0.635	73/383	75/379	0.044	0.835
	Total	8/125/396	17/135/379	3.994	0.136	0.256	141/917	169/893	2.840	0.092
rs11237200	Han	21/142/237	20/161/217	2.092	0.351	0.184	184/616	201/595	1.105	0.293
(A/G)	Zhuang	17/96/183	16/107/176	0.748	0.688	1.000	130/462	139/459	0.281	0.596
	Total	38/238/420	36/268/393	2.729	0.256	0.304	314/1078	340/1054	1.302	0.254

SNP, single nucleotide polymorphisms; A1, minor allele; A2, major allele; Case, subjects diagnosed with schizophrenia; P_{HWE}: P-value for Hardy-Weinberg equilibrium test; G, guanine; T, thymine; A, adenine. Bold text indicates a statistically significant difference (P<0.05) between the case and control groups.

genetic analyses were conducted using PLINK version 1.07 (The Center for Human Genetic Research, Massachusetts General Hospital and the Broad Institute of Harvard & MIT, Cambridge, MA, USA): Hardy-Weinberg equilibrium (HWE) was detected via a chi-square goodness of fit test; the genetic association of rs2844337 and rs11237200 polymorphisms with SCZ susceptibility was evaluated in terms of the four genetic models (additive, dominant, recessive and allelic models), through unconditional logistic regression analysis; and the correlation of rs2844337 and rs11237200 polymorphisms with PANSS scores was estimated through linear regression analysis. P-values were two tailed, and P<0.05 was considered to indicate a statistically significant difference. In addition, Bonferroni correction was used for multiple testing. Furthermore, log-odds (LOD) scores were also calculated using Haploview. LOD >1 was considered to indicate a linkage disequilibrium, while LOD <-2 was considered to indicate that a linkage disequilibrium did not exist.

Results

Subject characteristics. In the Chinese Han population, the SCZ patient group consisted of 269 males and 131 females with an average age of 32.29±11.56 years, whereas the healthy control group comprised 248 males and 152 females with an average age of 33.09±11.17 years. In the Chinese Han population, the patients with SCZ did not significantly differ from the healthy controls in terms of gender (χ^2=2.411; P=0.120) and age (t=0.992; P=0.321). In the Chinese Zhuang population, the SCZ patient group contained 207 males and 93 females with an average age of 33.68±11.99 years; the healthy control group included 198 males and 102 females with an average age of 32.37±12.27 years. In the Chinese Zhuang population, the patients with SCZ did not significantly differ from the healthy controls in terms of gender (χ^2=0.615; P=0.894) and age (t=1.319; P=0.188). In both populations, the patients with SCZ did not significantly differ from the healthy controls in terms of gender (χ^2=2.859; P=0.091) and age (t=-0.167; P=0.868).

A total of 591 patients with SCZ completed the PANSS assessment. Chinese Han patients included 385 individuals (216 males and 124 females) with an average age of 32.30±11.66 years; the Chinese Zhuang patients consisted of 206 individuals (151 males and 55 females) with an average age of 31.85±11.28 years. The Chinese Zhuang patients did not significantly differ from the Chinese Han patients in terms of the distribution of gender (χ^2=1.929; P=0.165) or age (t=0.449; P=0.654).

HWE evaluation. In the healthy control group, the genotypic distributions of the rs2844337 and rs11237200 polymorphisms did not significantly deviate from the HWE (P_{HWE}>0.05; Table II).

Distribution of genotype and allele frequencies. Genotypic (P=0.038) and allelic (P=0.033) frequencies of the 3'-UTR of the rs2844337 polymorphism in patients significantly decreased compared to that in controls in the Chinese Han population but not in the Chinese Zhuang population (Table II). By contrast, the genotypic and allelic frequencies of the rs11237200 polymorphism did not significantly differ in either population.

Table III. Association of SNP rs2844337and rs11237200 with schizophrenia risk in different ethnic group.

			Crude		Adjusted	
Ethnicity	SNP	Model	OR (95% CI)	P-value	OR (95% CI)	P_{adj}
Han	rs2844337	Additive	0.699 (0.501-0.977)	**0.036**	0.694 (0.497-0.970)	**0.032**
		Dominant	0.736 (0.508-1.066)	0.105	0.733 (0.505-1.062)	0.101
		Recessive	0.197 (0.043-0.905)	**0.037**	0.186 (0.040-0.860)	**0.031**
		Allelic	0.696 (0.498-0.973)	**0.033**	-	-
	rs11237200	Additive	0.880 (0.696-1.112)	0.284	0.879 (0.695-1.111)	0.279
		Dominant	0.824 (0.623-1.092)	0.178	0.822 (0.621-1.089)	0.172
		Recessive	1.047 (0.559-1.964)	0.886	1.050 (0.560-1.970)	0.879
		Allelic	0.884 (0.703-1.112)	0.293	-	-
Zhuang	rs2844337	Additive	0.964 (0.680-1.367)	0.836	0.985 (0.693-1.400)	0.933
		Dominant	0.973 (0.651-1.455)	0.894	1.005 (0.670-1.508)	0.980
		Recessive	0.849 (0.281-2.568)	0.773	0.828 (0.273-2.516)	0.740
		Allelic	0.963 (0.677-1.370)	0.835	-	-
	rs11237200	Additive	0.931 (0.712-1.218)	0.601	0.926 (0.708-1.212)	0.576
		Dominant	0.884 (0.636-1.227)	0.460	0.878 (0.631-1.220)	0.437
		Recessive	1.078 (0.534-2.176)	0.835	1.073 (0.531-2.169)	0.843
		Allelic	0.929 (0.708-1.219)	0.596	-	-

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; A1, minor allele; A2, major allele; Additive, A1A1/A1A2/A2A2; Dominant, (A1A1+A1A2)/A2A2; Recessive, A1A1/(A1A2+A2A2); Allelic, A1/A2; P_{adj}, P-value adjusted for age and gender. Bold text indicates a statistically significant association (P<0.05) between SNP and schizophrenia susceptibility.

Table IV. Linkage disequilibrium for rs2844337 and rs11237200.

Samples	D'	D' conf. bounds	LOD	r^2
Han	0.881	0.65-0.96	6.44	0.026
Zhuang	0.802	0.53-0.92	4.51	0.025

LOD, log-odds.

Table V. Association of haplotype rs2844337/rs11237200 with schizophrenia risk.

		Crude		Adjusted	
Ethnicity	Haplotype	OR	P-value	OR_{adj}	P_{adj}
Han	T-A	0.791	0.159	0.775	0.129
	G-G	0.709	**0.048**	0.72	0.062
	T-G	1.51	**0.003**	1.52	**0.003**
Zhuang	T-A	0.744	0.132	0.737	0.122
	G-G	0.992	0.963	1.01	0.952
	T-G	1.25	0.157	1.24	0.178

Adjusted, adjusted for gender and age; OR, odds ratio; P_{adj}, P-value adjusted for gender and age; T, thymine; A, adenine; G, guanine. Bold text indicates a statistically significant association (P<0.05) between haplotype and schizophrenia susceptibility.

Genetic association between polymorphism and SCZ susceptibility. In the Chinese Han population, the rs2844337 polymorphism was significantly associated with SCZ susceptibility in additive (P_{adj}=0.032), recessive (P_{adj}=0.031) and allelic (P=0.033) models; however, these differences were not statistically significant following Bonferroni correction (additive, P_{BC}=0.064; recessive, P_{BC}=0.062; allelic, P_{BC}=0.066). Conversely, the rs11237200 polymorphism was not significantly associated with SCZ susceptibility in any of the three genetic models. Likewise, these two polymorphisms were not significantly associated with SCZ susceptibility in the Chinese Zhuang population (Table III).

LD and haplotype analysis. LD analyses were performed with D' values of 0.881 and 0.802 in the Chinese Han and Chinese Zhuang samples, respectively, suggesting that LD may exist between rs2844337 and rs11237200. Detailed results are presented in Table IV. In addition, three haplotypes were constructed using the rs2844337 and rs11237200 polymorphisms. In the Chinese Han population, the TG haplotype posed a significantly higher SCZ risk compared with other haplotypes (OR_{adj}=1.52; P_{adj}=0.003). This risk remained statistically significant following Bonferroni correction (P_{BC}=0.009). By contrast, these haplotypes were not significantly associated with SCZ risk in the Chinese Zhuang population (Table V).

Association of the PAK1 polymorphisms with PANSS scores. In the Chinese Han population, rs11237200 was significantly

Table VI. Linear regression results for genotypic association between rs2844337/rs11237200 and PANSS scores in the Han population (adjusted for age and gender).

		Additive model		Dominant model		Recessive model	
SNP	Variables	β (95% CI)	P_{adj}	β (95% CI)	P_{adj}	β (95% CI)	P_{adj}
rs2844337	Total score	1.904 (-4.035-7.844)	0.530	2.072 (-4.159-8.304)	0.515	0.608 (-30.810-32.020)	0.970
	Positive	0.882 (-1.404-3.168)	0.450	0.875 (-1.524-3.274)	0.475	2.435 (-9.665-14.530)	0.694
	Negative	0.145 (-1.824-2.113)	0.886	0.076 (-1.989-2.142)	0.942	2.102 (-8.305-12.510)	0.693
	General psychopathology	0.852 (-2.014-3.719)	0.561	1.094 (-1.912-4.101)	0.476	-3.963 (-19.120-11.190)	0.609
rs11237200	Total score	0.818 (-2.936-4.573)	0.670	-1.733 (-6.305-2.838)	0.458	13.770 (3.977-23.560)	**0.006**
	Positive	0.583 (-0.828-1.994)	0.418	-0.130 (-1.849-1.590)	0.882	4.653 (0.963-8.342)	**0.014**
	Negative	-0.301 (-1.561-0.960)	0.640	-1.047 (-2.579-0.484)	0.181	2.813 (-0.496-6.122)	0.097
	General psychopathology	0.516 (-1.292-2.324)	0.576	-0.579 (-2.782-1.623)	0.606	6.283 (1.563-11.000)	**0.009**

SNP, single nucleotide polymorphisms; Additive model, A1A1/A1A2/A2A2; Dominant model, (A1A1+A1A2)/A2A2; Recessive model, A1A1/(A1A2+A2A2); A1, minor allele; A2, major allele; β, partial regression coefficent of the linear regression results; CI, confidence interval of partial regression coefficient; P_{adj}, P-value adjusted for age and gender; PANSS, Positive and Negative Syndrome Scale. Bold text indicates a statistically significant genotypic association (P<0.05) between SNP and PANSS for schizophrenia.

associated with the total score (P_{adj}=0.006), positive scale score (P_{adj}=0.014) and general psychopathology scale score (P_{adj}=0.009) in the recessive model. By contrast, rs2844337 was not significantly associated with the PANSS score in any of the three genetic models (Table VI). In the Chinese Zhuang population, the *PAK1* polymorphisms were not significantly associated with the PANSS scores in any of the three genetic models (Table VII).

Discussion

To the best of our knowledge, this research is the first to investigate the association of rs2844337 with susceptibility to SCZ, and the association of rs11237200 with the clinical symptoms of SCZ. The present results indicated that the rs2844337 polymorphism of *PAK1* was a risk factor of the susceptibility to SCZ in the Chinese Han, but not in the Chinese Zhuang, population. Moreover, the results of the quantitative trait locus analysis suggested that rs11237200 influenced the SCZ symptoms of the Chinese Han but not of the Chinese Zhuang population.

Notably, rs2844337 and rs11237200 are located in the *PAK1* gene, which encodes a family member of serine/threonine p21 activating kinases, namely PAK proteins (25). The dysregulation of PAK proteins contributes to the regulation of actin stabilization and the reduction of dendritic spines in patients with SCZ (26). PAK proteins can phosphorylate and inactivate LIM-kinase during actin depolymerization, and alter F-actin stabilization (27). Dominant negative *PAK1* can reduce the number of basal dendrites and the number of the primary branches on apical dendrites in immature cortical neurons, and can alter the morphological characteristics of hippocampal neurons in the spine (28). Huang *et al* (29) demonstrated that dendritic axons are markedly simplified, and synapse density is reduced in *PAK1*/*PAK3* double-knockout mice. Based on these results, the present hypothesis is that the *PAK1* gene may be involved in the molecular pathological mechanism of SCZ.

3'-UTR has an important role in the post-transcriptional regulation of gene expression; in particular, 3'-UTR binds to miRNA. The genetic variation in this region induces various pathophysiological changes (30). Rs10759, a genetic variation in the 3'-UTR of the regulator of G protein signaling 4 (*RGS4*), is associated with SCZ (31). In a previous *in vitro* luciferase reporter assay, rs10759 was demonstrated to influence SCZ susceptibility as miR-124 repressed the binding of the 3'-UTR of the *RGS4* gene to its target mRNA (32). Saetre *et al* (33) found that the polymorphism of *DISC1* in the 3'-UTR enhances susceptibility to SCZ. Rossi *et al* (17) conducted a functional study and demonstrated that genetic variation in the 3'-UTR of *DISC1* increases the gene expression levels and affects SCZ-associated phenotypes by disrupting miRNA-mRNA target recognition. In the present study, the 3'-UTR of the rs2844337 polymorphism of the *PAK1* gene was significantly associated with the susceptibility of the Chinese Han population to SCZ. However, the biological function of rs2844337 polymorphism is yet to be verified. Furthermore, studies have yet to determine whether rs2844337 participates in the SCZ pathomechanism by influencing the binding of miRNA to the *PAK1* gene, thereby affecting *PAK1* expression levels and stability. Therefore, further study is required to verify this mechanism.

Considering the rs11237200 polymorphism near the 5'-UTR, it was indicated that this polymorphism did not influence SCZ susceptibility; alternatively, this polymorphism affected the quantitative traits of SCZ susceptibility. Nadalin *et al* (34) demonstrated that the peroxisome proliferator-activated receptor α-L162V polymorphism is not associated with SCZ; however, it influenced the overall PANSS psychopathology score. Wang *et al* (35) reported that the allelic variant of rs66642155 is not significantly associated with SCZ; nevertheless, this variant

Table VII. Linear regression results for genotypic association between rs2844337/rs11237200 and PANSS scores in the Zhuang population (adjusted for age and gender).

SNP	Variables	Additive model		Dominant model		Recessive model	
		β (95% CI)	P_{adj}	β (95% CI)	P_{adj}	β (95% CI)	P_{adj}
rs2844337	Total score	-0.024 (-8.092-8.045)	0.995	0.744 (-7.720-9.207)	0.864	-23.010 (-69.140-23.130)	0.330
	Positive	0.673 (-2.409-3.756)	0.669	0.837 (-2.395-4.068)	0.613	-2.931 (-20.750-14.890)	0.748
	Negative	-0.397 (-2.983-2.188)	0.764	-0.177 (-2.889-2.535)	0.898	-7.896 (-22.790-7.000)	0.301
	General psychopathology	-0.395 (-4.432-3.642)	0.848	-0.026 (-4.262-4.209)	0.990	-12.210 (-35.280-10.870)	0.302
rs11237200	Total score	0.775 (-4.406-5.955)	0.770	-1.211 (-7.609-5.187)	0.711	10.350 (-2.881-23.580)	0.127
	Positive	-0.707 (-2.692-1.278)	0.486	-1.816 (-4.257-0.626)	0.147	3.196 (-1.896-8.288)	0.220
	Negative	1.695 (-0.028-3.418)	0.055	1.661 (-0.470-3.793)	0.128	3.998 (-0.449-8.446)	0.080
	General psychopathology	-0.228 (-2.789-2.332)	0.861	-1.082 (-4.236-2.072)	0.502	3.202 (-3.374-9.779)	0.341

SNP, single nucleotide polymorphisms; Additive model, A1A1/A1A2/A2A2; Dominant model, (A1A1+A1A2)/A2A2; Recessive model, A1A1/(A1A2+A2A2); A1, minor allele; A2, major allele; β, partial regression coefficent of the linear regression results; CI, confidence interval of partial regression coefficient; P_{adj}, P-value adjusted for age and gender; PANSS, Positive and Negative Syndrome Scale. Bold text indicates a statistically significant genotypic association (P<0.05) between SNP and PANSS for schizophrenia.

affects the positive symptoms of SCZ. SCZ is considered as a clinically heterogeneous disease, and the symptom heterogeneity of SCZ is defined as a symptom dimension (36). Symptom dimensions represent multiple sets of symptoms, which usually occur simultaneously and account for the majority of the phenotypic variations detected among patients (37). Different symptom dimensions are potentially under the control of a set of genes; these dimensions then facilitate the genetic distribution of SCZ (37-39). Therefore, the association between the genetic variations and the symptoms assessed by PANSS may indicate the genetic mechanism of SCZ. At present, the diagnosis of SCZ is primarily based on descriptive clinical criteria (40). Therefore, it can be suggested that traditional diagnostic criteria should be replaced with a novel classification and diagnostic criteria based on symptom dimensions. The genetic variations associated with the clinical symptoms of SCZ should also provide the genetic basis of novel criteria.

The present results showed that the rs2844337 and rs11237200 polymorphisms investigated herein influenced SCZ susceptibility and/or quantitative traits in the Chinese Han population, but not in the Chinese Zhuang population. This disparity is attributed to differing genetic effects on the Chinese Han and Chinese Zhuang populations. Genetic effects on complex diseases are heterogeneous among different ethnic groups (41). For instance, the Chinese Zhuang population has higher lipid levels, lower hyperlipidemia prevalence and higher blood pressure compared with the Chinese Han population (42-44). Studies on the genetic association of these two populations have revealed that multiple genetic variants, including polymorphisms in the lipoprotein lipase gene, hepatic lipase gene and sterol regulatory element-binding protein-2 gene, of the Chinese Zhuang population are distinct from those of the Chinese Han population (45-47). However, it is possible that in the present study this ethnic difference was confounded by the smaller sample size of the Chinese Zhuang subjects, which may result in a relatively weaker power of the Chinese Zhuang samples.

In the present study, several limitations should be considered. Firstly, rs2844337 is a functional site located in the 3'-UTR of the *PAK1* gene, and was significantly associated with SCZ susceptibility. However, the biological function of rs2844337 remains unclear. As such, further studies should explore the effect of rs2844337 on the binding force of miRNA to the *PAK1* gene, and on the expression and translation of the *PAK1* gene. Secondly, the sample size of the Chinese Zhuang was not equal to that of the Chinese Han. Therefore, the results should be interpreted cautiously; future studies should be conducted using a larger sample size of Chinese Zhuang individuals. Thirdly, only two SNPs on the *PAK1* gene were investigated in the analysis of the association of rs2844337 and rs11237200 with SCZ, which cannot represent all genetic variations in the 3'-UTR and/or 5'-UTR of the *PAK1* gene. Therefore, it is necessary for further studies to focus on whether other *PAK1* gene SNPs affect the susceptibility and severity of the clinical symptoms of SCZ in the Chinese Han population. Fourthly, genetic effects should be determined to explain the cause of SCZ. However, the role of environmental factors, including maternal exposure to viruses during pregnancy (48), social disorganization of neighborhoods (49) and prenatal stress (50) should be considered.

In conclusion, the present study supports the hypothesis that genetic variations in the *PAK1* gene influence the susceptibility and severity of the clinical symptoms of SCZ in the Chinese Han population. Further studies should be conducted to investigate the biological function of the rs2844337 polymorphism and the pathomechanism involved in SCZ.

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