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Influence of *NRGN* rs12807809 polymorphism on symptom severity in individuals with schizophrenia in the Han population but not the Zhuang population of south China

Su L, Long J, Pan R, Xie X, Mao X, Zhou Y, Chen Q, Wei B. Influence of *NRGN* rs12807809 polymorphism on symptom severity in individuals with schizophrenia in the Han population but not the Zhuang population of south China.

Background: *NRGN* is one of the most promising candidate genes for schizophrenia based on function and position. Therefore, this study aimed to examine the genetic association of this polymorphism with schizophrenia in the Zhuang and Han populations of south China.
Subjects and methods: A total of 282 patients (188 Han and 94 Zhuang) and 282 healthy subjects (188 Han and 94 Zhuang) were recruited. Of these, 246 schizophrenia patients underwent an assessment of psychotic symptoms using the Positive and Negative Syndrome Scale (PANSS). A TaqMan genotyping assay method was used to determine the genotypes.
Results: We did not find a significant association of rs12807809 polymorphism with schizophrenia in the total pooled samples, or in the separate ethnic groups. However, in Han schizophrenia patients, quantitative data analyses showed that the CC genotype of the rs12807809 polymorphism was associated with PANSS aggression subscale score and activation subscale score. Furthermore, carriers of the C allele of rs12807809 polymorphism among Han schizophrenia patients had higher scores of general, activation, depression, aggression, and global symptoms than the T allele carriers.
Conclusion: In conclusion rs12807809 polymorphism may not contribute to the risk of schizophrenia but influence the clinical symptoms of schizophrenia in the Han population.

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Significant outcomes

1. *NRGN* rs12807809 single nucleotide polymorphism (SNP) may not contribute to the risk of schizophrenia in Han and Zhuang groups.
2. *NRGN* rs12807809 SNP influence the clinical symptoms of schizophrenia among Han population.
3. *NRGN* rs12807809 SNP did not influence the clinical symptoms of schizophrenia in Zhuang population.

Limitations

1. We only genotyped the rs12807809 polymorphism, while this single nucleotide polymorphism cannot represent the complete *NRGN* gene variants.
2. Although we found an association between rs12807809 genotype and allele with the severity of clinical symptoms in Han schizophrenia patients, but not in Zhuang patients, the sample size was relatively small to clarify this question.

3. Schizophrenia has been divided into five subtypes (catatonic, paranoid, disorganised, undifferentiated, and residual), but our research groups were limited to one homogeneous type.
4. We cannot investigate other possibilities for the association with the symptom severity, such as duration of untreated psychosis, duration of treatment, age at onset, effects of medication, and hospital admission, etc.
5. We cannot describe what exactly different between Han patients and Zhuang patients in psychotic symptomatology.

Introduction

Schizophrenia is a chronic, serious, and disabling psychiatric disorder with a global incidence of 1% (1), characterised by positive symptoms, negative symptoms, and cognitive dysfunctions. Despite extensive research, the aetiology of this disorder remains poorly understood. Family, twin, and adoption studies indicate that schizophrenia and a wide range of phenotypic expressions can be largely attributed to a genetic component, with a heritability of ~80% (2). Moreover, genome-wide association studies (GWASs) provide evidence for a substantial genetic contribution to schizophrenia susceptibility, by defining a number of single nucleotide polymorphisms (SNPs) associated with schizophrenia. Although many genes have been implicated in the pathogenesis of schizophrenia (3), there is no complete consensus about the genetic variants involved (4) until recently.

Recently, Stefansson et al. (5) combined SNP data from several large GWASs and identified seven SNPs with *p* values smaller than the genome-wide significance in schizophrenia. Among these SNPs, we focus on rs12807809, a SNP located 3457 bases upstream of the neurogranin (*NRGN*) gene, because *NRGN* is considered a plausible candidate gene for schizophrenia. Chromosome 11q23.3-24 has been demonstrated to be linked to schizophrenia in a genome-wide genetic linkage analysis and *NRGN*, localised on chromosome 11q24.2, is consistent with this chromosomal region. Besides, *NRGN* encodes a substrate for postsynaptic protein kinase C that binds to calmodulin (CaM) in the absence of calcium (6). *NRGN* regulates the release of CaM and the activation of downstream CaM-Ca^{2+}-dependent enzymes, mediating N-methyl-D-aspartate (NMDA) receptor signalling that plays an important role in the neuroplasticity mechanisms of learning and memory and is also relevant to the proposed glutamate pathophysiology of schizophrenia (7,8). *NRGN* is a promising candidate gene for schizophrenia based on function and position, but only a few studies have investigated the association between *NRGN* polymorphism and schizophrenia. Ruano et al. (9) studied the association of *NRGN* with schizophrenia in two independent case-control samples from mainland Portugal and Brazil. The authors detected that the rs7113041-CG genotype was significantly different between male patients and male controls in the mainland Portuguese sample. However, a Bulgarian association study did not find a significant association between schizophrenia and three SNPs (rs7113041, rs1804829, and rs12541) of *NRGN* (10). For the genome-wide supported SNP, rs12807809, three separate studies reported no association between this variant and schizophrenia in Japanese, Chinese Han, and Taiwan populations (11–13). The inconsistency of association among the above studies could be due to ethnic differences and clinical heterogeneity.

China is a united multi-ethnic state officially composed of 56 ethnic groups (55 minorities plus the dominant Han). Second only to the Han Chinese, the Zhuangs form the largest minority in China. Zhuang Han have different customs, language, and even genetic background. Guangxi is a province of southern China in which >90% of the Zhuangs live. To date, association studies on genetic polymorphism with schizophrenia conducted in China have mostly focused on the Han population, but a few studies have focused on other ethnic groups. To the best of our knowledge, only one study, reported by our group recently, has focused on the genetic polymorphism of schizophrenia in the Zhuang group (14), which did not investigate relationship between genetic polymorphism and the severity of clinical symptoms. Taking into account ethnic differences and clinical heterogeneity, we performed this case-control study to investigate whether the rs12807809 polymorphism contributed to the susceptibility to schizophrenia or the severity of clinical symptoms of schizophrenia assessed by the Positive and Negative Syndrome Scale (PANSS) in the Han population and the Zhuang population of south China.

Methods and materials

A total of 282 schizophrenia patients, comprising 188 Hans and 94 Zhuangs, were recruited from the psychiatric inpatient department of Guangxi Brain Hospital from April to June 2010. Inclusion criteria were: (1) confirmed diagnosis of schizophrenia according to the ICD-10 by at least two experienced

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psychiatrists; (2) Han or Zhuang ethnicity, born and living in Guangxi, and all biological grandparents were of the same ethnic background. Exclusion criteria were: (1) mental disorders caused by cerebral diseases, physical diseases, medical, or other treatments; (2) nervous system diseases (cerebral apoplexy, epilepsy, etc.), or other severe physical diseases; (3) mental retardation; (4) history of severe head trauma; (5) substance abuse (alcohol or drug abuse); (6) uncontrolled excitement and impulsion or frequent suicide and autolesion attempts.

The 282 age- and gender-matched healthy controls also consist of two ethnic groups: 188 Hans and 94 Zhuangs. Among these controls, 188 Han controls and 30 Zhuang controls were recruited from the medical examination centre of the First Affiliated Hospital of Guangxi University of Chinese Medicine; the other 64 Zhuang controls were recruited from Liujiang county. Liujiang is a county populated by the Zhuang people located in Guangxi, and >70% of the population in this county is made up of the Zhuang people. All subjects were unrelated Hans or Zhuangs born and living in Guangxi, and all their biological grandparents were of the same ethnic background (according to Household Register). Subjects who had a past history or family history of the follow diseases were excluded: mental disorder and nervous system diseases, severe physical illness, familial heredity diseases, and severe head trauma or birth injuries.

None of the above 564 subjects had family histories of intermarriage among the last generation or had a genetic relationship with each other. All the participants were well informed of the object and procedure of our study. Written informed consent was obtained from all the participants and the study was carried out with prior approval from the Guangxi Medical University Ethnics Committee.

The severity of schizophrenia was assessed by a well-trained psychiatrist who was blinded to the genotype identification using the PANSS (15,16). A total of 246 schizophrenic patients (83 Zhuang and 163 Han) were assessed using the PANSS on the day of admission.

After informed consent was obtained from the subjects, peripheral venous blood samples (5 ml) were collected in ethylene diamine tetraacetie acid tubes and stored at 4°C until processed. Genomic DNA was extracted from blood samples within 3 days using the TIANamp Blood DNA Kit (catalogue number: DP318DNA; TIANGEN, Beijing, China), aliquoted into three tubes, and stored at −80°C. Genotyping of rs12807809 within the *NRGN* gene was carried out using the TaqMan SNP genotyping assay (Applied Biosystems Inc., Foster City, CA, USA). The amplification was performed using 10 µl reactants with 1.00 µl of the extracted DNA, 5.00 µl of 2× TaqMan Universal PCR Mix (Life Technologies Corporation), 0.25 µl of Assay-on-Demand SNP Genotyping Assay Mix (40×) (Life Technologies Corporation), and 3.75 µl of nuclease-free H_2O. The thermal cycling conditions for the initial Taq polymerase activation were at 95°C for 10 min, then 43 cycles of denaturation at 92°C for 15 s, and annealing at 60°C for 1 min. All genotyping was carried out 'blindly', that is, the clinical status of the samples was unknown to the genotyper. A random 5% of the samples were repeated to validate the genotyping procedures and the concordance rate was 100%.

The Hardy–Weinberg equilibrium (HWE) for the genotype distribution in healthy controls of Zhuang and Han groups was assessed by the χ^2 test. Different genetic models were adopted to assess the risk of schizophrenia with *NRGN* rs12807809 in the two ethnic groups by odds ratio (OR), along with the corresponding 95% confidence interval (CI). Meta-analysis was also performed to combine the data of the Zhuang and Han groups. Heterogeneity was measured using Q statistic test, ensuring that the studies were suitable for meta-analysis. Random effects model was used on a condition for which significant heterogeneity was observed; otherwise, a fixed effects model was adopted. Comparison between groups of enumeration data was performed by χ^2 test, while the comparison of the measurement data was performed by *t*-test or analysis of variance (ANOVA). A p value of <0.05 (two-tailed) was considered a statistically significant difference. All statistical analysis was performed using the STATA software version 11.1 (Stata, College Station, TX, USA) or SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

The study included 188 patients (mean age 33.86 ± 12.33 years, 119 males and 69 females) and 188 controls (mean age 33.78 ± 8.23 years, 116 males and 72 females) of Han ethnicity, along with 94 patients (mean age 33.00 ± 9.60 years, 52 males and 42 females) and 94 controls (mean age 32.03 ± 11.97 years, 60 males and 34 females) of Zhuang ethnicity. No differences were detected between patients with schizophrenia and healthy controls for the gender or age in the two separate ethnic groups. A total of 163 Han (mean age 32.59 ± 12.35 years, 94 males and 69 females) and 83 Zhuang patients (mean age 34.09 ± 12.61 years, 50 males and 33 females) completed the assessment of the severity of clinical symptoms using the PANSS. Gender and age were not statistically significantly different between Zhuang patients and Han patients who completed the PANSS assessment.

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The χ^2 goodness-of-fit test showed that the SNP rs12807809 genotype distribution in control groups of Zhuang and Han ethnicities was consistent with the HWE (Zhuang: $\chi^2 = 0.859$, $p = 0.354$; Han: $\chi^2 = 0.010$, $p = 0.923$). Table 1 lists the genotype and allele distributions of the SNP rs12807809 for patients with schizophrenia and control subjects in separate Zhuang and Han ethnicity groups. Compared by the χ^2 test, there were no statistically significant differences in genotype or allele distributions between patients and controls in the Zhuang population. Similarly, in the Han population, no significant differences were observed in the genotype or allele distributions between patients and controls. Moreover, using multivariate logistic regression (adjusting for age and gender), negative results were also found in the two separate ethnic groups. Subsequently, a meta-analysis was conducted of the Zhuang and Han groups pooled together. We did not find any positive associations in three genetic models (allele model: OR, 0.82, 95% CI, 0.63–1.05, $I^2 = 0\%$; dominant model: OR, 0.80, 95% CI, 0.57–1.11, $I^2 = 0\%$; recessive model: OR, 0.67, 95% CI, 0.27–1.67, $I^2 = 56.0\%$).

Table 1. Alleles and genotypes associations of *NRGN* rs12807809 with schizophrenia in Zhuang and Han population

	Genotype frequency (n, %)				
	Patient	Control	χ^2	*p*	OR (95% CI)*
Zhuang					
T/T	44(46.81%)	41(43.62%)			1.000
T/C	44(46.81%)	39(41.49%)	3.607	0.165	1.06(0.58–1.94)
C/C	6(6.38%)	14(14.89%)			0.41(0.14–1.18)
T	132(70.21)	121(64.36)			1.00
C	56(29.79)	67(35.64)	1.462	0.227	0.78(0.50–1.20)
Han					
T/T	104(55.32%)	91(48.40%)			1.000
T/C	67(35.64%)	80(42.55%)	2.016	0.365	0.73(0.45–1.13)
C/C	17(9.04%)	17(9.04%)			0.88(0.42–1.82)
T	275(73.14)	262(69.68)	1.101	0.844	1.00
C	101(29.86)	114(30.32)			0.85(0.60–1.16)

OR, odds ratio; 95% CI, 95% confidence interval.

* Adjusted ORs (95% CI) and adjusted *p* value were obtained with multivariate unconditional logistic regression analysis by adjusting for age and gender.

The mean PANSS scores of Zhuang patients for total, positive, and negative symptoms as well as anergia, thought disturbance, activation, paranoia, depression, and aggression according to the different genotypes and alleles are shown in Table 2. ANOVA results showed no significant differences in total, anergia, thought disturbance, activation, paranoia, depression aggression, or positive or negative symptoms scores for the different genotypes of the *NRGN* rs12807809 polymorphism. We also made an estimate on PANSS subscales in Zhuang patients with different alleles by *t*-test. We did not find any statistically significant differences between patients with the C and T alleles for any item.

The mean PANSS scores for the total and nine clinical symptoms in Han patients according to the different genotypes and alleles are shown in Table 3. Using ANOVA, we found significant associations between the rs12807809 genotypes and scores on two PANSS items: activation ($F = 5.156$, $p = 0.007$) and aggression ($F = 4.486$, $p = 0.013$). Furthermore, pairwise comparisons showed patients carrying the rs12807809 CC genotype were more likely to obtain a higher PANSS aggression subscale score than those carrying the TT genotype ($p < 0.05$) or the CT genotype ($p < 0.05$), while the PANSS aggression subscale score was not statistically significantly different between patients with the CC genotype and the CT or TT genotype ($p > 0.05$). Similar findings were observed for the PANSS activation subscale score; patients carrying the rs12807809 CC genotype were more likely to obtain a higher score than those carrying the TT genotype ($p < 0.05$) or the CT genotype ($p < 0.05$), but no statistically significant differences were found

Table 2. The mean scores of PANSS item in Zhuang schizophrenia according to NRGN rs12807809 genotype and allele ($\overline{X} \pm S$)

	Genotype					Allele			
PNASS scores	T/T (n = 41)	T/C (n = 36)	C/C (n = 6)	*F*	*P*1	T (n = 118)	C (n = 48)	*t*	*P*2
Global symptoms	73.02 ± 19.76	70.25 ± 19.39	77.83 ± 25.72	0.443	0.644	72.18 ± 19.52	72.15 ± 20.78	0.009	0.992
Positive symptoms	22.95 ± 8.51	22.50 ± 7.76	25.00 ± .32	0.242	0.786	22.81 ± 8.22	23.13 ± 7.79	−0.225	0.823
Negative symptoms	17.12 ± 7.05	16.61 ± 7.17	16.67 ± 5.43	0.053	0.948	16.97 ± 7.03	16.63 ± 6.68	0.287	0.775
General symptoms	32.95 ± 9.96	31.14 ± 11.47	36.17 ± 13.70	0.651	0.524	32.40 ± 10.39	32.40 ± 11.95	0.001	0.999
Anergia	8.29 ± 3.53	8.08 ± 3.59	6.67 ± 2.73	0.561	0.573	8.23 ± 3.52	7.73 ± 3.40	−0.837	0.404
Disturbance	10.83 ± 3.83	10.75 ± 3.19	10.83 ± 4.58	0.005	0.995	10.81 ± 3.62	10.77 ± 3.47	0.056	0.955
Activation	5.61 ± 3.24	5.03 ± 1.87	7.83 ± 3.49	2.728	0.071	5.43 ± 2.88	5.73 ± 2.59	−0.619	0.537
Paranoia	10.90 ± 4.80	9.92 ± 4.75	11.83 ± 5.04	0.640	0.530	10.60 ± 4.77	10.40 ± 4.79	0.252	0.801
Depression	6.27 ± 2.53	6.25 ± 2.93	7.73 ± 3.06	2.443	0.093	6.26 ± 2.64	6.90 ± 3.11	−1.330	0.185
Aggression	13.49 ± 5.47	12.56 ± 5.98	15.83 ± 6.46	0.899	0.411	13.20 ± 5.60	13.38 ± 6.13	−0.174	0.862

PNSS, Positive and Negative Syndrome Scale.

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Table 3. The mean scores of PANSS item in Han schizophrenia according to NRGN rs12807809 genotype and allele ($\overline{X} \pm S$)

	Genotype					Allele			
PNASS scores	T/T (n = 93)	T/C (n = 55)	C/C (n = 15)	*F*	*P*1	T (n = 241)	C (n = 85)	*t*	*P*2
Global symptoms	79.03 ± 23.60	83.62 ± 27.84	93.60 ± 34.17	2.179	0.116	80.08 ± 24.60	87.14 ± 30.17	−2.140	0.033*
Positive symptoms	24.96 ± 8.83	24.95 ± 8.86	29.60 ± 8.24	1.903	0.152	24.95 ± 8.08	26.59 ± 8.83	−1.470	0.143
Negative symptoms	18.48 ± 7.48	19.42 ± 8.25	19.93 ± 8.96	0.376	0.687	18.70 ± 7.64	19.60 ± 8.40	−0.913	0.362
Gerenal symptoms	35.59 ± 12.88*	39.25 ± 16.16	44.07 ± 20.37	2.623	0.076	36.43 ± 13.72	40.95 ± 17.65	−2.146	0.034*
Anergia	8.80 ± 3.97	9.49 ± 4.91	9.93 ± 5.40	0.690	0.503	8.95 ± 4.19	9.65 ± 5.03	−1.241	0.216
Disturbance	11.96 ± 4.29	12.40 ± 4.70	14.13 ± 4.97	1.533	0.219	12.06 ± 4.37	13.01 ± 4.81	−1.684	0.093
Activation	6.10 ± 2.92*	6.91 ± 3.58	8.93 ± 4.10	5.156	0.007*	6.28 ± 3.09	7.62 ± 3.85	−2.903	0.004*
Paranoia	11.16 ± 4.63	11.35 ± 4.69	13.13 ± 3.64	1.207	0.302	11.20 ± 4.63	11.98 ± 4.39	−1.342	0.181
Depression	7.57 ± 3.96	8.16 ± 4.63	10.07 ± 5.81	2.155	0.119	7.71 ± 4.11	8.84 ± 5.08	−2.043	0.042*
Aggression	14.54 ± 6.09	15.56 ± 6.67	19.87 ± 7.50	4.486	0.013*	14.77 ± 6.21	17.08 ± 7.18	−2.828	0.005*

PNSS, Positive and Negative Syndrome Scale.
*$p < 0.05$

between patients with the TT genotype and those with the CT genotype ($p > 0.05$). In addition, the *t*-test results showed that patients with the C allele at rs12807809 polymorphism were more likely to have a higher subscale score for general ($p < 0.05$), activation ($p < 0.05$), depression ($p < 0.05$), aggression ($p < 0.05$), and global symptoms ($p < 0.05$) than those with a T allele.

Discussion

In this study, we assessed the association of *NRGN* rs12807809 polymorphism with schizophrenia by conducting a case-control study in Guangxi, south China. No significant differences were observed between patients and controls for genotype and allele distributions, neither in separate analysis nor in pooled analysis of Zhuang and Han populations. In spite of the strong rationale for *NRGN* rs12807809 being a plausible candidate SNP for schizophrenia in Caucasians (5), we could not replicate this association in our population. Similar to our finding, three research teams found no evidence of a significant association between the rs12807809 SNP and schizophrenia in Asian populations (11–13). In the literature, a genome-wide genetic linkage analysis confirmed that the chromosomal region 11q23.3-24, including the *NRGN* gene, is linked to schizophrenia in British and Icelandic populations (17). Subsequently, Ruano et al. (9) found an association of *NRGN* rs7113041 with schizophrenia in males in a Portuguese series. However, Betcheva et al. (10) reported no association between three SNPs (rs7113041, rs1804829, and rs12541) of *NRGN* and schizophrenia in Bulgarian populations. The contradicting results of association among the previous studies along with the present study might result from ethnic differences.

Schizophrenia is recognised as a heterogeneous disease with diverse clinical presentations and different classifications. The phenotypic heterogeneity underlying schizophrenia and the overlap with other neuropsychiatric diseases make it difficult to identify patients with schizophrenia. Further, it may confuse the results and analysis of the experiments, thus preventing a more detailed understanding of the genetic architecture of schizophrenia (18). This can be overcome by narrowing the phenotype to a hopefully more homogeneous subgroup, such as by electrophysiological assessments, neuroimaging substrates, or carefully defined symptom subtypes (19). Several genes are reported to affect the clinical features of schizophrenia, but not increase susceptibility to the illness (20). Therefore, considering the clinical features in the association studies of schizophrenia may help detect such genes, because the diagnosis of schizophrenia is based on a series of clinical features and not on a singe pathophysiology. In recent years, association analyses have found that a number of SNPs are associated with certain syndromes of schizophrenia when assessed by the PANSS (21,22). Some studies have even shown that a considerable number of SNPs do not contribute to the risk of schizophrenia but influence the severity of the psychopathological symptoms of schizophrenia (23–25).

In the present study, we used the PANSS-scored psychotic symptoms as quantitative data to investigate whether the rs12807809 polymorphism could contribute to the severity of clinical symptoms in two separate schizophrenia patient groups (83 Zhuang and 163 Han). To our knowledge, this is the first association study to report on the association between rs12807809 polymorphism and the severity of clinical symptoms. Our results show that rs12807809 polymorphism is not associated with the severity of clinical symptoms in Zhuang schizophrenia patients. However, in Han schizophrenia patients, the CC genotype of the *NRGN* rs12807809 polymorphism is associated with a higher PANSS aggression subscale

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score and activation subscale score. Furthermore, carriers of the C allele of *NRGN* rs12807809 SNP among Han schizophrenia patients had higher scores of general, activation, depression, aggression, and global symptoms than carriers of the T allele. The results suggest that the effect of *NRGN* rs12807809 polymorphism on the severity of clinical symptoms is different between Zhuang and Han populations. The disparity could be accounted for by genetic differences among Zhuang and Han schizophrenia patients. On the other hand, we cannot exclude that this disparity could be spurious due to the smaller sample size of Zhuang patients (half of the sample size of the Han patients), which may have led to inadequate power to detect a significant association.

The mechanisms underlying *NRGN* rs12807809 polymorphism's influence on the severity of clinical symptoms of schizophrenia remain a mystery. *NRGN* is abundantly expressed in brain regions known to be important for cognitive functions and is especially enriched in CA1 pyramidal neurons in the hippocampus (26). It is involved in long-term potentiation, spatial learning, and hippocampal plasticity (27). The main function of *NRGN* may be to act as a CaM reservoir, mediating the Ca^{2+}-CaM signalling pathway. Glutamate stimulation of NMDA receptors leads to Ca^{2+} influx in neurons and *NRGN* oxidation. The oxidation of *NRGN* leads to the release of CaM and the postsynaptic activation of CaM-dependent protein kinase II (CaMKII) (7). CaMKII plays an important role in mediating NMDA receptor signalling, which is involved in synaptic plasticity and the formation of associative memories in the brain (28). Impaired cognitive function including memory is thought to be a main feature of the pathophysiology of schizophrenia.

In conclusion, our case-control association study did not provide evidence for *NRGN* rs12807809 polymorphism being associated with schizophrenia in both Zhuang and Han populations. However, the *NRGN* gene may contribute to the severity of clinical symptoms in Han schizophrenia patients. This finding remains preliminary due to the relatively small sample size and further studies verifying the present results should be performed on a larger sample of patients.

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Conflicts of Interest

All authors have no conflicts of interest to declare.

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.

References

1. Saha S, Chant D, Welham J, McGrath J. A systematic review of the prevalence of schizophrenia. PLoS Med 2005;**2**:e141.
2. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 2003;**60**:1187–1192.
3. Sun J, Kuo PH, Riley BP, Kendler KS, Zhao Z. Candidate genes for schizophrenia: a survey of association studies and gene ranking. Am J Med Genet B Neuropsychiatr Genet 2008;**147B**:1173–1181.
4. Craddock N, O'Donovan MC, Owen MJ. Genes for schizophrenia and bipolar disorder? Implications for psychiatric nosology. Schizophr Bull 2006;**32**:9–16.
5. Stefansson H, Ophoff RA, Steinberg S et al. Common variants conferring risk of schizophrenia. Nature 2009; **460**:744–747.
6. Ran X, Miao HH, Sheu FS, Yang D. Structural and dynamic characterization of a neuron-specific protein kinase C substrate, neurogranin. Biochemistry 2003;**42**:5143–5150.
7. Huang KP, Huang FL, Jager T, Li J, Reymann KG, Balschun D. Neurogranin/RC3 enhances long-term potentiation and learning by promoting calcium-mediated signaling. J Neurosci 2004;**24**:10660–10669.
8. Hakak Y, Walker JR, Li C et al. Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. Proc Natl Acad Sci U S A 2001;**98**:4746–4751.
9. Ruano D, Aulchenko YS, Macedo A et al. Association of the gene encoding neurogranin with schizophrenia in males. J Psychiatr Res 2008;**42**:125–133.
10. Betcheva ET, Mushiroda T, Takahashi A et al. Case-control association study of 59 candidate genes reveals the *DRD2* SNP rs6277 (C957T) as the only susceptibility factor for schizophrenia in the Bulgarian population. J Hum Genet 2009;**54**:98–107.
11. Ohi K, Hashimoto R, Yasuda Y et al. Functional genetic variation at the *NRGN* gene and schizophrenia: evidence from a gene-based case-control study and gene expression

https://doi.org/10.1017/neu.2015.13 Published online by Cambridge University Press

analysis. Am J Med Genet B Neuropsychiatry Genet 2012;**159B**:405–413.
12. Li T, Li Z, Chen P et al. Common variants in major histocompatibility complex region and *TCF4* gene are significantly associated with schizophrenia in Han Chinese. Biol Psychiatry 2010;**68**:671–673.
13. Shen YC, Tsai HM, Cheng MC, Hsu SH, Chen SF, Chen CH. Genetic and functional analysis of the gene encoding neurogranin in schizophrenia. Schizophr Res 2012;**137**:7–13.
14. Su L, Wei B, Chen Q et al. Association study of *PRODH* gene variant rs385440 with schizophrenia in Zhuang and Han nationality of Guangxi Chinese. J Behav Med Brain Sci 2012;**21**:36–39.
15. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 1987;**13**:261–276.
16. Lindenmayer JP1, Grochowski S, Hyman RB. Five factor model of schizophrenia: replication across samples. Schizophr Res 1995;**3**:229–234.
17. Gurling HM, Kalsi G, Brynjolfson J et al. Genomewide genetic linkage analysis confirms the presence of susceptibility loci for schizophrenia, on chromosomes 1q32.2, 5q33.2, and 8p21-22 and provides support for linkage to schizophrenia, on chromosomes 11q23.3-24 and 20q12.1-11.23. Am J Hum Genet 2001;**68**:661–673.
18. Cherlyn SY, Woon PS, Liu JJ, Ong WY, Tsai GC, Sim K. Genetic association studies of glutamate, GABA and related genes in schizophrenia and bipolar disorder: a decade of advance. Neurosci Biobehav Rev 2010;**34**:958–977.
19. Kim Y, Zerwas S, Trace SE, Sullivan PF. Schizophrenia genetics: where next? Schizophr Bull 2011;**37**:456–463.
20. Fanous AH, Kendler KS. Genetic heterogeneity, modifier genes, and quantitative phenotypes in psychiatric illness: searching for a framework. Mol Psychiatry 2005;**10**:6–13.
21. Li W, Wang X, Zhao J et al. Association study of myelin transcription factor 1-like polymorphisms with schizophrenia in Han Chinese population. Genes Brain Behav 2012;**11**:87–93.
22. Sun YH, Shen Y, Xu Q. *DTNBP1* gene is associated with some symptom factors of schizophrenia in Chinese Han nationality. Chin Med Sci J 2010;**25**:85–89.
23. Chang HA, Lu RB, Shy MJ, Chang CC, Lee MS, Huang SY. Brain-derived neurotrophic factor Val66Met polymorphism: association with psychopathological symptoms of schizophrenia? J Neuropsychiatry Clin Neurosci 2009;**21**:30–37.
24. Rethelyi JM, Bakker SC, Polgar P et al. Association study of *NRG1, DTNBP1, RGS4, G72/G30*, and *PIP5K2A* with schizophrenia and symptom severity in a Hungarian sample. Am J Med Genet B Neuropsychiatr Genet 2010;**153B**: 792–801.
25. Zhou DH, Yan QZ, Yan XM et al. The study of *BDNF* Val66Met polymorphism in Chinese schizophrenic patients. Prog Neuropsychopharmacol Biol Psychiatry 2010;**34**:930–933.
26. Huang FL, Huang KP, Boucheron C. Long-term enrichment enhances the cognitive behavior of the aging neurogranin null mice without affecting their hippocampal LTP. Learn Mem 2007;**14**:512–519.
27. Krug A, Krach S, Jansen A et al. The effect of neurogranin on neural correlates of episodic memory encoding and retrieval. Schizophr Bull 2011;**39**:141–150.
28. Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993; **361**:31–39.

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