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RESEARCH NOTE

Positive association between *NTNG1* and schizophrenia in Chinese Han population

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Introduction

Schizophrenia is a common mental disorder with a global incidence of 1% (Schultz and Andreasen 1999). Many families and twin studies revealed that genetic factors play an important role in this complex disease (Kruglyak 1997; Cardno *et al.* 1999). Some mild mental developmental disorders have been found in schizophrenia cases which arise from early neurodevelopment abnormalities, such as brain asymmetry development and neurons transitional obstacles (Chua and Murray 1996). Netrin G1 (*NTNG1*) is one of conserved families of axon guidance molecules. It plays an important role in axon migration as guidance cues in nervous system development (Kennedy 2000). *NTNG1* is located at chromosome 1p13.3 zone which is also the linkage zone of pathogenesis of schizophrenia (Kaufman *et al.* 1998; Lewis *et al.* 2003). Family and case–control studies of Japanese showed that the polymorphism and their haplotype of *NTNG1* were associated with schizophrenia (Aoki-Suzuki *et al.* 2005; Ohtsuki *et al.* 2008). In this study, we used Han Chinese case–control samples to investigate the relationship between genotype, haplotype and clinical feature of *NTNG1* in schizophrenia.

Materials and methods

In this study, 316 schizophrenia cases (169 males and 147 females, mean age ± S.D. = 37.75 ± 8.83 years) and 311 control subjects (161 males and 150 females, mean age ± S.D. = 38.54 ± 9.81 years) were recruited from Liaoning Province in the northeast of China. Clinical diagnosis was made strictly according to DSM-IV (American Psychiatric Association) criteria by at least two experienced psychiatrists on the basis of direct interviews, available medical records, and information provided by hospital staff and relatives. Age, sex, onset age, clinical classification, personality, family history were recorded for all cases and clinical symptoms for 252 first-episode cases (139 males and 113 females, mean age ± S.D. = 32.92 ± 8.08 years). The study was verified and approved by the Ethics Committee of the First Hospital of China Medical University. All participants in the study provided written informed consent. All subjects were unrelated northern Han Chinese in origin.

Based on the previous studies by transmission disequilibrium test (Ohtsuki *et al.* 2008) and presence of a restriction enzyme site, three SNPs (rs4132604-SNP1, rs2218404-SNP2 and rs1373336-SNP3) were genotyped in this study. The information of DNA sequences containing the SNPs was obtained from the SNPs database (http://www.ncbi.nlm.nih.gov/SNP). Genomic DNA was prepared from venous blood using standard phenol–chloroform extraction. Genotyping was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The primer sequences for rs4132604 were TCCCATTATTGCCACCA (forward) and ATCCAGATTTTAGGTGACCTC (reverse); for rs22184O4 were ACTAAAAGTGATATTTTGGACTC (forward) and GCTGTGATAATCGGGGTCT (reverse); for rs137336 were TATTTGTTACATAAGGTGAAGGGA (forward) and CTCCTTGCCTGGCTTAGTTT (reverse).

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For each SNP, PCR was performed in a 20 μL reaction mixture. The reaction mixture consisted of 50 ng genomic DNA, 1 U *Taq* polymerase, 1.5 μL of each primer (5 pM), 2 μL PCR buffer (10× Takara Biotechnology, Dalian, China), and 2 μL dNTPs (each 2.5 mM). Amplification was under the following conditions: an initial 5 min at 95°C, 35 cycles of 1 min at 94°C, 45 s at an appropriate annealing temperature, and 1 min at 72°C, followed by a final extension of 10 min at 72°C. The PCR products were completely digested with restriction enzyme and then separated by agarose gel electrophoresis (2–4% gel) stained with ethidium bromide.

For the case–control association study, SPSS 11.5 for Windows (http://www.broad.mit.edu/haploview) was used to analyse genotype, allele frequencies for all subjects. Chi square test was used for testing association of clinical features with genotype frequencies for the first-episode cases. Hardy–Weinberg equilibrium (HWE) and pairwise linkage disequilibrium (LD) were tested by Haploview 4.1.

Results

Cases and controls were well matched in terms of age and ethnicity. Mean age was 37.75 ± 8.83 years for cases and 38.54 ± 9.81 years for controls. No significant differences in the frequency distributions of age were found between cases and controls ($P = 0.355$).

Genotype frequencies did not deviate from HWE. The frequency of G allele of rs4132604 was 0.59 for cases and 0.51 for controls. For SNP rs4132604, the difference in the allele and genotype frequencies between cases and controls were statistically significant ($P = 0.022$ for genotypes and $P = 0.005$ for alleles). However, the allele and genotype frequencies of rs2218404 and those of rs1373336 did not show significant difference between cases and controls. SNP rs4132604 was associated with increased risk of schizophrenia (OR = 1.379, 95%CI: 1.101–1.727). However, SNP rs2218404 and rs1373336 were not significantly associated with the risk of schizophrenia (table 1).

A strong pairwise linkage disequilibrium was found between the three SNPs, rs4132604, rs2218404 and rs1373336 (all $|D'| > 0.60$). The three SNPs were therefore designated as an LD block. Significant differences of haplotype containing rs4132604 alleles between cases and controls were found, as shown in table 2, GG ($P = 0.013$) and TG ($P = 0.021$) between rs4132604 and rs2218404, GGT ($P = 0.000$), TGT ($P = 0.010$) among three SNPs.

As shown in table 3, symptoms with good stability and quality for diagnosis of schizophrenia were collected, and therefore 252 first-episode cases were divided into two groups according to those with or without each particular symptoms. The allele frequencies of rs4132604 of the cases with clinical symptoms such as delusion of reference ($P = 0.000$), delusion of persecution ($P = 0.004$), delusion of being revealed ($P = 0.014$) and delusion of jealousy ($P = 0.015$) were significantly different from those

Table 1. Genotype and allele frequencies of SNPs on *NTNG1* in cases and controls.

SNPs	Group	Genotype frequency (%)			χ^2	*P* value	Allele frequency (%)		χ^2	*P* value	OR (95%CI)
		AA	AB	BB			A	B			
rs4132604	Cases	110 (35.71)	141 (45.78)	57 (18.51)	7.667	0.022*	361 (58.60)	255 (41.40)	7.858	0.005*	1.379
	Controls	81 (26.30)	150 (48.70)	77 (25.00)			312 (50.65)	304 (49.35)			(1.101–1.727)
rs2218404	Cases	228 (73.08)	74 (23.72)	10 (3.20)	1.849	0.397	530 (84.94)	94 (15.06)	0.170	0.680	1.066
	Controls	217 (69.77)	87 (27.97)	7 (2.26)			521 (83.76)	101 (16.24)			(0.786–1.446)
rs1373336	Cases	62 (19.94)	146 (46.95)	103 (33.11)	0.909	0.635	270 (43.41)	352 (56.59)	3.762	0.052	1.247
	Controls	66 (21.22)	153 (49.20)	92 (29.58)			285 (45.82)	337 (54.18)			(0.998–1.560)

*P value less than 0.05. rs4132604 ($N = 308$), A:G, B:T; rs2218404 ($N = 312$), A:G, B:T; rs1373336 ($N = 311$), A:C, B:T.

Table 2. Frequencies of haplotype of SNPs on *NTNG1* in cases and controls.

Haplotype	rs4132604	rs2218404	rs1373336	Haplotype frequency (%)		χ^2	*P*	OR (95%CI)
				Cases	Controls			
1	G	G		343.78 (54.59)	296.02 (47.61)	6.107	0.013*	1.325 (1.061–1.654)
2	T	G		189.42 (30.13)	225.04 (36.63)	5.275	0.021*	0.756 (0.597–0.958)
3	T	T		72.53 (11.52)	82.80 (13.31)	0.932	0.334	0.850 (0.607–1.189)
4	G	T		24.27 (3.88)	18.24 (2.93)	0.815	0.367	1.329 (0.714–1.474)
5	G	G	T	312.67 (49.58)	244.83 (39.44)	13.405	0.000*	1.519 (1.214–1.901)
6	T	G	C	150.43 (23.91)	171.18 (27.49)	2.176	0.140	0.824 (0.639–1.063)
7	T	T	C	71.72 (11.43)	66.73 (10.72)	0.138	0.710	1.069 (0.751–1.521)
8	T	G	T	37.38 (5.87)	61.24 (9.83)	6.561	0.010*	0.574 (0.375–0.877)
9	G	G	C	32.53 (5.22)	43.89 (7.08)	1.972	0.160	0.725 (0.455–1.155)
10	G	T	C	19.53 (3.14)	22.32 (3.63)	0.230	0.632	0.893 (0.482–1.653)

The haplotype without rs4132604 alleles or less than 1% were eliminated. *P value less than 0.05.

Table 3. Association between allele frequencies of SNPs and clinical symptoms of first-episode cases.

Psychotic symptoms (%)	rs4132604 ($N = 252$)		rs2218404 ($N = 252$)		rs1373336 ($N = 252$)	
	χ^2	*P*	χ^2	*P*	χ^2	*P*
Genuine hallucination (83.09)	1.937	0.380	1.575	0.665	2.188	0.335
Delusion of reference (65.32)	20.328	0.000*	6.541	0.086	15.905	0.000*
Delusion of influence (58.25)	0.051	0.975	1.848	0.605	0.072	0.965
Delusion of persecution (50.95)	11.105	0.004*	4.600	0.204	14.367	0.001*
Nihilistic delusion (7.14)	2.957	0.228	2.712	0.438	4.891	0.087
Experience of being revealed (17.86)	8.531	0.014*	2.701	0.440	0.848	0.654
Delusion of guilt (23.02)	3.777	0.151	1.288	0.732	5.696	0.058
Delusion of jealousy (17.06)	8.334	0.015*	1.413	0.702	11.716	0.003*
Grandiose delusion (9.93)	4.452	0.108	5.340	0.149	4.191	0.123
Delusion of love (17.86)	0.274	0.872	1.073	0.784	2.272	0.321
Other delusion (35.56)	1.301	0.522	3.277	0.351	1.363	0.506
Logical thinking disorder (35.48)	0.469	0.791	2.660	0.447	0.064	0.968
Queer behaviour (52.14)	0.585	0.746	1.341	0.719	1.324	0.516
Poverty of thought (40.87)	1.338	0.512	7.531	0.057	3.563	0.168
Apathy (44.44)	4.103	0.129	1.896	0.594	2.820	0.244
Abulia (39.68)	1.671	0.434	7.153	0.067	1.762	0.414

*P value less than 0.05.

of the cases without the symptoms. Significant differences were also found in allele frequencies of rs1373336 between the cases with or without the following clinical symptoms: delusion of reference ($P = 0.000$), delusion of persecution ($P = 0.001$), and delusion of jealousy ($P = 0.003$).

Discussion

The aetiology and pathology of schizophrenia is not fully understood. However, at present, neurodevelopment disorder is an accepted aspect in pathogenesis of schizophrenia (Mjellem and Kringlen 2000). Many observations suggest that schizophrenia is due to a series of genetic anomalies in neural development and differentiation. Myelin related protein gene (Novak *et al.* 2002), *NOTCH4* gene (Zhang *et al.* 2004), neurotrophin-3 gene (Hattori *et al.* 2002) and neuregulin 1 gene (Stefansson *et al.* 2003) have shown association with schizophrenia. Based on 124 family studies, Aoki-Suzuki *et al.* (2005) suggested that *NTNG1* genes probably were relevant for pathogenesis of schizophrenia. Ohtsuki *et al.* (2008) have also reported rs4132604 and rs2218404 of *NTNG1* are positive associations with schizophrenia by transmission disequilibrium test and the haplotype contained rs2218404 and rs1373336 showed high positive association with schizophrenia in case–control study.

This study showed positive association between rs4132604 and schizophrenia on the basis of the alleles ($\chi^2 = 7.858$, $P = 0.005$) and genotype ($\chi^2 = 7.667$, $P = 0.022$) frequencies distribution between cases and controls. The frequency of allele G was significantly higher than alleles T frequency in rs4132604, which suggested the haplotype or chromosome contained allele G (OR = 1.379, 95%CI = 1.101–1.727) had significant effects on susceptibility to schizophrenia and allele T had resistant effects on susceptibility to schizophrenia. The findings in this study were different from the former study (Ohtsuki *et al.* 2008), in which the frequency of Allele T

was found higher than alleles G. The difference may be due to samples from different ethnic groups. However, we got the same results in rs2218404 and rs1373336 which showed no association with schizophrenia. Eastwood and Harrison (2008) reported *NTNG1* mRNAs *in situ* hybridization histochemistry were not influenced by rs1373336 in postmortem study. We did not find association between rs1373336 and schizophrenia in this study as well.

LD analysis showed that there were high positive correlations among the three SNPs. The haplotype analysis showed that GG (OR = 1.325, 95%CI = 1.061–1.654), TG (OR = 0.756, 95%CI = 0.597–0.958) between rs4132604 and rs2218404, GGT (OR = 1.519, 95%CI = 1.214–1.901), TGT (OR = 0.574, 95%CI = 0.375–0.877) of the three SNPs had significant differences between the cases and controls. All the OR values of positive haplotype contained allele G of rs4132604 were more than 1 while all the OR values that contained allele T of rs4132604 were less than 1. Further, the findings suggested that allele G of rs4132604 might be the risky gene of schizophrenia. There were no significant differences in the genotype and allele frequencies of the allele G of rs2218404 and allele T of rs1373336 but high proportion in the haplotype, which suggested that the above two SNPs might play synergistic roles in susceptibility to schizophrenia.

Significant differences of rs4132604 allele frequencies were found in the cases with the following clinic symptoms: delusion of reference, delusion of being revealed, delusion of persecution, delusion of jealousy. Significant differences were also found on the allele frequencies of rs1373336 on the cases with delusion of reference, delusion of persecution, delusion of jealousy. These findings suggested that rs4132604 and rs1373336 might contribute to multiple clinical symptoms, while many clinical symptoms were associated with them. However, the association of the three SNPs with negative clinical symptoms in schizophrenia was not observed in the present study. We cannot reject that just according to the above findings. It should be treated cautiously because of the sample size and genetic method. It is necessary to expand the sample size in further validation and the best method is family study.

In conclusion, our findings suggest that rs4132604 and the haplotype of the three selected SNPs are significantly associated with schizophrenia. Whether or not they play the main genetic role in pathogenesis in schizophrenia still needs further research.

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