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ORIGINAL ARTICLE

DTNBP1 Gene Is Associated with Some Symptom Factors of Schizophrenia in Chinese Han Nationality[△]

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Key words: schizophrenia; DTNBP1 gene; positive and negative symptoms; quantitative trait test

Objective To study the association of DTNBP1 gene with some symptom factors of schizophrenia.

Methods A total of 285 unrelated schizophrenic individuals were recruited from December 2004 to January 2009 for genetic analysis, and their symptom factors were assessed based on the Positive and Negative Syndrome Scale (PANSS). The quantitative trait test was performed by the UNPHASED program (version 3.0.12) to investigate the association between scored positive and negative symptoms and the single nucleotide polymorphisms (SNPs) in DTNBP1 gene.

Results The quantitative trait test showed allelic association of rs909706 with the excitement symptom of schizophrenia ($P < 0.05$, adjusted by 10 000 permutations), while the genotype C/G of rs2619539 with a negative symptom, lack of spontaneity and flow of conversation ($P < 0.05$, adjusted by 10 000 permutations).

Conclusion DTNBP1 variations are possibly associated with some symptoms of schizophrenia, which could partly explain the relationship between the susceptibility gene DTNBP1 and that disease.

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SCHIZOPHRENIA is a severe form of mental illness affecting about 1% of the adult population. It is often clinically described in terms of positive and negative symptoms, and cognitive deficits,

which are assessed with Positive and Negative Syndrome Scale (PANSS) in clinical practice.¹

DTNBP1 gene (6p22.3) was first reported to be associated with schizophrenia in 270 Irish high-density pedigrees.² In the next few years, DTNBP1 gene was recognized as one of the hottest schizophrenia susceptibility genes based on case-control and family-based association studies in different populations.³⁻¹⁰ Yet consistent polymorphisms or haplotypes were not identified until now. There are also evidences showing that dysbindin is involved in the pathology of schizophrenia at both mRNA and protein levels.¹¹⁻¹⁴ However, some ge-

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netic results previously reported showed no association between DTNBP1 gene and schizophrenia.¹⁵⁻¹⁸ In some other studies, that gene of interest was shown to be associated with negative symptoms of schizophrenia, cognitive deficits, working memory, and some other phenotypes.¹⁹⁻²⁶

In the present study, we selected four single nucleotide polymorphisms (SNPs) (rs742106, rs909706, rs1011313, and rs2619539) in DTNBP1 gene previously reported associated with schizophrenia^{6,10,24,27,28} to test whether these polymorphisms were associated with some symptom factors of that mental illness.

PATIENTS AND METHODS

Patients

A total of 284 unrelated schizophrenia patients (131 males and 154 females, aged 31.9 ± 10.9 years) of Han nationality were recruited by Department of Psychiatry, the First Hospital of Shanxi Medical University, from December 2004 to January 2009. These patients were diagnosed with schizophrenia by at least two consultant psychiatrists in a clinical interview based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 1994). Patients were excluded from this study if they suffered from any organic brain disorders, mental retardation, severe head trauma, or demonstrate psychotic symptoms due to medical conditions or treatments. The included patients all provided written informed consents to blood sample collection for genetic analysis. The protocol of this study was examined and approved by the Ethics Committee of Chinese Academy of Medical Sciences & Peking Union Medical College.

Clinical assessment of symptoms

For all the included patients, positive and negative symptoms were assessed according to the PANSS. The severity of each symptom was scored with a 7-grade scale (1: absent; 2: minimal; 3: mild; 4: moderate; 5: moderately severe; 6: severe; 7: extreme).

Genotyping of SNPs

Four SNPs present in the DTNBP1 gene were selected in the present study to be genotyped. Among those SNPs, rs909706 is located in intron 1, rs1011313 in intron 4, rs2619539 in intron 5, and rs742106 in intron 9.^{6,10,24,27,28}

Genomic DNA was extracted from peripheral blood leukocytes following the standard phenol-chloroform procedure. The primers used for polymerase chain reaction

(PCR) amplification were designed by the Primer Premier 5.0 software (Table 1). The reaction volume of PCR amplification was 25 μ L, containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 0.001% (w/v) gelatin, 200 μ mol/L of deoxyribonucleoside triphosphates (dNTPs), 0.4 μ mol/L of the primers, 1.0 unit of Taq DNA polymerase (Tiangen, Beijing, China), and 50 ng of the extracted genomic DNA. The conditions for thermal cycling included an initial denaturation at 94°C for 5 minutes, 35 cycles at 94°C for 30 seconds, at 50-56°C for 30 seconds, and at 72°C for 30 seconds, followed by a final elongation at 72°C for 10 minutes on GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA, USA). The PCR products were purified and then sequenced bidirectionally with an ABI 3700 DNA sequencer (Applied Biosystems).

Statistical analysis

Hardy-Weinberg equilibrium for the genotypic distributions of each SNP was tested with the chi-square (χ^2) goodness-of-fit test. Pair-wise linkage disequilibrium among these four SNPs was defined by the four-gamete rule using Haploview 4.2. The UNPHASED program (version 3.0.12) was applied to analyze genotype data.²⁹ The additive genetic value, which represents the difference in expected trait due to the allele compared with the reference, was estimated for determining the association between scored positive/negative symptoms and tested SNPs. The statistical significance level was set at 0.05 and adjusted by 10 000 permutations.

RESULTS

The χ^2 goodness-of-fit test showed that the genotypic distributions of the four studied SNPs were all in Hardy-Weinberg equilibrium in the schizophrenia patients (Table 2).

According to the plotting by Haploview 4.2, rs1011313 and rs2619539 were in one linkage disequilibrium block in our samples, while the other two were not.

The quantitative trait test showed allelic association of rs909706 with the excitement symptom of schizophrenia ($\chi^2=7.352$, $P<0.05$, adjusted by 10 000 permutations), and allele G with higher additive values than allele A (Table 3). In addition, rs2619539 was shown to be associated with lack of spontaneity and flow of conversation ($\chi^2=9.151$, $P<0.05$, adjusted by 10 000 permutations), and the genotype C/G with a higher additive values than the other genotypes (Table 4).

Table 1. Polymerase chain reaction primers for genotyping of DTNBP1 SNPs

SNP	Forward primers	Reverse primers	Tm (°C)	Fragment length (bp)
rs742106	5'-CAGTCACTTACGCATAAACCC-3'	5'-TGACCCAGCCCAAAGAAC -3'	56	507
rs909706	5'-ACGCTTGAGCACTTACCA -3'	5'-TGAGTTGTTCTCCACCAG -3'	51	655
rs1011313	5'-CGCATTGGAGTTACTTCTG-3'	5'-ACATTGCTGGATTGGTTTG -3'	55	667
rs2619539	5'-CTCTCACAGACGAGGTATCA-3'	5'-TATGTGATTATGGAACGCTTA -3'	52	653

SNP: single nucleotide polymorphism; Tm: melting temperature.

Table 2. Hardy-Weinberg equilibrium test of the four SNPs in schizophrenia patients

SNP	Observed			Expected			Allele count	χ^2	P
	1/1	1/2	2/2	1/1	1/2	2/2			
rs742106	99	226	154	93.8	236.3	148.8	958	0.917	0.338
rs909706	222	218	42	227.3	207.4	47.3	964	1.262	0.261
rs1011313	29	163	291	25.3	170.4	287.3	966	0.920	0.337
rs2619539	238	193	45	235.1	198.9	42.1	952	0.415	0.519

Table 3. Allelic association of four SNPs and excitement

SNP	Allele	Count	MarFreq	Addval	95%Lo	95%Hi	χ^2	P
rs742106	1	242	0.4246	0	0	0	0.9069	0.3409
	2	328	0.5754	-0.06649	-0.2035	0.7048		
rs909706	1	382	0.6702	0	0	0	7.352	0.006697*
	2	188	0.3298	0.1939	0.05268	0.3352		
rs1011313	1	128	0.2254	0	0	0	0.004145	0.9487□
	2	440	0.7746	-0.00534	-0.1679	0.1572		
rs2619539	1	386	0.6893	0	0	0	5.905	0.0151□
	2	174	0.3107	0.177	0.03332	0.3206		

MarFreq: marginal frequency; Addval: additive value; 95%Lo: 95% lower confidence limit for the additive value; 95%Hi: 95% upper confidence limit for the additive value.

*P=0.0238 adjusted by 10 000 permutations.

Table 4. Genotypic association of four SNPs and lack of spontaneity and flow of conversation

SNP	Allele	Count	MarFreq	Addval	95%Lo	95%Hi	χ^2	P
rs742106	1/1	55	0.193	0	0	0	0.0911	0.7628
	1/2	132	0.4632	0.09798	-0.1216	0.3175	2.793	0.09467
	2/2	98	0.3439	-0.06405	-0.3001	0.172	2.261	0.1327
rs909706	1/1	125	0.4386	0	0	0	3.823	0.05055
	1/2	132	0.4632	0.2181	0.04383	0.3923	7.989	0.004705
	2/2	28	0.09825	-0.111	-0.4292	0.2073	2.177	0.1401
rs1011313	1/1	15	0.05282	0	0	0	0.1183	0.7309
	1/2	98	0.3451	0.1486	-0.2389	0.5361	2.474	0.1157
	2/2	171	0.6021	0.01497	-0.3639	0.3939	1.879	0.1705
rs2619539	1/1	136	0.4857	0	0	0	4.509	0.03371
	1/2	114	0.4071	0.2423	0.06607	0.4185	9.151	0.002485*
	2/2	30	0.1071	-0.08669	-0.3889	0.2155	1.888	0.1695

*P=0.0227 adjusted by 10 000 permutations.

We also carried out quantitative trait tests for other symptoms covered in PANSS, yet no associations were found between those symptoms and the studied SNPs.

DISCUSSION

Schizophrenia is a genetically complex disorder with an unknown pathophysiology. It is suggested that to study its specific symptoms or clinical phenotypes could help to

establish a better understanding of this disorder.³⁰ The PANSS assessing the positive and negative symptoms of schizophrenia includes positive scales (delusions, conceptual disorganization, hallucinatory behavior, excitement, grandiosity, suspiciousness/persecution, and hostility), negative scales (blunted affect, emotional withdrawal, poor rapport, passive/apathetic social withdrawal, difficulty in abstract thinking, lack of spontaneity and flow of conversation, and stereotyped thinking), and general

psychopathology scale (somatic concern, anxiety, guilt feelings, tension, mannerisms and posturing, depression, motor retardation, uncooperativeness, unusual thought content, disorientation, poor attention, lack of judgment and insight, disturbance of volition, poor impulse control, preoccupation, and active social avoidance).¹

rs909706 and rs2619539, the two SNPs found in this study to be related with some symptoms of schizophrenia, are both intronic polymorphisms. rs909706 was found to contribute to schizophrenia,¹⁰ and associated with change of patients' response to anti-psychotic agent haloperidol.²⁸ And rs2619539 was associated with methamphetamine psychosis,³¹ less severe manic-type symptoms in psychosis,³² and cognitive functions.²³

Complementary to those previous findings, the quantitative trait test in our study showed association of some SNPs in DTNBP1 gene with some symptom factors of schizophrenia. In specific, rs909706 was associated with excitement, and rs2619539 with lack of spontaneity and flow of conversation. Their locations in different linkage disequilibrium blocks may explain the fact that those two symptoms belong to positive and negative scales respectively.

Our study revealed a possible association between DTNBP1 and the symptoms of schizophrenia, which perhaps could partly explain the relationship between DTNBP1 variations and that mental illness. Further investigation in large samples and different populations is still needed for clarifying the mechanisms of how the DTNBP1 gene affects the symptoms of schizophrenia.

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