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Regulators of G-protein signaling 9 genetic variations in Chinese subjects with schizophrenia

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ABSTRACT. To identify single-nucleotide polymorphisms that contribute to the genetic susceptibility to schizophrenia, we examined the potential association between schizophrenia and 9 single nucleotide polymorphisms (rs1530351, rs4791230, rs2869577, rs8077696, rs8070231, rs2292592, rs9916525, rs1122079, and rs4790953) in the G-protein signaling 9 gene. The participants included 395 schizophrenia subjects and 400 healthy controls. The selected single nucleotide polymorphisms were genotyped using mass spectrometry techniques. The allelic or genotypic frequencies of the rs4791230 (promoter region) polymorphisms in subjects with schizophrenia were significantly different from those in healthy controls. The subjects with schizophrenia had a significantly higher frequency of the G allele (P = 0.030, odds ratio = 1.589, 95% confidence interval = 1.042-2.422) of rs4791230. Strong linkage disequilibrium was observed in 4 blocks

(D' > 0.9). Significantly fewer T-A (rs1530351-rs4791230) haplotypes (P = 0.029) were found in subjects with schizophrenia. These findings suggest a role of G-protein signaling 9 polymorphisms in schizophrenia among Han Chinese and may be informative for future genetic or neurobiological studies on schizophrenia.

Key words: Regulators of G-protein signaling 9 gene; Schizophrenia; Single nucleotide polymorphisms

INTRODUCTION

Schizophrenia is a chronic, debilitating psychotic mental disorder that affects approximately 1% of the population worldwide (Mueser and McGurk, 2004). The disease is characterized by positive symptoms, such as delusions, hallucinations, and thought disorder, and by negative symptoms, such as social withdrawal, apathy, and cognitive impairment. Family, adoption, and twin studies have consistently demonstrated a substantial genetic influence on the development of schizophrenia, with inherited risk estimates in the range of 80% (Cannon, 2005; Kukshal et al., 2012). Previous studies have suggested that polymorphisms in the regulator of G-protein signaling 9 (RGS9) may be related to schizophrenia (Liou et al., 2009).

The receptors for dopamine are members of the G-protein coupled receptor superfamily (Missale et al., 1998), and G-protein coupled receptor signal termination can be enhanced by members of a family of proteins known as RGSs (Berman and Gilman, 1998). RGS9-2 is highly expressed in the striatum and plays a role in modulating dopaminergic-mediated signaling cascades (Gold et al., 1997; Rahman et al., 2003). The supersensitivity of D2 receptors, as observed in RGS9 knockout mice, is a common feature of psychosis (Rahman et al., 2003). Consistently, RGS9 expression was diminished in amphetamine-treated rat model of schizophrenia (Seeman et al., 2007). Previous studies have shown that the expression of RGS9-2 in the postmortem brain of patients with schizophrenia was lower than that in controls (Mirnics et al., 2001; Seeman et al., 2007). These findings provide additional support implicating RGS9 as a candidate gene in schizophrenia.

The RGS9 gene (17q21-25, 1.1 Mb) contains 18 exons with intron-exon junctions conforming to splice-site consensus sequences (Zhang et al., 1999), and linkage studies have demonstrated that the region is important because of its relationship with major mental illness (Rousset and Raymond, 1995; Escamilla et al., 2009; Clarke et al., 2011). A previous study found no association between the regulator of RGS9 and schizophrenia in a Jewish population (Greenbaum et al., 2010). However, a weak association between RGS9 (rs4790953, near the 3' region) and antipsychotic-induced tardive dyskinesia was reported in a sample from Taiwan (Liou et al., 2009). Using haplotype analyses, researchers found a significant association between the haplotype consisting of rs8077696, rs8070231, and rs2292593 in the RGS9 gene (Liou et al., 2009). Based on the crucial role of RGS9 in mental illness, we investigated 9 SNPs (rs8077696, rs8070231, rs2292593, rs2292592, rs9916525, rs11220799, rs1530351, rs4791230, and rs2869577) in a Chinese population to verify the putative association between RGS9 polymorphisms and schizophrenia.

MATERIAL AND METHODS

Subjects

A total of 395 unrelated patients with schizophrenia (N = 226 males and 169 females; mean age of onset, 32.3 ± 9.6 years) were recruited from the mental health center of the First Affiliated Hospital, Xi'an Jiaotong University and the Xi'an Mental Health Center. All diagnoses were assigned following a standard procedure. After providing written informed consent, each subject was assessed using the Structured Clinical Interview for DSM-IV Axis I disorder, which was administered by experienced psychiatrists. Standard diagnostic assessments were supplemented with clinical information obtained by reviewing medical records and interviewing family informants. Detailed information concerning the onset and course of clinical disorders, the presence of personality disorders and mental retardation, and a brief description of the subject's psychosocial and occupational functioning during the course of the illness were presented to a consensus diagnostic group, which included at minimum 3 trained psychiatrists with Diagnostic and Statistical Manual of Mental Disorders, 4th revision (DSM-IV) diagnostic experience, as well as other trained Structured Clinical Interview for DSM-IV Axis I disorder raters. All available information (personal history, hospital record, and family-history report) was used to reach a consensus-related DSM-IV diagnosis. Participants were excluded if they: met the DSM-IV criteria for an additional Axis I disorder; had a history of substance-induced psychotic disorders, learning disabilities, head injuries, and other symptomatic psychoses; were taking other prescribed medications that could affect the central nervous system; had a history of seizures, hematological diseases, or severe liver or kidney impairment; or were pregnant. The study complied with the guidelines of our local Medical Ethical Committee, and all participants recruited in this study provided written informed consent.

A total of 400 healthy blood donors (N = 232 males and 168 females; mean age, 33.1 ± 6.8 years) were recruited at the First Hospital Affiliated to the Medical College of Xi'an Jiaotong University. Subjects who had psychotic disorders, participated in other studies, or suffered from chronic brain diseases were excluded. All participants were Han Chinese from Shanxi Province and were not genetically related. Written informed consent was obtained from all participants. The study protocol was approved by the Ethical Committee of Xi'an Jiaotong University, Xi'an, China.

Single nucleotide polymorphism (SNP) selection

SNPs in the promoter region, untranslated regions, exons, introns, and near the 3' end of the RGS9 gene were systematically screened. The RGS9 gene with a genomic length of 1.1 Mb and 10 SNPs selected, which were selected for genotyping, are shown in Table 1. Preliminary analysis was performed using the HapMap data. Using the Haploview software v4.2 and a minor allele frequency cut-off ≥5% (HapMap Data Release 27), TagSNPs were examined in Chinese Han in Beijing population. The linkage disequilibrium pattern of this gene was determined in the Chinese population using the preliminary data from the HapMap. These SNPs were analyzed in an association study.

Table 1. Genotypic and allelic frequencies of RGS9 polymorphisms in the controls and patients with schizophrenia.

Variable/bp	Location	MAF	Controls (N = 400)		Schizophrenia (N = 395)		P value[a]	OR, 95%CI
			No.	%	No.	%		
rs1530351/63131609	Promoter	0.051	400		395		0.148	
TT			362	90.5	344	43.5	0.122	0.704, 0.451-1.099
CT			38	9.5	49	6.2	0.182	1.358, 0.867-2.218
CC			0	0.0	2	0.3	-	-
Per C allele			38	4.8	53	6.7	0.093	1.442, 0.939-2.214
rs4791230/63132328	Promoter	0.051	400		395		0.035	
AA			362	90.5	342	43.3	0.081	0.674, 0.433-1.049
GA			38	9.5	48	6.1	0.219	1.327, 0.845-2.084
GG			0	0.0	5	0.6	-	-
Per G allele			38	4.8	58	7.3	0.030	1.589, 1.042-2.422
rs2869577/63195592	Intron 1	0.298	400		393		0.335	
CC			204	51.0	213	54.2	0.441	1.116, 0.844-1.477
CG			154	38.5	150	38.2	0.924	0.986, 0.740-1.315
GG			42	10.5	30	7.6	0.157	0.702, 0.430-1.147
Per G allele			238	29.8	210	26.7	0.180	0.861, 0.692-1.072
rs8077696/63192561	Intron 12	0.333	398		393		0.116	
CC			187	47.0	196	49.9	0.434	1.118, 0.846-1.478
AC			157	39.4	162	41.2	0.602	1.079, 0.812-1.433
AA			54	13.6	35	8.9	0.504	1.146, 0.769-1.708
Per A allele			265	33.3	232	29.2	0.116	0.839, 0.678-1.038
rs8070231/63194945	Intron 13	0.331	400		394		0.114	
AA			189	47.3	192	48.7	0.725	1.051, 0.795-1.390
GA			157	39.3	167	42.4	0.377	1.137, 0.856-1.510
GG			54	13.5	35	8.9	0.504	1.146, 0.769-1.708
Per G allele			265	33.1	237	29.7	0.196	0.868, 0.703-1.073
rs2292592/63204209	Intron 16	0.270	400		392		0.384	
TT			220	55.0	222	56.6	0.763	1.044, 0.789-1.382
TC			144	36.0	145	37.0	0.819	1.034, 0.774-1.382
CC			36	9.0	25	6.4	0.484	1.153, 0.714-1.717
Per C allele			216	27.0	195	24.6	0.359	0.895, 0.715-1.121
rs9916525/63211437	Intron 17	0.295	400		393		0.200	
CC			208	52.0	193	49.1	0.363	0.879, 0.665-1.161
TC			148	37.0	167	42.5	0.131	1.246, 0.936-1.657
TT			44	11.0	33	8.4	0.228	0.746, 0.464-1.201
Per T allele			236	29.5	233	29.3	0.956	1.007, 0.812-1.249
rs1122079/63214503	Intron 17	0.248	400		393		0.264	
TT			234	58.5	218	55.5	0.325	0.868, 0.655-1.150
TA			134	33.5	151	38.4	0.155	1.235, 0.923-1.652
AA			32	8.0	24	6.1	0.304	0.750, 0.433-1.298
Per A allele			198	24.8	199	25.0	0.817	1.031, 0.821-1.294
rs4790953/63227030	3' near	0.248	400		392		0.105	
TT			236	59.0	213	54.3	0.134	0.806, 0.608-1.069
GT			130	32.5	154	39.3	0.052	1.335, 0.997-1.787
GG			34	8.5	25	6.4	0.257	0.733, 0.429-1.254
Per G allele			198	24.8	204	25.7	0.564	1.069, 0.853-1.341

[a]P value was calculated by 2 x 3 and 2 x 2 chi-squared tests based on codominant, dominant for the rare allele, heterosis and recessive for the rare allele models of inheritance. Alpha value is adjusted by Bonferroni correction and statistically significant results ($P < 0.05$).

Genotyping

Three to five milliliters peripheral blood was collected into tubes coated with EDTA. Genomic DNA was extracted from blood leukocytes using the EZNA™ Blood DNA Midi Kit (Omega Bio-Tek, Norcross, GA, USA), according to the manufacturer protocol. Schizophrenia cases and controls were mixed on the same plates, and a double-blind procedure was used. The DNA was stored at -80°C until SNP analysis. Genotyping was performed for all SNPs using the MassARRAY platform (Sequenom, San Diego, CA, USA). SNPs were genotyped using high throughput, matrix-assisted laser desorption ionization-time-of-flight mass spectrometry. Subsequently, the resulting spectra were processed using the Typer Analyzer software (Sequenom), and genotype data were generated from the samples. Because the final genotype call rate of each SNP was greater than 95% and the overall genotyping call rate was 98.6%, the reliability of additional statistical analysis was ensured.

Statistical analysis

Allele and genotype frequencies for each individual polymorphism and Hardy-Weinberg equilibrium were evaluated using the chi-squared test. The associations between the case-control status and each polymorphism were assessed using the Fisher exact test or Pearson chi-square test. Unconditional logistic regression was used to calculate the odds ratio and 95% confidence interval of the independent association between each locus and the presence of schizophrenia. The gender and age of the subjects were treated as covariants in binary logistic regression. P values were calculated based on the codominant, dominant for the rare allele, heterosis and recessive for the rare allele models of inheritance. Pairwise linkage disequilibrium statistics (D' and r^2) and haplotype frequencies were computed using Haploview 4.0 to construct haplotype blocks. All statistical analyses were performed using the SPSS 16.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

The distribution frequencies of 9 genotyped SNPs were consistent with Hardy-Weinberg equilibrium. The analysis of strong linkage disequilibrium in the schizophrenia patients and the healthy controls revealed that 2 SNPs (rs1530351, rs4791230), 4 SNPs (rs8077696, rs8070231, rs2869577, rs2292593) and 4 SNPs (rs2292592, rs9916525, rs1122079, rs4790953) were located in haplotype block 1, block 2, and block 3, respectively (D' > 0.9) (Figure 1). The genotype distribution, allelic frequencies, and haplotypes of schizophrenia patients and healthy controls are shown in Tables 1-4.

The difference in the distribution of genotype frequencies of rs4791230 (Promoter) between schizophrenia subjects and healthy controls was significant (P = 0.010). Schizophrenia subjects showed a significantly higher frequency of the G allele (P = 0.030, odds ratio = 1.589, 95% confidence interval = 1.042-2.422); however, this finding was insignificant after Bonferroni's correction.

The T-A (rs1530351-rs4791230) haplotype was observed significantly less frequently (P = 0.029) in subjects with schizophrenia. These differences retained statistical significance after Bonferroni's correction.

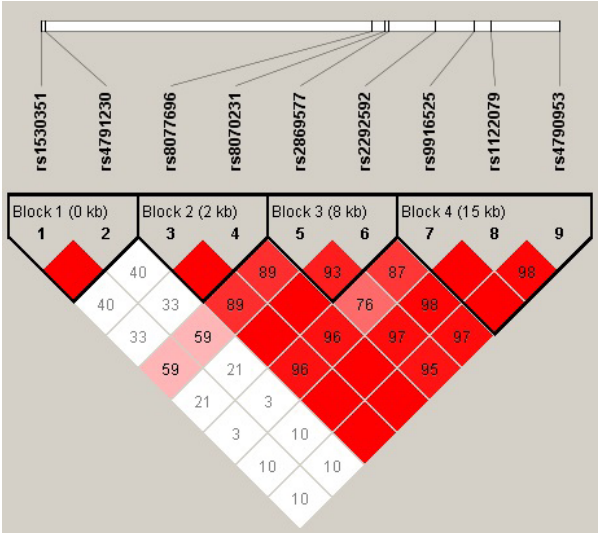

Figure 1. Linkage disequilibrium plot of the 9 SNPs in the RGS9 gene. Values in squares are the pair-wise calculation of r^2. Black squares indicate $r^2 = 1$ (i.e., perfect linkage disequilibrium between a pair of SNPs). Empty squares indicate D' = 1 (i.e., complete linkage disequilibrium between a pair of SNPs).

Table 2. RGS9 haplotype in block 1 frequencies and the results of their associations with risk of schizophrenia.

ID	Haplotype		Frequency (%)		P value[a]
	rs1530351	rs4791230	Cases	Controls	
HAP1	T	A	0.913	0.952	0.029
HAP3	C	G	0.082	0.048	0.052

[a]Based on comparison of frequency distribution of all haplotypes for the combination of SNPs. Alpha value is adjusted by Bonferroni's correction and statistically significant results (P < 0.025).

Table 3. RGS9 haplotype in block 2 frequencies and the results of their associations with risk of schizophrenia.

ID	Haplotype		Frequency (%)		P value[a]
	rs8077696	rs8070231	Cases	Controls	
HAP1	C	A	0.694	0.669	0.474
HAP3	A	G	0.293	0.331	0.269

[a]Based on comparison of frequency distribution of all haplotypes for the combination of SNPs. Alpha value is adjusted by Bonferroni's correction and statistically significant results (P < 0.025).

Table 4. RGS9 haplotype in block 3 frequencies and the results of their associations with risk of schizophrenia.

ID	Haplotype		Frequency (%)		P value[a]
	rs2869577	rs2292592	Cases	Controls	
HAP1	C	T	0.721	0.690	0.330
HAP2	G	C	0.236	0.257	0.471
HAP3	G	T	0.031	0.031	0.462
HAP4	C	C	0.012	0.012	0.984

[a]Based on comparison of frequency distribution of all haplotypes for the combination of SNPs. Alpha value is adjusted by Bonferroni's correction and statistically significant results (P < 0.0125).

DISCUSSION

The reduced levels of RGS9 expression in both the animal model of schizophrenia and the postmortem schizophrenia brain implicate RGS9 as a candidate gene in schizophrenia (Mirnics et al., 2001; Seeman et al., 2007). In addition, our results provide direct evidence that a genetic change in RGS9 is linked to schizophrenia in humans and extends the list of genetic variations in RGS9 that may affect schizophrenia development.

One of the key steps in linkage and association studies is to identify common risk variants in different populations. In a study by Okahisa et al. (2011), the subjects included 487 patients with schizophrenia and 464 age- and sex-matched healthy controls. We genotyped 2 nonsynonymous polymorphisms, rs12452285 (Leu225Ser) and rs34797451 (His498Arg), in the RGS9 gene. The 2 SNPs showed monomorphism in the present Han population. There were no significant differences in genotypic or allelic distributions of rs12452285 between the patients with schizophrenia and the corresponding controls. The results did not support the association of the RGS9 gene with schizophrenia in the Israeli Jewish population. In our study, there was a significant between-group difference in the genotype distribution of rs4791230. Subjects with schizophrenia showed a significantly higher frequency of the G allele of rs4791230. However, this association was not significantly different after Bonferroni's correction. Based on these observations, our data indicate that the difference in RGS9 rs4791230 genotype frequencies were not highly significant in this study. RGS9 was a single factor that likely had a modest effect on the risk of schizophrenia. This is the first study to identify a significant association between rs4791230 in the 5' regulatory region of the RGS9 gene and schizophrenia. Liou et al. (2009) found that an association was likely between the RGS9 gene and tardive dyskinesia using 7 SNPs, including rs8077696, rs8070231, rs2292593, rs2292592, rs9916525, rs1122079, and rs4790953. A weak association between RGS9 (rs4790953) and antipsychotic-induced tardive dyskinesia was reported in a sample in Taiwan. To some extent, this finding supports the role of RGS9 polymorphisms in schizophrenia.

In the haplotype analysis, a significant association between the T-A (rs1530351-rs4791230) haplotype of the RGS9 gene was found. The function of the T-A haplotype of the RGS9 gene is unknown; it may alter the binding efficiency of RGS9 to DRD2 or other genetic variations near any one of these SNPs and may play an important role in schizophrenia development. These results indicated that patients with T-A haplotypes of the RGS9 gene were less prone to schizophrenia, suggesting that they may show protective effects against schizophrenia. Additional studies are needed to explore the protective effect of the T-A haplotypes of the RGS9 gene on the risk for developing schizophrenia.

In a large and homogeneous sample, RGS9 gene polymorphisms (rs4791230) were associated with schizophrenia. These findings encourage future investigations into the functional polymorphisms within and close to the RGS9 gene using a systemic approach in large sample populations.

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