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Brief report

Evaluation of relationship between GRM3 polymorphisms and cognitive function in schizophrenia of Han Chinese

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ABSTRACT

Recently, the novel SNP rs12704290 in *GRM3* was identified in a genome-wide association study on schizophrenia susceptibility. Our study was to investigate the association of 29 selected SNPs (including rs12704290) with schizophrenia and to evaluate any possible relationship between them and cognition related to schizophrenia. The SNPs were analyzed in 1115 unrelated schizophrenic patients and 2289 healthy controls. The results showed significant associations between these SNPs and schizophrenia as well as with changes in cognition.

1. Introduction

Schizophrenia (SCZ) is a severe complex chronic psychiatric disorder with a heterogeneous clinical phenotype that affects approximately 1.1% of the population worldwide (Perala et al., 2007, Guan et al., 2012a). Although evidence from family, adoption, and twin studies suggests high heritability in the development of SCZ (80%), the etiology of SCZ remains unknown (Guan et al., 2013). Of the many glutamate receptors, the metabotropic glutamate receptor 3 (GRM3) has gained significant attention and been shown to be a promising candidate gene in SCZ. Recently, it has been reported that SNP rs12704290 in the *GRM3* gene was significantly associated with SCZ at genome-wide level in European subjects (Ripke et al., 2014), which was subsequently replicated by O'Brien et al. (2014) in another European population. Despite evidence of a strongly significant association in Europeans, the underlying biological mechanisms are largely unknown, and the genetic loci contributing to SCZ susceptibility have not been elucidated in other independent populations. Thus, the first aim of our study was to systematically explore the potential association between *GRM3* polymorphisms (including rs12704290) and SCZ in Han Chinese individuals. Additionally, there have been implications that the *GRM3* gene is involved with various domains of cognitive function (Roffman et al., 2006), but any associations between rs12704290 and cognitive dysfunction have yet to be evaluated. A previous study stated that perseverative error processing appears to be not only a marker of frontal lobe dysfunction in SCZ but also a vulnerability marker of SCZ (Baune et al., 2010). To better understand the interaction between the *GRM3* gene and perseverative error processing in pathological conditions, our secondary purpose was to evaluate the relationship between *GRM3* polymorphisms and perseverative error processing in SCZ patients.

2. Materials and methods

2.1. Subjects and genotyping

For this study, 1115 patients with schizophrenia (mean age = 36.5 ± 6.8 years; 48.0% male) and 2289 matched unrelated healthy controls (mean age = 36.4 ± 9.2 years; 48.4% male) were recruited. A diagnosis of SCZ was confirmed by at least two experienced psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for SCZ. All subjects are of Han descent, and all patients completed the Wisconsin Card Sorting Test (WCST) for cognitive assessment. The Ethics Committee of Xi'an Jiaotong University approved the study, and written informed consent was obtained from all subjects. Twenty-nine SNPs (rs187993, rs274618, rs274622, rs1468412, rs2282958, rs2282960, rs2299214, rs1989796, rs13242038, rs724226, rs2189814, rs917071, rs757656, rs2237562, rs6947784, rs6465084, rs2228595, rs12704290, rs1476455, rs17676277, rs2299225,

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rs2237558, rs6465088, rs1990040, rs2282966, rs7808623, rs7781414, rs17161070, rs148754219) were genotyped using the MassARRAY platform (Sequenom, San Diego, California, USA).

2.2. Statistical analysis

All Hardy–Weinberg equilibrium (HWE) calculations and association tests for each SNP between the case and control groups were conducted using PLINK v2.0. Haploview v4.2 software was utilized to investigate the linkage disequilibrium (LD) structure of the candidate markers. The haplotype frequency was calculated using GENECOUNTING v2.2 software with 10000 permutation tests. Given that a relationship was observed between body mass index (BMI) and cognition, we included BMI in our further analysis because it is likely to be correlated with weight and thus suggest colinearity. Multivariate analysis of covariance was performed to investigate the association between 28 SNPs and the WCST parameters in patients with regard to age, gender, BMI and duration of illness. A *p*-value of 0.05 was chosen as the threshold for significance in all analyses. In addition, we have calculated the statistical power using PGA v2.0 (Guan et al., 2014). Our sample size can detect SNP and haplotype associations with 92% and 83% power, respectively, at a false positive rate of 5%.

3. Results

There were no significant differences in the mean age, weight, height and BMI between the patient and control groups (Table S1). The SNP rs148754219 was excluded from the further analysis due to the lack of polymorphism in our cohort. The results of the allelic and genotypic association analysis of 28 SNPs are presented in Table 1 and Table S2. All SNPs were in HWE in the case and control subjects. Two SNPs (rs6465084 and rs12704290) were associated with SCZ ($p=0.02588$ and 0.000595, respectively). For the SNP rs12704290, the *P* value remained statistically significant after Bonferroni correction (corrected $p=0.01666$). Genotypic association analysis confirmed this result by a similar pattern, with a significant corrected *p* value of 0.041832.

The LD and haplotype analyses revealed 4 haplotype blocks (Fig. S1). Haplotypes in block 3 (rs6465084, rs2228595, and rs12704290) were significantly different between patients and healthy controls (global $p<0.001$, Table 1). HAP2, HAP3 and HAP4 were significantly associated with SCZ, and the frequency of HAP3 increased 2-fold in the SCZ group ($p<0.001$). Because of the higher frequencies observed in the control group, a protective effect might be associated with HAP2 (Table 1). We observed significantly more perseverative errors and fewer correct categories in the AA genotype of rs6465084, CC genotype of rs2228595 and GG genotype of rs12704290 compared to the non-AA genotypes, non-CC genotypes and non-GG genotypes, respectively. Furthermore, the significance of rs6465084 and rs2228595 increased with the greatest correlation associated with the SNP rs12704290 (Table 2). However, we observed no association between the other 25 SNPs and cognitive performance (Table S3).

4. Discussion

We compared our study with Ripke et al. (2014) and observed a significant association between rs12704290 and SCZ, with the G allele of rs12704290 as the risk allele. However, we also observed differences between these two studies. The OR of rs12704290 was 0.755 in our data compared with 0.904 in the report of Ripke et al. (2014), and the risk allele frequency was a slightly higher in Han Chinese individuals [0.896 in the patients from our study; 0.889 in the patients from the study of Ripke et al. (2014)]. Possible explanations for these discrepancies include differences in the ethnicity, genetic heterogeneity and sample size. Indeed, we cannot draw a conclusion simply from the analysis of a small selection of SNPs (Guan et al., 2012b). The results of our haplotype analysis indicated a significant association signal with SCZ. Thus, the consistency between two studies of different populations (Han Chinese and European) provides strong evidence that the SNP rs12704290 may be involved in SCZ susceptibility. A previous study has reported an exon skipping event in the splicing of GRM3 transcripts (Sartorius et al., 2006), and a synonymous exon3 SNP (rs2228595) in the *GRM3* gene was demonstrated to increase expression of the GRM3Δ4 splice variant (Sartorius et al., 2008). However, they emphasized that the SNP might influence splicing in a certain unpredictable manner or could be in LD with other undiscovered SNPs involved with the splicing machinery, conferring the risk for SCZ. Given that the notable association between GRM3 and SCZ exists in the SNP of rs12704290 located in a significant LD block with rs2228595, which was not associated with SCZ in our study, we could hypothesize that rs12704290 might play an important role in the pathogenesis of SCZ by impacting the splicing and expression of GRM3. Because of the lack of biological

Table 1
Allele, genotype and haplotype frequency of association analysis.

SNP markers	Allele freq. (%)		*p*-value (Chi-square)[a]	Genotype freq. (%)			*p*-value (Chi-square)[a]	H-WE	OR[b] 95%CI
rs6465084	A	G		AA	AG	GG			
SCZ	1966(88.16)	264(11.84)	***0.025880***(4.97)	866(77.67)	234(20.99)	15(1.35)	0.082921(4.98)	0.857	1.19
CTR	3947(86.22)	631(13.78)	0.72464	1701(74.31)	545(23.81)	43(1.88)		0.932	1.02–1.39
rs12704290	G	A		GG	GA	AA			
SCZ	1998(89.6)	232(10.4)	***0.000595*** (11.81)	891(79.91)	216(19.37)	8(0.72)	***0.001494*** (1.30)	0.191	1.32
CTR	3968(86.68)	610(13.32)	***0.01666***	1721(75.19)	526(22.98)	42(1.83)	***0.041832***	0.806	1.13–1.55

Haplotype frequency of association analysis

Haplotype	rs6465084-rs2228595-rs12704290	Genecounting (frequency %)			
		SCZ	CTR	*p*-value (*z*-value)[c]	Global p (Chi-square)[d]
HAP1	ACG	82.1	83.6	0.142 (−1.53)	< ***0.001*** (120.22)
HAP2	GTA	6.32	11.1	< ***0.001***(−6.33)	
HAP3	GTG	4.95	2.42	< ***0.001***(5.54)	
HAP4	ATA	3.51	1.89	< ***0.001***(4.08)	

SCZ: schizophrenia; CTR: control; CI: confidence interval; OR: odds ratio

[a] Significant *p* values are in italic bold, and corrected *p* values are underlined after Bonferroni correction. Chi-square values were represented within the parenthesis.
[b] OR refers to risk allele odds ratio in cases and controls. Haplotypes are not shown if frequency less than 1.5%.
[c] Based on 10,000 permutations; *z*-value given by Genecounting and represented within the parenthesis.
[d] Based on comparison of frequency distribution of all haplotypes for the combination of SNPs; Chi-square values were represented within the parenthesis.