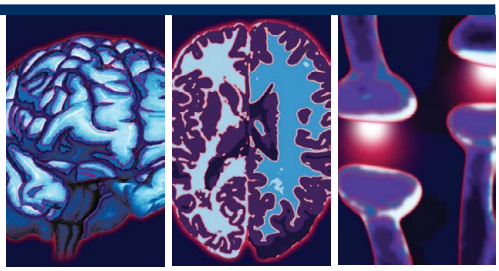

Association study of suppressor with morphogenetic effect on genitalia protein 6 (SMG6) polymorphisms and schizophrenia symptoms in the Han Chinese population

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ABSTRACT

Aim

Schizophrenia (SZ) is a complex psychiatric disorder that has a genetic component. Suppressor with morphogenetic effect on genitalia protein 6 (*SMG6*) encodes a protein that is involved in the nonsense-mediated mRNA decay pathway; the gene is located in the lissencephaly critical region of chromosome 17p13.3 that is linked to neuronal migration, the disturbance of which has been implicated in SZ pathogenesis. Several studies have found that *SMG6* is associated with SZ. The present case–control study sought to identify *SMG6* gene polymorphisms that may confer susceptibility to SZ in a Han Chinese population.

Methods

Paranoid SZ patients and control subjects (n=528 each) were genotyped for three single nucleotide polymorphisms. Genotype was determined by real-time polymerase chain reaction. SZ symptoms were evaluated by the Positive and Negative Symptom Scale.

Results

Significant associations were observed in genotypes between SZ and controls at rs1885986 (χ^2 = 8.89; P=0.011), in alleles in females at rs1885986 (χ^2 = 4.59; P = 0.032), and in genotypes between males and females at rs1885986 (χ^2=6.55 and 32.92, P=0.037 and 7.47×10^{-8}, respectively). In addition, rs216193 was significantly associated with total Positive and Negative Syndrome Score and positive symptom subscore in SZ (F=9.79 and 9.69, P=0.000 and 0.000, respectively).

Conclusions

These results provide evidence for an association between *SMG6* polymorphisms and SZ susceptibility and symptoms in the Han Chinese population.

Keywords

SMG6, Polymorphism, PANSS, Schizophrenia

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Introduction

Schizophrenia (SZ) is a common and complex psychiatric disorder that has a significant genetic component, affecting 1.0% of the global population [1], with heritability estimates ranging from 60%–80% [2–4]. Research into the genetic basis of SZ has mostly focused on identifying linkage regions, candidate genes, and polymorphisms; some studies have identified susceptibility genes that contribute to SZ, including two recent genome-wide association studies of a Chinese population [5,6], and one in which 108 SZ associated genetic loci were identified by examining 36,989 cases and 113,075 controls [7]. However, others have reported that multiple genes contribute to the pathogenesis of SZ, with each one contributing only weakly or moderately to predisposition [8]. Determining the major susceptibility genes among numerous candidates is an outstanding challenge.

Molecules involved in neuronal migration or positioning or synaptic connectivity during embryonic development and adulthood (e.g., neuregulin, reelin, disrupted in schizophrenia 1) have been linked to SZ, bipolar disorder, and epilepsy [9,10]. Suppressor with morphogenetic effect on genitalia protein *6* (*SMG6*) encodes a protein that participates in nonsense-mediated mRNA decay [11]; it is located in the lissencephaly critical region of chromosome 17p13.3, which is implicated in neuronal migration. Several studies have found an association between structural variants (deletions and insertions) of *SMG6* and SZ or a bipolar phenotype in a Spanish population [12,13], and *SMG6* was identified as a candidate gene for autism by sequencing balanced chromosomal abnormalities in patients with autism or related neurodevelopmental disorders [14].

Here we carried out a case–control study to investigate the association between *SMG6* single nucleotide polymorphisms (SNPs) and SZ in a Han Chinese population.

Materials

■ Subjects

This present study was carried out at the inpatient ward of the Second Affiliated Hospital of Xinxiang Medical University from March 2010 to December 2014. The study population consisted of 528 SZ patients (264 male and 264 female; mean age: 27.32±8.03 years) and 528 healthy controls (264 male and 264 female; mean age: 27.73±8.01 years). A diagnosis of SZ was made by at least two experienced psychiatrists according to Diagnostic and Statistical Manual of Mental Disorders, Edition IV (DSM-IV) criteria. Individuals with a history of other major psychiatric disorders, severe medical complications, organic brain disease, or substance dependence were excluded. In order to eliminate heterogeneity associated with the different clinical subtypes, only paranoid SZ patients were selected.

The age at first manifestation of positive symptoms was considered as the age of SZ onset [15] and was determined based on Comprehensive Assessment of Symptoms and History (CASH) [16]. A family mental health history (FH) was defined as at least one first- or second-degree relative of the proband who met the DSM-IV criteria for SZ or schizoaffective disorder, and was determined by questioning the patient or a family member during the initial interview. At the beginning of this study, evaluations were carried out by trained psychiatrists experienced in the administration of psychopathological tests such as the Positive and Negative Symptom Scale (PANSS) and CASH. In order to ensure inter-rater consistency, all participating psychiatrists underwent training every 6 months, at which time diagnoses and test results were compared based on videotaped interviews. Agreement among the raters was high for DSM-IV, CASH, and PANSS, with kappa values ranging from 0.762 to 0.843 and intraclass correlation coefficients (i.e., r values) range from 0.827 to 0.933.

Psychotic syndromes were evaluated using the PANSS [17] in 229 of the 528 SZ patients (116 male and 113 female, mean age: 27.53±6.01 years) who were not taking antipsychotic medications. Raters were trained to administer the PANSS using the Structured Clinical Interview for the PANSS and achieved an inter-rater reliability of 0.80 or greater. Five factors were rated for the PANSS [18]: positive symptoms (items P1, P3, P6 and P5), negative symptoms (N1–4, N6, G7 and G16), expression/anxiety (G2–4 and G6), cognition (P2, N5, G9, G10, G11, G13 and G15), and excitement/hostility (P4, P7, G8, G12 and G14).

Healthy controls were volunteers who were also screened by psychiatrists during non-structured interviews. Any individual with a personal or FH of mental health or neurological diseases

was excluded. Healthy controls were matched to patients in terms of gender ratio (1:1 for both groups) and age (F=0.699, P=0.403). All participants in this study were unrelated Han Chinese who were born and living in the North Henan province. Their biological grandparents were of Han Chinese ancestry.

■ SNP selection

We selected *SMG6* SNPs for study according to the following criteria. (1) All SNPs covering the genomic region chr17: 2149693–2150203 were included in functional analyses that were carried out using the FASTSNP online service (http://fastsnp.ibms.sinica.edu.tw) [19]. Only SNPs with highly ranked risk and a minor allele frequency (MAF)≥0.05 in the Beijing Chinese population according to the HapMap database were selected. (2) Tag SNPs were chosen based on the aggressive tagging algorithm (r^2≥0.80, MAF≥0.05) using genotype data from the HapMap database as implemented in Haploview v.4.1 (*http://www. broad.mit.edu/mpg/haploview/*) [20].

■ Genotyping

Peripheral blood samples from each subject were collected into Vacutainer tubes (BD Biosciences, Franklin Lakes, NJ, USA) containing the anticoagulant ethylenediaminetetraacetic acid. Genomic DNA was extracted from leukocytes using the RelaxGene Blood DNA System (Tiangen Biotech, Beijing, China). Genotype was determined by real-time polymerase chain reaction (PCR) (LC480; Roche, Basel, Switzerland). The PCR reaction volume was 5 µl and contained 1 µl of 20 ng/µl DNA template, 2.5 µl 2× Taqman Genotyping Master Mix, 0.125 µl of 40× Custom Taqman SNP Genotyping Assay, and 1.375 µl DNase-free H_2O. The reaction conditions were as follows: 95°C for 10 min, followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. For quality control, three SNPs were genotyped in duplicate (90 samples) by DNA sequencing.

Statistical analysis

Genotype and allele frequencies were compared using Haploview v.4.1 with Bonferroni corrections for multiple pairwise tests. Hardy-Weinberg equilibrium (HWE) was also calculated using this software. Single-SNP analyses for individual genotyping data were carried out using Pearson's χ^2 tests on allele and genotype counts. Odds ratios and 95% confidence intervals were calculated to evaluate the effect of different alleles and haplotypes. HWE was assessed by χ^2 tests with one degree of freedom. To evaluate interactions between genes and sex, a global test for interaction was performed. P<0.05 was considered statistically significant for tests in HWE. Power analyses were performed using the Genetic Power Calculator [21].

Genotype differences between SZ patients and healthy controls were compared with the χ^2 test (one degree of freedom). Associations between age-at-onset subgroups and different genotype carriers were tested by one-way analysis of variance (ANOVA) using SPSS v.13.0 (SPSS, Inc. Chicago, IL, USA). Differences between *SMG6* genotypes in the SZ group and scores of five factors from the PANSS were evaluated by ANOVA with age and gender as covariables. Fisher's least significant difference test was used for pairwise comparisons of the three genotypes after one-way ANOVA. Type I errors were excluded by Bonferroni corrections for multiple tests.

Results

To identify allelic variants of the *SMG6* gene that are associated with SZ, we analyzed the allele and genotype frequencies of three common SNPs in 528 SZ patients and 528 control subjects of Han Chinese descent. The sampling success rate for all subjects and SNPs was 99.94%. Power analyses revealed that the total sample size (n=1056) had a power of 0.86 to detect a small effect (r=0.1–0.23), and a power of 1.00 to detect both medium (r=0.24–0.36) and large (r>0.37) effects on genotype distributions. For example, for the SNP rs216193, we set the parameters as follows: high risk allele frequency (A)=0.25, prevalence=0.1 (prevalence of SZ), genotype relative risk Aa=1.2, genotype relative risk AA=3, number of cases=528. For a power above 80%, 229 cases are needed according to Genetic Power Calculator, so for genotype analyses, the sample size (n=528) had sufficient power (0.70–0.80) to detect effects. For allele frequency, the sample size (n=2112) had the power (0.91–1.00) to detect small, medium, and large effects. Evaluation of population structure using 10,000 iterations for the burn-in period and 10,000 repeats after burn-in revealed no evidence of population stratification in the control group (K=1, P=1).\

None of the genotype distributions of these three SNPs deviated from HWE. A significant association was found in genotypes between

SZ and controls at rs1885986 (P=0.011). After applying the Bonferroni correction, rs1885986 still showed a significant genotype association with SZ (P=0.035). However, there was no association in alleles between SZ and controls at rs1885986 (P=0.067), nor in genotypes and alleles between SZ and controls at rs216196 and rs216193 (P=0.842, 0.757; 0.960, and 0.920, respectively; **Table 1**). In addition, significant differences were detected in the genotypes and alleles of female SZ patients and controls at rs1885986 when the two groups were stratified by gender ($P=7.47 \times 10^{-8}$ and 0.032, respectively; **Table 2**). Significant differences were also found in the genotypes of male SZ patients and controls at rs1885986 (P=0.037; **Table 2**) and in the genotypes of rs1885986 between FH(+) and FH(–) in SZ (P = 0.011; **Table 3**).

To assess the haplotype structure of the subjects, we evaluated pairwise linkage disequilibrium of three SNPs in case and control groups using standardized D' and r2 values; however, haplotypes were not formed. To investigate the association between *SMG6* variants and symptoms, 228 first-onset SZ patients with complete PANSS scores were examined. As shown in **Table 4**, rs216193 genotypes were associated with total PANSS and positive factor scores; other SNPs did not exhibit these associations. There were significantly associated between rs216193 and total PANSS and five factor scores while age at onset and illness duration as covariables (P=0.000, data not shown).

Discussion

This study investigated *SMG6* mutations associated with SZ and psychotic symptoms in the Han Chinese population. Significant differences were detected in genotype frequencies of rs1885986 between SZ patients and controls that were present even after stratification by gender and FH and after Bonferroni correction.

Previous positional studies have provided evidence for one or more loci in the 17p11–13 region that influence susceptibility to SZ and bipolar disorder [22,23]. *SMG6*, which is located in this region, is associated with neuronal migration and implicated in SZ. A recent study identified *SMG6* as an autism candidate gene [14]. However, these findings have been controversial [24]. Several studies have reported that structural variants (i.e., additions, deletions, or copy number variants) are associated with SZ and bipolar disorder [12,25]; a similar association has been found between structural variants in the D17S22 marker of *SMG6* and SZ and bipolar phenotypes in a Spanish population, which was linked to deficits in executive control by the prefrontal cortex [12,13].

SZ is a highly heterogeneous disorder at both genetic and symptomatic levels. Studies of susceptibility genes are influenced by sample size, ethnicity, and clinical subtypes. To negate the impact of these factors, we adopted the following measures. Firstly, we selected only paranoid SZ patients and used a large sample size (528 patients vs. 528 controls), thereby improving the power to detect disease associations. Secondly, all of our subjects were Han Chinese living in the North Henan province of China who belonged to the same population group based on structure analyses.

SZ is characterized by domains of symptoms (positive, negative, disorganization of thoughts and behaviors, and cognitive deficits) [26,27] that reflect the psychotic phenotype. Several of the susceptibility loci and genes identified by positional studies of this phenotype may modulate neuronal migration and connectivity and maintenance of neural microcircuitry [28]. We therefore investigated this feature in SZ patients based on five PANSS factors. We found that rs216193 was associated with symptoms such as positive factors and total PANSS subscores in SZ patients, though not with SZ. These results suggest that variations in *SMG6*,

Table 1: Genotype and allele frequencies of three SNPs in the SMG6 gene in SZ patients and controls.

dbSNP ID	Allele (D/d)[a]	Patients						Controls						P-value	
		n[b]	HWE (P)	Genotype			MAF	n[b]	HWE (P)	Genotype			MAF		
				DD	Dd	dd				DD	Dd	dd		Genotype	Allele
rs1885986	C/G	528	0.016	368	136	24	0.174	528	0.000	358	122	48	0.206	0.011	0.067
rs216196	G/A	528	0.370	187	246	95	0.413	526	0.837	178	254	94	0.420	0.842	0.757
rs216193	G/A	528	0.521	302	191	35	0.247	528	0.816	298	196	34	0.250	0.960	0.920

[a]Major/minor alleles are denoted by D and d, respectively

[b]Number of samples that were well genotyped

HWE: Hardy-Weinberg Equilibrium; MAF: Minor Allele Frequency, SNP: Single Nucleotide Polymorphism

Table 2: Genotype and allele frequencies of association SNPs from the χ^2 test in female and male subjects.

Gender	dbSNP ID	Genotype			HWE	P value	Allele		P-value
Male	rs1885986	CC	CG	GG		0.037	C	G	0.629
	Patients	176	79	9	0.970		431	97	
	Controls	190	57	17	7.85e−005		437	91	
	rs216196	GG	GA	AA		0.663	G	A	0.359
	Patients	87	125	50	0.669		299	225	
	Controls	96	124	44	0.712		316	212	
	rs216193	GG	GA	AA		0.149	G	A	0.886
	Patients	145	105	14	0.368		395	133	
	Controls	155	87	22	0.057		397	131	
Female	rs1885986	CC	CG	GG		7.07×10^{-8}	C	G	0.032
	Patients	182	43	39	2.11e−018		407	121	
	Controls	178	79	7	0.613		435	93	
	rs216196	GG	GA	AA		0.700	G	A	0.662
	Patients	91	129	44	0.880		311	217	
	Controls	91	122	51	0.379		304	224	
	rs216193	GG	GA	AA		0.290	G	A	0.943
	Patients	153	91	20	0.216		397	131	
	Controls	147	104	13	0.319		398	130	

HWE: Hardy-Weinberg Equilibrium, SNP: Single Nucleotide Polymorphism.

Table 3: Genotype frequencies of association SNPs from the χ^2 test in FH(+) and FH(−) subjects.

SNP	Genotype						χ^2	P-value
	FH(−)			FH(+)				
rs216196	AA	AG	GG	AA	AG	GG	5.890	0.117
	77	216	137	17	38	41		
rs1885986	CC	CG	GG	CC	CG	GG	3.187	0.203
	298	98	35	60	24	13		
rs216193	AA	AG	GG	AA	AG	GG	9.078	0.011
	32	168	231	2	28	67		

FH: Family Mental Health History, SNP: Single Nucleotide Polymorphism.

Table 4: Association analysis between five PANSS factors and three SNPs of SMG6 in patients with SZ.

SNP	Genotype	Total PANSS	Positive	Negative	Depression/anxiety	Cognition	Excitement/hostility
rs216196	AA	89.31 ± 24.18	14.90 ± 3.85	24.02 ± 7.95	15.39 ± 5.51	14.04 ± 6.76	12.46 ± 4.70
	AG	93.34 ± 21.68	15.32 ± 3.38	26.42 ± 8.27	15.20 ± 5.26	14.98 ± 5.13	12.84 ± 4.99
	GG	90.99 ± 20.59	14.56 ± 2.84	26.14 ± 9.08	15.00 ± 4.58	14.98 ± 5.91	12.36 ± 4.34
rs1885986	CC	94.21 ± 21.71	15.05 ± 3.37	26.64 ± 8.61	15.59 ± 5.09	15.57 ± 5.87	12.82 ± 4.78
	CG	86.73 ± 21.40	15.16 ± 3.23	24.18 ± 8.60	14.33 ± 4.60	13.19 ± 4.93	12.20 ± 4.75
	GG	84.44 ± 19.79	13.75 ± 2.60	24.11 ± 6.58	13.59 ± 5.72	12.54 ± 4.82	11.85 ± 3.96
rs216193	AA	107.10 ± 7.99*	16.11 ± 4.97*	28.04 ± 8.23	19.24 ± 6.03	19.62 ± 7.60	14.68 ± 5.23
	AG	92.02 ± 20.12	15.26 ± 3.29	25.74 ± 8.24	15.10 ± 4.90	14.49 ± 4.98	12.67 ± 4.88
	GG	89.98 ± 21.45	14.67 ± 3.03	25.79 ± 8.76	14.75 ± 4.88	14.50 ± 5.71	12.33 ± 4.50

*P<0.05 vs. other genotypes (least significant difference test).

age at onset and illness duration influence the manifestation of SZ symptoms.

This study had some limitations. First, few SNP sites were selected and haplotypes failed to form. Secondly, there was no independent validation of the results. Additional studies are required to improve these aspects of our research.

Conclusion

In summary, this study provides evidence for an association between *SMG6* and SZ susceptibility and symptoms. Additional studies are needed to validate these findings in different populations and explore the role of *SMG6* in the manifestation of psychotic symptoms.

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Authors' contributions

LL and WL designed the study. HY, YY, GY and HZ collected the samples and participants' characteristics. YY, WL and XS analyzed and discussed the experimental results. HY, YY, WL and LL wrote the paper. All authors have read and approved the final manuscript.

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