Common Variants in Major Histocompatibility Complex Region and *TCF4* Gene Are Significantly Associated with Schizophrenia in Han Chinese

Tao Li, Zhiqiang Li, Peng Chen, Qian Zhao, Ti Wang, Ke Huang, JunYan Li, You Li, Jie Liu, Zhen Zeng, GuoYin Feng, Lin He, and YongYong Shi

Background: Schizophrenia is a complex major psychiatric disorder affecting ~1% of the world population. Recently, in a genome-wide association study and a follow-up in Caucasians, Stefansson *et al.* examined 7662 schizophrenic cases and 29053 normal control subjects and reported seven common single nucleotide polymorphisms (SNPs) that were significantly ($>10^{-8}$) associated with schizophrenia.

Methods: To investigate whether these risk SNPs were significantly associated in Han Chinese, we analyzed the seven SNPs in 2496 schizophrenia patients and 5184 normal control subjects. Because only three of the seven SNPs were polymorphic in Han Chinese, we genotyped two additional common SNPs from the same risk regions.

Results: Three SNPs, rs6932590 ($p = .00096$), rs3131296 ($p = 1.29 \times 10^{-6}$), and rs3130375 ($p = 1.76 \times 10^{-5}$), mapping to the major histocompatibility complex region and one SNP rs2958182 ($p = 3.64 \times 10^{-6}$) located in the *TCF4* gene were significant in our sample set. A meta-analysis using published genome-wide association study results also supported our findings.

Conclusions: Our results confirm that common risk factors in the major histocompatibility complex region and *TCF4* gene are associated with schizophrenia in Han Chinese, but our results fail to show an association with SNP rs12807809 in the *NRGN* gene.

Key Words: Common variants, Han Chinese, MHC region, *NRGN*, schizophrenia, *TCF4*

Genome-wide association studies (GWAS) accessing thousands of DNA samples from cases and control subjects is a powerful tool for identifying common risk factors for complex diseases. However, even apparently highly significant positive findings require replication, preferably in several sample sets from both the same and different ethnic groups. In 2009, Stefansson *et al.* (1) described a GWAS in schizophrenia. They examined DNA from 7662 schizophrenic cases and 29,053 normal control subjects recruited from eight European locations. They identified seven common risk single nucleotide polymorphisms (SNPs) showing genome-wide significance after correction for multiple testing (rs6913660, rs13219354, rs6932590, rs13211507, rs3131296, rs12807809, and rs9960767, respectively) (1). They then carried out a combined analysis using schizophrenia and control genotypes from the International Schizophrenia Consortium (ISC) and the Molecular Genetics of Schizophrenia GWAS studies (1–3). A further SNP, rs3130375, also in the major histocompatibility complex (MHC) region, was reported to be highly associated with schizophrenia in Caucasians by the ISC study alone (2). Therefore, in light of these findings, we decided to genotype these eight highly significant SNPs in Han Chinese. We examined DNA from 2496 schizophrenia patients and 5184 normal control subjects drawn from Han Chinese. Unfortunately, on genotyping, we found that four of the eight SNPs, rs6913660, rs13219354, rs13211507, and rs9960767, were not polymorphic in our Chinese samples. Because rs9960767 was the only SNP in the *TCF4* gene reported as significant by Stefansson *et al.* (1), we selected rs2958182, an SNP nearby to rs9960767 on the HapMap Han Chinese in Beijing (HCB) samples provided by the International HapMap Consortium that was polymorphic in our samples. It is the nearest common SNP to rs9960767 in HapMap HCB samples.

From the Bio-X Center and Affiliated Changning Mental Health Center, Bio-X Center (TL, ZL, PC, QZ, TW, KH, JYL, YL, JL, ZZ, LH, YYS), Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University; Institute for Nutritional Sciences (LH), Shanghai Institute of Biological Sciences, Chinese Academy of Sciences; Shanghai Institute of Mental Health (GYF); and Institute of Biomedical Sciences (LH), Fudan University, Shanghai, People's Republic of China.

Authors TL and ZL contributed equally to this work.

Address correspondence to YongYong Shi, Ph.D., Shanghai Jiao Tong University, Bio-X Center, 1954 Huashan Road, Shanghai 200030, People's Republic of China; E-mail: shiyongyong@gmail.com.

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Methods and Materials

We examined DNA from 2496 schizophrenia cases (1376 male and 1120 female cases, mean onset age 25.6 ± 8.1 years) and from 5184 control subjects (2685 male and 2499 female control subjects, mean age 56.2 ± 12.8 years). All subjects were volunteers and longstanding residents of Shanghai. Schizophrenia patients were given a standardized interview and diagnosed independently by two psychiatrists as meeting DSM-IV criteria for schizophrenia. All control subjects were randomly selected from the local resident population of Shanghai, having been screened for the absence of major mental illness. All participants gave written informed consent after the nature of the study had been fully explained. The study was reviewed and approved by the local Ethical Committee of Human Genetics Resources.

We genotyped in our laboratory nine markers, rs6913660, rs13219354, rs6932590, rs13211507, rs3131296, rs12807809, rs9960767, rs3130375, and rs2958182, in 7680 Han Chinese subjects by the polymerase chain reaction-ligase detection reaction method (4,5) (Supplement 1). Technical support was provided by the Shanghai Biowing Applied Biotechnology Company. Four SNPs, rs6913660, rs13219354, rs13211507, and rs9960767, showed no polymorphic variation in our samples; this was consistent with the HapMap HCB data. The average genotype call rate for all markers was 97.2%.

We carried out the case-control study and Hardy-Weinberg equilibrium test using the SHEsis software platform (http://analysis.

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bio-x.cn) (6,7). In our meta-analysis, we used the Mantel-Haenszel method with a fixed-effects model. The analysis was conducted by Comprehensive Meta-Analysis Version 2 (trial version; Biostat, Englewood, New Jersey) (8).

Results

Table 1 presents the results of the five polymorphic SNPs, rs6932590, rs3131296, rs12807809, rs3130375, and rs2958182. Four SNPs, rs6932590 [$p = .00096$, odds ratio of minor allele = .76, 95% confidence interval (CI) = (.65,.89)], rs3131296 [$p = 1.29 \times 10^{-6}$, odds ratio of minor allele = .68, 95% CI = (.58,.79)], rs3130375 [$p = 1.76 \times 10^{-5}$, odds ratio of minor allele = .69, 95% CI = (.59,.82)] in MHC region, and rs2958182 [$p = 3.64 \times 10^{-6}$, odds ratio of minor allele = .78, 95% CI = (.70,.86)] in *TCF4* were found to be significant in our Chinese samples. By contrast, rs12807809 was not significant [$p = .36$, odds ratio = 1.04, 95% CI = (.96,1.12)]. There was no deviation from Hardy-Weinberg equilibrium in the control subjects (Supplement 1).

We then carried out a meta-analysis of rs6932590, rs3131296, and rs3130375 (Table 1). Because the study by Stefansson *et al.* (1) reported the combined results of their own plus ISC and Molecular Genetics of Schizophrenia data, we also used these combined results. An exception was rs3130375, which was only documented in the ISC study. For meta-analysis of this locus, we only included ISC and our own data. From the meta-analysis, three SNPs showed increased significance (rs6932590 $p = 1.5 \times 10^{-14}$; rs3131296 $p = 1.5 \times 10^{-11}$; and rs3130375 $p = 2.1 \times 10^{-10}$).

Discussion

Our findings in this large Chinese schizophrenia case-control study broadly replicate the association findings in Caucasians for chromosome 6 and the *TCF4* locus but fail to confirm an association with SNP rs12807809 in the *NRGN* gene.

The SNP rs6932590 on chromosome 6 maps to the intergenic region between *PRSS16* and *POM121L2*. In both the Stefansson *et al.* (1) study and our own studies, the minor C allele is protective. The odds ratio was .76 in our data compared with .86 in the report by Stefansson *et al.* (1), and its frequency in cases was much lower in Han Chinese (.044 in our samples and .22 in the Stefansson *et al.* [1] samples).

The SNP rs3131296 is located in intron 18 of *NOTCH4*, which has been widely studied in schizophrenia but with conflicting results. The minor A allele is protective in both the Stefansson *et al.* (1) study and our own studies, and the odds ratio is .68 compared with .83 reported by Stefansson *et al* (1). Its frequency is lower in Han Chinese schizophrenia cases (.047) than in the cases reported by Stefansson *et al.* (1) (.13).

The SNP rs3130375 is located in the intergenic region between *TRIM39* and *HLA-E*. It was the most significantly associated with schizophrenia SNP in the MHC region reported in the ISC study (3). The minor A allele was protective in both the ISC and our own study, with odds ratios of .73 and .69, respectively.

The SNP rs2958182 maps to an intron region of *TCF4*, while rs9960767, which was not polymorphic in Han Chinese, is in the same intron. We searched for all SNPs with minor allele frequency > .05 between upstream 7.5 kilobase (kb) and downstream 7.5 kb (15 kb window) to rs9960767 in HapMap HCB database by Haploview (Broad Institute of MIT and Harvard, Cambridge, Massachusetts) (9), and we only found one SNP, rs2958182. The distance between rs2958182 and rs9960767 is ~ 6 kb pairs; linkage disequilibrium between the two markers in HapMap CEU samples (northern and western European ancestry living in Utah from the Centre d'Etude

Table 1. Association Studies and Meta-Analysis

Chr/Mb	SNP [Minor Allele]	Case MAF	This Study (2,496 Cases /5,184 Control Subjects)		Combined Results in Stefansson *et al.* (1) Study (12,945 Cases /34,591 Control Subjects)		Results Only Demonstrated by ISC Study (3,322 Cases/3,587 Controls)		Meta-Analysis of Existed Data		Region/Neighboring Gene
			OR (95% CI)	*p* Value	OR (95% CI)	*p* Value	OR	*p* Value	OR	*p* Value	
6/27.4	rs6932590[C]	.044	.76 (.65, .89)	.00096	.86 (.83, .90)	1.4×10^{-12}	—	—	.86 (.82, .89)	1.5×10^{-14}	MHC/*PRSS16*
6/32.3	rs3131296[A]	.047	.68 (.58, .79)	1.29×10^{-6}	.84 (.80, .88)	2.3×10^{-10}	—	—	.84 (.80, .89)	1.5×10^{-11}	MHC/*NOTCH4*
6/30.4	rs3130375 [A]	.038	.69 (.59, .82)	1.76×10^{-5}	—	—	.73	3.7×10^{-7}	.74 (.68, .82)	2.1×10^{-10}	MHC/–
11/124.1	rs12807809[C]	.275	1.04 (.96, 1.12)	.36	.87 (.83, .91)	2.4×10^{-9}	—	—	—	—	*NRGN*
18/51.3	rs2958182[A]	.103	.78 (.70, .86)	3.64×10^{-6}	—	—	—	—	—	—	*TCF4*

Table 1 shows the allelic OR (for minor allele) and *p* values (two-sided) and compared our results with Stefansson *et al.* (1).
Chr, chromosome; CI, confidence interval; ISC, International Schizophrenia Consortium; MAF, minor allele frequency; Mb, megabase; MHC, major histocompatibility complex; OR, odds ratio; SNP, single nucleotide polymorphism.

du Polymorphisme Humain [CEPH] collection) is 1. The SNP rs2958182 is significantly associated with schizophrenia in our Chinese samples ($p = 3.64 \times 10^{-6}$). This lends additional support to the positive association results for schizophrenia and the *TCF4* gene.

There are, however, limitations to the interpretation of our results. Firstly, there are considerable genetic differences between the Chinese and Caucasian populations. This is highlighted by the finding that while SNPs rs6913660, rs13219354, rs13211507, and rs9960767 were common polymorphic variants in the Caucasian sample sets, they showed no polymorphic variation in the Han Chinese sample sets. Also, the minor allele frequencies of SNPs rs6932590 and rs3131296 were much lower in our sample set compared with Stefansson *et al.* (1). The minor allele frequency of rs12807809 also differed between the two populations and also showed no association with schizophrenia in our study. Secondly, the MHC region is one of the most complex regions of the human genome and easily lends itself to population stratification. While this cannot be completely ruled out as an explanation of our positive association findings, this seems unlikely. Both our case and control samples were recruited from the same geographic area and we only included local term and not recently migrated residents; we might expect, therefore, that if there was any subtle population admixture it would be the same in cases and control subjects. We also compared our data with that from another Shanghai sample set recruited by the same criteria as this study; again, there was no obvious pattern suggesting population stratification in the MHC region (Supplement 1). Finally, the same alleles of the same SNPs in the MHC region as reported by Stefansson *et al.* (1) significantly replicated in our sample sets. By contrast, we found no association with rs12807809 in the *NRGN* gene. We did, however, obtain a strong positive signal with rs2958182, a marker close-by and in complete linkage disequilibrium with the original positive marker at the *TCF4* locus, rs9960767, which was not polymorphic in Han Chinese. All these interesting findings should therefore by considered preliminary; additional follow-up studies are required including high density mapping and deep sequencing in both Chinese and Caucasian populations to find possible causal variants.

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Supplementary material cited in this article is available online.

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