BIOMEDICAL AND ENVIRONMENTAL SCIENCES **18**, 31-35 (2005)

Searching for a Schizophrenia Susceptibility Gene in the 22q11 Region[1]

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Objective To investigate a genetic association for schizophrenia within chromosome 22q11 in a Chinese Han population. **Methods** The PCR-based restriction fragment length polymorphism (PCR-RFLP) analysis was used to detect three single nucleotide polymorphisms (SNPs), rs165655 (A/G base change) and rs165815 (C/T base change) present in the ARVCF (armadillo repeat gene deletion in velocardiofacial syndrome) locus, and rs756656 (A/C base change) in the LOC128979 (expressed sequence tags, EST) locus, among 100 Chinese family trios consisting of fathers, mothers and affected offspring with schizophrenia. Genotype data were analyzed by using linkage disequilibrium (LD) methods including haplotype relative risk (HRR) analysis, transmission disequilibrium test (TDT) and haplotype transmission analysis. **Results** The genotype frequency distributions of three SNPs were all in Hardy-Weinberg equilibrium (P>0.05). Both the HRR and the TDT analysis showed that rs165815 was associated with schizophrenia (χ^2=6.447, df=1, P=0.011 and χ^2=6.313, df=1, P=0.012, respectively), whereas the other two SNPs did not show any allelic association. The haplotype transmission analysis showed a biased transmission for the rs165655-rs165815 haplotype system (χ^2=17.224, df=3, P=0.0006) and for the rs756656-rs165655-rs165815 hapoltype system (χ^2=20.965, df=7, P=0.0038). **Conclusion** Either the ARVCF gene itself or a nearby locus may confer susceptibility to schizophrenia in a Chinese Han population.

Key words: Schizophrenia; 22q11; Single nucleotide polymorphisms (SNPs); ARVCF

INTRODUCTION

Schizophrenia is a serious mental illness affecting approximately 1% of the general population world-wide[1]. Linkage studies suggest that the long arm of chromosome 22 may bear a schizophrenia susceptibility gene[2-4]. Additional support was provided by the observation of a higher-than-expected frequency of 22q11 (chromosome 22 long arm region 1 band 1) microdeletions in patients with schizophrenia and the demonstration that 20%-30% of individuals with 22q11 microdeletions develop schizophrenia or schizoaffective disorders in adolescence and adulthood[5-7]. To address the possibility that one of individual genes located in the 22q11 microdeletion region may play a role in schizophrenia, the present study was designed to detect 3 single nucleotide polymorphisms (SNPs) present in the 22q11 region, rs165655 and rs165815 at the ARVCF (armadillo repeat gene deletion in velocardiofacial syndrome) locus and rs756656 at the LOC128979 (expressed sequence tags, EST) locus, among a Chinese population.

MATERIALS AND METHODS

Subjects

One hundred family trios of Chinese Han descent, consisting of fathers, mothers and affected offspring with schizophrenia, were recruited for the genetic analysis. The patients (65 men, 35 women) with a mean age of 26.8±6.7 years were admitted to a psychotic hospital in Jilin Province during the period between 2000 and 2002. The mean age of onset of the disease was 22.9±5.6 years and the course of the illness ranged from 3 to 276 months. They were diagnosed as having schizophrenia with the criteria of

[1]This work was supported by the National 863 program (grant No. 2004AA221070 and 2002BA711A07) and the grants from the National Natural Science Foundation of China (grant No. 30170343).

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0895-3988/2005
CN 11-2816/Q
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the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10) by at least two well-trained psychiatrists. Venous blood was taken from all the subjects after informed consent was given for the genetic analysis.

Genotyping of SNPs

We analyzed 3 SNPs present in a 379362 bp region containing the ARVCF gene and the LOC 128979 locus. These three SNPs were genotyped by PCR-based restriction fragment length polymorphism (PCR-RFLP) analysis[8]. The primers used for PCR amplification of a target DNA sequence, the predicted length of PCR products and the genetic information of polymorphic SNPs were given in Table 1.

TABLE 1

The Primers for PCR Amplification of SNPs

SNPs	Allele	Primers	Length of Products	RFLP
rs 756656	A/C	5'-AGAGACATTCCTCTCTGACTGG-3' 5'-CCTCAAGTAGCCATTTGTCCAGC-3'	276 bp	*Eco*RI
rs 165655	A/G	5'-CAGCATGGGCTGGAGAAAGG-3' 5'-GGGGCTGGGAAAGAACTTGG-3'	223 bp	*Hae*III
rs 165815	C/T	5'-TAGGCTGCTCTCCAGCAACC-3' 5'-TGTCTCCCAAAGCCCCTGTC-3'	256 bp	*Msp*I

Genomic DNA used for PCR amplification was extracted from the whole blood sample using a DNA extraction kit (Xingpu Ltd, Ningbo, China). The PCR amplification was performed at a 20 μL reaction volume 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 μmol/L of each dNTP, 0.4 μmol/L of each primer, 1.0 unit of Taq DNA polymerase (Promega, Beijing) and 20-40 ng of genomic DNA. The cycling conditions consisted of an initial denaturation of 94℃ for 5 min followed by 35-40 cycles of denaturation at 94℃ for 45 s, annealing at 58℃-60℃ for 1 min, and an elongation at 72℃ for 1 min. The cycling was followed by a final extension at 72℃ for 10 min. The reaction was performed in a thermal cycler TC-1 (PE, USA). The PCR products were electrophoresised on 2.5% agarose gels followed by ethidium bromide staining. Individual genotypes were then identified according to the fragment length of PCR products digested with the very restriction endonucleases (Promega, Beijing).

Statistical Analysis

The goodness-of-fit chi-square test was applied to test for Hardy-Weinberg equilibrium. Allelic association for a single SNP was analyzed by using family-based association tests including the haplotype relative risk (HRR) test and the transmission disequilibrium test (TDT)[9-11]. The TDT analysis was performed only with genotyping data from heterozygous parents. In such a family-based study, the allele transmitted by parents to affected offspring was used as a 'case' and that non-transmitted as a 'control' so as to form a genetically well-matched case-control sample. The transmissions of haplotypes consisting of 2 and 3 SNPs were performed by using the program Transmit v2.5 (David Clayton, MRC Biostatistics Unit, Cambridge, UK). The global χ^2 test for association on H-1 degree of freedom (df), where H is the number of haplotypes for which transmission data are available.

RESULTS

Hardy-Weinberg Equilibrium Test

The number of genotype and allele of SNPs was calculated by SPSS 10.0 software. The results were shown in Table 2. The goodness-of-fit test showed that the genotypic distributions of rs756656, rs165655 and rs165815 were all in Hardy-Weinberg equilibrium (P>0.05).

HRR Analysis

As shown in Table 3, the HRR analysis revealed an allelic association between rs165815 and schizophrenia (χ^2=6.447, df=1, P=0.011), whereas other two SNPs were not associated with schizophrenia.

TDT Analysis

As shown in Table 4, the TDT analysis also showed an association between rs165815 and schizophrenia (χ^2=6.313, df=1, P=0.012), whereas the other two SNPs did not show any association. The rs165815 is a C to T base change and heterozygous parents had excessively transmitted allele C to their affected offspring, suggesting that the haplotype containing rs165815(C) might carry a susceptibility allele for schizophrenia. The result was consistent

with that given by the HRR analysis.

Haplotype Transmission Analysis

The global χ^2 test for haplotype transmission analysis showed a strong association for the rs756656-rs165655-rs165815 haplotype system (χ^2=20.965, *df*=7, *P*=0.0038) and for the rs165655-rs165815 haplotype system (χ^2=17.224, *df*=3, *P*=0.0006), whereas the rest of haplotype systems showed no association with schizophrenia (Table 5).

TABLE 2

Genotype and Allele Frequencies of SNPs

Group		N	Genotype Numbers (%)			Allele Numbers (%)	
			1/1	1/2	2/2	1	2
rs756656	Parent	200	43(21.5)	100(50.0)	57(28.5)	186(46.5)	214(53.5)
	Patient	100	21(21.0)	53(53.0)	26(26.0)	95(47.5)	105(52.5)
rs165655	Parent	200	38(19.0)	101(50.5)	61(30.5)	177(44.3)	223(55.7)
	Patient	100	18(18.0)	54(54.0)	28(28.0)	90(45.0)	110(55.0)
rs165815	Parent	200	33(16.5)	99(49.5)	68(34.0)	165(41.3)	235(58.7)
	Patient	100	18(18.0)	59(59.0)	23(23.0)	95(47.5)	105(52.5)

Note. rs756656 1(A) 2(C), rs165655 1(G) 2(A), rs165815 1(C) 2(T); the numbers in the blank are the frequencies of genotype and allele. N: numbers.

TABLE 3

The HRR Analysis for a Genetic Association Between an SNP in the 22q11 Region and Schizophrenia

SNPs	Allele	HRR		χ^2	*df*	*P*
		Transmitted	Non-transmitted			
rs756656	A	95	91	0.161	1	0.688
	C	105	109			
rs165655	G	90	88	0.040	1	0.841
	A	110	112			
rs165815	C	95	70	6.447	1	0.011
	T	105	130			

TABLE 4

The TDT Analysis for a Genetic Association Between an SNP in the 22q11 Region and Schizophrenia

SNPs	Locus	Transmitted	χ^2	*df*	*P*
rs756656	LOC128979	A=52/C=48	0.160	1	0.689
rs165655	ARVCF	A=50/G=52	0.039	1	0.843
rs165815	ARVCF	C=62/T=37	6.313	1	0.012

TABLE 5

The Global Chi-square Test for Haplotype Transmission

Haplotypes	χ^2	*df*	*P*
rs756656-rs165655	1.3805	3	0.7101
rs756656-rs165815	6.3369	3	0.9634
rs165655-rs165815	17.2240	3	0.0006
rs756656-rs165655-rs165815	20.9650	7	0.0038

DISCUSSION

In previous studies, linkage signals were identified at D22S315 on 22q11-12 in eight Utah schizophrenic families[12]. Two dinucleotide repeats and six SNPs at the COMT locus were tested for transmission distortion in 198 schizophrenic Chinese family trios and an excess transmission of a five-marker haplotype was observed[13]. In recent years, a number of studies have described the relationship between velo-cardio-facial syndrome (VCFS) and schizophrenia[5-7]. VCFS is caused by microdeletions in the q11 band of chromosome 22. The high prevalence of psychiatric symptoms in patients with VCFS suggests that a locus on 22q11 may underlie mental disorders, such as schizophrenia. There are more than 30 known genes mapped to the VCFS region[14]. Of these known genes, 3 have been implicated to be associated with schizophrenia, including COMT (Catechol-O-Methyltransferase), UFD1L (Ubiquitin fusion degradation 1-like) and PRODH2 (Proline oxidase)[13,15-17], although inconsistent results have been reported. The present study was focusing on the region containing the COMT locus. We have totally genotyped rs165655 and rs165815 at the ARVCF locus and rs756656 at the LOC128979 locus in a Chinese population of Han descent. The three SNPs are all near to the COMT locus.

The goodness of fit test showed that the genotypic distributions of 3 SNPs were not deviated from the Hardy-Weinberg equilibrium, and thus this sample pool is suitable for the genetic analysis. The rs165815 showed an allelic association with schizophrenia in both the TDT and the HRR analysis although neither the HRR nor the TDT analysis showed allelic association of rs165655 and rs756656 with schizophrenia. The rs165815 is a missense SNP located in the ARVCF locus. Parents had excessively transmitted allele C to their affected offspring at rs165815. The haplotype transmission analysis showed that the rs165655-rs165815 haplotype system and the rs756656-rs165655-rs165815 haplotype system were associated with schizophrenia. The analysis for multi-SNPs haplotype transmission is essential to look for a specific haplotype or chromosome possibly carrying a variant for the disease. ARVCF is a member of the catenin family, which plays an important role in the formation of adherens junction complexes. It is thought to facilitate communications between the inside and outside environments of cells. The ARVCF gene contains a predicted nuclear-targeting sequence, suggesting that it may function as a nuclear protein[18].

Taken together, the present study shows a genetic association between the ARVCF gene and schizophrenia in a Chinese population. This finding implies that the ARVCF gene itself or a nearby locus may confer susceptibility to schizophrenia. Since the ARVCF gene is immediately telomeric of the COMT gene, the ARVCF association may represent linkage disequilibrium (LD) with a disease variant in the COMT gene. Further work remains needed to replicate the initial finding with a large sample size in order to clarify if the ARVCF/COMT locus contains a variant underlying susceptibility to schizophrenia.

ACKNOWLEDGEMENT

The authors wish to thank Dr. H. SANG for collecting the blood samples.

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(*Received December* 16, 2003 *Accepted June* 21, 2004)