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A family-based association study of kinesin heavy chain member 2 gene (*KIF2*) and schizophrenia

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Abstract

Schizophrenia is a multifactorial disease characterized by multiple genetic susceptibility elements. The human *KIF2* gene represents an orthologue of the murine *Kif2a*, which plays an important role in the transport of various membranous organelles and protein complexes on microtubules. To examine whether this gene is involved in schizophrenia etiology, we undertook studies of transmission disequilibrium in a cohort of affected family samples to test for association. Although, we failed to detect any positive results in single markers, a common two-SNP haplotype (rs2289883/rs464058, G/A) showed a significant association with the disease and a four-SNP haplotype (T/G/A/G) with a frequency of 23.4% was identified in parental chromosomes and showed a significant association with the disease ($P = 0.00795$). Our results demonstrate that the *KIF2* gene, located at 5q12.1, is a potential schizophrenia susceptibility gene.

Keywords: Schizophrenia; *KIF2*; Genetic polymorphisms; Association study; Chinese population

Schizophrenia (SCZD; MIM 181500) is a chronic, severe mental disorder that affects approximately 1% of the world's population. The disorder is characterized by psychotic symptoms and by cognitive, affective, and psychosocial impairment. Studies of family, twin, and adoption reveal that genetic factors play an important role in the etiological complex of schizophrenia. Although lots of evidence demonstrated high heritability of schizophrenia (~80%), the exact etiology and the genetic mechanism of the disorder are still unknown. Multiple studies have consistently demonstrated that the risk to relatives of a proband with schizophrenia is higher than that to relatives of controls [13–15,27].

In mammalian neurons, only two kinds of mechanochemical motor proteins (kinesin and brain dynein) transport various kinds of membranous components in neuronal axons. Kinesins, such as KIF2, are microtubule-associated motor proteins, which plays a significant role in the transport of various membranous organelles and protein complexes on microtubules [9]. The kinesin heavy chain member 2 gene (*KIF2*), the orthologue of *Kif2a*, maps on 5q12.1, and its expression products (KIF2) is a 716 amino acid middle-type member of kinesin superfamily (KIFs). KIF2 forms homodimers and acts as a plus-end-directed motor [18]. Northern blot analysis reveals that KIF2 is expressed in the brain as well as in other tissues [1]. However, its expression decreases significantly in mature neurons.

Weinberger et al. stated a hypothesis that schizophrenia is associated with structural deformities of the hippocampus [28]. Csernansky et al. provided additional support for the hypothesis using the high dimensional brain mapping method [5]. Homma et al. reported that *Kif2a* knockout mice showed many brain abnormalities, especially in the CA1 and CA3 fields of hippocampal pyramidal cells [10], which are associated with many mental disorders. Thus, on the basis that *KIF2* may be a potential candidate gene for schizophrenia, we studied the gene using 11 common SNPs (Fig. 1) in unrelated Chinese schizophrenia

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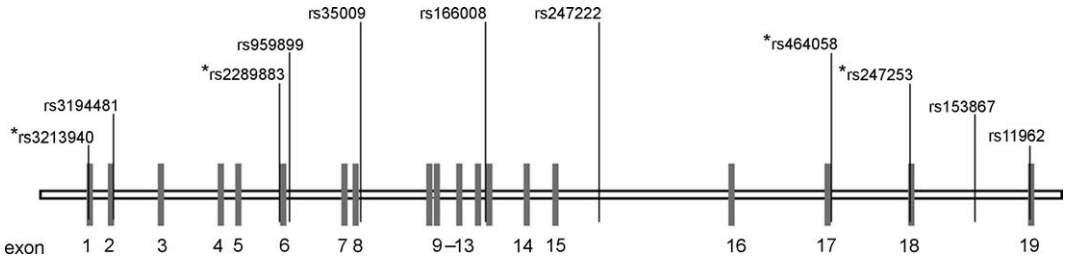

Fig. 1. Genomic structure of the human *KIF2* gene and locations of polymorphisms analyzed in the present study. Exons are denoted by gray rectangle boxes. Four SNPs (with '*') were genotyped in larger scale samples. Other SNPs revealed low minor allele frequencies (<0.3) in tests of the 24 trios.

trios. Four (rs3213490, rs2289883, rs464058 and rs247253) of the 11 SNPs, which exhibited high heterozygosity (minor allele frequencies >0.3), were selected for more detailed analysis. This analysis identified four SNP haplotypes (T/G/A/G), which had a frequency of 23.4% in parental chromosomes and had a significant association with schizophrenia ($P = 0.00795$).

In this study, 303 unrelated schizophrenia patients were screened. Of these, 23 were excluded because of the lack of complete genotypes and the remaining 280 patients (163 male and 117 female, with a mean age of 30.19 years, S.D. = 7.50) and their biological parents were recruited. The average onset age of all patients was 21.98 years (S.D. = 6.31). All subjects were of Han Chinese origin and from Shanghai. The probands were diagnosed strictly according to the criteria of the Diagnostic and Statistical Manual of Mental Disorder, Third Revised Edition (DSM-III-R; American Psychiatric Association, 1987). All the patients were inpatients and before they were include in the study they were interviewed by psychiatrists with the diagnoses being independently verified and checked by two senior psychiatrists who reviewed the patients' medical case notes. Written, informed consent was obtained from all the those tested. A standard informed consent to the protocol, which was reviewed and approved by the Shanghai Ethics Committee of Human Genetic Resources, was provided by the participating subjects after the nature of study had been fully explained.

SNPs used for genotyping were found from the NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP). We genotyped SNPs with SYBR Green I fluorescence in kinetic real-time PCR on an ABI7900 system (Applied Biosystem), in which primers were designed to specifically amplify the reference allele or its variant in separate PCR reactions. All primers were designed using the tetra-primer methods [29]. The standard PCR reactions of 5 μL were carried out using Taqman Universal PCR Master Mix reagent kits according to the supplier's guidelines. The assay combining kinetic (real-time quantitative) PCR with allele-specific amplification was performed as described by Germer et al. [7]. Detailed information of PCR amplification conditions is listed in supplementary table.

Hardy–Weinberg equilibrium (HWE) tests were performed for each polymorphism on an online calculator (http://www.kursus.kvl.dk/shares/vetgen/_Popgen/genetik/applets/kitest.htm). The mean age of each group was calculated using SPSS (version 12.0). The FBAT program, version 1.5.5, was used for transmission/disequilibrium test analysis of individual SNPs and haplotypes in families. The program estimates haplotype frequencies with the EM-algorithm and corrects for multiple testing [11,16,22]. The standardized measure of LD for each pair of markers, denoted as D', was estimated using software 2LD for TDT analysis [30]. All tests were two tailed and significance was accepted at $P < 0.05$. To confirm the results of the 2LD and the FBAT, the Haploview program, version 3.2, was used to perform the linkage disequilibrium and haplotype analysis [3]. The statistical power of our sample size was estimated using an online calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/) [21]. We used the option "TDT for discrete traits".

To determine whether alterations in this gene could contribute to schizophrenia susceptibility, we genotyped four SNPs in the *KIF2* gene region (Fig. 1) in the schizophrenia patients, and studied transmission disequilibrium to examine association of this gene with the disease in a large sample of affected families based on the orthologue of *Kif2a* between mice and humans [18].

We selected 11 common SNPs in the *KIF2* and genotyped them in the sample of 24 tested trios using the method described above. Among these SNPs, rs3194481 situated in exon 2 of the *KIF2* was the only one to result in a nonsynonymous change from threonine residue at position 56 to an alanine residue, but this locus exhibited no polymorphisms in the 24 tested trios. A fair assessment for significance of this mutation will require more detailed work.

Table 1
Frequencies of all markers and their individual analysis using the program FBAT

Markers	Distance	Type	Informative families (no.)	Minor allele frequencies	HW result	*Z* score	*P* value
rs3213940	−37	T/C	194	0.323	0.053	−0.250	0.803
rs2289883	7,869	G/A	217	0.375	0.914	1.037	0.300
rs464058	30,696	G/A	204	0.489	1.774	−0.600	0.549
rs247253	33,928	G/T	221	0.398	0.669	0.422	0.673

HW result, result of χ^2 test for deviation from Hardy–Weinberg equilibrium.

Based on the genotyping data of the 24 trios, we selected four SNPs with minor allele frequencies >0.3 from the 11 SNPs for genotyping, and analyzed the trios transmission of haplotypes composed of different combinations of the four SNPs. All the markers showed obvious differences in allele frequencies in our sample. Genotypes of the markers were in Hardy–Weinberg equilibrium in parents and had no Mendelian inheritance errors. Allele frequencies and single marker analysis are shown in Table 1. None of the four markers revealed transmission distortion in the trios. The results of pairwise linkage disequilibrium between SNPs are shown in Table 2. We then performed ordinal two-, three- and four-locus haplotype analysis. In the two-locus haplotypes analysis, only combination rs2289883–rs464058 showed association with schizophrenia with $P = 0.0363$. In multilocus haplotype analyses, combination rs3213940–rs2289883–rs464058, rs2289883–rs464058–rs247253 and rs3213940–rs2289883–rs464058–rs247253 showed significant association with the disease. In total, four haplotypes with frequencies >3% were observed to be significant (Table 3). They all share a common haplotype that extends from rs2289883 to rs464058. The haplotype, T/G/A/G, showed most significant difference ($P = 0.00795$). Using the Haploview program to perform haplotype analysis, we found the results consistent with FBAT (Table 3). Global P values form single-locus and multilocus association analysis using the FBAT program are shown in Table 4.

On the basis of the genotype data, the power of our sample was 0.94 at alpha = 0.05.

Table 2
Pairwise linkage disequilibrium between SNPs

D' coefficient	rs3213940	rs2289883	rs464058	rs247253
rs3213940		0.886	0.795	0.890
rs2289883	0.911		0.630	0.950
rs464058	0.649	0.479		0.657
rs247253	0.957	0.920	0.451	

Above the diagonal: results of Haploview analysis; below the diagonal: results of 2LD analysis.

Table 3
Estimated haplotype frequencies and association significance

Marker haplotype				Individual haplotype frequencies	FBAT[a]				Haploview P value
rs3213940	rs2289883	rs464058	rs247253		S[b]/E(S)[c]	Var(S)[d]	Z score	P value[e]	
2SNPs									
T	G			0.349	176.282/169.519	59.633	0.876	0.381	0.364
T	A			0.333	164.718/169.481	65.128	−0.590	0.555	0.521
C	A			0.298	144.282/148.019	60.719	−0.480	0.632	0.674
	A	G		0.355	175.383/174.662	67.364	0.088	0.930	0.898
	A	A		0.276	132.617/141.838	54.593	−1.248	0.212	0.205
	G	A		0.258	144.383/130.162	46.131	2.094	**0.0363**	**0.0404**
	G	G		0.111	53.617/59.338	24.902	−1.146	0.252	0.240
		G	T	0.344	173.900/171.300	68.644	0.315	0.753	0.719
		A	T	0.273	135.100/141.200	56.561	−0.811	0.417	0.396
		A	G	0.261	141.900/130.800	47.011	1.619	0.106	0.107
		G	G	0.122	59.100/66.700	27.294	−1.455	0.146	0.140
3SNPs									
T	G	A		0.251	144.326/129.489	45.351	2.203	**0.0282**	**0.0327**
C	A	G		0.239	125.794/125.996	51.164	−0.028	0.978	0.971
T	A	A		0.216	110.159/116.073	44.192	−0.890	0.374	0.403
T	A	G		0.117	63.606/63.209	29.346	0.073	0.942	0.910
T	G	G		0.099	45.908/53.229	22.968	−1.528	0.127	0.111
C	A	A		0.060	24.440/27.222	10.245	−0.869	0.385	0.368
	A	G	T	0.334	172.353/167.035	65.467	0.657	0.511	0.444
	A	A	T	0.252	126.494/129.335	49.861	−0.402	0.687	0.668
	G	A	G	0.237	133.919/116.934	42.811	2.596	**0.00944**	**0.0088**
	G	G	G	0.101	51.928/54.436	22.981	−0.523	0.601	0.577
4SNPs									
T	G	A	G	0.234	134.199/116.922	42.366	2.654	**0.00795**	**0.0057**
C	A	G	T	0.231	124.113/121.549	50.203	0.362	0.718	0.655
T	A	A	T	0.195	98.381/100.969	38.476	−0.417	0.674	0.691
T	A	G	T	0.104	58.441/55.883	27.075	0.491	0.623	0.610
T	G	G	G	0.091	47.099/50.538	21.429	−0.743	0.458	0.363
C	A	A	T	0.056	27.279/27.168	10.180	0.035	0.972	0.952

Haplotypes were omitted from analysis if the estimated allele frequencies were less than 3%.

[a] FBAT, family-based association test.

[b] S, observed transmissions of haplotype to affected offspring.

[c] E(S), expected transmission under Mendelian inheritance.

[d] Var(S), variance of (S).

[e] P values were based on χ^2 test with degree of freedom = 1.

Table 4
Global *P* values form single-locus and multilocus (two to four) association analysis produced by the FBAT program

	1	2	3	4
rs3213940	0.803			
		0.686		
rs2289883	0.300		0.190	
		0.140		**0.0314**
rs464058	0.549		**0.00828**	
		0.249		
rs247253	0.673			

KIF2 belongs to the kinesin-like protein family and regulates microtubule dynamics at the growth cone edge by depolymerizing microtubules [18]. It plays an important role in the suppression of collateral branch extension [10]. On the other hand, KIF2 is a motor for anterograde transport, and plays an important role in expansion at the nerve growth cone [20]. Recent study indicates that abnormal microtubular dynamics in cerebral cortical development underlies dysfunctions occurring in schizophrenia. These abnormalities are due to dysfunction of the dynein protein complex [12]. Kinesin and dynein have similar function as transporters of protein along the microtubule. Therefore, the abnormality of the microtubule-associated KIF2 motor complex may contribute to psychiatric disorder.

KIF2 is not included among the main susceptibility loci identified by linkage studies in families with schizophrenia [6,8,17,19,24,26]. However, genome-wide linkage scans for genetically heterogeneous disorders are sometimes unable to produce reliable signals when the contributing alleles are associated with moderate increase in risk or are present at low frequencies. The brain abnormalities observed in the *Kif2a* knockout mice [10] prompted us to utilize transmission studies to test for genetic association of this gene with schizophrenia. Our study was designed to observe whether common genetic polymorphisms spanning *KIF2* are associated with an increase of susceptibility to schizophrenia, and it is based on the common disease/common variant (CD/CV) hypothesis [23,25]. Under this hypothesis and LD mapping method [2,4], susceptibility alleles might also be detected indirectly using genotyped markers and LD information. Although, we failed to detect any positive results in single SNP markers, a significant overtransmission of a common *KIF2* haplotype in a sample of the 280 affected trios' families was found. This analysis identified a four-SNPs haplotype (T/G/A/G) with a frequency of 23.4% in parental chromosomes and showed a significant association with schizophrenia ($P = 0.00795$). We also investigated four haplotypes, which share a common haplotype extending from rs2289883 to rs464058. Our finding reveals the *KIF2* gene as a potential schizophrenia susceptibility gene, and provides the evidence that the *KIF2* is positively associated with schizophrenia in the Chinese Han population.

KIF2A depolymerizes microtubules (MTs) at the growth cone edge and suppresses the growth of axonal collateral branches. This kind of function is necessary to the neurite extension, even though it is not the regulator of this complicated process. In our study, few patients had the A allele at the site of rs3194481 that contributes amino displacement in the corresponding protein of KIF2. This evidence may indicate that the structure of the KIF2 protein fulfils a conservative function, or that the abnormality at this site induces more severe neuropathy or death in infancy and therefore that the mutation is not relevant. Abnormal presentation of a high-risk haplotype in individuals with schizophrenia may partially account for differences in the brain. Further haplotype-specific expression tests may be needed to clarify this issue.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2006.08.025.

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