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# Is KPNB3 Locus Associated With Schizophrenia?[1]

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**Objective** To reconfirm the association of KPNB3 with schizophrenia in Chinese population. **Methods** Two single nucleotide polymorphisms (SNPs), rs2588014 and rs626716 at the KPNB3 locus, were genotyped in 304 Chinese Han family trios consisting of fathers, mothers, and affected offsprings with schizophrenia. These 2 SNPs were detected by PCR-based restriction fragment length polymorphism (RFLP**)** analysis. The Hardy-Weinberg equilibrium for genotypic distributions was estimated by the goodness-of-fit test. The UNPHASED program was used to perform transmission disequilibrium test (TDT), haplotype analysis, and pair-wise measure of linkage disequilibrium (LD) between these 2 SNPs. **Results** The genotypic distributions of both rs2588014 and rs626716 were in the Hardy-Weinberg equilibrium ($P$>0.05). The TDT revealed allelic association with rs626716 ($\chi^2$ =9.31, $P$=0.0023) but not with rs2588014 ($\chi^2$ =3.44, $P$=0.064). The global $P$-value was 0.0099 for 100 permutations. The haplotype analysis also showed a disease association ($\chi^2$=25.97, df=3, $P$=0.0000097). **Conclusion** The present study provides further evidence in support of the KPNB3 association with schizophrenia in Chinese population.

**Key words**: Chromosome 13; KPNB3; Single nucleotide polymorphisms (SNPs); Schizophrenia

## INTRODUCTION

There is no doubt that a genetic component underlies schizophrenia, but the mechanism of inheritance remains unknown. Because the mode of transmission of the disease does not show a Mendelian pattern of inheritance, schizophrenia is thought as a complex disease involving multiple genes showing moderate effects. Linkage studies suggest that the long arm of chromosome 13 is very likely to bear a gene susceptible to schizophrenia[1-8]. A question to be addressed here is why the chromosomal region containing a schizophrenia-susceptibile gene can be localized by linkage studies as the linkage analysis is not sufficiently powerful to map a complex trait gene showing a moderate effect? It is possible that a strong linkage signal may result from several disease-underlying variants coupled with one another on the same chromosome. It could mean that each chromosomal region indicated by a linkage analysis may contain more than one distinct disease-causing variant.

Chromosome 13q14-33 is the favoured region linked to schizophrenia[2-8]. Association studies have demonstrated that the HTR2A gene located on 13q14 is associated with susceptibility to schizophrenia[9-13] although replication of the HTR2A finding has been inconsistent[14-27]. Recent studies indicate that the G72 gene on 13q34 may underlie susceptibility to schizophrenia[28-33]. However, the physical distance between the HTR2A and G72 loci is approximately 60 Mb of DNA. Such a long distance cannot be covered fully by linkage signals from a single DNA marker. Most linkage analyses showed that the peak LOD score is at markers present in the 13q32 region[2,4,8]. An additional gene susceptible to schizophrenia may therefore harbour within this chromosomal region. In a recent study, Wei and Hemmings[34] found that the KPNB3 gene located on 13q32 is associated with schizophrenia in British

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population. The positive single nucleotide polymorphism (SNP) marker detected is rs626716, a synonymous SNP present in exon 8 of the KPNB3 gene. This initial finding, however, resulted only from 124 family trios. Further replication of the KPNB3 finding is needed both in a large sample size and in different ethnic populations. Accordingly, the present study was then undertaken to reconfirm the KPNB3 association with schizophrenia in Chinese population.

## MATERIALS AND METHODS

### *Subjects*

A total of 304 Chinese parent-offspring trios were recruited for the genetic analysis at the Jilin University Research Center for Genomic Medicine, Changchun, China. These family trios did not include those showing Mendelian errors rectified by genotyping more than 10 highly informative SNPs (heterozygosity is greater than 0.25). These subjects originally came from the northeast of China. They were all Chinese of Han descent. The patients (194 males and 110 females), aged 25.5±6.8 years, were admitted to a psychiatric hospital between 2000 and 2004. They were diagnosed having schizophrenia using the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10). All the subjects gave their written informed consent for the genetic analysis. The whole blood sample was then taken from them for extraction of genomic DNA.

### *Genotyping of SNPs*

We detected 2 SNPs at the KPNB3 locus with PCR-based restriction fragment length polymorphism (RFLP) analysis, including the disease-associated SNP rs626716 previously reported in British population[34] and an additional SNP rs2588014, which is 3539 bp away from rs626716. Genomic DNA used for PCR amplification was extracted from the whole blood sample using a DNA extraction kit (Promega, Beijing, China). The primers specifically annealed to a target DNA sequence are as follows: 5′-CCAGAAAACATAATGGGGATTCAC-3′ and 5′-CACAAAGGTTCTCTACAGCTTGC-3′ for genotyping rs2588014 (RsaI site), and 5′-TGTT GAGGCTAGTGTCTCCAC-3′ and 5′-TAGGCGG TAAATGACTCGTGC-3′ for genotyping rs626716 (PstI site). PCR amplification was performed in a 25 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, China), and 30-50 ng of genomic DNA. The conditions used for PCR amplification included an initial denaturation at 94℃ for 5 min, followed by 35-40 cycles at 94℃ for 45 s, at 55℃-60℃ for 1 min and at 72℃ for 1 min, and a final elongation at 72℃ for 10 min. A 15 μL aliquot of the PCR products was completely digested with 6-8 units of restriction enzymes. The digested PCR products were then separated on ethidium bromide-stained agarose gels.

### *Statistical Analysis*

The Hardy-Weinberg equilibrium for genotypic distributions was tested using the chi-square ($\chi^2$) goodness-of-fit test. Transmission disequilibrium test (TDT), haplotype analysis, and pair-wise measure of linkage disequilibrium (LD) between these 2 SNPs were performed with the UNPHASED program (Frank Dudbridge, MRC Human Genome Mapping Project Resource Centre, Hinxton, UK). Only heterozygous parents were used for TDT analysis. In such a family-based study, the allele transmitted by parents to affected offsprings was treated as “case” and that not transmitted as “control”. Haplotype analysis included two $\chi^2$ tests, global test for association on H-1 degree of freedom, where H is the number of haplotypes for which transmission data are available, and the 1-df test for excess transmission of each haplotype. The *P*-value given by the 1-df test was corrected by Bonferroni corrections. Pair-wise measure (D’) was used to represent the strength of LD.

## RESULTS

The $\chi^2$ goodness-of-fit test showed that the genotypic distributions of these 2 SNPs detected were not deviated from Hardy-Weinberg equilibrium in both patient group ($\chi^2$=3.20, df=2, *P*=0.20) and parent group ($\chi^2$=0.41, df=2, *P*=0.82). The TDT revealed allelic association for rs626716 ($\chi^2$=9.31, *P*=0.0023) but not for rs2588014 ($\chi^2$=3.44, *P*=0.064). The global *P*-value was 0.0099 for 100 permutations (Table 1). The haplotype analysis also showed a disease association ($\chi^2$=25.97, df=3, *P*=0.0000097). The rs2588014-rs626716 haplotype system consists of 4 individual haplotypes (Table 2), of which the rs2588014(C)-rs626716(C) haplotype was excessively transmitted ($\chi^2$=9.8, corrected *P*=0.0068) and the rs2588014(C)-rs626716(T)) haplotype was not excessively transmitted ($\chi^2$=18.41, corrected *P*=0.000072). The LD measure (D’) between rs2588014 and rs626716 was 0.969.

TABLE 1

TDT Analysis for Allelic Association

| SNP | N[a] | Allele | | Transmitted[b] | | $\chi^2$ | *P* |
|---|---|---|---|---|---|---|---|
| | | Major | Minor | Major | Minor | | |
| rs2588014 | 608 | T=834 | C=384 | 116 | 146 | 3.44 | 0.064 |
| rs626716 | 608 | T=832 | C=386 | 110 | 160 | 9.31 | 0.0023 |

*Note*. The gobal *P*-value was 0.0099 for 100 permutations. [a] The number of parents genotyped in this study. [b] The number of alleles transmitted only by heterozygous parents.

TABLE 2

Chi-square Test for Transmission of Rs2588014-Rs626716 Haplotypes

| Haplotypes | Transmitted | Non-transmitted | $\chi^2$ | *P* (df=1) |
|---|---|---|---|---|
| CC | 197 | 148 | 9.8 | 0.0017[a] |
| CT | 4 | 20 | 11.94 | 0.000018[b] |
| TC | 6 | 12 | 0.26 | 0.609 |
| TT | 73 | 84 | 2.14 | 0.143 |

*Note*. The global $\chi^2$ test showed $\chi^2$=25.97, df=3, *P*=0.000097. [a] Corrected *P*=0.0068. [b] Corrected *P*=0.000072.

## DISCUSSION

The present results provide further evidence for the KPNB3 association with schizophrenia, which was initially found in British population[34], although the allele frequencies of SNPs detected vary between these two populations. In the British sample, for example, there were only 20 heterozygotes (8.1%) at rs626716 among 248 parents of affected individuals with schizophrenia. These 20 heterozygous parents transmitted 16 C-alleles and 4 T-alleles to their affected offsprings. In contrast, of the 608 Chinese parents genotyped in this study, 270 were heterozygous (heterozygosity of 44.4%) and transmitted 160 C-alleles and 110 T-alleles to their affected offsprings (Table 1). Possibly, the disease-underlying variant may be rs626716 itself or a nearby variant.

The KPNB3 gene products belong to the family of karyopherins. This is a multigene family responsible for the transport of proteins into and out of the nuclei through the nuclear pore complex[35]. The nuclear transport of different subsets of proteins may proceed *via* several distinct import and export pathways. Proteins containing classical nuclear localization signals are bound to a heterodimer consisting of karyopherin alpha proteins and karyopherin beta-1, which then dock at the nuclear pore complex[35]. The second pathway regarding the import of mRNA-binding proteins is mediated by karyopherin beta-2[36]. Karyopherin beta-3 may be capable of mediating the third pathway involved in the import of a set of ribosomal proteins[37]. Control of the nuclear localization of specific proteins is an important mechanism for regulating many signal transduction pathways. Because the present work gives strong evidence supporting the hypothesis of the KPNB3 association with schizophrenia, disturbances of the nuclear transport of proteins may be involved in such a mental disease.

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