Letter to the Editors

Lack of association of the KPNB3 locus in case–control samples

Dear Editors,

A couple of family-based studies suggest that the gene coding for karyopherin beta-3 (KPNB3) is associated with schizophrenia in both a British population and a Chinese population (Wei and Hemmings, 2004; Hu et al., 2005). The major finding is that a synonymous single nucleotide polymorphism (SNP), rs626716 present in exon 10 of the gene, shows disease association. Because high false positive rate is a common problem with the association study of human diseases, it is very important to replicate an initial finding with different samples and experimental designs. The present work, therefore, was undertaken to replicate the KPNB3 association in a case–control sample.

We recruited a total of 780 patients with schizophrenia and 909 healthy controls for the genetic analysis at the Research Center for Neuroscience & MH Radiobiology Research Unit, Changchun, China. Of 780 patients, 417 (53.5%) were male, aged 34.0±11.3 years, and 363 (46.5%) were female, aged 36.0±12.6 years. Of 909 healthy controls, 587 (65.6%) were male, aged 35.3±11.4 years, and 322 were female, aged 34.2±10.8 years. These participants were all Chinese of Han origin and came from the Northeast area of China. Patients were admitted to Changchun Kaixuan Hospital, a psychiatric hospital in Jilin Province, China, in the period between 2004 and 2006. Diagnosis of schizophrenia was made independently by two well-trained psychiatrists using the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10). Detailed information regarding a history of illness, duration and medication was noted. The healthy control subjects were recruited from the same areas and they did not have a history of schizophrenia, affective disorders and other severe forms of mental diseases. All subjects gave written informed consent before their blood samples were taken. This study was approved by the ethics committee of Jilin University, Changchun, China.

About 5 ml of venous blood sample was drawn from each participant for extraction of genomic DNA. Genotyping of rs626716 (Pst I site) was conducted by PCR-based restriction fragment length polymorphism (RFLP) analysis. The primers used for PCR amplification are as follows: 5′-ACGATCCTTAAGGGCAAG-3′ and 5′-GGGTAAATGACTCGTGTCT-3′. PCR amplification was performed in a 25-μl reaction volume containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.001% (w/v) gelatin, 200 μM of each dNTP, 0.4 μM of each primer, 1.0 unit of Taq DNA polymerase (Promega, Beijing, China), and 30–50 ng of genomic DNA. A 15-μl aliquot of the PCR products was completely digested with 6–8 units of Pst I restriction enzyme and then separated on an agarose gel followed by ethidium bromide staining.

The chi-square ($\chi^2$) goodness-of-fit test was applied to test whether or not the genotypic distributions of rs626716 would be in Hardy–Weinberg equilibrium. SPSS for windows (version 14.0) was used to apply an $\chi^2$ test for allelic associations and SPSS SamplePower 2.0 was applied to test a power of the case–control sample.

The $\chi^2$ goodness-of-fit test showed that the genotypic distributions of rs626716 were in Hardy–Weinberg equilibrium in both the patient group ($\chi^2 = 0.30$, $p = 0.589$) and the control group ($\chi^2 = 0.14$, $p = 0.705$). SNP rs626716 is a T to C base change. The two family-based studies showed that the C allele was preferentially transmitted by parents to their offspring affected with schizophrenia. However, the present work did not suggest such an association at all in the case–control samples (Table 1). The sample tested with frequency distribution of the C allele gave a power of 92% to detect a small effect size.

There is no doubt that a genetic component contributes to the etiology of schizophrenia although the mode of transmission remains unclear. A polygenic mechanism is very likely to be involved although genetic heterogeneity cannot be ruled out. Under such a hypothesis, the gene of small effect is not sufficient to
cause illness on its own. Additive effects of a few or many genes are needed to make a high risk of diseases. The present study has failed to replicate the KPNB3 association with schizophrenia although the family-based analysis produced a strong association in the Chinese population (Hu et al., 2005). There are two major possibilities for the controversial results. The effect size of the KPNB3 gene may be too small or the initial finding may be just a chance. Further replication with independent sample collection of Chinese family trios is needed to draw a firm conclusion.

Acknowledgements

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Table 1
Allelic association of rs626716 at the KPNB3 locus with schizophrenia

<table>
<thead>
<tr>
<th>Group</th>
<th>C allele (%)</th>
<th>T allele (%)</th>
<th>$\chi^2$</th>
<th>p</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>265(0.318)</td>
<td>569(0.682)</td>
<td>0.058</td>
<td>0.810</td>
<td>0.977</td>
</tr>
<tr>
<td>Control</td>
<td>379(0.323)</td>
<td>795(0.677)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>248(0.342)</td>
<td>478(0.658)</td>
<td>1.347</td>
<td>0.246</td>
<td>1.143</td>
</tr>
<tr>
<td>Control</td>
<td>201(0.312)</td>
<td>443(0.688)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>513(0.329)</td>
<td>1047(0.671)</td>
<td>0.369</td>
<td>0.543</td>
<td>1.046</td>
</tr>
<tr>
<td>Control</td>
<td>580(0.319)</td>
<td>1238(0.681)</td>
<td></td>
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References


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