Original Article
Association between polymorphism of the norepinephrine transporter gene rs2242446 and rs5669 loci and depression disorders

Yu Pan1,2*, Qi Cheng3*, Mo-Shui Shan4*, Jin Yan1

1Department of Psychology and Mental Health, Second Military Medical University, Shanghai, China; 2Department of Medical Psychology, General Hospital of PLA, Beijing, China; 3Department of Juvenile and Children’s Behavioral Medicine, 102 Hospital of PLA, Changzhou, China; 4Department of 215 Clinical, Sleeping Center, Dalian Nursing Home, Dalian, China. *Equal contributors.

Received July 19, 2015; Accepted September 3, 2015; Epub October 15, 2015; Published October 30, 2015

Abstract: Objective: To explore the association between polymorphism of the norepinephrine transporter (NET) gene rs2242446 and rs5569 loci and depression in Chinese Han population. Methods: A case-control study was carried out, the gene types and allele distributions of NFT gene rs2242446 and rs5569 loci in 302 depression patients and 302 healthy controls were detected by Taqman SNP genotyping technology. Results: The gene types and allele frequency distributions of NFT gene rs2242446 and rs5569 loci had significant differences between case group and control group (rs2242446, \(\chi^2=26.045, P<0.05\), \(\chi^2=8.827, P<0.05\), rs5569, \(\chi^2=42.47, P<0.05\), \(\chi^2=20.9, P<0.05\)). The CC genotype of NET gene rs2242446 locus and rs5569 loci was a protective factor of depression compared with the CT and TT genotypes. Conclusion: The NET genotype polymorphism of rs2242446 and rs5569 loci was a associated with depression in Chinese Han population, in which the CC genotype of rs2242446 and rs5569 loci was a protective factor of depression.

Keywords: Depression, norepinephrine transporter gene

Introduction

Depression is a kind of common mental disorders, and its mainly clinical symptom is lasting mood depression, which may develop from unhappiness, grief to inferiority and even suicide attempt or suicidal behavior. According to WHO statistics, the disease burden of depression will be ranked second and account for 5.7% of the total disease burden in 2020 [2]. Because of the high prevalence, high suicidal rate and high disease burden of depression, it has become a globally concerned socio-economic issue [1]. The annual incidence of depression is about 3%-5% in China, of which 10%-15% patients have suicidal tendency, and the recurrence rate rises with the increased prevalence of illness [2]. Experts predict that depression is likely to become the second major disease with huge disease burden after cardiovascular disease.

The prevention and treatment of depression have become one of the hot topics in the society, of which the etiology mechanism is the key of the hot researches. Studies show that there is obvious genetic predisposition to depression, involving three major gene system, and the candidate genes mainly include serotonin (5-HT), dopamine (DA) and norepinephrine (NE) [3-7]. Norepinephrine transporter (NET) has been widely concerned due to its role in the treatment of antidepressant drugs [11-14]. However, with regard to the NET gene, whether it serves as the pathogenic gene for depression has been controversial. The study of Zill P, Owen D, et al. shows that the NET gene has no correlation with depression, but the result of Sun et al. is opposite [8-10]. This study was a case control study, which included Chinese Han population as the subjects and compared the NET gene polymorphism of rs5569 and rs2242446 loci between case group and control group, aiming to explore association between the NET gene
polymorphism of rs2242446 and rs5669 loci with depression.

Subjects and methods

Subjects

The patient group: Patients who visited outpatient department in the Second Military Medical University from April 2013 to April 2015 were recruited. The inclusion criteria of depression were based on the Fourth Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). All the enrolled patients were Chinese Han population and the scores of Hamilton Depression Scale (HAMD) (17 item version) were greater than 17 points. The exclusion criteria were as follows: genetic diseases; mental disorders due to severe physical disease; secondary mental disorders caused by other various causes. After oral informed consent, all the patients were voluntary to participate in this study.

The control group: We recruited health control subjects who underwent health examination in Physical Examination Center A hospital from April 2013 to April 2015. All the subjects were Chinese Han in nationality, without history of mental disorders and family history of mental disorders. They were all voluntary to participate in this study.

Methods

DNA extraction: 2 ml venous blood of subjects were extracted with EDTA anticoagulant tube. Qiagen reagent kit (Germany) was used to extract the DNA according to the instructions.

The detection of NET gene polymorphism of rs5569 and rs2242446 loci: The allele genes of rs5569 and rs2242446 were detected by TaqMan SNP gene typing technique, reacted by standard gene SNP typing analysis reagent (Taqman® SNP genotyping Assays, ID: 4351379). The PCR reaction system was 5 ul, which contained 5 ng DNA template, and it was placed in a 384-well plate for reaction. The reaction conditions of PCR were as follows: pre degeneration at 95°C for 10 min, denaturation at 92°C for 15 s, annealing at 60°C for 1 min, 45 cycles in total.

Statistical analysis

All data were analyzed with SPSS16.0 software. The differences of genotype and allele frequency among the groups were compared by X² test.

Results

Comparison of the demographic data and gene typing of the depression case group and the control group

This study enrolled 302 cases, including 148 males and 154 females, who were aged from 18 to 65 years. A total number of 302 health controls were recruited, including 150 males and 152 females, who were aged from 18 to 63 years. T-test and X² test were employed to compare the age and sex distributions between case group and control group, and it was found that the differences were not statistically significant (Table 1).

Taqman SNP genotyping results indicated that the NET gene polymorphism of the rs2242446 and rs5569 loci were shown in Figures 1 and 2. In case group, CC genotype, TT genotype, and TT genotype of rs5569 accounted for 30%, 50%, and 20%, respectively, while in control group, CC genotype, TT genotype, and TT genotype of rs5569 accounted for 56%, 34%, and 10%, respectively. In control group, CC genotype, TT genotype, and TT genotype of rs2242446 accounted for 6%, 40%, and 54%, respectively, while in control group, CC genotype, TT genotype, and TT genotype of rs2242446 accounted for 20%, 35% and 45%, respectively.

Hardy-Weinberg genetic equilibrium test

Hardy-Weinberg genetic equilibrium was tested by X² test, and the results showed that the gene distributions of rs5569 and rs2242446 in case group had no significant differences with the theoretical distributions of Hardy-Weinberg
NT and depression disorders

Figure 1. The genotyping map of NET gene rs2242446 locus (Blue: CC genotype; Red: CT genotype; Green: TT genotype).

Figure 2. The genotyping map of NET gene rs5569 locus (Blue: TT genotype; Red: CT genotype; Green: CC genotype).
NT and depression disorders

Table 2. The genotype and allele frequency distribution of rs2242446 in case group and control group

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Allele (%)</th>
<th>Genotype (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>T</td>
</tr>
<tr>
<td>Case group</td>
<td>302</td>
<td>79 (26.0)</td>
<td>223 (74.0)</td>
</tr>
<tr>
<td>Control group</td>
<td>302</td>
<td>113 (37.5)</td>
<td>189 (62.5)</td>
</tr>
</tbody>
</table>

Note: Take CC as reference, the OR values of TC and TT were separately 0.26 (95% CI 0.15-0.47, \( \chi^2 = 0.27, P < 0.05 \)) and 0.25 (95% CI 0.14-0.44, \( \chi^2 = 0.26, P < 0.05 \)).

Table 3. The genotype and allele frequency distribution of rs5569 in case group and control group

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Allele (%)</th>
<th>Genotype (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>T</td>
</tr>
<tr>
<td>Case group</td>
<td>302</td>
<td>166 (55.0)</td>
<td>136 (45.0)</td>
</tr>
<tr>
<td>Control group</td>
<td>302</td>
<td>220 (72.8)</td>
<td>82 (27.2)</td>
</tr>
</tbody>
</table>

Note: Take CC as reference, the OR values of TC and TT were separately 0.37 (95% CI 0.26-0.53, \( \chi^2 = 0.25, P = 0.05 \)) and 0.27 (95% CI 0.16-0.45, \( \chi^2 = 0.28, P < 0.05 \)).

Association between rs2242446 and rs5569 gene polymorphism and depression

The study found that the CC genotype of rs2242446 and rs5569 gene was a protective factor for depression. By setting CC genotype as the reference gene and calculating the risk degree of CT and TT genotype, the OR values of rs2242446 were 0.26 (95% CI 0.15-0.47, \( \chi^2 = 0.27, P < 0.05 \)) and 0.25 (95% CI 0.14-0.44, \( \chi^2 = 0.26, P < 0.05 \)), respectively. The OR values of rs5569 were 0.37 (95% CI 0.26-0.53, \( \chi^2 = 0.25, P < 0.05 \)) and 0.27 (95% CI 0.16-0.45, \( \chi^2 = 0.28, P < 0.05 \)), respectively.

Discussion

Depression is a disease with complex etiology and higher genetic predisposing, which severely reduces the people’s quality of life and makes them have suicidal tendency. It has a large proportion of disease burden and becomes a common social and economic problem in China. The possible pathogenic factors of depression include genetics, social psychology and personality, but the exact cause is not clear [15]. A large number of studies have demonstrated that NEdys function is closely related to severe depression, and NET can reabsorb synaptic norepinephrine to maintain the body noradrenaline level. Therefore, the imbalance of norepinephrine caused by NET level changes in brain may be associated with the occurrence of depression. As a result, NET gene is one of the three major genes in the genetic etiology of depression, which is quite important in the etiology study of depression.

NET gene contains 14 exons, about 45 kb [16]. Rs5569 and rs2242446 gene are located at...
exon 9 1287G/A of NET gene, which is a silent mutation. The significance of this locus to depression has been controversial, which was a hot topic in the world during the recent years. We performed a case-control study and aimed to investigate the variation of rs5569 and rs2242446 gene polymorphism distribution between case group and control group, and find the potential association between rs5569 and rs2242446 gene polymorphism and depression.

The results showed that the genotype distribution and allele frequency of NET gene rs5569 and rs2242446 loci had significant differences between case group and control group, suggesting that both genes were indeed correlated with depression. This study also found that CC genotype of rs5569 and rs2242446 gene was a protective factor for depressive episodes, consistent with the conclusion drawn from Yu Yan et al.’s study that was performed on Han population of south China, but contrary to the results of Ryush et al.’s study conducted in Korea [17, 18]. Therefore, more systematic studies with larger sample size are still required to determine the correlation between the NET gene and depression.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jin Yan, Department of Psychology and Mental Health, Second Military Medical University, No. 800, Xiangyin Road, Shanghai 200433, China. Tel: +8602125070351; Fax: +8602125070351; E-mail: yanjiun975@126.com

References

Norepinephrine Transporter. Neuropsychopharmacology 2006; 31: 2424.


