ASSOCIATION OF GPX4-718C/T POLYMORPHISM WITH TOURETTE SYNDROME IN A CHINA POPULATION

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Abstract: Tourette syndrome (TS) is an inherited neuro developmental disorder. However, it has no definite susceptibility genes. In order to research the genetic susceptibility, GPX4-718C/T was as a candidate single nucleotide polymorphism (SNP) to study. We recruited 100 TS nuclear family trios. Alleles of GPX4-718C/T were detected by TaqMan real-time PCR. We analyzed the tag SNP in GPX4 using the transmission disequilibrium test (TDT). TDT shown no over-transmission in the GPX4-718C/T polymorphism (χ²=0.910, P=0.397, OR=1.535, 95%CI=0.875-2.693). Haplotype relative risk (HRR) was used to ensure the accuracy of the TDT result and no statistical difference was found in the HRR result (χ²=2.261, P=0.133, OR=0.635, 95%CI=0.350-1.150). Although this finding indicated an unlikely association between GPX4-718C/T and TS in a China population, a potential role for GPX4 cannot be ruled out in TS genetic susceptibility.

Keywords: Tourette syndrome; GPX4; Transmission disequilibrium test; Haplotype relative risk; Single nucleotide polymorphism.

1. Introduction

Tourette syndrome (TS) is an early onset inherited neuropsychiatric disorder with an estimated prevalence of 0.1–1% in children and adolescents between 5 and 18 years old(Robertson 2008). Following decades of extensive studies, the exact susceptibility genes remain poorly understood. Numerous literature shown that DRD1 (dopamine receptor D1) (Chou et al. 2004), ADR (adrenergic receptor) (Brett et al. 1997), 5-HT (5-hydroxytryptamine receptor) (Niesler et al. 2005), SLITRK1 (slit and trk-like family member 1) (Abelson et al. 2005), and NLGN4 (neuroligin 4) (Lawson-Yuen et al. 2008) may be association with TS genetic susceptibility. The results of these studies, however, were not satisfactory. Children with Tourette syndrome have high levels of biochemical indices of oxidative stress in previous study (Landau et al. 2012). And Glutathione Peroxidase (GPX) 4 is a unique membrane-associated
selenium-dependent antioxidant enzyme that can directly reduce phospholipid hydroperoxides and protect against oxidative stress (Imai and Nakagawa 2003). GPX4-718C/T polymorphism as a tag SNP was related to certain disorders, such as Kashin-Beck disease (Du et al. 2012). Now we designed this experiment to study the relationship between GPX4-718C/T polymorphism and TS genetic susceptibility in a China population.

2. Methods and Statistical Analysis

We recruited 100 TS nuclear family trios (patients and their parents) from the Affiliated Hospital of Qingdao University. Clinical material including age and gender were collected in a clinical database. TS patients comprised 79 male and 21 female, aged between 5 and 21 years old. Mean ± SD age of patients was 12.8±17.1 years old. They were diagnosed based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, text revision (DSM-IV). Genomic DNA was extracted from peripheral blood using standard chloroform method. GPX4-718C/T polymorphism was conducted by real-time PCR. Taqman probe and primer were synthesized by Applied Biosystems of Life Technologies. Forward and reverse primers were 5-CCGCCCGAGCCCCTGCCCACGCCCT-3 and 5-GGAGCCTTCCACCGGCACCTCATGAC-3. PCR mixture contained 1.25 μl 20×SNP Genotyping Assay, 12.5 μl 2×PCR Master Mix, and 11.25 μl DNA and DNase-free water. The Amplifications were carried out in a C1000™ thermal cycler system and CFX96™ real-time system under the following conditions: 95°C for 3 min, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. For each cycle, fluorescence signals were detected by VIC/FAM-labeled probes. Genotype data of parents were tested by Hardy-Weinberg equilibrium (HWE). Then all the data was counted using statistical software package SPSS 21.0. TDT and HRR were used to test the transmission disequilibrium. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to show the relative risk degree. All of the Statistical significance was set at P<0.05.

3. Results

Genotype of parents was consistent with HWE and can undertake the genetics research (χ²=2.143, P=0.143). The transmitted and non-transmitted groups were not statistically different in the TDT (χ²=0.910, P=0.397, OR=1.535, 95% CI=0.875-2.693) (Table 1). The HRR also indicated no statistically difference between transmitted and non-transmitted groups
Table 1: The TDT result of the GPX4-718C/T polymorphism

<table>
<thead>
<tr>
<th>Groups</th>
<th>Non-transmitted allele</th>
<th>Transmitted allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>T</td>
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<tr>
<td>Transmitted allele</td>
<td>47</td>
<td>40</td>
</tr>
<tr>
<td>TDT result</td>
<td>$\chi^2=0.910$, P=0.397, OR=1.535, 95%CI=0.875-2.693</td>
<td></td>
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</table>

Table 2: The HRR result of the GPX4-718C/T polymorphism

<table>
<thead>
<tr>
<th>Groups</th>
<th>C(+)</th>
<th>C(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmitted allele</td>
<td>62</td>
<td>38</td>
</tr>
<tr>
<td>Non-transmitted allele</td>
<td>72</td>
<td>28</td>
</tr>
<tr>
<td>HRR result</td>
<td>$\chi^2=2.261$, P=0.133, OR=0.635, 95%CI=0.350-1.150</td>
<td>95%CI=0.350-1.150</td>
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4. Discussion

The factors that contribute to the pathogenesis of TS are poorly defined. Twins and families studies (Pauls et al. 1981, Price et al. 1985) provided strong evidence that genetic factors, particularly polygenic hereditary patterns, are involved in the vertical transmission of TS. More recent studies suggested that the pattern of inheritance is more complex and that some genes have a major effect while others act as modifiers (Pauls 2001). Through too many researches in the field of genetics, the investigators were still not clear about its main predisposing genes, so the follow-up study of the genetics is still highly necessary. Oxidative stress is suspected to be important in neurodegenerative diseases including Alzheimer's disease, Huntington's disease, Depression and Multiple sclerosis (Patel and Chu 2011). And TS, as a neurodevelopmental disorder, has studied the role of oxidative stress in its pathogenesis (Landau et al. 2012, Shen et al. 2014). The protein encoded by GPX4 belongs to the glutathione peroxidase family, members of which catalyze the reduction of hydrogen peroxide, organic hydroperoxides and lipid hydroperoxides, and thereby protect cells against oxidative damage.
Mutations in this gene are associated with Sedaghatian type of spondylometaphyseal dysplasia (SMDS) (Smith et al. 2014). These fully show that the GPX4 plays a fatal role in the process of oxidative stress. GPX4-718C/T (GPX4 3'untranslated region, chromosome 19) as a tag SNP was association with diverse disorders by changing glutathione peroxidase family enzyme activity, such as Kidney disease, Kashin-Beck disease, Colorectal cancer (Du et al. 2012, Monteiro et al. 2013, Meplan et al. 2010). We guessed that there was a relationship between GPX4-718C/T polymorphism and TS genetic susceptibility. We collected 100 nuclear family trios to study, which could be favorable to exclude irrelevant factors. TDT and HRR ensure the reliability of the experimental results. Nevertheless, our statistical results of TDT and HRR shown that the P values were greater than 0.05 (P_{TDT}=0.397, P_{HRR}=0.133). These demonstrated that the GPX4-718C/T polymorphism may be no relation with TS genetic susceptibility in a China population. Certainly, there are some limitations in our study. Small sample size, single race and sole SNP are likely to result in the deviation of the results. And we will make up for these limitations as far as possible in the future research.

Acknowledgements
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References


