Original article

Association analysis between Tourette’s syndrome and two dopamine genes (DAT1, DBH) in Taiwanese children

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\textbf{A B S T R A C T}

Background: Recent research suggests that Tourette’s syndrome (TS) may result from a defect in the dopamine system. Several candidate gene polymorphisms have been implicated in attention deficit hyperactivity disorder, including the dopamine transporter (DAT1) and dopamine β-hydroxylase (DBH) genes. A high rate of comorbidity between attention deficit hyperactivity disorder and TS indicates that they may share the same pathophysiology.

Purpose: We aimed to test the hypothesis that the dopamine gene might play a role in TS.

Methods: An association study, using an independent sample of patients from the midland region of Taiwan, was performed to investigate whether DAT1 and DBH gene polymorphisms can be used as markers of susceptibility to TS. A total of 160 children with TS and 83 normal control participants were included in the study. Polymerase chain reaction was used to identify polymorphisms in the DAT1 (40 bp VNTR) and DBH (TaqI A2) genes. Genotypes and allelic frequencies for the DAT1 and DBH gene polymorphisms in both groups were compared.

Results: The results showed that genotypes and allelic frequencies in both groups were not significantly different. The most common genotype for DAT1 (40 bp VNTR) was the 10,10 homozygote in both groups. The most common genotype for DBH (TaqI A2) was the T homozygote in both groups.

Conclusion: These data suggest that the DAT1 and DBH genes may not be useful markers to predict susceptibility to TS.

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1. Introduction

Gilles de la Tourette syndrome (TS) is a neuropsychiatric disorder characterized by both motor and vocal tics. In addition, affected individuals frequently display symptoms such as attention deficit hyperactivity disorder (ADHD) and/or obsessive–compulsive disorder. In the 1970s, investigators first demonstrated that TS has a familial concentration [1]. TS was
then shown to be transmitted vertically from generation to generation, and studies of twin pairs confirmed a genetic influence [2,3]. To date, the gene search in TS has been unsuccessful [4], which is illustrative of the many factors that can complicate genetic analysis of complex human traits [5].

The pathogenesis of TS remains obscure. Current evidence suggests that TS may result from a defect in the dopamine system [6–10]. Studies have focused mainly on the dopamine transporter gene [DAT1 40 bp variable tandem nucleotide repeat (VNTR)], and the dopamine beta hydroxylase gene [DBH (TaqI A2)] in ADHD [11–13]. ADHD is common in TS probands and is reported to affect about 50–70% of referred TS cases [14–16]. These observations led us to test the polygenic hypothesis by examining the potential effect of DAT1 and DBH in TS. We previously used single nucleotide polymorphisms (SNPs) as a tool in genetic studies of polygenic disorders [17–21]. SNPs are markers that may provide a new way to identify complex gene-associated diseases such as TS. In this study, we tested the hypothesis that genetic variation in the DAT1 (40 bp VNTR) and DBH (TaqI A2) genes confers susceptibility to TS. Two SNP markers have been identified in these genes, allowing researchers to detect disease-causing gene associations [22].

2. Materials and methods

The study included Taiwanese children with TS (n = 100 in the DAT1 group and n = 160 in the DBH group, respectively) and normal control participants (n = 83). This study was approved by the Ethics Committee of the China Medical University Hospital, Taichung, Taiwan. All parents signed informed consent before blood tests were performed. TS patients and the controls were both recruited from the midland regions of Taiwan. Diagnosis of TS followed the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) [23]. The criteria for TS are as follows: the presence of multiple motor and at least one vocal tic (not necessarily concurrently); a waxing and waning course with tics evolving in a progressive manner; the presence of tic symptoms for at least 1 year; the onset of symptoms before 21 years of age; the absence of a precipitating illness (e.g., encephalitis, stroke, or degenerative disease) or medication; the observation of tics by a knowledgeable neurologist; and marked distress or significant impairment in social, occupational, or other important areas of functioning. A pediatric neurologist (I-C.C.) examined the children and made sure that all cases were unrelated. The 83 controls were healthy volunteers with no history of psychiatric treatment.

All children underwent peripheral blood sampling for genotype analyses. Genomic DNA was isolated from peripheral blood by means of a DNA extractor kit (Genomaker DNA extraction kit; Blossom, Taipei, Taiwan). A total of 50 ng of genomic DNA was mixed with 20 pmol of each polymerase chain reaction (PCR) primer in a total volume of 25 μL containing 10 mM Tris-hydrochloride, pH 8.3; 50 mM potassium chloride; 2.0 mM magnesium chloride; 0.2 mM each deoxyribonucleotide triphosphate; and 1 U of DNA polymerase (Amplitaq; Perkin Elmer, Foster City, CA, USA). Four PCR primers were used to amplify the correlated gene. The sequences of these primers were as following (from the 5’ to 3’ end): DBH (444 g/a): upstream, CCTGGACGCCCAGTGGTGC; downstream, ACGCCCTCTGGGTACTGCC; and DAT1: upstream, TGTGGTGTAGGGAACGGCCTGAGA; downstream, AAATTCGATGGGTCCTCCTCGT. The PCR conditions were as follows: 35 cycles of: 95°C for 30 seconds, 60°C for DBH (444 g/a) or 66.5°C for DAT1 for 30 seconds, and 72°C for 45 seconds, followed by 72°C for 7 minutes, and then held at 4°C. The polymorphisms were analyzed by PCR amplification followed by restriction analysis with EcoNI for DBH (444 g/a). The PCR products were directly analyzed on 3% agarose gel by electrophoresis, and each allele was identified according to its size.

Allelic frequencies were expressed as a percentage of the total number of alleles. The genotypes and allelic frequencies for DAT1 and DBH polymorphisms in both groups were compared. Using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) with the χ² test was used for statistical analyses. A p value of <0.05 was considered statistically significant.

3. Results

Genotype proportions and allele frequencies for DAT1 and DBH were not significantly different between the groups (Tables 1 and 2). The most common genotype for DAT1 was the 10,10 homozygote in both groups. The allele 10 frequency for DAT1 in TS patients was 87% and in controls it was 88.6% (Table 1).

The most common genotype for DBH was the T homozygote in both groups. The proportions of T homozygotes, T/C heterozygotes, and C homozygotes for DBH were: in TS patients, 75.6%, 22.5%, and 1.9%, respectively; and in controls, 85.6%, 14.2%, and 0%. The p-values were calculated using the χ² test.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tourette patients, n (%) (n = 100)</th>
<th>Controls, n (%) (n = 83)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>11,11</td>
<td>1 (1)</td>
<td>0</td>
<td>0.795</td>
</tr>
<tr>
<td>10,13</td>
<td>0</td>
<td>1 (1.2)</td>
<td></td>
</tr>
<tr>
<td>10,11</td>
<td>2 (2)</td>
<td>2 (2.4)</td>
<td></td>
</tr>
<tr>
<td>10,10</td>
<td>75 (75)</td>
<td>66 (79.5)</td>
<td></td>
</tr>
<tr>
<td>10,9</td>
<td>19 (19)</td>
<td>11 (13.3)</td>
<td></td>
</tr>
<tr>
<td>10,8</td>
<td>1 (1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10,7</td>
<td>1 (1)</td>
<td>1 (1.2)</td>
<td></td>
</tr>
<tr>
<td>10,6</td>
<td>1 (1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>9,9</td>
<td>0</td>
<td>1 (1.2)</td>
<td></td>
</tr>
<tr>
<td>9,7</td>
<td>0</td>
<td>1 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Allelic frequency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele 13</td>
<td>0</td>
<td>1 (0.6)</td>
<td>0.858</td>
</tr>
<tr>
<td>Allele 11</td>
<td>4 (2)</td>
<td>2 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Allele 10</td>
<td>174 (87)</td>
<td>147 (88.6)</td>
<td></td>
</tr>
<tr>
<td>Allele 9</td>
<td>19 (9.5)</td>
<td>14 (8.4)</td>
<td></td>
</tr>
<tr>
<td>Allele 8</td>
<td>1 (0.5)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Allele 7</td>
<td>1 (0.5)</td>
<td>2 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Allele 6</td>
<td>1 (0.5)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

The p-values were calculated using the χ² test.
Genotypes and allele frequencies for DBH (TaqI A2) polymorphisms in children with Tourette’s syndrome and in normal individuals.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tourette patients, n (%) (n = 160)</th>
<th>Controls, n (%) (n = 83)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>T/T</td>
<td>121 (75.6)</td>
<td>65 (78.3)</td>
<td>0.900</td>
</tr>
<tr>
<td>C/T</td>
<td>36 (22.5)</td>
<td>17 (20.5)</td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>3 (1.9)</td>
<td>1 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Allelic frequency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele T</td>
<td>278 (86.9)</td>
<td>147 (88.6)</td>
<td>0.700</td>
</tr>
<tr>
<td>Allele C</td>
<td>42 (13.1)</td>
<td>19 (11.4)</td>
<td></td>
</tr>
</tbody>
</table>

The p-values were calculated using the $\chi^2$ test.

78.3%, 20.5%, and 1.2%, respectively. The allele T and C frequencies for DBH in TS patients were 86.9% and 13.1%, respectively; and in controls, 88.6% and 11.4%, respectively (Table 2).

4. Discussion

Dopamine transport was first described 40 years ago [24]. DAT was itself identified and its molecular structure described more than 20 years later [25]. The human DAT gene is localized on chromosome 5p15.3 [26]. A genetic polymorphism of a 40 bp VNTR polymorphic sequence in the 3’ untranslated region of exon 15 of the gene has been described [27]. This VNTR of exon 15 is repeated 3–11 times, most typically 10 times. The 10-repeat shows ethnic heterogeneity with a frequency of 0.7 among Caucasians and Hispanics in the USA, 0.54 in African Americans, and 0.9 in Asians [28-30]. DATs are expressed in a small number of neurons in the brain, mainly in the striatum and nucleus accumbens, but also in the globus pallidus, cingulate cortex, olfactory tubercle, amygdala, and midbrain [31]. DAT, like the transporters for norepinephrine and serotonin, is a Na⁺/Cl⁻ dependent transmembrane transport protein [32] which regulates the concentration of dopamine in the synaptic cleft.

DBH appears to be a strong candidate for investigation in TS, because it catalyzes the conversion of dopamine to norepinephrine and therefore influences both the dopaminergic and adrenergic systems. Serum DBH levels are under strong genetic control and show large interindividual variation [30]. Alleles of several polymorphisms at the DBH locus have been found to be associated with serum DBH levels.

In the present study we did not find significant evidence for association in our TS samples. The role of the dopaminergic system in the pathogenesis of TS is still not known. Preliminary studies have suggested that the pathogenesis of tics involves neuronal activity within subcortical neuronal circuits [33]. Therefore, this raises the possibility that classic neurotransmitters, dopamine and serotonin, may be involved in the pathobiology of TS. However, other investigators have emphasized that abnormalities of dopamine fail to explain many clinical and laboratory observations, including the description of unchanged tics in four adults who developed parkinsonism and received treatment with L-dopa [34].

Our review of the literature found that recent linkage studies have not provided any positive results regarding: dopamine D1-5 receptors [35-37], glycine ρ-1 subunit, GABAA receptor ρ-1, ρ-6, and γ-2 subunits (GABRA1, GABRA2, GABRG2), GABAA receptor α-1 and α-2 subunits (GABRB1, GABRA2), glutamate receptor GLUR1, the α-adrenergic receptor ADRA1, the β-adrenergic receptor ADRB1, and the glucocorticoid receptor GR [38]; norepinephrine transporter gene [39]; or catechol-o-methyltransferase [40]. Other investigators have sought to identify associations between TS and other movement disorders [41]. Further studies will be required to confirm these assertions.

The etiology of TS is therefore unknown. In fact, TS in children may involve a complex interaction between environmental influences, especially infection, autoimmune contributions, epigenetic factors, and genetic factors. Our study suggests that the DBH and DAT1 genes may not contribute to the etiology of TS. Further studies could focus on the analysis of other dopaminergic genes in TS patients. Our results could provide the database for a further survey of DBH and DAT1 gene polymorphisms.

Acknowledgments

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