Original Article
Association study of dopamine transporter gene (DAT1) variable tandem repeat sequence (VNTR) with obsessive-compulsive disorder in Chinese Han Population

Shaoyan Zhang1*, Weihua Jiang2*, Xiuming Tang3, Quanchen Xu4, Jingli Wang5, Rui Gui6, Xinhua Zhang2, Shiguo Liu5,7

1Clinical Laboratory, The Affiliated Hospital of Qingdao University, Qingdao 266003, China; 2Department of Psychiatry, Medical College, Qingdao University, Qingdao, 266021, China; 3Reproductive Medicine Centre, The Affiliated Hospital of Qingdao University, Qingdao 266003, China; 4The Affiliated Hospital of Qingdao University, Qingdao 266003, China; 5Prenatal Diagnosis Center, The Affiliated Hospital of Qingdao University, Qingdao, 266003, China; 6Clinical Laboratory, Pingyi Hospital Chinese Medicine, Shandong, China; 7Genetic Laboratory, The Affiliated Hospital of Qingdao University, Qingdao 266003, China. *Equal contributors.

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Abstract: Objective: Multiple evidence suggests an involvement of the dopamine neurotransmitter system in Obsessive-compulsive disorder (OCD). Therefore, we explore the association of 3'UTR region of 40 bp variable tandem repeat (VNTR) polymorphism in Dopamine Transporter Gene (DAT1) in Chinese Han population. Methods: A total of 305 OCD patients and 435 healthy individuals were recruited for the study. OCD was diagnosed with the Forth Edition (DSM-IV) diagnostic criteria. After polymerase chain reaction of VNTR was used to evaluate the 40 bp VNTR polymorphism in DAT1, a case-control association analysis was performed by the \( \chi^2 \) test. Results: The results showed that no association was found between OCD patients and controls for the genotype distribution (\( \chi^2 = 0.743, \) \( P=0.690, \) df=2) as well as allelic (\( \chi^2 = 0.172, \) \( P=0.678, \) OR=0.928, 95% CI=0.885-1.224) distribution. Conclusions: Our data suggest that the 40 bp VNTR polymorphism in DAT1 may not be associated with susceptibility to OCD in the Chinese Han population studied. However, this result needed to be replicated from different populations.

Keywords: OCD, DAT1, 40 bp VNTR, susceptibility, association analysis

Introduction

Obsessive-compulsive disorder (OCD), which has an estimated incidence of approximately 1.1%-3.3% [1], is the common revalent psychiatric disorder, characterized by force concept, forced impulse or compulsive behavior, etc. OCD usually onsets from 20 to 24 years old [2], which has serious affected the normal living, work and sociality badly and brought heavy burden to the society. Many studies showed that OCD is a much more complex factors disease and genetic factors may play a significant role [3-7]. But until now, no common vulnerability genetic variants which clearly associated with the disorder have been reported.

Previous studies showed several lines of evidence illustrated that dopamine system may be related to OCD. Vandenbergh et al. reported that reduced serotonin function and increased norepinephrine activity in the brain lead to aggressive and impulsive behavior in animal and human studies [8]. In addition, some anti-psychotic drugs such as selective serotonin re-uptake inhibitors (SSRIs), which adjust dopamine function activities in brain, can be used as a kind of aggrandizement lonely auxiliary instrument for OCD treatment [9]. As one of important dopamine transporters, dopamine transporter gene (DAT1, SLC6A3), located on 5p15.3, is responsible for the bulk of dopamine degradation in the striatum and the prefrontal cortex and the transfer of dopamine in the synaptic cleft, thus affecting the signaling transmission mediated by postsynaptic membrane. Some unit of length 40 bp variable tandem repeat (VNTR) repeat sequence in the 3'end of the
non-translated region (3’UTR) of DAT1 may affect its expression. Mill et al. observed DAT1 expression of the 10-repeat allele of VNTR polymorphism was increased in the cerebellum, frontal lobe, and lymphocytes. This polymorphism has been associated with susceptibility to Tourette syndrome (TS) and Attention Deficit and Hyperactivity Disorder (ADHD).

In the present study, we hypothesized the association between OCD and the 40 bp VNTR polymorphism in DAT1 and performed a case-control association analysis to assess whether the polymorphism in this gene could be implicated in susceptibility to OCD in a Chinese Han population.

Materials and methods

Study population

A total of 305 OCD patients [mean age (SD)=31.4 (12.4) years] and 435 control subjects [mean age (SD)=31.6 (10.8) years] were included in this study from the Affiliated Hospital of Medical College, Qingdao University (Table 1). The affected individuals included 154 male and 151 female patients and control included 232 male and 203 female. All patients were diagnosed according to Diagnostic and Statistical Manual of mental disorders (DSM-IV) diagnostic criteria, and the severity of the disorder was evaluated with the Yale-Brown Obsessive Compulsive Scale. The control subjects were selected among healthy volunteers and all had no history of any psychiatric disorder. The patients with OCD and healthy controls were Chinese people from the same geographic region and of the same ethnic origin. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Affiliated Hospital of Medical College, Qingdao University.

Table 1. Demographic Characteristics of OCD Patients and Controls

<table>
<thead>
<tr>
<th></th>
<th>OCD patients</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean [SD]</td>
<td>31.4 [12.4]</td>
<td>31.6 [10.8]</td>
<td>0.15</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>154</td>
<td>232</td>
<td>0.45</td>
</tr>
<tr>
<td>male</td>
<td>151</td>
<td>203</td>
<td></td>
</tr>
</tbody>
</table>

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes using standard methods. The segments of polymorphism were replicated using the primers as follows: 5-TCTGCTACAAGTTCTGGC-3 and 5-CTGCAGCTTTTTTCTCTAG-3. PCR reactions were carried out in a final volume of 10 μl containing 2 × PCR MasterMix, 0.5 μl primer and 50 ng of DNA. The reaction of DAT1 involved a starter step at high temperature of 94°C for 5 min followed by 35 cycles with the following cycle profile: denaturation at 94°C for 1 minute, annealing temperature 66°C for 1 min and elongation at 72°C for 30 s and a final elongation at 72°C for 10 min. The PCR products were directly detected by 3.5% agarose gel electrophoresis. A 50 bp DNA ladder was used to mark the relative molecular mass of PCR product. The allele produces present five fragments of 520, 480, 440, 400 and 360 bp, which were visualized under ultraviolet (UV) light.

Statistical analysis

For all data of patients and controls, the Hardy-Weinberg equilibrium of the genotype distribution was tested using the homogeneity chi-squared test. A case-control study was performed using the homogeneity chi-squared test, which was carried out using the Statistical Package for Social Sciences (Version 12.0 for Windows; SPSS, Inc., Chicago, IL, USA) and Microsoft Excel 2000.

Results

We found that this VNTR polymorphism consisted of the 11, 10, 9, 8 and 7 repeats units, respectively. Each allele consisted of the 520, 480, 440, 400, and 360 bp nucleotide sequences through sequencing, respectively. Nine genotypes which were 10-10, 11-10, 11-9, 9-10, 8-10, 7-10, 7-9, 9-9 were found in all the participants. Major allele is 10 repeat and minor alleles are 11, 9, 8 and 7 repeat. X represents allele of 11, 9, 8, 7 repeat. In the VNTR loci, the subjects were categorized into three genotypes: major allele homozygote (10-10), heterozygote with major and minor alleles (10-X), and minor allele homozygote (X-X), respectively.

VNTR polymorphism followed the Hardy-Weinberg equilibrium (HWE) in the control group.
Association of DAT1 VNTR and obsessive-compulsive

Table 2. The associations in the distributions of genotypes and allele frequency for VNTR polymorphism in DAT1 in cases and controls

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>(1) Case, N=305</th>
<th>(2) Control, N=435</th>
<th>(1) VS (2) X², df, P-value, AND (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td>X² = 0.743, df = 2, P = 0.690, OR = 0.928, 95% CI = 0.653-1.320</td>
</tr>
<tr>
<td>10-10</td>
<td>249</td>
<td>358</td>
<td></td>
</tr>
<tr>
<td>10-X</td>
<td>52</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>X-X</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td>X² = 0.172, df = 1, P = 0.678, OR = 0.928, 95% CI = 0.653-1.320</td>
</tr>
<tr>
<td>10</td>
<td>550</td>
<td>790</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>60</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

(cutoff p-value = 0.697). The genotype frequencies and allelic frequency distributions in cases and controls are listed in Table 2. Evaluation of VNTR polymorphism in DAT1 showed that the prevalence of the 10/10 genotype was 249 (81.64%) in patients and 358 (82.30%) in controls, the frequency of the 10/X genotype was 52 (17.05%) and 74 (17.01%) in patients and controls, respectively, and the values for the X/X genotype in the patient group was 4 (1.31%) and in controls was 3 (0.69%) (Table 2). Statistical analysis showed no difference between groups regarding these genotypes (X² = 0.743, df = 2, P = 0.690, OR = 0.928, 95% CI = 0.653-1.320). The frequency of the 10 allele was 550 (90.16%) and 790 (90.80%) in patients and controls, respectively. 60 (9.84%) the X alleles were seen in patients, but the frequency of this allele was 80 (9.20%) in controls. No significant difference were found in these alleles (X² = 0.172, P = 0.678, OR = 0.928, 95% CI = 0.653-1.320, DF = 1).

Discussion

Due to its high morbidity and a chronic course lead to serious consequences [14, 15], OCD is paid more attention by the National Institute of Mental Health. In the past few years, many researches were performed for OCD etiology such as family survey and candidate genes [16, 17], and there are many evidence of genetic contribution to its etiology, but environmental risk factors also are likely to be involved. Animal experiments and pharmacological studies have indicated that serotonin and dopamine play an important role in OCD [18]. As a core factor in dopamine transmission, DAT1 plays a key role in the transfer of dopamine in the synaptic cleft [19]. The VNTR polymorphism 40 bp core sequence in the 3’-UTR of DAT1 may occur 3 to 11 times, repeating the common is 9 (440 bp allele 9), 10 repeats (480 bp, allele 10). Fuke et al. indicated this VNTR polymorphism affected its expression, when the 3’UTR contains 10 repetitions of the sequence, the gene expression levels rise [20].

Previous studies were performed for DAT1 40 bp VNTR polymorphism with susceptibility to TS and ADHD. Tarnok Z and his colleague found that the DAT1 40 bp VNTR showed an association with the peak tic-severity by means of transmission disequilibrium test (TDT) design in 103 TS trios in Hungary population and patients with at least one copy of the 9-repeat allele had significantly more severe symptoms than individuals with the homozygous 10/10 genotype [21], while no significant association were found in mixed European descent [22]. Hawi et al. indicated a significantly association between ADHD and 10-repeat allele of DAT1 in 187 trios in Omani population by HHRR studies [23]. Chen et al. reported evidence of increased transmission of the 10-repeat allele using TRANSMIT in a sample of 110 Taiwanese probands with a DSM-IV diagnosis of ADHD [24]. However, Shang et al. examined the linkage disequilibrium structure of the DAT1 and investigated whether the DAT1 was associated with ADHD in Chinese family-based association sample in 273 probands and found although a haplotype rs27048 (C)/rs429699 (T) was significantly associated with the inattentive subtype, no association between DAT1 40 bp VNTR and ADHD [18]. There are also several negative association studies [25, 26].

In this present study, we explored the association of VNTR polymorphism of DAT1 and OCD in the Chinese Han population. We found that this tandem repeat polymorphism consisted of the 11, 10, 9, 8 and 7 repeats units. Major allele is
10 repeat while minor alleles are 11, 9, 8 and 7 repeat, which were represented by X allele. So the VNTR loci were categorized into three genotypes: major allele homozygote (10-10), heterozygote with major and minor alleles (10-X), and minor allele homozygote (X-X), respectively. However, our results showed that no association was found between OCD patients and controls for the genotype and alleles distribution. Our results did not confirm an involvement of the VNTR polymorphism of DAT1 in conferring susceptibility to OCD in Chinese Han population. Walitza S et al. investigated the association between this VNTR polymorphism and OCD by 69 OCD trios in German population and found similar result with us [27]. Miguita et al. also found lack of association analysis between a VNTR intron 8 polymorphism of DAT1 and OCD in a Brazilian sample [28]. However, two disadvantages can be pointed in our study: on the one hand, this study was limited by the small size of the available samples. On the other hand, ethnic difference in genetic polymorphisms may determine their functions in different populations. Therefore, our finding remains to be confirmed by further studies that should consider larger specimens in more area and update method adopting.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Shiguo Liu, Genetic Laboratory, The Affiliated Hospital of Qingdao University, 16 Jiangsu Road, Qingdao 266003, China. E-mail: liushiguo2002@126.com; Dr. Xinhua Zhang, Department of Psychiatry, Medical College, Qingdao University, 16 Jiangsu Road, Qingdao 266003, China. E-mail: xzhang@126.com

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