Analysis of the MRPL3, DNAJC13 and OFCC1 variants in Chinese Han patients with TS-CTD

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A B S T R A C T
Tourette syndrome/chronic tic phenotype (TS-CTD) is a neurological disorder manifested particularly by motor and vocal tics and associated with a variety of behavioral abnormalities. Recently, the mitochondrial ribosomal protein L3 gene (MRPL3) 75SN, the DnaJ (Hsp40) homolog subfamily C member 13 gene (DNAJC13) A2057S, the orofacial cleft 1 candidate 1 gene (OFCC1) R129G variants were reported to be associated with Tourette syndrome/chronic tic phenotype (TS-CTD) in patients of European ancestry. To evaluate whether these variants are associated with TS-CTD in Chinese Han patients, we screened 132 Chinese Han patients from Mainland China. None of the 132 samples from patients with TS-CTD showed the MRPL3 75SN, DNAJC13 A2057S, OFCC1 R129G and c.-5A>G variants, and these variants probably are a rare cause of TS-CTD in a Chinese Han ethnic group. Genetic heterogeneity of TS should be considered and tests designed to detect these variants in Chinese Han ethnic group probably will not have a diagnostic utility in clinical practice.

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1. Introduction

Tourette syndrome/chronic tic phenotype (TS-CTD) is a neurological disorder manifested particularly by motor and vocal tics and associated with a variety of behavioral abnormalities. While environmental causes have been proposed to play a role, genetic factors are believed to be the main determinants of the disorder and its clinical manifestations. There may be different patterns of inheritance including polygenic mechanisms and monogenetic model [9], and many TS-CTD gene loci including 2p11, 3q, 7q31, 7q25-q36.2, 8q22.1, 9p, 13q31, 15q13-q22.3, 16q, 17p11, 17q25, 18q22, and 22q11 have been identified by cytogenetic and linkage analysis [1.3–6,12–14,17,20–23,26]. We have performed genetic analysis of the coding region of the histidine decarboxylase (HDC) gene in Chinese Han patients with TS and found variants in the HDC gene may play little role in TS [16]. Recently, Sundaram et al. reports that the mitochondrial ribosomal protein L3 gene (MRPL3) 75SN, the DnaJ (Hsp40) homolog subfamily C member 13 gene (DNAJC13) A2057S and the orofacial cleft 1 candidate 1 gene (OFCC1) R129G nonsynonymous variants co-segregated with TS-CTD phenotype in a three-generation nonconsanguineous family of European ancestry by exome sequencing, and extended analysis showed that a novel variant (c.-5A>G) was found in the 5′-untranslated region of the OFCC1 gene in 2 out of 94 TS-CTD patients, whereas absents in dbSNP/1000 genomes variant calls [24], suggesting these four variants may be potential candidate factors for the development of TS-CTD.

To determine the frequency of these variants in the Chinese Han patients with TS, we recruited 132 TS-CTD patients of Han ethnicity from Mainland China.

2. Materials and methods

2.1. Study subjects

One hundred and thirty-two unrelated Chinese Han patients with TS-CTD (mean age 10.7 ± 5.4 years; mean age at onset: 8.0 ± 4.5 years, male/female: 106/26; 36 familial cases, 96 sporadic cases) from Mainland China were enrolled in this study. They were diagnosed by two independent investigators according to common diagnostic criteria described [11,25]. A detailed family history was obtained from all individuals. Seventy-six percent (100/132) of the patients were screened and found to be negative for mutation in the coding region of the HDC gene [16]. This study was approved by the Institutional Review Board of the Third Xiangya Hospital, and all

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participating individuals or their guardians signed their informed consents.

2.2. Genetic analysis

Genomic DNA was isolated from whole blood using standard phenol/chloroform method and genetic analysis was conducted [8]. Polymerase chain reaction (PCR) products were generated with 50 ng DNA template in 2.5 μL 10 × PCR buffer, 2.0 μL of 25 mmol/L each dNTP, 1.5 μL of 25 mmol/L MgCl2, 10 pmol of each primer (Table 1) and 1 unit Taq polymerase in a total volume of 25 μL PCR amplified the fragments covering MRPL3 5′UTR, DNAJC13 2′UTR, OFCC1 3′UTR and c.-5A>G variants using by a 9700 Thermal Cycler System (Applied Biosystems Inc., Foster City, CA, USA), for 35 cycles at 95°C for 30 s, 57°C for 30 s, 72°C for 40 s, and a final extension step at 72°C for 5 min. 8.5 μL PCR products were digested by 0.8 U shrimp alkaline phosphatase (SAP) and 8 U exonuclease I (Fermentas) in a 10 μL reaction volume, and sequenced directionally using an 8-capillary 3500 genetic analyzer (Applied Biosystems Inc.) [10].

3. Results

None of the 132 samples showed the MRPL3 5′UTR, DNAJC13 2′UTR, OFCC1 3′UTR and c.-5A>G variants. However, we found that one individual with familial TS was heterozygous for a known nucleotide variant rs79534309 (c.C102T, nt1280, AC_000046). This patient was a 12-year-old boy with an onset of 4 years and he presented a typical TS. The rs79534309 variant neither changed the amino acid (Ile34Ile) in the protein nor altered mRNA splicing site (predicted by http://www.fruitfly.org/seq_tools/splice.html), suggesting it is a non-pathogenic variant.

4. Discussion

TS is a childhood-onset disorder that is characterized by persistent motor and vocal tics fluctuating in severity. Family studies have repeatedly demonstrated that TS is highly familial, and twin studies provide strong evidence for genetic nature of TS. Our knowledge of TS-CTD has advanced in recent years as a result of discoveries of mutations or disruption in genes, including the inner mitochondrial membrane peptidase 2 like gene (IMMP2L) [18,20], the contactin associated protein-like 2 gene (CNTNAP2) [2,27], the Slit and Trk-like 1 gene (SLITRK1) [1], the neuroglian X-linked gene (NLGN4X) [15] and HDC [9]. These genes may be responsible for TS phenotype with a autosomal dominant Mendelian fashion [9]. However, the exact role of these genes are not well confirmed by following studies or all of these probably represents a minority of TS [7,15]. Given that four variants (MRPL3 5′UTR, DNAJC13 2′UTR, and OFCC1 3′UTR) and c.-5A>G) were found in patients with chronic tic phenotype, whereas they were absent in normal controls, we investigated these variants in our well-characterized cohort of 132 Chinese Han subjects with TS-CTD. Although with a great interest, none of these 132 patients were found to carry these four variants, except that a known rs79534309 variant was found. Thus, MRPL3 5′UTR, DNAJC13 2′UTR, and OFCC1 3′UTR and c.-5A>G variants may not represent common causes of TS-CTD in Chinese Han ethnicity. Our study suggests that a recent report of those variants in the MRPL3, DNAJC13 and OFCC1 gene are rare cause of TS-CTD in Chinese Han group. To our knowledge, this is the first study which assesses the role of MRPL3 5′UTR, DNAJC13 2′UTR, and OFCC1 3′UTR in a large cohort of Chinese Han patients with TS-CTD. However, this study does not exclude that mutations in other exons and regulatory regions in the MRPL3, DNAJC13 and OFCC1 gene may result in TS-CTD in our patients. Probably, MRPL3 5′UTR, DNAJC13 2′UTR, and OFCC1 3′UTR and c.-5A>G variants concern a small set of patients with TS-CTD, originating from some focal localities. Also, it implies that genetic screening of these four recently identified variants may not be of widespread use for diagnostics in clinical practice in Chinese Han TS-CTD population [7].

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References


Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>Forward primer (5′ → 3′)</th>
<th>Reverse primer (5′ → 3′)</th>
<th>Product size (bp)</th>
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<td>MRPL3</td>
<td>5′UTR</td>
<td>ATGGTGGAAGTACGATCTTTT</td>
<td>TAATACCTGTTCCAAAGG</td>
<td>144</td>
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<tr>
<td>DNAJC13</td>
<td>3′UTR</td>
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<td>AAGGCGATGATAACCGGAAT</td>
<td>132</td>
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<tr>
<td>OFCC1</td>
<td>3′UTR</td>
<td>GCTGCGAATACTGACCTTTT</td>
<td>GCTGCCAGCTTCAATTTCGTTT</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td>c.-5A&gt;G</td>
<td>TGGTGAATTTGACATTTT</td>
<td>CAAACTGAAAGCTCTGCTC</td>
<td>175</td>
</tr>
</tbody>
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