Short communication

No evidence of association between the LINGO4 gene and essential tremor in Chinese Han patients

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ABSTRACT

Essential tremor (ET) is shown an autosomal dominant mode of inheritance, with no disease-causing gene has been found. Genetic variations in the leucine-rich repeat and Ig domain containing nogo receptor-interacting protein genes (LINGO1 and LINGO2) were reported to be associated with an increased risk of developing ET. To explore whether the LINGO4 gene (a homologous gene of the LINGO1 and the LINGO2 genes) plays a role in ET susceptibility, we performed genetic analysis of coding region of the LINGO4 gene in 100 patients with ET from Mainland China. Two nucleotide variants had been identified: (1) T > A transition (rs61746299), predicted to lead to the amino acid change Thr444Ser, and (2) C > T transition (rs1521179), located 12 bp downstream to the end of coding region. To evaluate whether these variants are related to ET susceptibility, we investigated a total of 150 Chinese Han ET patients (77 familial ET and 73 sporadic ET) and 300 sex, age and ethnicity matched normal controls. No significant differences in genotypic and allele distributions between patients and control subjects for the two variants (p = 0.531 and p = 0.867 for genotypic distributions; p = 1.000 and p = 0.844 for allele distributions) were observed, suggesting variants in coding region of the LINGO4 gene may play litter or no role in the risk of ET susceptibility.

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1. Introduction

Essential tremor (ET) is one of the most common neurological disorders, characterized with tremor in posture and movement. The prevalence of ET has been reported to range from 4% in the population aged 40 years or older to 14% in those 65 years or older [1]. Family studies frequently show a pattern consistent with an autosomal dominant mode of inheritance and three gene loci (ETM1 on 3q13.3, ETM2 on 2p24.1, and ETM3 on 6p23) have been identified as susceptibility loci for ET [1–3]. Though genes causing the associated neurological disorders have provided insight into the genetics of ET, no causative gene for monogenic form has been found [1]. Furthermore, the previously reported association of the dopamine D3 receptor gene (DRD3) Ser9Gly variant and ET has also not been consistently replicated [4]. Recently, a genome-wide association study found variant rs9652490 in the leucine-rich repeat and Ig domain containing nogo receptor-interacting protein 1 gene (LINGO1) was associated with the risk of ET [5]. Although some subsequent studies were unable to show this correlation in some ethnicities including Chinese [6], most reports indicated that variants in the LINGO1 gene were associated with ET susceptibility in Caucasians, especially variant rs9652490 [5,7]. Additionally, the LINGO2 gene, a homologue of LINGO1, was also found to be associated with ET [8,9]. Intriguingly, the LINGO4 gene, a member of Lingo/LEARN gene family, was detected in the neural tube in a subset of progenitors adjacent to the motor neurons, and its encoding protein LINGO4 protein was 44% of amino acid consistency with LINGO1 [10]. Given that the LINGO4 gene is a potential candidate gene for ET susceptibility, we evaluated a large cohort of Chinese Han patients with ET from Mainland China.

2. Methods

2.1. Study subjects

One hundred and fifty unrelated Chinese Han patients (male/female = 85/65; age 51.8 ± 18.1 years; onset age 41.6 ± 19.3 years) with ET, and 300 age, gender and ethnicity matched normal controls (male/female = 173/127; age 52.3 ± 16.6 years; health check-up or community volunteers) without any family history of
neurological disorders were included in this study. All subjects underwent a medical history questionnaire and a family history questionnaire. Among the 150 ET patients, 77 had first- or second-degree relatives affected with ET (familial, male/female: 39/38) and 73 cases (sporadic, male/female: 46/27) had no family history. All subjects were enrolled in the sequence and evaluated at Department of Neurology, the Third Xiangya Hospital of Central South University by two independent neurologists. The diagnosis of ET was made based on internationally accepted clinical criteria [11]. The presence of bradykinesia or any other sign of parkinsonism (except isolated rest tremor) was an exclusionary criterion for ET. The protocol of this study was approved by the Ethics Committee of the Third Xiangya Hospital, Central South University and all the examinees signed informed consent.

2.2. Mutation analysis

A two-step screening strategy was conducted in this study. In the first step, mutation in the coding region and flanking sequence of the LINGO4 gene was screened in 100 ET patients (male/female = 50/50; age 33.4 ± 15.8 years; onset age 43.3 ± 18.1 years). In the second step, the risk of two variants (rs61746299 and rs1521179) was evaluated between enlarged ET group (150 cases including 100 cases from the first study) and normal controls (300 cases) to increase statistical sensitivity. Genomic DNA was isolated from lymphocytes using standard phenol-chloroform method. PCR amplification of the LINGO4 gene using a 9700 Thermal cycler System (ABI), for 35 cycles at 95°C for 30 s, 58°C for 35 s, 72°C for 40 s, and a final extension step at 72°C for 5 min, 100 ng of DNA and 10 pmol primers were used in a 25 μl reaction volume. The primers used for PCR amplification cover all coding regions of LINGO4 (Table 1), and single-strand conformation polymorphism (SSCP) was employed. PCR products exhibiting the abnormally shifted bands in SSCP were sequenced bi-directionally using ABI 3500 genetic analyzer [12]. Sequenced controls (include wild-type, heterozygous and homozygous) and negative control (with no DNA sample) were set in every experiment for case-control study of these two variants.

2.3. Statistical analyses

The power of the study was calculated a priori using Power and Sample Size Program. The power to detect association with the disorder in 150 cases and 300 controls was estimated over 0.8 (0.878 for rs61746299 and 0.812 for rs1521179). A two-step screening strategy was conducted in this study. In the first step, mutation in the coding region and flanking sequence of the LINGO4 gene was screened in 100 ET patients (male/female = 50/50; age 33.4 ± 15.8 years; onset age 43.3 ± 18.1 years). In the second step, the risk of two variants (rs61746299 and rs1521179) was evaluated between enlarged ET group (150 cases including 100 cases from the first study) and normal controls (300 cases) to increase statistical sensitivity. Genomic DNA was isolated from lymphocytes using standard phenol-chloroform method. PCR amplification of the LINGO4 gene using a 9700 Thermal cycler System (ABI), for 35 cycles at 95°C for 30 s, 58°C for 35 s, 72°C for 40 s, and a final extension step at 72°C for 5 min, 100 ng of DNA and 10 pmol primers were used in a 25 μl reaction volume. The primers used for PCR amplification cover all coding regions of LINGO4 (Table 1), and single-strand conformation polymorphism (SSCP) was employed. PCR products exhibiting the abnormally shifted bands in SSCP were sequenced bi-directionally using ABI 3500 genetic analyzer [12]. Sequenced controls (include wild-type, heterozygous and homozygous) and negative control (with no DNA sample) were set in every experiment for case-control study of these two variants.

3. Results

Two variants (rs61746299 and rs1521179) were identified by the first step PCR-SSCP and sequencing analysis of the LINGO4 gene. Rs61746299 is a coding variant, predicted to lead to a change from threonine into serine at amino acid position 444 (Thr444Ser). Whereas rs1521179, located 12 bp downstream to the end of coding region, with no slicing site change. In our extended study of rs61746299 and rs1521179 variants in 150 Chinese Han ET patients (77 familial ET and 73 sporadic ET) and 300 age, gender and ethnicity matched normal controls, no statistically significant differences between the patients and the controls were found in terms of the mean age and sex. Distributions of the genotypes in

Table 1 Primer sequences for the LINGO4 gene.

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Forward primer (5’→3’)</th>
<th>Reverse primer (5’→3’)</th>
<th>Product size (bp)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>CCGTCATCTGAAAGCCACTT</td>
<td>GCTGACTGTCGACGTGCAACT</td>
<td>254</td>
</tr>
<tr>
<td>2</td>
<td>CCGTCATCTGAAAGCCACTT</td>
<td>GCTGACTGTCGACGTGCAACT</td>
<td>254</td>
</tr>
<tr>
<td>3</td>
<td>CAGTGCCCTCAAAAGCTAC</td>
<td>AGGCACCTGCAGTTGGTGTTG</td>
<td>277</td>
</tr>
<tr>
<td>4</td>
<td>CAACACTGCTGGATTTCGTC</td>
<td>TCTAGATTGGACGGACTGAT</td>
<td>300</td>
</tr>
<tr>
<td>5</td>
<td>CATCTGCTGGAGGTCGGCGA</td>
<td>GTCCTGAAGGGCTTCTGCTG</td>
<td>298</td>
</tr>
<tr>
<td>6</td>
<td>ATGGCCTACTCTCTGCTG</td>
<td>CACGACGGGCTGATTCGTC</td>
<td>277</td>
</tr>
<tr>
<td>7</td>
<td>CATCTGCTGGAGGTCGGCGA</td>
<td>GTCCTGAAGGGCTTCTGCTG</td>
<td>298</td>
</tr>
<tr>
<td>8</td>
<td>TACGCTACTCTGCTCTGCT</td>
<td>ATGGCCTACTCTCTGCTG</td>
<td>277</td>
</tr>
<tr>
<td>9</td>
<td>TACTGGCAACACACTACAC</td>
<td>GGTATCTGAAAGCCACTG</td>
<td>297</td>
</tr>
</tbody>
</table>

Table 2 Genotypic and allelic distributions of the LINGO4 gene variants in ET patients and controls.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Allele</th>
<th>Total ET (n = 150)</th>
<th>Familial ET (n = 77)</th>
<th>Sporadic ET (n = 73)</th>
<th>Controls (n = 300)</th>
<th>p-value (c2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>T</td>
<td>39</td>
<td>13</td>
<td>29</td>
<td>93</td>
<td>0.531(1.265)</td>
</tr>
<tr>
<td>rs61746299</td>
<td>AC</td>
<td>T/C</td>
<td>42</td>
<td>25</td>
<td>17</td>
<td>123</td>
<td>0.472(1.502)</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>C</td>
<td>12</td>
<td>13</td>
<td>9</td>
<td>72</td>
<td>0.709(0.871)</td>
</tr>
<tr>
<td>rs1521179</td>
<td>TT</td>
<td>T</td>
<td>12</td>
<td>13</td>
<td>9</td>
<td>72</td>
<td>0.580(1.025)</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>T/C</td>
<td>12</td>
<td>13</td>
<td>9</td>
<td>72</td>
<td>0.580(1.025)</td>
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<td>TT</td>
<td>T</td>
<td>12</td>
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<td>150</td>
<td>77</td>
<td>73</td>
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</table>

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ET and control groups were both in Hardy–Weinberg equilibrium. There was no significant difference in genotypic distributions between disease group and control subjects ($\chi^2 = 1.265, p = 0.531$ for rs61746299; $\chi^2 = 0.285, p = 0.867$ for rs1521179), and the allele frequencies of disease group did not differ significantly ($\chi^2 = 0.000, p = 1.000$ for rs61746299; $\chi^2 = 0.039, p = 0.844$ for rs1521179) compared with the panel of healthy controls (Table 2). The rs61746299 variant was in linkage with the rs1521179 variant ($\chi^2 = 0.78$). After stratifying by family history and age of onset, no significant differences in genotypic and allele distributions for both SNPs were found between ET groups (family ET, sporadic ET; younger ET, age $\leq 40$; older ET, age $> 40$) and matched controls (Table 2).

4. Discussion

Variant rs9662490 of the LINGO1 gene has been reported to confer risk of ET in a genome-wide association study, and subsequent studies indicated that this association was detectable in ET patients from Iceland, Germany, France, Austria, North America, etc [5,7]. Other single-nucleotide polymorphisms (SNPs) in this gene, including rs177088, rs13313467, and rs80288908, were also found to be associated with subtypes of ET [7,13], although several research groups were unable to identify the same association between the LINGO1 gene variants and ET patients from certain geographical regions including China [6]. Intriguingly, variants (rs1412229, rs108812774, and rs7033345) in the LINGO2 gene were also reported to be associated with ET [9]. There are three homologues of LINGO1 in the NCBI database, designed LINGOs 2–4. In mouse embryo, Lingo1 mRNA is expressed in both central nervous system (CNS) and peripheral nervous system (PNS). Lingo2 and Lingo4 are detected in both CNS and nasal placode though the level of expression was lower than Lingo1. Lingo3 mRNA was not detected in either CNS or PNS in the study by Homma et al., though detectable Lingo3 expression in a specific tissue or stage was reported by Haines et al. [10].

The LINGO4 gene, mapped on chromosome 1q21.3 contains 2 exons spanning at least 5 kb. The open reading frame (ORF) consists of 1782 nucleotides coding for a protein of 593 amino acids. The protein has an Ig-like C2-type (immunoglobulin-like) domain and 13 LRR (leucine-rich) repeats, locating in membrane and single-pass type I membrane protein [10].

The LINGO4 gene is one of the homologues of LINGO1 gene, and as it is known that members of a gene family often have the same or analogous structures and functions. The homologous genes may play similar roles in development of the disorders [9]. We have, therefore, screened 100 Chinese Han ET patients to determine whether ET is pathogenically related to variants in the LINGO4 gene. We identify SNPs, not potentially producing an acceptor site (predicted by http://www.hgsc.bcm.tmc.edu). Further analysis of these two variants in 150 ET patients and 300 normal controls indicates that these variants do not increase or decrease the risk of ET. Our results suggest that polymorphisms in the coding region and flanking sequence of the LINGO4 gene do not seem to be a major genetic cause of ET in Chinese Han subjects. To our knowledge, this is the first study of possible link between the LINGO4 gene and human disorder. However, given that variant in noncoding region may exert a role on the regulation of gene expression or affect transcription factor binding, native splicing, etc., we can not exclude variants in noncoding region and epigenetic factor of the LINGO4 gene may be involved in the development of ET. Despite our negative results, studies of untranslated regions in the LINGO4 gene, and other candidate genes which regulate expression of this gene, candidate genes in the ETM region, construction of the LINGO4 gene deficiency animal model may shed new light on the genetic factors involved in ET [1].

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgement

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