EIF4G1 Ala502Val and Arg1205His variants in Chinese patients with Parkinson disease

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HIGHLIGHTS
• The EIF4G1 p.Ala502Val and p.Arg1205His variants are a rare cause of PD in Chinese.
• The first study of p.Ala502Val and p.Arg1205His in a large cohort of Chinese PD.
• The findings will stimulate researches about pathogenic role of the EIF4G1 gene.

A B S T R A C T
Growing evidences show that genetic abnormalities play an important role in the etiopathogenesis of Parkinson disease (PD). At least 18 genetic loci and 13 disease-related genes for parkinsonism have been identified. Recently, the p.Ala502Val and p.Arg1205His variants in the eukaryotic translation initiation factor 4-gamma 1 gene (EIF4G1) were found to be associated with PD. To evaluate whether the EIF4G1 p.Ala502Val and p.Arg1205His variants are related to PD in Chinese Han population, we conducted genetic examination of these two variants in 425 PD patients from Mainland China and none was found in our patients. We did identify a known non-pathogenic polymorphism c.3660C>T (p.Ala1220Ala, rs143852330) in a 73-year-old male patient. Our results, consistent with other recent reports, suggest that the EIF4G1 p.Ala502Val and p.Arg1205His variants are a rare cause of PD, at least in Chinese population.

Parkinson disease (PD; MIM 168600) is the second most common neurodegenerative disorder after Alzheimer’s disease. It is characterized by the loss of dopamine-producing neurons in the substantia nigra and other brainstem nuclei, culminating in troublesome symptoms, including rest tremor, bradykinesia, rigidity, postural instability, and other motor and non-motor symptoms [5,6,13]. There is growing evidence that genetic abnormalities play an important role in the etiopathogenesis of PD [3,5,7,9,10]. To date, at least 18 genetic loci and 13 disease-related genes for parkinsonism have been identified through linkage analyses of family members (PARK1-15) or genome-wide association studies of index patients (PARK16-18) [3]. Recently, the eukaryotic translation initiation factor 4-gamma 1 gene (EIF4G1; MIM 600495), was reported responsible for PARK18. Two variants of the EIF4G1 gene, p.Ala502Val and p.Arg1205His were identified in four and eight different families from different countries, sharing the same ancestral founder, respectively [2]. To determine the frequency and the pathogenicity of the EIF4G1 p.Ala502Val and p.Arg1205His variants in Chinese Han patients with PD, we conducted genetic examination of 425 patients with PD from Mainland China.

Four hundred and twenty-five unrelated Chinese Han patients with PD from Mainland China were enrolled in this study. The mean age of the 425 PD patients was 66.1 ± 10.9 years (male/female: 283/162) and the mean age at onset of PD symptoms was 62.6 ± 12.2 years. Among the 425 PD patients, 119 (28%) had first- or second-degree relatives affected with PD (familial PD; mean age at onset 59.8 ± 12.9 years; male/female: 72/47), 306 cases (72%) had no family history (sporadic PD; mean age at onset 63.1 ± 12.0 years; male/female: 191/115). 44.7% (190/425) of the patients were
screened and found to be negative for the vacuolar protein sorting 35 gene (VPS35) mutation [4]. The diagnosis of PD was made based on clinical criteria, and rest tremor, bradykinesia, rigidity and postural instability were generally considered the cardinal signs of PD. Several features, such as tremor, early gait abnormality, postural instability, pyramidal tract findings and response to levodopa, were used to differentiate PD from other parkinsonian disorders [13]. All participants have signed an informed consent, approved by the Ethics Committee of the Third Xiangya Hospital, Central South University, China.

Genomic DNA was extracted from lymphocytes using standard phenol–chloroform method [5]. Genetic analysis of the EIF4G1 p.Ala502Val and p.Arg1205His variants was performed in 425 PD patients and the method was described previously [15]. The primers used for PCR amplification are listed in Table 1. PCR products were generated with 100 ng of gDNA in 2.5 μL 10× PCR buffer, 2.0 μL of 2.5 mmol/L each dNTP, 1.5 μL of 25 mmol/L MgCl2, 1 μL of 10 μmol/L each primer and 1 μL Taq polymerase in a total volume of 25 μL. PCR amplified the EIF4G1 gene by using a GeneAmp 9700 thermal cycler system (Applied Biosystems, Foster City, CA, USA), and PCR conditions were 95 °C for 3 min, followed by 35 cycles of 95 °C for 40 s, 58 °C for 35 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, Fermentas) and 8 U exonuclease I (Fermentas) in a 10 μL reaction volume, and sequenced directionally using an 8-capillary 3500 genetic analyzer (Applied Biosystems, Foster City, CA, USA) [8].

We did not detect any p.Ala502Val or p.Arg1205His variant in the EIF4G1 gene in any of the 425 PD patients, but we did identify a known variant c.3660C>T (p.Ala1220Ala, rs143852330) in a 73-year-old male patient. The variant was also predicted to not result in an altered splicing site (http://www.fruitfly.org/seq_tools/splice.html), indicating that it was not a pathogenic variant.

Accumulating evidence indicates that genetic abnormalities play an important role in the etiopathogenesis of PD. The past 15 years has witnessed dramatic progress in the genetic basis of PD by discovery of at least 13 disease-related genes for parkinsonism [3,5,7,9,10,14,23].

Human EIF4G1 gene, located on chromosome 3q27.1 spanning over 20 kb, is a 33-exon gene which includes 31 coding exons encoding 1599 amino acids. The protein elf4G1, existing as multiple isoforms generated by alternative translation initiation codons or alternative splicing, regulates the translation initiation of mRNAs encoding bioenergetic, cell survival and growth genes in response to different stresses [1,2,18]. The complex comprised by elf4A, elf4E and elf4G1 is referred to as elf4F [17]. It facilitates the recruitment of mRNA to the ribosome, which is the rate-limiting step for protein synthesis under normal physiological conditions. Recognition of the mRNA cap structure and the ATP-dependent unwinding of mRNA secondary structure during the initiation phase of protein synthesis are catalyzed by initiation factors of the elf4F complex [24]. Binding of the elf4F to the cap structure can be hindered by elf4E-binding proteins (4E-BPs), which compete with elf4F for a shared binding site on elf4E, consequently 4E-BPs inhibit cap-dependent translation. 4E-BP binding to elf4E is controlled by phosphorylation through the mammalian target of rapamycin (mTOR), and phosphorylation of 4E-BP weakens its interaction with elf4E [2,21]. elf4G1 contains binding sites for other members of the elf4F and serves as a scaffold for elf4E and elf4A to coordinate their functions [12]. It has three domains roughly equivalent in size: the N-terminal part as defined by its cleavage by picornaviral proteases, the middle ‘core’ domain critical for assembly of the translation machinery and the carboxy-terminal domain playing a modular role in translation [17]. The N-terminal can bind the poly(A)-binding protein and inhibit poly(A)-dependent translation, but has no effect on translation of a deadenylated mRNA [11]. elf4G1 is also the target for cleavage during picornavirus infection, an event responsible for the inhibition of host cellular mRNA translation [1,24]. Its depletion phenocopies nutrient starvation or inhibition of protein kinase mTOR, and promotes autophagy [18]. In a mouse model of PD constructed by 6-hydroxydopamine, inhibition of mTOR complex 1 signaling by rapamycin may prevent L-DOPA-induced dyskinesia [19].

Recently, variants in the EIF4G1 gene were reported to be responsible for PARK18. p.Ala502Val or p.Arg1205His substitution of the elf4G1 protein affected its scaffold function and disrupted elf4E or elf4F binding, consequently it impaired the ability of cells to rapidly and dynamically respond to stress, presumably through changes in the translation of existing mRNAs essential to cell survival [2], supporting these two variants play a role in the development of PD. However, these two variants were also observed in several normal controls [20,22], and it may be explained by the possibility of development of PD in the future, incomplete penetrance, or conferring a risk for PD. In this study, we investigated the p.Ala502Val and p.Arg1205His variants in the EIF4G1 gene in 425 Chinese subjects with PD. Although with a great interest, we did not find these variants in the EIF4G1 gene in our well-characterized cohort of 425 PD patients, consistent with the recent reports that these two variants were rare in patients with PD (Table 2) [16,20,22,25]. We did identify a known polymorphism c.3660C>T (p.Ala1220Ala) in a 73-year-old

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### Table 1

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Variant</th>
<th>Exon</th>
<th>Forward primer (5′ → 3′)</th>
<th>Reverse primer (5′ → 3′)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p.Ala502Val</td>
<td>10</td>
<td>GCAAGCCAGCACGACGACGA</td>
<td>CTAGCCGCCCTATTCTTCAC</td>
<td>161</td>
</tr>
<tr>
<td>2</td>
<td>p.Arg1205His</td>
<td>24</td>
<td>GCCCTCAGCAAGGAATTGACAG</td>
<td>TCAGTCTCTCCAGCAACA</td>
<td>212</td>
</tr>
</tbody>
</table>

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### Table 2

<table>
<thead>
<tr>
<th>Reference</th>
<th>Detection region</th>
<th>Case</th>
<th>Geographic distribution/ethnic background</th>
<th>Variant detected</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chartier-Harlin et al. [2]</td>
<td>Coding regions</td>
<td>4483 PD</td>
<td>United States, Poland, etc.</td>
<td>p.Ala502Val</td>
<td>4/4483</td>
</tr>
<tr>
<td></td>
<td>Coding regions</td>
<td>4719 PD</td>
<td>France, United States, Canada, Ireland, Italy, Tunisia, etc.</td>
<td>p.Arg1205His</td>
<td>19/4719</td>
</tr>
<tr>
<td>Tucci et al. [22]</td>
<td>Exon 10 and exon 24</td>
<td>150 FPD</td>
<td>United Kingdom</td>
<td>No</td>
<td>0/150</td>
</tr>
<tr>
<td>Lesage et al. [16]</td>
<td>Coding regions</td>
<td>251 FPD</td>
<td>French</td>
<td>No</td>
<td>0/251</td>
</tr>
<tr>
<td>Schulte et al. [20]</td>
<td>33 exons</td>
<td>376 PD</td>
<td>German</td>
<td>No</td>
<td>0/376</td>
</tr>
<tr>
<td>Zhao et al. [25]</td>
<td>33 exons</td>
<td>96 PD</td>
<td>Asian</td>
<td>No</td>
<td>0/96</td>
</tr>
<tr>
<td>Our study</td>
<td>p.Ala502Val and p.Arg1205His</td>
<td>119 FPD, 306 SPD</td>
<td>Chinese Han</td>
<td>No</td>
<td>0/425</td>
</tr>
</tbody>
</table>

PD: Parkinson disease; FPD: familial PD; SPD: sporadic PD.
male patient, reported in SNP database (rs1438522330), with a frequency of 0.1%, derived from population cohorts participating in the National Heart, Lung, and Blood Institute (NHBLI) Exome Sequencing Project (http://www.ncbi.nlm.nih.gov/), suggesting it was a non-pathogenic variant.

Our study showed that the EIF4G1 p.Ala502Val and p.Arg1205His variants were rare in Chinese Han patients. To our knowledge, this is the first study of the EIF4G1 p.Ala502Val and p.Arg1205His variants in a large cohort of Chinese patients with PD from Mainland China. However, our study does not exclude other variants in the EIF4G1 gene causing PD in our subjects. Further investigations are required to evaluate whether these variants have diagnostic utility.

Acknowledgments

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References