

## Research Article

# Mutation Analysis of *HTRA2* Gene in Chinese Familial Essential Tremor and Familial Parkinson's Disease

Ya-Chao He, Pei Huang, Qiong-Qiong Li, Qian Sun, Dun-Hui Li, Tian Wang, Jun-Yi Shen, Juan-Juan Du, Shi-Shuang Cui, Chao Gao, Rao Fu, and Sheng-Di Chen

Department of Neurology and Collaborative Innovation Center for Brain Science, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

Correspondence should be addressed to Sheng-Di Chen; [chen\\_sd@medmail.com.cn](mailto:chen_sd@medmail.com.cn)

Received 9 November 2016; Revised 16 December 2016; Accepted 26 December 2016; Published 24 January 2017

Academic Editor: Hélio Teive

Copyright © 2017 Ya-Chao He et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Background.** *HTRA2* has already been nominated as PARK13 which may cause Parkinson's disease, though there are still discrepancies among these results. Recently, Gulsuner et al.'s study found that *HTRA2* p.G399S is responsible for hereditary essential tremor and homozygotes of this allele develop Parkinson's disease by examining a six-generation family segregating essential tremor and essential tremor coexisting with Parkinson's disease. We performed this study to validate the condition of *HTRA2* gene in Chinese familial essential tremor and familial Parkinson's disease patients, especially essential tremor. **Methods.** We directly sequenced all eight exons, exon-intron boundaries, and part of the introns in 101 familial essential tremor patients, 105 familial Parkinson's disease patients, and 100 healthy controls. **Results.** No exonic variant was identified, while one exon-intron boundary variant (rs2241028) and one intron variant (rs2241027) were detected, both with no clinical significance and uncertain function. There was no difference in allele, genotype, and haplotype between groups. **Conclusions.** *HTRA2* exonic variant might be rare among Chinese Parkinson's disease and essential tremor patients with family history, and *HTRA2* may not be the cause of familial Parkinson's disease and essential tremor in China.

## 1. Introduction

As two of the most prevalent tremor disorders, essential tremor (ET) and Parkinson's disease (PD), which are estimated to constitute 0.9% and 0.3% of worldwide population, respectively, are considered as distinctively different entities formerly [1, 2]. Several lines of evidence showed that there are remarkable overlaps in clinical features, epidemiology, imaging, genetics, and pathology between PD and ET, including a fourfold increase of risk developing Parkinson's disease in essential tremor cases [3, 4].

ET is widely regarded as caused by genetic with no disease-causing gene ever been focused; Contrarily, though PD is mainly sporadic, up to now 22 PARK loci have been identified [5, 6]. To be specific, 50% of ET patients demonstrate familial aggregation, while less than 15% of PD patients have affected first-degree relatives [7–9]. Due to the overlap phenomena between ET and PD, investigations into the relationship between PD risk variants and ET patients have

been done, involving *LINGO1*, *LINGO2*, *LRRK2*, *SLCIA2*, and *HTRA2* genes [3, 10–12].

*HTRA2* has already been nominated as PARK13 which may cause Parkinson's disease, though there are still discrepancies among these results. Recently, a research by Gulsuner and colleagues examining a six-generation family segregating ET and ET coexisting with PD revealed that *HTRA2* p.G399S is responsible for hereditary essential tremor and homozygotes for this allele develop Parkinson's disease [13]. Replications conducted in Western Norway and Asian population to address the association between p.G399S and ET, PD, ET/PD, and tremulous cervical dystonia failed to reach a consensus [14, 15]. In addition, report from a small sample (29 FETs) in Germany adopting coding exon Sanger sequencing did not reconfirm it either [16]. To validate the condition in Chinese familial essential tremor (FET) and familial Parkinson's disease (FPD) patients, we performed a Sanger sequencing of eight exons and exon-intron boundaries of *HTRA2* instead of just one variant (p.G399S).

TABLE 1: Demographics of participants.

| Details                               | FET                         | FPD                         | ET-PD                    | Control                  |
|---------------------------------------|-----------------------------|-----------------------------|--------------------------|--------------------------|
| Total                                 | 101                         | 105                         | 15                       | 100                      |
| Age <sup>a</sup> (range, $p^b$ value) | 61.24 ± 12.62 (28–90, 0.12) | 59.28 ± 11.21 (36–84, 0.86) | 67.80 ± 8.65 (56–79, NA) | 59.06 ± 6.21 (49–74, NA) |
| Male/female, $p^b$ value              | 51/50, 0.52                 | 53/52, 0.52                 | 12/3, N/A                | 46/54, N/A               |

N/A: not applicable; <sup>a</sup>data are mean ± SD; <sup>b</sup>data are compared with control; FPD: familial PD; FET: familial ET; ET-PD: ET coexisting with PD.

TABLE 2: Primers of HTRA2.

| Name | Forward                        | Reverse                     | Products                                   |
|------|--------------------------------|-----------------------------|--|
| 1    | GTC TCA CAA CTC GCG TCC G      | GCC TGA AAT GGA GGG AAA GCA | Exon 1 and boundaries                      |
| 2    | TCG AGA TCC TGG ACC GGT AA     | GGC CAC ATT TTT GCA GCC TAA | Exons 2, 3 and intron 2; boundaries        |
| 3    | GCA GCT ATT GAT GTG CGT CC     | TGA AGG GAG ACA GCT CTT GTG | Exons 4, 5, 6 and introns 4, 5; boundaries |
| 4    | ACT CAG CCA ACC TGA TTT CCT AC | TTC AGA GCC CAG GAG TCA GT  | Exons 7, 8 and intron 7; boundaries        |

## 2. Methods

**2.1. Patients.** This study enrolled 221 unrelated Chinese patients, including 105 PD patients with autosomal dominant inheritance (2 or more affected relatives in 2 consecutive generations), 101 ET patients with family history, and 15 patients of ET coexisting with PD. All patients were from the Movement Disorder Clinic of Department of Neurology at Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine. PD and ET patients were diagnosed by senior movement disorder specialist on the basis of MDS clinical diagnostic criteria for Parkinson's disease and Consensus Statement on Tremor of the Movement Disorders Society, respectively [17, 18]. Patients presenting secondary Parkinsonism, Parkinson-plus syndrome, or hyperthyroidism were excluded from the study. We also included 100 healthy controls without any symptom of movement disorders. The demographic information of patients is shown in Table 1. We received approval from the Ethics Committee of Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine. Written informed consents were obtained from all patients and controls participating in the study as well.

**2.2. DNA Sequencing and Mutation Analysis.** Genomic DNA was extracted from venous blood applying standardized phenol/chloroform extraction method from patients and controls. The 8 coding sequences, exon-intron boundaries, and part of introns were sequenced by Sanger sequencing in 4 products of PCR (polymerase chain reaction) amplification using 4 pairs of primers (Table 2). DNASTAR Lasergene MegAlign (v7.1.0) and Chromas (v2.33) were used to conduct sequence alignment, and the chromatograms were double checked to avoid missing any variants. Variants detected were searched in NCBI to get access to their clinical significance and MAF in ExAC and 1000 Genomes Projects database.

**2.3. Statistical Analysis.** Statistical analysis was performed with Statistical Analysis System V8 (SAS V8). Difference of age was assessed applying  $t$ -test or  $t'$ -test. Hardy-Weinberg equilibrium (HWE) was calculated by Chi-square analysis. Chi-square or Fisher's exact test was used to test the differences in genotype and gender between groups. Odds ratios

(ORs) and 95% confidence intervals (95% CI) were evaluated by Mantel-Haenszel Chi-squared test to verify the association between variants and FPD or FET. The evaluation of the association was also conducted using logistic regression under different genetic models adjusted for age and gender. Online SHEsis program was used to conduct haplotype analysis [19]. Two-tailed  $p$  value < 0.05 was considered significant. The statistical power was performed using Quanto.

## 3. Results

The patients and controls in the study are well matched for mean age ( $p = 0.12$  for FET and  $p = 0.86$  for FPD) and sex distribution ( $p = 0.52$  for FET and  $p = 0.52$  for FPD) (Table 1). By sequencing all the four products in all 221 patients (FET, FPD, and ET-PD) and 100 controls, no exonic variant was identified, while one exon-intron boundary variant (rs2241028) and one intron variant (rs2241027) were detected. In NCBI SNP database, MAF of rs2241027 and rs2241028 were 0.05/0.10, 0.06/0.07, respectively, from ExAC/1000 Genomes Project, both with no clinical significance. The function of both variants was defined as uncertain by MyGenostics. The variants distribution was within the range of Hardy-Weinberg equilibrium in controls ( $p = 0.82$ , 0.71 resp., Table 3). Given the present sample sizes, we have 80% power to detect an odds ratio of 1.83 in both PD and ET for rs2241027 adopting an additive model and OR of 1.91 in both PD and ET for rs2241028 adopting an additive model. What is worth noting is that there are big differences in MAFs between our control and database in both two variants, which may be caused by ethnical diversity, so we calculate the power considering MAFs of 0.28 and 0.21, respectively, in our control, which is higher than in database; otherwise, it would require much bigger sample sizes. Additionally, we only have 34% power to detect an OR of 1.44 (the OR in Krüger et al.'s study) for rs2241028.

As for allele and genotype distribution of both variants, we failed to detect any significant differences either in FET versus controls or in FPD versus controls (Tables 3 and 4). No significant difference was observed in the logistic regression either (data not shown). Moreover, haplotypes of two variants showed hardly any association with the risk

TABLE 3: Statistics of minor allele frequency (MAF).

| RS number | Position             | Function  | FET  | MAF  |       |             | Control   | ExAC/1000   |                        | HWE ( $p^a$ )          | FET versus control | OR (95% CI), $p$ value |
|-----------|----------------------|-----------|------|------|-------|-------------|-----------|-------------|------------------------|------------------------|--------------------|------------------------|
|           |                      |           |      | FPD  | ET-PD | Genomes MAF |           | Genomes MAF | FPD versus control     |                        |                    |                        |
| rs2241027 | Intron               | Uncertain | 0.33 | 0.29 | 0.33  | 0.28        | 0.05/0.10 | 0.82        | 1.28 (0.83-1.96), 0.26 | 0.80 (0.47-1.35), 0.40 |                    |                        |
| rs2241028 | Exon-intron boundary | Uncertain | 0.14 | 0.16 | 0.23  | 0.21        | 0.06/0.07 | 0.71        | 1.08 (0.70-1.66), 0.73 | 0.70 (0.42-1.16), 0.17 |                    |                        |

<sup>a</sup>HWE for controls.

TABLE 4: Statistics of genotype.

| Participants | Genotype (rs2241027/rs2241028) |       |     | $p^a$ value |           |
|--------------|--------------------------------|-------|-----|-------------|-----------|
|              | GG                             | GA    | AA  | rs2241027   | rs2241028 |
| FET          | 44/75                          | 48/23 | 9/3 | 0.50        | 0.29      |
| FPD          | 53/73                          | 43/31 | 9/1 | 0.77        | 0.14      |
| ET-PD        | 8/9                            | 4/5   | 3/1 | N/A         | N/A       |
| Control      | 51/64                          | 43/30 | 6/6 | N/A         | N/A       |

<sup>a</sup>  $p$  value compared with controls.

TABLE 5: Haplotype analysis.

| Haplotype | FET (%) | FPD (%) | Control (%) | $\chi^2$ value <sup>a/b</sup> | Fisher's $p^{a/b}$ | Pearson's $p^{a/b}$ | OR (95% CI) <sup>a/b</sup>        |
|-----------|---------|---------|-------------|-------------------------------|--------------------|---------------------|-----------------------------------|
| A-A       | 0       | 0       | 0           | —                             | —                  | —                   | —                                 |
| A-G       | 33      | 29      | 28          | 1.28/0.12                     | 0.26/0.73          | 0.26/0.73           | 1.28 (0.83–1.96)/1.08 (0.70–1.66) |
| G-A       | 14      | 16      | 21          | 3.05/1.92                     | 0.08/0.17          | 0.08/0.17           | 0.63 (0.38–1.06)/0.70 (0.42–1.16) |
| G-G       | 53      | 55      | 52          | 0.09/0.58                     | 0.77/0.45          | 0.77/0.45           | 1.06 (0.72–1.57)/1.16 (0.79–1.71) |

<sup>a/b</sup> value for FET versus controls/FPD versus controls.

of FET or FPD (Table 5). Regarding ET-PD, in which we attempted to investigate the situation of *HTRA2* in case there were some dramatic mutations, owing to the limitation of sample size, we quitted further statistical analysis.

#### 4. Discussion

The high temperature requirement A2 (*HTRA2*), known as a mitochondria protein, plays distinct different roles in mitochondria homeostasis and cellular apoptosis regulation [20]. As one study indicated, deficiency of *HTRA2* can cause damage and mutation of mitochondria DNA [21]. Another study revealed that *HTRA2* was regulated by *PINK1*, which might contribute to early-onset PD, in the proteolytic activity [22].

Many researches concerning the association of PD with *HTRA2* variants have been done. The earliest mutation screening of *HTRA2* in PD patients was done in a German population after the finding that targeted disruption of *HTRA2* can cause neurodegeneration and a Parkinsonian phenotype in mice, which resulted in the identification of two mutations (G399S and A141S) related to the risk of PD [23, 24]. Later on, replications with contradictory consequences have been conducted [25–31], and one large scale genetic association study is worth noting, which showed no evidence for an overall association of common variants in *HTRA2* with PD [32], while Gulsuner et al.'s study of a six-generation family provides further evidence for the probability of *HTRA2* acting as a cause for PD and ET, especially those with family history [13]. So the aim of our study is to investigate the situation of *HTRA2* by Sanger sequencing of the whole coding sequence in FET, FPD, and ET-PD in Chinese population, especially FET and ET-PD.

Our study detected two variants (rs2241028, rs2241027). Variant rs2241028 has been reported in several studies with similar negative results except for Krüger et al.'s study, in which rs2241028 was considered as a susceptible factor for PD in the Scandinavian population and their descent from

USA [32], while there is no report of this variant in studies about ET. Since rs2241028 is near the splicing region, it may affect the transcript efficiency of *HTRA2* to some extent or influence the expression of *HTRA2* in some other way, so it would be promising to do some research into the function of this variant and the relationship with PD. Variant rs2241027 has never been mentioned in the previous study no matter about PD or ET. Our study showed that neither of two variants was related to the risk of developing ET or PD, and two variants were defined as no clinical significance in database. Meanwhile, we have not detected mutations (G399S and A141S) mentioned in other studies. So we provided no evidence of association of *HTRA2* with FET and FPD. As for ET-PD, the result of our study was not so convincing due to the sample size though we found nothing significant as well. Admittedly, there are some limitations in our study. On the one hand, the sample sizes were only able to detect a moderate correlation with enough power and not for a relatively weaker correlation, which may cause false negative error. On the other hand, it would be more persuasive if the promoter of *HTRA2* gene has been sequenced as well.

In conclusion, *HTRA2* might not be a cause of familial ET or PD in China. Studies with larger sample size are needed to investigate thoroughly the role of *HTRA2* in ET and ET-PD in China and other places in the world.

#### Competing Interests

The authors report no competing interests.

#### Authors' Contributions

Ya-Chao He and Pei Huang contributed equally to this work as first authors.

#### Acknowledgments

The authors are grateful to all the participants recruited in this study for their donation of blood samples without which

the research could not be conducted. The study was supported by National Natural Science Fund (81430022, 91332107, and 81371407). The Sanger sequencing was performed by MyGenostics and Biosune.

## References

- [1] E. D. Louis and J. J. Ferreira, "How common is the most common adult movement disorder? Update on the worldwide prevalence of essential tremor," *Movement Disorders*, vol. 25, no. 5, pp. 534–541, 2010.
- [2] J.-F. Smouh, P. A. Dion, and G. A. Rouleau, "Genetics of essential tremor: from phenotype to genes, insights from both human and mouse studies," *Progress in Neurobiology*, vol. 119–120, pp. 1–19, 2014.
- [3] M. A. Thenganatt and J. Jankovic, "The relationship between essential tremor and Parkinson's disease," *Parkinsonism and Related Disorders*, vol. 22, supplement 1, pp. S162–S165, 2016.
- [4] J. Benito-León, E. D. Louis, and F. Bermejo-Pareja, "Risk of incident Parkinson's disease and parkinsonism in essential tremor: A Population Based Study," *Journal of Neurology, Neurosurgery and Psychiatry*, vol. 80, no. 4, pp. 423–425, 2009.
- [5] K. Kalinderi, S. Bostantjopoulou, and L. Fidani, "The genetic background of Parkinson's disease: current progress and future prospects," *Acta Neurologica Scandinavica*, vol. 134, no. 5, pp. 314–326, 2016.
- [6] M. Funayama, K. Ohe, T. Amo et al., "CHCHD2 mutations in autosomal dominant late-onset Parkinson's disease: a genome-wide linkage and sequencing study," *The Lancet Neurology*, vol. 14, no. 3, pp. 274–282, 2015.
- [7] S. K. McDonnell, D. J. Schaid, A. Elbaz et al., "Complex segregation analysis of Parkinson's disease: the Mayo Clinic Family Study," *Annals of Neurology*, vol. 59, no. 5, pp. 788–795, 2006.
- [8] N. R. Whaley, J. D. Putzke, Y. Baba, Z. K. Wszolek, and R. J. Uitti, "Essential tremor: phenotypic expression in a clinical cohort," *Parkinsonism and Related Disorders*, vol. 13, no. 6, pp. 333–339, 2007.
- [9] C. Wider, O. A. Ross, and Z. K. Wszolek, "Genetics of Parkinson disease and essential tremor," *Current Opinion in Neurology*, vol. 23, no. 4, pp. 388–393, 2010.
- [10] Y. X. Chao, E. Y. Ng, L. Tan et al., "Lrrk2 R1628P variant is a risk factor for essential tremor," *Scientific Reports*, vol. 5, article 9029, 2015.
- [11] S.-W. Yu, C.-M. Chen, Y.-C. Chen et al., "SLC1A2 variant is associated with essential tremor in taiwanese population," *PLoS ONE*, vol. 8, no. 8, article e71919, 2013.
- [12] E. García-Martín, C. Martínez, H. Alonso-Navarro et al., "No association of the SLC1A2 rs3794087 allele with risk for essential tremor in the Spanish population," *Pharmacogenetics and Genomics*, vol. 23, no. 11, pp. 587–590, 2013.
- [13] H. U. Gulsuner, S. Gulsuner, F. N. Mercan et al., "Mitochondrial serine protease HTRA2 p.G399S in a kindred with essential tremor and Parkinson disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 51, pp. 18285–18290, 2014.
- [14] C. Tzoulis, T. Zayats, P. M. Knappskog et al., "HTRA2 p.G399S in Parkinson disease, essential tremor, and tremulous cervical dystonia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 112, no. 18, Article ID E2268, 2015.
- [15] Y. X. Chao, E. Y. Ng, J. N. Foo, J. Liu, Y. Zhao, and E.-K. Tan, "Mitochondrial serine protease HTRA2 gene mutation in Asians with coexistent essential tremor and Parkinson disease," *Neurogenetics*, vol. 16, no. 3, pp. 241–242, 2015.
- [16] F. Hopfner, S. H. Müller, D. Lorenz et al., "Mutations in HTRA2 are not a common cause of familial classic ET," *Movement Disorders*, vol. 30, no. 8, pp. 1149–1150, 2015.
- [17] R. B. Postuma, D. Berg, M. Stern et al., "MDS clinical diagnostic criteria for Parkinson's disease," *Movement Disorders*, vol. 30, no. 12, pp. 1591–1601, 2015.
- [18] G. Deuschl, P. Bain, and M. Brin, "Consensus statement of the Movement Disorder Society on Tremor. Ad Hoc Scientific Committee," *Movement Disorders*, vol. 13, supplement 3, pp. 2–23, 1998.
- [19] Z. Li, Z. Zhang, Z. He et al., "A partition-ligation-combination-subdivision em algorithm for haplotype inference with multiallelic markers: update of the SHESis (<http://analysis.bio-x.cn>)," *Cell Research*, vol. 19, no. 4, pp. 519–523, 2009.
- [20] L. Vande Walle, M. Lamkanfi, and P. Vandenabeele, "The mitochondrial serine protease HtrA2/Omi: an overview," *Cell Death and Differentiation*, vol. 15, no. 3, pp. 453–460, 2008.
- [21] H.-G. Goo, M. K. Jung, S. S. Han, H. Rhim, and S. Kang, "HtrA2/Omi deficiency causes damage and mutation of mitochondrial DNA," *Biochimica et Biophysica Acta (BBA)—Molecular Cell Research*, vol. 1833, no. 8, pp. 1866–1875, 2013.
- [22] H. Plun-Favreau, K. Klupsch, N. Moiso et al., "The mitochondrial protease HtrA2 is regulated by Parkinson's disease-associated kinase PINK1," *Nature Cell Biology*, vol. 9, no. 11, pp. 1243–1252, 2007.
- [23] K. M. Strauss, L. M. Martins, H. Plun-Favreau et al., "Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease," *Human Molecular Genetics*, vol. 14, no. 15, pp. 2099–2111, 2005.
- [24] J. M. Jones, P. Datta, S. M. Srinivasula et al., "Loss of Omi mitochondrial protease activity causes the neuromuscular disorder of mnd2 mutant mice," *Nature*, vol. 425, no. 6959, pp. 721–727, 2003.
- [25] V. Bogaerts, K. Nuytemans, J. Reumers et al., "Genetic variability in the mitochondrial serine protease HTRA2 contributes to risk for Parkinson disease," *Human Mutation*, vol. 29, no. 6, pp. 832–840, 2008.
- [26] O. A. Ross, A. I. Soto, C. Vilariño-Güell et al., "Genetic variation of Omi/HtrA2 and Parkinson's disease," *Parkinsonism and Related Disorders*, vol. 14, no. 7, pp. 539–543, 2008.
- [27] J. Simón-Sánchez and A. B. Singleton, "Sequencing analysis of OMI/HTRA2 shows previously reported pathogenic mutations in neurologically normal controls," *Human Molecular Genetics*, vol. 17, no. 13, pp. 1988–1993, 2008.
- [28] C.-H. Lin, M.-L. Chen, G. S. Chen, C.-H. Tai, and R.-M. Wu, "Novel variant Pro143Ala in HTRA2 contributes to Parkinson's disease by inducing hyperphosphorylation of HTRA2 protein in mitochondria," *Human Genetics*, vol. 130, no. 6, pp. 817–827, 2011.
- [29] C.-Y. Wang, Q. Xu, L. Weng et al., "Genetic variations of Omi/HTRA2 in Chinese patients with Parkinson's disease," *Brain Research*, vol. 1385, pp. 293–297, 2011.
- [30] J.-Y. Tian, J.-F. Guo, L. Wang et al., "Mutation analysis of LRRK2, SCNA, UCHL1, HtrA2 and GIGYF2 genes in Chinese patients with autosomal dominant Parkinson's disease," *Neuroscience Letters*, vol. 516, no. 2, pp. 207–211, 2012.

- [31] C.-M. Chen, C.-H. Wu, C.-H. Hsieh et al., "HTRA2 variations in Taiwanese Parkinson's disease," *Journal of Neural Transmission*, vol. 121, no. 5, pp. 491–498, 2014.
- [32] R. Krüger, M. Sharma, O. Riess et al., "A large-scale genetic association study to evaluate the contribution of Omi/HtrA2 (PARK13) to Parkinson's disease," *Neurobiology of Aging*, vol. 32, no. 3, pp. 548.e9–548.e18, 2011.



**Hindawi**  
Submit your manuscripts at  
<https://www.hindawi.com>

