INTRODUCTION

Essential tremor (ET) is a common adult-onset movement disorder with an unknown etiology. The prevalence increases with advancing age, being 0.9% at all ages and 4% in individuals aged ≥ 65 years.¹ Once considered a benign monosymptomatic motor disease, ET is now known to include many other nonmotor symptoms, with cognitive, psychiatric, dementia, and sensory features,² which are similar to Parkinson's disease (PD). However, unlike PD, there is still no breakthrough in this field except for classical drugs such as...

Abstract

Introduction: Essential tremor (ET) is one of the most prevalent movement disorders. The genetic etiology of ET has not been well defined although a significant proportion (≥50%) are familial cases. Linkage analysis and genome-wide association studies (GWASs) have identified several risk variants. In recent years, whole-exome sequencing of ET has revealed several specific causal variants in FUS (p.Q290X), HTRA2 (p.G399S), and TENM4 (c.4324 G>A, c.4100C>A, and c.3412G>A) genes.

Objective: To investigate the genetic contribution of these three genes to ET, the protein-coding sequences of FUS, HTRA2, and TENM4 were analyzed in a total of 238 ET patients and 272 controls from eastern China using direct Sanger sequencing.

Results: We identified two synonymous coding single nucleotide polymorphisms (SNPs), rs741810 and rs1052352 in FUS, and three previously reported synonymous SNPs, rs11237621, rs689369, and rs2277277 in TENM4. No nonsynonymous exonic variants were identified in these subjects. We found that the frequency of the rs1052352C allele was significantly higher (P = .001) in the ET group than in the control group.

Conclusion: Overall, our findings suggest that rs1052352 of FUS might contribute to ET risk in Chinese population.

Keywords

essential tremor, FUS, genetic analysis, HTRA2, TENM4
propranolol. Deficiency of disease-specific biomarkers for ET has resulted in high misdiagnosis rates; usually, it is misdiagnosed as PD.

Despite its high rates of prevalence and frequent cases of misdiagnosis, our understanding of the pathogenesis of ET continues to be limited. Although ≥50% of patients have family history, the genetic etiology of ET is still unclear. This situation highlights a need for more genetic studies on this complex disorder to improve our understanding with the purpose of seeking better treatments.

Although a strong genetic hereditary component exists, only a few pathogenic risk loci or genes have been discovered. Genome-wide scanning of several ET families has identified the first three associated loci (ETM1, ETM2, and ETM3). Later, another five potential ET susceptibility genes (LINGO1, SLC1A2, PPARGC1A, STK32B, and CTNNA3) were also found by genome-wide association studies (GWAS). Since 2012, pathogenic variants of FUS, HTRA2, and TENM4 have been reported to be responsible for familial ET through whole-exome sequencing.

Overall, to explore the roles of these three genes in ET, we sequenced all exons of FUS, HTRA2, and TENM4 in 238 ET and 272 healthy control subjects from eastern China.

## Materials and Methods

A total of 238 unrelated Han Chinese patients were recruited at the Department of Neurology, The Second Affiliated Hospital of Zhejiang University, with a diagnosis of ET based on recommendations of the Consensus Statement of the Movement Disorders Society. Definite ET required bilateral postural tremor (with or without kinetic tremor) of the hands and forearms, with a duration of longer than 5 years. Exaggerated physiological tremor, psychogenic-like tremor, the presence of other abnormal neurological signs, and some identified causes of other disease were all excluded. However, there are some changes in the diagnostic criteria in 2018. Among the 238 cases, 49.58% of patients exhibited a positive family history. Another 272 healthy controls, matched for age and sex (Table 1), were recruited. The study had been conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each subject, and the current study was approved by the local ethics committees.

Genomic DNA was extracted from white cells using standard phenol-chloroform method. Polymerase chain reaction (PCR) amplification and sequencing of FUS, HTRA2, and TENM4 gene coding region were performed using the ABI 3100 Automated Sequencer for both 238 patients and 272 controls. Primer and amplification conditions are available upon request.

Statistical analysis was performed using SPSS 16.0 (SPSS Inc.). Descriptive statistics (range, mean, and standard deviation) for both cases and controls were determined (Table 1). Pearson's chi-squared test was used to compare the allele frequency between ET cases and controls for each SNP (Table 2). The odds ratio (OR) with 95% confidence interval for each SNP was calculated. P < .05 was considered statistical significant for all analysis. And the obtained five P values were corrected by false discovery rate (FDR) test. The statistical power was performed by the Genetic Power Calculator software (Gauderman, 2002). All of the above five SNPs were in Hardy-Weinberg equilibrium.

## Results

Two synonymous coding SNPs (Table 2, rs741810 and rs1052352) were detected after sequencing of the FUS gene. For rs1052352, a significant difference in allelic distribution was found between ET and controls (P = .001), both for familial ET vs controls and for sporadic ET vs controls (Table 2). After correction by FDR test, the difference was still significant (P = .005) (Table S1). The minor allele (allele C) of rs1052352 seemed to be less common in controls than in patients (12.32% vs 20.17%) (Table 2). And the statistical power of rs1052352 between ET and controls was more than 0.8 (Table 3), while the statistical power between familial/sporadic ET and controls also reached the level of 0.8 (Tables S2 and S3). The minor allele frequencies for rs741810 observed in the present study appeared to be similar to the SNP database (https://www.internationalgenome.org/1000-genomes-browsers/).

Our sequencing study of the HTRA2 gene in 238 patients and 272 controls did not reveal any exonic variant. We did not observe the HTRA2 S399 mutation and the A141S polymorphism that have been described in previous studies.

Three previously reported synonymous SNPs in TENM4 were also identified in our study, rs11237621, rs689369, and rs2277277.

### Table 1 Case-control dataset included in this study

|                  | ET | Controls |
|------------------|----|----------|
|                  | FET | SET | Controls 1 | Controls 2 |
| Number of subjects | n = 118 | n = 120 | n = 132 | n = 140 |
| Mean age at onset (y) ± SD (range) | 44.81 ± 15.17 (6-71) | 49.11 ± 15.33 (16-79) |
| Mean disease duration (y) ± SD (range) | 12.35 ± 12.30 (1-54) | 9.54 ± 11.17 (1-54) |
| Gender           |     |     |           |           |
| Male             | 58  | 63  | 62        | 75        |
| Female           | 60  | 57  | 70        | 65        |
### TABLE 2  Allele frequencies for the five SNPs of FUS and TENM4 genes in ET patients and controls

| SNP      | Alleles | FET, Number of alleles (frequency) | Control1, Number of alleles (frequency) | P, OR (95% CI) | SET, Number of alleles (frequency) | Control2, Number of alleles (frequency) | P, OR (95% CI) | ET, Number of alleles (frequency) | Control, Number of alleles (frequency) | P, OR (95%) |
|----------|---------|------------------------------------|----------------------------------------|----------------|-----------------------------------|----------------------------------------|----------------|-----------------------------------|----------------------------------------|-------------|
| rs741810 | Allele C| 204 (86.44%)                       | 222 (84.09%)                           | .460, 1.028 (0.956-1.106) | 187 (77.92%)                     | 231 (82.50%)                           | .189, 0.944 (0.866-1.030) | 391 (82.14%)                     | 453 (83.27%)                         | .634, 0.986 (0.932-1.044) |
|          | Allele A| 32 (13.56%)                        | 42 (15.91%)                            | .460, 0.852 (0.557-1.304) | 53 (22.08%)                      | 49 (17.50%)                            | .189, 1.262 (0.891-1.787) | 85 (17.86%)                      | 91 (16.73%)                          | .634, 1.068 (0.816-1.397) |
| rs1052352| Allele T| 187 (79.23%)                       | 233 (88.26%)                           | .006, 0.898 (0.830-0.971) | 193 (80.42%)                     | 244 (87.14%)                           | .037, 0.923 (0.854-0.997) | 380 (79.83%)                     | 477 (87.68%)                          | .001, 0.910 (0.862-0.962) |
|          | Allele C| 49 (20.76%)                        | 31 (11.74%)                            | .006, 1.768 (1.169-2.675) | 47 (19.58%)                      | 36 (12.86%)                            | .037, 1.523 (1.023-2.269) | 96 (20.17%)                      | 67 (12.32%)                          | .001, 1.638 (1.229-2.181) |
| rs1123762| Allele C| 104 (44.07%)                       | 132 (50.00%)                           | .185, 0.881 (0.731-1.063) | 125 (52.08%)                     | 158 (56.43%)                           | .321, 0.923 (0.787-1.082) | 229 (48.11%)                     | 290 (53.31%)                          | .097, 0.902 (0.799-1.020) |
|          | Allele T| 132 (55.93%)                       | 132 (50.00%)                           | .185, 1.119 (0.948-1.320) | 115 (47.92%)                     | 122 (43.57%)                           | .321, 1.100 (0.912-1.327) | 247 (51.89%)                     | 254 (46.69%)                          | .097, 1.111 (0.981-1.259) |
| rs689369  | Allele C| 196 (83.05%)                       | 234 (88.64%)                           | .072, 0.937 (0.872-1.007) | 203 (84.58%)                     | 239 (85.36%)                           | .805, 0.991 (0.922-1.066) | 399 (83.82%)                     | 473 (86.95%)                          | .157, 0.964 (0.916-1.015) |
|          | Allele T| 40 (16.95%)                        | 30 (11.36%)                            | .072, 1.492 (0.961-2.315) | 37 (15.42%)                      | 41 (14.64%)                            | .805, 1.053 (0.699-1.586) | 77 (16.18%)                      | 71 (13.05%)                          | .157, 1.239 (0.920-1.670) |
| rs2277277 | Allele C| 195 (82.63%)                       | 230 (87.12%)                           | .160, 0.948 (0.880-1.022) | 209 (87.08%)                     | 242 (86.43%)                           | .826, 1.008 (0.942-1.078) | 404 (84.87%)                     | 472 (86.76%)                          | .387, 0.978 (0.930-1.029) |
|          | Allele T| 41 (17.37%)                        | 34 (12.88%)                            | .160, 1.349 (0.887-2.052)  | 31 (23.92%)                      | 38 (13.57%)                            | .826, 0.952 (0.612-1.481) | 72 (15.13%)                      | 72 (13.24%)                          | .387, 1.143 (0.844-1.547) |

**Note:** Characters in boldface represent the P value reached the significance level (P < .05).
They have been reported in the NCBI database, dbSNP. No novel missense coding variants of TENM4 were detected in our study. The allele frequency of these SNPs between ET patients and controls did not reach statistical significance in our samples (all \( P > .05 \)) (Table 2).

### 4 | DISCUSSION

In this study, FUS, HTRA2, and TENM4 genes were sequenced in 238 ET patients and 72 controls of Chinese origin. Five reported SNPs, rs741810 and rs1052352 in FUS, and rs11237621, rs689369, and rs2277277 in TENM4, were detected. The FUS gene, once considered as a fusion oncogene, is located on chromosome 16. Apart from its role in various cellular functions, it is also proved to be associated with the etiology of certain neurodegenerative diseases. A nonsense mutation (p.Q290X) in this gene was supposed to cause familial ET in a large Canadian family through exome sequencing. Another two novel variants (p.R216C and p.P431L) were also identified in this study. In 2013, a novel FUS gene variant of p.Met392Ile was detected by Tan et al, which caused increased risk of ET in patients of Chinese ethnicity. Other researchers detected a heterozygous p.Arg377Trp substitution in an ET family, while some other studies detected various known SNPs, such as rs741810, rs1052352, and rs138901914. In our present study, we similarly did not detect any novel FUS mutations, except for two synonymous exonic SNPs, rs741810 and rs1052352. Allele frequencies of rs741810 did not differ significantly between cases and controls, which was consistent with previous observations. However, the frequency of rs1052352 was significantly higher in cases than in controls. Our findings suggest that rs1052352 might be a risk factor for ET in eastern China, but this link is not proved by other studies. Nonetheless, whether this synonymous variant contributes to ET is still unknown. The number of our ET sample is not large enough to reach a solid conclusion.

The mitochondrial high-temperature–regulated A2 (HtrA2) was found to play essential roles in mitochondrial function and cellular apoptosis regulation. Gulsuner et al reported a six-generation family with ET and ET with parkinsonism carrying HTRA2 p.G399S variant, suggesting its role in both PD and ET. Additionally, some novel variants, such as c.427C>G, c.106C>T, IVS5+29T>A, and c.G77A were identified in PD patients. However, the study conducted by Tzoulis et al did not identify an association between the p.G399S mutation and ET and their study also found no exonic variants in the Chinese PD patients. In our study, no exonic mutation was detected in ET patients. Thus, HTRA2 mutations are unlikely to be a causative gene for ET or PD in eastern China.

TENM4, mostly distributed in the cerebellum, plays roles in the regulation of axon guidance and central myelination. In 2015, three distinct pathogenic variants in TENM4 (c.4100C>A, c.4324G>A, and c.3412G>A) were identified in three families of Spanish origin. One year later, Houle et al detected numerous rare missense variants in patients, and no significant association was discovered between ET patients and controls in a Canadian population. In the current study, we detected three known SNPs, rs11237621, rs689369, and rs2277277 in TENM4. The allele frequencies of these SNPs did not differ significantly between ET patients and controls (all \( P > .05 \)).

### 5 | CONCLUSION

In conclusion, our data show that rs1052352 in the FUS gene increases the risk for ET in the eastern China and that the other two genes, HTRA2 and TENM4, do not show an association with ET. Our study is the first to reveal that rs1052352 (FUS) is associated with ET in eastern China. However, due to the limited number of our sample and racial heterogeneity, more studies are needed to completely elucidate whether FUS gene is a risk factor for ET in other populations. Additionally, as the criteria of ET varied, the new diagnostic criteria should be used by more neurologists. Further functional studies are also required to elucidate the potential role played by FUS variants in the development of this disease.

| SNP       | Model  | MAF    | Control | ET     | CON per case | OR    | Power |
|-----------|--------|--------|---------|--------|--------------|-------|-------|
| rs741810  | Dominant | 0.173  | 272     | 238    | 1.143        | 1.118 | 0.139 |
|           | Recessive | 0.855  | 10.651  | 0.999  |              |       |       |
| rs1052352 | Dominant | 0.160  | 1.800   | 0.996  |              |       |       |
|           | Recessive | 1.225  | 0.298   | 1.382  | 0.660        |       |       |
| rs11237621| Dominant | 0.491  | 1.301   | 0.508  |              |       |       |
|           | Recessive | 1.333  | 0.318   | 1.733  |              |       |       |
| rs689369  | Dominant | 0.145  | 1.327   | 0.563  |              |       |       |
|           | Recessive | 0.321  | 0.506   |        |              |       |       |

Note: The positive locus identified in this study was marked in bold font.

Abbreviations: CON, controls; ET, essential tremor; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism.

Additional table: Power calculation of five loci in ET patients and controls (dominant and recessive models)
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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.