**H1-MAPT and the Risk for Familial Essential Tremor**

Elena García-Martín¹, Carmen Martínez², Hortensia Alonso-Navarro³,⁴, Julián Benito-León⁵,⁶, Oswaldo Lorenzo-Betancor⁷,⁸, Pau Pasto⁶,⁷,⁸, Tomás López-Alburquerque⁹, Lluís Samaranch⁷, Elena Lorenzo⁷, José A. G. Ágúndez¹⁰, Félix Javier Jiménez-Jiménez³,⁷,⁸

¹ Department of Biochemistry and Molecular Biology, Universidad de Extremadura, Cáceres, Spain, ² Department of Pharmacology, Universidad de Extremadura, Badajoz, Spain, ³ Department of Medicine-Neurology, Hospital “Príncipe de Asturias” Universidade de Alcalá, Alcalá de Henares, Madrid, Spain, ⁴ Section of Neurology, Hospital Universitario del Sureste, Arganda del Rey, Madrid, Spain, ⁵ Service of Neurology, Hospital Doce de Octubre, Department of Medicine, Universidad Complutense, Madrid, Spain, ⁶ Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas, Instituto de Salud Carlos III, Madrid, Spain, ⁷ Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, Universidad de Navarra, Universidad de Navarra School of Medicine, Pamplona, Spain, ⁸ Department of Neurology, Clinica Universidad de Navarra, University of Navarra School of Medicine, Pamplona, Spain, ⁹ Department of Neurology, Hospital Universitario de Salamanca, Salamanca, Spain, ¹⁰ Department of Pharmacology, Universidad de Extremadura, Cáceres, Spain

**Abstract**

The most frequent MAPT H1 haplotype is associated with the risk for developing progressive supranuclear palsy and other neurodegenerative diseases such as Parkinson’s disease. A recent report suggests that the MAPT H1 is associated with the risk for developing essential tremor. We wanted to confirm this association in a different population. We analyzed the distribution of allelic and genotype frequencies of rs1052553, which is an H1/H2 SNP, in 200 subjects with familial ET and 291 healthy controls. rs1052553 genotype and allelic frequencies did not differ significantly between subjects with ET and controls and were unrelated with the age at onset of tremor or gender, and with the presence of head, voice, chin, and tongue tremor. Our study suggests that the MAPT H1 rs1052553 is not associated with the risk for developing familial ET in the Spanish population.

**Introduction**

Essential tremor (ET) is characterized by postural or kinetic 4–12 Hz tremor involving mainly the hands and forearms, although it can also affect the head, chin, voice, and other body regions. Family history of tremor in ET subjects ranges from 17.4% to 100%, and it is significantly more frequent than in a healthy population. Linkage studies identified three susceptibility loci for familial ET mapped at chromosomes 3q13, 2p24.1, and 6p23, but the responsible genes have not been clearly identified [1].

In recent years, it has been reported that mutations in the microtubule-associated protein tau gene (MAPT) cause frontotemporal dementia with parkinsonism linked to the chromosome 17 [2]. Single nucleotide polymorphisms (SNPs) across a chromosomal region expanding 1.3 Mb of the MAPT H1 haplotype have shown association with the risk for developing Parkinson’s disease (PD) [3–8], progressive supranuclear palsy [3,9,10], corticobasal degeneration [3], and multiple system atrophy [11].

There are many clinical, epidemiologic, genetic, neuroimaging and neuropathological data suggesting a relationship between ET and PD [12–14]. A recent study been suggested that the SNP rs1025253, which discriminates between MAPT H1 and H2 haplotypes, is associated with the risk for developing ET, with an odds-ratio of 1.32 (1.03–1.67) [11]. We attempted to replicate this finding in the Spanish population with ET compared with healthy controls.

**Methods**

We studied 200 unselected and unrelated patients with diagnostic criteria for definite ET [15] (99 men,101 women, mean age 65.7±16.1, mean age at onset of ET 48.2±18.1 years), and 291 sex-matched controls (146 men, 145 women, mean age 46.5±12.6 years). The absence of previous neurological diseases, normality of thyroid function, and positive family history of ET (at least 1 first-degree relative affected) were obligatory requisites for inclusion. The patients were recruited from the Movement Disorders Units of 3 Hospitals. Controls were healthy unrelated gender-matched Caucasian Spanish individuals, most of them students or professors from the University of Extremadura, who did not have tremor or other movement disorders. Although our control group was not age-matched, age is not a determinant factor for polymorphisms of drug-metabolising enzymes [16], and the mean age of onset of ET did not differ significantly from the mean age of controls. According to the prevalence rates of ET in Spain [17], the percentage of controls with the risk genotypes that could develop ET in the lapse between the mean age of controls and the mean age of cases should not influence the results of the study.
All the participants were included in the study after giving written informed consent. This study was approved by the Ethics Committee of the University Hospital “Príncipe de Asturias”, University of Alcalá, (Carretera de Alcalá Meco s/n, Alcalá de Henares E28805 Spain). The study was conducted according to the principles expressed in the declaration of Helsinki.

Genotyping for rs1052553 allelic variant was performed in genomic DNA obtained from venous blood samples of participants using TaqMan Assays (C_7563736_10, Applied Biosciences Hispania, Alcobendas, Madrid, Spain) designed to detect the SNP rs1052553. Detection was carried out by qPCR in an Eppendorf realplex thermocycler. The amplification conditions were as follows: after a denaturation time of 10 min at 96°C, 45 cycles of 92°C 15 sec 60°C 90 sec were carried out and fluorescence was measured at the end of each cycle and at endpoint. All samples were determined in triplicate and genotypes were assigned both by gene identification software (RealPlex 2.0, Eppendorf, Madrid, Spain) and by analysis of the reference cycle number for each fluorescence curve, calculated by the use of the CalQPlex algorithm (Eppendorf, Madrid, Spain).

The intergroup comparison values were calculated by using the chi-square or Fisher tests when appropriate. The 95% confidence intervals were also calculated. We calculated the statistical power for the sample sizes in this study, in the Vilarín-Guell et al. study [11], and in the pooled data from both studies, which was determined from allele frequencies with a genetic model analyzing Table 1. MAPT rs1052553 genotypes and allelic variants of patients with essential tremor (ET) and healthy volunteers.

| DATA FROM THE PRESENT STUDY | ET PATIENTS (N = 200, 400 alleles) | CONTROLS (N = 291, 582 alleles) | OR (95% CI), p | Negative predictive value (95% CI) |
|----------------------------|---------------------------------|-----------------------------|----------------|-----------------------------|
| GENOTYPES                  |                                 |                             |                |                             |
| AA                         | 104 (52.0; 45.1–58.9)           | 158 (54.3; 48.6–60.0)       | 0.91 (0.62–1.33), 0.617 | 0.58 (0.53–0.63) |
| AG                         | 75 (37.5; 30.8–44.2)            | 111 (38.1; 32.6–43.7)       | 0.98 (0.66–1.43), 0.885 | 0.59 (0.56–0.63) |
| GG                         | 21 (10.5; 6.3–14.7)             | 22 (7.6; 4.5–10.6)          | 1.43 (0.73–2.81), 0.258 | 0.60 (0.59–0.62) |

| ALLELES                    |                                 |                             |                |                             |
| A                          | 283 (70.8; 66.3–75.2)            | 427 (73.4; 69.8–77.0)       | 0.88 (0.64–1.18), 0.368 | 0.60 (0.58–0.62) |
| G                          | 117 (29.3; 24.8–33.7)            | 155 (26.6; 23.0–30.2)       | 1.13 (0.85–1.53), 0.368 | 0.57 (0.52–0.62) |

| DATA FROM THE PRESENT STUDY COMBINED WITH VILARÍN-GUELL ET AL. STUDY [11] | ET PATIENTS (N = 539, 1078 alleles) | CONTROLS (N = 697, 1394 alleles) | OR (95% CI), p | Negative predictive value (95% CI) |
|------------------------------------------------------------------------|---------------------------------|-----------------------------|----------------|-----------------------------|
| GENOTYPES                                                              |                                 |                             |                |                             |
| AA                                                                     | 325 (60.3; 56.2–64.4)           | 389 (55.8; 52.1–59.5)       | 1.20 (0.95–1.52), 0.113 | 0.59 (0.56–0.62) |
| AG                                                                     | 175 (32.5; 28.5–36.4)           | 259 (37.2; 33.6–40.7)       | 0.81 (0.64–1.04), 0.087 | 0.55 (0.53–0.57) |
| GG                                                                     | 39 (7.2; 5.0–9.4)               | 49 (7.0; 5.1–8.9)           | 1.03 (0.65–1.63), 0.889 | 0.56 (0.56–0.57) |

| ALLELES                                                                |                                 |                             |                |                             |
| A                                                                      | 825 (76.5; 74.0–79.1)            | 1037 (74.4; 72.1–76.7)      | 1.12 (0.93–1.36), 0.221 | 0.59 (0.55–0.62) |
| G                                                                      | 253 (23.5; 20.9–26.0)            | 357 (25.6; 23.3–27.9)       | 0.89 (0.74–1.08), 0.221 | 0.56 (0.55–0.57) |

The values in each cell represent: number (percentage; 95% confidence intervals).

doi:10.1371/journal.pone.0041581.t001

Table 2. MAPT rs1052553 genotypes and allelic variants of patients with essential tremor and healthy volunteers distributed by gender.

| ET WOMEN (N = 101, 202 ALLELES) | CONTROL WOMEN (N = 146, 292 ALLELES) | INTERGROUP COMPARISON VALUES OR (95% CI) P | ET MEN (N = 99, 198 ALLELES) | CONTROL MEN (N = 145, 290 ALLELES) | INTERGROUP COMPARISON VALUES OR (95% CI) P |
|--------------------------------|-------------------------------------|----------------------------------------|----------------------------|-----------------------------------|----------------------------------------|
| GENOTYPES                      |                                     |                                        |                            |                                   |                                        |
| AA                             | 57 (56.4; 46.8–66.1)                | 79 (54.1; 46.0–62.2)                   | 1.10 (0.64–1.89), 0.718 | 47 (47.5; 37.6–57.3)              | 79 (55.4; 46.4–62.6)                  | 0.76 (0.44–1.30), 0.283 |
| AG                             | 31 (30.7; 21.7–39.7)                | 57 (39.0; 31.1–47.0)                  | 0.69 (0.39–1.23), 0.179 | 44 (44.4; 34.7–54.2)              | 54 (37.2; 29.4–45.1)                  | 1.35 (0.78–2.35), 0.261 |
| GG                             | 13 (12.9; 6.3–19.4)                 | 10 (6.8; 2.8–10.9)                    | 2.01 (0.78–5.20), 0.110 | 8 (8.1; 2.7–13.4)                 | 12 (8.3; 3.8–12.8)                    | 0.97 (0.35–2.69), 0.957 |

| ALLELES                        |                                     |                                        |                            |                                   |                                        |
| A                               | 145 (71.8; 65.6–78.0)               | 215 (73.6; 68.6–78.7)                 | 0.91 (0.60–1.39), 0.650 | 138 (69.7; 63.3–76.1)             | 212 (73.1; 68.0–78.2)                 | 0.85 (0.56–1.29), 0.412 |
| G                               | 57 (28.2; 22.0–34.4)                | 77 (26.4; 21.3–31.4)                  | 1.10 (0.72–1.67), 0.650 | 60 (30.3; 23.9–36.7)              | 78 (26.9; 21.8–32.0)                  | 1.18 (0.78–1.80), 0.412 |

The values in each cell represent: number (percentage; 95% confidence intervals).

doi:10.1371/journal.pone.0041581.t002
the frequency for carriers of the disease gene taking as reference value the OR reported in the original study (1.32) [11]. The respective values with P = 0.05 for one-tailed and two-tailed associations were, respectively 61.8% and 49.4% in the present study, 76.9% and 66.3%, in the original study [11], and 92.4% and 86.8% in the pooled analysis of the two studies. The negative predictive value was calculated as d/r2 (d = number of control individuals with the risk factor absent; r2 = sum of ET patients and controls with the risk factor absent). The Hardy-Weinberg equilibrium was confirmed by means of Arlequin software Ver. 2.000.

Results

The frequencies of rs1052553 genotypes and allelic variants in ET patients did not differ significantly from those of healthy controls (Table 1), and were in Hardy-Weinberg’s equilibrium. An analysis of the pooled data of the present study with those of the Vilariño-Guell et al. [11] report, did not show significant differences between the frequencies of rs1052553 genotypes and allelic variants in ET patients compared with controls (Table 1).

Allele and genotype frequencies of rs1052553 were not influenced either by gender or by the age at onset of tremor (Table 2). Mean ±SD age at onset of tremor did not differ among the ET rs1052553-LI, rs1052553MG and rs1052553GG carriers (46.3 ± 16.8; 49.4 ± 19.4, and 54.7 ± 17.7 years, respectively).

The respective frequencies of the rs1052553GG genotype and the rs1052553G allelic variant found in the ET patients with head (n = 45 [8.9%]; 95% CI, 0.6–17.2%; and [25.6%]; 95% CI, 16.5–34.6%), voice (n = 36 [11.1%]; 95% CI, 0.8–21.4%; and [29.2%], 95% CI, 18.7–39.7), tongue (n = 16 [12.5%]; 95% CI, 3.7–28.7; and [37.5%]; 95% CI, 20.7–54.3), and chin tremor (n = 11 [0%] and [18.2%]; 95% CI, 2.1–34.3%) did not differ significantly from those found in the control group.

Discussion

To date, neither linkage studies nor case-control association studies have been able to identify conclusively any gene responsible for ET. Some association studies suggested a possible relationship of the risk of developing ET with the methylenetetrahydrofolate reductase, alpha-synuclein, CYP2C19, and CYP2C9/8 polymorphisms, whereas others did not find any association with alpha-synuclein, CYP2D6, alcohol-dehydrogenase 2 (ADH2), glutathione-transferase P1 (GSTP1) (revised in reference [1]), and paraoxonase 1 (PON-1) [18] polymorphisms. Our group reported association between the rs1052553G allele with the risk for ET, although both variants showed a weak association with the risk for developing familial ET [29].

In the present study, we found no significant differences either in the frequencies of the rs1052553 genotypes or in the frequencies of the allelic variants of this SNP in patients with familial ET when compared with healthy controls. In addition, rs1052553 was unrelated with age at onset of ET, and with the presence of head, voice, tongue or chin tremor.

The rs1052553 variant allele frequency observed in control individuals in this study (26.6%) is almost identical to that reported by Vilariño-Guell et al. [11] (24.9%). However, we did not observe a decreased rs1052553 allelic frequency among ET patients reported by Vilariño-Guell et al. [11], but a slight non-significant increase in the variant allele frequency (Table 1). While in the present series we only used familial cases, in the report by Vilariño-Guell et al. [11] the percentage of patients with familial and sporadic ET is not specified.

The results of the present study suggest that the rs1052553 SNP is not related with the risk for developing familial ET. Despite the small sample size in the present study, it is of note that the analysis of the pooled data of this study with those of the report by Vilariño-Guell et al. [11], which includes a total of 539 ET patients and 697 controls, show lack of association between the rs1052553 SNP and the risk for ET. However, this result should be interpreted with caution, since the pooled analysis could be unreliable when the status of the ET (familial or sporadic) in the original study is unknown.

Acknowledgments

We thank Gara Esquevillas for technical assistance, and Prof. James McCue for assistance in language editing.

Authors Contribution

Conceived and designed the experiments: EGM CM PP JAGA FJJJ. Performed the experiments: EGM CM HAN JBL OLB PP TLA LS EL. Analyzed the data: EGM CM PP JAGA FJJJ. Contributed reagents/materials/analysis tools: EGM PP JAGA FJJJ. Wrote the paper: HAN JAGA FJJJ. Critical revision of the manuscript: EGM HAN JBL OLB PP TLA JAGA FJJJ. Administrative, technical, and material support: EGM HAN PP JAGA FJJJ. Supervision: EGM HAN PP JAGA FJJJ.
7. Wider C, Vilariño-Güell C, Heckman MG, Jasinska-Myga B, Ortolaza-Soto AI, et al. (2011) SNCA, MAPT, and GSK3B in Parkinson disease: a gene-gene interaction study. Eur J Neurol 18: 876–881.

8. Elbaz A, Ross OA, Ioannidis JP, Soto-Ortolaza AI, Moisan F, et al. (2011) Independent and joint effects of the MAPT and SNCA genes in Parkinson disease. Ann Neurol 69: 778–792.

9. Pastor P, Pastor E, Garnero C, Vela R, García T, et al. (2001) Familial atypical progressive supranuclear palsy associated with homozygosity for the delN296 mutation in the tau gene. Ann Neurol 49: 263–267.

10. Rademakers R, Melquier S, Cruts M, Theuws J, Del-Favero J, et al. (2005) High-density SNP haplotyping suggests altered regulations of tau gene expression in progressive supranuclear palsy. Hum Mol Genet 13: 3201–3212.

11. Vilariño-Güell C, Soto-Ortolaza AI, Rajput A, Maib DC, Papapetropoulos S, et al. (2011) MAPT H1 haplotype is a risk factor for essential tremor and multiple system atrophy. Neurology 76: 670–672.

12. LaRoia H, Louis ED (2011) Association between essential tremor and other neurodegenerative diseases: what is the epidemiological evidence. Neuroepidemiology 37: 1–10.

13. Fekete R, Jankovic J (2011) Revisiting the relationship between essential tremor and Parkinson’s disease. Mov Disord 26: 391–398.

14. Jiménez-Jiménez FJ, Alonso-Navarro H, García-Martín E, Agúndez JAG (2012) The Relationship between Parkinson’s Disease and Essential Tremor: Review of Clinical, Epidemiologic, Genetic, Neuroimaging and Neuropathological Data, and Data on the Presence of Cardinal Signs of Parkinsonism in Essential Tremor. TOHM, in press.

15. Deuschl G, Bain P, Brin M (1998) Consensus statement of the Movement Disorder Society on Tremor. Ad Hoc Scientific Committee. Mov Disord 13 (Suppl 3): 2–23.

16. Agúndez JA, Rodríguez I, Olivera M, Ladero JM, García MA, et al. (1997) CYP2D6, NAT2 and CYP2E1 genetic polymorphisms in nonagenarians. Age Ageing 26: 147–151.

17. Benito-León J, Benuje-Pareja F, Morales JM, Vega S, Molina JA (2003) Prevalence of essential tremor in three elderly populations of Central Spain. Mov Disord 18: 389–394.

18. García-Martín E, Martínez C, Alonso-Navarro H, Benito-León J, Puertas I, et al. (2010) Paraoxonase 1 (PON1) polymorphisms and risk for essential tremor. Eur J Neurol 17: 879–881.

19. Ledesma MC, García-Martín E, Alonso-Navarro H, Martínez C, Jiménez-Jiménez FJ, et al. (2008) The nonsynonymous Thr105Ile polymorphism of the histamine N-methyltransferase is associated to the risk of developing essential tremor. Neuromolecular Med 10: 356–361.

20. Keeling BH, Vilariño-Güell C, Soto-Ortolaza AI, Ross OA, Uitti RJ, et al. (2010) Histamine N-methyltransferase Thr105Ile is not associated with Parkinson’s disease or essential tremor. Parkinsonism Relat Disord 6: 112–114.

21. García-Martín E, Martínez C, Alonso-Navarro H, Benito-León J, Puertas I, et al. (2009) Dopamine receptor D3 (DRD3) genotype and allelic variants and risk for essential tremor. Mov Disord 24: 1910–1915.

22. Lorenz D, Kléhe S, Stevanin G, Thier S, Nebel A, et al. (2009) Dopamine receptor D3 gene and essential tremor in large series of German, Danish and French patients. Eur J Hum Genet 17: 766–773.

23. Deng H, Xie WJ, Le WD, Huang MS, Jankovic J (2006) Genetic analysis of the GABRA1 gene in patients with essential tremor. Neurosci Lett 401: 16–19.

24. García-Martín E, Martínez C, Alonso-Navarro H, Benito-León J, Lorenzon-Betancor O, et al. (2011) Gamma-aminobutyric acid (GABA) receptors rho (GABRR) polymorphisms and risk for essential tremor. J Neurol 258: 203–211.

25. García-Martín E, Martínez C, Alonso-Navarro H, Benito-León J, Lorenzon-Betancor O, et al. (2011) Gamma-aminobutyric acid GABRA4, GABRE and GABRQ receptor polymorphisms and risk for essential tremor. Pharmacogenet Genomics 21: 436–439.

26. Thier S, Kuhlenhäuser G, Lorenz D, Nothnagel M, Nebel A, et al. (2011) GABA(A) receptor and GABA transporter polymorphisms and risk for essential tremor. Mov Disord 26: 1098–1100.

27. Stensson H, Steinberg S, Petronson H, Gustafsson O, Gudbjonsson BH, et al. (2009) Variant in the sequence of the LINGO1 gene confers risk of essential tremor. Nat Genet 41: 277–279.

28. Jiménez-Jiménez FJ, García-Martín E, Lorenzon-Betancor O, Pastor P, Alonso-Navarro H, et al. (2012) LINGO1 and risk for essential tremor: Results of a meta-analysis of rs9652490 and rs11856808. J Neurol Sci 317: 52–57.