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Exome-wide rare variant analysis in familial essential tremor

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Conflicts of interest

The authors declare that they have no conflict of interest.

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Abstract

Introduction: Essential tremor (ET) is one of the most common movement disorders. Despite its high prevalence and heritability, its genetic aetiology remains elusive with only a few susceptibility genes identified and poorly replicated. Our aim was to find novel candidate genes involved in ET predisposition through whole exome sequencing.

Methods: We studied eight multigenerational families (N=40 individuals) with an autosomaldominant inheritance using a comprehensive strategy combining whole exome sequencing, followed by case-control association testing of prioritized variants in a separate cohort comprising 521 ET cases and 596 controls. We further performed gene-based burden analyses in an additional dataset comprising 789 ET patients and 770 healthy individuals to investigate whether there was an enrichment of rare deleterious variants within our candidate genes.

Results: Fifteen variants co-segregated with disease status in at least one of the families, among which rs749875462 in *CCDC183*, rs535864157 in *MMP10* and rs114285050 in *GPR151* showed a nominal association with ET. However, we found no significant enrichment of rare variants within these genes in cases compared with controls. Interestingly, MMP10 protein is involved in

the inflammatory response to neuronal damage and has been previously associated with other neurological disorders.

Conclusions: We prioritized a set of promising genes, especially *MMP10*, for further genetic and functional studies in ET. Our study suggests that rare deleterious coding variants that markedly increase susceptibility to ET are likely to be found in many genes. Future studies are needed to replicate and further infer biological mechanisms and potential disease causality for our identified genes.

Keywords

essential tremor; genetic risk; WES; rare variants; MMP10

1. Introduction

Essential tremor (ET) is one of the most frequent movement disorders with an estimated prevalence of 0.9% in the general population and up to 4.6% in individuals at age 65 or older [1]. ET subjects show action tremors affecting primarily the upper limbs, although tremors can be present in other body regions. Owing to its clinical heterogeneity, ET is now considered a syndrome that may have multiple genetic and environmental etiologic factors [2].

Many studies have reported a high frequency of familial aggregation of tremor among patients with ET [3], suggesting a high heritability for this disorder [4,5]. Historically, most studies have assumed a Mendelian pattern of inheritance; however, it remains unclear whether such strong familial aggregation is either due to a single highly penetrant rare variant, many low penetrant common variants, or a combination of both.

Despite its high prevalence and frequent familial aggregation, the genetic basis of ET remains poorly understood with only a handful of susceptibility genes identified and not consistently replicated (Table 1). The first studies pointed out three distinct susceptibility loci under autosomal dominant (AD) inheritance models [6–8], in which no candidate gene has been found yet. Under the ‘*common disease-common variant*’ hypothesis, one could postulate that the genetic component of ET is likely due to a large number of common low-risk alleles [9]. However, genome-wide association studies (GWAS) have only reported five common, low-penetrance genetic variants associated with risk for sporadic ET with low disease odds ratios [10–12]. As in other complex diseases, the identified GWAS hits do not account for the total ET genetic variance [13], suggesting that rare penetrant variants with larger effects may also play a substantial role in the disease etiology, particularly in familial cases [14]. Indeed, whole exome sequencing (WES) has proven to be fruitful in identifying putative causal genes in ET families – such as *FUS* [15], *HTRA2* [16], *SORT1* [17], *TENM4* [18], *SCN4* [19], *NOS3*, *KCNS2*, *HAPLN4*, and *USP46* [20] (Table 1) – which have provided clues to disease pathogenesis. For instance, targeted expression of *FUS* p.Gln290* in both dopaminergic and serotonergic neurons in transgenic *Drosophila* resulted in motor dysfunction together with impairment in the GABAergic pathway [21], which has been extensively linked to ET pathogenesis [22]. Similarly, *Tenm4* knockout mice model showed severe action tremor phenotype and hypomyelination of small-diameter axons in the

central nervous system [23]. However, further genetic evidence is needed to consider these genes as causative for ET. We hypothesized that a family-based exome sequencing study would help to identify rare genetic determinants for hereditary ET.

We performed WES in 8 families (n=40 individuals) with an AD inherited form of ET, followed by a targeted association testing of prioritized variants, in an attempt to identify novel candidate genes involved in ET.

2. Materials and methods

2.1. Study participants

Eight families of Spanish origin with strong ET aggregation, defined as having at least three affected relatives, were included in the study. Families were selected based on the following criteria: (1) the proband had a diagnosis of definite ET; (2) there were at least two first-degree affected relatives in the family in addition to the proband; and (3) the families had affected members in different generations. The final sample selected for WES included a total of 40 individuals, comprising 29 affected, 9 unaffected relatives, and two individuals with unclear ET diagnosis. The pedigrees for the eight families, indicating the family members that underwent WES, are detailed in Figure 1.

All participants were examined by at least one movement disorders specialist. Neurological exam included the assessment of rest (assessed with the hands lying on the lap), postural (assessed with the arms extended), and kinetic tremor (assessed with the finger to nose & finger to finger tests), and presence/absence of rigidity and bradykinesia. ET diagnosis was made according to the TRIG classification of essential tremor not following the inclusion criterion “duration of tremor more than 5 years” [24]. Other entities such as dystonia, Parkinson’s disease (PD) and drug-induced tremor were specifically excluded. Enhanced physiological tremor was excluded according to the expert guidelines [25]. All subjects fulfilled the criteria for definite ET, except for individuals II:1 and II.2 from family F who were labeled as having possible ET, since they showed mild parkinsonian signs. Further clinical and demographic details are included in Figure 1. Two of the families included (D and F) had been previously reported (as family 11 and 4 respectively) elsewhere [26]. The study protocol was approved by the Ethics Committee of University Hospital Mutua de Terrassa and University of Navarra as well as the local ethics committees at the different centers. Written informed consent was obtained from all enrollees.

2.2. Whole exome sequencing, variant calling and annotation

Genomic DNA from all individuals was isolated from whole blood using standard procedures. Whole exome sequencing for the 40 individuals was performed at the University of Washington Center for Mendelian Genomics (Seattle, WA, USA). In brief, exomes were captured using the Nimblegen SeqCap EZ Human Exome Library v2.0 (~44 Mb target) (Roche NimbleGen, Inc., Madison, WI, USA). The captured DNA was sequenced with 75bp paired-end reads on an Illumina HiSeq 4000 sequencing platform according to manufacturer’s instructions (Illumina, Inc., San Diego, CA, USA). Exome-captured sequencing reads were aligned to the human reference genome assembly GRCh37 using

BWA-MEM (v.0.7.12). PCR duplicates were removed using Picard-tools v1.137 (<http://picard.sourceforge.net>) and local realignment and base quality score recalibration using Genome Analysis Toolkit (GATK) v3.4–46. Finally, single-nucleotide variants (SNVs) and insertions/deletions (indels) were called using GATK HaplotypeCaller. Additional hard filters applied at variant, genotype and individual levels are detailed in Supplementary Material.

Variants were annotated using hg19, SnpEff and SnpSift. Functional impact was calculated according to Combined Annotation Dependent Depletion (CADD); pathogenicity predictions were calculated according to MutationAssessor, MutationTaster, Polyphen-2 and SIFT algorithms; and population allele frequencies were based on non-Finnish European (NFE) exome samples from the Genome Aggregation Database (gnomAD v.2.1.1) (Supplementary Material).

2.3. Segregation analysis and variant prioritization

Annotated variants were assessed for segregation within the families. Using an AD model of inheritance, only perfectly-segregating variants (present in affected individuals and absent in unaffected relatives) within each family were selected. In addition, only coding, non-synonymous variants with a minor allele frequency (MAF) below 1% and with predicted high impact on protein function, were selected for further analyses. A flow chart of our multi-step filtering strategy is shown in Supplementary Figure 1 and detailed in Supplementary Material.

We obtained a list of “candidate” variants that were exclusively shared by affected ET individuals within a family (Supplementary Table 2). These variants were visually inspected through the Integrative Genomics Viewer and discarded when any sequencing artifact due to strand bias was detected. All variants of interest were confirmed by Sanger sequencing when DNA was available and selected for further analyses (Supplementary Material).

In addition, we checked for presence and segregation of rare variants in the genes previously reported in association with ET in both Mendelian forms of disease and GWAS (Table 1). No rare exonic deleterious variants in ET related genes were found to cosegregate with disease within the families.

2.4. Genetic association analysis with ET status

Next, we performed targeted genotyping for the candidate variants and case-control association testing in a Spanish cohort (*Dataset 1*) comprising 521 unrelated ET cases and 596 age-matched healthy individuals (Supplementary Materials, Supplementary Table 1). Follow-up genotyping was performed using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry with Sequenom iPLEX Gold custom assays designed using MassARRAY assay design software (Sequenom, San Diego, CA, USA). For variants that could not be genotyped by Sequenom due to specific biochemistry of DNA sequence boundaries where the variant is located, we used the microfluidic-based BioMark™ HD array platform (Fluidigm Corporation, CA, USA). Details of the genotyping procedures are provided in Supplementary Material. After genotyping, we performed an

allelic Fisher's Exact Test in R (v1.2). All reported p-values are two-tailed and the significant threshold was set after Bonferroni correction.

2.5. Gene burden analysis

In order to investigate whether there was an enrichment of rare deleterious variants within our candidate genes in cases compared to controls, we performed gene-based burden analyses for disease trait in a separate cohort (*Dataset 2*) comprising 789 ET cases and 770 controls of European and North American ancestry (Supplementary Materials, Supplementary Table 1). Molecular inversion probes (MIPs) were used to capture all exons and exon/intron boundaries (5bp flanking) of the genes harboring variants nominally-associated in the case-control association analysis or seen in cases but absent in controls. MIPs were designed, targeted and amplified as previously described [27]. Briefly, the MIPs library was sequenced using Illumina HiSeq 4000 platform at the McGill University and Genome Québec Innovation Centre. Sequencing processing was done using BWA-MEM, Varscan (v2.3.9; samtools v1.9) for variant calling, and ANNOVAR for annotation (Supplementary Material). Quality control was performed as described in Supplementary Material. We then performed fixed threshold aggregation methods (BurdenBT tests) including only exonic, non-synonymous variants with a MAF < 1% as registered in gnomAD using Variant Association Tools (<http://varianttools.sourceforge.net/Association/Aggregation>).

3. Results

Basic demographic characteristics of the family members that were exome sequenced are shown in Supplementary Table 1. The average age at onset of tremor in ET patients was 38.8 years (± 19.5) and average duration of disease was 16.6 years (± 11.8). The average age for healthy relatives was 49 years (± 22.2). The number of affected individuals enrolled per family ranged from two to seven (mean=3.6).

All 40 samples sequenced passed quality control filters and the total number of SNVs/indels numbered 177,970. After annotating for functional impact, pathogenicity and allele frequencies, we assessed variants for segregation in ET pedigrees. Using an AD model of inheritance, 47,286 variants in 29,089 genes segregated with ET affection status in at least one pedigree, of which 10,726 (22.7%) were seen in more than one family. Following our multi-step filtering strategy, we identified a total of 15 rare variants predicted to affect protein function that segregated perfectly with the disease in seven out of the eight families (Supplementary Table 2). Among these variants, 13 were private (only seen in one family) and two were present in two different families (A and H), and included five stopgained SNVs, three insertions and seven deletions. Twelve variants (80%) were predicted as having a high functional impact (CADD score > 20) and five (33.3%) as being disease causing. A total of five genes contained segregating novel variants.

We then performed single-variant association analysis using an allelic Fisher's Exact Test. Of note, one variant (rs11288615 in *OR5K4*) could not be genotyped due to DNA sequence boundaries, being excluded from the analysis. We found no significant results after Bonferroni correction (significance threshold = 0.0036). However, we identified three

variants nominally associated (p-value < 0.05) with ET, which included rs749875462 in *CCDC183* (p-value = 0.0087), rs535864157 in *MMP10* (p-value = 0.0220) and rs114285050 in *GPR151* (p-value = 0.0425) (Table 2). **Family A.** The variant identified in *CCDC183* gene (c.1025G>A; rs749875462) results in a nonsense amino-acid substitution (p.Trp342*) predicted as disease-causing. Sanger sequencing in an additional unaffected relative (IV1) confirmed that the variant was only present in affected individuals within the family. We also found this variant in heterozygous state in 10 independent ET cases and in homozygous state in two ET patients, and in heterozygous state in four controls (Table 2). Although the *CCDC183* variant identified is at a higher frequency in the African population (0.07%), our ancestry analysis showed no African admixture (Supplementary Figure 1). **Family F.** We found a possible pathogenic frameshift variant (c.32_33delGT; rs535864157) in *MMP10* present in all three affected individuals but absent in one healthy relative. This variant was also present in heterozygous state in additional 5 ET patients but absent in 596 controls (Table 2). The *MMP10* rs535864157 variant is situated in the N-terminal signal peptide of the protein, which directs the protein to the secretory pathway and therefore it may impact in its release into the extracellular space. **Family H.** A SNV (c.283C>T; rs114285050) in *GPR151* was found in all four affected individuals and resulted in a nonsense amino-acid substitution (p.Arg95*) predicted as disease-causing. In addition, we found this variant in heterozygous state in 10 unrelated ET cases and 6 controls, and in homozygous state in 2 ET patients (Table 2). The rs114285050 is located in the transmembrane domain, one of the most conserved parts of the protein. G-protein coupled receptors have seven hydrophobic regions, each of which most probably spans the membrane. The N-terminus is located on the extracellular side of the membrane and is often glycosylated, while the C-terminus is cytoplasmic and generally phosphorylated. Three extracellular loops alternate with three intracellular loops to link the seven transmembrane regions.

CCDC183, *GPR151* and *MMP10* genes have observed/expected (o/e) scores of 0.66 (90%CI 0.460-0.98), 0.93 (90%CI 0.6–1.5) and 0.99 (90%CI 0.71–1.41), indicating that they are highly tolerant to loss-of-function (LOF) variants, which are unlikely to be pathogenic (Supplementary Materials). We also used GeneMANIA software (<https://genemania.org/>) to identify how these genes may impact tremor circuits leading to tremor but found no potential interactions with proteins involved in ET brain circuits such as GABAergic associated proteins, voltage-gated calcium channels or LINGO1 [28].

Next, we performed gene-based analyses. MIPs sequencing and subsequent variant calling identified 184 SNVs within *GPR151*, *CCDC183*, *MMP10*, *GEMIN5*, and *MS4A14* genes. After filtering for only exonic, non-synonymous variants with a MAF < 1%, 88 rare protein-altering variants remained and were included in gene-based burden analyses (Supplementary Materials). We did not find a significant enrichment of rare deleterious variants within the candidate genes in cases compared to controls (Table 3). However, we found additional rare and potential deleterious variants in those genes in other unrelated ET patients (Supplementary Table 7).

4. Discussion

In this study, we sought to identify novel genetic candidates for ET using a comprehensive strategy combining WES, followed by case-control association testing and gene burden analyses. Our stringent filtering approach for only protein-altering variants and subsequent targeted genotyping resulted in a small number of candidate genes nominally-associated with ET risk, which include *CCDC183* (family A), *MMP10* (family F) and *GPR151* (family H). The absence of genes with rare functional variants segregating in more than two families evidences the genetic heterogeneity of ET.

It is of special interest the putative association between *MMP10* and ET. *MMP10*, also called stromelysin-2, belongs to the matrix metalloproteinases (MMPs) family, which are zinc-dependent endoproteases involved in tissue remodeling and degradation of components of the extracellular matrix. *MMP10* is also known to play an important role in cell proliferation, migration, differentiation and angiogenesis, as well as vascular integrity [29]. *MMP10* is induced by inflammation, known to be highly expressed by activated microglia, and may be involved in the inflammatory response to neuronal damage [30]. Interestingly, some studies have suggested a potential involvement of *MMP10* in Alzheimer's disease pathophysiology. Higher *MMP10* levels are found in cerebrospinal fluid (CSF) of Alzheimer's disease patients compared to both controls and patients with vascular dementia, which indicates a brain-specificity of this protein. *MMP10* expression was also increased in neurons of the ischemic brain but not in healthy areas in humans [31] and mice [32], suggesting that its expression is linked to brain injury and to the subsequent inflammatory response. CSF *MMP10* levels correlate with both CSF total tau and phosphorylated tau [33] and with cognitive performance [34]. Similarly, a recent study found a positive correlation of CSF *MMP10* levels with PD progression [35]. Also, *MMP10* is the only metalloproteinase that directly cleaves the huntingtin protein and specific *MMP10* inhibitors may have a therapeutic benefit in Huntington's disease (HD), thus pointing *MMP10* as a potential genetic modifier for HD [36]. However, in order to increase the evidence supporting this gene as a causative gene for ET, further replication and functional studies are needed.

We are aware that our association and gene-based analyses are likely underpowered, as rare variant analyses require thousands of individuals to reach acceptable statistical power [37]. A reasonable replication strategy is to select a few genes based on strength of association and perform sequencing and rare-variant associations on new samples, using multiple-test correction threshold applied only to the smaller set of candidate genes [37], as we did in this study. However, we did not identify new genes consistently significant across all of our analyses. Recent sequencing of thousands of exomes from across the world has revealed that the vast majority of genetic variation is rare and highly population-specific [14,38], meaning that our lack of replication might also be explained by genetic differences across populations. In addition, familial clustering in some ET cases may not be due to a single highly-penetrant variant with large effects but a high genetic load of common risk variants. It would have been very useful to perform complementary mapping gene strategies such as linkage analysis – especially in pedigrees G and H – but for most individuals there was not enough DNA available.

Our study has several limitations. First, we did not account for the potential contribution of common variants to the etiology of ET; although it could be argued that common variants may have a reduced impact in multiplex families with strong aggregation of a disease. Second, WES does not cover noncoding or structural variants, which are believed to play a major role in complex trait genetics despite having small effect sizes. Indeed, structural variants have been recently reported to be associated with familial ET [39]. Future long-sequencing analyses might unravel disease-causing structural variants associated with disease, and whole genome sequencing (WGS) may help reveal intronic variants with potential regulatory effects. Indeed, WGS recently identified candidate genes for ET in a subset of families in which WES analysis previously failed to identify the causative variant due to incomplete coverage of the entire coding region of the genome [40]. Third, the power of segregation analysis of individual rare variants in small families is limited and presence of phenocopies within a family could have an impact on the interpretation of apparently non-segregating but pathogenic variants. We cannot exclude the contribution of pathogenic variants found that partially segregate with the disease and that were not further studied. Fourth, some unaffected subjects within the families were young and we could not rule out the possibility that they would develop the disease in the future. Due to the high clinical heterogeneity in ET, we are aware that collecting more samples from additional affected and unaffected relatives would have been very helpful to confirm the causative variants. We argue that more detailed phenotypic data could also help to identify subjects who may have varying amounts of genetic risk.

In summary, here we studied a unique cohort of pedigrees with strong disease aggregation to identify rare variants of high penetrance contributing to ET susceptibility. Our analysis prioritized a set of promising genes, especially *MMP10*, for further genetic and functional studies in ET, but also suggests that rare deleterious coding variants that markedly increase susceptibility to ET are likely to be found in many genes with low effect size. Future genetic studies including a more detailed clinical characterization of ET patients and larger scale GWAS are needed to better understand the underlying etiology of such a common disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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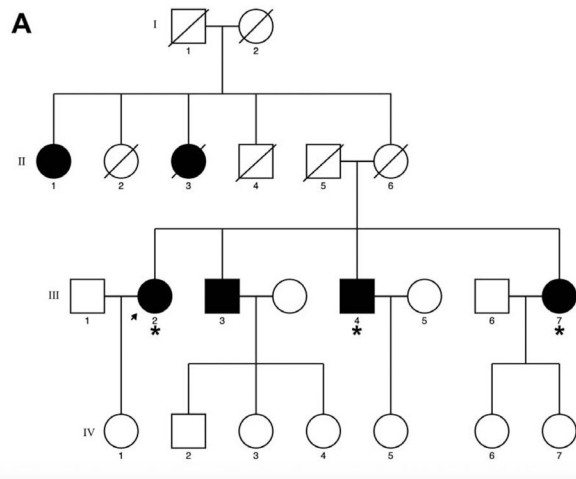
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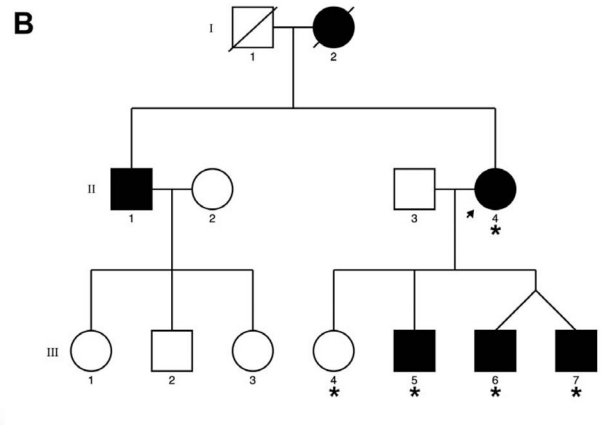
Highlights

- The genetic aetiology of essential tremor remains elusive.
- Whole exome sequencing was performed in 8 multigenerational Spanish ET families.
- *CCDC183*, *MMP10* and *GPR151* genes were nominally associated with ET.
- *MMP10* has been associated with other neurological disorders such as AD and PD.
- ET is likely to be caused by variants in different genes with small effect sizes.

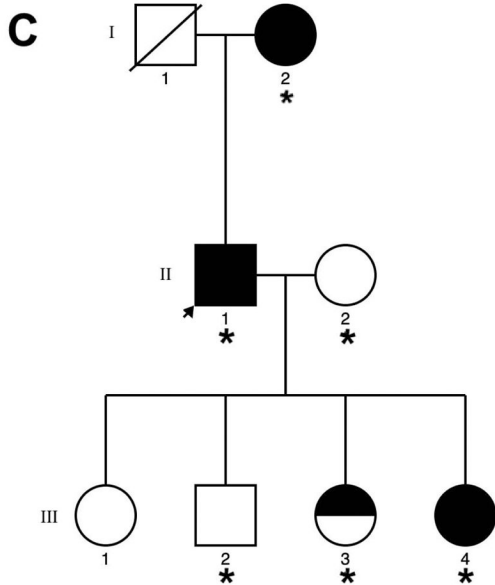


Family A	III-2	III-4	III-7
Age	72	64	55
AAO	50	54	Unknown
CCDC183 c.1025G>A	G A	G A	G A

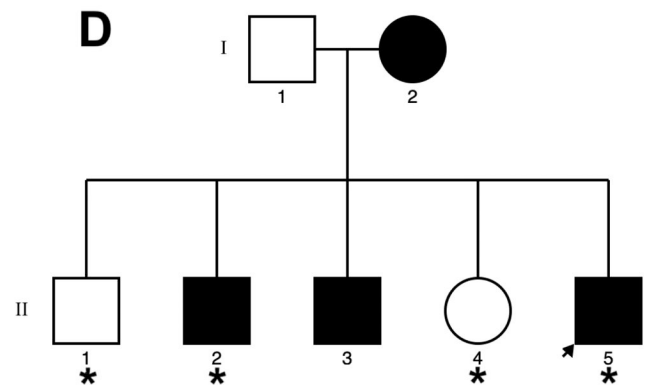
Sanger sequencing performed also on relative IV-1 and confirmed genotype G|G



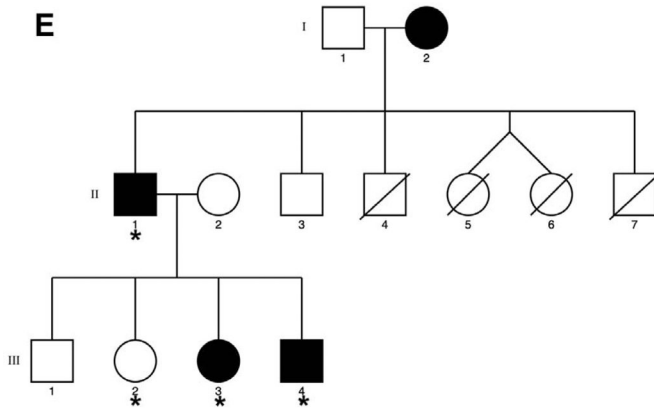
Family B	II-4	III-4	III-5	III-6	III-7
Age	78	41	39	36	36
AAO	55	-	Unknown	26	Unknown



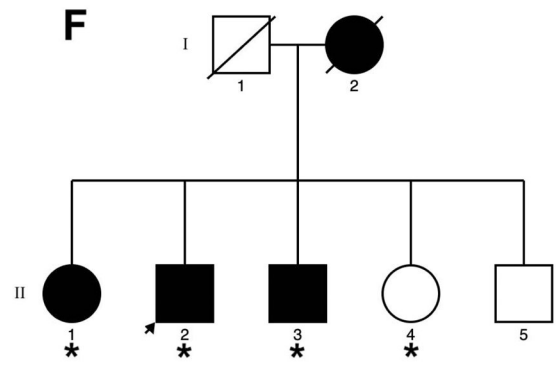
Family C	I-2	II-1	II-2	III-2	III-3	III-4
Age	79	51	50	29	24	19
AAO	60	20	-	-	Unknown	10



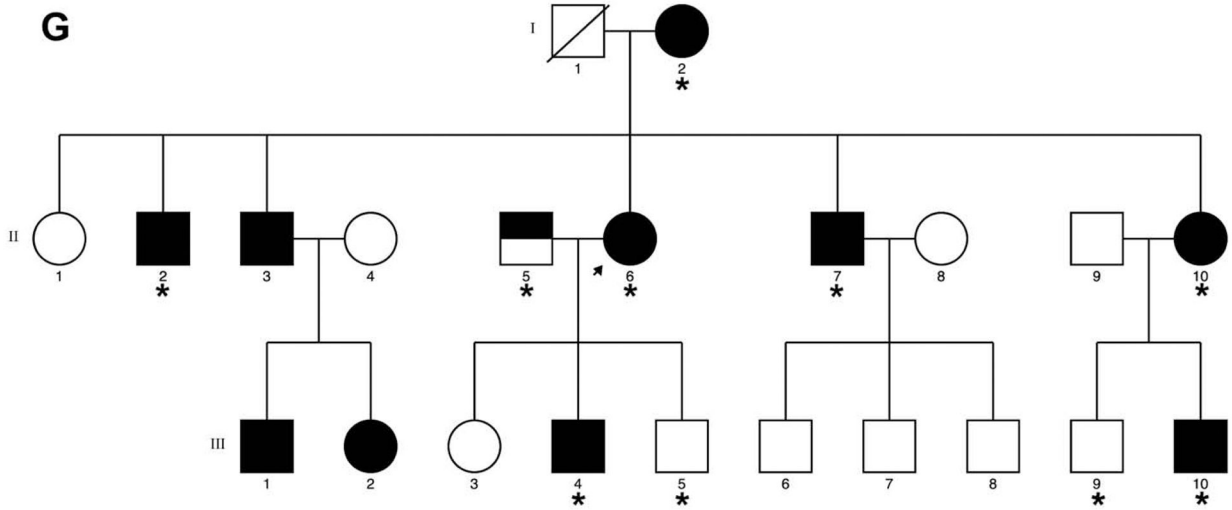
Family D	II-1	II-2	II-4	II-5
Age	87	86	77	72
AAO	-	48	-	50



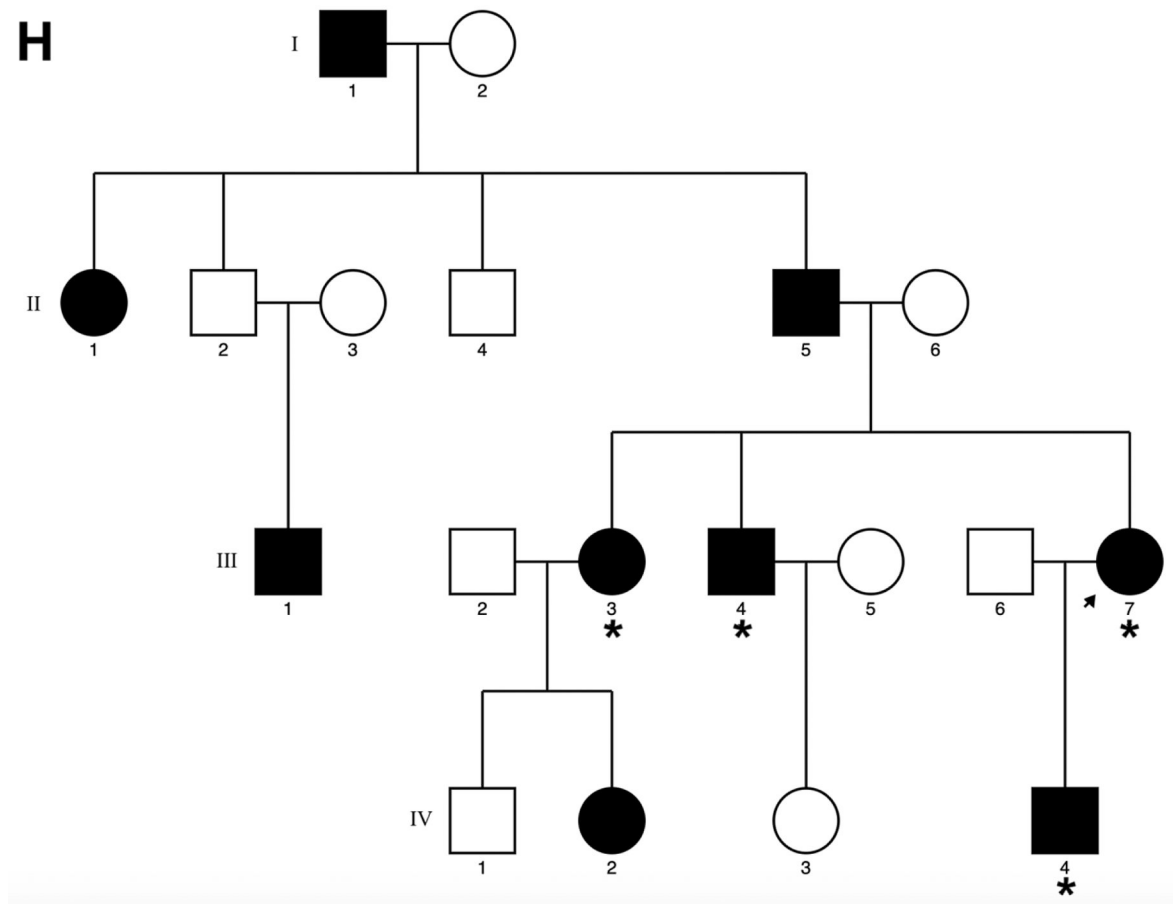
Family E	II-1	III-2	III-3	III-4
Age	65	37	32	31
AAO	60	-	22	18



Family F	II-1	II-2	II-3	II-4
Age	71	69	66	64
AAO	Unknown	66	Unknown	-
<i>MMP10</i> c.32_33delGT	GAC G	GAC G	GAC G	GAC GAC



Family G	I-2	II-2	II-5	II-6	II-7	II-10	III-4	III-5	III-9	III-10
Age	87	65	62	62	60	58	29	27	29	28
AAO	80	20	62	30	45	30	20	-	-	22



Family H	III-3	III-4	III-7	IV-4
Age	54	51	49	21
AAO	42	Unknown	25	15
<i>GPR151</i> c.283C>T	G A	G A	G A	G A

Figure 1. Pedigrees for the families that underwent WES, asterisk indicates the family members sequenced.

Genetic pedigrees plots of the eight families (A-H) included in the study are shown. The generation in each pedigree is indicated by roman numerals. The proband is indicated by an arrow. Red asterisk indicates those subjects that were exome sequenced (N=40). Below each pedigree, age at enrollment, age at tremor onset (for cases, if known), tremor region and severity are shown in a table. Tremor region (localization of the tremor): Head (H), Tongue (T), Chin (C), Upper limbs (UL), Lower limbs (LL). Tremor severity corresponds to scores in the Part A of the more affected body region according to the Fahn-Tolosa-Marin Clinical Rating Scale for Tremor (FTM) [41]. Shading legend: Symbols completely black indicate individuals with clinical definite ET diagnosis; Symbols half vertical black fill indicate individuals with unclear ET diagnosis; Symbols completely blank indicate unaffected

individuals; Symbols with a diagonal line indicated deceased relatives. Segregation within families for the three candidate variants (rs749875462 in *CCDC183*, rs535864157 in *MMP10* and rs114285050) is also shown in families A, F and H.

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Table 1.

Summary of genes and *loci* that have been previously associated with essential tremor.

Genes or <i>loci</i> (chr position)	Ethnic or geographic distribution	Study sample size (cases/controls)	Methodology used	References
<i>ETM1</i> (3q13)	Icelandic	16 families	Genome-wide scan	Gulcher et al., 1997 [6]
<i>ETM2</i> (2p24.1)	American of Czech ancestry	1 family	Genome-wide scan	Higgins et al., 1997 [7]
<i>ETM3</i> (6p23)	North American	7 families	Genome-wide scan	Shatunov et al., 2006 [8]
<i>HST1-BP3</i>	North American	2 families	Fine mapping studies	Higgins et al., 2005 [*]
<i>DRD3</i>	French	30 families	Candidate-gene approach	Lucotte et al., 2006 [†]
<i>LINGO1</i>	Icelandic, European and American	752/15,797	GWAS	Stefansson et al., 2009 [10]
<i>SLC1A2</i>	European	658/1,490	GWAS	Thier et al., 2012 [11]
<i>FUS</i>	French-Canadian	1 family and 270 cases	Exome sequencing	Merner et al., 2012 [15]
<i>HTRA2</i>	Turkish	1 family	Exome sequencing	Unal Gulsuner et al., 2014 [16]
<i>SORT1</i>	Spanish	1 family and 151 cases	Exome sequencing	Sanchez et al., 2015 [17]
<i>TENM4</i>	Spanish	1 family and 299 familial cases	Exome sequencing and targeted resequencing	Hor et al., 2015 [18]
<i>SCN4A</i>	Spanish	1 family, 76 sporadic and 47 familial cases	Exome sequencing	Bergareche et al., 2015 [19]
<i>NOS3, KCNS2, HAPLN4, USP46</i>	North American	37 families	Exome sequencing	Liu et al., 2016 [20]
<i>STK32B, PPARGC1A, CTNNA3</i>	Northern European and North American	2,807/6,441	GWAS	Müller et al., 2016 [12]

Key: chr, chromosomal; GWAS, Genome-wide association study.

* J.J. Higgins, R.Q. Lombardi, J. Pucilowska, J. Jankovic, E.K. Tan, J.P. Rooney, A variant in the HST1-BP3 gene is associated with familial essential tremor, *Neurology*. 64 (2005) 417–421.

† G. Lucotte, J.P. Lagarde, B. Fumalot, P. Sokoloff, Linkage with the Ser9Gly DRD3 polymorphism in essential tremor families, *Clin. Genet*. 69 (2006) 437–440.

Table 2.

Single-variant case-control association analysis in 521 ET cases and 596 healthy individuals.

Variant (HGVS)	Gene	rs ID	Reference allele	Alternate allele	ET [n = 521] (AF)	Controls [n = 596] (AF)	P-value* (OR) [95% CI]
NM_001145710.1: c.906delC	<i>FAM228B</i>	Novel	C	DEL	35 (0.034)	26 (0.022)	0.0922 (1.56) [0.91–2.72]
NM_003241.3: c.806G>A	<i>TGM4</i>	rs139860990	G	A	18 (0.017)	16 (0.013)	0.4918 (1.29) [0.62–2.72]
NM_001005516.1: c.904delA	<i>OR5K3</i>	rs79045298	A	DEL	0 (0)	0 (0)	1 (nan)
NM_183375.2: c.127_131delGTCAG	<i>PRSS48</i>	Novel	GTCAG	DEL	0 (0)	0 (0)	1 (nan)
NM_194251.2: c.283C>T	<i>GPR151</i>	rs114285050	G	A	14 (0.013)	6 (0.005)	0.0425 (2.69) [0.97–8.58]
NM_015465.4: c.2519_2522delTCAA	<i>GEMIN5</i>	rs769616395	TTGA	DEL	2 (0.002)	0 (0)	0.2174 (nan)
NM_004999.3: c.2751insA	<i>MYO6</i>	rs551348450	DEL	A	0 (0)	0 (0)	1 (nan)
NM_001039374.4: c.1025G>A	<i>CCDC183</i>	rs749875462	G	A	14 (0.013)	4 (0.003)	0.0087 (4.04) [1.26–16.91]
NM_002425.2: c.32_33delGT	<i>MMP10</i>	rs535864157	AC	DEL	5 (0.005)	0 (0)	0.0220 (nan)
NM_001261828.1: c.167_168insTT	<i>MS4A14</i>	Novel	DEL	TT	2 (0.002)	0 (0)	0.2174 (nan)
NM_144666.2: c.2839G>T	<i>DNHDI</i>	rs180918289	G	T	27 (0.026)	47 (0.039)	0.0768 (0.65) [0.39–1.07]
NM_001005182.1: c.244delA	<i>OR6C1</i>	Novel	A	DEL	0 (0)	0 (0)	1 (nan)
NM_181536.1: c.3691_3694delCAAAA	<i>PKD/L3</i>	Novel	TTTTG	DEL	0 (0)	0 (0)	1 (nan)
NM_032423.2: c.1282C>T	<i>ZNF528</i>	rs150257846	C	T	2 (0.002)	2 (0.002)	1 (1.14) [0.08–15.81]

Key: AF, allele frequency; CI, confidence interval; DEL, deletion; HGVS, Human Genome Variation Society; nan, not a number/not available; OR, odds ratio.

* P-values derived from Fisher's Exact test. All p-values were two-tailed and significant threshold was set at 0.0036 after Bonferroni correction (0.05/14 variants). Nominal associations (p < 0.05) are highlighted in bold.

Gene-based burden association tests of candidate genes in 789 ET cases and 770 healthy individuals.

Table 3.

Gene name	Sample size	N of variants*	Total MAC [‡]	Beta	P-value	Wald statistic
<i>CCDC183</i>	1559	15	48	-0.2842	0.8599	-1.0798
<i>MMP10</i>	1559	13	37	0.1209	0.3589	0.3615
<i>GPR151</i>	1559	13	19	-0.1075	0.5964	-0.2442
<i>MS4A14</i>	1559	16	43	0.2237	0.2317	0.7332
<i>GEMIN5</i>	1559	31	138	-0.2798	0.9512	-1.6564

Key: ET, Essential tremor; MAC, minor allele count; N, number.

Only exonic deleterious variants (missense, non-sense SNVs) with a minor allele frequency below 1% in gnomAD non-Finnish Europeans exomes were used for the analysis. Bonferroni corrected p-value threshold for MAF<1% is 0.01.

* The number of variants in each group is adjusted for specified MAF; in this case, below 0.01.

[‡]The total minor allele count (MAC) in a group adjusted for mode of inheritance.