Supporting Information

Unal Gulsuner et al. 10.1073/pnas.1419581111

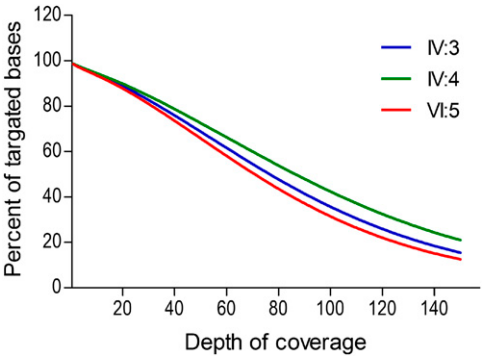

Fig. S1. Percentage of targeted bases covered at particular depths.

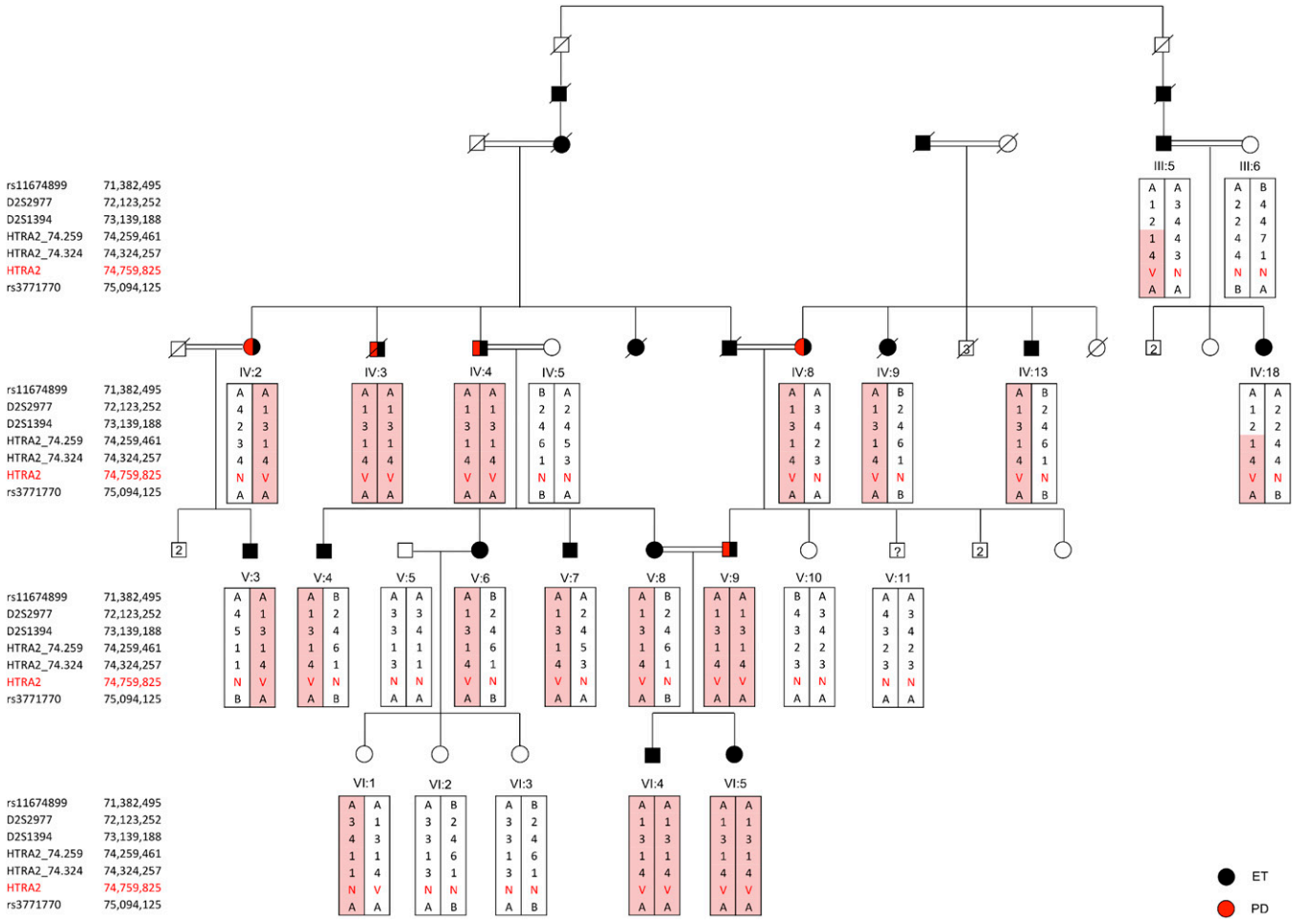

Fig. S2. Haplotype structure at the *HTRA2* locus for the ET-1 family. All participating family members were genotyped for six informative markers spanning 3.7 mb flanking *HTRA2* and haplotypes constructed by direct pedigree analysis. The 12 subjects heterozygous for HTRA2 p.G399S harbor 9 different haplotypes for their alternate allele, providing strong evidence against a second pathogenic mutation closely linked to *HTRA2*.

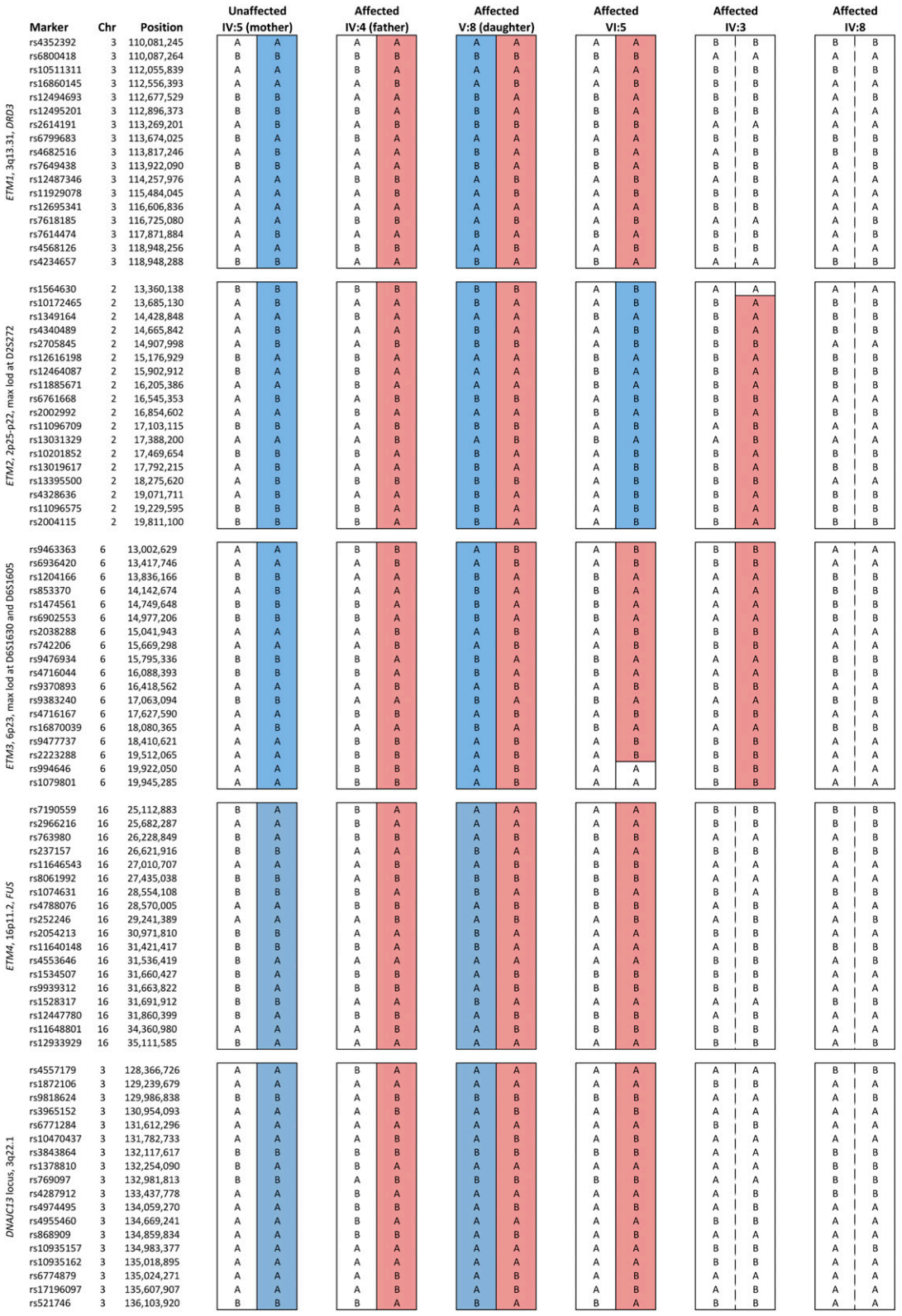

Fig. S3. Essential tremor in family ET-1 is not linked to *DNAJC13* or to genomic regions previously reported to be associated with essential tremor. DNA samples from five affected subjects (IV:3, IV:4, IV:8, V:8, and VI:5) and from one unaffected subject (IV:5) were genotyped by using GeneChip mapping 250K NspI SNP arrays and analyzed by using GTYPE software (Affymetrix). Haplotypes were generated to evaluate the possibility of a disease-causing mutation in any of these regions (1–5). At each region, the haplotype shared by the affected father-daughter pair IV:4 and V:8 is indicated in pink. At none of these regions was this haplotype also shared by the other affected family members.

1. Gulcher JR, et al. (1997) Mapping of a familial essential tremor gene, FET1, to chromosome 3q13. *Nat Genet* 17(1):84–87.

2. Higgins JJ, Pho LT, Nee LE (1997) A gene (ETM) for essential tremor maps to chromosome 2p22-p25. *Mov Disord* 12(6):859–864.
3. Shatunov A, et al. (2006) Genomewide scans in North American families reveal genetic linkage of essential tremor to a region on chromosome 6p23. *Brain* 129(Pt 9):2318–2331.
4. Merner ND, et al. (2012) Exome sequencing identifies FUS mutations as a cause of essential tremor. *Am J Hum Genet* 91(2):313–319.
5. Rajput A, et al. (2014) VPS35 and DNAJC13 disease-causing variants in essential tremor. *Eur J Hum Genet*, 10.1038/ejhg.2014.164.

Table S1. Clinical characteristics of affected individuals of family ET-1

			Tremor									Archimedes spiral test		Bradykinesia		Rigidity			
			Resting		Postural		Kinetic												
Individual	Age at Onset	Age at Examination	R	L	R	L	R	L	Head	Chin	Voice	R	L	R	L	R	L	Postural instability	Hypomimia
III:5	50	78	—	+	+	++	+++	+++	Yes	No	Yes	+++	+++	—	—	+	+	—	+
IV:2	40	79	++	+	++	++	++	++	Yes	No	Yes	+++	+++	+	+	—	—	—	—
IV:3	<30	81	—	—	++	+++	++	+++	No	No	No	+++	+++	++	++	+++	++	+++	++
IV:4	<30	89	—	—	++	+++	++	+++	No	No	No	+++	+++	++	++	+++	++	+++	++
IV:8	40	81	++	+	++	+++	++	+++	No	Yes	No	++	+	+++	++	+++	++	+	+
IV:13	59	67	—	—	+	++	+	++	No	No	No	+	+	—	—	+	—	—	—
IV:18	10	45	+	+	+++	+++	+++	+++	No	Yes	Yes	+++	+++	—	—	—	—	—	+
V:3	48	56	—	—	+	+	+	+	No	No	No	+	+	—	—	+	—	—	—
V:4	30	61	—	+	+	+	+	+	No	No	No	+	+	—	+	+	+	—	—
V:6	50	60	—	—	+	+	—	—	No	No	No	—	—	—	—	+	—	—	—
V:7	<55	59	—	—	++	+	+	++	No	No	No	+	+	—	—	—	—	—	—
V:8	12	50	+	+	+++	+++	+++	+++	Yes	No	No	+++	+++	—	+	—	+	—	+
V:9	15	56	+	++	++	+++	+	++	No	No	No	+++	++	++	+	+	+	—	+
VI:5	10	25	—	+	+++	++	++	++	No	No	No	++	++	—	—	—	—	—	+

For tremor +, low amplitude or barely perceivable tremor; ++, moderate amplitude tremor (1-2 cm); +++, large amplitude, severe tremor (>2 cm) (1). For Archimedes spiral test, bradykinesia, rigidity, postural instability and hypomimia: +, mild; ++, moderate; +++, severe. L, left hand; R, right hand. None of the subjects had intentional tremor. Subject IV:2 developed postural and action tremor at age 40 and subsequently bradykinesia and resting tremor. Subjects IV:3, IV:4, and V:9 developed mild to moderate postural and action tremor before age 30, and bradykinesia, rigidity, resting tremor, and postural instability with increasing age. Subject IV:8 developed postural and action tremor at age 40 and subsequently resting tremor, bradykinesia, rigidity, and postural instability. Subject IV:13 was using propranolol at the time of examination. Subjects V:3, V:4, V:6, and V:7 had mild tremor of at least 4-y duration. Subject V:8, whose clinical features of essential tremor were particularly severe, was diagnosed with essential tremor at age 12 and with Hashimoto's thyroiditis at age 49. Subject IV:9 was deceased, V:11 declined clinical examination, and VI:4 was not available for clinical examination but was assessed based on prior clinical history. These three subjects are not included in the table.

1. Louis ED, Ford B, Lee H, Andrews H, Cameron G (1998) Diagnostic criteria for essential tremor: A population perspective. *Arch Neurol* 55(6):823–828.

Table S2. Results of whole exome sequencing of three affected relatives from family ET-1

Feature	IV:3	IV:4	VI:5
Total number of reads	82,905,251	88,364,432	83,032,868
% of mapped reads	98.1	98.3	97.6
% of targeted bases covered ≥ 8X	95.1	95.4	94.6
Joint coverage at ≥ 8X,* %		93.6	
Average coverage, X	90	101	83
All coding variants	18,729	18,839	18,198
Shared by all 3 exomes		11,639	
Rare variants[†]		129	
Predicted damaging to protein function[‡]		13	
Cosegregation with essential tremor in the family		1 (HTRA2 p.G399S)	

*Joint coverage was defined as fold coverage for the least well covered of the three samples.
[†]Variants were excluded if minor allele frequency ≥ 0.005 on dbSNP138, the 1000 Genomes Project, or the NHLBI Exome Sequencing Project.
[‡]Alleles predicted damaging to protein function were of the following classes: truncating mutations; splice site mutations predicted to lead to altered transcripts; whole gene deletions; and missense variants with all of Polyphen-2 score ≥ 0.8, SIFT score ≤ 0.05, and Mutation Assessor score ≥ 1.95.

Table S3. Homozygous regions shared by three affected relatives

Chromosome	Start	End	Size, bp	All SNVs	Rare potentially damaging SNVs and indels*
2	73,518,867	75,115,108	1,596,241	48	1
14	94,912,799	96,157,331	1,244,532	41	0
22	16,953,727	18,650,682	1,696,955	77	0

Homozygosity mapping from whole exome data were performed by using PLINK V1.07 with 200 kb minimum segment size (1). In the shared homozygous regions, 98% of all coding bases were sequenced at least four times in at least one subject with average coverage of 76X.
*Alleles predicted damaging to protein function were of the following classes: truncating mutations; splice site mutations predicted to lead to altered transcripts; whole gene deletions; and missense variants with Polyphen-2 score ≥ 0.8, SIFT score ≤ 0.05, and Mutation Assessor score ≥ 1.95.

1. Purcell S, et al. (2007) PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81(3):559–575.

Table S4. All rare coding sequence variants predicted to be damaging and shared by three affected relatives of family ET-1

												Subjects with essential tremor		
Chr	Position	Ref	Var	Gene	Effect	Genotype	PPH2	SIFT	MA	GERP	ESP	1000G	NN	NV	VV
2	55,194,157	C	T	EML6	R1839C	Het	0.998	0.022	2.25	6.17	—	0.0009	4	10	2
2	**74,759,825**	**G**	**A**	**HTRA2**	**G399S**	**Homoz**	**0.986**	**0.020**	**2.39**	**3.99**	**0.0034**	**0.0023**	**0**	**11**	**5**
3	13,359,234	G	A	NUP210	R1871C	Het	0.999	0.021	2.07	4.58	—	—	11	5	0
4	1,343,416	T	C	UVSSA	L68P	Het	1.000	0.001	3.02	4.98	—	—	10	6	0
6	13,306,697	A	G	TBC1D7	L243S	Het	1.000	0	2.67	5.87	—	—	9	7	0
9	104,190,765	T	C	ALDOB	E122G	Het	0.997	0	3.36	5.87	0.0001	—	9	7	0
9	135,203,279	C	A	SETX	V1236F	Het	0.868	0.003	1.95	4.82	—	—	5	11	0
12	52,284,475	C	T	ANKRD33	R124W	Het	1.000	0.001	2.28	—	0.0005	—	11	5	0
15	41,797,248	C	T	LTK	R647Q	Het	1.000	0	2.50	3.79	0.0003	—	11	5	0
15	58,004,256	G	A	GCOM1	R675Q	Het	0.999	0.036	1.99	2.79	—	—	6	10	0
15	90,328,681	G	A	ANPEP	R935W	Het	1.000	0.013	3.12	5.31	0.0009	0.0005	11	5	0
17	31,098,168	T	C	MYO1D	Y230C	Het	0.998	0	3.48	5.82	0.0008	—	10	6	0
19	48,565,262	G	A	PLA2G4C	P417L	Het	0.819	0.013	2.48	2.79	—	—	9	7	0

The variant segregating with essential tremor in the ET-1 family is shown in bold. 1000G, 1000 Genomes Project; Chr, chromosome; ESP, NHLBI Exome Sequencing project; MA, MutationAssessor; N, wild-type allele; PPH2, Polyphen2; Ref, reference base; V, variant allele; Var, variant base.

Table S5. Primers used for PCR amplification and haplotype analysis

Primer name	Forward	Reverse
Segregation		
EML6	CTGAGCTTGGGTTTGGAGAA	CAGATCCTGCACAGACTTGG
HTRA2	ATGCCTGGGTTTGGCTAATA	CAACTGGGATTGGGTTCG
NUP210	GTGTGAAGAGACGGCAGTGA	TGTGAGAGTGTCCTGGGTGA
UVSSA	AATGAAGATGGGAAGGCAGT	AGTGGTAGCCCAAGGCAAG
TBC1D7	AAACTAACCCTCAGGCCAAC	GGGTTTGGGATAAAGTTGTGAG
ALDOB	GCTTCCTTCTTTACTTGCCTTC	GGGTCCCTCGCACTAATACA
SETX	GGCTCAGGACACTGACGAA	CCAATGGCTGAAGATCCTGT
ANKRD33	CCCACATCAGTCTTGCTCCT	GTGGCAGTCACAAGGTGGTC
LTK	TACAGGAGGGAGGAGGTGAA	TCATCCACAGGTTAGGAGCA
GCOM1	GGCCATTTCCTTATGTTCCA	GCTGTTTCTGAAGTGCCAAG
ANPEP	GGCTGGAGACTTTGTCCTTG	AGCTCCTCCTCAAGGCTGTT
MYO1D	TGGAGCAATCTCAAAGAGGA	CTAAGCAAGCAACCACCACA
PLA2G4C	CAGAAGTTCGTTGGATGTGG	CCTGGTGGATGCTGGTTTAG
Coding regions		
HTRA2_1	GTCCTACTGTCCGCCTGCT	CTGTGACCACGGCCTCATAC
HTRA2_2	TGTGGTGGAGAAGACAGCAC	AGAGCTAACAATGCCGGATG
HTRA2_3	CGCTGAGGATTCAGACTAAGG	CTTGGAAGGAAGGATGTCTCA
HTRA2_4	TCAGTGTGGGAAGGGTAGGT	GACAGAAATGAGAACAAAGCTCA
HTRA2_5	TTGATGAGAGACTTGAGGTGGA	AGGAGTCAGTGCTGGTGGTT
Regulatory regions		
HTRA2_3UTR	TGAGGCTCCTGCTCTGATTT	AGCATGGGAATCTTTGCTC
HTRA2_5UTR1	CCTCGTGGAAGCACAGAATC	GGACACAGGAGGTGGTGACT
HTRA2_5UTR2	TCACGGTGCTACAGGTGGTA	GAGCGGCTCTTTGACTCG
HTRA2_5UTR3	CGAGCAGTAGGAAGCAGTCA	AGCAGGCGGACAGTAGGAC
STRs		
HTRA2_74.259	FAM-GGGAGGAGTTGTGAGAATGC	GTTTCTTAGCACACAAGAACCAGGTCA
HTRA2_74.324	HEX-AGCAGGTCACAGAATAGCATGT	GTTTCTTTTTAGATGACTACCCAGACATTTG
D2S2977	FAM-GGCAGCACAGTGACAGATAA	GTTTCTTCTTCCCCAATCAACTCTCCT
D2S1394	FAM-GGCATCTTTATCCTTAGCCC	GTTTCTTCGGGGTCTGCATTACAGTAT
SNPs		
rs11674899	TTCAAGTGACATTCAAGAGAGAGC	GTGAGGGTGGAGAGACCAGA
rs3771770	AGTGGCCTGACTCTTGTCGT	GACTACCTCCTCAGTGCTTTCA