Whole Exome Sequencing in Essential Tremor

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Background

Essential tremor (ET) is a common movement disorder, affecting about 5% of the population older than 65 years of age. The etiology of ET remains unclear. Given that up to 75% of patients have a positive family history and there is high concordance among monozygotic twins, ET is likely to have a genetic etiology. However, the search for genes has largely proven elusive. Genes previously implicated in ET are shown in Table 1, but most require replication.

Whole exome sequencing (WES) is a promising tool to elucidate the genetics of ET, potentially identifying genetic variants that could contribute to ET pathophysiology. Identifying genes associated with ET can aid in diagnosis, risk prediction and possibly targeted therapeutic strategies.

In this study, we performed WES in families and individual cases with ET and analyzed for pathogenic variants that may be associated with disease.

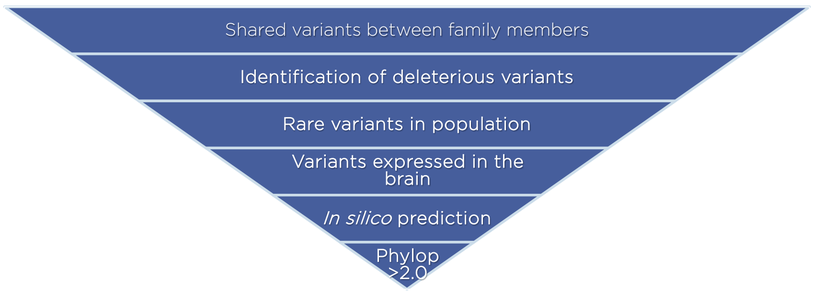


Figure 1. Schematic representation of analysis of variants.

Methods

Three families and 14 individual ET cases were included in our WES cohort. We initially evaluated the presence of variants in genes previously described in the literature (Table 1). None were detected in our cohort. Next, we focused on two related subjects from each of three families. The common variants in each family were then filtered and ranked.

The variant filtering strategy is described in Figure 1. First, we identified shared variants among subjects from individuals in the same family. We identified: frameshift insertions/deletions, stopgain single nucleotide variants (SNVs), non-synonymous SNVs, and splicing variants. Next, population-level filters were applied to enrich for rare variants. Cohorts with healthy controls (ARIC-Atherosclerosis Risk in Communities) and individuals not known to have a diagnosis of ET (ExAC-Exome Aggregation Consortium) were used to determine the frequency of candidate variants. Only variants with ARIC scores <10 and allele frequencies <0.001 in ExAC were included. Then, the Genotype-Tissue Expression (GTEx) project was used to select genes expressed in the brain.

Subsequently, nonsynonymous SNVs variants were ranked based on *in silico* predictions of pathogenicity (SIFT, Polyphen2, Mutation Taster, LRT). Within each group, the variants were further ranked by Phylop score (>2.0), based on conservation of protein in 46 vertebrate species and by known function of the protein (PubMed). Splice site variants were ranked in order of distance from the closest exon because variants closer to the exon are expected to be more deleterious.

Individual cases were evaluated for variants in the top prioritized genes within each family.

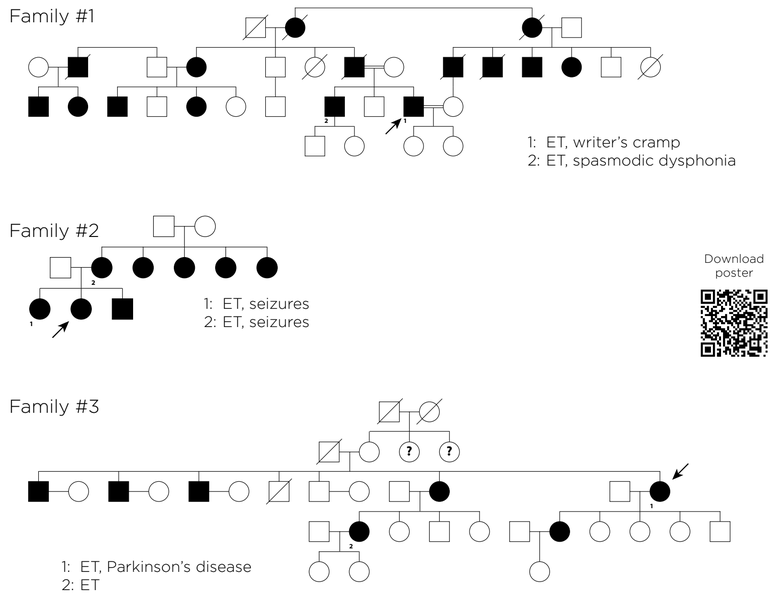
Table 1. Genes associated with ET.

LINGO1	*HAPLN4*	*HTRA2*	*FUS*	*SLC1A2*	*PPARGC1A*
DNAJC13	*USP46*	*HS1BP3*	*DRD3*	*SORT1*	*MAPT*
STK32B	*TENM4*	*KCNS2*	*SCN4A*	*CTNNA3*	*NOS3*

Subjects / Families

Our familial ET WES cohort of 20 cases (55% female) includes six subjects from three families (two related subjects per family), and 14 individual cases (one subject per family). The median age at tremor onset is 19.5 years (range 4-57 years) and the median age at enrollment was 52.5 years (range 38-75 years). For all three pedigrees, we assumed an autosomal dominant pattern of inheritance, but we cannot exclude X-linked inheritance for family #2.

Figure 2. Pedigrees



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Criteria	Number of Variants in Family #1	#2	#3
Shared variants between family members	477	489	406
Frameshift insertions/deletions, stopgain SNVs, nonsynonymous SNVs, splice site	432	425	273
1. Allele frequency < 0.001 in ExAC 2. ARIC score < 10 3. Expressed in brain	137	103	103
Frameshift, stopgain	1	3	2
Nonsynonymous SNVs	37	24	16
Splice site	13	21	5

	Gene	Variant	ExAC freq.	Function	Present in other members of the cohort
Family #1	*GSTM5*	c.C214T:p.Q72X	0	Glutathione-S-Transferase, role in drug metabolism, role in macular degeneration, modifier of Parkinson's disease age of onset	c.A649G:p.S217G
	CCT4	c.G1033A:p.V345I	0.0000082	Chaperone protein, related to sensory neuropathy in rats	N/A
	FARSB	c.C379T:p.R127C	0.000033	Phenylalanine-tRNA synthetase, no known relationship to human disease	c.C292T:p.R98W
	PHLDB1	c.G275A:p.G92D	0.000050	Pleckstrin homology domain-like protein, risk factor for glioma	N/A
	AKAP11	c.C1007T:p.P336L	0.00030	Protein kinase A anchor protein, role in microtubule polymerization and cell migration	N/A
Family #2	*DFNA5*	c.1174_1175insC:p.A392fs	0.000025	Role in programmed cell death, autosomal dominant deafness, possible tumor suppressor gene	N/A
	KIAA1407	c.2539_2543del:p.N847fs	0	Unknown	c.G1193A:p.R398Q
	FAM120B	c.C424T:p.R142X	0	Transactivator of PPARG and ESR1; functions in adipogenesis through PPARG activation	c.A1289G:p.D430G c.T1291C:p.S431P
	BAI2	c.C4339T:p.R1447C	0.0000092	Role in brain angiogenesis inhibition	c.C329T:p.P110L c.C4594T:p.R1532C
	TAF1	c.C2402T:p.T801M	0.000011	X-linked dystonia-parkinsonism	N/A
Family #3	*SLC44A1*	c.G932A:p.R311H	0	Choline transporter - may be involved in membrane synthesis and myelin production	N/A
	SART3	c.C1351T:p.R451C	0.0001	RNA binding nuclear protein, role in splicing	c.G37C:p.E13Q
	COL19A1	c.A2845C:p.K949Q	0	Collagen 19 A1, function unknown	N/A
	SZT2	c.A4102G:p.S1368G	0.00013	May function as a component of the nuclear pore complex	c.G2578A:p.E860K c.C4171T:p.R1391C
	PANX3	c.G238A:p.V80M	0.00031	Structural component of the gap junctions	N/A

Conclusions / Future Directions

- We identified several promising candidate variants potentially associated with ET.
- Family-based WES analysis of subjects with ET is a promising approach to discover variants that contribute to disease.
- Additional sequencing of affected and unaffected family members as well as larger ET cohorts will be required to determine if the variants have a definite role in the disease risk.

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Funding source: R01NS073872 (NIH / NINDS)