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Investigating the role of *FUS* exonic variants in Essential Tremor

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Abstract

Essential Tremor is the most common form of movement disorder. Aggregation in families suggests a strong genetic component to disease. Linkage and association studies have identified several risk loci but the specific causal variants are still unknown. A recent study using whole exome sequencing identified a rare nonsense variant in the *FUS* gene (p.Q290X) that segregated with Essential Tremor in a large French Canadian family. In addition, two other rare *FUS* variants were identified (p.R216C and p.P431L) in Essential Tremor patients however co-segregation analysis with disease was not possible. In the present study, we sequenced all 15 exons of *FUS* in 152 familial probands with Essential Tremor and genotyped three reported *FUS* variants in 112 sporadic Essential Tremor patients and 716 control subjects recruited at Mayo Clinic Florida. Only known synonymous SNPs unlikely to be pathogenic were detected in our sequencing and not any of the recently identified mutations or novel ones. We conclude that the *FUS* mutations associated with risk of Essential Tremor are probably a rare occurrence.

Keywords

Essential tremor; Fused in Sarcoma; Parkinson disease; genetic

Introduction

Essential tremor (ET) is the most common movement disorder among adults [1]. Patients typically present tremor of the hands of 4 to 12 Hz but the disorder may also affect the head, voice, trunk, and legs [2]. A meta-analysis of 28 population-based studies calculated the average lifetime prevalence to be 0.9%; however, age is a major risk factor for ET and prevalence in individuals aged 65 years increases to 4.6% [1]. A majority of ET patients report family history of tremor and this observation supported studies into the identification of genetic risk factors for ET [3]. Subsequent linkage approaches have nominated three familial loci, ETM1 on Chr3q13, ETM2 on Chr2p22–25, and ETM3 on Chr6p23, although to date no specific gene or variant has been linked to disease [4]. Two genome-wide association studies have been performed, leading to the identification of a variant in the

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leucine rich repeat and Ig domain containing 1 (*LINGO1*) gene and suggestive association to a variant in the solute carrier family 1 member 2 (*SLC1A2*) gene [5, 6]. Of note, these loci do not overlap with the results of the linkage studies and functional variants at all these loci have yet to be identified to confirm a role in ET.

Recently, Merner *et al.* published the results of a whole exome sequencing study in a large ET family from Quebec [7]. The authors identified a rare nonsense variant (c.868C>T, p.Q290X) in exon 9 of the fused in sarcoma (*FUS*) gene shared exclusively by affected individuals. They subsequently screened 270 ET cases and identified two additional non-synonymous variants in exon 6 (c.646C>T; p.R216C) and exon 12 (c.1292C>T; p.P431L). Interestingly, *FUS* variants are known to cause two other neurological disorders, amyotrophic lateral sclerosis (ALS)[8, 9] and frontotemporal lobar degeneration (FTLD) [10]. However, the authors suggest that the underlying biological mechanisms implicating *FUS* variants are different in ALS and ET. Specifically, the ET-related *FUS* nonsense variant is located in the nuclear export sequence (NES) and the transcript is degraded by the nonsense-mediated decay pathway, whereas the reported nonsense mutations causing ALS variants are in the arginine-glycine-rich domains and produce a truncated protein product [7].

Although intriguing, these findings have yet to be validated in an independent population and it is now crucial to ascertain the population impact of *FUS* variants on disease risk. A recent replication study looking at *FUS* variants in 116 early-onset ET patients failed to identify any mutations [11]. In the present study, we sequenced all fifteen exons of the *FUS* gene in 152 independent patients with familial ET recruited at Mayo Clinic Florida.

Material and Methods

Subjects

In order to assess the impact of *FUS* mutations on the risk of developing ET, we performed bidirectional sequencing of the fifteen exons in *FUS* in 152 probands with familial ET. Their mean age was 73 (± 12) years and they had a mean age at diagnosis of 64 (± 15) years. We then genotyped 112 sporadic ET patients (mean age 75 years (± 12), age at onset 66 (± 14)) and 716 controls (mean age 72 years (± 13)). All subjects are unrelated, non-Hispanic Caucasians of mixed European ancestry, recruited at Mayo Clinic Florida. Demographics for each series are given in Table 1. All patients received a clinical examination by a movement disorder neurologist (ZKW) and were given a research diagnosis of ET using the criteria of the Consensus Statement of the Movement Disorder Society on Tremor [12]. The institutional review board approved the study and all participants provided written informed consent.

Sequencing

Genomic DNA was extracted from peripheral blood lymphocytes using Autogen FlexStar (AutoGen, Inc, Holliston, MA, USA). PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA) on a Biomek FX^P Laboratory Automation Workstation (Beckman Coulter, Brea, CA, USA) and incorporated into a 10ul sequencing reaction using BigDye terminator v3.1 chemistry (Applied Biosystems, Foster City, CA, USA). Sequencing products were purified using Agencourt CleanSeq beads (Beckman Coulter, Brea, CA, USA) on a Biomek FX^P Laboratory Automation Workstation (Beckman Coulter, Brea, CA, USA). All sequencing reads were done using an ABI 3730XL DNA sequencer (Applied Biosystems, Foster City, CA, USA) and analyzed using SeqScape v2.5 software (Applied Biosystems, Foster City, CA, USA). All primer sequences and amplification conditions are available upon request.

Genotyping

Variants in *FUS* exon 6 c.646C>T (rs267606832; p.R216C), exon 9 c.868C>T (p.Q290Stop), and exon 12 c.1292C>T (rs186547381; p.P431L) were genotyped using a TaqMan Allelic Discrimination Assay on an ABI 7900HT Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Positive controls were included to confirm assays were optimized for both alleles. Data analysis was performed using SDS 2.2.2 software. Positive or ambiguous results in the TaqMan assay were also confirmed / resolved with direct sequencing as described above. All primer and probe sequences are available on request.

Results

All fifteen *FUS* exons were sequenced bidirectionally in 152 probands with familial ET. We identified five synonymous coding SNPs and one intronic SNP (Table 2), none appeared to be pathogenic, all variants are reported in the NCBI database, dbSNP. The minor allele frequencies for the variants observed in our series match the described frequencies for Caucasian populations in the public database. The variants previously identified by Merner et al.[7] were not detected in our sequencing study. We performed an additional screening of 112 patients with sporadic ET and 716 control subjects for the three putative pathogenic variants reported (p.R216C, p.Q290X and p.P431L) using ABI Taqman chemistry. Positive control DNA confirmed allelic discrimination for our assays however no additional carriers were observed within our patient-control series.

Discussion

Exome sequencing in an expanded French-Canadian pedigree identified a nonsense mutation (p.Q290X) in the *FUS* gene which has been proposed to cause familial ET [7]. The study of Merner et al. also identified two other rare variants (p.R216C and p.P431L) in additional patients. The nomination of a novel gene for disease warrants replication and a recent follow-up, including members of the original study, did not identify any further *FUS* mutations in an additional sequencing screen of 116 early-onset ET patients [11]. Herein we sequenced 152 patients with familial ET for the complete coding region of *FUS* and did not detect any putative pathogenic mutations. Combining the replication with the present study, 268 patients with familial or early-onset ET have now been gene sequenced without identifying any additional mutations. Furthermore, Parmalee *et al.*[11] and our group have also individually genotyped additional patients (a total of 255) with none of the three *FUS* mutations detected.

Our study detected five synonymous exonic SNPs unlikely to be disease causing variants. Our further screening of the three identified mutations in our sporadic ET and control subjects did not identify any carriers. It is possible that the specific *FUS* mutation reported (p.Q290X) is private to the large ET family studied or is a rare cause of familial ET. Of note, the original study was performed in French Canadian families from Quebec a population known for a founder effect [13]. As a consequence, disease carrying mutations may have different frequencies in other Caucasian subpopulations. Additional, sequencing studies are needed in order to fully understand the impact of *FUS* variants on the risk of developing ET in other populations.

The application of next generation sequencing technology is becoming more commonplace within the clinical setting. Exome sequencing approaches have now been used to identify mutations for a number of neurodegenerative disorders. However the results of exome sequencing must be treated with caution as many forms of genetic variation can be missed, for example copy number, structural and DNA repeat variations are not detected by exome

capture and sequence approaches. In addition, the non-coding sequence within genes contains many conserved motifs which regulate function and expression as highlighted by the recent Encyclopedia of DNA Elements (ENCODE) consortium that suggests 80% of the human genome serves some biochemical purpose [14]. As it might be also the case for ET, a combination of detection methods and extending the search to the entire gene, might be useful in order to catalog potential pathogenic variants in *FUS* and other genes.

The genetics of ET is still in its infancy and the biological pathways leading to the development of the disease remain unresolved. Further genetic and functional studies are warranted in order to fully understand the pathogenesis of ET, and the potential role played by *FUS*.

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Table 1

Subject Demographics

	ET Familial	ET Sporadic	Controls
	n=152	n=112	n=716
Age	73 ± 12 (34–95)	75 ± 12 (45–97)	72 ± 13 (23–96)
Gender			
Male	69 (45%)	53 (47%)	297 (41%)
Female	83 (55%)	59 (53%)	419 (59%)
Age at onset	64 ± 15 (18–88)	66 ± 14 (20–89)	NA

ET= Essential Tremor, The sample mean ± SD (minimum – maximum) is given for age and age at onset.

Table 2

FUS variants identified in subjects

Position on Chr. 16 (GRCh37)	Exon	rs#	Nucleotide Change	AA	MAF
311193942	3	rs741810	C>A	G49G	0.336
311193948	3	rs61733962	C>T	G51G	0.007
311193994	Intron 3	rs73530283	C>T		0.007
311195186	4	rs144853447	T>C	Y66Y	0.003
311195279	4	rs1052352	T>C	Y97Y	0.464
31202744	15	rs138901914	G>A	R522R	0.003

AA= amino acid, MAF= minor allele frequency