This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the author's institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier’s archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/authorsrights
Negative results

Genetic analysis of the fused in sarcoma gene in Chinese Han patients with essential tremor

Wen Zhenga,b, Xiong Denga, Hui Lianga, Zhi Songb, Kai Gaoa, Yan Yangb, Hao Denga,b,*

Abstract

We conducted genetic analysis of the fused in sarcoma gene (FUS) in Chinese Han patients with essential tremor (ET) in a case–control association study. One hundred eighty unrelated patients with ET were screened for mutations in the coding region and exon–intron boundaries of FUS. Reverse transcriptase polymerase chain reaction analysis was performed to evaluate if the c.1176G>A variant results in change of splice site. Two hundred seventy-three normal control subjects were also analyzed when DNA variants were identified in ET cohort. A novel missense mutation, c.1176G>A (p.M392I), in FUS was identified in a 62-year-old patient. Four known variants (c.52C>A, p.P18T; c.147C>T, p.G49G; c.291T>C, p.Y97Y; c.684C>T, p.G228G) were observed in the case–control study without statistically significant differences in genotype and allele distributions. Mutation(s) in FUS might be associated with a small subset of ET cases in the Chinese population.

1. Introduction

Essential tremor (ET) is 1 of the most common adult-onset movement disorders and it was defined by the Movement Disorder Society Consensus Statement on Tremor (1998) as “a bilateral, largely symmetric postural or kinetic tremor involving hands and forearms that is visible and persistent” (Deng et al., 2007; Deuschl et al., 1998; Merner et al., 2012). The prevalence of ET increases with aging, from 4% in individuals aged 40 years to 13% in individuals older than 65 years (Deng et al., 2007; Deuschl et al., 1998; Merner et al., 2012). Twin and family studies have provided evidence for a genetic basis for ET, and 50–70% of cases have a positive family history (Deng et al., 2007). Recently, the first exome sequencing study on a large ET family was performed, and mutations in the fused in sarcoma gene (FUS) were reported to be responsible for ET in the family (Merner et al., 2012). However, sequencing analysis of 116 patients with early-onset ET in North America did not yield supportive results (Parmelee et al., 2012). No confirmatory data have been reported to date. In this study, we tested whether mutations in FUS are linked to ET in Chinese Han patients.

2. Methods

Genomic DNA was isolated from lymphocytes using a standard phenol-chloroform method. Primers covered all coding regions and exon–intron boundaries of FUS (Supplementary Table 1). Genetic analysis was performed using the method described and polymerase chain reaction (PCR) products were sequenced using an ABI 3500 genetic analyzer (Liang et al., 2012). Variants identified in ET patients were further tested in 273 normal control subjects. To determine whether variant c.1176G>A affects FUS messenger RNA (mRNA) splicing, we analyzed FUS mRNA isolated from lymphocytes of the patients with this variant. The complementary DNA was generated and analyzed using reverse transcription PCR. The PCR products were analyzed using gel purification and sequencing (full methods and cohort description are available in the Supplementary data).

3. Results

In our ET cohort of 180 cases, we identified a novel heterozygous missense mutation, c.1176G>A (p.M392I) (Supplementary Fig. 1), in FUS in a sporadic ET case. The patient was a 62-year-old man and his age of onset was 61 years. This missense mutation was not annotated in either dbSNP or the 1000 Genomes Project database; moreover, it was not present in the Exome-Sequencing Project cohort population with 4550 chromosomes sampled, likely precluding that it represents a benign or more common
polymorphism. Furthermore, we did not identify this missense mutation in 273 ethnic matched Chinese control samples. The amino acid M291 is located in a functional G/R-rich domain of the protein and is strictly conserved throughout evolution (Supplementary Fig. 2), compatible with an important functional role for this residue. Altogether, our genetic data strongly suggest a causative role for the M392I missense mutation in ET. Because the c.1176G>A variant might alter the splicing site (http://www.fruitfly.org/seq_tools/splice.html), we therefore analyzed mRNA from lymphocytes of the patient. Our data suggest that this variant does not lead to change in the splicing site. Additional family members were not available for segregation analysis.

Four validated nucleotide variants c.52C>A (p.P18T; rs144888138), c.147C>A (p.G49G; rs741810), c.291T>C (p.Y97Y; rs1052352), and c.684C>T (p.G228G; rs151073460) were found in ET case and control samples. No significant differences were observed between 180 patients and 273 control subjects for genotype and allele distributions for these 4 variants (all p > 0.05) (Supplementary Table 2), indicating that these variants were polymorphisms and not associated with ET. Distributions of the genotypes in the ET and control groups were in Hardy–Weinberg equilibrium.

4. Discussion

Mutations in FUS have been previously shown to account for approximately 4% of familial amyotrophic lateral sclerosis (ALS) and some sporadic ALS cases through an unknown mechanism (Kwiatkowski et al., 2009; Vance et al., 2009). The first exome sequencing study of ET revealed that a Q290X mutation in FUS causes ET in a French Canadian family, and 2 heterozygous mutations in 3 unrelated patients among 270 additional ET probands (Merner et al., 2012). Although a subsequently analysis of 116 early-onset ET cases in North America did not yield supportive results (Parmalee et al., 2012), our data presented here might provide the first line of genetic evidence supporting the notion that mutations in FUS cause or increase the risk to ET, which might account for approximately 0.5% of ET cases in the Chinese Han population. The case with the FUS mutation is apparently sporadic, suggesting a low penetrance of this mutation-associated disease.

Pseudus in sarcoma (FUS) is a nucleoprotein with multiple functions in DNA and RNA metabolism, including DNA repair and transcription regulation, RNA splicing, and RNA export to the cytoplasm. It is interesting to note that mutations in FUS can cause either ALS or ET, 2 distinct types of neurological disorder. However, this is not unusual, because mutations in the same gene have been previously shown to cause clinically different diseases. For example, mutations in VCP might lead to inclusion body myopathy with Paget disease of bone and frontotemporal dementia (Watts et al., 2004), or ALS (Johnson et al., 2010); and a long expansion of polyglutamine repeats (>36) in ataxin-2 cause spinocerebellar ataxia type 2 (Pulst et al., 1996), but intermediate length repeats (27–33) can lead to ALS (Elden et al., 2010). These data suggest that different neuronal populations have their unique ways to handle assaulting signals from the same molecules, resulting in different sensitivities to extrinsic and intrinsic damage.

The M392I of FUS is highly conserved and the M392I mutation is not present in all the DNA databases consisting of more than 6700 alleles analyzed or in our control cohort. These data favor a pathogenic role for the M392I mutation in ET.

The pathogenic mechanism of FUS-linked ALS is not well understood. However, the identification of a premature Q290X mutation and nonsense-mediated mRNA decay of the mutant FUS mRNA vigorously argue for a loss of function mechanism in FUS-linked ET. A number of premature stop codon mutations of FUS have also been identified in ALS. However, most of these mutations result in stop codons in exon 15, the last exon of FUS (Waibel et al., 2010; Yan et al., 2010). Therefore, it is unlikely that these mutations lead to nonsense-mediated mRNA decay, suggesting that FUS-linked ALS and ET have different pathogenic mechanisms. However, a recurrent premature stop codon mutation, R495X, located in exon 14, has been reported in different ALS populations (Waibel et al., 2010; Yan et al., 2010; Zou et al., 2013). Its mRNA has potential to be degraded via nonsense-mediated mRNA decay. Thus, loss of function mechanisms cannot be completely ruled out in FUS-linked ALS. Further genetic studies of FUS in ET and ALS populations and function analysis of mutant FUS are warranted to understand pathogenic mechanisms of the FUS-linked ET and ALS.