No association with the *ETM2* locus in Czech patients with familial essential tremor

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Abstract

OBJECTIVES: Essential tremor (ET) is one of the most common neurological movement disorders. In more than half of the cases, ET is inherited in an autosomal dominant manner, but no causative ET gene has been identified. However, several candidate loci have been reported, including the *ETM2* locus that was originally found in a large American family of Czech descent.
METHODS: To ascertain the association with *ETM2*, we performed a genetic analysis of three polymorphic loci, *etm1231*, *etm1234*, and *etm1240*, located within the *ETM2* candidate region in 61 Czech patients with a family history of ET and 68 healthy controls.
RESULTS: The allele frequencies were not significantly different between the patients and the controls, and we did not observe any haplotype specifically associated with ET.
CONCLUSIONS: This is the first report on the genetics of familial essential tremor in Czech patients. Our findings provide further evidence of genetic heterogeneity for ET.

INTRODUCTION

Essential tremor (ET) is one of the most common movement disorders, with an overall estimated prevalence between 0.4 and 0.9% (Louis *et al.* 2010). ET prevalence increases with age, potentially reaching more than 6% in people 60 years of age or older (Moghal *et al.* 1994; Findley 2000; Louis *et al.* 2010). The disorder is characterized by a postural and kinetic tremor in the both upper limbs; the head, the voice, the face, the tongue, the trunk, and the lower limbs are less commonly affected (Jankovic 2000). ET is suggested to be a genetically

Abbreviations:

DRD3	- dopamine receptor 3 gene
ET	- essential tremor
HS1BP3	- HS1-binding protein 3 gene
LINGO1	- leucine-rich repeat and Ig domain containing 1 gene
PCR	- polymerase chain reaction
STR	- short tandem repeat

ORIGINAL ARTICLE

heterogeneous disorder with variable expression and reduced penetrance. Most of the cases are familial, with an autosomal dominant mode of inheritance (Tanner *et al.* 2001; Jankovic 2002). However, the interactions between environmental and genetic factors may also cause ET.

At least three candidate loci are linked to ET. The *ETM1* locus (also called *FET1*, OMIM 190300) on chromosome 3q13 was shown to be associated with ET in 16 Icelandic families (Gulcher *et al.* 1997). Later, association between risk and age-at-onset of ET and p.Ser9Gly variant in the *DRD3* gene located in the *ETM1* region was reported (Jeanneteau *et al.* 2006), but the study by Blair *et al.* failed to confirm such an association (Blair *et al.* 2008). The *ETM2* locus (OMIM 602134) on chromosome 2p24.1 was originally identified in a large American family of Czech descent (Higgins *et al.* 1997; 2003). Subsequently, the association with *ETM2* was reported in other American and Asian ET patients (Higgins *et al.* 2003; 2004; 2006). The proposed association with the *HS1BP3* gene located within the *ETM2* locus (Higgins *et al.* 2005; Shatunov *et al.* 2005) has not yet been definitively confirmed. Finally, linkage to the *ETM3* locus (OMIM 611456) on chromosome 6p23 was revealed in two American families (Shatunov *et al.* 2006). No specific causative gene within these loci has been identified despite the high prevalence of ET and intense research efforts. The results of various studies often yield contradictory results, potentially due to the genetic heterogeneity of ET. Recently, an association of genetic variant rs9652490 in *LINGO1* with an increased risk of developing ET was found (Stefansson *et al.* 2009) and confirmed by several groups (Tan *et al.* 2009; Thier *et al.* 2010; Vilarino-Guell *et al.* 2010). The variant, which is located in the non-coding region of *LINGO1*, may affect proper mRNA splicing and protein function, and most probably is located in the vicinity of unknown functional variants (Tan 2010).

In this study, we present association analysis of three short tandem repeats (STRs), *etm1231* (UniSTS 256382), *etm1234* (UniSTS 256383), and *etm1240* (UniSTS 256384), located in a 133-kb region within the *ETM2* locus in Czech patients with ET.

METHODS

Diagnostic criteria and demographics

Informed consent was obtained from each participant before clinical and genetic tests. The study was carried out in accordance with the World Medical Association's Declaration of Helsinki and was approved by the Committee of Medical Ethics at the First Faculty of Medicine, Charles University in Prague and General Teaching Hospital in Prague. The criteria for study participation included a family history of ET consistent with a dominant mode of inheritance and a diagnosis of definite ET according to National Institutes of Health diagnostic criteria for ET (Jankovic 2000). Subjects with other movement disorders, such as Parkinson's disease, dystonia, myoclonus, peripheral neuropathy, or restless leg syndrome, were not included in the study. All ET patients and controls were examined by a movement disorders specialist to confirm the diagnosis of ET and to exclude any clinical signs of tremor or any other movement disorders in the controls. All affected individuals had at least two generations of family members with a tremor. Only a single individual from each family was represented in the sample. The control group was recruited from individuals older than age 55 because of their low risk of having the familial form of ET (Bain PG *et al.* 1994).

Molecular studies

The genomic DNA was extracted from peripheral blood lymphocytes using a standard desalting procedure. The following primers were used to amplify regions flanking each STR: etm1231Fw 5′-TGTCAGTCTATCAGAGACGGAC-3′, etm1231Rev (labeled with 6-FAM) 5′-TGCTCATTAGTTTGTGGGATTTAG-3′, etm1234Fw (labeled with 6-FAM) 5′-TTCAGGGATAATAAGTGTGG-3′, etm1234Rev 5′-ATGTCTTTTGGTATCTGGATTA-3′, etm1240Fw 5′-TGCTTGCCTGTAGTCCTAGC-3′, etm1240Rev (labeled with 6-FAM) 5′-TGCACCCACCCTAGGTACA-3′. Polymerase chain reactions (PCR) were performed in a total volume of 12.5 µl containing 50 ng of genomic DNA, 1 mM of the relevant forward and reverse primer, and 1x Plain Combi PP Master Mix (Top-Bio, Czech Republic). The cycling conditions were as follows: initial denaturation at 95 °C for 2 min, 25 cycles of 95 °C for 30 sec, 61 °C (*etm1231*, *etm1234*) or 64 °C (*etm1240*) for 30 sec, 72 °C for 45 sec, and a final elongation at 72 °C for 7 min. 0.5 µl of each PCR product was mixed with 0.5 µl of GeneScan ROX-500 size standard (Applied Biosystems, USA) and 9 µl of Hi-Di formamide (Applied Biosystems, USA) and immediately analyzed by capillary electrophoresis using a 3100-Avant Genetic Analyzer (Applied Biosystems, USA). The allele sizes were determined using the original Peak Scanner software v1.0 (Applied Biosystems, USA).

Haplotype analysis and statistics

The association analysis for multiple allele markers was performed using the software package UNPHASED 3.1.1 (Dudbridge 2008). Global association was tested for individual markers and haplotypes formed by respective pairs and all three markers. Permutation tests were performed to confirm suggestive association signals and to control for a random effect in multiple individual haplotypes (70 for all three markers).

RESULTS

Demographics

The ET group included 61 patients, 27 (44%) males and 34 (56%) females, of mean age 65 ± SD 15 years. The

control group consisted of 68 individuals, 29 (43%) males and 39 (57%) females, of mean age 67 ± 9 years, with no clinical symptoms or family history of a tremor.

Genotyping

The genotypes of three STRs, *etm1231*, *etm1234*, and *etm1240*, were determined in all 129 participants. The *etm1231* marker had seven alleles corresponding to 15, 16, 17, 18, 19, 20, and 21 repeats of $(CA)_n$ dinucleotide (180–192 bp). $(CA)_{18}$ was the prevailing allele in both groups. The *etm1234* marker showed eight alleles corresponding to 11, 12, 14, 15, 16, 17, 18, and 19 repeats of $(CA)_n$ dinucleotide (160–176 bp). The most common alleles in the patients and the controls were $(CA)_{17}$ and $(CA)_{11}$. Eight alleles were identified at locus *etm1240* with 8, 10, 11, 12, 13, 14, 15, and 16 repeats of $(AAT)_n$ trinucleotide (269–293 bp). The most frequent alleles in both groups were $(AAT)_{11}$ and $(AAT)_{15}$. The distribution of the alleles in the patients and the controls is presented in Figure 1.

Allele frequency studies

For each marker alone and all tested alleles, we did not observe any significant association. The same results were found when testing haplotypes formed by all possible combinations of the markers. The haplotype formed by all three markers showed a significant global association (p=0.00076). However, no individual combination of alleles was significantly associated alone. Moreover, a permutation test using random reassignments of affection status with our sample could not confirm the association (p=0.9208 after 100 permutations).

DISCUSSION

In this report, we present the results of an association study between three *ETM2* polymorphic markers and ET in 61 unrelated patients with a family history of ET. The distances between the markers on a physical map of the candidate region are *etm1240* – 58 kb – *etm1231* – 75 kb – *etm1234* (Higgins *et al.* 2004). Our patients were of Czech origin, and therefore originated from the same geographical area as the family in which the *ETM2* locus was first described (Higgins *et al.* 1997). Several studies reported an association of the *ETM2* locus with familial ET (Higgins *et al.* 1997; 1998; Kim *et al.* 2005). However, we did not observe any significant association between the presence of ET and the *ETM2* locus. A decrease in the number of dinucleotide repeats within the *etm1234* was noticed among ET patients by Kim *et al.* (Kim *et al.* 2005) but we did not observe such a tendency (Figure 1). Similar to our findings, there have been studies that did not find any particular allele or genotype that would have increased frequency in the ET patients compared to in the controls (Kovach *et al.* 2001; Aridon *et al.* 2008). The studies on genetics of ET are complicated by genetic heterogeneity, incomplete penetrance, and a high phenocopy rate. Our findings support the theory that familial ET is a genetically heterogeneous movement disorder. Although the involvement of a gene located within *ETM2* may be present in some patients, other genetic factors are also likely to cause the same phenotype in various families with ET.

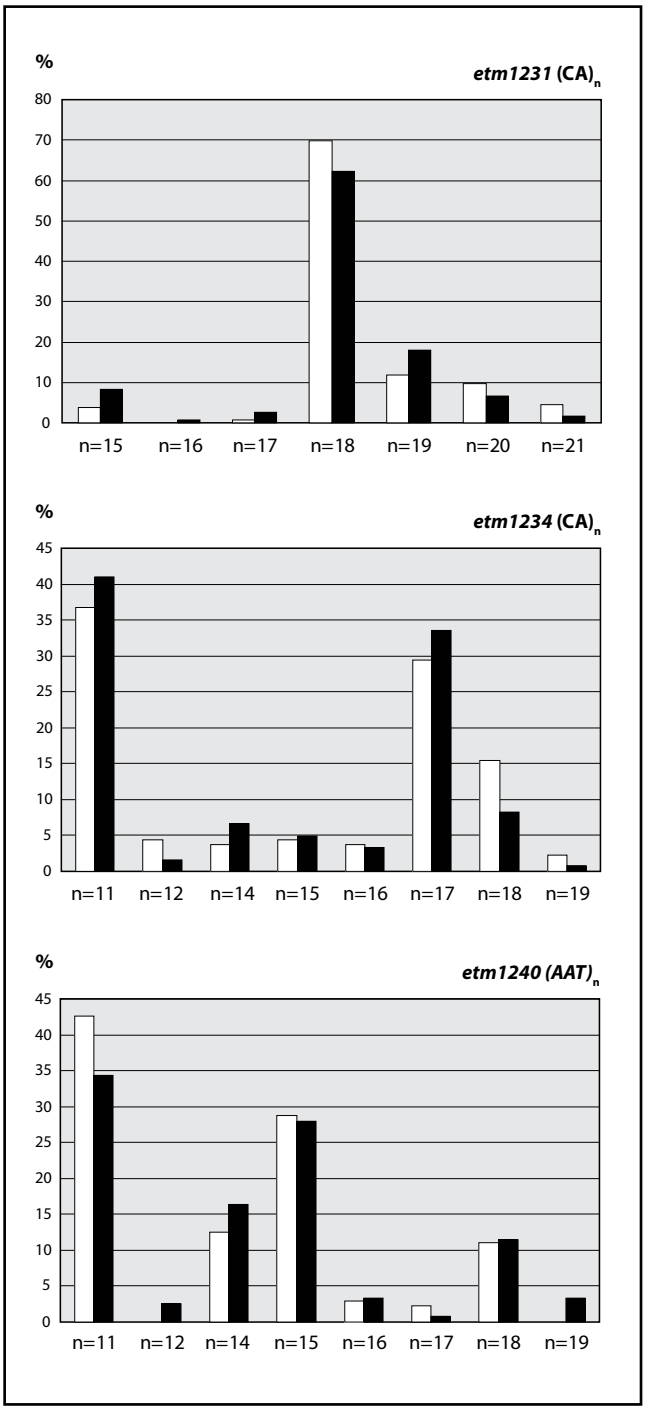

Fig. 1. Distribution of the alleles of *etm1231*, *etm1234*, and *etm1240* markers in healthy controls (white bars) and ET patients (black bars).

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