ORIGINAL ARTICLE

Whole-Genome Study of a Multigenerational Family with Essential Tremor

Ming Zhang, Mohammad Rohani, Mahdi Montazer Haghighi, Christine Sato, Ekaterina Rogaeva, Alfonso Fasano

ABSTRACT: ***Background:*** Essential tremor (ET) is a common movement disorder with ~5% prevalence in individuals above the age of 65, but in rare cases, it arises during childhood. Growing evidence suggests the role of cerebellum in the disease mechanism. ET is highly heritable, however, poor replication of risk loci point to its significant heterogeneity. Thus, it is important to genetically investigate kindreds with a strong aggregation of ET. ***Methods:*** We conducted a clinical and whole-genome investigation of a large Caucasian Canadian family, in which six out of eight patients are affected by childhood-onset ET in four consecutive generations. Eight family members were available for study, including three patients affected by ET. Whole-genome sequencing (WGS) was conducted for the four most informative individuals, followed by Sanger sequencing in the entire kindred. ***Results:*** We searched for rare variants absent in the eldest unaffected individual, but present in the patients (two siblings and their third-degree relative). Our stringent whole-genome filtering approach revealed a rare heterozygous p. Arg90Gln substitution in *TCP10L* (rs151233771) in all three investigated patients. Sanger sequencing confirmed the p. Arg90Gln variant and revealed its absence in the rest of the family members. ***Conclusions:*** Whole-genome data of the family with ET resulted in a single candidate gene mapped to 21q22.11 locus (*TCP10L*) with the highest brain expression in cerebellum. Our study encourages future replication studies to validate the genetic link between *TCP10L* and ET, and suggests the p. Arg90Gln variant for functional investigation.

RÉSUMÉ : Étude du génome complet d'une famille multigénérationnelle dont les membres sont atteints de tremblements essentiels. ***Contexte*** **:** Les tremblements essentiels (*essential tremor*) sont des troubles du mouvement courants dont la prévalence est de ~5 % chez les personnes âgées de plus de 65 ans. Dans des rares cas, ils peuvent survenir pendant l'enfance. Des preuves de plus en plus nombreuses suggèrent à cet égard le rôle du cervelet dans le mécanisme d'apparition de ces troubles. Ajoutons en outre que les tremblements essentiels sont fortement héréditaires. Toutefois, une faible réplication des *loci* à risque a mis en évidence une hétérogénéité significative. De ce point de vue, il est important d'étudier sur le plan génétique les familles présentant une forte incidence de tremblements essentiels. ***Méthodes*** **:** Nous avons effectué une étude clinique et génomique complète d'une famille canadienne nombreuse de descendance européenne dans laquelle six patients sur huit sont affectés par des tremblements essentiels dont les premières manifestations remontent à l'enfance, et ce, au fil de quatre générations consécutives. Au total, huit membres de cette famille étaient disponibles dans le cadre de cette étude, ce qui inclut trois patients atteints de tremblements essentiels. Le séquençage complet des génomes a été effectué pour les quatre individus les plus révélateurs. Il a été ensuite complété par le séquençage de tous les membres de la famille au moyen de la méthode de Sanger. ***Résultats*** **:** Nous avons essayé de repérer des variants rares absents chez le sujet le plus âgé ne souffrant pas de tremblements essentiels mais présents chez ces trois patients (deux enfants d'une même fratrie ainsi qu'un parent du troisième degré). Notre approche rigoureuse de filtrage de la totalité des génomes a ainsi révélé chez eux une substitution hétérozygote inhabituelle de p.Arg90Gln dans *TCP10L* (rs151233771). Le séquençage à l'aide de la méthode de Sanger a par ailleurs confirmé la présence du variant p.Arg90Gln et a révélé son absence chez le reste des membres de la famille. ***Conclusions*** **:** Des données portant sur le génome complet d'individus d'une même famille atteints de tremblements essentiels ont donc fait émerger un seul gène candidat cartographié au *locus* 21q22.11 (*TCP10L*) avec la plus forte expression cérébrale située dans le cervelet. Les résultats de notre étude encouragent ainsi la poursuite de travaux de réplication afin de valider le lien génétique entre *TCP10L* et les tremblements essentiels. Ils suggèrent aussi de mener des recherches fonctionnelles au sujet du variant p.Arg90Gln.

Keywords: Essential tremor, Genetic variation, Movement disorders, TCP10L

doi:10.1017/cjn.2021.104 Can J Neurol Sci. 2022; 49: 381–386

From the Shanghai First Rehabilitation Hospital, School of Medicine, Tongji University, Shanghai, China (MZ); Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, ON, Canada (MZ, MMH, CS, ER); Clinical Center for Brain and Spinal Cord Research, Tongji University, Shanghai, Shanghai, China (MZ); Institute for Advanced Study, Tongji University, Shanghai, China (MZ); Department of Neurology, Hazrat Rasool Hospital, Iran University of Medical Sciences, Tehran, Iran (MR); Division of Neurology, Department of Medicine, University of Toronto, Toronto, Canada (ER, AF); Edmond J. Safra Program in Parkinson's Disease and Morton and Gloria Shulman Movement Disorders Clinic, Toronto Western Hospital and Division of Neurology, University Health Network, Toronto, ON, Canada (AF); and Krembil Brain Institute, Toronto, ON, Canada (AF)

Received March 12, 2021. Final Revisions Submitted April 26, 2021. Date of Acceptance April 29, 2021.

Correspondence to: Alfonso Fasano, Chair in Neuromodulation and Multidisciplinary Care, UHN and UoT, Movement Disorders Centre, Toronto Western Hospital, 399 Bathurst St, 7McL410 – Toronto, ON, M5T 2S8, Canada. Email: alfonso.fasano@uhn.ca
Ming Zhang and Mohammad Rohani contributed equally to this work.

Introduction

Essential tremor (ET) is a common neurological movement disorder characterized by a bilateral upper limb action tremor for at least 3 years in the absence of other neurological signs, except for the cogwheel phenomenon during passive limb movements.[1] Over the years, ET can involve the head, legs, trunk, voice, but seldom the jaw or facial muscles, causing different degrees of functional disability.[2] ET patients can develop some non-motor symptoms (e.g., cognitive dysfunction, sensory abnormalities, or depression).[1] ET is a progressive disorder that shares features with other neurodegenerative conditions such as Parkinson's disease (PD). The recent classification of tremors by the International Parkinson and Movement Disorder Society (IPMDS) emphasizes that ET is an axis I diagnosis, namely it is a syndrome with many possible biological underpinnings.[1]

Growing evidence points to the role of GABAergic systems and cerebellum as a common disease mechanism. For instance, abnormalities in the ET brain are centered on the Purkinje cells and connected neurons.[3] Notably, an effective ET treatment (thalamic deep brain stimulation) points to possible abnormal cerebellar outflow pathways to the thalamus.[3]

Age at onset of ET may span from childhood (3–12 years) to late ages, however, only about 1 in 20 ET cases arises during childhood.[4] The estimated prevalence of ET in the general population is ˜1%, and up to 5% in individuals over the age of 65 with some reports suggesting a slightly higher prevalence of ET among men.[5] Furthermore, ethnicity could be a risk factor for ET. For instance, Caucasians are five times more likely to be diagnosed with ET than African Americans.[6]

ET might be influenced by both genetic and environmental risk factors including neurotoxic compounds (e.g., β-carboline alkaloids or ethanol).[7] The high heritability of ET was shown by twin studies, which revealed that the concordance of ET in monozygotic twins is 69%–93% versus 27%–29% in dizygotic twins.[8] ET is often inherited in an autosomal dominant mode.[9] However, it remains unclear whether strong familial aggregation of ET is the result of a highly penetrant mutation or the combined action of several low-risk variants. Up to 50 genes/loci have been reported to be associated with ET[9] and some genes have been shown to produce a different phenotype in the same family, such as *HTRA2*[10] and *TNR*.[11,12]

Very few studies have identified mutations segregating with ET-affected family members, most of which were not confirmed in other families.[9] Poor replication of ET loci points to the high genetic heterogeneity of ET, and the continued investigation of kindreds with a strong aggregation of ET is important. Hence, we conducted a comprehensive genome-wide analysis of a large Canadian family affected by ET with mainly childhood onset.

Materials and Methods

Participants

The structure of the investigated Canadian family with ET is presented in Figure 1(A). Eight of the family members were recruited at the Movement Disorders Centre (Toronto Western Hospital) and directly examined in 2016 by two neurologists (MR and AF). All available subjects were videotaped and evaluated with parts A–C of the Fahn–Tolosa–Marin Tremor Rating Scale (TRS)[13] and the Montreal Cognitive Assessment (MoCA).[14] This study was performed in accordance with the University Health Network Research Ethics Board-approved protocol (UHN-REB 08-0615-AE) and informed consent was obtained from all study participants.

Genetic Analyses

Genomic DNA was isolated from blood samples of eight family members using a QIAGEN kit and genotyped on the NeuroX array (Illumina Inc., San Diego, CA, USA) at the Clinical Genomics Centre (Toronto). NeuroX has the standard exome content of ˜240,000 variants, as well as ˜24,000 custom variants related to neurologic diseases.[15] Genotype data were loaded to Genome Studio (Illumina Inc.), which confirmed a call frequency of >0.96 for all samples, and GenTrain score of >0.35 for all variants. NeuroX markers with GenTrain scores between 0.35 and 0.70 were visually inspected and those with a cluster separation <0.2 were removed.[16]

Whole-genome sequencing (WGS) was conducted for four of the most informative family members (Figure 1(A)) at Genome Quebec (Montreal, Canada). In brief, 1 µg of blood genomic DNA was used for PCR-free library preparation using the NxSeq® AmpFREE Low DNA Library Kit (Lucigen, Middleton, WI, USA). WGS was performed using the NovaSeq 6000 (Illumina Inc.) PE150, with a minimum average sequencing depth of 30. The raw FASTQ data were aligned to UCSC hg19 reference genome to generate SAM and BAM files, using the Burrows–Wheeler Aligner (http://bio-bwa.sourceforge.net) and SAMTOOL (http://samtools.sourceforge.net). The Genome Analysis Toolkit (GATK) pipeline (https://gatk.broadinstitute.org/hc/en-us) was used to call single-nucleotide variants (SNVs) and insertion/deletions (InDels). We used CNVnator (https://github.com/abyzovlab/CNVnator) to call copy number variants (CNVs). Using the ExpansionHunter tool (https://github.com/Illumina/ExpansionHunter), we also searched for repeat expansions in 24 genes that are known to cause neurological diseases (Supplementary Table 1). To find rare short tandem repeats (STRs), we used the ExpansionHunter Denovo (https://github.com/Illumina/ExpansionHunterDenovo) with the outlier mode, and a Z-score >10, and compared against 150 controls with STR profiles extracted from the Illumina Polaris dataset (https://github.com/Illumina/Polaris/wiki/HiSeqX-Diversity-Cohort).

Rs151233771 genotypes for the eight family members were obtained by Sanger sequencing of PCR products generated using the forward (5′-ataccgcgtcattcagcaat) and reverse (5′-gagggcttgagtattttgcag) primers with 1X Q solution (Qiagen, Venlo, the Netherlands) and an annealing temperature of 58° C. For RT-PCR, RNA was isolated from cerebellum of three control brains, using a QIAGEN kit and converted to cDNA using the Affinity-Script cDNA Synthesis Kit (Agilent, CA, USA). Primers for the *CFAP298-TCP10L* transcript were 5′-tacgtggggaagaatgaaaa and 5′-gcaagaatcgcacactctg. Primers for the *TCP10L* transcript were 5′-gggctgtcatggagaagac and 5′-gcaagaatcgcacactctg. The annealing temperature used for RT-PCR was 56°C and 58°C, respectively.

Bioinformatic Analyses

To analyze SNVs and InDels, we used the ANNOVAR package (https://doc-openbio.readthedocs.io/projects/annovar/en/latest/) to annotate variants derived from WGS by applying the GATK pipeline. We selected exonic and splicing SNVs/InDels with a minor allele frequency <0.0001 in public datasets (gnomAD_all,

https://doi.org/10.1017/cjn.2021.104 Published online by Cambridge University Press

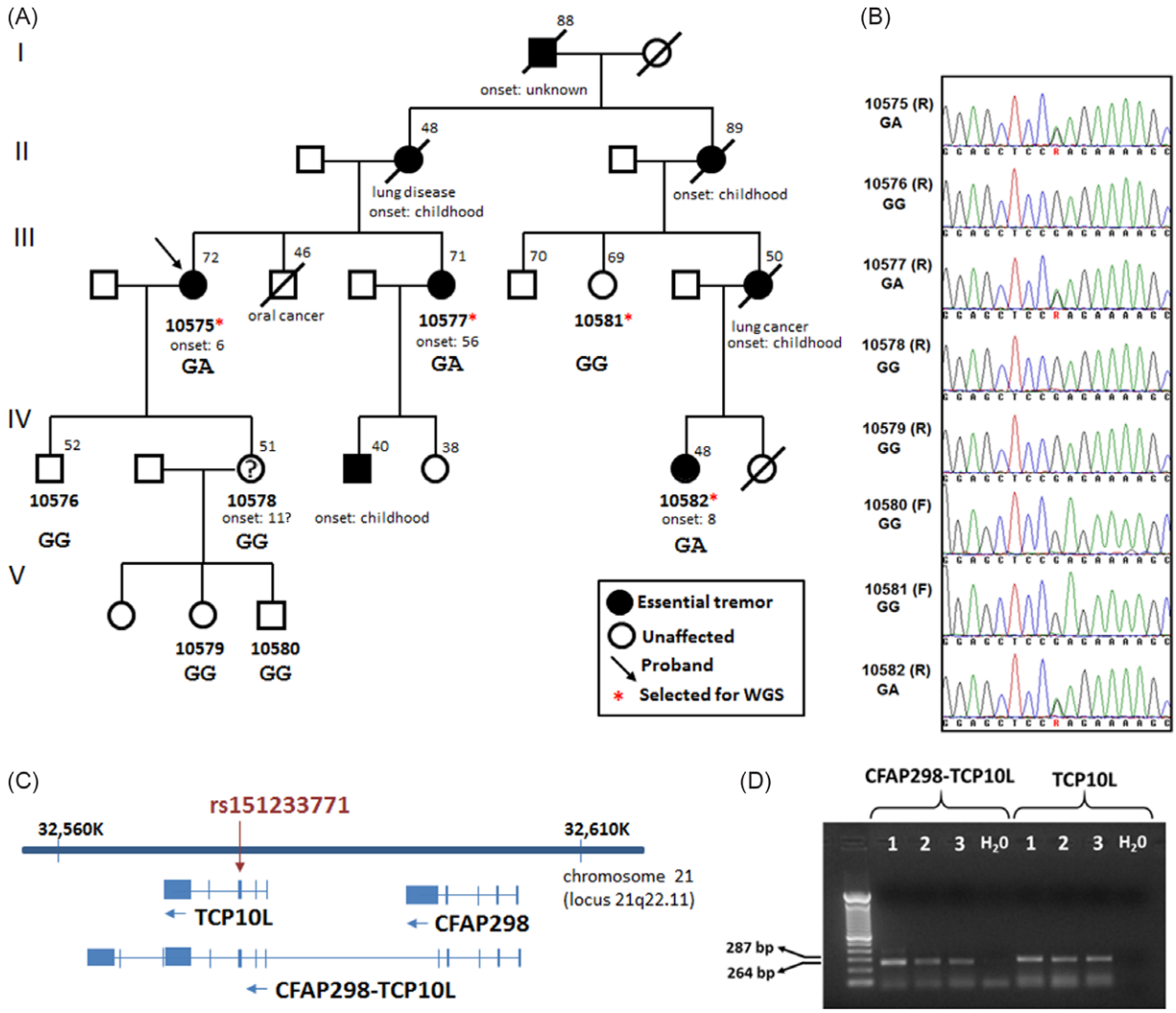

Figure 1: (A) Pedigree of the family with essential tremor (ET) with proband indicated by an arrow (each generation is marked by roman numbers). The family members selected for whole-genome sequencing (WGS) are indicated by *. Blank symbols indicate unaffected individuals and black symbols indicate individuals with an ET diagnosis. The subject with unconfirmed ET is marked by a question mark. A diagonal line indicates deceased relatives with the diagnosis at death beneath the symbol (if known). Age at sample collection or age at death is shown above the symbol. Age at onset of ET and Sanger sequencing results for the p. Arg90Gln substitution in TCP10L (rs151233771) are indicated beneath the symbol. (B) Sanger sequencing diagrams for rs151233771 obtained with either the forward (F) or reverse (R) primer for all study participants. (C) Gene region around the rs151233771 variation based on the UCSC Human Dec. 2013 (GRCh38/hg38) Assembly. (D) Gel image of RT-PCR results confirming the expression of the TCP10L and CFAP298-TCP10L transcripts in cerebellum of three control cases.

gnomAD_NFE, EXAC_all, and EXAC_NEF). To analyze CNVs and filter out common CNVs in public databases, we used AnnotSV (https://lbgi.fr/AnnotSV/), which is a tool that compiles relevant functional, regulatory, and clinical information for structural variants.[17] We selected CNVs with a frequency <0.001 in both the Genome Aggregation Database (gnomAD) and Database of Genomic Variants (>500 tested samples).

Results

Clinical Findings

The proband (10,575) belongs to a Caucasian Canadian family with nine family members diagnosed with ET affecting four consecutive generations; however, the ET diagnosis of subject 10,578 is in question, as discussed below. With two exceptions, all affected individuals had childhood onset of ET, starting as early as age 6 (Figure 1(A)). The early onset of disease supports the possibility of a genetic cause of ET in the family. Samples from eight individuals of this kindred were available for genetic study, including three patients with a confirmed ET diagnosis collected from two generations.

The proband is a 72-year-old right-handed woman who was referred for surgical treatment of her hand tremor, which started at the age of 6 and has progressed very slowly over the years. At the age of 40, the patient began using propranolol with initial benefit, increased till the maximum tolerated dose of 80 mg/day. Over the years, the tremor spread to the upper body also affecting the voice, face, and head, eventually leading to her retiring from her business as a florist at the age of 60. The patient also complained of mild depressive symptoms and memory problems, although

https://doi.org/10.1017/cjn.2021.104 Published online by Cambridge University Press

Table 1: Clinical and demographic characteristics of the family members diagnosed with ET and assessed at the Toronto Western Hospital

Patient #	Sex	Handedness	Age at onset	ET duration	Site of onset	Alcohol responsiveness	TRS A/B/C/ total	Head tremor	Voice tremor	Soft signs	Notes (treatment)
10,575	F	R	6	66 years	Hands	Unknown	15/14/18/47	Yes	Yes	L torticollis	Good effect of high doses of propranolol, but didn't tolerate primidone or gabapentin
10,577	F	R	56	15 years	R hand	Unknown	6/11/10/27	Yes	Yes	R torticollis	Didn't tolerate propranolol
10,578	F	R	11	40 years	Hands	yes	2/1/0/3	No	No	none	On 20 mg of propranolol since ET diagnosis by a local neurologist, but our examination didn't confirm ET
10,582	F	L	8	40 years	Hands	Unknown	4/9/5/18	No	No	L hand dystonia	No treatment

ET=essential tremor; F=female; L=left; R=right; SE=side effect; TRS=Tremor Rating Scale (higher score indicating more severe tremor).

her MoCA score was 29/30. Brain magnetic resonance imaging at the age of 70 was unremarkable. On examination at the age of 72, the proband had mostly a postural and kinetic hand tremor affecting the right side slightly more than the left. The patient also had a facial tremor (chin, lips, and periocular), "no-no" head tremor, as well as a moderate voice tremor. No parkinsonian or cerebellar signs were detected, and gait was normal, including tandem. There was a mild left torticollis (Video 1). Re-evaluation at the age of 74 revealed that the tremor had only minimally worsened (TRS total from 47 to 51) and the MoCA score was 28. The patient continued to be independent; however, the tremor started to interfere with daily activities limiting the patient's housework and public encounters. She was then referred to neurosurgery for unilateral thalamic deep brain stimulation. Her other medical history includes type II diabetes and hypothyroidism.

Clinical features of the proband and the rest of the assessed family members are shown in Table 1. All investigated patients are women (two male ET patients were not available for study) and share similar clinical features, namely a very slowly progressive tremor lasting for most of their lives, spreading from the upper limb to the head and voice in most patients. The upper limb tremor was largely symmetrical. Dystonic soft signs with mild torticollis were noticed on examination of patient 10,577 (Video 2). Possible action dystonia of the left hand during drawing was also noticed in subject 10,582 (Video 3). Of note, subject 10,578 was also diagnosed with ET by a local neurologist and treated with propranolol (20 mg/day), however, our exam (by AF and MR) did not reveal any tremor despite a putative disease duration of 40 years (Video 4). In addition, this patient was affected by severe insomnia and arthritis with elevated chronic ferritin. We concluded that this patient is not affected by ET.

Results of Genome-Wide Analyses

Relatedness between the family members was confirmed using the NeuroX data and PLINK.[16] None of the rare coding NeuroX variations co-segregated with ET. Next, we conducted a WGS analysis of the four most informative family members (Figure 1(A)). We searched for rare variants present in ET-affected siblings (10,575, 10,577) and their third-degree affected relative (10,582), but is absent in the eldest (age 69) unaffected individual (10,581).

We did not detect any novel or known pathogenic STRs (Supplementary Table 1). Rare SNVs, InDels, or CNVs found in at least two ET patients and absent in the unaffected individual 10,581 are listed in Supplementary Tables 2–4, respectively. Our stringent WGS filtering approach resulted in a single variant detected in all three ET patients: a heterozygous missense substitution (*TCP10L*, c.G269A:p. Arg90Gln; rs151233771) mapped to 21q22.11 locus (Supplementary Table 4). Sanger sequencing confirmed the rs151233771 genotypes obtained by WGS and revealed genotypes in the rest of the family members (Figure 1(B)). The *TCP10L* p. Arg90Gln variant was present in all three patients with a confirmed diagnosis of ET and absent in five unaffected, including subject 10,578 with unverified ET (Figure 1(A)).

The p. Arg90Gln substitution is very rare (minor allele frequency is 0.000015 in gnomAD_NFE) and has a combined annotation-dependent depletion score of 13, representing the top 5% of deleterious variants in the human genome.[18] This variant mapped to the leucine zipper domain of TCP10L (residues 75–96), as well as to an overlapping *CFAP298-TCP10L* transcript, which represents a readthrough transcription between neighboring genes (*TCP10L* and *CFAP298*) and may encode a fusion protein that shares sequence identity with both genes (Figure 1(C)).

Nothing is known about the *CFAP298-TCP10* transcript, while *TCP10L* encodes the T-complex protein 10A homolog 1 protein, which is highly expressed in testis and liver. In the brain, the highest expression of *TCP10L* is observed in cerebellum (specifically in the granular layer and Purkinje cells), according to the Human Protein Atlas (https://www.proteinatlas.org/).[19] The expression in cerebellum of both the *TCP10L* and *CFAP298-TCP10L* transcripts was confirmed by an *in-house* RT-PCR (Figure 1(D)). However, we are not able to assess p. Arg90Gln carriers because none of them came to autopsy.

Discussion

We conducted a clinical and WGS evaluation of a unique multigenerational Canadian family, in which six out of eight

https://doi.org/10.1017/cjn.2021.104 Published online by Cambridge University Press

patients are affected by childhood-onset ET. Due to the occurrence of mild dystonic signs, these patients should be more properly diagnosed with ET-plus. In fact, the recent IPMDS tremor classification recognizes the existence of "ET-plus" in the case of "soft" neurological signs, including dystonia.[1]

One of the strengths of the study is the comprehensive assessment of WGS data, including the search for structural variants (e.g., tandem repeats), which are rarely evaluated in ET even though multiple repeat expansions were associated with neurological disorders. For instance, the GGC expansion in *NOTCH2NLC* was recently linked to familial ET in Chinese kindreds.[20]

WGS data of the Canadian ET family resulted in a single candidate gene. We detected a very rare p. Arg90Gln variation in *TCP10L* co-segregating with ET. TCP10L is highly expressed in testis and liver, based on three public datasets (Human Protein Atlas, GTEx, and FANTOM5). In the brain, cerebellum has a relatively high expression of *TCP10L* (GTEx). It remains to be explored if the p. Arg90Gln variation affects tissue-specific expression of *TCP10L*.

TCP10L is a primate-specific gene encoding the TCP10L protein with a subcellular localization in nuclei,[21] which was reported to be involved in transcriptional regulation and acts as a tumor suppressor.[22] Currently, the structure of TCP10L is not solved, and it is unclear how the p. Arg90Gln variant might affect protein stability. Its leucine zipper motif mediates the homodimerization of the TCP10L protein, which could be abolished in a cell culture experiment by either deletion of residues 72–108 in TCP10L or the p. Leu89Pro mutation.[21] Notably, the p. Arg90Gln variant detected in the investigated ET family is directly adjacent to the critical p. Leu89 residue in TCP10L. Hence, there is a need to conduct an investigation of the effect of the p. Arg90Gln on homodimerization of TCP10L in future functional studies.

Our study limitations include an absence of cerebellum tissues from carriers of the p. Arg90Gln variation to assess its functional consequences (the study participants are alive). Moreover, a single family has limited statistical power in the context of genome-wide data, and future study of a large cohort is required to assess the frequency of p. Arg90Gln in ET cases versus controls. Of note, the*TCP10L* locus was not previously reported to be associated with ET, and it is possible that the rare p. Arg90Gln variation is family-specific. Indeed, a recent whole-exome sequencing of eight autosomal-dominant ET families of Spanish origin revealed the absence of rare functional variants co-segregating with ET in more than two families.[23] Specifically, the study identified 15 ET-segregating rare variants with 13 of them family-specific, which indicates significant genetic heterogeneity of ET even within the same ethnic group.

In conclusion, our study suggests the *TCP10L* p. Arg90Gln variant for further genetic and functional studies in ET.

Acknowledgments

The authors are grateful to the patients and family members who made this study possible, as well as to Dr. Brett Trost and Dr. Stephen W. Scherer at the University of Toronto for their guidance in the analysis of structural variants.

Funding

This study in part was funded by the Chair in Neuromodulation and Multidisciplinary Care at the University of Toronto and University Health Network (AF), the Canadian Consortium on Neurodegeneration in Aging (ER), the Shanghai Pujiang Program 19PJ1410300 (MZ), the National Natural Science Foundation of China (82071430) (MZ, ER), and the Fundamental Research Funds for the Central Universities (MZ).

Conflict of Interest

Nothing to report.

Statement of Authorship

MZ: Research project: Execution, Statistical Analysis: Design, Statistical Analysis: Execution, Manuscript: Writing of the first draft. MR: Research project: Execution, Statistical Analysis: Review and Critique, Manuscript: Writing of the first draft. MMH: Research project: Execution, Statistical Analysis: Review and Critique, Manuscript: Review and Critique. CS: Research project: Execution, Statistical Analysis: Review and Critique, Manuscript: Review and Critique. ER: Research project: Conception, Research project: Organization, Statistical Analysis: Review and Critique, Manuscript: Writing of the first draft. AF: Research project: Conception, Research project: Organization, Statistical Analysis: Review and Critique, Manuscript: Writing of the first draft.

Supplementary Material

To view supplementary material for this article, please visit https://doi.org/10.1017/cjn.2021.104.

References

1. Bhatia KP, Bain P, Bajaj N, et al. Consensus Statement on the classification of tremors. from the task force on tremor of the International Parkinson and Movement Disorder Society. Mov Disord. 2018;33:75–87.
2. Louis ED. The primary type of tremor in essential tremor is kinetic rather than postural: cross-sectional observation of tremor phenomenology in 369 cases. Eur J Neurol. 2013;20:725–27.
3. Louis ED. Essential tremor and the cerebellum. Handb Clin Neurol. 2018;155:245–58.
4. Louis ED, Dure LSt, Pullman S. Essential tremor in childhood: a series of nineteen cases. Mov Disord. 2001;16:921–23.
5. Louis ED, Ferreira JJ. How common is the most common adult movement disorder? Update on the worldwide prevalence of essential tremor. Mov Disord. 2010;25:534–41.
6. Louis ED, Fried LP, Fitzpatrick AL, Longstreth WT, Newman AB. Regional and racial differences in the prevalence of physician-diagnosed essential tremor in the United States. Mov Disord. 2003;18:1035–40.
7. Ong YL, Deng X, Tan EK. Etiologic links between environmental and lifestyle factors and Essential tremor. Ann Clin Transl Neurol. 2019;6:979–89.
8. Lorenz D, Frederiksen H, Moises H, Kopper F, Deuschl G, Christensen K. High concordance for essential tremor in monozygotic twins of old age. Neurology. 2004;62:208–11.
9. Siokas V, Aloizou AM, Tsouris Z, et al. Genetic risk factors for essential tremor: a review. Tremor Other Hyperkinet Mov. 2020;10:4.
10. Unal Gulsuner H, Gulsuner S, Mercan FN, et al. Mitochondrial serine protease HTRA2 p.G399S in a kindred with essential

tremor and Parkinson disease. Proc Natl Acad Sci USA. 2014;111:18285–90.
11. Saenz-Farret M, Munhoz RP, Fasano A, Zuniga-Ramirez C. TNR gene mutation in familial parkinson's disease: possible implications for essential tremor. J Mov Disord. 2020; (In the Press).
12. Renaud M, Marcel C, Rudolf G, et al. A step toward essential tremor gene discovery: identification of extreme phenotype and screening of HTRA2 and ANO3. BMC Neurol. 2016;16:238.
13. Fahn S, Tolosa E, Marín C. Clinical rating scale for tremor. In: Jankovic J, Tolosa E, editors. Parkinson's Disease and Movement Disorders, 2nd ed. Baltimore: Williams & Wilkins; 1993, pp. 225–34.
14. Nasreddine ZS, Phillips NA, Bedirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 2005;53:695–99.
15. Ghani M, Lang AE, Zinman L, et al. Mutation analysis of patients with neurodegenerative disorders using NeuroX array. Neurobiol Aging. 2015;545:e549–14.
16. Guo Y, He J, Zhao S, et al. Illumina human exome genotyping array clustering and quality control. Nat Protoc. 2014;9:2643–62.
17. Geoffroy V, Herenger Y, Kress A, et al. AnnotSV: an integrated tool for structural variations annotation. Bioinformatics. 2018;34: 3572–74.
18. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res. 2019;47:D886–94.
19. Uhlen M, Fagerberg L, Hallstrom BM, et al. Proteomics. Tissue-based map of the human proteome. Science. 2015;347:1260419.
20. Sun QY, Xu Q, Tian Y, et al. Expansion of GGC repeat in the human-specific NOTCH2NLC gene is associated with essential tremor. Brain. 2020;143:222–33.
21. Zhong Z, Qiu J, Chen X, et al. Identification of TCP10L as primate-specific gene derived via segmental duplication and homodimerization of TCP10L through the leucine zipper motif. Mol Biol Rep. 2008;35:171–78.
22. Shen S, Feng H, Liu L, Su W, Yu L, Wu J. TCP10L negatively regulates alpha-fetoprotein expression in hepatocellular carcinoma. BMB Rep. 2020;53:431–36.
23. Diez-Fairen M, Houle G, Ortega-Cubero S, et al. Exome-wide rare variant analysis in familial essential tremor. Parkinsonism Relat Disord. 2020;82:109–16.

https://doi.org/10.1017/cjn.2021.104 Published online by Cambridge University Press