No rare deleterious variants from *STK32B*, *PPARGC1A*, and *CTNNA3* are associated with essential tremor

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Supplemental data at Neurology.org/ng

ABSTRACT

Objective: To assess the contribution of variants in *STK32B*, *PPARGC1A*, and *CTNNA3* as essential tremor (ET) predisposing factors following their association in a 2-stage genome-wide association study (GWAS).

Methods: The coding regions of these genes was examined for the presence of rare variants using two approaches: (1) Looking at whole-exome and whole-genome sequencing data of 14 autosomal dominant multiplex ET families. (2) Conducting a targeted massive parallel sequencing to examine the three genes in cohorts of 269 ET cases and 287 control individuals. The cumulative impact of rare variants was assessed using SKAT-O analyses using (1) all variants, (2) only rare variants, and (3) only the rare variants altering the mRNA.

Results: Thirty-four variants were identified. No difference emerged regarding the distributions of individual variants (or gene) between cases and controls.

Conclusion: No rare exonic variants further validated one of these genes as a risk factor for ET. The recent GWAS offers promising avenues, but the genetic heterogeneity of ET is nonetheless challenging for the validation of risk factors, and ultimately larger cohorts of cases should help to overcome this task. ***Neurol Genet* 2017;3:e195; doi: 10.1212/NXG.0000000000000195**

GLOSSARY

ET = essential tremor; **ExAC** = Exome Aggregation Consortium; **GWAS** = genome-wide association study; **QC** = quality control; **SKAT-O** = sequence kernel association test; **WES** = whole-exome sequencing; **WGS** = whole-genome sequencing.

With a worldwide prevalence of 0.9% across age groups (≤4.6% in individuals older than 65 years),[1] essential tremor (ET) is one of the most common human movement disorders. ET is characterized by involuntary oscillations of a body part, primarily in upper limbs, during postural control and voluntary motion.[2,3] Despite strong evidence supporting ET to be an inherited predisposition, very few predisposing genes have been identified.[4] Recently, a 2-stage genome-wide association study (GWAS) using 2,807 cases and 6,441 controls of European descent[5] was reported. This study revealed disease associations for intronic variants within 3 genes: a serine/threonine kinase (*STK32BI*), a transcriptional coactivator (*PPARGC1A*), and a cell-adhesion molecule (*CTNNA3*). The authors performed linkage disequilibrium analysis and showed no additional associated markers in neighboring genes. This study aims to establish whether coding variants from these genes might be associated with ET. Because low-frequency and rare variants are not tagged by conventional genome-wide genotyping arrays, they may represent an important and understudied component of complex trait genetics. Using higher

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Funding information and disclosures are provided at the end of the article. Go to Neurology.org/ng for full disclosure forms. The Article Processing Charge was funded by the authors.

resolution methods to interrogate variants across the entire frequency spectrum, this study has the potential to find additional evidence to support the role of the genes in the disease etiology.

METHODS Patients were recruited in different centers across Canada, and a senior neurologist trained to evaluate movement disorders reviewed their ET diagnoses. Exclusion criteria included (1) an identified cause of exaggerated physiologic tremor, (2) presence of other neurologic deficits (parkinsonisms, polyneuritis, and others), and (3) an orthostatic tremor or (4) a psychogenic-like tremor. Signed written informed consent forms were obtained from each individual studied.

In an effort to identify potentially deleterious variants in the genes recently associated with ET by GWAS, we first examined the whole-exome and whole-genome sequencing (WES/WGS) data from 54 cases across 14 multiplex families with autosomal dominant ET. Secondly, we selected 269 unrelated patients with ET and 287 ethnically matched unrelated individuals with no neurologic disorder is known for a case-control study. European decent participants were recruited from Canadian movement disorder clinics. Targeted massive parallel sequencing was performed across the coding regions of *STK32B* (NM_018401), *PPARGC1A* (NM_013261), and *CTNNA3* (NM_013266). Read mapping, variant calling, and quality controls (QCs) are described in the supplementary material at Neurology.org/ng. A total of 34 variants passed the QC validation.

Single-variant case-control associations were analyzed using a Fisher exact test (PLINK v1.90).[6] In addition, a gene-based, variance-component test was performed using an optimal sequence kernel association test (SKAT-O).[7,8] Results were considered statistically significant when p values were ≤ 0.05 after Bonferroni correction for multiple testing.

RESULTS All familial ET samples for which WES or WGS data were available had ≥97% of the targeted sequences covered at ≥15X. After genotype and variant QC, 12 variants were identified and 7 of these altered the amino acid sequence. Only 1 variant (*CTNNA3* c.1453A>T) was rare in the general population (frequency <0.01 in Exome Aggregation Consortium [ExAC]) databases,[8] but it was observed only in 3 of the 4 affected individuals of a single family. Despite the absence of segregating rare deleterious variants across familial cases, we proceeded with the analysis of the targeted sequencing in cases and controls. After removing poorly captured samples (n = 8), the remaining ones had ≥80% of the targeted sequences covered at >15X, and 34 coding variants were identified. Briefly, 20 nonsynonymous variants were found, among which 3 were common ones (frequency >0.01 in ExAC) and 17 were rare. In addition, we found 1 rare nonframeshifting deletion in *STK32B* of a control individual; unfortunately, no DNA from family members was available to test for co-segregation with the phenotype (see table e-1 for a detailed list of variants).

Of the 34 single nucleotide polymorphisms identified, none had a significantly different allelic distribution between cases and controls (Fisher exact test after Bonferroni correction). To assess the cumulative impact of rare variants, we performed SKAT-O analyses using (1) all variants, (2) only rare variants, and (3) only the rare variants altering the mRNA. Using individual genes as bin delimiters, none of the SKAT-O tests led to a rejection of the null hypothesis ($p > 0.05$ after Bonferroni correction); thus, no difference in variant distribution for any of the genes was observed between the ET cases and the controls.

DISCUSSION In this study, we performed a combination of exonic and targeted DNA sequencing of 3 genes. ET-affected cases and matching controls from European descents were recruited to identify rare variants associated with ET. Genes were chosen for analysis on the basis of a recently published study that showed association between variants located in their intronic regions and ET. It is important that this previous study relied on GWAS approaches, which generally do not interrogate rare genetic variants.

Both an examination of WES/WGS data obtained from a cohort of familial ET cases and a case-control study (Canadians of European decent) analysis failed to identify additional *STK32B*, *PPARGC1A*, and *CTNNA3* variants that are associated with ET. Although a few rare coding variants were identified across the genes, SKAT-O did not reveal those to have a cumulative effect toward ET. Rare variants are inherently uncommon, and the size of the cohorts examined here was modest. Making an allowance for the genetic heterogeneity of ET and that up to 50% of individuals diagnosed with ET have been suggested to be misdiagnosed,[9] it is likely that the increased power of detection of a larger cohort might be warranted to further validate these genes. Nonetheless, this study identified coding variants in 3 genes recently associated with ET.[5]

AUTHOR CONTRIBUTIONS

Ms. Houle: design or conceptualization of the study; analysis or interpretation of the data; and drafting or revising the manuscript for intellectual content. Drs. Ambalavanan and Schmouth: analysis or interpretation of the data. Dr. Leblond: design or conceptualization of the study. Mr. Spiegelman, Ms. Laurent, and Ms. Bourassa: analysis or interpretation of the data. Drs. Grayson, Panisset, Chouinard, Dupré, Vilariño-Güell, Rajput, and Girard: drafting or revising the manuscript for intellectual content. Drs. Dion and Rouleau: design or conceptualization of the study; analysis or interpretation of the data; and drafting or revising the manuscript for intellectual content.

STUDY FUNDING

This study was fully supported by a Foundation Scheme application (#332971) from the Canadian Institutes of Health and in part Xenon® Pharmaceutical which of reference sequencing data but not of the targeted sequencing presented in this manuscript.

DISCLOSURE

G. Houle signed an acknowledgment of Research Agreement for her involvement in the study conducted within the research agreement concluded between Xenon Pharmaceutical and Dr. Rouleau and has received research support from the Canadian Institute of Health Research (CIHR). A.

Ambalavanan and J. Schmouth report no disclosures. C. Leblond has received research support from ALS Canada. D. Spiegelman, S. Laurent, and C. Bourassa report no disclosures. C. Grayson serves as a Research Scientist in Clinical Genetics for Xenon Pharmaceuticals Inc and acted as a project manager in a separate essential tremor study conducted within a research agreement concluded between Xenon Pharmaceutical and Dr. Rouleau (September 25, 2014, to February 1, 2016). A part of the high-throughput sequencing data used in this study were provided by Xenon Pharmaceuticals to be freely used by the team of Dr. Rouleau outside their Research Agreement. M. Panisset has served on the scientific advisory boards of Allergan and Merz and has received research support from Allergan, Merz, Medtronic, and the Weston Foundation. S. Chouinard reports no disclosures. N. Dupré has received travel funding/speaker honoraria from Actelion Pharmaceuticals and has served on the editorial board of *Cerebellum & Ataxias*. C. Vilariño-Güell reports no disclosures. A. Rajput has served on a scientific advisory board sponsored by Ipsen Biopharmaceuticals Canada Inc.; has received speaker honoraria from Teva and Parkinson Society Canada; has served on the editorial boards of *Canadian Journal of Neurological Sciences* and *Parkinsonism and Related Disorders*; and receives research support from the Dr. Ali Rajput Endowment for Parkinson's Disease and Movement Disorders. S. Girard reports no disclosures. P. Dion signed an acknowledgment of Research Agreement for his involvement in the study conducted within the research agreement concluded between Xenon Pharmaceutical and Dr. Rouleau. G. Rouleau has received research support from the CIHR Foundation Scheme, ALS Society of Canada, and ALS Association and signed a research agreement (via The Royal Institution for Advancement of Learning/McGill University) regarding a separate essential tremor study conducted between his laboratory and Xenon Pharmaceutical (September 25, 2014, to February 01, 2016). Go to Neurology.org/ng for full disclosure forms.

Received April 14, 2017. Accepted in final form July 24, 2017.

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