Rare Genetic Variants Contribute to Essential Tremor Disease Risk

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Abstract

Essential tremor (ET) is a prevalent neurological condition characterized by bilateral kinetic tremor of a body part, mostly the hands, but it can also affect the head, voice or lower limbs. Patients sometimes also experience additional neurological signs such as impaired tandem gait, dystonic posturing or memory impairment. Although not life threatening, ET symptoms can affect daily activities such as writing, eating; these symptoms are also a source of social anxiety.

ET is well-known to have a strong genetic component; familial, twin and genotyping studies have all provided strong evidence to support high heritability for the disorder. Despite decades of research, the genetic basis of ET remains largely unknown. Hence, the present thesis aims at identifying novel gene associations as well as following up on loci previously implicated with the disorder.

Using a whole gene collapsing approach, we identified three new genes potentially associated with ET. By employing direct DNA sequencing of a panel of high priority genes, we demonstrated that some carried a higher burden of rare variants in their protein coding region than expected in the general population. We concluded that *VPS13D*, *CACNA1A* and *KCNN2* sequence disruptions could be associated with increased risk of ET. The results presented in this thesis contribute to our understanding of the genetic origins the disorder.

Résumé

Le tremblement essential (TE) est un trouble neurologique commun caractérisé par un tremblement bilatéral d'une partie du corps, affectant généralement les mains, mais il peut aussi toucher la tête, la voix ou les membres inférieurs. Les patients éprouvent parfois des signes neurologiques additionnels comme une démarche en tandem précaire, une posture dystonique ou des troubles de la mémoire. Malgré qu'il ne s'agit pas d'une condition fatale, les symptômes du TE peuvent affecter les activités quotidiennes des patients tels qu'écrire et manger, en plus de d'être la source d'anxiété sociale.

Le TE est bien établi pour avoir une importante composante génétique; des études familiales, de jumeaux et de génotypage ont toutes permis de mettre en évidence la grande héritabilité de la maladie. Malgré des décennies de recherche, les bases génétiques du TE sont toujours largement inconnues. Par conséquent, la présente thèse a pour but d'identifier de nouvelles associations génétiques et de faire le suivi sur des loci précédemment impliqués dans la maladie.

En utilisant une approche de regroupement de variants pour l'ensemble d'un gène spécifique, nous avons identifié trois nouveaux gènes potentiellement associés avec TE. En employant le séquençage direct de l'ADN d'un panel de gènes prioritaires, nous avons démontré que certains d'entre eux portaient plus de variants rares dans leurs régions codantes qu'attendu dans la population générale. Nous avons conclu que des perturbations de séquence dans *VPS13D*, *CACNA1A* et *KCNN2* pourraient être associées à un plus grand risque de TE. Les résultats présentés dans cette thèse contribuent à notre compréhension des origines génétiques du TE.

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List of Abbreviations

ALS	Amyotrophic lateral sclerosis
APBA1	Amyloid beta precursor protein binding family A member 1
BK	High-conductance calcium-activated potassium channel
Ca^{2+}	Calcium ions
CACNA1A	Calcium Voltage-Gated Channel Subunit Alpha1 A
CACNA1G	Calcium Voltage-Gated Channel Subunit Alpha1 G
CASK	Calcium/Calmodulin Dependent Serine Protein Kinase
Cav	Voltage-gated calcium channel
ChIP-seq	Chromatin immunoprecipitation sequencing
CNS	Central nervous system
cRNA	Coding RNA
CTNNA3	Catenin Alpha 3
DNA	Deoxyribonucleic acid
DNAJC13	DnaJ Heat Shock Protein Family (Hsp40) Member C13
DOI	Digital object identifier
DRD3	Dopamine Receptor D3
DZ	Dizygotic twins
EA2	Episodic Ataxia Type2
EAAT2	Excitatory amino acid transporters 2
EBIO	1-Ethyl-1,3-dihydro-2H-benzimidazol-2-one
eQTL	Expression quantitative trait loci
ET	Essential tremor
ETM1	Essential tremor, hereditary, 1
ETM2	Essential tremor, hereditary, 2
FUS	Fused-in-sarcoma
GABA	Gamma-Aminobutyric acid
GWAS	Genome wide association study
HAPLN4	Hyaluronan And Proteoglycan Link Protein 4
HS1BP3	HCLS1 binding protein 3
HTRA2	HtrA Serine Peptidase 2
HTS	High-Throughput Sequencing
IK	Intermediate-conductance calcium-activated potassium channel
K_{Ca}	Calcium activated potassium channel
KCNN2	Potassium Calcium-Activated Channel Subfamily N Member 2
KCNS2	Potassium Voltage-Gated Channel Modifier Subfamily S Member 2
LINGO1	Leucine Rich Repeat And Ig Domain Containing 1
LINGO2	Leucine Rich Repeat And Ig Domain Containing 2
LRRK2	Leucine Rich Repeat Kinase 2

MAPK	Mitogen-activated protein kinase
Miro	Mitochondrial Rho
mRNA	Messenger RNA
MZ	Monozygotic twins
Nav	Voltage-gated sodium channel
NMD	Nonsense-mediated mRNA decay
NOS3	Nitric Oxide Synthase 3
NOTCH2NLC	Notch 2 N-Terminal Like C
PD	Parkinson's disease
PPARGC1A	Peroxisome proliferator-activated receptor *γ* coactivator 1*α*
RIM1	Regulating Synaptic Membrane Exocytosis 1
RIT2	Ras Like Without CAAX 2
RNA	Ribonucleic acid
SCA6	Spinocerebellar Ataxia Type6
SCAR4	Autosomal Recessive Spinocerebellar Ataxia Type 4
SCN11A	Sodium Voltage-Gated Channel Alpha Subunit 11
SCN4A	Sodium channel protein type 4 subunit alpha
SK	Small-conductance calcium-activated potassium channel
SK2	Small-conductance calcium-activated potassium channel type 2
SLC1A2	Solute Carrier Family 1 Member 2
SNAP25	Synaptosomal-Associated Protein, 25kDa
SNARE	Soluble N-ethylmaleimide-Sensitive Fusion Protein Attachment Protein Receptor
SNP	Single-nucleotide polymorphism
SORT1	Sortilin 1
STK32B	Serine/Threonine Kinase 32B
TENM4	Teneurin transmembrane protein 4
TUB	Tubby protein homolog
USP46	Ubiquitin Specific Peptidase 46
VGCC	Voltage gated calcium channel
VPS13	Vacuolar Protein Sorting 13
VPS13A	Vacuolar Protein Sorting 13 Homolog A
VPS13B	Vacuolar Protein Sorting 13 Homolog B
VPS13C	Vacuolar Protein Sorting 13 Homolog C
VPS13D	Vacuolar Protein Sorting 13 Homolog D

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Acknowledgements

First and foremost, I would like to acknowledge and give my warmest thanks to my supervisors Drs Guy Rouleau and Patrick Dion. They generously introduced me to the world of academic research by allowing me to do my first internship in their lab when I was still an undergraduate student. Since then, Guy always supported my scientific creativity by providing the assistance to design and execute my experiments. He helped push my scientific reflection and structure its conceptual expression. In a similar fashion, Patrick was always present to provide guidance, instruction and encouragement in my research activities. His intellect, dedication, humility, and passion are a great source of inspiration.

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Finally, I would like to thank my friends and family for their unconditional love and support. My parents always encouraged me to accomplish my dreams and this thesis is one of them. All was well.

Contribution to Original Knowledge

The work presented in this thesis embodies original contributions that will significantly expand to our understanding of genetic factors contributing to ET. The different studies included here are follow-up reports examining loci that were previously implicated in ET, and reports of previously unreported genetic associations with ET. Overall this work is the first to use a whole gene rare variant collapsing approach for the identification of ET genetic risk factors.

Chapter 2 follows up 13 genes previously proposed to have a role in ET. This chapter describes a thorough replication study of all newly proposed ET genes since the advent of high throughput sequencing. No study previously attempted to meticulously replicate such a large pool of ET candidate genes, and as such this study ensures the genotype-phenotype associations that were reported actually represent credible relationships with ET.

Chapter 3 follow up on three genes carrying a maker reported to be associated with ET in a GWAS. This study was the first to attempt to expand the role for these genes in a large case/control cohort. It was published in *Neurology Genetics* in 2017 under the title "No rare deleterious variants from *STK32B*, *PPARGC1A*, and *CTNNA3* are associated with essential tremor".

In chapter 4, we explored the role of 16 dystonia causing genes in ET. Although ET and dystonia have long had a complex relationship, this study is the first to investigate the possible pleiotropy and report a possible role for *VPS13D* in ET. The finding of this chapter will help to shed light on the possible genetic relationship between these two movement disorders.

In chapter 5, we investigated the bi-directional relationship between ET and migraine. This involved the sequencing of five genes that were reported to be relevant for this non-

movement disorder. While the co-morbidity between the two conditions has not been the subject of many reports, our study describing a positive association between ET and the migraine gene, *CACNA1A,* is the first to suggest a genetic overlap between ET and migraine.

Finally, in chapter 6, we sequenced the protein coding region of *KCNN2*, a gene recently reported to present a missense variant leading to an ET-like phenotype in a rodent model. Our follow-up study is the first to probe the possible contribution of *KCNN2* variants in patients with ET. This reverse genetic approach led to the identification of rare *KCNN2* variants which be the cause of ET in humans.

Format of the Thesis

The present thesis is prepared following the thesis preparations guidelines by McGill's department of Graduate and Postdoctoral Studies under the manuscript-based format. The worked presented was performed under the supervision of Drs Guy A. Rouleau and Patrick A. Dion.

This thesis contains ten chapters. Chapter 1 is a introduction to the thesis with the overall rationales, objectives and hypotheses. Chapter 3 is a study published at *Neurology: Genetics*. While only the work presented in chapter 3 has been published in a peer-reviewed journal, manuscripts synthesizing our findings in chapters 2, 4, 5 and 6 are currently under preparation and will be submitted in the following weeks.

Contribution of Authors

Chapter one contains a review of literature covering current knowledge on the genetic and molecular bases underlying Essential Tremor, as well as general knowledge on disease history, symptoms and pathophysiology. This chapter includes a manuscript published in 2018 in eLS journal. The writing and review of literature was performed by the thesis author under the supervision of Drs Guy A. Rouleau and Patrick A. Dion.

Chapter two represents a manuscript currently under preparation for eminent submission and authored by Gabrielle Houle, Sandra Laurent, Charles-Étienne Castonguay, Calwing Liao, Jay Ross, Daniel Rochefort, Dan Spiegelman, Inge A. Meijer, Owen A. Ross, Félix Javier Jiménez-Jiménez, Pau Pastor, Carles Vilariño-Güell, Alex Rajput, Gregor Kuhlenbaümer, Guy A. Rouleau and Patrick A. Dion. Experimental design, samples preparation, data and statistical analyses, as well as manuscript preparation was performed by the thesis author. CÉC, CL, JR, PAD and GAR conceived and designed the analyses. CL, IAM, OAR, FJJJ, PP, CVG, AR, and GK contributed to sample collection. JR, SL, DR, DS participated in experimental procedures. All authors will review the final manuscript. PAD and GAR co-supervised the project.

Chapter three was published in 2018 in Neurology Genetics and is authored by Gabrielle Houle, Amirthagowri Ambalavanan, Jean-François Schmouth, Claire S. Leblond, Dan Spiegelman, Sandra B. Laurent, Cynthia V. Bourassa, Celene Grayson, Michel Panisset, Sylvain Chouinard, Nicolas Dupré, Carles Vilariño-Güell, Alex Rajput, Simon L. Girard, Patrick A. Dion and Guy A. Rouleau. Experimental design, data QC, analysis and interpretation and manuscript writing were performed by the thesis author. CSL, PAD and GAR contributed design or conceptualization of the study. AA, JFS, DS, SBL, CVB, PAD and GAR performed analysis or interpretation of the data. CG, MP, SC, ND, CVG, AR, SG, PAD and GAR contributed to drafting or revising the manuscript for intellectual content. PAD and GAR co-supervised the project.

Chapter four is a manuscript under preparation for submission. Authors include Gabrielle Houle, Calwing Liao, Charles-Étienne Castonguay, Jay Ross, Sandra Laurent, Daniel Rochefort, Dan Spiegelman, Owen A. Ross, Félix Javier Jiménez-Jiménez, Pau Pastor, Carles Vilariño-Güell, Alex Rajput, Gregor Kuhlenbaümer, Patrick A. Dion, Guy A. Rouleau and Inge A. Meijer. The present thesis author designed and conducted the study, including samples preparation, bioinformatic analyses, data interpretation and manuscript preparation. Experimental design was conducted by PAD, IAM and GAR. CL, IAM, OAR, FJJJ, PP, CVG, AR, and GK contributed to sample collection. JR, SL DS and DR performed experimental procedures. All authors will revise the final manuscript. IAM and GAR co-supervised the project.

Chapter five is also a manuscript under preparation. Authors will include Gabrielle Houle, Charles-Étienne Castonguay, Sandra Laurent, Calwing Liao, Jay Ross, Daniel Rochefort, Dan Spiegelman, Inge A. Meijer, Owen A. Ross, Félix Javier Jiménez-Jiménez, Pau Pastor, Carles Vilariño-Güell, Alex Rajput, Gregor Kuhlenbaümer, Patrick A. Dion, Guy A. Rouleau. The author of the thesis carried out conception and design, acquisition, analysis and interpretation of data as well as manuscript drafting for this study. CL, IAM, OAR, FJJJ, PP, CVG, AR, and GK contributed to sample collection. SL, JR and DR contributed to sample preparation and acquisition of data. CÉC performed essential unpublished experimental procedures. DS participated to data processing. GAR and PAD contributed to study design, data interpretation, manuscript revisions and supervised the project.

Chapter six is a manuscript soon to be submitted. Authors will include Gabrielle Houle, Sandra Laurent, Calwing Liao, Charles-Étienne Castonguay, Jay Ross, Daniel Rochefort, Dan Spiegelman, Inge A. Meijer, Owen A. Ross, Félix Javier Jiménez-Jiménez, Pau Pastor, Carles Vilariño-Güell, Alex Rajput, Gregor Kuhlenbaümer, Patrick A. Dion. Guy A. Rouleau. The thesis author conceived and performed the experiments, analyzed results, and prepared manuscript draft. SL performed sample and library preparation. DR and DS contributed to data processing. CL, CÉC, JR helped with data interpretation. CL, IAM, OAR,

FJJJ, PP, CVG, AR, and GK contributed to patient recruitment and diagnosis. GAR and PAD designed the study, provided revisions of manuscript intellectual content and supervised the project.

Chapter 1: Introduction

Preface

Essential tremor (ET) was characterized as early as 1887[1]. At that time, is was described as "a fine tremor, constantly present in typical cases during waking hours, voluntarily controlled for a brief time affecting nearly all the voluntary muscles, chronic beginning in very early in life, not progressive, not shortening life, not accompanied with paralysis or any other disturbances of nervous function". Since then, the consensus criteria for ET has evolved to an isolated action tremor syndrome of the upper limbs, sometimes affecting other locations (e.g., head, voice, or lower limbs)[2]. Additional neurological signs are observed in some patients, such as impaired tandem gait, dystonic posturing or mild memory impairment.

Interestingly, the important genetic component of the disease was already observed at that time. The author noted "a most striking clinical feature is its marked hereditary or family type". Due to the rapid advances in genomics, great efforts have been made to unravel the underlying mechanisms of the disorder. By harnessing the genetic techniques available through the years, important developments have been made to understand genetic factors implicated in the disorder.

The literature review presented in section 1.2 focuses on the early genetic developments, prior to 2018[3]. These studies mainly focus on analyses of family genetic inheritance, using linkage studies and high-throughput sequencing (mainly exomes) to identify disease-causing mutations in large families with ET clustering. Additionally, several studies leverage the close relationship between ET and Parkinson's disease, another important tremor disorder.

Section 1.3 reviews the most recent literature on the genetic of ET, issued after the publication of the article presented in section 1.2. Further studies have taken advantage of high throughput DNA sequencing in familial cohorts and suggested novel genes associated with the disorder. Additionally, RNA sequencing studies on post mortem brain

tissues allowed the identification of additional genes and pathways involved in the disorder.

Section 1.2

Molecular genetics of essential tremor

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***Advanced article**

Abstract: Essential tremor (ET) is a movement disorder characterized by involuntary, rhythmic shaking, especially in the hands. The primary symptom associated with ET is an action tremor, which occurs with the purposeful use of a body part, like the hand. ET has a strong genetic component as 50-70% of cases have a familial history where transmission is typically autosomal dominant. The high rate of misdiagnosis, which is in part due to the absence of biomarkers, imaging or genetic markers, complicates the identification of genetic risk factors. Another challenging aspect is the overlapping clinical and pathological features between ET and Parkinson's disease (PD), along the evidence of increased odds of developing PD when affected by ET. Decades of research has identified several ET loci

and candidate genes, albeit replication studies, when available, yielded mixed results and in most cases, evidence supporting variant causality remains poor. Nonetheless, these associations provide valuable insights into the complexity of the genetics underlining of the disorder.

Key words : Essential tremor; Genetics, Movement Disorder; Heritability; Parkinsonism; Linkage Study; Genome-Wide Association Study; High-Throughput Sequencing

Key Concepts :

-Essential tremor is a neurological disorder characterized by involuntary and rhythmic shaking.

-The causes of essential tremor are unknown.

-Strong evidence supports high heritability for essential tremor.

-Decades of research have suggested multiple **loci** and genes associated with the disorder.

-Few of these genetic associations have been replicated in independent cohorts.

Introduction

Phenotype

Essential tremor (ET) is neurological disorder characterized by the slow and progressive development of involuntary and rhythmic shakings of a body part[1,2]. Tremors generally affect the hands and upper limbs but the head, larynx, tongue and chin can also be affected. While the occurrence of tremors may initially be intermittent, the frequency of their occurrence typically increases over time and they can become persistent. The amplitude of the tremors can also vary over time and can be worsened by stress, fatigue, hunger and temperature.

The primary symptom associated with ET is an action tremor, which is produced by the voluntary contraction of muscles[1,2]. By definition action tremors are the opposite of rest tremors, which are tremors that occur in a body part while it is not voluntary activated;

not only are rest tremors considered to be a separate entity, they are in fact more symptomatic of Parkinson's disease (PD). Despite this difference between ET and PD, recent epidemiologic studies suggest a link between them since the risk of developing PD is around 2x higher in ET patients than in the general population[3].

ET is the most frequent type of tremor reported in humans, estimates of its prevalence range between 0.4 and 6 percent of the general population and it is higher in individuals over 65 years of age[1]. Indeed, while ET is observed in people of all ages, its prevalence increases with age; the typical *ages* of *onset* are either the early 20s and 30s or the late 60s and 70s.

While not considered life threatening, the tremor can restrict basic activities of everyday life; it often leaves individuals with ET unable to perform very simple tasks such as tying shoelaces, drinking from a cup, brushing their teeth or writing legibly[4]. Some individuals become obliged to quit their work and/or become embarrassed by their tremor in public, as it is often associated by lay people to excessive alcohol intake[1]. Current diagnosis is solely based on clinical findings, which poorly differentiate ET from similar **phenocopies** and other conditions associated with tremors. ET cannot be cured and current therapies do not effectively control symptoms.

Pathology

The pathophysiological origin of ET remains controversial but numerous studies have suggested that ET is caused by a functional disturbance of the oscillatory network comprising the **cerebello-thalamo-cortical pathway**[5]. The reasons for the disturbance of this network are currently unknown. Studies conducted using either animal models, human tissues and brain imaging have suggested abnormalities of the **Gamma-Aminobutyric acid** (GABA) neurotransmitter[6]. Nevertheless, the lack of efficacy of GABA-ergic medication and the absence of genetic association between GABA(A) receptor and GABA transporter genes with ET do not support this hypothesis[3].

Some studies suggest the occurrence of neurodegeneration in ET, which is certainly compatible with the association of ET with advanced aging (i.e., both prevalence and

incidence increase with age). Neuroimaging and post-mortem studies have reported neuronal loss (reduced number and axonal morphometric changes of Purkinje cells in the cerebellum) and abnormal aggregates of protein (Lewy body inclusions)[5]. However, these observations remain controversial and additional studies are required to elucidated the elusive pathophysiological origin of ET. In addition, it is likely that ET is more a syndrome than a single disease, so neurodegeneration may occur only in a fraction of cases, making such studies more difficult.

Genetic Epidemiology

ET is considered to have a strong genetic component. Estimates of genetic and environmental contributions were obtained from twin studies. Two independent studies have revealed a concordance rates of 60–93% in monozygotic twins (MZ) and 27–29% in dizygotic twins (DZ)[2]. A higher concordance in MZ pairs than DZ pairs suggest that genetic factors are implicated in vulnerability to the disorder (see DOI: 10.1002/9780470015902.a0005242.pub2). These data suggest a heritability in the order of 60-90%. Moreover, relative risk for ET was approximately five times greater in a first-degree relative of an ET patient than in a first-degree relative of a control subject[7]. Finally, 50-70% of case have a familial history where transmission is typically autosomal dominant[8].

Genetics

Linkage studies

Genetic linkage studies use a classical approach for the identification of disease causative genes across pedigrees (preferably large) of multiple individuals who have a specific condition with a traceable phenotype. The mapping of a specific chromosomic region shared by multiple affected *individuals who are related* provides strong evidence regarding the locations of functionally important *genes. (see* DOI: 10.1038/npg.els.0005397*). When the disease mapping of a specific chromosomic region is found to be shared across unrelated pedigrees segregating the phenotype, the causative nature of the region is further reinforced.*

DRD3

Dopamine Receptor D3 (encoded by *DRD3*) is a member of the dopamine receptor G-protein-coupled receptor family. The gene is located within the ET linkage locus ETM1 on chromosome 3q13[9]. The role of *DRD3* variants in the pathogenesis of ET is controversial but originally a missense variant, p.S9G (rs6280), was reported to co-segregate with the phenotype in 23 out of 30 families of French origin[10]. Additionally, the authors noted the homozygous carriers of the variant to have a significantly younger age of onset than the heterozygous carriers. However, subsequent meta-analyses failed to replicate these findings in independent cohorts[11].

D_3 receptors are expressed both as autoreceptors and postsynaptic receptors. They are mainly expressed in the limbic system of the human brain which contributes emotional, cognitive, and endocrine functions. The substitution of serine with glycine is deemed to alter the dopamine-binding affinity of D3 autoreceptors and lead to a more robust intracellular signaling[12]. Overall, we estimate that the evidence for the implication of *DRD3* in ET pathogenesis is weak.

HS1BP3

A missense variant located in the *HS1BP3* gene (p.A265G), located in the ETM2 locus on chromosome 2p24, was first found to segregate with ET in two unrelated families of American origin[13,14]. Subsequently, after the genotyping of an additional 73 patients, 12 individuals with ET (16.4%) were found to be heterozygous carriers of the mutant allele, whereas all healthy controls (n=304) were homozygous for the wild-type allele[15]. Nonetheless, subsequent analyses showed that the allele frequency of p.A265G in ET is not statistically higher than that in the normal population and the frequency of the alternative allele in the general population is much higher than initially reported[11].

The *HS1BP3* gene encodes for the HCLS1 binding protein-3 and plays a role in macroautophagy, the intracellular degradation system for cytoplasmic contents (e.g., abnormal intracellular proteins, excess or damaged organelles). *HS1BP3* negatively regulates autophagy by modulating the levels of phosphatidic acid. The impact of the

p.A265G variant on the protein function is unknown. Overall, the evidence for association between *HS1BP3* and ET remains limited.

Although linkage analyses have been successful in delineating ET susceptibility loci, subsequent efforts investigating *DRD3* and *HS1BP3* have met with little success. While specific variants of these two genes segregated in an autosomal dominant manner within a small number of large ET families, additional studies appear to be warranted to further support their pathogenic contribution. Moreover, the genetic architecture of different defects underlying unrelated familial ET cases is likely to be heterogeneous, considering that the few loci thus far identified were subsequently excluded when additional families were examined in the wake of the original reports.

Genome Wide Association Studies (GWAS)

GWAS have become a widely adopted approach to identify associations between genetic regions (loci) and traits (including diseases). The method allows the interrogation of hundreds of thousands of individual variants across the entire genome. Doing such an examination can identify specific variants that are disproportionally present within a cohort of unrelated individuals who share a phenotype, by comparison to what can be observed in a matched cohort of individuals without the phenotype being studied. *(see eLS* DOI: 10.1002/9780470015902.a0021458*)*

LINGO1

The first ET GWAS was performed by the deCODE group. The authors found an association between the G allele of marker rs9652490, which is located in an intron of the *LINGO1* gene, and ET[16]. Depending on the origin of the cohorts being examined in follow-up studies, contradictory results have emerged. Nonetheless a meta-analysis has eventually concluded that *LINGO1* could not be excluded as a risk factor for ET. This being said the most recent, and largest to date, GWAS failed to observe an association between variants present within in *LINGO1* and ET[17].

LINGO1 (Leucine Rich Repeat And Ig Domain Containing 1) is a gene known to encode for a protein that plays an important role in negative regulation of oligodentrocyte

differentiation and axonal myelination. Because animal models have demonstrated that *LINGO1* contributes to the survival of dopaminergic neurons (an important pathological characteristic of PD), many studies have investigated the associations between PD and LINGO1. However, no convincing association was found[18].

SLC1A2

A second GWAS study later revealed an association between the marker rs3794087 in intron 4 of *SLC1A2*, a gene which encodes the main glial glutamate transporter, and ET in a European population[19]. Independent teams subsequently attempted to replicate this finding in cohorts of various origins. A meta-analysis of the five association studies (1,925 ET patients, 4,914 controls) failed to identify a significant differences for this specific variant when it was examined across independent cohorts of ET cases and control individuals after the exclusion of data from the discovery series (which was responsible for a high degree of heterogeneity)[11]. Finally the most recent, and thus far largest, GWAS conducted for ET did not support the association of rs3794087 with ET[17].

SLC1A2 encodes for the excitatory amino acid transporters 2 (EAAT2) which is a transporter that is expressed throughout the brain and the spinal cord; the protein is one of the major glial glutamate transporters as it is responsible for >90% of glutamate uptake. Glutamate is the primary excitatory amino acid neurotransmitter in the central nervous system and it participates to the signaling process through different types of glutamatergic receptors. Although some studies have showed glutamate dysregulation in different ET patient brains regions[3], evidence for the genetic association between *SLC1A2* and ET is weak.

PPARGC1A, STK32B, CTNNA3

The largest ET GWAS to date was recently performed using a two-stage design[17]. A total of 2,807 cases and 6,441 control individuals of European ancestry were included. The joint analysis of the two stages of the study showed genome-wide significant association for three distinct loci. Albeit these results are promising, further replication studies in other cohorts are now needed to confirm the associations.

The first associated locus was captured through a marker (rs10937625) that is located in an intron of the serine/threonine kinase *STK32B*. Very little is known about the product of this gene, other than the fact that like other serine/threonine kinase it transphosphorylase serine and threonine residues of target protein upon its activation. Interestingly, the authors showed an increase of *STK32B* mRNA expression in lateral cerebellar cortex of patients with ET. Moreover, the Braineac eQTL database shows an association of the protective minor allele with a reduction of *STK32B* expression in cerebellar cortex. Finally, a subsequent replication study confirmed the association between rs10937625 and ET in a Chinese population[20]. These results further support a role for *STK32B* in the pathogenesis of ET.

A second association was observed with an intronic variant (rs17590046) of the gene encoding the peroxisome proliferator-activated receptor *γ* coactivator 1*α* (PGC-1*α*, encoded by the gene *PPARGC1A*). *PPARGC1A* is a coactivator of transcription factors that is involved in multiple metabolic processes, including mitochondrial biogenesis and respiration, hepatic gluconeogenesis, and muscle fiber-type switching. Recently the association between ET and rs17590046 was tested in an independent cohort of Chinese origin but it failed to replicated the original finding[20].

The third locus associated was captured by three markers located in intron seven of *CTNNA3*. Although located close to each other, the authors noted that they are not in strong linkage disequilibrium ($r^2 < 0.8$). *CTNNA3* encodes for the cell adhesion protein, Catenin Alpha 3 which is involved in formation of stretch-resistant cell-cell adhesion complexes. Although all three markers were tested, only the G allele of rs7903491 was recently reported to be a risk factor for ET in Chinese population[20].

In conclusion, recent GWAS have identified a few putative genetic polymorphisms associated with ET (see table 1). To date, all risk variants are located in non-coding intronic regions, thus making it more challenging to define how they contribute to the pathogenesis at play in ET. Non-coding regions often comprise critical DNA sequences that are important for gene regulation, gene splicing, transcription, translation, chromosomal organization, and generation of untranslated RNAs. It has been shown that

intronic variants can induce aberrant splicing (e.g. exon skipping or cryptic splice site utilisation) or influence the expression of the genes that host them[21]. It is also possible that current sequencing and genotyping methods did not pinpoint the actual causative variants in these genes but instead identified closely located genetic markers that are in linkage disequilibrium with one another (as yet unidentified) functional variant.

High Throughput DNA sequencing

Since the completion of the Human Genome Project, DNA sequencing has greatly advanced, and the cost of sequencing a human genome has significantly decreased. Massive parallel sequencing instruments, which are the core of High-Throughput Sequencing (HTS), are now easily accessible for independent research labs to address biological questions at a genome-wide scale. HTS instruments can detect variants in unanticipated genes because these are examined without a preconceived notion as what kind of genes might be critical; albeit targeted candidate genes studies are still conducted they can now be done at a lower cost and higher scale.

FUS

The first disease causing variant identified in ET was a nonsense coding variant (p.Q290*) located in the gene *FUS*[22]. It was first identified in 2012 using HTS (whole exome sequencing) of a large pedigree of French-Canadian origin. Further screening of 270 ET cases identified two additional rare missenses in the gene. Independent studies subsequently examined cohorts of cases in additional populations and reported additional rare missense variants in *FUS*, these variants were tested in a meta-analysis[23]. The meta-analysis failed to reveal any of these to be significantly enrichment in ET, by comparison to the general population, but variants affecting the coding sequence of *FUS* are very rare and the lack of association might be due to the small number of cases used. Thus at this point we cannot exclude that additional rare variants in of *FUS* might explain additional cases of monogenic ET.

The gene *FUS*, or fused-in-sarcoma, encodes an RNA-binding protein. Variants in this gene have been identified as causative or risk factors for other neurological diseases such as amyotrophic lateral sclerosis (ALS) and rare forms of frontotemporal lobar degeneration.

Variants causing ALS have been shown to cluster in the C-terminal region of the protein coding for the nuclear localization signal. In contrast, no apparent difference in variant distribution was observed for ET[23].

Upon identification of the nonsense variant in the large Canadian family, the authors reported the variant to create a premature termination codon causing the resulting *FUS* messenger RNA to be degraded by **nonsense-mediated mRNA decay** (NMD). An observation that leads to believe that rare deleterious *FUS* variants of ET would involve **haploinsufficiency,** and thus suggest that by opposition to what was reported for FUS in ALS, it is a loss-of-function disease mechanism that is at play in ET. In contrast, the generation of *transgenic Drosophila showed that hFUS-Q290X lines have similar expression levels to the wildtype line, suggesting that variant carrier mRNAs are not degraded through NMD[24]. However, the p*henotypic characterisation of this *Drosophila* model established that *hFUS-Q290X* expression recapitulates some characteristics that are observed in individuals with the disease, such as an increased motor dysfunction with age. While the observations of penetrant variants of *FUS* only explain a small subset of cases, they suggest that *FUS* is the first ET causative gene identified.

HTRA2

Whole exome sequencing was performed across individuals from a six generations consanguineous family of Turkish origin and a missense variant (p.G399S) was identified in a gene encoding for a mitochondrial serine protease *HTRA2[25]. The variant was observed to be either in heterozygous of homozygous states in different cases of ET. Interestingly,* several lines of evidence suggested that the number of copies of *HTRA2* p.G399S influenced severity of the phenotype. The homozygous carriers showed earlier age at onset and more severe tremor. Three out of five homozygous carriers later developed parkinsonism at middle age, whereas only two out of eleven heterozygous carriers developed it past 70 years of age. However, no convincing follow-up study have yet been able to replicate these findings[26-29].

HTRA2 encode for an enzyme that is targeted to the intermembrane space of mitochondria. It is involved in the regulation of mitochondrial physiology as well

apoptosis modulation and signaling. Heterozygous variants in this gene have already been associated with PD, notably the same p.G399S missense. It was suggested that that variant affect the regulation of the enzymatic activity and induced mitochondrial dysfunction. Follow-up studies could not conclude that variability at *HTRA2* contributes to risk of PD[30]. Finally, mice lacking HtrA2 develop a neurodegenerative phenotype with Parkinsonism[31].

SORT1

SORT1 was proposed to be a cause for ET following the identification of a missense variant (p.G171A) in a small family of Spanish origin[32]. The variant was carried by the two affected members of the family, a father and his daughter, as well as an unaffected brother. *SORT1* encodes for Sortilin, a receptor located at the surface of the cell and its compartments. In the central and peripheral nervous system, *SORT1* regulates neuronal viability and function[33]. In order to assess the pathogenicity of the variant, the authors used *in vitro* transfection to show that mRNA and protein expression was reduced for the p.G171A allele by comparison to the wildtype allele[32]. No additional replication studies have thus far been reported.

TENM4

In 2015 a rare deleterious variant in the Teneurin transmembrane protein 4 (*TENM4*) gene has been described in a Spanish family[34]. Using HTS, the authors detected a missense variant (p.T1367N) carried by all five affected family members for which DNA was available. Targeted resequencing of *TENM4* in 299 index cases of familial ET found 12 additional carriers of rare missense variants. However, a follow-up study showed that one of these variant proposed to be linked to ET (p.A1442T) was found in two healthy Chinese individuals (n=398) but not in the 379 ET cases genotyped[35]. In an additional study, targeted sequencing of the entire coding sequence of *TENM4* in a cohort Canadian origin could not find an enrichment of rare missense variants in ET patients compared to ethnically matched healthy controls[36].

TENM4 encodes a cell surface signaling protein and *in silico* experiments have shown that the product encoded by gene carrying the ET variants is mislocalized[34]. The injection a

morpholino oligonucleotide into zebrafish embryos to knockdown the orthologue of *TENM4* gene produces an axonal guidance defect; interestingly the injection of a human cRNA encoding a TENM4 with the A1442T variant in led to a similar defect[34]. It should be noted that characterizations performed at the embryonic stage of a model organism might not faithfully represent the molecular perturbations in ET, considering that in human it is more often an adult onset disease that progresses with age. Interestingly, Tenm4 knockout mice (*Tenm4*$^{-/-}$) were reported to display a severe action tremor phenotype which is suggested to be the consequence of the hypomyelination of small-diameter axons in the CNS of *Tenm4*$^{-/-}$animals [37]. Although these mice display a ET-like phenotype, these abnormalities were found in homozygous mice, whereas the missense variants found in ET patients are all heterozygous. *All together*, *these results suggest* a possible *role* for the *TENM4* in ET pathogenesis but further functional analyses are necessary to confirm this association.

SCN4A

A disease-segregating variant (p.G1537S) in *SCN4A* was discovered in a family of Spanish origin[38]. *SCN4A* encodes the Nav1.4 pore-forming subunit of the main sodium channel present in skeletal muscles[39]. Variants of *SCN4A* lead to different muscular channelopathies. Although predominantly expressed in the muscle tissue, the authors showed that $Na_v1.4$ is also expressed in the mouse and human brain. Moreover, using whole-cell patch clamp, they showed a significantly faster inactivation of mutated channels as well as a potential change in ion selectivity. These results suggest a functional significance of p.G1537S on the protein function. This family is the first to suggest a role for *SCN4A* in ET and no other variants in have been identified in unrelated cases.

NOS3*, *KCNS2*, *HAPLN4* and *USP46

A genetic analysis of 37 early-onset ET families highlighted four potential susceptibility genes[40]. Two different families had a rare variant in nitric oxide (NO) synthase 3 gene (*NOS3).* Three independent families were also carriers of a single rare variant in *KCNS2*, *HAPLN4* or USP46 respectively. Although all four genes are highly expressed in the human cerebellum, and *in silico* analyses predicted all variants to be deleterious and

damaging, no functional analysis has been performed to assess their pathogenicity. While this genetic study might have opened novel avenues for ET research, additional evidence will be required to confirm their involvement in ET.

SCN11A

A large four-generations Chinese family presenting multiple individuals with early-onset episodic pain and adult onset ET was recently described[41]. The use of HTS revealed a rare *SCN11A* variant (p.R225C) carried by all eight affected members of the family. *SCN11A* encodes for the voltage-gated sodium channel $Na_V1.9$. Genetic and functional findings illustrate that $Na_V1.9$ variants cause various human pain disorders[42]. Although this phenotype might represent a distinct form of ET, the identification of a $Na_V1.9$ variant may contribute to a better understanding of ET and eventually reveal shared pathogenic routes.

The advancement of HTS technologies has led to the identification of multiple potential disease-causing variants in ET families (see table 2.). However, very few of these candidate genes have been replicated in independent cohorts. Hence, well-powered genetic and molecular follow-up studies are necessary to confirm the impact of these variants on the disease etiology. As the volume of patient HTS data increases, it is critical that candidate genes are subjected to rigorous validation to prevent mis-annotation of the pathogenicity of variants in public databases.

Genetic overlap between ET and PD

The strong overlaps of clinical and pathological features between ET and PD, along with evidence of increased odds developing PD when affected by ET, has prompted investigations to test the possible associations of PD risk variants in ET patients. Genetic links between ET and PD includes *LINGO2*, *LRRK2*, *DNAJC13* and *RIT2*.

As previously discussed, *LINGO1* has been linked to both ET and PD. Given the high degree of homology between *LINGO1* and its paralog *LINGO2*, independent teams have investigated the potential contribution of *LINGO2* variants in ET. Two different groups reported an association with two distinct variants, rs1412229[43] and rs10812774[44]

respectively. Additionally, an earlier age at onset by 4 to 5 years was also observed for two variants (rs10812774 and rs7033345)[43].

Multiple variants in the coding region of Leucine Rich Repeat Kinase 2 (*LRRK2*) are well established to cause autosomal dominant late-onset PD[45]. Although different groups have investigated multiple known disease causing variants for PD in ET patients, only a single variant (p.R1628P) was found to be a risk factor for ET in a Chinese population[46].

DnaJ (Hsp40) Homolog, Subfamily C, Member 13 (*DNAJC13*) plays a role in regulation of clathrin dynamics and endosomal trafficking. A variant located in this gene (p.N855S) has been described to cause autosomal dominant PD and Lewy body pathology in a large Mennonite family[47]. Interestingly, this same variant was afterwards observed in patients with ET[48].

Ras Like Without CAAX 2 (*RIT2*) plays a role in neurodevelopment, neuron cell differentiation, and survival. A meta-analysis of five GWAS identified the G allele of rs12456492(A/G) variant in *RIT2 as a risk factor for PD[49]*. Subsequent genotyping of the same variant in an ET cohort also revealed it too be significantly associated with ET[50].

The identification of variants associated in both disorders suggests a level of heterogeneity in the manifestation of clinical signs associated to these genes (see table 3). While ET and PD are distinct genetic puzzles, current observations suggest that some parts of these are not exclusive to only one of them. This being said each of condition has its unique parts considering their respective genetic heterogeneity. A better understanding of how some genetic elements can give rise to the two conditions could shed light on shared mechanisms at play in both conditions.

Discussion

ET is the most common movement disorder, but the identification of causative genes has remained elusive. Decades of genetic research has identified several ET loci and candidate genes, albeit replication studies, when available, yielded mixed results; In most cases, evidence supporting variant causality remains poor. Nonetheless, these

associations provide valuable insights into the complexity of the genetic underlining of the disorder. Despite these recent advances, known causative variants only explain a small proportion of cases; no definite cellular pathway has been linked to ET.

Although several studies are supporting the highly inherited nature of ET and such an evidence bodes well for the success of future genetic studies, we think multiple factors are plaguing the genetic analysis of the disorder. The relatively high prevalence and multiple distinct susceptibility loci increase the possibility of phenocopies within a family. Moreover, the high rate of misdiagnosis due to the absence of very definite biomarkers, imaging or genetic parameters also complicate the identification of genetic risk factors. Finally, variable clinical manifestations and age at onset within a family suggest reduced variant penetrance and gene expression. While in some cases a specific variant in a single but critical gene might trigger the onset and progression of ET symptoms in a highly penetrant manner, it is most likely that environmental or additional genetic factors are at play.

Further Reading

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Glossary

Locus: Genetic position on a chromosome (plural: loci)

Phenocopy: Individual displaying similar or identical phenotype due to different underlying genotypes or environmental factors.

Cerebello-thalamo-cortical pathway: Neuronal projection from the cerebellum to the motor cortex used to influence voluntary movement.

Gamma-Aminobutyric acid (GABA): Principal inhibitory neurotransmitter in the mammalian central nervous system.

Nonsense-mediated mRNA decay: Cellular surveillance pathway that eliminates mRNAs containing premature translation-termination codons.

Haploinsufficiency: Situation that occurs when only a single functioning copy of a gene is not enough for normal function, so that loss-of-function mutations cause a dominant phenotype.

Table 1. Suggested genome wide association with ET risk.

Variant	Gene Symbols	Variant location	Publication	Year published	Method	Population studied	PANTHER Protein Class
rs9652490	LINGO1	Intronic	Stefansson et al.	2009	GWAS	Icelandic	extracellular matrix protein; receptor
rs3794087	SLC1A2	Intronic	Thier et al.	2012	GWAS	European	cation transporter
rs10937625	STK32B	Intronic	Müller et al.	2016	GWAS	Caucasian	annexin; calmodulin; non-receptor serine/threonine protein kinase; transfer/carrier protein
rs17590046	*PPARGC1A*	Intronic	Müller et al.	2016	GWAS	Caucasian	transcription cofactor
rs12764057; rs10822974; rs7903491	CTNNA3	Intronic	Müller et al.	2016	GWAS	Caucasian	cell adhesion molecule; non-motor actin binding protein

Table 2. Suggested disease causing variants in ET families.

Variant	Gene Symbols	Amino Acid Change	Publication	Year published	Method	Population studied	PANTHER Protein Class
rs387907274	FUS	p.Q290*	Merner et al.	2012	HTS	French-Canadian	DNA binding protein; mRNA splicing factor; transcription factor
rs72470545	HTRA2	p.G399S	Unal Gulsuner et al.	2014	HTS	Turkish	chaperone; serine protease
rs750957839	SORT1	p.G171A	Sánchez et al.	2015	HTS	Spanish	Receptor ; transporter
rs763485258	TENM4	p.T1367N	Hor et al.	2015	HTS	Spanish	-
rs571210585	SCN4A	p.G1537S	Bergareche et al.	2015	HTS	Spanish	voltage-gated calcium channel; voltage-gated sodium channel
rs368332097; rs753511043	NOS3	p.G16S; p.P55L	Liu et al.	2016	HTS	Unknown	nuclease
-	KCNS2	p.N379E	Liu et al.	2016	HTS	Unknown	-
rs781390304	HAPLN4	p.G350R	Liu et al.	2016	HTS	Unknown	extracellular matrix glycoprotein
-	USP46	p.A133V	Liu et al.	2016	HTS	Unknown	-
rs138607170	SCN11A	p.R225C	Leng et al.	2017	HTS	Chinese	voltage-gated calcium channel; voltage-gated sodium channel

Table 3. Potential genetic overlap between ET and PD risk.

Variant	Gene Symbols	Variant location	Publication	Year published	Method	Population studied	PANTHER Protein Class
rs1412229; rs10812774	*LINGO2*	(Intronic)	Vilarino-Guell *et al.; Wu et al.*	2010; 2011	Genotyping	Caucasian; Asian	extracellular matrix protein; receptor
rs72470545	HTRA2	p.G399S	Unal Gulsuner et al.	2014	HTS	Turkish	chaperone; serine protease
rs33949390	*LRRK2*	p.R1628P	Chao *et al.*	2015	Genotyping	Asian	-
rs387907571	*DNAJC13*	p.N855S	Rajput *et al.*	2015	Genotyping	European	-
rs12456492	*RIT2*	(Intronic)	Emamalizadeh *et al.*	2017	Genotyping	Iranian	small GTPase

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Section 1.3

New developments

Since the publication of our past review[3], new studies have suggested additional genes associated with increased risk of ET, as well as molecular pathways deemed to be relevant to the development of ET. Two complementary approaches have been used to elucidate the genetic basis of ET: High Throughput DNA sequencing and RNA sequencing of post-mortem tissues.

High Throughput DNA sequencing

CACNA1G

Analyses of 40 ET patients from eight unrelated families using whole genome sequencing led to the suggestion of a new candidate gene, *CACNA1G*[4]. Using a kernel association test adjusted for familial structure and phenotype prioritization, this gene was among the top priority gene identified in the cohort. Three small families carried a different rare missense variant co-segregating with the disorder.

CACNA1G encodes the pore forming subunit of low voltage gated calcium channel Cav3.1. Upon membrane depolarization of excitable cells, Cav3.1 mediates calcium entry resulting in activation of downstream targets. However, electrophysiological studies found little difference in voltage dependence between the HEK293T cells expressing the variants found in ET families and the wildtype. Nonetheless, this gene is interesting because of its high expression in Purkinje neurons and deep cerebellar nuclei[5,6] as well as its previous association with spinocerebellar ataxia type 42[7].

NOTCH2NLC

Pathogenic expansions of GCC repeat in the *NOTCH2NLC* gene were found to co-segregate with ET in eleven Chinese families[8]. Using a combination of linkage analysis and long read sequencing, two families we found to carry a repeat expansion in the 5' region of the gene. Repeat primed PCR confirmed those results, as the ET patients had alleles ranging from 81 to 138 GCC repeats compared to only 7 to 23 for the controls. Examination of 195 additional ET index cases identified nine other families with repeat expansion in *NOTCH2NLC*.

This report generated a significant interest given the very high prevalence of the novel repeat expansion that was observed: expansions were detected in 11 of 197 (5.58%) of the families studied. However, replication studies yielded mix results. Screening of 462 Asian ET patients and 200 controls identified four additional patients with >60 GCC

repeats[9]. A second study on a cohort of 228 East-Chinese ET patients in turn detected repeat expansion in three families[10]. Two independent reports on of 111 and 204 European descent patients respectively did not find any case with GCC expansion, suggesting very low prevalence in this population[11,12]. Together, these results suggest a role for *NOTCH2NLC* pathogenic GCC repeat in ET pathogenesis in, at least, Asian populations.

NOTCH2NLC encodes for "Notch 2 N-Terminal Like C" protein, a regulator of Notch signaling pathway. By promoting expansion of cortical progenitor, NOTCH2NLC stimulates neuronal number in the cortex[13,14]. Pathogenic GCC repeat in the 5' region of *NOTCH2NLC* was first found in patient with neuronal intranuclear inclusion disease (NIID), a rare but fatal progressive neurogenerative disorder with prominent dementia[15]. Compared to NIID, the repeat expansion in ET was significantly shorted and the frequency of GGA interruption was lower, which could explain part of the phenotypic difference between the carriers[8].

TUB

Using whole exome sequencing, a large family with apparent autosomal dominance inheritance pattern was found to carry a missense variant (p.V431I) in *TUB*[16]. The variant is relatively rare in European population (MAF_{gnomAD}=0.01) and was carried by nine out of ten affected members of this family. Targeted resequencing of 820 ET cases and 630 controls found and enrichment of variants predicted to be deleterious according to the dbNSFP functional predictions and scores database.

TUB encodes for Tubby protein homolog, a membrane bound transcription regulator activated by phosphoinositide hydrolysis[17]. Following the identification of *TUB* variants in ET patients, chromatin immunoprecipitation sequencing (ChIP-seq) was performed to identify TUB binding sites in five different mouse brain regions[16]. The cerebellum had the most binding sites and affected a wide range of regulatory pathways including some

involved in neuronal regulation. Considering the relative high frequency of the p.V431I variant in the general population and its imperfect segregation in the large family studied, it is possible rare *TUB* missenses with incomplete penetrance could increase the risk of susceptibility to ET.

RNA Sequencing of post-mortem tissues

RNA sequencing has emerged as a complementary approach to discover genes and pathways associated with complex traits. By detecting dysregulated gene expression, RNA sequencing can discover defects in individuals affected by genetic disorders. Recently, two independent studies used such an approach to identify differential gene expression in brain regions that are relevant to ET[12,18].

The first study compared post-mortem RNA levels in the cerebellar cortex of 33 ET patients and 21 controls[18]. Differential gene expression analysis identified 231 dysregulated transcripts, most of them having no known relationship to ET pathogenesis or cerebellar function. Clustering analysis identified four main pathways associated to ET: axon guidance, microtubule motor activity, endoplasmic reticulum to Golgi transport and calcium signaling/synaptic transmission.

A second study examined the gene expression of 16 ET patients and 16 controls in two different brain regions, the cerebellar cortex and the dentate nucleus[19]. A total of six different genes were differentially expressed in the cerebellar cortex and clustering analysis found several significant pathways including axon guidance, olfactory receptor activity and voltage-gated calcium channel activity. In turn, two genes were significantly dysregulated in the dentate nucleus and different pathways were enriched, including olfactory transduction and MAPK signaling.

Overall, these RNA-sequencing studies underscore the complexity of gene expression regulation in ET. Interestingly, the two RNA sequencing studies on ET cerebellar cortex

both found axon guidance and calcium channel activity to be among the top dysregulated pathways. These results suggest and important role for these molecular mechanisms in the development of ET.

Hence, although two decades of research have tried to elucidate the genetic basis of ET, few genetic factors have been associated with strong confidence. Knowledge of the molecular biology underlying the disorder has substantially increased and various studies have shed light on the genetic complexity of the condition The work presented in this thesis aims to build on current knowledge and suggests new genes associated with ET and approaches to identified additional loci in the future.

Hypotheses and Objectives

Strong lines of evidence are suggesting ET to be a highly heritable disease; heritability assessments based on twins studies and genome-wide estimates range between 45 to 90%[20-22]. However, despite years of research, current knowledge of genetic factors contributing to ET is limited. Loci identified by GWAS only explain a small proportion of disease heritability and disease-causing variants only explain a small proportion of cases. Hence, multiple factors could explain the "missing heritability", such as gene/gene or gene/environment interaction, multiple common variants each with a small effect, epigenetics or rare variants.

Multiple genes and loci contribute to ET development, and it is likely that most remain to be identified. Based on the previous literature and current knowledge, we hypothesized that rare functional variants in various selected genes could confer an increased risk for ET. As such a rare variants collapsing analysis could identify genes in which such variants are, in aggregate, associated with ET. The primary objective of this work is to investigate the role of 38 high priority genes as genetic risk factors for ET.

In chapter two, we followed up on 13 genes previously proposed to contribute to ET. The advent of high throughput sequencing has led to the suggestions of several candidate genes for ET, however replication studies, when available, yielded mixed results. In fact, the evidence supporting variant causality remains poor for most of these candidate genes. Our objective was to replicate these significant associations in an independent cohort to validate genetic risk factors of value. At the same time our effort had the capability to invalidate associations that would have been spurious or false positive findings.

In chapter three, we sequenced the entire protein coding regions of three genes for which intronic markers were found to be associated with ET in a GWAS. The hypothesis is that genomic loci containing common small effect variant, such as identified in GWAS, can harbor genes in which rare variants are also associated with disease susceptibility. We aimed to expand the role for these genes beyond the common SNPs.

In order to potentially identify novel ET genes, we first took advantage genetic pleiotropy. Because genetic loci can affect multiple phenotypes, we hypothesized the observation of comorbidity with other complex traits can be leveraged to identified shared heritability and underlying causal mechanisms. Hence, in chapter four, we aimed to explore the genetic overlap between ET and dystonia, two disorders known to have a complex relationship. We screened 16 dystonia causing genes to assess their role in an ET cohort.

Similarly, in chapter five, our objective was to evaluate the role of five hereditary migraine genes in ET. The rationale behind this examination is the bi-directional co-morbidity that has been described between the two disorders. We therefore hypothesized that common genetic factors might underly this relationship between a movement disorder and a non-movement disorder.

Finally, in chapter six, we aimed to characterize the role of a gene causing a phenotype of whole-body tremor with a pharmacological response mimicking ET in a rat model. The underlying idea was that animal models with useful annotation for functional genomics could help elucidate the genetic factors behind the disorder. Thus, the mechanisms causing ET-like phenotype in this mutant rat could also play a role in the development of ET in humans.

Chapter 2: Whole gene rare variant association study in Essential Tremor

Preface

Since the advent of modern genetics, researchers have tried to pinpoint the genetic causes of ET. Over the years, multiple genes and loci have associated with the disorder. However, in most cases, we cannot say with high levels of confidence which genes are truly playing a role in disease development due to the lack of replication studies in independent cohorts. Thus, in this chapter, we aim to explore the role of 13 genes based on their previous identification as genetic factors between 2012 and 2018. The post 2012 period corresponds to the beginning of high throughput DNA sequencing era, where this new technology allowed exponential identification of new genes and markers. The selection of genes ended in 2018, when the study presented in this chapter was first designed. The main finding of this study was that no suggested association with the disease could be replicated in our cohort, which suggest a limited role for these genes in ET.

Whole gene rare variant association study in Essential Tremor

Authors

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Introduction

Essential tremor (ET) is the most prevalent movement disorder and is estimated to affect around 1% of the population[1]. It is characterised by involuntary rhythmic tremor of a body part, generally the upper limbs, but can also affect the head, voice and other locations[2]. Patients generally have a family history (50-70%)[3] and high concordance rate in twins[4] suggest a strong genetic contribution to the development of the disorder.

It is projected that genetics explain much of the variability in ET and several loci and candidate genes have been proposed as contributing to the disorder. However, replication studies, when available, yielded mixed results and in most instances the evidence supporting variant causality remains poor. Replication of significant association in an independent cohort is important to avoid spurious or false positive findings.

Single marker rare variant associations tend to be underpowered because of their very nature; the rarer the variant, the more samples need to be genotyped to find carriers. Thus, gene-based test can be performed to account for allelic heterogeneity. Multiple rare alleles in a unit are collapsed into a single score and tested for association.

In order to explore the relationship between suggested genes and ET, we performed gene-based association testing. By sequencing the entire protein coding region of genes reported to be of interest, not only were we able to replicate the association with a single variant but also to implicate the entire gene.

Method

DNA sequencing was carried out in two phases. Phase 1 comprised 932 cases and 864 controls. Phase 2 of our study included an additional 939 cases and 978 controls. Diagnosis of ET was based on clinical examination or self-reported questionnaires. Only candidates with self-reported ethnicity listed as Caucasian or European descent were included and none had known familial relatedness. Control DNA samples were prepared from individuals with no known neurological disorders. Informed consent was obtained from all participants and the study was approved by MUHC (McGill University Health Centre) research ethic committee (Roubank protocol no: 14051). Some samples included in this study may overlap the primary studies exploring the association between the gene of interest and ET.

Review of literature was performed to identify positive genetic association for ET reported between August 2012 and January 2018, which corresponds to the period between the first high-throughput sequencing study of ET and the design of our current study. A total of 13 genes were included in our analysis: *FUS*[5], *HTRA2*[6], *SORT1*[7], *SCN4A*[8], *SCN11A*[9], *NOS3*[10], *KCNS2*[10], *HAPLN4*[10], *USP46*[10], *LRRK2*[11], *DNAJC13*[12], *RIT2*[13] and *CHCHD2*[14] *(table 1).* A separate study had already been performed on *TENM4* in our cohort and is not included here.

Targeted DNA capture was performed using Molecular Inverted Probes (MIPs); this method allows the sequencing of thousands of 150bps segments of genomic DNA simultaneously. MIPs are single stranded DNA molecules containing unique target specific arms and universal linkers[15]. After hybridisation to the target, gap filling and ligation

results in a circular DNA molecule containing the target region. A total of 708 probes were designed using MIPgen[16] to capture the entire protein coding region +5bp exon flanking regions of the 13 genes. Probes were synthesized by Integrated DNA Technologies and hydrated in TE (tris-EDTA) buffer with a final concentration of 100 µM. MIP captures were performed as described previously[17], with the use of 800 MIPs copies per genome (0.044 fmol) for 100 ng of DNA. The resulting library was sent for massive parallel sequencing at the Genome Quebec-McGill Innovation Center using Illumina HiSeq platform.

Sequencing data was processed using a custom pipeline. Quality control and filtering of *sequencing reads was performed using* FastQC[18]. BWA-mem algorithm[19] was used to align reads to the human reference genome h19. Aligned reads were then converted to pileup format using Samtools[20], keeping only the reads with MQ>13 and bases with BQ>40. Variant calling was performed using Varscan2 using the following parameters: min-coverage 3, min-reads2 1, min-var-freq 0.28, p-value 0.05, min-freq-for-hom 0.94, strand-filter 0. Variant quality control was performed using GATK SelectVariant[21] and vcftools: GQ>54, max-missing <0.9, max-maf <0.1, HWE<$10e^{-5}$. After QCing, variants were annotated using Annovar[22].

After a list of high-quality variants and samples was obtained, only variants with a minor allele frequency of <1% in European exomes from gnomAD[23] were used for further analyses. Novel variants (not previously reported in public databases) carried by cases were validated using Sanger sequencing, contingent on DNA availability. Single markers were tested for association using Firth logistic regression adjusted for sex, with the use of Plink2[24].

Gene-based rare variant association analyses were performed using Weighted Burden as implemented in VariantTools[25]. Briefly, variants are attributed a differential weight with the assumption that rarer variants contribute more to the phenotype[26]. Variants are then grouped by gene and collapsed into a single score that is tested for association using

logistic regression. 20,000 permutations were performed to control for multiple testing and sex was included as a covariate.

Results

Combined analysis of our cohort resulted in 3,408 samples passing all quality filters; of those 1,689 are cases (51.7% female) and 1,719 are controls (58.9 % female). The 13 genes of interest had 74.1%-99.9% of their protein coding region covered at a minimum of 10x (Table 1). A total of 730 high quality variants were retained for analyses.

After excluding variants with a minor allele frequency of more than 1% in gnomAD European exomes, a total of 407 variants were included in association analysis (Supplementary Table 1). This corresponds to 1,130 minor alleles found across all 13 genes. Using Firth bias-corrected logistic regression test adjusted for sex in PLINK2, the only nominal association found was with HTRA2 p.G399S (OR=0.28; SE=0.49; P=0.01), which is suggestive of protective effect for the minor allele. These results are in clear contrast with the original reports of the deleterious effect of p.G399S in ET[6] and Parkinson's disease (PD)[27]. Similar to other replication studies[28-33], our results do not suggest additional evidence for *HTRA2* implication in the disease. No single marker passed the significance threshold of 2.5×10^{-6} for genome-wide search.

The only other previously reported variant found in our cohort is a missense, p.R225C, in *SCN11A.* It was previously suggested as the cause of a combined phenotype of ET and familial episodic pain[9]. One ET patient was carrier of this variant in our cohort, but no further information about the presence of episodic pain was available.

Gene burden analyses were performed by first importing variants in Vtools, where variants were collapsed per genes and tested for association using Weighted Burden BT test including sex as covariate and 20,000 permutations (Table 2). No gene reached

statistical significance, meaning that no gene had a significantly higher burden of rare variants in ET cases as compared to the controls.

Discussion

Our study evaluates the role of rare variants in a large cohort of ET patients. A total of 13 genes with a previously suggested role in the disease have been tested for association at the variant and gene level. No significant association was found. Although some of the same variants previously reported in ET patients were also found in our cohort, no association with the disease could be replicated. While some of these variants could cause a rare monogenic form of the disease in some families, our results could not find additional evidence expending the role of these genes in the disease etiology.

While we do not report specific roles for rare variants in ET, such variant could still explain a significant proportion of the missing heredity observed in ET. Conventional genome-wide association studies (GWAS) only examine the role of common variants, as rare variants are not tagged by genotyping arrays; rare variants may represent an understudied but important component of common diseases such as ET. While our study did not extend to the interrogation of the entire genome, it shed light on a selected list of candidate gene across a large cohort of cases in a cost-effective manner. Additional studies of large size in other gene panels or whole genome sequencing will be needed to unravel the role of rare variants in ET.

A second limitation of our cohort was the inconsistent diagnostic criteria for participants. Our patients were recruited across various center in North America and Europe, with some samples being crowdsourced (24.2%); these patients were directly recruited based on a self-reported or previous diagnosis of ET by their treating physician who are not necessarily a movement disorder specialist. In addition, although our controls had no known neurological conditions at the time of recruitment, they weren't consistently exanimated by a neurologist. Also, due to their young age, it cannot be excluded that

some of them develop neurodegenerative diseases later in life. Additionally, the ethnicity of our participants is only self-reported, as the targeted sequencing doesn't allow quality control for potential population stratification. Finally, some samples in our cohort were overlapping in some of the studies discussed above.

In conclusion, our study underscores the importance of replication studies for genetic associations in large independent cohorts to delineate true relationships from spurious association. The search for the genetic source of ET is still ongoing and future investigation of larger gene panels or whole exomes in large and well powered are expected to provide answers to this state of affairs.

Table 1: Gene selection, average coverage and original study.

Gene	Isoform	10x coverage (%)	Study
FUS	NM_001170634	93.02	Merner, 2012[5]
HTRA2	NM_013247	86.68	Unal Gulsuner, 2014[6]
SORT1	NM_001205228	98.56	Sanchez, 2015[7]
SCN4A	NM_000334	93.51	Bergareche, 2015[8]
SCN11A	NM_014139	97.95	Leng, 2017[9]
NOS3	NM_000603	83.53	Liu, 2016[10]
KCNS2	NM_020697	96.52	Liu, 2016[10]
HAPLN4	NM_023002	74.07	Liu, 2016[10]
USP46	NM_001134223	99.23	Liu, 2016[10]
LRRK2	NM_198578	98.94	Chao, 2015[11]
DNAJC13	NM_015268	99.30	Rajput, 2015[12]
RIT2	NM_001272077	99.86	Emamalizadeh, 2017[13]
CHCHD2	NM_016139	99.26	Funayama, 2015[14]

Table 2: Gene-based rare variant association results

Gene	Total Variants	$MA_{count\ cases}$	$MA_{count\ controls}$	Beta	P	SE
CHCHD2	7	7	4	0.75	0.27	1.29
DNAJC13	67	178	160	0.26	0.13	0.24
FUS	13	12	9	0.2	0.34	0.60
HAPLN4	11	7	7	0.09	0.44	0.67
HTRA2	11	26	45	-0.60	0.82	0.69
KCNS2	12	11	16	-0.27	0.68	0.61
LRRK2	95	109	105	0.11	0.29	0.20
NOS3	36	32	39	-0.15	0.71	0.35
RIT2	3	2	2	-0.16	0.63	11.13
SCN11A	68	54	62	-0.10	0.65	0.25
SCN4A	62	78	113	-0.28	0.87	0.25
SORT1	18	24	23	0.22	0.33	0.51
USP46	4	2	3	-0.25	0.59	8.42

Notes: MAF: minor allele frequency; $MA_{count\ cases}$: minor allele count in essential tremor cases; $MA_{count\ controls}$: minor allele count in controls; Beta: Beta coefficient; P: P-value; SE:standard error

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Chapter 3: Absence of evidence for a role for rare genetic variants in STK32B, PPARGC1A, CTNNA3 in the risk for Essential Tremor in a cohort of Canadians of European decent.

Preface

In chapter 2, we followed up on 13 genes previously implicated in ET. Most of the selected genes were first identified using familial based studies, where a rare variant was found to co-segregate with the phenotype in large ET families. However, another type of study design has been used to detect loci associated with ET; In 2016, Müller et al. performed the largest ET GWAS to date, which led to the identification of three loci with suggestive association to ET. These markers were located in the intronic regions of three different genes: *STK32B*, *PPARGC1A* and *CTNNA3*. In chapter 3, in order to potentially expand the role for these genes in ET, we screened their entire protein coding region in a cohort of 265 ET cases and 283 control individuals. The underlying idea was that both common and rare variants (which are not detected by GWAS) can collectively play a role in the molecular mechanisms leading to ET. While we did find rare and potentially damaging variants in these three genes in our ET cohort, their frequency did not differ significantly between the cases and the controls.

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

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Abstract

OBJECTIVE: A two-stage genome-wide association study (GWAS) that included 2,807 cases and 6,441 controls of European descent recently reported associations between essential tremor (ET) and specific intronic variants within three genes: *STK32B, PPARGC1A*, *CTNNA3*.

METHOD: To further assess their role as ET predisposing factors we screened the coding regions of these genes for rare variants. Looking at exome and whole genome sequencing data no rare deleteriousvariant were found to segregate with the disease in 14 autosomal dominant multiplex ET families. Nextwe used a targeted massive parallel sequencing approach to examine the protein-coding region of thesegenes in 269 ET cases and 287 control individuals.

RESULTS: Thirty-four variants were identified. No difference emerged regarding the distributions ofindividual 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, ultimately larger cohorts of cases should help to overcomethis task.

Introduction

With a worldwide prevalence of 0.9% across age groups (≤4.6% in individuals >65 years)[1] essential tremor (ET) is one of the most common human movement disorder. 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]. Müller *et al.* recently reported a two-stage genome-wide association study (GWAS) using 2,807 cases and 6,441 controls of Europeandescent[5]. This study revealed disease associations for intronic variants within three genes: a serine/threonine kinase (*STK32B*), a transcriptional coactivator (*PPARGC1A*) and a cell-adhesion molecule (*CTNNA3*). The present study aims to establish if coding variants from these genes might be associated with ET.

Subjects and Method

ET diagnoses were reviewed by a senior neurologist. Exclusion criteriaincluded (i) an identified cause of exaggerated physiological tremor, (ii) presence of other neurological deficits (parkinsonisms, polyneuritis, other), and (iii) an orthostatic tremor or (iv) a psychogenic-like tremor. Signed consent forms were obtained from each individual studied.

In an effort to identify potentially deleterious variants in the genes from Müller *et al.*, we first examined the whole exome and genome sequencing (WES/WGS) data from 54 cases across 14 multiplex families with autosomal dominant ET. Secondly, we selected 269 unrelated ET patients and 287 ethnically matched unrelated individuals with no neurological disorder is known for a case control study. European decent participants were recruited from Canadian movement disorder clinics. Targetedmassive parallel

sequencing was done across the coding regions of *STK32B* (NM_018401), *PPARGC1A* (NM_013261) and *CTNNA3* (NM_013266). Read mapping, variant calling and quality controls are described in the supplementary material. A total of 34 variants passed the quality control (QC) validation.

Single variant case-control associations were analyzed using a Fisher exact test (PLINK v1.90)[6]. Additionally, a gene-based, variance-component test was performed using an optimal sequence kernel association tests (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 was available had ≥97% of the targeted sequences covered at ≥15X. After genotype and variant quality control 12 variants were identified and 7 of these altered the amino acid sequence. Only one variant (*CTNNA3* c.1453A>T) was 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 remainingones had ≥80% of the targeted sequences covered at >15X and 34 coding variants were identified.

Briefly, 20 nonsynonymous variants were found, among which 3 common ones (frequency>0.01 in ExAC) and 17 rare. Additionally, we found one rare non-frameshifting deletion in *STK32B* of a controlindividual; unfortunately no DNA from family members was available to test for co-segregation with the phenotype (see supplementary table 1 for detailed list of variants).

Of the 34 SNP identified, none had a significantly different allelic distribution between cases and controls (Fisher's 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 rarevariants 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 three genes. ET affected cases and matching controls from European descents were recruited to identifyrare 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. Importantly, this previous study relied on GWAS approaches, which generally do not interrogate rare genetic variants.

In summary both an examination of WES/WGS data obtained from a cohort of familial ET cases and acase-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. an allowance for the genetic heterogeneity of ET, it likely the increased power of detection of a larger cohort might be warranted to further validate these genes. Nonetheless this is the first study to look forcoding variants in three genes recently associated with ET[5].

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Chapter 4: Genetic contribution of dystonia related genes in an essential tremor cohort.

Preface

While chapters 3 and 4 aimed at following up on ET loci proposed by our and other groups, chapter 5 dives in new genetic association discovery. As discussed in chapter 2, some genes with positive association with ET were first considered because of their role in Parkinson's disease (PD), another tremor disorder. Because of the relationship between the two disorders, research groups aimed to explore the role for PD genetic variants in ET and successfully found an association. The genetic basis underlying this phenomenon is pleiotropy, were a genetic locus influences multiple traits. To further take advantage of this concept, we decided to investigate the role of 16 dystonia causing gene in our ET cohort. Dystonia is another movement disorder that can coexist with ET individual patients or families. Using this approach, we reported the novel *VPS13D* association, as ET patient carried a higher burden of rare protein altering variants in this gene than observed in our control cohort.

Genetic contribution of dystonia related genes in an essential tremor cohort.

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Introduction

Essential tremor (ET) is a movement disorder characterised by an isolated action tremor of a body part. While hands are mostly affected, the tremor can also occur in the upper limbs, head, tongue, trunk and more rarely lower limbs[1,2]. While the genetic factors predisposing to ET are poorly understood, evidence from twin, family, and genetic studies support a role for an inherited predisposition to the development of ET[3-5].

Although ET is reported to be one of the most common movement disorders[6], biological markers or specific brain morphological changes have not yet been reliably associated with the disease. The genetic risk factors thus far reported explain, at best, only a very small percentage of cases. Consequently, the diagnosis of ET is essentially based on patients' family history and clinical examination, which can make it difficult to distinguish ET from other related disorders and lead to suboptimal or inappropriate treatment.

To unravel the mechanisms underlying ET pathophysiology, numerous studies have, over the recent years, explored the genetic overlap between different brain disorders and found shared genetic loci between them. This shared contribution is particularly well documented for psychiatric disorders where both correlation with common variant and

enrichment of rare variants in shared genes have been described[7-9]. At a smaller scale, some evidence of overlapping genetic mechanism in movement disorders have been reported. This has been shown for the *MAPT* gene, for which different variants have been found to cause different disorders, including frontotemporal dementia, frontotemporal dementia with parkinsonism and progressive supranuclear palsy[10].

To explore the biological mechanisms underlying ET and to understand its relationship with another closely related movement disorder, dystonia, we propose to investigate the genetic overlap between the two disorders. Dystonia is a movement disorder characterised by involuntary muscle spasms or abnormal posture, which can be tremulous[11]. Although dystonia is considered an exclusion criterion criteria in the most recent version of the Movement Disorder Society diagnosis guideline[2], the association between the two disorders has long been the subject of debate[12]. Notably, some studies have reported the coexistence of both diseases in patients with a familial history of ET[13-16].

The screening of 16 well documented dystonia-related genes in ET patients will potentially unravel shared genetic etiologies between the two disorders, as variants in a given gene can be associated with several phenotypes.

Method

A total of 932 cases and 864 controls were recruited for the discovery cohort of this study (Table 1), followed by an additional 939 cases and 978 controls for the replication cohort. Informed consent was obtained from all participants and the study was approved by MUHC (McGill University Health Centre) research ethic committee (protocol no: 14051). Patient ethnicity was self-reported as Caucasian/European descent and had no known genetic relatedness. Diagnosis of ET for the discovery cohort is based on clinical examination (53.4%) or self-reported questionnaires (46.6%). For the replication cohort, 95.7% were recruited after clinical examination and 4.3% are self-reported. Control

samples were selected to not have a history of other neurological disorders. DNA samples were extracted from blood or saliva.

Sixteen genes were chosen for this study based on their involvement in hereditary dystonia[17]. Five of these (*TOR1A*, *TAPH1*, *GNAL*, *ANO3* and *KMT2B*) cause isolated dystonia. Another eleven genes are associated with dystonia and other signs (*GCH1*, *TH*, *SPR*, *TAF1*, *PRKRA*, *ATP1A3, SGCE, GNAO1, TBCD24, KCTD17, VPS13D*)[18] (Table 2).

A custom targeted DNA sequencing panel was created using molecular inversion probes (MIPs) designed using MIPgen[19]. Briefly, MIPs are circular oligonucleotides containing unique targeting arms and universal linkers, allowing the selective capture of genomic regions. Combined with massive parallel sequencing, this cost-effective method allows the simultaneous interrogation of thousands of 150bps genomic segments. A total of 798 probes were designed to capture the entire protein coding region +5bp exon flanking regions of the 16 genes. Sequencing was performed at the Genome Quebec-McGill Innovation Center using Illumina HiSeq platform.

Raw sequencing data was processed using an in-house pipeline. Briefly, reads were pre-processed using FastQC[20] and aligned to the human reference genome h19 using BWA-mem algorithm[21]. Aligned reads were then converted to pileup format using Samtools[22], keeping only the reads with MQ>13 and bases with BQ>40. Variants were called using Varscan2[23] using the following parameters: min-coverage 3, min-reads2 1, min-var-freq 0.28, p-value 0.05, min-freq-for-hom 0.94, strand-filter 0. The resulting vcf was filtered in order to keep only high quality variants and samples. Using GATK SelectVariant[24], variants with GQ<54 and heterozygote calls with AD/DP >0.65 were excluded. Using vcftools[25], variants and samples with missing call rate >0.1 or HWE<$10e^{-5}$ were also excluded. After QCing, variants were annotated using Annovar[26]. Variants are considered “previously reported” if they are listed in The Movement Disorder Society Genetic mutation database (MDSGene)[27] or associated to dystonia in The Human Gene Mutation Database (HGMD)[28].

Only rare protein-altering variants were retained for further statistical analysis. Synonymous variants, variants outside coding regions and variants with MAF >0.01 in Non-Finish European (NFE) exomes gnomAD[29] were filtered out. Genotype-phenotype association was performed using Weighted Burden BT as implemented in VariantTools[30]. Variants within genes from the analyzed panel were aggregated for each gene before they were assigned a weight with the assumption that "rarer" variants tend to be more important. To correct for the occurrence of false positives, 20,000 permutations were performed to control for multiple testing. P-value for genes harboring <3 variants were not calculated.

Results

A total of 789 cases and 770 controls from our discovery cohort passed quality control and were included in the subsequent analysis (Table 1). Genes had between 69%-99% of their coding sequence covered at a minimum of 10x (Supplementary Table 1). Together, they carried a total of 369 high quality variants. Of those, 181 were missense or indels with minor allele frequency <0.01 in the general population, as reported in the public database gnomAD for non-Finnish European samples (gnomAD exome NFE version 20170311)[29].

To establish whether ET patients carried an increased load of rare missense variants in the protein coding region of dystonia associated genes, we performed a weighted burden analysis (Table 3). This method allows the aggregation of all variants in a genomic region (in this case the protein coding region of each gene of interest) into a summary dose variable[31]. Only genes with >3 rare variants across all samples were retained for association testing.

The analysis of our discovery cohort revealed a significant higher burden of rare variant (MAF<0.01) in *VPS13D* (P=0.02; beta=0.49; SE=0.23) (Table 3). A total of 80 cases carried

a rare allele in this gene, contrasting with the 56 found in controls (Supplementary Table 2). In order to replicate these results, the entire protein coding region of the gene was re-sequenced in an independent cohort of 900 cases and 949 controls. This did not confirm our initial finding of increased risk, as 102 additional cases and 87 controls carried a rare allele in this cohort (P=0.09; beta=0.28; SE=0.21). Nonetheless, joint analysis of the combined cohorts is still suggestive of an association (P=0.007; beta=0.42; SE=0.17) (table 4).

Although the other genes studied did not show an enrichment of variants in the cases, some rare and potentially damaging variants were found (Supplementary Tables 3 to 6). In addition, some rare variants previously reported in dystonia patients were also found in our ET cohort.

Notably, the recurrent *TOR1A* heterozygous in-frame disease causing deletion in exon 5 (c.904_906delGAG; p.302/303delE) was found in a patient with bilateral hand, head and leg tremor (age at onset = 20 years old). In addition, three individuals, two cases as well as one control, were carriers for *TOR1A* missenses previously reported as disease causing for dystonia[32-35]. The cases reported age at onset between 53 and 68 years old.

One of the ET patient was carrier of the dystonia-myoclonus null variant p.7W>X[36]. This patient had first noticed tremor in his late teens and the tremor is located in his hands only. Other previously reported variants include one population control with a missense in THAP1, p.132F>S[37] and two controls with known *GCH1 variants*[38-45], suggesting incomplete penetrance of these variants.

Discussion

Overall, this study allowed the investigation of the possible role of dystonia causing genes in a cohort of 780 ET cases and 770 controls using a unique MIP approach. The screening of 16 dystonia causing genes in our discovery cohort found an increase burden of rare

exonic variants in *VPS13D* in ET (p-value=0.02). However, the resequencing of *VPS13D* in our independent follow-up cohort of 900 ET cases and 949 controls did not confirm this association (p-value=0.09). Nonetheless, combined analysis of both cohorts is still suggestive of an association (p=0.007)

A previously discussed, a rare, early onset Mendelian movement disorder is cause by bi-allelic loss-of-function variants in *VSP13D*, which contrast with the heterozygous variant of reduced penetrance found in ET cases. In recent years, increase evidence have suggested the role of multiple rare variants with moderate penetrance in susceptibility to common disorders[46]. The allelic imbalance between ET cases and controls suggest that exonic variants in *VPS13D* can contribute to polygenic risk of developing ET.

VPS13D encodes for the vacuolar protein sorting-associated protein 13D, a very large protein of 492 kDa. VPSs proteins are required for proper trafficking of endocytic and biosynthetic proteins to the vacuole and plays an important role in intracellular sorting and delivery of soluble vacuolar proteins[47,48]. VPS13D play a key role in bilayer lipids trafficking between organelles and membrane growth promotion[49]. Disruption of VPS13D results in enlarged mitochondria, autophagy defects and peroxisome loss in cellular models[50,51].

Although we did not find an association between the remaining 15 genes and an increased risk of developing ET, some patients were carriers of rare protein altering variants. Interestingly, we also report some ET diagnosed patients, as well as controls, carrying specific variants previously reported to cause dystonia.

The detection of known dystonia disease causing variants in ET patients suggests diagnostic errors or perhaps the need to expand the phenotype associated with certain genes. For example, in our cohort, the molecular identification of a well-documented in-frame deletion in *TOR1A* in a patient with head tremor points to a re-classification to

isolated dystonia. Similarly, the available clinical information for the patient carrying the p.192D>N variant in *THAP1* points towards a probable case of dystonia, since the patient reported onset in speech at a very young age as well as trunk tremor. Although, after closer examination, some dystonia samples might have been misclassified as ET, the contamination of our cohort seems to be minimal. It is to note that ET is a lot more common than dystonia, having an estimated prevalence of ~1%[52] and ~0.01%[53] respectively.

Nonetheless, without extensive family history or molecular experiments, evaluating the impact of the variants on the protein function and their role in the disease is challenging. In that sense, although some rare variants have been found in our ET cases, we also demonstrate that such variants are found in our population controls at a similar frequency, and therefore are unlikely to have a large impact on ET etiology. It is also worth mentioning that although the controls do not have any known neurological disorder, most of them were not subjected to extensive neurological examination; it is therefore possible that some of them had mild neurological signs or developed a disease later in life.

The present study did not explore the full potential genetic overlap between ET and dystonia as it is limited to the contribution of rare variants in sixteen well documented dystonia genes; in the recent years, with the advent of next generation sequencing, additional putative dystonia causing genes have been reported, but they are still pending confirmation or explain a very small portion of cases. Also, we did not evaluate the contribution of common variants on shared genetic risk between the two disorders. Despite recent advances in genome-wide association studies (GWAS), the two most recent GWAS for isolated dystonia, musician dystonia[54] and cervical dystonia[55], did not yield genome wide significant loci that could be interrogated in our ET cohort.

Finally, our study power is limited by the size of our cohort; some genes had very few rare protein coding variants in their sequence, making it impossible to perform rare variant

association testing without recruiting more patients. Nonetheless, it allowed the investigation of the frequency of such variants in a cohort of ET which, to our knowledge, has never been done to this extend.

Table 1: Discovery cohort description

		ET cases	Controls
N		789	770
Sex	Male	0.42	0.47
	Female	0.58	0.53
Age	mean	62.93	59.63
	sd	14.32	15.34
	unknown	0.45	0.04
AOO	mean	43.86	NA
	sd	21.76	NA
	<25	0.23	NA
	>50	0.38	NA
	unknown	0.12	NA

Notes: SD: Standard Deviation; AOO: Age of onset; NA: Not applicable

Table 2: Selected gene causing isolated or combined dystonia

Form of dystonia	Gene	Designation	Inheritance
Isolated	TOR1A	DYT1	AD
	THAP1	DYT6	AD
	ANO3	DYT24	AD
	GNAL	DYT25	AD
	KMT2B	DYT28	AD
combined with parkinsonism	GCH1	DYT5a	AD
	ATP1A3	DYT12	AD
	TH	DYT5b	AR
	SPR	DYT-SPR	AR
	PRKRA	DYT16	AR
combined with myoclonus	SGCE	DYT11	AD
	KCTD17	DYT26	AD
combined with other hyperkinetic movements	GNAO1	-	AD
	ADCY5	-	AD
	TBC1D24	-	AR
	VPS13D	-	AR

Notes: AD: Autosomal Dominant; AR: Autosomal Recessive

Table 3: Weighted burden analysis of 16 dystonia genes in a cohort of ET

Gene	Total Variants	$MA_{count\ cases}$	$MA_{count\ controls}$	Beta	SE	P
ADCY5	17	12	14	-0.3	0.49	0.74
ANO3	15	21	22	-1.01	0.55	0.97
ATP1A3	10	4	8	-1.50	1.96	0.98
GCH1	2	0	2	-25.22	17.52	0.81
GNAL	3	3	1	0.95	11.63	0.31
GNAO1	2	1	1	-0.02	17.70	0.29
KCTD17	2	1	1	-0.08	17.90	0.69
KMT2B	25	21	20	0.16	0.40	0.34
PRKRA	0	0	0	NA	NA	NA
SGCE	6	2	6	-0.84	4.19	0.80
SPR	5	9	5	1.11	1.12	0.16
TBC1D24	7	6	6	0.13	0.86	0.45
TH	15	7	21	-1.54	0.56	1.00
THAP1	3	2	1	0.70	11.82	0.15
TOR1A	7	5	2	0.91	3.18	0.08
VPS13D	71	80	56	0.49	0.23	0.02

Notes: $MA_{count\ cases}$: minor allele count in essential tremor cases; $MA_{count\ controls}$: minor allele count in controls; Beta: beta coefficient; SE: standard error; P: P-value

Table 4: Weighted burden analysis of rare coding variants in VPS13D for two independent ET cohorts

Gene	Total Variants	$MA_{count\ cases}$	$MA_{count\ controls}$	Beta	SE	P
Discovery	71	80	56	0.49	0.23	0.02
Follow-up	88	114	89	0.28	0.21	0.09
Combined	131	182	143	0.42	0.17	0.007

Notes: $MA_{count\ cases}$: minor allele count in essential tremor cases; $MA_{count\ controls}$: minor allele count in controls; Beta: beta coefficient; SE: standard error; P: P-value

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Chapter 5: Rare CACNA1A coding variants increase risk of Essential Tremor

Preface

Chapter 5 uses a similar approach to chapter 4, in the sense that we were also interested in leveraging comorbidity knowledge to possibility identify new gene associations. Although the connection between ET and migraine is not as well established as dystonia, we sought to explore this relationship, as it could inform us on genetic factors contributing to both diseases. Once again, using targeted sequencing, we investigated the role of five genes associated with migraine; Heterozygous protein altering variants in these genes are known to cause familial forms of migraine. Interestingly, we reported ET patients to carry an increased burden of rare variants in the protein coding sequence of *CACNA1A*.

Rare CACNA1A coding variants increase risk of Essential Tremor

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Introduction

Essential tremor (ET) is a chronic disease characterized by involuntary tremor of a body part[1,2]. Localized generally in the hands, it can also affect other body parts such as the head or the voice. It is one of the most common movement disorders, affecting around 1% of the world population[3] and is eight times more common than Parkinson's disease[4]. Although ET is not a life threatening it can significantly both the physical and psychosocial aspects of the quality of life[5].

Twin- and family-based studies have shown that ET has a strong genetic basis with heritability estimates ranging from 45 to 90%[6-8]. Despite the known substantial genetic contribution to the etiology of ET few loci or pathways have been reliably implicated in the disease etiology[9]. Validated disease causing variants can only explain, at best, a very small proportion of the genetic risk for ET.

Some studies have found an association between ET and other disorders[5,10]. Notably, a relationship between ET and migraine has been debated since the 1990s when one of the earliest studies found a higher lifetime prevalence of migraine in ET patients by comparison to controls[11]. The same study also found that individuals with migraine headaches were more likely to also suffer from ET. Since then, the association between

the two disorders have been explored and although studies have yield mixed results[12-15], the current evidence points towards a possible relationship.

Bidirectional association between ET and migraine suggests a shared physiopathology underpinning the two disorders. Recent evidence suggests that dysregulated calcium signaling pathways are involved in both diseases[16-18]. Like ET, a strong genetic component is known to contribute to migraine disease risk; familial and twin studies estimate migraine heritability to be between 30 to 60%[19-21]. Using traditional linkage studies and more recently high throughput DNA sequencing, a handful of genes has been established to cause familial migraine[22]. In addition, recent genome wide association studies (GWAS) have associated several genetic markers to increased migraine risk[23,24].

No study has examined the potential for shared genetic influence on disease development. These shared genetic factors may underlie the comorbidity of ET and migraine. By examining the contribution of five known migraine genes in an ET cohort, we investigated to what extent these genes are involved in ET.

Method

In order to explore the influence of migraine associated gene in the development of ET, we selected six genes implicated in migraine risk[22]. These include *CACNA1A*, *ATP1A3*, *SCN1A* and *PRRT2*, four genes with a well-defined role in the development of Familial Hemiplegic Migraine. Two other genes, *CSNK1D* and *KCNK18*, were in turn selected based on their role in migraine with aura.

Targeted sequencing was carried out on two separate cohorts. The discovery cohort was comprised of 932 ET cases and 864 controls. Of the cases, 46.6% self-reported their ET diagnosis. The replication cohort included 939 independent cases and 978 controls. Of those, 4.3% were self-reported. All samples self-identified as Caucasian and none had

known familial relatedness. The others were directly examined by a clinical neurologist. All participants provided signed consent.

The protein coding sequence of our genes of interest and the genomic segments around the loci were capture using molecular inverted probes (MIPs)[25,26]. This method allows targeted enrichment of the genomic regions of interest for the purpose of library construction prior to massive parallel DNA sequencing. This cost-effective approach allows the interrogation of the large number of samples necessary for well powered association studies.

Using MIPgen[27], a total of 345 MIPs were designed to each capture 150 bps segments of our regions of interest. All protein coding exons and +10 bp gene flanking regions were targeted. Library preparation was performed as previously described[26]. In brief, probes are composed of a 30 bp universal backbone flanked by 16-29 bps primers specific to the region of interest. Once individual probes were pooled together at equimolar concentration (0.044 fmol), hybridization, gap filling and ligation was carried out by incubating genomic DNA (100 ng) with the MIP pool, Taq polymerase and Ampligase for 24 hrs at 60 °C. The resulting circular DNA was used as a template to introduce sample-specific index sequence and illumina adaptors by PCR amplification. Sequencing was performed at the Genome Quebec-McGill Innovation Center using Illumina Hiseq2500 platform.

Raw sequencing data was pre-processed using Trimmomatic before being aligned by BWA-MEM[28] to the human reference genome (hg19). The proportion of coding sequence covered at a minimum of 10x was calculated per gene for each sample using GATK's DepthOfCoverage[29]. One gene, *PRRT2*, had to be excluded because of poor capture (65%).

Variants we called using SAMtools (1.9)[30] followed by Varscan(v2.3.9)[31] using the following parameters: -d 1000, -Q 13, -q 40, -B; --min-coverage 3, --min-reads2 1,--min-

var-freq 0.28, --p-value 0.05, --min-freq-for-hom 0.94. Low quality variants were excluded if their GQ score was lower than 54, more than 10% sample had missing genotype, or not respecting Hardy Weinberg equilibrium (p<10e-5). Samples with more than 15% missingness were also excluded. The remaining high quality variants were annotated using ANNOVAR[32].

Single marker association tests were performed in Plink2[33] using Firth logistic regression with sex as a covariate. To identify genes in which rare variants, in aggregate, are associated with ET, we performed gene-based collapsing analysis. Only variants with a minor allele frequency (MAF) <1% per the Genome Aggregation Database (gnomAD NFE exome) were kept. Rare variant burden was achieved using WeightedBurdenBT as implemented in VariantTools[34] using genes as aggregation bins, sex as a covariate and 20,000 permutations to control for type I error. Pending DNA availability, candidate variants in *CACNA1A* were validated using Sanger sequencing.

Variant localization analysis was performed based on the NCBI Reference Sequence NP_001120693. *CACNA1A* variants previously found in migraine patients were included if listed under "missense/nonsense" and "Phenotype = Hemiplegic Migraine" in The Human Gene Mutation Database (HGMD)[35].

Results

Using MIPs sequencing we captured the protein coding sequence of our candidate genes in our discovery cohort comprising 789 ET patients and 770 controls. Using 345 MIPs, we captured the protein coding sequencing of *CACNA1A, ATP1A3, SCN1A, CSNK1D* and *KCNK18*. On average, the genes had a minimum of 10x coverage for 88.5% to 99.5% of their bases across samples.

Following variant calling and quality control, only variants with a minor allele frequency less than 1% in the public database gnomAD[36] were retained for further analyses. This

represented a total of 55 unique variants in our discovery cohort. To test for single variant association with the phenotype, we used Firth logistic regression adjusted for sex, as implemented in PLINK2. No variants were found to have allelic imbalance between cases and controls (Supplementary table 1).

We next evaluated the cumulative effects of multiple rare variants on the potential association between the genes of interest and ET by performing rare variant burden analysis. Briefly, this method consists into collapsing information for multiple genetic variants into a single score, followed by testing for association between this score and the phenotype. Using VTools[34] package, 123 minor alleles were imported and association test was carried out using weighted aggregation test[37].

Gene-based analysis of protein sequence altering, rare variants revealed *CACNA1A* as possibly associated with ET (beta=1.10; p-value=0.01 ; SE=0.45) in our discovery cohort. While no individual variant presented allelic imbalance, a total of 30 minor alleles were found in our ET cohort compared to only 19 in the controls. A number of rare and potentially deleterious variants were also found in the other five genes studies, although at a similar rate in ET patients and controls (Table 1).

In order to replicate our findings in an independent dataset, we re-sequenced *CACNA1A* in an unrelated cohort of 900 ET cases and 949 controls. Using the same gene burden approach, we once again found a significant enrichment of rare sequence altering variants in *CACNA1A* in our ET samples (beta=1.32; p-value=0.005; SE=0.48) (Table 2). While our controls only carried 17 minor alleles in the *CACNA1A* gene, 34 were found in the ET cases. Although our findings were replicated in two independent cohorts, combined analysis of our two cohorts did not reach genome wide significance (p-value $<5x10^{-6}$) (beta=1.32; p-value=$2x10^{-4}$; SE=0.35). No single marker in *CACNA1A* was associated with the phenotype (Supplementary table 2).

CACNA1A encodes for the pore-forming subunit of $Ca_v2.1$ voltage-gated Ca^{2+} channels. These calcium channels contribute to vesicle release at the synaptic terminals by translating neuronal excitation to neurotransmitter release[38,39]. Each channel subunit consists of four homologous domains containing six putative alpha helical membrane-spanning segments (Figure 1A). These contain important functional domains such as the voltage sensor and channel pore[40]. The analysis of the positions of the ET associated variants revealed that the vast majority of minor alleles (59/64) are located in the cytoplasmic segments of the channel. Only five rare variants were located in one of the transmembrane domains. This is similar to the position of variants found in controls (35/36 being in cytoplasmic domains) but contrast with the missense variants previously reported in hemiplegic migraine patients, as they are mostly located in the one of the four membrane-spanning segments[41,42] (Figure 1B). None of the variants previously described in migraine patients were found in our cohort.

Discussion

Pleiotropy is the genetic phenomenon whereby a single locus or gene can affect multiple traits. In recent years, leveraging this information has become common and several approaches have been developed to cross-phenotype genetic association. In the present study, we explored the role of five genes known to be involved in the development of migraine in a cohort of essential tremor. Using targeted gene sequencing, we captured the protein coding region of *CACNA1A, ATP1A3, SCN1A, CSNK1D* and *KCNK18* in 789 ET patients and 770 matching controls.

Although no individual variant was found to be associated with the disorder, the whole-gene collapsing method showed that rare coding variants in *CACNA1A* are, in aggregate, associated with an increased risk of disease (beta=1.10; p-value=0.01 ; SE=0.45). We found that ET patients are more likely to carry potentially disease-causing variant in *CACNA1A* than controls ($MA_{count\ cases}$=30; $MA_{count\ controls}$=19). This association was

subsequently replicated in an independent set of 900 cases and 949 controls (beta=1.32; p-value=0.005; SE=0.48).

CACNA1A is composed of four homologous domains, each with six transmembrane helices, connected by large intracellular linkers. These linkers, in addition to the amino- and carboxy terminal region, serve as a platform to bind different regulatory proteins that carry out or regulate calcium signaling[43]. For example, the loop between segment II and III is known to bind syntaxin-1, SNAP25 and synaptotagmin-1, three SNARE proteins essential for vesicle docking and fusion[44]. In turn, the C-terminal domain contains binding sites for calmodulin to mediate Ca^{2+}-dependent inactivation and facilitation[45]. Interestingly ET patients carried most of their variants in the intracellular segments of the protein.

The rare variants found in ET patients could disrupt the binding sites of the regulatory interacting proteins, which in turn may perturbate channel activities. Although only functional studies would confirm this hypothesis, previous studies have shown that disruption of sites such as the SNARE interaction domain compromises vesicle exocytosis and channel viability[44]. One variant found in our cohort, p.E1018K, carried by 23 ET cases and 17 controls, causes impairment in protein function by depolarizing shift in channel activation[46]. Therefore, protein sequence altering variants in *CACNA1A* might perturbate the important role of Cav2.1 in neurotransmitter release and neuronal excitability, and lead to the development of ET.

Our study is not the first to suggest a role for *CACNA1A* in ET. Earlier RNA-sequencing on post-mortem tissues from ET patients found *CACNA1A* to be one of the most dysregulated gene in the cerebellar cortex[17]. This was consistent with another transcriptomic study which found that calcium signaling and synaptic transmission to be within the top 5 most dysregulated processes in ET cerebellum[16].

Interestingly, missenses or truncating variants *CACNA1A* are also known to cause episodic ataxia type 2[47]. Although the primary motor feature of ET is an action tremor, patient can also have other signs of cerebellar dysfunction such as intention tremor[48], impaired gait[49] and dysmetria[50]. Numerous postmortem and imaging studies have confirmed cerebellar morphological changes and abnormal activity in ET[51]. Isolated ablation of *CACNA1A* in Purkinje cells causes mostly ataxic movements but also tremors (trunk, limbs, neck, face) in mice[52]. Thus, disruption of calcium regulation at synaptic terminals via Cav2.1, one of the most important calcium channel in Purkinje neurons[53], could explain the development of ET symptoms.

Our study is limited in several aspects. First, the lack of additional clinical information didn't allow us to describe possible neurological features associated with *CACNA1A* variants in ET. Second, although the controls did not have known neurological conditions, they did not undergo clinical examination. Additionally, although replicated in two independent cohorts, the association between the gene and ET did not reach genome-wide significance. Finally, targeted sequencing does not interrogate the entire genome and cannot control for possible biases such as population stratification and cryptic relatedness. Nonetheless, our results suggest a role for *CACNA1A* in ET pathology and could, in part, explain its comorbidity with migraine.

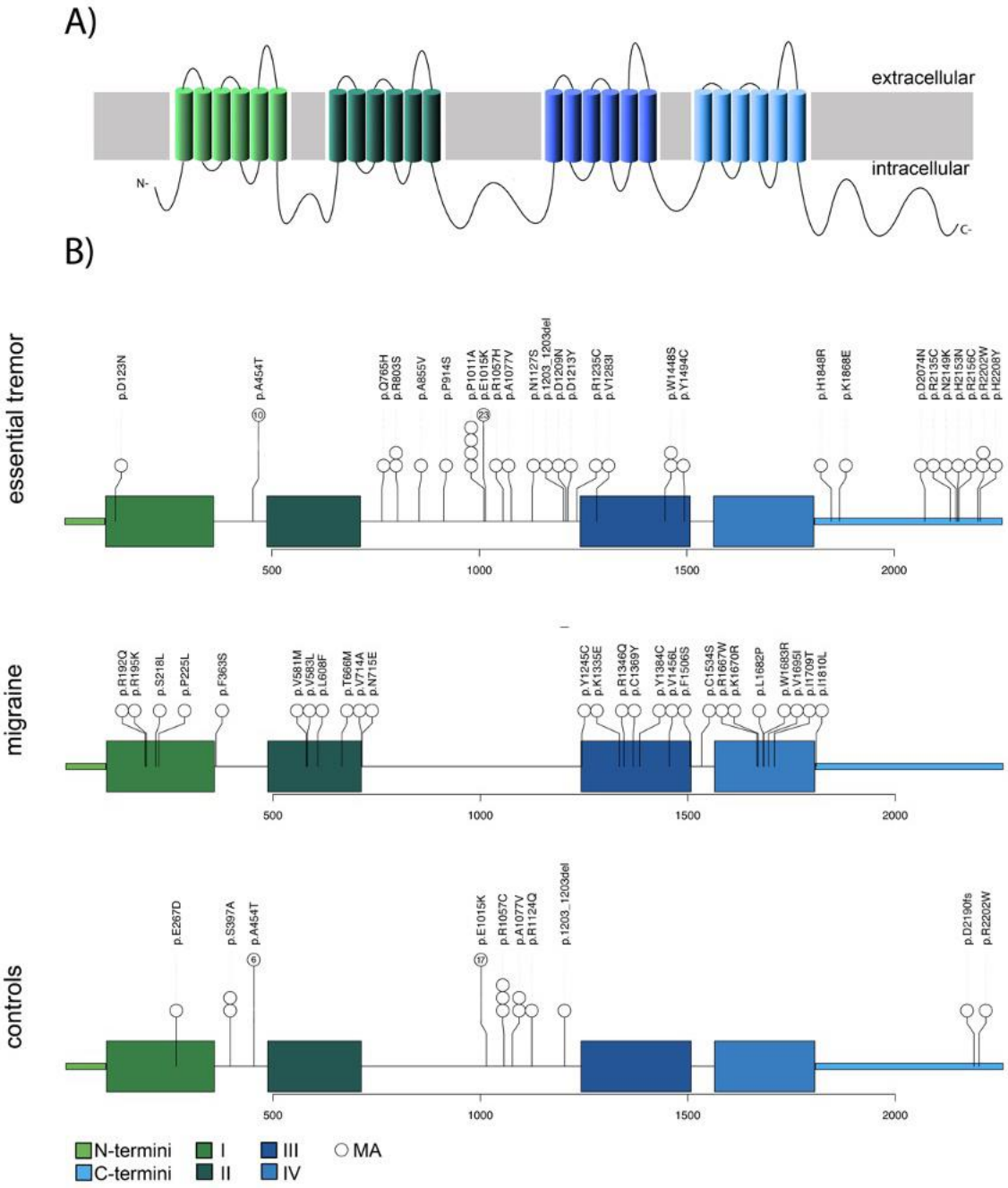

Figure 1: CACNA1A structure and variant localization. A) *CACNA1A* encodes for the pore forming subunit of Cav2.1 calcium channel. It is composed of four homologous domains (I-IV), each containing six transmembrane alpha helices. B) Schematic localization of *CACNA1A* rare variants found in our ET cohort (top panel), healthy controls (middle panel) or published literature on familial hemiplegic migraine patients (lower panel). While migraine patients mostly carry their variants in one of the four transmembrane domains

(I-IV), variants in ET patients are mostly located in the intracellular segments of the channel.

Note: N: N-termini; C-termini; I-IV: transmembrane domains I to IV; MA: minor allele

Table 1: Discovery cohort rare variant burden results

Gene	Total Variants	$MA_{count\ cases}$	$MA_{count\ controls}$	Beta	P	SE
ATP1A2	8	11	11	0.22	0.40	0.79
CACNA1A	21	30	19	1.10	0.01*	0.45
CSNK1D	3	1	9	-3.99	0.99	1.34
KCNK18	8	8	3	1.04	0.06	0.76
SCN1A	15	16	19	-1.92	0.83	1.90

Notes: MAF: minor allele frequency; $MA_{count\ cases}$: minor allele count in essential tremor cases; $MA_{count\ controls}$: minor allele count in controls; Beta: Beta coefficient; P: P-value; SE: standard error, *:Statistically significant.

Table 2: CACNA1A all cohorts burden results

Cohort	Total Variants	$MA_{count\ cases}$	$MA_{count\ controls}$	Beta	P	SE
Discovery	21	30	19	1.10	0.01*	0.45
Replication	19	34	17	1.32	0.005*	0.48
Combined	33	64	36	1.32	0.0002*	0.35

Notes: MAF: minor allele frequency; $MA_{count\ cases}$: minor allele count in essential tremor cases; $MA_{count\ controls}$: minor allele count in controls; Beta: Beta coefficient; P: P-value; SE: standard error, *:Statistically significant.

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Chapter 6: Rare variants in KCNN2 increase risk of Essential Tremor

Preface

Similar to chapters 4 and 5, the work presented in chapter 6 uses a targeted sequencing approach followed by whole gene rare variant collapsing tests to examine the role of a novel candidate gene in ET, *KCNN2*. This gene is of particular interest because a heterozygous missense causes a phenotype clinically and pharmacologically mimicking ET in a rodent model. Thus, by screening the entire protein coding region of the human ortholog gene in a cohort of ET cases and matching controls, we demonstrated patients carry a higher burden of variants in this *KCNN2* than expected in the general population which in turn suggests a role for this gene in the molecular pathways underlying ET.

Rare variants in KCNN2 increase risk of Essential Tremor

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Introduction

Essential tremor (ET) is a common movement disorder characterized by postural or kinetic tremor of the upper limbs, sometimes affecting other body parts such as the head, voice or lower limbs[1]. Although twin and familial studies have shown the important contribution of genetics to the disease etiology, few genetic factors have been found[2].

Recently, a N-ethyl-N-nitrosourea (ENU) -induced mutant rat was described as a model for ET[3]. The tremor dominant Kyoto rat (F344-Trdk/+) is characterized by autosomal dominant whole-body tremor, beginning around 2 weeks of age and continued throughout life. Administration of anti-tremor drugs showed that the phenotype of Trdk rats pharmacologically resembled that of human ET; tremor duration and intensity were significantly reduced with administration of propranolol or phenobarbital, two drugs used in the treatment of ET.

Genetic linkage analysis and targeted sequencing led to the identification of the F344-Trdk/+ phenotype causative heterozygote mutation (c. 866T>A, p. I289N) in *Kcnn2*. This gene encodes for the small conductance calcium-activated potassium channel protein 2 (SK2). *Kcnn2* is expressed thorough the brain at the synaptic membrane where it regulates neuronal excitability and synaptic transmission[4]. Modulated by intracellular calcium, the activation of this voltage-independent channel leads to potassium efflux resulting in repolarizing/hyperpolarizing membrane potential[5,6].

Considering that a heterozygote mutation in *Kcnn2* causes a pharmacological phenotype similar to ET in the F344-Trdk/+ rat, we hypothesised that rare deleterious variants in the human orthologue could contribute to the phenotype in ET patients. To explore the contribution of *KCNN2* in ET, we screened the entire protein coding region of the gene in a cohort of ET patients and controls.

Method

Participants were recruited at nine centers across North America and Europe. All patients received a diagnosis of ET, 75.6% by direct clinical evaluation and 24.4% by self-reported questionnaire. Controls had no known history of neurological disorders. Written informed consent was obtained from all participants prior to sample collection and the study was approved by the McGill University Health Center (MUHC) ethics committee (protocol no: 14051). All participants reported to be of Caucasian descent and did not have any known relatedness. DNA samples were extracted from blood or saliva.

Targeted gene sequencing was carried out using a custom molecular inversion probes (MIPs)[7] followed by high throughput sequencing. Using MIPgen[8], 26 MIPs were designed to capture the entire protein coding region of uc003kqo. DNA capture and amplification was performed as previously described[9]. Sequencing was performed at the Genome Quebec-McGill Innovation Center using Illumina Hiseq2500 platform.

Raw sequencing data was pre-processed using Trimmomatic[10] and the resulting reads were aligned to the human reference genome (hg19) using BWA-MEM[11]. Variants we called using two different callers: 1- GATK (3.9)'s[12] HaplotypeCaller followed by GenotypeGVCFs, where only variants with a QUAL>1000 were kept, and 2-SAMtools (1.9)[13] followed by Varscan(v2.3.9)[14] using the following parameters: -d 1000, -Q 13, -q 40, -B; --min-coverage 3, --min-reads2 1,--min-var-freq 0.28, --p-value 0.05, --min-freq-

for-hom 0.94. Resulting variants we then concatenated into a single vcf using BCFtools[13]. Low quality variants we excluded using GATK's SelectVariant[12] (GQ<54) and Vcftools[15] (--max-missing 0.9, --hwe 10e-5). Samples with high missingness were also excluded (>0.15).

All variants were annotated using ANNOVAR[16]. Single marker association tests were achieved using Firth logistic regression with sex as a covariate as implemented in Plink2[17]. Whole gene burden analyses were performed using Weighted Burden BT by VariantTools[18]. Briefly, the variants are assigned a weight for rarity, so that rarer variants carry a higher weight. Variants are then aggregated per locus of interest (gene) into a single score for each sample and tested for association with the phenotype. Type I error was controlled using 20,000 permutations. All protein altering variants found in cases were validated using Sanger sequencing. Variant localization analysis is based on NP_067627.2.

Results

To explore the potential contribution of *KCNN2* variants to ET etiology, we sequenced the entire protein coding region of this gene in our cohort. An average of 94.7% of the sequence of interest was covered at a minimum of 10x. A total of 1,679 unrelated ET cases and 1,694 controls passed all quality controls, resulting in 20 unique variants identified (Table 1). Of those, 6 were predicted to affect the protein coding sequence (missense or indel) and had a minor allele frequency of <0.01 in public databases (gnomAD).

A total of 54 ET cases and 35 controls carried a minor allele in *KCNN2*. We first tested if any of the variant were individually associated with the phenotype. Using Firth generalized linear model, we did not find a significant difference in allele frequency between the cases and the controls for any of the 20 variants present (Table 1).

Second, we performed whole gene burden association analysis using VariantTools' WeightedBurdenBT. Briefly, this test uses a weighted sum approach where all variants found in the gene sequence are collapsed into a single score for each sample[19]. Cases and

controls are compared to identify an excess of variants in the affected samples. Using this method, we found a significant enrichment of minor alleles in the protein coding region of *KCNN2* in our cohort (Beta= 4.35; SE=2.24; p-value=0.03) (Table 2).

When only variants affecting the protein sequence (i.e. missenses or indels) were selected, our ET cohort showed a significantly higher burden of variants than the control individuals cohort (Beta=2.43; SE=1.22; p-value=0.03). This enrichment was not observed when we only selected synonymous variants (Beta=1.92; SE=1.86; p-value=0.15). Our results suggest that protein sequence altering variants contribute the ET disease risk.

Of the 8 protein altering alleles (missenses and indels) found in our cohort, 7 were carried by ET patients and only one was carried by a healthy control. All variants carried by ET patients were missenses and were predicted to deleterious by CADD[20] (CADD Phred score >20). The only control carried a non-frameshifting 3 bp deletion in exon 1. Interestingly, all the sequence altering variants found in our cohort are located in the N- and C-terminal intracellular regions of the channel (Figure 1).

Discussion

Through a forward genetic approach, rats with a pathogenic variant in *Kcnn2* were found to have ET-like physical and pharmacological phenotype[3]. Based on these findings, we sought to explore if potentially pathogenic variants in the human orthologue *KCNN2* could contribute to ET in humans.

Using targeted DNA sequencing, we found a higher burden of minor alleles in the coding sequence as well as significantly more protein altering variants in our cohort of 1,679 unrelated ET patients compared to unrelated controls. Of these ET patients, seven had variants that altered the protein sequence compared to only one controls. Although we

did not find any marker individually associated with the phenotype, our whole gene aggregation method suggests that *KCNN2* may play a role in ET etiology.

KCNN2 encodes for the SK2 ion channel, a voltage independent, calcium activated, potassium channel. They are widely expressed in the central nervous system and are solely activated by intracellular Ca^{2+}, resulting in potassium efflux[21,22]. SK2 channels play a critical role in synaptic transmission and neuron excitability by repolarizing or hyperpolarizing the membrane.

Like other members of the SK channel family, SK2 are tetrameric and the each subunit is composed of six transmembrane domains with cytoplasmic N- and C-terminal domains[23,24]. Interestingly, all protein sequence altering variants found in our cohort where located in the terminal domains (Figure 1). These intracellular regions are known to contain binding domains for regulatory proteins that play a role in modulating the channel activity and localization[22,25].

Two variants (p.D412G and p.K435R) found in our ET cohort are located in the highly conserved calmodulin-binding domain (CaMBD). This portion is in the cytoplasmic C-terminal region of the protein is where calmodulin intrinsically binds and modulates the channel sensitivity to Ca^{2+}[26]. It is therefore possible that the protein altering variants found in our ET patients could disrupt normal protein regulation.

KCNN2 has previously been associated with neurological diseases. First, a heterozygous missense was found in a kindred with autosomal dominant myoclonus-dystonia[27]. The family had an onset in infancy with writer's cramp and/or hand tremor. The disease typically progressed to tremulous dystonia, primary affecting the hands and the neck, with myoclonus and sometimes nystagmus. Whole-exome sequencing led to the identification of a heterozygous variant (c.1112G>A:p.Gly371Glu) co-segregating with the disease in the family.

Subsequently, loss-of-function, *de novo* variants in *KCNN2* were found in a cohort of ten unrelated individuals presenting motor and language development delay, intellectual disability and behavioural disturbances[28]. Six out of ten patients also had movement disorders, including cerebellar ataxia, extrapyramidal symptoms and tremors.

In that sense, although our study is the first to associate *KCNN2* with a common disease, the identification of genes overlapping monogenic disorder and complex traits is well documented[29]. For example, it has been shown that Parkinson's disease patients are more likely to carry variants in GBA, a gene known to, when deficient, cause Gaucher disease, a rare lysosomal storage disorder[30]. We could hypothesize that, unlike the highly disruptive *de novo KCNN2* variants found in the infancy onset development delay patients, the variants we identified in ET could have a milder effect on the normal protein function and cause a milder action tremor.

Nonetheless, our study is limited by the phenotypic information available for each patient. Only a diagnosis of ET was made and no supplementary clinical information on possible additional neurological features is available for most patients. Similarly, our controls did not systematically undergo a thorough neurological examination or could develop the disorder later in life. Additionally, the targeted DNA sequencing approached used does not allow for population stratification control or identification of cryptic relatedness. Finally, although we found a significantly higher *KCNN2* exonic variants burden in our ET cohort, the association found did not reach the genome wide gene-level significant threshold of $p<2.5 \times 10^{-6}$.

It is possible that the variants identified in ET patients are not responsible for the tremor. Functional analyses exploring the impact of these variants on protein function could help determine if they are disease predisposing. Nonetheless, given the gene's very low tolerance for loss of function variants (pLoF=0.99)[31], its known phenotype in rats[3] and its previous implication in human movement disorders[27,28], we conclude rare variants in *KCNN2* likely contribute to ET pathology.

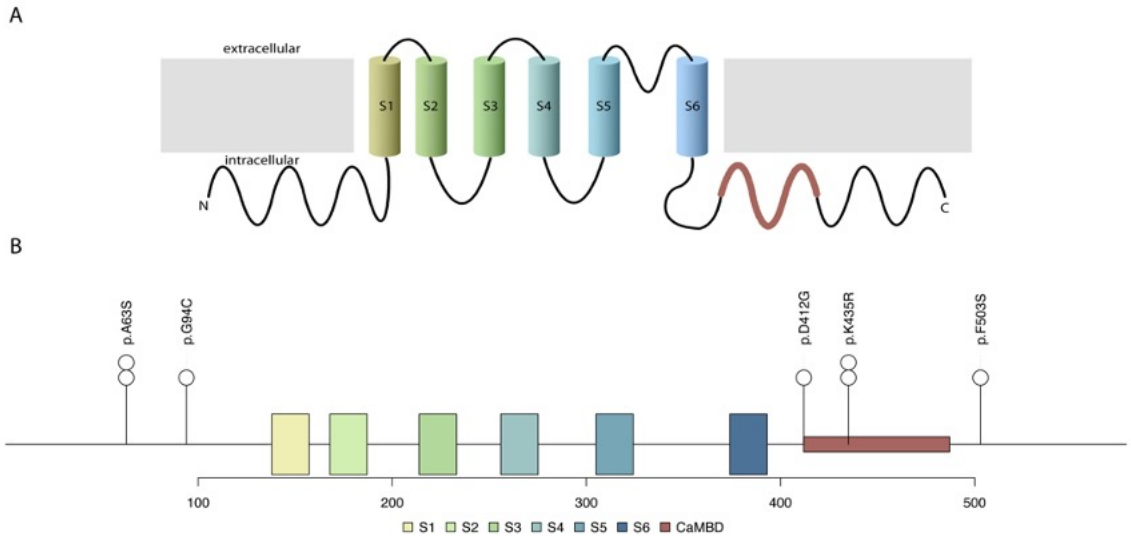

Figure 1: Schematic representations of SK2. A) SK2 consists of six transmembrane segments (S1-6) and large intracellular N- and C- terminal domains. Functional SK2 channels assemble to form tetramers. B) Distribution of rare coding variants found in our ET cohort on the protein sequence. All variants are located in the N- and C- intracellular segments. The C-Terminal domain is known to contain the calmodulin binding domain (CaMBD), where calmodulin intrinsically binds the channel and servers as a CA^{2+} sensor to modulate channel activity.

Table 1: Variants present in KCNN2 in our ET cohort and single marker association results

Variants Characteristics									Association Results		
Position	REF	ALT	NA	AA	MAF	CADD	$MA_{count\ cases}$	$MA_{count\ controls}$	OR	SE	P-Value
5:113698659	G	T	c.G187T	p.A63S	2.104e-05	22.8	2	0	5.12	1.9	0.39
5:113698689	CACA	C	c.218_220del	p.73_74del	4.144e-05		0	1	0.31	2.31	0.61
5:113698697	C	T	c.C225T		1.025e-05		1	0	3.45	2.31	0.59
5:113698752	G	T	c.G280T	p.G94C	0	23.0	1	0	3.45	2.31	0.59
5:113698763	C	T	c.C291T		2.786e-05		1	0	3.45	2.31	0.59
5:113698808	G	A	c.G336A		0.0002		0	1	0.29	2.31	0.59
5:113698817	G	A	c.G345A		0		0	1	0.34	2.31	0.64
5:113698844	G	A	c.G372A		0		2	5	0.74	0.65	0.64
5:113699683	C	T	c.C567T		0.0009		2	0	5.76	1.9	0.36
5:113740257	C	T	c.C705T		8.968e-06		1	0	3.45	2.31	0.59
5:113740341	C	T	c.C789T		0		3	0	7.54	1.75	0.25
5:113798857	A	T	c.A1113T		4.515e-05		0	2	0.23	1.9	0.44
5:113808842	A	G	c.A1235G	p.D412G	0	25.4	1	0	3.45	2.31	0.59
5:113822782	A	T	c.A1290T		3.598e-05		3	3	1.07	0.82	0.93
5:113822796	A	G	c.A1304G	p.K435R	2.697e-05	25.0	2	0	5.21	1.9	0.39
5:113822848	A	G	c.A1356G		8.99e-06		0	1	0.38	2.31	0.68
5:113831647	T	C	c.T1508C	p.F503S	0	23.6	1	0	3.45	2.31	0.59
5:113831696	T	C	c.T1557C		0.0005		2	2	1.09	1	0.93
5:113831756	C	T	c.C1617T		0.0050		31	19	1.44	0.3	0.23
5:113831822	G	A	c.G1683A	p.S561S	1.797e-05		1	0	2.79	2.31	0.66

Notes: REF: reference allele; ATL: alternative (i.e., effect) allele; NA: nucleique acid change; AA: amino acid change; MAF: minor allele frequency; CADD: CADD phred score; $MA_{count\ cases}$: minor allele count in essential tremor cases; $MA_{count\ controls}$: minor allele count in controls; OR: odds ratio; SE: standard error

Table 2: Gene-based rare variant association results

	total variants	$MA_{count\ cases}$	$MA_{count\ controls}$	Beta	SE	P
All variants	20	54	35	4.35	2.24	0.03
Exonic	6	7	1	2.43	1.22	0.03
Synonymous	14	46	34	1.92	1.86	0.15

Notes: $MA_{count\ cases}$: minor allele count in essential tremor cases; $MA_{count\ controls}$: minor allele count in controls; Beta: beta coefficient; SE: standard error; P: P-value

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Chapter 7: Discussion and Conclusion

Discussion

Three novel genes are associated with ET risk

Since the completion of the first draft of the human genome in 2001[23], major advances have been made in understanding the genetic origins of complex traits. Although over two decades of research have attempted to map the genetic contribution to ET, only a few genes and markers were associated with a variable disease risk[3].

The present study aimed to delineate the potential contribution of 38 genes to ET risk. These genes were prioritized based on five different hypotheses: [1] previously associated ET genes, [2] genes carrying associated GWAS markers, [3] pleiotropy with dystonia, [4] pleiotropy with migraine, and [5] rodent model. We anticipated that sequencing high priority genes in a large case-control cohort would allow us to identify potentially deleterious variants contributing to ET disease risk.

Chapters two and three of this thesis intended to further explore the role of genes which were previously suggested to be associated with ET; investigating these genes in our large cohort could expand our understanding of their potential contribution to the disease. First, we sequenced three genes following their association in a GWAS composed of 2,807 cases and 6,441 controls[24]. Second, we captured an additional 13 genes based on their suggested contribution in family-based studies. Neither single variant or gene-based association study provided evidence for a role for these genes in our cohort.

Chapters four and five aimed to explore the potential genetic overlap between ET and two comorbidities; 16 dystonia causing genes and five migraine genes were sequenced in our cohort. Two genes, *VPS13D* and *CACNA1A*, associated with dystonia and migraine respectively, were shown to have a significantly higher burden of rare variants in the coding sequence of ET patients by comparison to control. These results suggest a conceivable role for these genes in disease development.

The last chapter proposed to investigate the role of rare variants in *KCNN2*, a gene causing a phenotype of whole body tremor with a pharmacological profile resembling ET in a rat model[25]. Whole-gene sequencing revealed a small proportion of ET patients had a sequence altering and potentially deleterious variant in this highly conserved gene. Our results suggest that carrying a missense in this gene increases the risk of developing ET.

Overall, by means of five different hypotheses, we identified three new genes associated with increased risk of ET. Using collapsing analyses, we demonstrated that ET patients carry more rare variants in the protein coding sequence of these genes than control individuals. These results suggest that the dysregulation of these genes could contribute to the development of ET.

VPS13D causes movement disorder via mitochondria dysfunction

The first gene identified was *VPS13D*. It encodes for the vacuolar protein sorting 13 homolog D, one of the four family members of human Vps13. First described in *Saccharomyces cerevisiae*, Vps13 is key player in intracellular lipid transport[26]. It locates at their membrane contact site between the reticulum endoplasmic (where most lipids are synthesized) and other organelles such as nucleus, vacuole, endosome, mitochondria[27]. Through its hydrophobic groove, Vps13 functions as a conduit for the lipids to slide between membrane[28], allowing trafficking of bilayer lipids and promotion of membrane growth.

The four members of VPS13 family have been associated with different neurological disorders. Homozygous or compound heterozygous loss of function variants lead to chorea-acanthocytosis (VPS13A)[29,30], Cohen syndrome (VPS13B)[31], Parkinson's disease (VPS13C)[32,33] and childhood onset complex movement disorder (VPS13D)[34,35]. These findings highlight the importance of VPS13 family members in neuronal integrity.

Intracellular localization varies between the four Vps13 human paralogs. VPS13D is found in the inter-organelle contact site between the Golgi complex, the mitochondria and the peroxisome[36]. Cellular models have shown that disruption of VPS13D function leads to enlarged mitochondria, autophagy defects and peroxisome loss[37,38]. In neuron, the depletion of this gene caused incomplete mitophagy, leading to toxic intermediate accumulation and cell death[39]. Homozygous deletion of VPS13D in animal models leads to embryonic lethality[34,37].

In humans, disruption of VPS13D function also leads to mitochondrial defects. Cell lines derived from patients with biallelic variants in this gene exhibit altered mitochondrial morphology and reduced protein markers[35]. Although no specific mitochondrial defect has been previously been described in ET, a broad spectrum of movement disorders has been associated with mitochondrial dysfunction, including dystonia, parkinsonism, myoclonus and tremor[40-42]. Thus, a new association between ET and a peroxisome and mitochondria essential gene is not surprising.

CACNA1A is a key player in neurotransmitter release

The second gene our study identified as a newly associated with ET disease risk factor is *CACNA1A*. We demonstrated that ET patients carried rarer, and potentially deleterious variants, in the protein coding region of this gene than what was observed in control individuals. Re-sequencing of this gene in an unrelated cohort confirmed the association.

CACNA1A encodes for the pore forming, alpha subunit, of the voltage gated calcium channel (VGCC) Cav2.1. VGCCs are ion channels acting as transducers between the electrical signal of the membrane potential and calcium signaling. When action potential passes along the cell membrane, VGCCs activate which leads to a change in conformation and channel opening. This allows Ca^{2+} ions to flux into the cell and activate various calcium

activated targets and processes. VGCCs can be classified into high ($Cav_{1.2\text{-}1.4}$ and $Cav_{2.1\text{-}2.3}$) and low ($Cav_{3.1\text{-}3.3}$) threshold channels, according to the strength of depolarization required to reach activation threshold[43].

In humans, ten different genes encode for the alpha subunit of VGCCs. Each have a different pattern of distribution and specific regulatory properties. The alpha subunits are composed of four homologous domains containing six putative alpha helical membrane-spanning segments. Those contain important functional domains such as the voltage sensor and channel pore. In addition, large intracellular domain linkers and N- and C-termini domains serve as a platform to bind different regulatory proteins that carry out or regulate calcium signaling[44].

CACNA1A forms the alpha subunit of Cav2.1, also known as P/Q type. The channel is also composed of three subunits, the intracellular $\alpha_2\delta$, the extracellular β and the transmembrane γ[44,45]. These play a role in channel biophysical properties such as modulating maximum current density and inactivation rate, as well as correct localization[44-46]. In addition, increasing evidences suggest additional roles for the subunits outside the calcium channel complexes[46].

Cav2.1 is located across the nervous system and has highest expression in the cerebellum[47]. It is the most abundant high-threshold VGCCs of Purkinje cells[48,49]. Located at the presynaptic membranes of the neuron terminal, Cav2.1 control neurotransmitter release by modulating local intracellular Ca^{2+} at the active zone[50]. When action potential reaches a nerve terminal, Cav2.1 open which in turns allows Ca^{2+} to trigger fusion of neurotransmitter containing vesicles with the presynaptic plasma membrane, resulting in neurotransmitter release in the synaptic cleft and activation of postsynaptic receptors[44,51-53]. Synaptic vesicles docking, and exocytosis is mediate by SNARE proteins, a Ca^{2+} dependent process; In response to elevated Ca^{2+}, calcium sensitive synaptotagmin-

1 and Munc13 join vesicular and plasma membranes together and initiate fusion pore opening[54,55].

Three other neurological phenotypes have previously been associated with Cav2.1 defects. Loss of function mutations such as frameshifting, large deletions or leading to aberrant splicing were first identified as causing episodic ataxia type 2[56]. Poly-glutamine expansion in the protein C-terminus domain has in turn been identified as the cause of another type of ataxia, spinocerebellar ataxia type 6[57]. Finally, deleterious missense variants cause familial hemiplegic migraine, a subtype of migraine with classic characteristics of unilateral headache and nausea but accompanied by other neurological symptoms such as muscle weakness and paresthesia[56]. These conditions can occasionally overlap and present additional symptoms such as epilepsy, intellectual disability and psychiatric manifestations[58-61].

Deleterious variants previously described in *CACNA1A* phenotypes have been shown to change the channel biophysical characteristics[62-67]. Since the variants found in ET patient are not predicted to cause a complete loss of function, and the majority is not located in the pore forming or voltage sensing functional domains of the protein, it is possible that the ET variants might not only affect the biophysical channel characteristics but also influence the downstream signaling pathways and molecular environment.

The majority of variants found in ET patients are located in the intracellular linker between homologous domains II-III and the C-terminal domain. The II-III linker in known to contain the “synprint” site, a synaptic protein interaction site which bind SNARE proteins including syntaxin1, SNAP25 and synaptotagmin1[68]. Disruption of this site has been shown to compromise vesicle exocytosis and channel viability[69].

Via the C-terminal domain, Calmodulin pre-associates with Cav2.1 and, upon Ca^{2+} stimulation, inactivates the channel[70]. Additionally, modular adaptors CASK and Mint1

interact with this portion of the protein to target and anchor channels to their proper location[71]. Similarly, RIM1 binds to the C-terminal domain to anchor vesicles containing neurotransmitter to the channel in addition to modulating the channel inactivation[72].

Overall, we can conclude that Cav2.1 is tightly regulated by a various interacting proteins and disruption of their binding sites by rare variants could result in perturbated channel activities. Rare variants in the protein coding region of this gene could perturbate the important role of Cav2.1 in neurotransmitter release and neuronal excitability and in turn lead to the development of ET.

KCNN2 regulates neuronal membrane excitability

The third and last gene found to have a high rate of rate sequence altering variants in our ET cohort was *KCNN2*. This gene encodes for the small conductance, calcium activated, potassium channel SK2. This ion channel responds to elevated intracellular calcium, allowing potassium influx to cause membrane hyperpolarization. In the nervous system, this unique ability contributes to regulation of neuron excitability, spike frequency and neurotransmitter release.

Calcium activated potassium channels (K_{Ca}) are divided into three subfamilies; high conductance (BK), intermediate conductance (IK) and small conductance (SK). Through their high calcium sensitivity, K_{Ca} channel regulate Ca^{2+} signaling pathways in both excitable and non-excitable cells. Over the years, various diseases have been associated with variants in genes from the K_{Ca} channels family including epilepsy, schizophrenia and hypertension[73].

KCNN2 is expressed extensively in the central nervous system[74], more specifically in the neuronal cell soma, presynaptic and postsynaptic compartment[75-77]. Distinct physiological roles are carried out depending on the subcellular position and coupling with different

Ca^{2+} source. For example, in the hippocampus, co-localization of SK2 channel and NMDA receptor at postsynaptic membrane of glutamatergic synapses led to the extensive study of SK2 role in learning and memory[78].

Variants in *KCNN2* have been reported to be implicated in various diseases. First, common polymorphism were reported to result in increased risk of cardiovascular diseases, coronary artery aneurysms in Kawasaki disease[79] and cardiac tachyarrythmias[80]. More recently, whole exome sequencing allowed the identification a rare missense co-segregating with myoclonus-dystonia in a multigeneration family[81]. Finally, *de novo* variants in *KCNN2* were associated with dominant neurodevelopmental movement disorders[82].

Cav2.1 and SK2 form complexes in neurons

In various cells, Ca^{2+} entry via VGCC selectively activate calcium activated potassium channels. Because elevated Ca^{2+} signal is precisely localized in time and space, the location on VGCCs' post signaling targets are found in close special proximity with the channels[83]. Although various VGCC subtypes are found in the Purkinje cells, only Cav2.1 has been shown to colocalize with SK2 in these neurons[84,85]. Hence, Cav2.1 directly provides the Ca^{2+} needed for rapid and precise activation of SK2.

Moreover, Cav2.1 and SK2 have been found to co-immunoprecipitate, along with $mGlu_{1\alpha}$, in cerebellar membranes. The receptor $mGlu_{1\alpha}$ is known to modulate Ca^{2+} via lipid rafts upon glutamate activation[86]. Hence, the co-assembly of all three receptors suggest their tight association provide the Ca^{2+} regulation that ensures neuronal membrane excitability in Purkinje cells.

The simultaneous identification of increased burden of rare variants in two direct interactor suggests the importance of thigh regulation of this pathway in the

development of ET symptoms. It is interesting to note that in models of ataxic Cav2.1 mutant mouse, normal Purkinje cell pacemaking activity is lost due to reduced activation of K_{CA}[87,88]. Motor performance of ataxic mice was significantly improved upon perfusion of EBIO, an agonist of SK channels[73]. These results could represent an interesting avenue in the context of new pharmacological treatments for ET symptoms.

Calcium signaling contributes to ET pathogenesis

Accumulating evidences point towards a role for calcium signaling dysregulation in ET. Two different transcriptome analyses on post-mortem ET patients revealed abnormal expression levels for many genes involved in calcium signaling and homeostasis[18,19]. First, an RNA-sequencing study on cerebellar cortex tissue from 33 ET patients and 21 controls revealed that calcium signaling, and synaptic transmission was among the top 5 most dysregulated processes[18].

A second independent study, this time in 16 ET cases and 16 controls in the cerebellar cortex and dente nucleus, in turn reported voltage-gated calcium channel activity pathway to be significantly differentially expressed in the cerebellar cortex but not the dentate nucleus[19]. The same study also performed gene-base genome wide association study (GWGAS) and found "calcium ion-dependent exocytosis" gene cluster to be genetically associated with ET. Interestingly, *CACNA1A* was found to be one of the most dysregulated gene in the cerebellar cortex, having significantly lower levels of expression in ET patients.

Perturbances in CACNA1A activity can have a major impact on calcium signaling. This channel, along with Cav2.2, is the most abundant VGCCs in the mammalian brain[89]. In the cerebellar Purkinje cells, Cav2.1 contributes to up to 90% of high-threshold VGCCs activity, making it one of the major Ca^{2+} input in these cells[49]. By modulating calcium influx, Cav2.1

modulates the binding of ions to exocytosis control proteins needed for neurotransmitter release.

SK2 is a direct target and regulator of calcium signaling. Via the intrinsically bound calmodulin protein, increase in intracellular Ca^{2+} results in channel activation. The resulting K^+ ions release contributes to after hyperpolarization thus regulates firing rate of neurons. Thus, SK2 effectively forms calcium mediated feedback loop with calcium channels, by exerting a repolarizing conductance in response to increased intracellular Ca^{2+}.

No specific role for VPS13D has been established in calcium signaling. However, it is known that VPS13D is positioned at the membrane contact site, the location where organelles come in contact to exchange small molecules and is involved in protein mediated lipid transfer. Three main functions have been linked with the membrane contact site: lipid synthesis and lipid transfer, mitochondrial and endosomal fission as well as calcium homeostasis[90].

Recent research has also shown that, like vesicle trafficking, lipid transfer can be regulated by Ca^{2+} intracellular messenger via extended synaptotagmins[91]. Additionally, VPS13D has been shown to specifically bind to mitochondria and peroxisome via the GTPase Miro[36]. This protein contains two Ca^{2+} binding domain and has been mostly involved in mitochondrial mobility in mammalian cell[92-94]. Based on these finding, it is possible that, VPS13D function is modulated by Ca^{2+} via its organelle membrane anchor Miro.

<u>Calcium channels perturbances in the olivary hypothesis of ET</u>

A role for VGCCs has previously been suggested in ET, however evidences pointed towards the low threshold calcium channel $Cav_{3.1}$. First, it was suggested that low threshold calcium current could be relevant in ET pathology due to its role in the rhythmic

firing of the inferior olive cells[95]; synchronized activities of the inferior olive have been associated with motor coordination[96,97]. However, this theory is now disfavored because of the normal appearance of the inferior olive in neuroimaging and post-mortem studies[98].

Studies on the impact of the alkaloid harmaline on the CNS has shown the compound to produce acute postural and kinetic tremor by causing changes in the electrophysiological properties of inferior olive neurons, climbing fibers and deep cerebellar nuclei[99,100]. Recent studies on harmaline induced tremors have pinpoint a role for $Cav_{3.1}$ in these structures[101].

Harmaline induced tremor in mammals is a commonly used animal model for ET[100,102]. It produces an 8-16 Hz action tremor in mice, rats, cats and monkey that is alleviated by propranolol and ethanol, two pharmacological agents widely used to reduce ET symptoms[103]. Upon injection of harmaline, inferior olive neurons exhibit increased rebound of low threshold calcium spikes. Such increased excitability results in the conversion of their normal sub-threshold rhythmic firing to pathological oscillation in olivocerebellar pathway, resulting in muscle synchronized oscillations[100,102,104].

Earlier studies have found the high molecular weight alcohol 1-octanol to alleviate harmaline induced tremors in animal models[103,105]; the same effect was observed in ET patients[106,107]. Octanol acts as an inhibitor of the low threshold calcium channels in the inferior olive in rats[105]. Subsequently, five additional low threshold calcium channels blockers were reported to suppress tremor with various degree of efficacy in harmaline models[108].

Cav3.1 is the most abundant low threshold calcium channels in the inferior olive and is encoded by *CACNA1G*. This gene has been recently suggested as an ET susceptibility gene following the report of three small families carrying a rare missense[4]. However, *Cacna1g*

knockout mice do not exhibit tremor or motor dysfunction[101,109]. Contradicting results on the impact of harmaline in Cav3.1 knockout mice were reported; where one study found no phenotypic difference between wildtype and Cav3.1 KO mice upon administration of harmaline[108], another report found that Cav3.1 KO mice inferior olive failed to produce rhythmic firing, as in seen in wildtype mice[101].

Although most research on the pathological origin of ET in the inferior olive has focused on the role of low threshold calcium channels, other molecular components are at play in the normal synchronized, sub-threshold oscillation. It is thought that three main ion channels are at play: a high voltage calcium channel (Cav2), a calcium activated potassium conductance (SK) and a low threshold calcium channel (Cav3)[95].

When an action potential reaches the neuron dendrites, it activates the high voltage calcium channels. The resulting influx of Ca^{2+} ions will in turn activate the calcium dependent potassium channel, resulting in long-lasting hyperpolarization of the membrane. This consecutively activate the low threshold calcium channel which promotes low-threshold spike and rebound burst firing[95,110]. Thus, disruption of the normal function of one of these ionic channels could be the molecular basis of the olivo-cerebellar circuit model of ET.

The cerebellar origin of ET and disturbances in ataxia associated genes

As second hypothesis gaining traction in the past decade on the disease model of ET points towards a cerebellar origin. Numerous post-mortem and imagining studies have found metabolic and structural changes in the cerebellum of ET patients[111]. Notably, histopathological examination of changes in the Purkinje cell population of the cerebellar cortex include: axon thickening and increased branching, axonal torpedoes increase, dendritic swelling and decrease pruning, cell body mis-localization and abnormal

organization of climbing fibers[111]. Although some studies have reported Purkinje cell death, the neurodegenerative aspect of ET remains controversial[112-116].

Various neuroimaging techniques have been used to unravel changes in ET brains *in vivo*[117-119]. Most studies have reported altered connectivity in the cerebellum and cerebello-thalamico-cortical circuitry. Once again, findings indicating possible neuro-degenerescense are controversial; while some studies reported cerebellar atrophy, others did not find morphological cerebellar changes. Nonetheless, microstructural alterations of cerebellar peduncles and dentate nuclei are more consistently reported. Additionally, functional alterations of the cerebello-motor cortical projections were reported. Hence, numerous lines of evidence indicate that the cerebellum is instrumental within a multiple oscillator tremor network which triggers pathological tremor in ET[117-119].

Since most post-mortem and imagining studies indicate a key role for the cerebellum in ET, it is of interest to mention all three genes associated with increased risk of disease identified in this study are established to cause cerebellar ataxia. Ataxia is a group of disorders characterized by impaired balance, coordination of voluntary movements or lack of muscle control[120]. It is caused by pathological processes affecting the cerebellum and/or its networks[121].

Although the hallmark motor feature of ET is a 4-12 Hz kinetic tremor, additional neurological signs are often observed. Multiple studies have reported gait impairment in ET patient, often in the form of abnormalities in tandem gait, reduced speed, impaired balance and slower walk[122-127]. Other signs cerebellar dysfunction such as intention tremor[128], abnormal timing during finger tapping[129], dysmetria[130] and oculomotor deficits[131] are sometimes observed in ET patients and point to a common pathomechanism.

Mutation in *CACNA1A* were identified as a cause of hereditary ataxia 25 years ago[56]. Loss-of-function variants in this gene causes episodic ataxia type2 (EA2), an autosomal dominant disorder characterized by cerebellar ataxia and migraine-like symptoms, nystagmus and cerebellar atrophy[132]. In addition, polyglutamine expansions in CACNA1A cause spinocerebellar ataxia type6 (SCA6), an adult onset subtype causing ataxia, dysarthria and dysphagia[57]. Various animal model for CACNA1A dysfunction have been developed over the years and the majority display ataxia and other cerebellar signs[133,134].

Variants disrupting *KCNN2* have recently been described as causing autosomal dominant developmental delay with intellectual disability in a cohort of ten unrelated patients[82]. More than half also presented signs of cerebellar ataxia and/or extrapyramidal symptoms. Kcnn2-null mice develop ataxia and /or abnormal gait, as well as whole body tremor[135-137].

VPS13D disruption causes autosomal recessive spinocerebellar ataxia type 4 (SCAR4)[34,35]. A multiplex family with autosomal recessive cerebellar ataxia and extra pyramidal signs was first described with compound heterozygous mutation in the gene[34]. The identification of twelve additional patients expanded the phenotype to hyperkinetic movement disorder with, among other signs, gait instability, caused by spastic ataxia[34,35].

Knowing that all three genes identified in the present study have been described as causing cerebellar degeneration when disrupted, it is reasonable to hypothesize that the increased burden of genetic variant we observed can contribute to the cerebellar defects present in ET patients. Possibly less disruptive than those identified in hereditary ataxia, the variants reported in ET patient can cause slight perturbances in these tightly regulated genes and cause the phenotypic consequences observed in ET.

<u>ET is a polygenic disorder</u>

ET was first thought to be a monogenic disorder following an autosomal dominant mode of inheritance[138], mostly due to its apparent excess prevalence within certain kindreds[139]. Several large families with multiple consecutive generations of affected individuals have been reported[140].

However, recent evidences point towards a complex genetic architecture. Sequencing technologies have confirmed the etiology of the disorder to be highly heterogenous et multigenic; no variant have been identified so far to be present in a significant percentage of afflicted individuals. In addition, the recent analysis of GWAS dataset has highlighted the important contribution of common variants to ET disease risk by estimating the heritability explained by common variant to 75%[22]. Finally, our results provide additional evidence that the average ET case is a product of many susceptibility-increasing genetic variants, each with a low to moderate effect size.

Conclusion and Future Directions

ET is a highly heterogeneous disorder in many ways; symptoms, disease onset, progression and response to medication all vary among patients. The variability in the disease presentation is partly due to the lack of disease-specific biomarker and morphological changes. Hence, the identification of genetic variants and genes associated with the disorder is needed to better understand the molecular mechanisms underlying the disorder and potentially improve diagnosis and treatment.

Despite the high heritability of ET, few variants or genes are confidently and reproducibly implicated with the disease. Decades of research utilizing various approaches like linkage studies, GWAS and whole exome sequencing led to the suggestion of several loci with various levels of confidence. Nonetheless, using a candidate gene approach, we showed that whole-gene collapsing analyses are a promising avenue for the identification of new genes associated with increased risk of ET.

The follow up of this study would be to apply our novel approach at the genome scale. Instead of using targeted sequencing of high priority gene, whole genome DNA sequencing would allow the interrogation of the all genomic regions in a hypothesis-free approach. This could allow the identification of unsuspected genes associations. In addition, the rare variant collapsing method could be expanded to genomic regions other than protein coding genes; Variants could be grouped based on their location in functionally relevant regions or in related molecular pathways.

Finally, the work presented in this study is the first to employ rare genetic variant burden to identify ET susceptibility loci. Major findings include the identification of three novel genes implicated with the disorder. These contributions are instrumental to a better understanding of the molecular changes underlying ET pathogenicity which could in turn potentially help improve ET diagnosis and treatment.

Chapter 8: References

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Chapter 9: Appendices

Appendix 1: Significant contributions by the thesis author to other projects

1. Diez-Fairen, M., **Houle, G.**, Ortega-Cubero, S., Bandres-Ciga, S., Alvarez, I., Carcel, M., Ibañez, L., Fernandez, M.V., Budde, J.P., Trotta, J.R., Tonda, R., Chong, J.X., Bamshad, M.J., Nickerson, D.A., Aguilar, M., Tartari, J.P., Gironell, A., García-Martín, E., Agundez, J.A., Alonso-Navarro, H., Jimenez-Jimenez, F.J., Fernandez, M., Valldeoriola, F., Marti, M.J., Tolosa, E., Coria, F., Pastor, M.A., Vilariño-Güell, C., Rajput, A., Dion, P.A., Cruchaga, C., Rouleau, G.A. & Pastor, P. Exome-wide rare variant analysis in familial essential tremor. *Parkinsonism Relat Disord* **82**, 109-116 (2021).

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Gene-based rare variant association results

Appendix 2: Chapter 2 Supplementary Material

Chapter 2: Whole gene rare variant association study in Essential Tremor

Supplementary Table 1: Single marker association results

Gene	Position	REF	ALT	AA	MAF	$MA_{count\ cases}$	$MA_{count\ controls}$	OR	P	SE
CHCHD2	7:56171984	C	T	p.A79T	8.992e-06	0	1	0.39	0.68	2.31
CHCHD2	7:56172073	G	A	p.A49V	9.21e-05	2	0	5.25	0.38	1.9
CHCHD2	7:56172095	C	A	p.A42S	0	0	1	0.31	0.61	2.31
CHCHD2	7:56172118	G	A	p.P34L	0.0003	1	0	3.5	0.59	2.31
CHCHD2	7:56174067	G	A	p.P14S	0.0002	0	1	0.39	0.68	2.31
CHCHD2	7:56174097	C	T	p.G4R	2.696e-05	1	0	3.05	2.31	0.63
CHCHD2	7:56174102	G	A	p.P2L	0.0022	3	1	2.44	0.41	1.09
DNAJC13	3:132166169	C	T	p.P50L	0	1	0	2.81	0.65	2.31
DNAJC13	3:132166309	G	A	p.A97T	2.038e-05	1	1	1.16	0.92	1.41
DNAJC13	3:132169614	A	G	p.R154G	0	1	0	2.81	0.65	2.31
DNAJC13	3:132172498	C	T	p.P267L	4.5e-05	1	0	3.48	0.59	2.31
DNAJC13	3:132175152	C	G	p.P336A	1.798e-05	1	0	3.48	0.59	2.31
DNAJC13	3:132175595	C	T	p.A423V	0.0002	0	1	0.39	0.68	2.31
DNAJC13	3:132179897	C	G	p.H521D	1.793e-05	2	0	5.25	0.38	1.9
DNAJC13	3:132180027	G	A	p.R564K	1.792e-05	1	0	2.81	0.65	2.31
DNAJC13	3:132182555	A	G	p.I596V	0	0	1	0.31	0.62	2.31
DNAJC13	3:132182654	A	G	p.T629A	0	1	0	2.81	0.65	2.31
DNAJC13	3:132185182	G	A	p.V670I	4.621e-05	2	0	5.25	0.38	1.9
DNAJC13	3:132185195	A	C	p.D674A	0.0004	2	2	1.16	0.88	1.00
DNAJC13	3:132185236	A	G	p.M688V	3.802e-05	0	1	0.39	0.68	2.31
DNAJC13	3:132186033	A	G	p.N695S	9.007e-06	1	2	0.65	0.72	1.19
DNAJC13	3:132186072	C	T	p.A708V	8.061e-05	1	0	2.81	0.65	2.31
DNAJC13	3:132186113	G	T	p.V722L	3.581e-05	1	0	3.4	0.6	2.31
DNAJC13	3:132186121	G	A	p.M724I	0.0005	2	0	5.23	0.38	1.9
DNAJC13	3:132191992	A	G	p.I738V	9.026e-06	1	1	1.04	0.98	1.42
DNAJC13	3:132193825	C	G	p.R781G	0	1	0	3.48	0.59	2.31
DNAJC13	3:132193853	C	A	p.S790Y	0.0073	29	20	1.48	0.19	0.3
DNAJC13	3:132193894	G	A	p.A804T	1.806e-05	1	0	3.48	0.59	2.31
DNAJC13	3:132196684	A	G	p.R833G	2.689e-05	1	0	2.81	0.65	2.31
DNAJC13	3:132196685	G	A	p.R833K	0.0002	1	0	2.81	0.65	2.31
DNAJC13	3:132196715	A	G	p.E843G	8.075e-05	0	2	0.23	0.44	1.9
DNAJC13	3:132196735	T	C	p.S850P	0	0	2	0.23	0.44	1.9
DNAJC13	3:132196839	A	G	p.N855S	9.231e-06	2	0	5.25	0.38	1.9

DNAJC13	3:132196956	C	T	p.T894I	0	1	1	1.16	0.92	1.41
DNAJC13	3:132196983	G	A	p.R903K	0.0036	26	17	1.54	0.17	0.32
DNAJC13	3:132198096	G	C	p.R912T	0	1	0	2.81	0.65	2.31
DNAJC13	3:132199295	G	A	p.R952Q	0.0001	1	0	3.48	0.59	2.31
DNAJC13	3:132202375	C	G	p.L1046V	0.0001	1	0	3.49	0.59	2.31
DNAJC13	3:132203494	C	T	p.T1082I	0.0007	1	1	0.94	0.97	1.42
DNAJC13	3:132207177	G	C	p.K1101N	0	1	0	2.81	0.65	2.31
DNAJC13	3:132207194	A	G	p.Y1107C	1.796e-05	0	1	0.31	0.61	2.31
DNAJC13	3:132207226	C	T	p.R1118C	8.977e-06	0	1	0.39	0.68	2.31
DNAJC13	3:132207272	G	A	p.G1133D	9.897e-05	0	1	0.39	0.68	2.31
DNAJC13	3:132211341	A	G	p.Y1236C	0.0005	1	0	2.81	0.65	2.31
DNAJC13	3:132211361	A	G	p.N1243D	8.958e-06	0	1	0.31	0.61	2.31
DNAJC13	3:132213030	G	A	p.V1290I	0	1	0	3.48	0.59	2.31
DNAJC13	3:132213034	A	G	p.E1291G	0.0055	23	21	1.15	0.65	0.3
DNAJC13	3:132213081	A	T	p.N1307Y	8.971e-06	0	3	0.17	0.3	1.75
DNAJC13	3:132215447	C	T	p.P1366S	0	1	3	0.42	0.43	1.09
DNAJC13	3:132218012	G	A	p.R1400Q	0.0003	1	5	0.29	0.22	1.00
DNAJC13	3:132218591	C	G	p.A1452G	0	1	0	2.81	0.65	2.31
DNAJC13	3:132218650	C	A	p.Q1472K	0	0	1	0.39	0.68	2.31
DNAJC13	3:132219669	T	C	p.V1483A	0	0	1	0.39	0.68	2.31
DNAJC13	3:132221142	C	T	p.R1516C	3.596e-05	2	0	5.25	0.38	1.9
DNAJC13	3:132221143	G	A	p.R1516H	0.0032	14	7	1.89	0.18	0.47
DNAJC13	3:132221145	G	T	p.V1517L	0	0	1	0.39	0.68	2.31
DNAJC13	3:132222197	G	A	p.R1619K	0	0	1	0.31	0.61	2.31
DNAJC13	3:132224222	A	C	p.E1654A	9.024e-05	0	1	0.31	0.61	2.31
DNAJC13	3:132226195	G	T	p.E1705X	0	0	1	0.31	0.61	2.31
DNAJC13	3:132230013	G	C	p.E1740Q	0.0016	3	2	1.56	0.63	0.91
DNAJC13	3:132233444	C	G	p.D1805E	9.034e-06	0	1	0.39	0.68	2.31
DNAJC13	3:132235602	G	A	p.R1872Q	2.698e-05	1	1	1.16	0.92	1.41
DNAJC13	3:132241682	C	T	p.T1895M	0.0026	8	11	0.79	0.62	0.47
DNAJC13	3:132242012	A	C	p.N1952T	9.87e-05	0	1	0.31	0.61	2.31
DNAJC13	3:132242018	A	G	p.Q1954R	8.972e-06	2	3	0.73	0.73	0.91
DNAJC13	3:132242468	C	A	p.Q1991K	0	1	0	2.81	0.65	2.31
DNAJC13	3:132242480	G	C	p.V1995L	0.0012	6	7	0.94	0.91	0.56
DNAJC13	3:132244474	G	C	p.L2027F	1.797e-05	0	2	0.23	0.44	1.9
DNAJC13	3:132244562	G	T	p.A2057S	0.0012	7	5	1.42	0.55	0.59
DNAJC13	3:132245007	C	G	p.S2088C	0	1	0	3.49	0.59	2.31
DNAJC13	3:132247160	T	G	p.L2170W	0.0041	8	5	1.76	0.28	0.53
DNAJC13	3:132249908	A	G	p.I2198V	3.583e-05	0	1	0.31	0.61	2.31

DNAJC13	3:132249932	T	C	p.Y2206H	2.687e-05	2	1	1.94	0.58	1.19
DNAJC13	3:132257069	G	A	p.M2225I	0.0029	9	19	0.51	0.1	0.4
FUS	16:31193983	A	G	p.N63S	0.0002	1	0	3.48	0.59	2.31
FUS	16:31195284	G	C	p.G98A	0	1	0	3.09	0.63	2.31
FUS	16:31195598	G	A	p.S134N	0.0002	6	2	2.42	0.28	0.82
FUS	16:31195678	A	C	p.N161H	8.953e-06	0	2	0.21	0.41	1.9
FUS	16:31196382	C	T	p.R215C	5.702e-05	0	1	0.39	0.68	2.31
FUS	16:31196385	G	C	p.G216R	0	0	1	0.31	0.61	2.31
FUS	16:31196414	CGGCGGCGGT	C	p.226_228del	0.0003	0	1	0.39	0.68	2.31
FUS	16:31196427	G	C	p.G230R	0	0	1	0.31	0.61	2.31
FUS	16:31200536	G	A	p.G308S	0	0	1	0.39	0.68	2.31
FUS	16:31201423	C	T	p.R376W	8.976e-06	1	0	3.48	0.59	2.31
FUS	16:31201719	C	T	p.P430L	0.0001	1	0	2.81	0.65	2.31
FUS	16:31202077	T	G	p.M435R	0	1	0	2.81	0.65	2.31
FUS	16:31202145	C	G	p.P458A	0	1	0	3.57	0.58	2.31
HAPLN4	19:19368787	C	G	p.G350R	8.309e-05	1	0	2.77	0.66	2.31
HAPLN4	19:19368807	C	T	p.R343K	0.0004	1	1	0.92	0.95	1.42
HAPLN4	19:19368927	A	T	p.V303E	0	1	0	2.81	0.65	2.31
HAPLN4	19:19368990	C	T	p.R282Q	0.0003	1	1	1.16	0.92	1.41
HAPLN4	19:19369368	G	A	p.R261C	9.184e-06	0	1	0.39	0.68	2.31
HAPLN4	19:19369406	C	T	p.G248D	4.603e-05	0	2	0.19	0.38	1.9
HAPLN4	19:19369631	C	T	p.R173Q	0	0	1	0.4	0.69	2.31
HAPLN4	19:19369645	G	T	p.H168Q	1.141e-05	0	1	0.4	0.69	2.31
HAPLN4	19:19371642	A	C	p.M155R	8.988e-06	1	0	2.82	0.65	2.31
HAPLN4	19:19371851	C	A	p.K85N	9.226e-06	1	0	3.5	0.59	2.31
HAPLN4	19:19372299	G	A	p.P26L	0	1	0	2.81	0.65	2.31
HTRA2	2:74757335	C	T	p.P68S	0	0	1	0.39	0.68	2.31
HTRA2	2:74757348	T	C	p.L72P	0.0031	14	18	0.77	0.48	0.37
HTRA2	2:74757393	C	T	p.T87I	9.722e-06	1	0	2.81	0.65	2.31
HTRA2	2:74757411	G	C	p.R93P	0	0	1	0.39	0.68	2.31
HTRA2	2:74757443	C	G	p.R104G	1.025e-05	1	3	0.47	0.49	1.09
HTRA2	2:74757485	CTGT	C	p.118_119del	6.708e-05	1	0	2.98	0.64	2.31
HTRA2	2:74757567	G	A	p.S145N	6.613e-05	1	1	1.04	0.98	1.42
HTRA2	2:74757875	G	T	p.G213V	0	1	0	2.81	0.65	2.31
HTRA2	2:74759004	G	A	p.G356E	8.952e-06	1	0	3.49	0.59	2.31
HTRA2	2:74759825	G	A	p.G399S	0.0041	6	20	0.28	0.01	0.49
HTRA2	2:74760053	C	T	p.Q440X	0	0	1	0.39	0.68	2.31
KCNS2	8:99440262	A	T	p.I19F	0	1	0	3.53	0.58	2.31

KCNS2	8:99440646	T	C	p.S147P	0	0	1	0.31	0.61	2.31
KCNS2	8:99440818	T	C	p.M204T	0.0004	1	4	0.34	0.3	1.03
KCNS2	8:99441012	G	A	p.V269I	8.951e-05	2	2	1.1	0.92	1.00
KCNS2	8:99441057	A	G	p.T284A	0.0018	3	6	0.56	0.35	0.63
KCNS2	8:99441108	C	T	p.R301W	0	1	0	3.48	0.59	2.31
KCNS2	8:99441117	C	T	p.R304C	8.953e-06	0	1	0.39	0.68	2.31
KCNS2	8:99441370	A	C	p.K388T	0	1	0	3.49	0.59	2.31
KCNS2	8:99441490	G	A	p.R428H	4.512e-05	0	1	0.31	0.61	2.31
KCNS2	8:99441510	G	A	p.G435R	0.0001	1	0	2.81	0.65	2.31
KCNS2	8:99441528	T	C	p.S441P	0	1	0	3.06	2.31	0.63
KCNS2	8:99441637	G	A	p.R477H	9.084e-06	0	1	0.39	0.68	2.31
LRRK2	12:40626083	G	T	p.W82L	0	1	0	2.81	0.65	2.31
LRRK2	12:40626147	G	C	p.Q103H	3.581e-05	0	1	0.31	0.61	2.31
LRRK2	12:40629436	T	C	p.L119P	0.0022	14	18	0.89	0.74	0.34
LRRK2	12:40629462	A	G	p.S128G	1.792e-05	0	2	0.23	0.44	1.9
LRRK2	12:40634345	C	T	p.A211V	0.0003	0	1	0.39	0.68	2.31
LRRK2	12:40634396	G	C	p.C228S	0.0002	0	1	0.31	0.61	2.31
LRRK2	12:40643645	C	G	p.L286V	0.0002	1	1	1.16	0.92	1.41
LRRK2	12:40645075	G	A	p.E334K	0.0012	6	1	4.62	0.11	0.97
LRRK2	12:40645106	A	G	p.E344G	0	1	0	2.81	0.65	2.31
LRRK2	12:40645148	C	T	p.T358M	0.0002	1	0	2.81	0.65	2.31
LRRK2	12:40645300	A	G	p.M379V	1.815e-05	2	0	5.81	0.35	1.9
LRRK2	12:40646786	C	T	p.A419V	0.0002	1	1	1.04	0.98	1.42
LRRK2	12:40653293	T	G	p.L477R	0	0	1	0.39	0.68	2.31
LRRK2	12:40668679	C	G	p.L609V	0.0001	0	1	0.31	0.61	2.31
LRRK2	12:40668728	A	G	p.H625R	8.972e-06	1	1	1.04	0.98	1.42
LRRK2	12:40671773	A	G	p.I675M	0	0	1	0.31	0.61	2.31
LRRK2	12:40671794	T	A	p.N682K	8.96e-05	2	0	5.25	0.38	1.9
LRRK2	12:40671894	T	C	p.F691S	3.591e-05	1	0	2.75	0.66	2.31
LRRK2	12:40677734	C	T	p.R767C	0.0001	1	0	2.81	0.65	2.31
LRRK2	12:40677735	G	A	p.R767H	0	0	1	0.31	0.61	2.31
LRRK2	12:40677813	G	T	p.R793M	0.0007	2	5	0.49	0.39	0.82
LRRK2	12:40677861	G	A	p.C809Y	1.792e-05	0	3	0.17	0.3	1.75
LRRK2	12:40681165	C	T	p.T838I	0	1	0	2.82	0.65	2.31
LRRK2	12:40681195	T	C	p.M848T	1.8e-05	0	1	0.39	0.68	2.31
LRRK2	12:40681246	C	T	p.S865F	9.89e-05	3	2	1.43	0.69	0.91
LRRK2	12:40681264	A	G	p.K871R	0	1	0	2.81	0.65	2.31
LRRK2	12:40687358	T	C	p.S901P	9.21e-06	0	2	0.21	0.41	1.9
LRRK2	12:40687359	C	T	p.S901L	9.2e-06	0	2	0.21	0.41	1.9

LRRK2	12:40687426	G	C	p.Q923H	0.0001	1	4	0.34	0.29	1.03
LRRK2	12:40688656	T	G	p.F940V	9.023e-06	1	0	3.48	0.59	2.31
LRRK2	12:40689235	C	T	p.S962L	0	0	2	0.19	0.38	1.9
LRRK2	12:40689265	A	G	p.D972G	9.896e-05	2	0	5.25	0.38	1.9
LRRK2	12:40689268	G	A	p.S973N	8.999e-06	0	1	0.39	0.68	2.31
LRRK2	12:40689369	A	G	p.S1007G	1.798e-05	1	0	2.81	0.65	2.31
LRRK2	12:40689437	G	T	p.Q1029H	9.021e-06	1	0	2.81	0.65	2.31
LRRK2	12:40692063	C	T	p.H1039Y	0	1	0	3.48	0.59	2.31
LRRK2	12:40692281	G	T	p.Q1111H	1.793e-05	1	2	0.21	0.41	1.9
LRRK2	12:40692979	A	T	p.N1139I	0	0	1	0.3	0.6	2.31
LRRK2	12:40692994	T	A	p.L1144Q	0	1	0	2.81	0.65	2.31
LRRK2	12:40696668	A	G	p.I1192V	9.034e-06	1	0	2.81	0.65	2.31
LRRK2	12:40696683	C	T	p.H1197Y		0	1	0.31	0.61	2.31
LRRK2	12:40697770	G	A	p.S1204N	8.971e-06	1	0	2.81	0.65	2.31
LRRK2	12:40697802	G	A	p.A1215T	0.0002	1	2	0.6	0.67	1.19
LRRK2	12:40697842	G	C	p.S1228T	0.0003	2	1	1.81	0.62	1.19
LRRK2	12:40699590	C	T	p.P1261S	0	1	0	3.38	0.6	2.31
LRRK2	12:40699593	C	G	p.P1262A	4.485e-05	1	0	2.76	0.66	2.31
LRRK2	12:40699655	A	T	p.R1282S	2.693e-05	0	1	0.31	0.61	2.31
LRRK2	12:40699748	T	A	p.C1313X	4.518e-05	1	0	2.81	0.65	2.31
LRRK2	12:40702283	G	A	p.R1325Q	0.0003	1	0	3.48	0.59	2.31
LRRK2	12:40702366	C	A	p.Q1353K	8.992e-05	1	6	0.25	0.15	0.97
LRRK2	12:40702369	T	G	p.L1354V	0	0	1	0.31	0.61	2.31
LRRK2	12:40702403	A	G	p.Q1365R	8.987e-06	1	0	2.81	0.65	2.31
LRRK2	12:40702453	G	C	p.D1382H	0	0	1	0.31	0.61	2.31
LRRK2	12:40702947	C	T	p.T1410M	0.0001	1	0	3.48	0.59	2.31
LRRK2	12:40704242	T	G	p.S1443A	8.976e-06	1	0	2.81	0.65	2.31
LRRK2	12:40704317	A	G	p.K1468E	0	1	0	3.48	0.59	2.31
LRRK2	12:40707778	G	A	p.R1514Q	0.0069	21	14	1.54	0.21	0.35
LRRK2	12:40707808	C	T	p.P1524L	9.006e-06	0	1	0.31	0.61	2.31
LRRK2	12:40707852	A	G	p.K1539E	1.798e-05	0	1	0.39	0.68	2.31
LRRK2	12:40707876	G	A	p.V1547I	0	1	0	2.81	0.65	2.31
LRRK2	12:40707950	C	G	p.H1571Q	8.985e-06	0	1	0.31	0.61	2.31
LRRK2	12:40709017	T	G	p.V1581G	2.696e-05	0	1	0.39	0.68	2.31
LRRK2	12:40713797	C	T	p.T1612I	0	0	1	0.31	0.61	2.31
LRRK2	12:40713845	G	A	p.R1628H	8.083e-05	3	1	2.58	0.39	1.09
LRRK2	12:40713932	A	C	p.Q1657P	0	0	1	0.39	0.68	2.31
LRRK2	12:40714924	A	G	p.M1702V	0	1	0	3.48	0.59	2.31
LRRK2	12:40715848	C	A	p.R1728S	0	1	0	2.81	0.65	2.31

LRRK2	12:40715858	G	A	p.R1731K	0	0	1	0.39	0.68	2.31
LRRK2	12:40715870	G	A	p.R1735Q	0	1	0	3.48	0.59	2.31
LRRK2	12:40716270	C	A	p.Q1823K	6.283e-05	1	0	2.81	0.65	2.31
LRRK2	12:40716992	G	A	p.R1847K	2.689e-05	0	2	0.23	0.44	1.9
LRRK2	12:40717006	A	G	p.I1852V	7.169e-05	0	1	0.39	0.68	2.31
LRRK2	12:40717058	T	C	p.M1869T	0.0004	2	0	5.25	0.38	1.9
LRRK2	12:40717067	A	G	p.N1872S	8.961e-06	1	0	3.48	0.59	2.31
LRRK2	12:40728838	C	T	p.R1943W	0	1	0	2.73	0.66	2.31
LRRK2	12:40728866	A	G	p.K1952R	8.954e-06	1	0	3.36	0.6	2.31
LRRK2	12:40728880	C	T	p.R1957C	2.686e-05	1	0	2.73	0.66	2.31
LRRK2	12:40728934	G	A	p.A1975T	0	1	0	2.81	0.65	2.31
LRRK2	12:40734202	G	A	p.G2019S	0.0002	1	1	0.94	0.96	1.42
LRRK2	12:40740628	ACTACT	A	p.L2062fs	0.0004	1	0	3.48	0.59	2.31
LRRK2	12:40740660	G	C	p.R2072T	0	1	0	2.81	0.65	2.31
LRRK2	12:40745387	G	A	p.R2143H	0.0001	1	0	2.81	0.65	2.31
LRRK2	12:40745476	C	G	p.H2173D	8.952e-06	0	1	0.31	0.61	2.31
LRRK2	12:40745525	A	G	p.Y2189C	0.0002	1	0	2.8	0.66	2.31
LRRK2	12:40753117	T	C	p.L2300S	0	1	0	3.48	0.59	2.31
LRRK2	12:40753146	A	G	p.T2310A	7.172e-05	0	1	0.31	0.61	2.31
LRRK2	12:40753147	C	T	p.T2310M	0.0001	0	1	0.31	0.61	2.31
LRRK2	12:40753180	C	T	p.T2321I	0	0	1	0.31	0.61	2.31
LRRK2	12:40757321	A	C	p.K2382N	0	1	0	2.81	0.65	2.31
LRRK2	12:40757322	C	A	p.L2383I	0	1	0	2.81	0.65	2.31
LRRK2	12:40757328	G	A	p.G2385R	3.593e-05	1	1	1.16	0.92	1.41
LRRK2	12:40757343	G	A	p.V2390M	3.599e-05	0	1	0.39	0.68	2.31
LRRK2	12:40758647	G	GGT	p.E2395fs	4.552e-05	1	0	2.81	0.65	2.31
LRRK2	12:40758844	C	T	p.A2461V	5.393e-05	1	3	0.44	0.46	1.09
LRRK2	12:40761453	A	C	p.Q2490H	0	0	1	0.31	0.61	2.31
NOS3	7:150690922	C	A	p.P11T	0.0007	0	2	0.21	0.41	1.9
NOS3	7:150690926	G	A	p.G12E	9.606e-06	1	0	3.49	0.59	2.31
NOS3	7:150690939	C	CCTGGGG	p.G16delinsGLG	5.779e-05	0	1	0.39	0.68	2.31
NOS3	7:150691010	G	A	p.R40Q	6.483e-05	1	0	3.49	0.59	2.31
NOS3	7:150692291	C	A	p.S53R	0	1	1	0.95	0.97	1.42
NOS3	7:150692333	G	C	p.K67N	0.0001	1	0	3.49	0.59	2.31
NOS3	7:150693897	G	A	p.E156K	0.0004	1	1	1.07	0.96	1.42
NOS3	7:150695463	T	A	p.C201S	0	0	1	0.39	0.68	2.31
NOS3	7:150695500	A	G	p.N213S	1.803e-05	0	2	0.21	0.41	1.9
NOS3	7:150695652	C	T	p.R234C	6.531e-05	0	1	0.39	0.68	2.31
NOS3	7:150695715	C	T	p.R255W	2.866e-05	0	1	0.39	0.68	2.31

NOS3	7:150696076	G	A	p.D287N	0.0020	4	2	1.97	0.43	0.85
NOS3	7:150696369	G	A	p.A350T	0.0001	0	1	0.31	0.61	2.31
NOS3	7:150697626	C	T	p.S391L	2.693e-05	0	1	0.39	0.68	2.31
NOS3	7:150698332	C	T	p.T416I	0	1	0	2.82	0.65	2.31
NOS3	7:150698349	G	A	p.A422T	0.0051	6	11	0.59	0.29	0.51
NOS3	7:150698352	G	A	p.A423T	0.0012	1	1	0.94	0.96	1.42
NOS3	7:150698396	CA	C	p.R438fs	4.485e-05	1	0	2.81	0.65	2.31
NOS3	7:150698401	G	C	p.G439A	0	0	1	0.39	0.68	2.31
NOS3	7:150698430	G	A	p.V449M	4.502e-05	2	1	1.56	0.71	1.19
NOS3	7:150698511	C	T	p.Q476X	0	0	1	0.41	0.7	2.31
NOS3	7:150699009	G	A	p.A535T	8.988e-06	0	1	0.31	0.61	2.31
NOS3	7:150699334	C	T	p.T565M	3.978e-05	1	0	3.48	0.59	2.31
NOS3	7:150699375	C	A	p.P579T	0	1	0	2.81	0.65	2.31
NOS3	7:150704198	T	C	p.V649A	1.801e-05	1	0	3.51	0.59	2.31
NOS3	7:150704215	C	T	p.R655W	0	0	1	0.39	0.68	2.31
NOS3	7:150706321	C	T	p.R764C	0.0007	1	0	2.93	0.64	2.31
NOS3	7:150707299	G	A	p.S870N	8.976e-06	1	0	3.53	0.58	2.31
NOS3	7:150707344	G	T	p.R885M	0.0003	2	1	1.81	0.62	1.19
NOS3	7:150707844	C	T	p.H949Y	9.042e-06	0	1	0.32	0.62	2.31
NOS3	7:150707889	A	C	p.T964P	8.359e-05	1	1	1.04	0.98	1.42
NOS3	7:150707890	C	G	p.T964S	1.857e-05	0	1	0.39	0.68	2.31
NOS3	7:150709453	G	A	p.R1000Q	0.0008	2	3	0.79	0.8	0.91
NOS3	7:150709464	G	A	p.D1004N	0	1	0	3.49	0.59	2.31
NOS3	7:150710430	T	G	p.V1073G	0	1	0	2.89	0.65	2.31
NOS3	7:150711241	A	T	p.D1199V	1.823e-05	0	1	0.39	0.68	2.31
RIT2	18:40613828	C	T	p.M36I	7.333e-05	1	0	3.07	2.31	0.63
RIT2	18:40695436	C	T	p.G17R	2.694e-05	1	1	1.16	0.92	1.41
RIT2	18:40695438	C	G	p.G16A	2.694e-05	0	1	0.31	0.61	2.31
SCN11A	3:38888195	T	C	p.H1789R	8.99e-06	1	0	3.56	0.58	2.31
SCN11A	3:38888202	T	C	p.K1787E	0.0001	1	0	1.76	1.15	0.62
SCN11A	3:38888346	T	C	p.I1739V	4.477e-05	1	0	2.81	0.65	2.31
SCN11A	3:38888354	C	A	p.G1736V	0.0022	8	1	6.03	0.05	0.93
SCN11A	3:38888412	G	A	p.L1717F	0	1	0	3.49	0.59	2.31
SCN11A	3:38888417	T	C	p.N1715S	8.969e-06	0	1	0.39	0.68	2.31
SCN11A	3:38888445	T	C	p.M1706V	8.985e-06	0	1	0.39	0.68	2.31
SCN11A	3:38888451	T	C	p.K1704E	8.987e-06	0	2	0.19	0.38	1.9
SCN11A	3:38888588	C	T	p.R1658H	0.0003	1	1	1.04	0.98	1.42
SCN11A	3:38888682	A	G	p.Y1627H	0.0002	1	0	3.48	0.59	2.31
SCN11A	3:38889062	A	C	p.I1500S	0	0	1	0.39	0.68	2.31

SCN11A	3:38889191	A	G	p.F1457S	0	0	1	0.39	0.68	2.31
SCN11A	3:38891998	C	A	p.C1434F	9.005e-06	0	1	0.31	0.61	2.31
SCN11A	3:38892017	C	T	p.G1428S	0.0004	0	6	0.09	0.13	1.59
SCN11A	3:38892089	C	T	p.V1404I	0	0	1	0.39	0.68	2.31
SCN11A	3:38892224	C	T	p.V1359M	3.648e-05	1	0	3.61	0.58	2.31
SCN11A	3:38904747	T	C	p.Y1332C	0	0	1	0.39	0.68	2.31
SCN11A	3:38908898	C	T	p.V1289I	8.972e-06	1	0	3.49	0.59	2.31
SCN11A	3:38912234	G	T	p.A1254E	1.804e-05	2	2	1.1	0.92	1.00
SCN11A	3:38912986	T	C	p.K1237E	0	1	0	3.48	0.59	2.31
SCN11A	3:38913057	G	A	p.S1213L	0	0	1	0.39	0.68	2.31
SCN11A	3:38913189	T	C	p.N1169S	0.0003	2	0	5.25	0.38	1.9
SCN11A	3:38913694	T	C	p.E1162G	5.406e-05	0	3	0.17	0.3	1.75
SCN11A	3:38913706	A	G	p.L1158P	0.0006	4	1	3.08	0.28	1.03
SCN11A	3:38913709	G	A	p.A1157V	0	0	1	0.31	0.61	2.31
SCN11A	3:38913733	A	G	p.L1149P	8.986e-06	0	1	0.31	0.61	2.31
SCN11A	3:38924797	C	T	p.C1049Y	8.965e-06	0	1	0.39	0.68	2.31
SCN11A	3:38924811	G	C	p.N1044K	0.0003	0	2	0.23	0.44	1.9
SCN11A	3:38926829	C	T	p.W1005X	4.481e-05	1	2	0.65	0.72	1.19
SCN11A	3:38926850	T	C	p.D998G	3.585e-05	1	0	3.48	0.59	2.31
SCN11A	3:38927663	T	C	p.M968V	0	1	0	2.81	0.65	2.31
SCN11A	3:38927714	G	A	p.Q951X	0	0	1	0.39	0.68	2.31
SCN11A	3:38936100	G	A	p.A920V	6.273e-05	0	1	0.39	0.68	2.31
SCN11A	3:38936154	G	T	p.S902Y	3.582e-05	0	3	0.14	0.27	1.75
SCN11A	3:38936328	CA	C	p.C844fs	0	1	0	2.81	0.65	2.31
SCN11A	3:38936329	A	G	p.C844R	4.482e-05	1	0	2.81	0.65	2.31
SCN11A	3:38936440	T	C	p.I807V	1.826e-05	1	0	3.48	0.59	2.31
SCN11A	3:38938421	A	G	p.M773T	0	1	0	3.48	0.59	2.31
SCN11A	3:38938428	C	T	p.E771K	8.961e-06	0	2	0.23	0.44	1.9
SCN11A	3:38938446	G	A	p.L765F	0	0	1	0.39	0.68	2.31
SCN11A	3:38938451	C	T	p.R763H	9.859e-05	2	0	5.25	0.38	1.9
SCN11A	3:38938517	G	A	p.P741L	2.691e-05	0	1	0.31	0.61	2.31
SCN11A	3:38938526	G	A	p.P738L	8.973e-06	1	0	3.48	0.59	2.31
SCN11A	3:38938556	T	A	p.N728I	0	0	1	0.39	0.68	2.31
SCN11A	3:38938644	C	T	p.G699R	0.0003	1	0	2.81	0.65	2.31
SCN11A	3:38938646	A	G	p.V698A	4.501e-05	0	1	0.39	0.68	2.31
SCN11A	3:38941498	A	C	p.F637V	0	0	1	0.39	0.68	2.31
SCN11A	3:38945454	C	T	p.A582T	0.0003	2	2	1.16	0.88	1.00
SCN11A	3:38945468	G	A	p.P577L	0.0003	1	0	2.81	0.65	2.31
SCN11A	3:38945492	A	G	p.V569A	0	0	1	0.31	0.61	2.31

SCN11A	3:38945584	T	G	p.K538N	0	0	1	0.31	0.61	2.31
SCN11A	3:38946710	C	G	p.V526L	8.976e-06	1	0	3.49	0.59	2.31
SCN11A	3:38946726	C	A	p.Q520H	2.693e-05	1	0	3.49	0.59	2.31
SCN11A	3:38946761	G	A	p.H509Y	0	1	0	3.48	0.59	2.31
SCN11A	3:38950530	C	A	p.K419N	0.0012	0	1	0.31	0.61	2.31
SCN11A	3:38950538	T	C	p.K417E	0	0	1	0.39	0.68	2.31
SCN11A	3:38950645	A	G	p.I381T	1.794e-05	0	1	0.31	0.61	2.31
SCN11A	3:38950678	C	T	p.R370H	9.881e-05	1	0	3.49	0.59	2.31
SCN11A	3:38951602	C	T	p.M352I	0	0	1	0.31	0.61	2.31
SCN11A	3:38962716	C	T	p.R248H	9.01e-06	1	1	1.04	0.98	1.42
SCN11A	3:38962732	C	T	p.V243M	9.03e-06	1	0	3.48	0.59	2.31
SCN11A	3:38966906	G	A	p.R238C	0.0004	2	3	0.8	0.8	0.91
SCN11A	3:38966945	G	A	p.R225C	8.958e-06	1	0	2.81	0.65	2.31
SCN11A	3:38966993	A	C	p.S209A	0	0	1	0.39	0.68	2.31
SCN11A	3:38988299	T	C	p.I123V	0	0	3	0.14	0.27	1.75
SCN11A	3:38991682	C	A	p.A58S	0	1	0	2.81	0.65	2.31
SCN11A	3:38991735	G	A	p.S40F	0	1	0	2.81	0.65	2.31
SCN11A	3:38991759	G	A	p.A32V	0.0011	4	3	1.41	0.66	0.76
SCN4A	17:62018216	G	C	p.P1809R	8.993e-06	1	0	3.48	0.59	2.31
SCN4A	17:62018217	G	C	p.P1809A	8.992e-06	1	0	3.48	0.59	2.31
SCN4A	17:62018218	C	T	p.M1808I	1.799e-05	0	1	0.31	0.61	2.31
SCN4A	17:62018232	T	TG	p.T1804fs	8.976e-06	1	0	3.48	0.59	2.31
SCN4A	17:62018241	C	T	p.A1801T	9.875e-05	1	1	0.94	0.96	1.42
SCN4A	17:62018308	C	A	p.K1778N	0	1	0	2.81	0.65	2.31
SCN4A	17:62018310	T	C	p.K1778E	0	1	0	2.81	0.65	2.31
SCN4A	17:62018349	C	G	p.A1765P	0	1	0	3.49	0.59	2.31
SCN4A	17:62018427	G	T	p.R1739S	0	1	0	3.54	0.58	2.31
SCN4A	17:62018726	C	T	p.R1639H	7.162e-05	1	0	2.81	0.65	2.31
SCN4A	17:62018931	G	A	p.P1571S	8.057e-05	0	1	0.31	0.61	2.31
SCN4A	17:62018952	C	T	p.V1564I	0.0016	6	10	0.68	0.45	0.52
SCN4A	17:62019033	C	T	p.G1537S	0.0001	0	2	0.23	0.44	1.9
SCN4A	17:62019088	G	T	p.N1518K	0	0	1	0.31	0.61	2.31
SCN4A	17:62019099	C	T	p.D1515N	0	0	1	0.39	0.68	2.31
SCN4A	17:62019157	G	C	p.I1495M	0	1	0	3.48	0.59	2.31
SCN4A	17:62019213	T	C	p.M1477V	2.685e-05	0	1	0.31	0.61	2.31
SCN4A	17:62019270	C	A	p.V1458F	8.986e-06	0	1	0.39	0.68	2.31
SCN4A	17:62020204	C	T	p.V1424I	0	0	1	0.39	0.68	2.31
SCN4A	17:62020294	T	C	p.I1394V	1.79e-05	2	0	5.82	0.35	1.9
SCN4A	17:62021187	C	G	p.M1312I	0	1	0	3.48	0.59	2.31

SCN4A	17:62022812	C	T	p.E1210K	8.952e-06	1	1	1.16	0.92	1.41
SCN4A	17:62025395	C	T	p.R1058Q	1.798e-05	0	1	0.31	0.61	2.31
SCN4A	17:62026852	C	T	p.V964I	0.0004	1	1	1.19	0.9	1.41
SCN4A	17:62028825	C	T	p.E938K	8.994e-06	1	0	3.48	0.59	2.31
SCN4A	17:62028915	C	T	p.E908K	5.371e-05	0	1	0.31	0.61	2.31
SCN4A	17:62028992	G	T	p.P882Q	7.31e-05	0	2	0.21	0.41	1.9
SCN4A	17:62029014	G	A	p.P875S	0.0020	2	4	0.58	0.52	0.85
SCN4A	17:62029074	T	C	p.M855V	0.0003	1	1	0.94	0.96	1.42
SCN4A	17:62034552	G	T	p.F782L	0	0	1	0.39	0.68	2.31
SCN4A	17:62034557	C	T	p.V781I	0.0077	23	28	0.86	0.6	0.28
SCN4A	17:62034710	C	T	p.V730M	0.0011	3	5	0.64	0.55	0.73
SCN4A	17:62034745	C	T	p.G718D	0	1	0	2.81	0.65	2.31
SCN4A	17:62034875	G	A	p.R675W	9.258e-06	1	0	2.81	0.65	2.31
SCN4A	17:62038645	T	C	p.T585A	8.954e-06	1	0	2.91	0.64	2.31
SCN4A	17:62041062	C	T	p.G526R	1.865e-05	0	1	0.31	0.61	2.31
SCN4A	17:62041176	C	T	p.A488T	0.0009	4	7	0.65	0.5	0.62
SCN4A	17:62041926	C	T	p.E452K	0.0001	0	3	0.15	0.28	1.75
SCN4A	17:62043565	C	T	p.R380Q	9.739e-05	1	1	1.04	0.98	1.42
SCN4A	17:62045467	A	G	p.W318R	0.0013	3	5	0.61	0.49	0.73
SCN4A	17:62045556	T	C	p.N288S	0	0	1	0.39	0.68	2.31
SCN4A	17:62045574	C	T	p.R282H	2.688e-05	0	1	0.31	0.61	2.31
SCN4A	17:62045589	C	T	p.R277K	8.964e-06	1	0	2.81	0.65	2.31
SCN4A	17:62045607	A	G	p.L271P	0	0	1	0.31	0.61	2.31
SCN4A	17:62048546	G	A	p.L227F	2.843e-05	0	1	0.39	0.68	2.31
SCN4A	17:62049125	G	A	p.R190W	1.831e-05	1	0	3.72	0.57	2.31
SCN4A	17:62049574	A	G	p.M135T	1.797e-05	1	1	1.16	0.92	1.41
SCN4A	17:62049575	T	G	p.M135L	0.0004	1	2	0.65	0.72	1.19
SCN4A	17:62049718	A	G	p.I129T	8.068e-05	0	2	0.21	0.41	1.9
SCN4A	17:62049739	C	T	p.R122H	7.166e-05	0	1	0.39	0.68	2.31
SCN4A	17:62049740	G	A	p.R122C	8.062e-05	0	1	0.39	0.68	2.31
SCN4A	17:62049749	C	T	p.V119I	0.0041	5	10	0.56	0.28	0.55
SCN4A	17:62049945	T	C	p.Y86C	3.159e-05	1	0	3.48	0.59	2.31
SCN4A	17:62049961	C	G	p.E81Q	1.007e-05	0	1	0.39	0.68	2.31
SCN4A	17:62049973	C	T	p.G77S	2.922e-05	1	0	3.48	0.59	2.31
SCN4A	17:62050036	T	C	p.S56G	0	1	0	3.51	0.59	2.31
SCN4A	17:62050057	C	T	p.E49K	0.0002	1	0	2.81	0.65	2.31
SCN4A	17:62050110	C	A	p.R31L	1.799e-05	0	1	0.39	0.68	2.31
SCN4A	17:62050120	T	G	p.I28L	4.515e-05	0	7	0.07	0.1	1.56
SCN4A	17:62050149	C	T	p.R18H	0	1	1	1.04	0.98	1.42

SCN4A	17:62050197	G	A	p.A2V	3.699e-05	1	0	2.81	0.65	2.31
SCN4A	17:62050198	C	T	p.A2T	0	1	0	3.49	0.59	2.31
SORT1	1:109856899	T	C	p.H685R	8.969e-06	1	0	2.82	0.65	2.31
SORT1	1:109857337	C	T	p.V635I	4.477e-05	1	0	3.48	0.59	2.31
SORT1	1:109857384	G	T	p.S619Y	5.375e-05	0	1	0.39	0.69	2.31
SORT1	1:109859485	C	T	p.S609N	4.527e-05	4	1	3.08	0.28	1.03
SORT1	1:109859575	C	T	p.R579Q	0.0002	0	2	0.23	0.44	1.9
SORT1	1:109865641	C	T	p.R509H	8.955e-06	0	2	0.19	0.38	1.9
SORT1	1:109867536	T	C	p.I470V	0	0	1	0.31	0.61	2.31
SORT1	1:109867662	C	T	p.D428N	0	1	0	2.81	0.65	2.31
SORT1	1:109867673	G	A	p.T424M	0	1	0	2.81	0.65	2.31
SORT1	1:109869627	T	C	p.I407V	2.703e-05	1	0	3.48	0.59	2.31
SORT1	1:109878893	T	C	p.E310G	0.0015	2	1	1.73	0.65	1.19
SORT1	1:109878920	G	A	p.T301M	0.0001	1	2	0.65	0.72	1.19
SORT1	1:109883399	A	T	p.F267Y	2.686e-05	0	1	0.31	0.61	2.31
SORT1	1:109883405	G	A	p.T265M	4.477e-05	1	0	2.81	0.65	2.31
SORT1	1:109884746	T	G	p.Q196P	0	1	0	2.82	0.65	2.31
SORT1	1:109884777	T	C	p.T186A	0	1	0	2.81	0.65	2.31
SORT1	1:109888432	T	C	p.K165E	0.0028	9	11	0.89	0.79	0.45
SORT1	1:109897147	A	C	p.L48V	5.402e-05	0	1	0.39	0.68	2.31
USP46	4:53494162	C	T	p.V89I	0	1	0	2.81	0.65	2.31
USP46	4:53494215	G	A	p.T71M	9.861e-05	0	2	0.21	0.41	1.9
USP46	4:53494270	G	A	p.R53W	1.794e-05	0	1	0.39	0.68	2.31
USP46	4:53494278	C	T	p.R50H	3.588e-05	1	0	2.81	0.65	2.31

Notes: REF: reference allele; ATL: alternative (i.e., effect) allele; AA: amino acid change; MAF: minor allele frequency; $MA_{count\ cases}$: minor allele count in essential tremor cases; $MA_{count\ controls}$: minor allele count in controls; OR: odds ratio; P: P-value; SE: standard error

Appendix 3: Chapter 3 Supplementary Material

Chapter 3: Absence of evidence for a role for rare genetic variants in *STK32B, PPARGC1A*, *CTNNA3* in the risk for Essential Tremor in a cohort of Canadians of European decent.

1. SUBJECTS AND METHOD

1.1 Patient recruitment

Approval was obtained from all responsible ethics committees: MUHC (McGill University Health Centre) (Roubank protocol no. 14051), CRCHUM (Centre de Recherche du Centre Hospitalier de l'Université de Montréal) (project no. ND043076). Each participant gave a written consent to allow blood withdrawal for DNA extraction and the subsequent molecular studies. All diagnoses were reviewed by a senior neurologist. Exclusion criteria included: (i) an identified cause of exaggerated physiological tremor, (ii) other neurological deficits (parkinsonisms, polyneuritis, other) were present, and (iii) an orthostatic tremor or (iv) a psychogenic-like tremor.

1.2 Multiplex families with autosomal dominant ET

In an effort to identify potentially deleterious variants in the genes reported by Müller et al., we first mined whole exome sequencing (WES) and whole genome sequencing (WGS) data from 14 multiplex families with autosomal dominant ET. 12 of those families identified their ethnicity as French-Canadian origins, one as American and one as Chilean. For each of these families, data on ≥2 ET cases were available. In total, data from 54 ET cases were analyzed.

1.3 Cases-controls cohort

We selected 269 unrelated ET patients for case-control analyses. In order to focus our analysis on the hereditary form of ET (as opposed to senile tremor) we selected patients with a reported disease onset at <50 years of age. These comprise enrolled individuals from both French-Canadian and western Canada descents (cohort details, see table 1). We also selected 287 ethnically matched unrelated individuals for which no neurological disorders is known. Since ET is an adult onset disease, we only included controls >70 years old, in order to control the possibility of including individuals who will develop the disorder later in life. The patient DNA samples included in this study were also part of the original GWAS study[1].

1.4 High-throughput sequencing

Genomic DNA was extracted from peripheral blood samples following to the manufacturer's protocol (Puregene; Gentra Systems Inc). High-throughput sequencing was carried out on 2 to 5 affected members per family; a key criterion was to have the greatest genetic distance possible between them so they would share a minimal number of variants. Exome library preparation was carried out using Human All Exon V4 (SureSelect, Agilent) or SeqCap EZ Exome Library v3 kit (Nimblegen, Roche) with 1 µg of genomic DNA and were sequenced on the Illumina HiSeq2500 with a paired-end 100 bp length configuration. Genome libraries were prepared using TruSeq Nano DNA library kit with 100 ng of genomic DNA and sequenced on the Illumina HiSeq X with a paired-end 150 bp length configuration. Reads were mapped against the Human Reference Genome

hg19 using the Burrows-Wheeler Aligner (BWA) (v0.7.5)[2]. Single nucleotide variants and indels were called using HaplotypeCaller from Genome Analysis Toolkit (GATK) (v2.6)[3] and annotated using ANNOVAR[4]. All identified variants had a minimum genotype quality (GQ) >60 and a minimum read depth (DP) >15. Coverage of the target sequence was assessed using GATK's DepthOfCoverage[3].

1.5 Genes Screening

Targeted massive parallel sequencing was performed in order to capture the entire coding regions of *STK32B* (NM_018401), *PPARGC1A* (NM_013261) and *CTNNA3* (NM_013266) respectively. Molecular inversion probes (MIPs) were design using scripts developed in Dr. Jay Shendure laboratory[5] (http://krishna.gs.washington.edu/mip_pipeline). A total of 274 MIPs spanning all the coding exons of the previously mentioned genes were synthesized by Integrated DNA Technologies (IDT) and hydrated in TE buffer with a final concentration of 100 uM. MIPs captures were performed as described previously[5,6] with the use of ~800 MIPs copies per genome (0.044 fmol) for 100 ng of DNA. Sequencing was carried out using the Illumina HiSeq 2000 platform from "McGill University and Génome Québec Innovation Centre".

Eight samples (4 cases and 4 controls) failed to meet our threshold for minimum coverage (>80% of bases covered ≥15×) and were removed prior to analysis. MIP data were analyzed with an in-house analysis pipeline. Briefly, MIP extension and ligation arms were removed and reads were trimmed using Trimmomatic[7]. Subsequently, reads were mapped against the Human Reference Genome v37 using the Burrows-Wheeler Aligner

(BWA) (v0.7.5)[2]. Input files were prepared using Samtool Mpileup (v.0.1.18)[8] using only alignments with mapping quality >10 and base quality >30. Single nucleotide variants and indels were called using VarScan (v2.3.9)[9] default parameters and annotated using ANNOVAR[4].

From the identified variants, using VCFtools (v.0.1.15)[10], only those with a genotype quality (MQ) higher than 30 and coverage of more than 15X were considered. Moreover, we excluded variants with <90% call rate across samples. Using PLINK[11], we extracted the data to create input files for SKAT[12] and tested the variants for deviation from Hardy–Weinberg equilibrium (HWE). No deviations from HWE were observed in controls or cases.

1.6 Statistical analyses

The PLINK[11] v1.90 whole genome analysis tool set was used for the case–control association study of the thirty four variants that passed our QC steps. Genotype and allele frequencies of the three genes between patients with ET and healthy controls were ascertained using Fisher exact test. Multiple testing was corrected using the Bonferroni correction considering 34 tests. Additionally, a gene-based, case-control analysis was performed using an optimal sequence kernel association tests (SKAT-O)[12,13] to detect a potential imbalance of deleterious variants associated with increased risk of ET in our genes of interest.

Supplementary table 1. List of variants extracted from the case-control cohort

Chromosomal position	Gene	Nucleotide change	Amino acid change	Type of mutation	MAF in ExAC	Number of occurrences in 265 cases	Number of occurrences in 284 controls
10:67680275	*CTNNA3*	c.2501G>A	p.R834Q	M	8.238e-06	1	0
10:67829100	*CTNNA3*	c.2125T>G	p.C709G	M	8.24e-06	0	1
10:67862920	*CTNNA3*	c.1972G>A	p.D658N	M	0	0	1
10:67862992	*CTNNA3*	c.1900G>A	p.E634K	M	0.0005	2	0
10:68040240	*CTNNA3*	c.1872C>A	p.V624V	S	0.0380	6	10
10:68040289	*CTNNA3*	c.1823A>T	p.D608V	M	5.784e-05	0	1
10:68138987	*CTNNA3*	c.1655C>T	p.T552M	M	0.0077	1	1
10:68139039	*CTNNA3*	c.1603C>T	p.R535C	M	0.0118	5	7
10:68526127	*CTNNA3*	c.1176G>A	p.T392T	S	2.474e-05	1	0
10:68940123	*CTNNA3*	c.999C>A	p.N333K	M	4.948e-05	1	0
10:68979585	*CTNNA3*	c.623G>A	p.R208Q	M	8.286e-06	1	0
10:69281639	*CTNNA3*	c.540G>A	p.E180E	S	4.945e-05	1	0
10:69407239	*CTNNA3*	c.33C>T	p.I11I	S	0.0017	3	1
4:5333091	*STK32B*	c.405G>A	p.E135E	S	0.0455	9	13
4:5448429	*STK32B*	c.592A>G	p.R198G	M	0.1814	59	63
4:5458607	*STK32B*	c.741_743del	p.247_248del	NFD	3.841e-05	0	1
4:5461848	*STK32B*	c.802G>A	p.E268K	M	0.0003	1	0
4:5500714	*STK32B*	c.1149C>G	p.T383T	S	0.0004	1	0
4:23803861	*PPARGC1A*	c.2127T>C	p.N709N	S	0.0002	0	1
4:23814707	*PPARGC1A*	c.1835C>T	p.T612M	M	0.0828	17	33
4:23815399	*PPARGC1A*	c.1707A>G	p.S569S	S	2.477e-05	1	0
4:23815506	*PPARGC1A*	c.1600T>C	p.F534L	M	9.895e-05	0	1

4:23815522	*PPARGC1A*	c.1584G>A	p.T528T	S	0.3720	177	181
4:23815681	*PPARGC1A*	c.1425C>T	p.D475D	S	0.0486	8	6
4:23815793	*PPARGC1A*	c.1313T>C	p.L438S	M	0.0026	1	0
4:23815878	*PPARGC1A*	c.1228C>A	p.L410I	M	0.0020	2	0
4:23815924	*PPARGC1A*	c.1182A>G	p.T394T	S	0.8365	255	272
4:23816064	*PPARGC1A*	c.1042G>A	p.G348R	M	2.472e-05	1	0
4:23830051	*PPARGC1A*	c.729C>A	p.H243Q	M	6.59e-05	0	1
4:23833261	*PPARGC1A*	c.348C>T	p.D116D	S	8.236e-05	0	1
4:23833277	*PPARGC1A*	c.332C>T	p.A111V	M	4.118e-05	1	0
4:23833287	*PPARGC1A*	c.322T>A	p.S108T	M	0	0	1
4:23833312	*PPARGC1A*	c.297T>C	p.L99L	S	0.0001	0	1
4:23886388	*PPARGC1A*	c.221C>T	p.S74L	M	0.0010	0	2

Notes: MAF :Minor Allele Frequency, S :Synonymous, M :Missense, NFD :Non-Frameshifting Deletion

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Appendix 4: Chapter 4 Supplementary Material

Chapter 4: Genetic contribution of dystonia related genes in an essential tremor cohort.

Supplementary Table 1: Average percentage of coding sequence covered at minimum of 10X per sample

Gene	Isoform	Bases covered at 10X
ADCY5	NM_183357	80.5
ANO3	NM_031418	99.8
ATP1A3	NM_001256213	81.4
GCH1	NM_000161	96.8
GNAL	NM_182978	94.1
GNAO1	NM_138736	98.0
KCTD17	NM_024681	69.2
KMT2B	NM_014727	74.2
PRKRA	NM_003690	99.8
SGCE	NM_001099400	86.5
SPR	NM_003124	94.4
TBC1D24	NM_001199107	88.9
TH	NM_199292	78.3
THAP1	NM_018105	99.8
TOR1A	NM_000113	85.9
VPS13D	NM_018156	98.7

Supplementary Table 2: VPS13D variants found in our combined cohorts

Gene	Position	NA	AA	Frequency	$MA_{Count\ Cases}$	$MA_{Count\ Controls}$
VPS13D	1:12294325	c.T2G	p.M1R		1	0
VPS13D	1:12294342	c.G19A	p.A7T		1	0
VPS13D	1:12304323	c.C196T	p.L66F	2.7e-05	0	1
VPS13D	1:12304411	c.A284T	p.Q95L		0	1
VPS13D	1:12304452	c.C325T	p.R109C	8.96e-06	0	1
VPS13D	1:12309291	c.A459C	p.Q153H	0.0017	17	9
VPS13D	1:12309326	c.A494G	p.N165S	6.273e-05	1	2
VPS13D	1:12309344	c.C512G	p.A171G	6.273e-05	1	1
VPS13D	1:12309350	c.G518A	p.G173D	0.0003	1	0
VPS13D	1:12309363	c.G531C	p.K177N		0	1
VPS13D	1:12313805	c.A591C	p.Q197H	8.961e-06	1	0
VPS13D	1:12313827	c.A613G	p.I205V	0.0034	11	11
VPS13D	1:12313828	c.T614C	p.I205T	0.0001	2	0
VPS13D	1:12316393	c.G673A	p.A225T	0.0005	6	4
VPS13D	1:12316549	c.A829G	p.K277E		1	0
VPS13D	1:12318153	c.A1103T	p.D368V	1.793e-05	0	1
VPS13D	1:12320753	c.G1114A	p.E372K	3.584e-05	1	1
VPS13D	1:12320804	c.C1165T	p.R389C	1.794e-05	2	0
VPS13D	1:12321021	c.A1229T	p.Q410L	0.0003	3	1
VPS13D	1:12321059	c.C1267T	p.P423S	0.0002	0	1
VPS13D	1:12321994	c.C1451T	p.S484L		1	0
VPS13D	1:12322081	c.A1538G	p.K513R	0.0006	3	7
VPS13D	1:12327012	c.C1669G	p.L557V		0	1
VPS13D	1:12327948	c.C1772G	p.T591R		0	1
VPS13D	1:12328771	c.C1810A	p.P604T		1	0
VPS13D	1:12328799	c.A1838G	p.Y613C		0	1
VPS13D	1:12328811	c.C1850T	p.P617L	0.0003	1	0
VPS13D	1:12328823	c.A1862T	p.H621L		0	1
VPS13D	1:12328834	c.C1873T	p.R625W		0	1
VPS13D	1:12328835	c.G1874A	p.R625Q	3.587e-05	0	1
VPS13D	1:12333081	c.G2125C	p.V709L		0	1
VPS13D	1:12333084	c.C2128T	p.R710W	0.0002	1	0
VPS13D	1:12333156	c.G2200A	p.V734M	1.791e-05	0	1
VPS13D	1:12333163	c.T2207C	p.L736P	8.958e-06	0	1
VPS13D	1:12336093	c.G2448T	p.R816S	8.977e-06	0	1
VPS13D	1:12336098	c.C2453T	p.S818L	8.08e-05	1	1

VPS13D	1:12336223	c.C2578T	p.R860C	8.968e-06	2	0
VPS13D	1:12336287	c.A2642C	p.D881A	8.976e-06	1	0
VPS13D	1:12336377	c.C2732T	p.T911I	8.98e-06	1	0
VPS13D	1:12336481	c.C2836T	p.R946C	0.0003	1	1
VPS13D	1:12336683	c.G3038A	p.G1013D		1	0
VPS13D	1:12336806	c.A3161C	p.D1054A	0.0003	1	0
VPS13D	1:12336817	c.C3172G	p.L1058V	4.481e-05	1	1
VPS13D	1:12336823	c.G3178A	p.D1060N	0.0006	1	2
VPS13D	1:12336873	c.A3228C	p.Q1076H		0	1
VPS13D	1:12336923	c.C3278T	p.S1093L	2.692e-05	1	0
VPS13D	1:12337562	c.C3917G	p.S1306C	5.396e-05	1	0
VPS13D	1:12337564	c.A3919C	p.M1307L	8.992e-06	0	1
VPS13D	1:12337610	c.C3965T	p.A1322V		1	0
VPS13D	1:12337667	c.C4022T	p.S1341L	0.0051	11	14
VPS13D	1:12337694	c.G4049A	p.R1350Q	0.0002	2	0
VPS13D	1:12337754	c.T4109C	p.L1370S	5.378e-05	1	0
VPS13D	1:12337807	c.A4162G	p.I1388V	8.955e-06	0	1
VPS13D	1:12337814	c.G4169A	p.R1390Q	1.791e-05	2	0
VPS13D	1:12342856	c.C4697T	p.T1566I		0	1
VPS13D	1:12343031	c.A4872G	p.I1624M		0	1
VPS13D	1:12343282	c.A5123C	p.Q1708P		0	1
VPS13D	1:12343326	c.C5167T	p.R1723W	8.962e-06	1	0
VPS13D	1:12343428	c.G5269A	p.D1757N	0.0002	0	1
VPS13D	1:12343452	c.T5293C	p.S1765P	8.961e-06	1	0
VPS13D	1:12343476	c.A5317T	p.I1773L	5.378e-05	3	1
VPS13D	1:12343493	c.T5334A	p.S1778R	0.0025	10	3
VPS13D	1:12343540	c.A5381G	p.K1794R		1	0
VPS13D	1:12343573	c.G5414A	p.R1805Q	8.962e-06	1	0
VPS13D	1:12348340	c.T5695G	p.S1899A	2.686e-05	1	0
VPS13D	1:12353621	c.A5893T	p.I1965F		1	0
VPS13D	1:12353678	c.G5950A	p.A1984T		0	1
VPS13D	1:12364596	c.C6250A	p.P2084T	8.971e-06	1	1
VPS13D	1:12364649	c.T6303G	p.H2101Q	0.0006	1	3
VPS13D	1:12364656	c.G6310A	p.G2104R	0.0005	1	0
VPS13D	1:12364666	c.C6320T	p.T2107M	3.587e-05	0	2
VPS13D	1:12364687	c.G6341A	p.S2114N	8.98e-06	1	0
VPS13D	1:12364696	c.G6350A	p.S2117N	3.594e-05	1	0
VPS13D	1:12364744	c.C6398T	p.P2133L	3.627e-05	2	0
VPS13D	1:12364752	c.A6406G	p.T2136A	0.0003	0	1

VPS13D	1:12368514	c.G6466A	p.V2156I	8.961e-06	1	0
VPS13D	1:12371633	c.C6773A	p.P2258H	1.793e-05	0	1
VPS13D	1:12371635	c.A6775G	p.I2259V	8.963e-06	1	0
VPS13D	1:12371895	c.C6848T	p.T2283I	8.958e-06	1	0
VPS13D	1:12371963	c.G6916C	p.G2306R		1	0
VPS13D	1:12374238	c.G7002T	p.L2334F	8.963e-06	0	2
VPS13D	1:12374320	c.A7084G	p.I2362V		1	0
VPS13D	1:12378213	c.T7233G	p.H2411Q	7.166e-05	1	0
VPS13D	1:12378223	c.C7243T	p.H2415Y	0.0031	2	4
VPS13D	1:12378247	c.C7267G	p.Q2423E		1	0
VPS13D	1:12378274	c.C7294T	p.R2432C	0.0032	11	7
VPS13D	1:12378337	c.C7357T	p.R2453W	0.0001	1	0
VPS13D	1:12379644	c.G7505A	p.R2502Q	1.79e-05	1	0
VPS13D	1:12381884	c.G7567A	p.G2523R		1	0
VPS13D	1:12382769	c.A7881T	p.R2627S	8.968e-06	0	1
VPS13D	1:12383810	c.T7963A	p.C2655S		1	0
VPS13D	1:12387780	c.G8066A	p.S2689N	3.581e-05	2	0
VPS13D	1:12387797	c.G8083T	p.A2695S	0.0004	2	3
VPS13D	1:12393304	c.C8309T	p.P2770L	6.284e-05	0	3
VPS13D	1:12395791	c.A8458G	p.M2820V	6.276e-05	1	1
VPS13D	1:12395830	c.G8497C	p.A2833P		1	0
VPS13D	1:12401857	c.C8572T	p.R2858C	0.0027	5	3
VPS13D	1:12414272	c.G9598A	p.E3200K	5.382e-05	1	0
VPS13D	1:12416519	c.T9861G	p.H3287Q		1	1
VPS13D	1:12416557	c.A9899G	p.Y3300C	9.076e-06	0	1
VPS13D	1:12422813	c.T10104A	p.D3368E		1	0
VPS13D	1:12423273	c.A10343G	p.N3448S	0.0003	1	2
VPS13D	1:12423276	c.A10346T	p.K3449M	0.0034	6	3
VPS13D	1:12428644	c.A10495G	p.K3499E	1.804e-05	1	0
VPS13D	1:12429576	c.G10552A	p.V3518M		1	0
VPS13D	1:12429585	c.A10561G	p.M3521V	0.0001	1	0
VPS13D	1:12433908	c.A10837G	p.K3613E		1	0
VPS13D	1:12438483	c.A10844C	p.E3615A		1	0
VPS13D	1:12439550	c.C11015T	p.P3672L	1.795e-05	1	0
VPS13D	1:12439634	c.A11099G	p.H3700R	8.96e-06	1	0
VPS13D	1:12443036	c.C11117T	p.A3706V	0.0002	1	0
VPS13D	1:12445338	c.G11315A	p.R3772Q		0	1
VPS13D	1:12445407	c.A11384T	p.N3795I	0.0006	3	1
VPS13D	1:12446358	c.A11524C	p.S3842R		0	1

VPS13D	1:12460308	c.A11630G	p.E3877G	3.581e-05	0	2
VPS13D	1:12460343	c.G11665A	p.A3889T	3.581e-05	1	0
VPS13D	1:12475185	c.G12001A	p.A4001T	8.958e-06	1	0
VPS13D	1:12475249	c.A12065G	p.N4022S	0.0002	1	0
VPS13D	1:12475255	c.T12071G	p.I4024S	0.0001	1	0
VPS13D	1:12475267	c.T12083G	p.F4028C		1	0
VPS13D	1:12476735	c.G12113A	p.R4038Q	8.963e-06	0	1
VPS13D	1:12476827	c.G12205T	p.V4069F	8.962e-06	0	1
VPS13D	1:12520355	c.T12491C	p.L4164P		0	2
VPS13D	1:12520436	c.C12572T	p.T4191I	0.0014	5	7
VPS13D	1:12557561	c.G12595A	p.A4199T		1	0
VPS13D	1:12557660	c.C12694G	p.L4232V		1	1
VPS13D	1:12566914	c.G12727A	p.A4243T		0	1
VPS13D	1:12566939	c.G12752A	p.C4251Y		0	1
VPS13D	1:12567112	c.G12925C	p.V4309L	0.0001	1	0
VPS13D	1:12567127	c.C12940G	p.P4314A	4.485e-05	2	1
VPS13D	1:12567133	c.C12946T	p.H4316Y	2.693e-05	0	1

Notes: NA: nucleic acid change; AA: amino acid change; MAF: minor allele frequency; $MA_{count\ cases}$: minor allele count in essential tremor cases; $MA_{count\ controls}$: minor allele count in controls

Supplementary Table 3: Variants found in genes causing isolated dystonia

Gene	Position	NA	AA	Frequency	$MA_{Count\ Cases}$	$MA_{Count\ Controls}$
TOR1A	9:132576288	c.C962T	p.T321M	1.79e-05	0	1
TOR1A	9:132576340	c.907_909del	p.303_303del	7.161e-05	1	0
TOR1A	9:132576387	c.G863A	p.R288Q	0.0002	1	0
TOR1A	9:132580832	c.G715A	p.A239T	8.951e-06	1	0
TOR1A	9:132581156	c.C488T	p.A163V	0.0003	1	0
TOR1A	9:132584961	c.A343G	p.I115V	1.79e-05	1	0
TOR1A	9:132586241	c.C124T	p.L42F	1.976e-05	0	1
THAP1	8:42693173	c.G574A	p.D192N	8.96e-06	1	0
THAP1	8:42693310	c.G437A	p.R146Q		1	0
THAP1	8:42693352	c.T395C	p.F132S	8.952e-06	0	1
ANO3	11:26463467	c.A49G	p.M17V	3.635e-05	0	1
ANO3	11:26463582	c.C164T	p.S55F	0.0081	18	11
ANO3	11:26538392	c.G610A	p.D204N		1	0
ANO3	11:26547193	c.A704G	p.Y235C	0.0005	1	0
ANO3	11:26547204	c.G715A	p.G239R	0.0004	1	0
ANO3	11:26547211	c.G722T	p.S241I		0	1
ANO3	11:26552755	c.742delC	p.Q248fs		0	1
ANO3	11:26556057	c.A924G	p.I308M	1.797e-05	0	1
ANO3	11:26563513	c.A1052G	p.Q351R		0	1
ANO3	11:26563563	c.C1102T	p.R368C		0	1
ANO3	11:26581288	c.G1409C	p.G470A		0	1
ANO3	11:26620466	c.C1592T	p.T531M		0	1
ANO3	11:26620488	c.G1614T	p.Q538H		0	1
ANO3	11:26621140	c.G1715A	p.R572H		0	1
ANO3	11:26663480	c.C2179T	p.R727W	1.793e-05	0	1
GNAL	18:11689600	c.G38A	p.G13E		0	1
GNAL	18:11689891	c.G329A	p.R110H	7.53e-05	2	0
GNAL	18:11881088	c.G1331A	p.R444H		1	0
KMT2B	19:36210429	c.T422G	p.L141R		0	1
KMT2B	19:36210847	c.G598A	p.A200T	8.103e-05	0	1
KMT2B	19:36211135	c.G886A	p.G296S	0.0002	1	0
KMT2B	19:36211211	c.T962C	p.L321S		0	1
KMT2B	19:36211354	c.1106_1108del	p.369_370del	0.0002	1	0
KMT2B	19:36211418	c.T1169C	p.M390T	0.0027	2	3
KMT2B	19:36211720	c.G1471A	p.G491S	0.0001	1	0
KMT2B	19:36212510	c.C2261T	p.P754L	8.279e-05	1	0

KMT2B	19:36213292	c.C2489A	p.A830D	0.0007	2	5
KMT2B	19:36213300	c.G2497A	p.V833I	0.0007	1	1
KMT2B	19:36213348	c.G2545A	p.V849M		2	0
KMT2B	19:36214671	c.G3097A	p.D1033N		1	0
KMT2B	19:36214866	c.G3292A	p.G1098R	1.923e-05	0	1
KMT2B	19:36214884	c.C3310T	p.R1104W		1	0
KMT2B	19:36214891	c.G3317A	p.R1106Q	0.0008	0	1
KMT2B	19:36215556	c.C3353T	p.P1118L	1.017e-05	1	0
KMT2B	19:36217156	c.G3905A	p.R1302H	1.792e-05	0	1
KMT2B	19:36218426	c.G4205A	p.R1402Q	9.529e-06	1	0
KMT2B	19:36218440	c.G4219A	p.G1407R	0.0004	3	2
KMT2B	19:36218608	c.G4312C	p.D1438H	9.075e-06	1	0
KMT2B	19:36218660	c.G4364A	p.R1455H	8.991e-06	0	1
KMT2B	19:36218851	c.C4462T	p.R1488W	5.378e-05	1	0
KMT2B	19:36218852	c.G4463A	p.R1488Q	1.083e-05	1	0
KMT2B	19:36219898	c.A4700G	p.K1567R		0	1
KMT2B	19:36221258	c.G5092A	p.D1698N	4.48e-05	0	1

Notes: NA: nucleic acid change; AA: amino acid change; MAF: minor allele frequency; $MA_{\text{count cases}}$: minor allele count in essential tremor cases; $MA_{\text{count controls}}$: minor allele count in controls

Supplementary Table 4: Variants found in genes causing combined dystonia with parkinsonism

Gene	Position	NA	AA	MAF	$MA_{Count\ Cases}$	$MA_{Count\ Controls}$
GCH1	14:55310817	c.A671G	p.K224R	0.0005	0	1
GCH1	14:55312502	c.G610A	p.V204I	0.0002	0	1
ATP1A3	19:42471461	c.A2986G	p.S996G		0	1
ATP1A3	19:42482395	c.A1747G	p.N583D	0.0001	1	0
ATP1A3	19:42482863	c.T1558G	p.S520A		0	1
ATP1A3	19:42482868	c.G1553A	p.R518H	0.0002	1	1
ATP1A3	19:42485704	c.C1420T	p.R474C	0.0009	0	1
ATP1A3	19:42489303	c.C793T	p.R265C	1.815e-05	0	1
ATP1A3	19:42492666	c.C88T	p.R30C	0.0001	0	1
ATP1A3	19:42492696	c.G58A	p.D20N		0	1
TH	11:2185488	c.C1562T	p.A521V		1	0
TH	11:2186541	c.G1348A	p.V450M	0.0003	1	4
TH	11:2186559	c.G1330A	p.D444N	2.704e-05	1	0
TH	11:2187252	c.G1177A	p.E393K	1.519e-05	0	1
TH	11:2187275	c.C1154T	p.A385V	4.85e-05	0	1
TH	11:2188228	c.G823T	p.A275S	5.816e-05	0	1
TH	11:2189335	c.G655C	p.D219H	2.692e-05	0	1
TH	11:2189733	c.567delC	p.P189fs		1	0
TH	11:2189756	c.T545C	p.V182A	0.0001	0	2
TH	11:2189810	c.G491A	p.R164H	0.0001	0	1
TH	11:2189817	c.T484G	p.F162V	0.0003	0	1
TH	11:2189835	c.G466T	p.G156C		0	1
TH	11:2189864	c.C437T	p.T146I	3.755e-05	0	1
TH	11:2192973	c.G44A	p.R15H	9.097e-06	0	1
TH	11:2193001	c.G16A	p.A6T	0.0060	3	6
SPR	2:73114673	c.G112A	p.V38I	0.0069	6	4
SPR	2:73114754	c.C193T	p.R65W	0.0001	0	1
SPR	2:73114755	c.G194A	p.R65Q		1	0
SPR	2:73115586	c.A448G	p.R150G	6.274e-05	1	0
SPR	2:73118523	c.G643T	p.D215Y	8.953e-06	1	0

Notes: NA: nucleic acid change; AA: amino acid change; MAF: minor allele frequency; $MA_{count\ cases}$: minor allele count in essential tremor cases; $MA_{count\ controls}$: minor allele count in controls

Supplementary Table 5: Variants found in genes causing combined dystonia with myoclonus

Gene	Position	NA	AA	MAF	$MA_{Count\ Cases}$	$MA_{Count\ Controls}$
SGCE	7:94228183	c.C1130G	p.T377R	8.967e-05	1	0
SGCE	7:94229967	c.G1028A	p.R343Q	8.15e-05	0	1
SGCE	7:94232735	c.C692T	p.P231L	2.698e-05	0	1
SGCE	7:94252690	c.G410A	p.R137H	1.809e-05	0	2
SGCE	7:94285352	c.G59A	p.R20Q	6.308e-05	0	1
SGCE	7:94285390	c.G21A	p.W7X	0.0001	1	0
KCTD17	22:37453444	c.C418T	p.P140S	1.793e-05	1	0
KCTD17	22:37455399	c.C554T	p.A185V	8.96e-06	0	1

Notes: NA: nucleic acid change; AA: amino acid change; MAF: minor allele frequency; $MA_{count\ cases}$: minor allele count in essential tremor cases; $MA_{count\ controls}$: minor allele count in controls

Supplementary Table 6: Variants found in genes causing combined dystonia with other hyperkinetic movement disorders

Gene	Position	NA	AA	MAF	$MA_{Count\ Cases}$	$MA_{Count\ Controls}$
GNAO1	16:56374792	c.A770G	p.N257S	3.581e-05	0	1
GNAO1	16:56377830	c.G1033A	p.A345T	0.0001	1	0
ADCY5	3:123008701	c.T3428C	p.V1143A	8.951e-06	0	1
ADCY5	3:123008791	c.A3338G	p.E1113G	2.687e-05	1	0
ADCY5	3:123010023	c.G3264C	p.E1088D		0	1
ADCY5	3:123010118	c.C3169T	p.R1057W		0	1
ADCY5	3:123016214	c.C2916A	p.N972K	8.952e-06	1	1
ADCY5	3:123019001	c.G2866A	p.D956N	0.0003	1	0
ADCY5	3:123019049	c.G2818A	p.E940K	0.0012	4	3
ADCY5	3:123021981	c.C2645T	p.A882V	0.0004	1	1
ADCY5	3:123022062	c.C2564T	p.T855M	9.052e-05	0	1
ADCY5	3:123036874	c.G2347A	p.V783M	0.0014	0	1
ADCY5	3:123044215	c.A2042G	p.Y681C	1.79e-05	0	1
ADCY5	3:123047641	c.G1655A	p.R552Q		0	1
ADCY5	3:123071389	c.G1174A	p.V392M	0.0003	0	1
ADCY5	3:123166411	c.G982A	p.G328S	7.37e-05	1	1
ADCY5	3:123166416	c.C977T	p.S326F	5.548e-05	1	0
ADCY5	3:123167153	c.237_239del	p.79_80del	0.0002	1	0
ADCY5	3:123167153	c.237_239del	p.79_80del		1	0
TBC1D24	16:2546328	c.G442T	p.E148X	0.0003	0	1
TBC1D24	16:2546591	c.G641A	p.R214H	1.804e-05	1	0
TBC1D24	16:2546790	c.C866T	p.A289V	0.0012	1	3
TBC1D24	16:2547015	c.G871A	p.A291T		1	0
TBC1D24	16:2547020	c.C877T	p.R293C	8.972e-05	1	1
TBC1D24	16:2547026	c.G878A	p.R293H	8.974e-06	0	1
TBC1D24	16:2547027	c.G878A	p.R293H	0.0003	2	0

Notes: NA: nucleic acid change; AA: amino acid change; MAF: minor allele frequency; $MA_{count\ cases}$: minor allele count in essential tremor cases; $MA_{count\ controls}$: minor allele count in controls

Appendix 5: Chapter 5 Supplementary Material

Chapter 5: Rare coding variants in CACNA1A increase risk of Essential Tremor

Supplementary Table 1: Rare variants found in our discovery cohort and single marker association results

Gene	Isoform	Position (hg19)	REF	ALT	AA	MAF	$MA_{Count\ Cases}$	$MA_{Count\ Controls}$	OR	P	SE
ATP1A2	NM_000702	1:160090708	T	A	p.Y9N	0.0031	5	6	0.80	0.72	0.61
ATP1A2	NM_000702	1:160093017	G	T	p.Q64H	0	1	0	2.73	0.66	2.31
ATP1A2	NM_000702	1:160093018	C	T	p.R65W	0.0001	1	1	0.91	0.95	1.42
ATP1A2	NM_000702	1:160093165	G	A	p.G114S	0.0008	1	0	3.26	0.61	2.31
ATP1A2	NM_000702	1:160098841	G	A	p.V430I	9.062e-06	0	1	0.30	0.61	2.31
ATP1A2	NM_000702	1:160099904	G	A	p.E492K	0.0007	1	2	0.58	0.64	1.19
ATP1A2	NM_000702	1:160106751	G	A	p.V924M	5.376e-05	1	1	0.91	0.95	1.42
ATP1A2	NM_000702	1:160109717	C	T	p.L993F	8.956e-06	1	0	3.26	0.61	2.31
SCN1A	NM_006920	2:166847820	C	T	p.V1978M	2.694e-05	0	1	0.30	0.60	2.31
SCN1A	NM_006920	2:166848003	G	C	p.R1917G	0.0016	6	6	0.99	0.99	0.58
SCN1A	NM_006920	2:166866307	T	A	p.E1297D	0.0012	1	1	0.99	1.00	1.42
SCN1A	NM_006920	2:166872146	G	C	p.T1163S	0.0028	1	1	0.91	0.95	1.42
SCN1A	NM_006920	2:166892767	C	T	p.D1063N	8.979e-06	0	1	0.36	0.66	2.31
SCN1A	NM_006920	2:166892889	A	G	p.F1022S	9.289e-06	0	1	0.30	0.61	2.31
SCN1A	NM_006920	2:166894462	C	T	p.A913T	0.0002	1	0	2.73	0.66	2.31
SCN1A	NM_006920	2:166900411	C	T	p.R604H	0.0013	1	1	1.08	0.95	1.42
SCN1A	NM_006920	2:166900483	C	T	p.R580Q	5.383e-05	0	2	0.22	0.42	1.90
SCN1A	NM_006920	2:166901590	C	T	p.R542Q	0.0014	4	2	1.74	0.52	0.85
SCN1A	NM_006920	2:166901633	T	A	p.S528C	8.956e-06	1	0	3.26	0.61	2.31
SCN1A	NM_006920	2:166903291	C	G	p.E456Q	9.002e-06	0	1	0.30	0.60	2.31
SCN1A	NM_006920	2:166903378	C	T	p.E427K	0	1	0	3.27	0.61	2.31
SCN1A	NM_006920	2:166903473	G	A	p.A395V	2.698e-05	0	1	0.31	0.61	2.31
SCN1A	NM_006920	2:166908256	C	G	p.D313H	0	0	1	0.30	0.60	2.31
KCNK18	NM_181840	10:118957052	G	A	p.G18E	8.963e-05	1	1	0.99	1.00	1.42
KCNK18	NM_181840	10:118957057	C	G	p.L20V	0.0004	1	0	2.73	0.66	2.31
KCNK18	NM_181840	10:118960769	T	C	p.F108S	8.953e-06	0	1	0.36	0.66	2.31
KCNK18	NM_181840	10:118969058	C	T	p.L135F	6.267e-05	1	0	2.73	0.66	2.31
KCNK18	NM_181840	10:118969064	GCT	G	p.A137fs	0.0008	2	0	4.99	0.40	1.90
KCNK18	NM_181840	10:118969353	C	A	p.A233E	0.0001	1	1	0.91	0.95	1.42
KCNK18	NM_181840	10:118969616	C	A	p.L321I	0	1	0	2.73	0.66	2.31
KCNK18	NM_181840	10:118969688	A	T	p.I345F	0	1	0	2.73	0.66	2.31

CSNK1D	NM_001893	17:80202704	G	C	p.P401A	0.0013	1	2	0.63	0.70	1.19
CSNK1D	NM_001893	17:80207454	C	T	p.A304T	0.0024	0	5	0.09	0.13	1.62
CSNK1D	NM_001893	17:80223660	G	A	p.A30V	0	0	2	0.18	0.37	1.90
CACNA1A	NM_001127221	19:13319731	G	A	p.H2208Y	0	1	0	1.75	0.63	1.16
CACNA1A	NM_001127221	19:13319749	G	A	p.R2202W	5.576e-05	2	1	1.74	0.64	1.19
CACNA1A	NM_001127221	19:13320189	G	A	p.R2156C	0.0001	1	0	2.82	0.65	2.31
CACNA1A	NM_001127221	19:13320208	G	T	p.N2149K	8.712e-05	1	0	2.79	0.66	2.31
CACNA1A	NM_023035	19:13338337	G	A	p.P1849L	9.543e-06	0	1	0.30	0.60	2.31
CACNA1A	NM_001127221	19:13370426	C	G	p.W1448S	0	1	0	2.73	0.66	2.31
CACNA1A	NM_001127221	19:13387921	C	T	p.V1283I	8.962e-06	1	0	2.73	0.66	2.31
CACNA1A	NM_001127221	19:13394203	G	A	p.R1235C	0	1	0	3.25	0.61	2.31
CACNA1A	NM_001127221	19:13395940	C	A	p.D1213Y	9.153e-06	1	0	2.73	0.66	2.31
CACNA1A	NM_001127221	19:13395952	C	T	p.D1209N	2.746e-05	1	0	3.26	0.61	2.31
CACNA1A	NM_001127221	19:13395967	CCTT	C	p.1203_1203del	0.0011	1	0	3.25	0.61	2.31
CACNA1A	NM_001127221	19:13397502	C	T	p.R1124Q	9.345e-05	0	1	0.30	0.61	2.31
CACNA1A	NM_001127221	19:13397643	G	A	p.A1077V	0.0003	0	2	0.21	0.42	1.90
CACNA1A	NM_001127221	19:13409407	C	T	p.E1015K	0.0060	9	11	0.83	0.67	0.45
CACNA1A	NM_001127221	19:13409419	G	C	p.P1011A	0.0002	2	0	4.63	0.42	1.90
CACNA1A	NM_001127221	19:13409710	G	A	p.P914S	4.078e-05	1	0	2.77	0.66	2.31
CACNA1A	NM_001127221	19:13409886	G	A	p.A855V	0	1	0	1.65	0.67	1.16
CACNA1A	NM_001127221	19:13410043	G	T	p.R803S	7.185e-05	1	0	2.74	0.66	2.31
CACNA1A	NM_001127221	19:13410155	C	G	p.Q765H	9.142e-06	1	0	2.73	0.66	2.31
CACNA1A	NM_001127221	19:13428124	C	T	p.A454T	0.0054	4	2	1.75	0.51	0.85
CACNA1A	NM_001127221	19:13470597	C	G	p.E267D	0	0	1	0.30	0.60	2.31

Notes: REF: reference allele; ATL: alternative (i.e., effect) allele; AA: amino acid change; MAF: minor allele frequency; $MA_{\text{count cases}}$: minor allele count in essential tremor cases; $MA_{\text{count controls}}$: minor allele count in controls; OR: odds ratio; P: P-value; SE: standard error

Supplementary Table 2: CACNA1A combined cohort association results

Gene	Isoform	Position (hg19)	REF	ALT	AA	MAF	$MA_{Count\ Cases}$	$MA_{Count\ Controls}$	OR	P	SE
CACNA1A	NM_001127221	19:13319731	G	A	p.H2208Y	0	1	0	1.93	0.57	1.15
CACNA1A	NM_001127221	19:13319749	G	A	p.R2202W	5.576e-05	2	1	1.70	0.66	1.19
CACNA1A	NM_001127221	19:13319784	TC	T	p.D2190fs	0	0	1	0.39	0.68	2.31
CACNA1A	NM_001127221	19:13320189	G	A	p.R2156C	0.0001	1	0	3.54	0.58	2.31
CACNA1A	NM_001127221	19:13320198	G	T	p.H2153N	0	1	0	2.87	0.65	2.31
CACNA1A	NM_001127221	19:13320208	G	T	p.N2149K	8.712e-05	1	0	3.52	0.59	2.31
CACNA1A	NM_001127221	19:13320252	G	A	p.R2135C	9.89e-05	1	0	2.89	0.65	2.31
CACNA1A	NM_001127221	19:13323003	C	T	p.D2074N	0	1	0	3.54	0.58	2.31
CACNA1A	NM_023035	19:13338337	G	A	p.P1849L	9.543e-06	0	1	0.39	0.68	2.31
CACNA1A	NM_001127221	19:13339539	T	C	p.K1868E	0	1	0	2.81	0.65	2.31
CACNA1A	NM_001127221	19:13339598	T	C	p.H1848R	0	1	0	2.82	0.65	2.31
CACNA1A	NM_001127221	19:13368276	T	C	p.Y1494C	0	1	0	3.17	0.62	2.31
CACNA1A	NM_001127221	19:13370426	C	G	p.W1448S	0	2	0	5.81	0.35	1.90
CACNA1A	NM_001127221	19:13387921	C	T	p.V1283I	8.962e-06	1	0	3.49	0.59	2.31
CACNA1A	NM_001127221	19:13394203	G	A	p.R1235C	0	1	0	2.81	0.65	2.31
CACNA1A	NM_001127221	19:13395940	C	A	p.D1213Y	9.153e-06	1	0	3.48	0.59	2.31
CACNA1A	NM_001127221	19:13395952	C	T	p.D1209N	2.746e-05	1	0	2.81	0.65	2.31
CACNA1A	NM_001127221	19:13395967	CCT	C	p.1203_1203del	0.0011	1	1	0.94	0.97	1.42
CACNA1A	NM_001127221	19:13397493	T	C	p.N1127S	2.098e-05	1	0	2.82	0.65	2.31
CACNA1A	NM_001127221	19:13397502	C	T	p.R1124Q	9.345e-05	0	1	0.39	0.68	2.31
CACNA1A	NM_001127221	19:13397643	G	A	p.A1077V	0.0003	1	2	0.69	0.76	1.20
CACNA1A	NM_001127221	19:13397703	C	T	p.R1057H	9.157e-05	1	0	2.81	0.65	2.31
CACNA1A	NM_001127221	19:13397704	G	A	p.R1057C	0	0	3	0.17	0.30	1.75
CACNA1A	NM_001127221	19:13409407	C	T	p.E1015K	0.0060	23	17	1.34	0.37	0.32
CACNA1A	NM_001127221	19:13409419	G	C	p.P1011A	0.0002	4	0	7.89	0.24	1.75
CACNA1A	NM_001127221	19:13409710	G	A	p.P914S	4.078e-05	1	0	3.21	0.61	2.31
CACNA1A	NM_001127221	19:13409886	G	A	p.A855V	0	1	0	1.86	0.59	1.15
CACNA1A	NM_001127221	19:13410043	G	T	p.R803S	7.185e-05	2	0	5.68	0.36	1.90
CACNA1A	NM_001127221	19:13410155	C	G	p.Q765H	9.142e-06	1	0	3.48	0.59	2.31
CACNA1A	NM_001127221	19:13428124	C	T	p.A454T	0.0054	10	6	1.74	0.28	0.52
CACNA1A	NM_001127221	19:13445201	A	C	p.S397A	1.791e-05	0	2	0.19	0.38	1.90
CACNA1A	NM_001127221	19:13470597	C	G	p.E267D	0	0	1	0.39	0.68	2.31
CACNA1A	NM_001127221	19:13565953	C	T	p.D123N	0	1	0	3.48	0.59	2.31

Notes: REF: reference allele; ATL: alternative (i.e., effect) allele; AA: amino acid change; MAF: minor allele frequency; $MA_{count\ cases}$: minor allele count in essential tremor cases; $MA_{count\ controls}$: minor allele count in controls; OR: odds ratio; P: P-value; SE: standard error

Appendix 6: Ethics Approval Forms

neuro
Institut-Hôpital neurologique de Montréal
Montreal Neurological Institute-Hospital

MUHC REB – NEUPSY / CÉR CUSM – NEUPSY
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McGill

Annual renewal submission form - Harmonized

Titre du protocole : **Essential Tremor/Dystonia**
Chercheur principal (au CER Éval) : **Dr. Guy Rouleau**
Submit date: **2021-09-07 11:23**
Project's REB approbation date: **2016-11-22**
Project number(s): **2017-322**
Form status: **Approved**
Submitted by: **Zaharieva, Vessela**
Nagano identifier: **Essential Tremor/Dystonia**
Form: **F9H-83119**

Administration - REB

1. **MUHC REB Panel & Co-chair(s):**
   Neurosciences-Psychiatry (NEUPSY)
   Co-chairs: Judith Marcoux, Franca Cantini

2. **REB Decision:**
   Approved - REB delegated review

3. **Renewal Period Granted:**
   From 2021-11-23 Until 2022-11-22

4. **Date of the REB final decision & signature**
   2021-09-08
   Signature

   

   Sonia Cantini
   MUHC REB Coordinator
   For MUHC Co-chair mentioned above

   2021-09-08 11:06

5. FWA 00000840 - FWA 00004545

6. **Local REB number**
IRB00010120

General information

1. **Indicate the name of the Principal Investigator in our institution (MUHC)**

Roufeau, Guy

From which department is the principal investigator?

Neurology

Required information for renewal

1. **Date when the research project is expected to end at your institution:**

Date unknown

2. **Indicate the current status of the research project at your institution:**

Project is in progress and recruitment is ongoing

3. **Briefly describe in a few lines, the current status of the project:**

The project is ongoing

4. **Please indicate the type of "participants" implicated in your research project**

Human samples

Did you obtain all the data / samples you needed for the realization of your project as described in the protocol?

No

Please specify

The project is a Bank. We wish to collect as many samples as possible.

5. **In terms of what you are responsible to report, over the past year, relative to the situation at the time of the last REB renewal (or initial approval):**

Have there been any unreported changes to the REB affecting the study documents?

No

Were there unanticipated problems, serious adverse reactions, major deviations or other events or information altering the ethical acceptability or balance between risks and benefits of the project that were not reported to the REB?

No

Were there any temporary interruptions to the project?

No

Have the results of the project been submitted for publication, presented or published?

No

Should the REB be notified of a conflict of interest situation (of any kind) affecting one or more members of the research team, that was not reported at the time of the last approval of the project?

No

Has there been an allegation related to a breach in ethical compliance (eg: complaint from a participant, non-compliance with rules relating to ethics or integrity) concerning one or more researchers?

No

Does the sponsor require the submission of minor deviations from the protocol or other report that does not identify any impact on participant safety?

No

Signature

Answer of: Zaharieva, Vessela

1. **I certify that the information provided on this form is correct.**

Vessela Zaharieva
2021-09-07 11:23