Genetic and molecular susceptibility factors for essential tremor

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ABSTRACT

Essential tremor (ET) is one of the most common movement disorders characterized by a bilateral, largely symmetric postural or kinetic tremor. The disorder affects up to 5% of population by the age of 65 and can greatly debilitate quality of life. Previous studies have demonstrated that both genetic and environmental risk factors contribute towards ET susceptibility, with a relatively high contribution from heritable factors. Despite the large genetic influence, there have not been many studies that have identified robust and replicable genetic and molecular risk factors. Several studies have determined that the dysregulation in the cerebellum is associated with ET. Given the lack of replicable risk factors and the association with the cerebellum, we sought to identify novel risk factors through genome-wide and cerebellar transcriptome-wide studies in this body of work. Here, we conducted a genome-wide association study (GWAS) that identified 5 genome-wide significant loci associated with ET. Additionally, we demonstrated ET has a single-nucleotide polymorphism (SNP) heritability of 0.18 and has a strong genetic correlation with Parkinson's disease and depression. We additionally conducted a case-control study investigating the cerebellar transcriptome to identify differentially expressed genes associated with ET. We identified several risk genes and found convergence for calcium- and axon- related pathways. Furthermore, we found that one of the dysregulated genes, *SHF*, was significantly associated with blood pressure medication, which is commonly used to treat ET. Treatment with propranolol, a first-line ET treatment and blood pressure medication, demonstrated that propranolol increased *SHF* levels, reinforcing its importance in ET biology. Overall, the results presented in this thesis represent significant

contributions towards understanding the genetic and molecular underpinnings of ET etiology.

RÉSUME

Le tremblement essentiel (TE) est l'un des plus communs troubles du mouvement. Il est caractérisé par un tremblement postural bilatéral et symétrique des membres supérieurs. Le trouble affecte jusqu'à 5% de la population à l'âge de 65 ans et peut considérablement affaiblir la qualité de vie. Des études antérieures ont démontré que les facteurs de risque génétiques et environnementaux contribuent à la susceptibilité au TE, avec une contribution relativement élevée des facteurs héréditaires. Malgré l'importante influence génétique, peu d'études ont identifié des facteurs de risque génétiques et moléculaires robustes et reproductibles. Plusieurs études ont déterminé que la dérégulation du cervelet est associée à la TE. Compte tenu de l'absence de facteurs de risque reproductibles et de l'association avec le cervelet, nous avons cherché à identifier de nouveaux facteurs de risque par le biais d'études à l'échelle du génome et du transcriptome cérébelleux. Nous avons mené une étude d'association pangénomique (GWAS) qui a identifié 5 loci associés au TE. De plus, nous avons démontré que le TE a une héritabilité de polymorphisme mononucléotidique (SNP) de 0,19 et présente une forte corrélation génétique avec la maladie de Parkinson et la dépression. Nous avons en outre mené une étude cas-témoins sur le transcriptome cérébelleux pour identifier les gènes différentiellement exprimés associés au TE. Nous avons identifié plusieurs facteurs de risques génétiques de même qu'une convergence pour les voies liées au calcium et aux axones. De plus, nous avons découvert que l'un des gènes dérégulés, *SHF*, était significativement associé aux médicaments contre l'hypertension, qui sont couramment utilisés pour traiter la TE. Le traitement au propranolol, un traitement de première intention contre la TE et un médicament contre la pression artérielle, a démontré que le

propranolol augmentait les taux de *SHF*, renforçant son importance dans la biologie du TE. Dans l'ensemble, les résultats présentés dans cette thèse représentent des contributions importantes à la compréhension des fondements génétiques et moléculaires de l'étiologie du TE.

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獻給我的母親陳煥嫦以及我的姐姐廖嘉琪，為了讓我有機會追逐我的夢想，她們為我犧牲良多。

LIST OF ABBREVIATIONS

Aβ	amyloid-β
ANGEL2	*Angel Homolog*
BACE2	Beta-Secretase 2
BUB1	Budding Uninhibited By Benzimidazoles 1 Homolog
CACNA1A	Calcium Voltage-Gated Channel Subunit Alpha1 A
cDNA	Complementary deoxyribonucleic acid
CMC	CommonMind Consortium
CTNNA3	Catenin Alpha 3
DEG	Differentially expressed genes
DRD3	Dopamine Receptor D3
eQTL	Expression quantitative trait locus
ET	Essential tremor
ETM1	Tremor, Familial Essential, 1
ETM2	Tremor, Familial Essential, 2
EMEM	Eagle's Minimum Essential Medium
FOCUS	Fine-mapping of causal gene sets
FUMA	Functional Mapping and Annotation of Genome-Wide Association Studies
FUS	RNA-binding protein FUS/TLS
FUSION	Functional Summary-based Imputation
GO	Gene ontology

GTEx	Genotype-Tissue Expression Project
GWAS	Genome-wide association study
GWGAS	Genome-wide gene association study
HS1BP3	HCLS1 Binding Protein 3
ICP	Inferior cerebellar peduncles
LD	Linkage disequilibrium
LDSC	Linkage Disequilibrium Score Regression
LINGO1	Leucine Rich Repeat and Ig Domain Containing 1
MAGMA	Multi-marker Analysis of GenoMic Annotation
MDCS	Movement Disorder Clinic Saskatchewan
MHC	Major histocompatibility complex
MiXeR	Bivariate causal mixture model
MTAG	Multi-trait analysis of GWAS
mtCOJO	Multi-trait-based conditional & joint analysis using GWAS summary data
NOTCH2NLC	Notch 2 N-Terminal Like C
PC	Principal components
PCR	Polymerase chain reaction
PD	Parkinson's disease
PheWAS	Phenome-wide association study
PMI	Postmortem interval
POLR2A	polymerase [RNA] II [DNA-directed] polypeptide
PPARGC1A	PPARG Coactivator 1 Alpha

PRKG1	Protein Kinase CGMP-Dependent 1
QC	Quality control
QQ	Quantile-quantile
RNA	Ribonucleic acid
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
SE	Standard error
SLC1A2	Solute Carrier Family 1 Member 2
SNP	Single nucleotide polymorphism
STK32B	Serine/Threonine Kinase 32B
SHF	Src Homology 2 Domain Containing F
TENM4	Teneurin transmembrane protein 4
TWAS	Transcriptome-wide association study

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ORIGINAL CONTRIBUTION TO KNOWLEDGE

The work presented in this thesis represents several significant contributions towards understanding the molecular and genetic risk factors of essential tremor (ET). The main approaches were agnostic hypothesis-free methods, that focused on high-throughput genome- and transcriptome- wide exploration.

One of the studies described herein (Chapter 2), is published in the journal *Movement Disorders*, entitled "Multiomics Analyses Identify Genes and Pathways Relevant to Essential Tremor". The studied compared cerebellar transcriptomic signatures of ET cases against matched controls using RNA sequencing (RNA-seq), for a total of 64 samples. This investigation identified differentially expressed genes (DEG) including *SHF* and *CACNA1A*, and calcium-related pathways were significantly enriched. Previous genome-wide association study (GWAS) data was used to further validate enriched pathways and found convergence of calcium implication. A phenome-wide association of the top differentially expressed genes also found *SHF* to be significantly associated with blood pressure medication, which is used to reduce tremor in ET patients. Treatment of cerebellar DAOY cells with beta-blocker medication found increases of *SHF* expression, suggesting potential therapeutic restoration.

The second study described in (Chapter 3), is published in *JAMA Neurology*, entitled "Genome-wide association study of essential tremor identifies novel loci". This study consisted of 7,177 ET cases and 475,877 controls. This investigation identified 5 novel independent genome-wide risk loci for ET and demonstrated that ET had a SNP heritability of 0.1829 (SE=0.0141). Functional enrichment implicated the cerebellar hemisphere, cerebellum and axonogenesis pathways. A transcriptome-wide association study (TWAS) identified several genes such as *BACE2*. Genetic correlation also identified significant correlation with Parkinson's disease (PD) and depression. Mendelian randomization analyses also identified that PD may be a potential risk factor for ET.

CONTRIBUTION OF AUTHORS

Chapter 2. Multiomics Analyses Identify Genes and Pathways Relevant to Essential Tremor

Calwing Liao contributed to the original concept of the project and conducted the molecular and cell culture experiments, data analysis, and wrote and oversaw revisions of the manuscript. Faezeh Sarayloo and Daniel Rochefort assisted with molecular experiments. Gabrielle Houle, Fulya Akçimen, Qin He, Alexandre D. Laporte and Dan Spiegelman provided expertise for data analysis. Werner Poewe, Daniela Berg, Stefanie Muller, Franziska Hopfner, Gunther Deuschl and Gregor Kuhlenbaeumer provided data. Alex Rajput conducted brain dissections. Patrick A. Dion and Guy A. Rouleau supervised the project.

Chapter 3. Genome-wide association study of essential tremor identifies several risk loci.

Calwing Liao conducted molecular work, data analysis, and wrote and oversaw revisions of the manuscript. Charles-Etienne Castonguay helped with analysis and writing. Karl Heilbron helped with data analysis. Veikko Vuokila helped with molecular work. Miranda Medeiros, Gabrielle Houle, Fulya Akçimen and Jay P Ross helped with data analysis and/or provided experimented. Helene Catoire helped with molecular work. Monica Diez-Fairen helped with molecular work. Jooeun Kang provided expertise. Stefanie H. Mueller, Simon L. Girard, Franziska Hopfner, Delia Lorenz, Lorraine N. Clark, Alexandra I. Soto-Beasley, Stephan Klebe, Mark Hallett, Zbigniew K. Wszolek, Manuela Pendziwiat, Oswaldo Lorenzo-Betancor, Klaus Seppi, Daniela Berg, Carles Vilariño-Güell, Ronald B. Postuma, Geneviève Bernard, Nicolas Dupré, Joseph Jankovic, Claudia M. Testa, Owen A. Ross, Thomas Arzberger, Sylvain Chouinard, Elan D. Louis, Paola Mandich, Carmine Vitale, Paolo Barone, Elena García-Martín, Hortensia Alonso-Navarro, José AG Agúndez, Félix Javier Jiménez-Jiménez, Pau Pastor, Alex Rajput, Günther Deuschl, Gregor Kuhlenbaümer and Inge A. Meijer MD provided samples and/or

contributed towards data generation. Patrick A. Dion and Guy A. Rouleau supervised the project.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Essential tremor (ET) is neurological movement disorder that affects approximately 1% of the worldwide population and up to 5% of the population by the age of 65[1]. The majority of studies find no difference in prevalence between females and males. The age of onset can be variable, but primarily has a bimodal distribution which peaks in the 20s and 60s[2]. The disorder is defined by an isolated bilateral upper-limb action tremor that has been present for at least 3 years[3]. ET commonly manifests with other neurological traits such as ataxia and memory impairment.

One common way of diagnosing ET is through the Archimedes Test, where patients are required to draw a continuous spiral once with each hand. This allows clinicians to assess any existing tremors and understand the severity of the tremor. The spiral typically presents with a smaller amplitude, higher frequency and more symmetry compared to other movement disorders such as dystonia[3,5]. The frequency of ET is typically 4 to 11 Hz, depending on which region is affected[3,6]. Typically, the proximal have a lower frequency whereas distal regions have a higher frequency.

The “essential” term suggests that the tremor cause is unknown and implies that there is only tremor and it is a crucial component of the disease[3]. However, the term is commonly used when cases have other clinical features such as dystonia and isolated tremors in the head or voice. The 2018 consensus statement by the Movement Disorder Society on tremor recently created a new term: essential tremor-plus (ET-plus)[5,8]. ET-plus is defined as tremor with similar characteristics but may have further neurological signs such as memory impairment, dystonia, impaired tandem gait, and other mild neurologic signs. Isolated head, orthostatic, voice, position and task specific tremor are therefore not considered part of ET[8]. However, this separation has been controversial and may oversimplify the ET phenotype as it is difficult to determine whether these comorbid symptoms are a function of having ET. Throughout this thesis, all studies consider ET to include ET-plus.

COMPLEXITIES WITH PARKINSON’S DISEASE

Parkinson’s disease

Parkinson’s disease (PD) and ET are both common movement disorders that can exhibit similar clinical features[9]. Patients can have both ET and PD, which can further complicate diagnoses and increases heterogeneity. Approximately 3% of ET cases eventually develop PD after a median follow-up duration of 3.3 years, translating to a four-to-five-fold risk increase of developing PD compared to controls[9–11]. In one study, it was found that one-third of patients were misdiagnosed with ET, but instead had PD[10–13]. There have additionally been studies that suggest ET may be a risk factor for PD,

however, the directionality of this relationship is still unclear as other studies have found PD to be a risk factor for ET. With approaches like mendelian randomization, there is now opportunity to potentially tease apart the relationship between ET and PD and inform on this epidemiological relationship.

TREATMENT

Pharmacotherapy

Two of most common first-line treatments for ET are propranolol and primidone, with the highest evidence of reducing tremor severity[14,15]. Propranolol is a non-selective beta-blocker, which has been shown to have high efficacy in randomized, controlled clinical trials at doses from 120–240 mg per day[14]. Accelerometry measures found that tremor amplitudes were reduced by an average of 55%[3,6,14]. However, several side effects include bronchospasm and bradycardia. One smaller controlled and randomized clinical trial found that long-acting propranolol had similar efficacy to short-acting forms for reducing tremor amplitude of ET patients[16]. Primidone is a barbiturate that is metabolized to phenylethylmalonamide and phenobarbital. The therapeutic dose from clinical studies have found it to range from 250–750 mg a day, with an approximately 60% reduction in the amplitude of tremors[6]. Several side effects include dizziness, fatigue and discomfort in one-third of patients, but typically disappear after 1 to 4 days[6]. A randomized controlled clinical trial of primidone-propranolol combination therapy compared to placebo found that there was an efficacy of 70% decrease in the amplitude of tremor[17]. Several

other medications such as topiramate, gabapentin, and other beta-blockers are often prescribed, but there is limited data from randomized, controlled trials on these medications.

Ablation and neurostimulation

Several studies have investigated the use of deep-brain stimulation and unilateral thalamotomy to medically treat upper-limb tremor in ET[18]. A randomized trial found that tremor patients (with some ET included) treated with deep-brain stimulation had more improvements and less side effects compared to thalamotomy[18]. A 5 year follow-up found that approximately half of the ET patients who had deep-brain stimulation had an attenuated therapeutic effect, which may either be due to tolerance to stimulation or disease progression[18–20]. In a randomized, controlled clinical trial of 76 ET patients, unilateral thalamic thermoablation through focused ultrasound with magnetic resonance imaging guidance led to significantly decreased hand tremor and improved quality life[21]. The main side effects were discomfort, post-operational paresthesia or numbness.

PATHOLOGICAL AND NEUROPHYSIOLOGICAL FACTORS

Despite ET being one of the most common movement disorders, little progress has been made for understanding ET pathophysiology. It was hypothesized that there may be a central pacemaker for tremor, supported by harmaline-induced tremor in animal models. It was suggested that a perturbation in the inferior olivary nucleus, which projects

to the cerebellar cortex and has pacemaker properties, was the driver in ET, although this was primarily theoretical. In the 1990s, positron emission tomography of the inferior olivary nucleus in ET patients did not find any abnormalities. Similarly, postmortem brain tissue did not identify any structural changes[22].

Cerebellum

Emerging clinical studies began identifying support for cerebellar correlates with ET. Several studies have identified abnormalities in balance and gait in ET patients[23–26]. Furthermore, some studies have found that over 50% of ET patients have cerebellar (intention) tremor in the arms and 10% with intention tremor in other areas such as the head[27,28]. Many other movement abnormalities such as aberrant limb motor behaviour and oculomotor deficits have been shown[29–34]. Several neuroimaging studies have also found structural and metabolic abnormalities in the cerebellum of ET patients[35,36]. These clinical reports point to a latent cerebellar abnormality in ET patients.

Postmortem studies

In parallel, there have been many emerging postmortem brain studies in ET patients compared to controls. The vast number of ET cases have been shown to have neurodegeneration in the cerebellum. Particularly, there has been evidence of dendritic and axonal changes in Purkinje cells, which have a pacemaker nature[37–40]. One study has found that the axons have six-to-seven-fold increase in Purkinje cell swelling compared to controls[41]. There have been similar findings for the dendrites.

GENETICS

Linkage studies

Essential tremor is commonly familial with an autosomal dominant pattern. The disease is progressive and twin studies have demonstrated that ET has a concordance of 69–93% in monozygotic twins and 27–29% in dizygotic twins, highlighting that both genetics and environment contribute towards disease etiology[42]. Several genetic linkage studies have identified putative risk loci across. The first ET linkage locus (ETM1) was identified by scanning 16 Icelandic families with 75 affected individuals with an autosomal dominant inheritance[43]. The scan mapped to a genome-wide significant locus on chromosome 3q13. In this locus, a missense variant (p.S9G, rs6280) in the Dopamine Receptor D3 (*DRD3*) gene, was segregating in 23 out of 30 French families[44]. However, subsequent follow-up studies failed to replicate these findings in independent cohorts[45]. A second ET linkage locus (ETM2) was identified on chromosome 2p24, and a missense variant in the *HS1BP3* gene (p.A265G) in that linkage locus was found to segregate in two unrelated American families[46,47]. Similarly, this failed to replicate in larger case-control studies[45].

Genome-wide association studies

Genome-wide association studies (GWAS) seek to agnostically assess regions of the genome that are associated with a specific phenotype. Common approaches include using genotyping arrays, due to the lower cost compared to approaches such as whole-genome sequencing. However, there is emerging evidence that low-coverage whole-

genome sequencing can reduce bias, especially for ancestrally diverse GWAS. There have been several studies that have investigated the common variant architecture of ET. Genome-wide association studies are widely adopted approaches to identify loci associated with phenotypes agnostically. The first ET GWAS consisted of 452 cases and 14,394 controls, implicating an intronic variant in the Leucine Rich Repeat and Ig Domain Containing 1 (*LINGO1*) gene[48]. A second genome-wide association study with 436 cases implicated an intronic variant in *SLC1A2*[49]. A larger subsequent two-stage GWAS failed to replicate the *LINGO1* and *SLC1A2* associations and did not find any genome-wide significant loci, likely due to small sample size (N=1778 cases)[2]. The top loci in the two-stage study were near the following genes: *CTNNA3*, *STK32B*, and *PPARGC1A*. However, the replicability of these loci in independent cohorts were inconclusive. The study also found *STK32B* to have increased expression in ET cerebellar tissue compared to matched controls[2]. Given that certain phenotypes tend to converge on a common and rare variant level, a resequencing experiment was done to assess the rare variant burden of *CTNNA3*, *STK32B*, and *PPARGC1A*[50]. The results failed to find any significant enrichment amongst these genes compared to controls. The small sample size and inconsistent results across genome-wide scans was motivation for conducting a larger GWAS to identify robust loci.

Sequencing studies

In parallel, there have been efforts to study rare risk variants for ET using next-generation sequencing. Specifically, whole-exome and whole-genome sequencing of large multiplex ET families have been done to identify risk genes. In 2012, the first

disease-associated variant was identified in a large French-Canadian family. Through whole-exome sequencing, a protein-truncating variant (p.Q290*) located in the Fused-in-sarcoma (*FUS*) gene segregated in the pedigree[51]. However, case-control follow-up studies failed to replicate the association, which may be due to lower power[52,53]. In a separate whole-exome study of a consanguineous Turkish family, a missense variant (p.G399S) was identified in *HTRA2*[54]. Similarly, no convincing replication has been established for this gene. In 2015, a rare variant (p. T1367N) in the Teneurin transmembrane protein 4 (TENM4) gene was found to segregate in a large Spanish family[55]. Resequencing of TENM4 in 299 familial ET probands found 12 additional rare missense carriers. However, an independent rare variant burden follow-up study failed to replicate this enrichment[56].

Several other genes such as *SORT1*, *SCN4A*, *NOS3*, *KCNS2*, *HAPLN4*, *USP46*, and *SCN11A* have been implicated through similar studies[53]. But similarly, there have been no consistent replication of these genes, highlighting the need for understanding ET genetics. Overall, the genetics of ET are unclear and inconclusive, giving reason to conduct additional studies that leverage larger sample size, more homogenous phenotypes, and alternative methods such as transcriptomics.

Expansions

In a recent study, a GGC repeat *NOTCH2NLC* expansion in the 5' region was found to be significantly associated with Asian-ancestry ET risk[57]. Specifically, 11 out of 197 Chinese pedigrees had abnormal GGC expansions that co-segregated with the

disease with genetic anticipation. This would be an estimate of ~5-6% of ET cases having a pathogenic expansion. Several independent follow-ups found that this expansion was not a risk factor for European-ancestry ET patients, highlighting the importance of increasing diversity in genetic studies to identify disease genes[58,59]. For instance, in one study that looked at 204 European-ancestry beta-blocker responsive ET patients, none of the individuals had a pathogenic repeat[58].

RATIONALE AND OBJECTIVES

Rationale

Despite decades of research into the genetics of ET, our understanding of risk factors and putative risk genes that confer susceptibility remains limited. Essential tremor is a highly heritable disorder demonstrated by twin studies, which strongly debilitates the quality of life for many individuals. However, there still have not been any replicable genes and loci. Several reasons include small sample size, and lack of integration across several different datasets. Furthermore, pharmacotherapy often has many common effects that make it difficult to stay on medication long-term. Given these important public health concerns, improving our understanding of ET can ultimately lead to better drug targets and provide potential avenues for alternative ways to manage and treat the disorder. The work presented in this thesis aims to identify genetic risk factors for ET and implicate molecular pathways that are perturbed for the disease through agnostic questions of the genome and transcriptome. First, we propose to ask what genes may be differentially expressed in ET tissue compared to controls and assess transcriptomic correlates with the phenome and genome. Transcriptomics insights offer an opportunity to overcome some limitations of genetics, such as low power for assessing genes on sex chromosomes. Second, we propose to ask the question whether there are common loci that significantly associate with ET compared to controls in a larger GWAS, and how this informs on risk genes and ET epidemiology. Given the amount of conflicting genetic results in the literature and candidate gene studies, a large agnostic GWAS could help better understand the genetics of ET.

Objectives and hypotheses

The first objective of this thesis was to investigate the transcriptomic differences in ET patients compared to controls. This was done by sequencing the RNA in post-mortem cerebellar tissue of clinically ascertained cases and matched controls. Firstly, we sought to assess whether there were significant differentially expressed genes. Next, we aimed to determine what molecular pathways are significantly enriched amongst the differentially expressed genes. After, we sought to use independent genome-wide and phenome-wide association data to validate any results, as replication has been an issue in understanding ET genetics.

The second objective of this work was to determine whether there was an underlying common variant architecture that contribute towards ET susceptibility. This was done by using an effective sample size ~5 times larger than the previous GWAS using genotyping arrays. Firstly, we sought to determine whether there were genome-wide significant loci that associate with disease. Then, we aimed to assess the heritability and the degree of polygenicity captured by common variation. Next, we leveraged this common variation to identify putative risk genes through transcriptomic imputation and inform on the epidemiology of ET through genetic correlation and mendelian randomization.

CHAPTER 2: MULTI-OMICS ANALYSES IDENTIFY GENES AND PATHWAYS RELEVANT TO ESSENTIAL TREMOR

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Abstract

Introduction:

The genetic factors and molecular mechanisms predisposing to essential tremor (ET) remains largely unknown.

Objective: The objective of this study was to identify pathways and genes relevant to ET by integrating multi-omics approaches.

Methods: Case-control RNA sequencing of two cerebellar regions was done for 64 samples. A phenome-wide association study (pheWAS) of the differentially expressed genes (DEGs) was conducted, and a genome-wide gene association study (GWGAS) was done to identify pathways overlapping with the transcriptomic data. Finally, a transcriptome-wide association study (TWAS) was done to identify novel risk genes for ET.

Results: We identified several novel dysregulated genes including *CACNA1A* and *SHF*. Pathways including axon guidance, olfactory loss, and calcium channel activity were significantly enriched. The ET GWGAS data found calcium ion-regulated exocytosis of neurotransmitters to be significantly enriched. The TWAS also found calcium and olfactory pathways enriched. The pheWAS identified that the underexpressed DEG, *SHF*, is associated with a blood pressure medication (P=9.3E-08), which is used to reduce tremor in ET patients. Treatment of cerebellar DAOY cells with the ET drug propranolol identified increase in *SHF* when treated, suggesting it may rescue the underexpression.

Conclusion: We found that calcium-related pathways were enriched across the GWGAS, TWAS, and transcriptome. *SHF* was shown to have significantly decreased expression and the pheWAS showed it was associated with blood pressure medication. Treatment of cells with propranolol showed that the drug restored levels of *SHF*. Overall, our findings highlight the power of integrating multiple different approaches to prioritize ET pathways and genes.

Introduction

Essential tremor (ET), one of the most common movement disorders, involves rhythmic shaking during voluntary movements, particularly in the hands[1]. Although the disease is not fatal, it can have large negative effects on daily life and psychological well-being. Familial clustering suggests that genetic factors have an important role in ET. Twin studies have shown that ET has a concordance of 69–93% in monozygotic twins and 27–29% in dizygotic twins, suggesting that both genetics and environmental factors drive the phenotype[2]. Despite dozens of studies investigating the genetic etiology of ET, the heritability has largely remained unexplained. This is likely due to the misdiagnosis of ET as other similar movement disorders (e.g. Parkinson's disease and dystonia), phenocopies, genetic heterogeneity and incomplete penetrance of risk alleles, greatly reducing statistical power of linkage studies[3,4].

By comparison to other neurological conditions, there have been relatively few genetic studies of ET. These studies have used approaches that range from screening of function-based candidate genes, linkage and gene associations, and high-throughput sequencing of familial cases. In 2016, the largest genome-wide association study (GWAS) thus far reported used ET cases of European descent and identified three genomic loci associated with ET[5]. Since this last study, replications were undertaken across cohorts of ET cases of different ethnic origin (e.g. Han-Chinese), yet only a few successfully replicated the association of a single locus. For instance, *LINGO1* has been replicated in a few studies[5–9]. The failure to replicate more than a locus is possibly due to relatively small cohorts and haplotype structures that were too different from the one originally used in 2016. A form of study that is absent from the ET literature is a high-

throughput transcriptomic-wide approach to identify gene dysregulations across the expression profile of disease relevant brain tissue. The cerebellum has been previously implicated in ET through clinical and histological studies. Specifically, ataxic features as clinical symptoms and atrophy and dysregulation of Purkinje cells have largely been associated with ET[10–12]. Since little is known about the underlying biology of ET and genomic studies have not found adequate evidence for the suggested heritability of ET, we conducted RNA sequencing to identify dysregulated genes and pathways. We sequenced two distinct regions of the cerebellum: the cerebellar cortex and the dentate nucleus, in 16 cases and 16 age- and sex-matched controls. Additionally, we conducted a genome-wide gene association study (GWGAS) using ET GWAS data to narrow down relevant pathways. Several interesting genes such as *PRKG1* and *CACNA1A* were differentially expressed. A phenome-wide association study (pheWAS) of the differentially expressed genes (DEGs) identified blood pressure medication as a relevant phenotype for the DEG, *SHF*. Both RNA sequencing and GWGAS identified calcium-channel relevant pathways suggesting that dysregulation in these pathways contribute towards the ET phenotype. Finally, integration of tissue-relevant transcriptomic and GWAS data identified several novel loci and putative causal genes.

Methods

Sample selection and criterion

Patients attending MDCS (Movement Disorder Clinic Saskatchewan) are offered autopsy at no cost to the family/estate. Autopsy consent is granted by the next-of-kin after death of the patient. The body is transported to Saskatoon and autopsy is performed within 24

h of death. The autopsy consent is approved by the Saskatoon Health Region Authority and the use of brain for research is approved by the Bioethics Board of the University of Saskatchewan and is therefore in concordance with the 1964 Declaration of Helsinki. Further details on patient recruitment can be found from Rajput et al. (2015) and Rajput et al. (2016)[13,14].

ET brains (N=16) were dissected to obtain the dentate nucleus and cerebellar cortex. Samples were selected based on the following criteria: grossly unremarkable cerebellum, staining showed no noticeable degeneration, no other neurological disorders or movement disorders (i.e. Parkinson's disease or dystonia), no signs of dementia or mild/moderate Alzheimer's changes, and have definite or probable ET. An independent neuropathologist reviewed supplementary table 4 and concluded that all morphological abnormal findings were completely non-specific and are seen in the majority of deceased cases >70. Controls (N=16) were age- and sex- matched. Additionally, the controls did not have any noticeable neurodegenerative or psychiatric disorders. The pH of all brains was neutral. Samples were matched to have an average RIN of 5 and all samples had a DV200 > 70%. The mean age across samples was 81.81 with a 1.35 standard error of mean. For sex, there was a male to female ratio of 1:1.66. Patients were reported as European. Case and controls groups did not significantly differ for PMI and we tried to prioritize samples <24 Hr PMI. One case, did not have a specific PMI value but was listed as <24 Hr, making unavailable to use as a covariate (Mann-Whitney U Test, U=75, P=0.54).

RNA extraction and sequencing

RNA was extracted from 64 samples using the RNeasy Lipid Tissue Mini Kit (Qiagen). Two samples were removed due to low quality. The RNA concentration was measured on the Synergy H4 microplate reader. RNA was sent to Macrogen Inc. for sequencing. Library preparation was done using the TruSeq Stranded Total RNA Kit (Ilumina) with Ribo-Zero depletion. Sequencing was done on the NovaSeq 6000 at 150bp paired-end reads with a total of 200M reads. Samples were randomized for tissue dissection, RNA extraction, Ribo-Zero depletion, library preparation and sequencing to account for potential batch effects.

Data processing, differential expression, splicing and eQTL analyses

The FASTQ files were pseudo-aligned using Salmon using the Ensembl v94 annotation of the human genome[15]. For data processing and parameters of Salmon, please refer to Liao et al. (2019)[16]. Sleuth was used to identify DEGs[17]. We focused on transcript level associations to avoid potential bias from single transcripts driving a gene aggregate association. The data was analyzed with the following full model for the likelihood ratio test: Gene expression ~ disease status + sex + age + sex:disease status + age:sex + sex:disease status. The reduced model for the likelihood ratio test is: Gene expression ~ sex + age + sex:age. A Wald test was used to get beta values, which is a bias estimator. Beta approximates the extent at which estimated counts is affected by the disease status rather than technical and biological variability. It can be used to estimate the magnitude and direction of fold change. P-values were corrected using the Benjamini-Hochberg procedure to account for false discovery rate (FDR). Q-value (p-

values corrected for FDR) significance was set for <0.05. Splicing analyses were done with SUPPA2 and rMATs[18,19].

Validation with reverse transcriptase qPCR

To validate the significant DEGs, reverse transcriptase qPCR (RT-qPCR) was done. Following the manufacturer's protocol, the SuperScript Velo cDNA Synthesis Kit (ThermoFisher Scientific) was used to convert 1 µg of RNA into cDNA. A standard curve was made for each TaqMan probe to determine PCR efficiency. The gene *POLR2A* (polymerase [RNA] II [DNA-directed] polypeptide) was used as an endogenous control.

Pathway enrichment analyses in brain tissue

Gene clustering was done using the GeneNetwork v2.0 RNA sequencing database (N=31,499). Clusters were independently analyzed for different enriched pathways in databases such as Reactome and GO. FUMA was used to identify enrichment in BrainSpan and GTEx 53 v7[20]. Tissue specificity was tested in GTEx 53 v7. Briefly, DEG sets were pre-made and the input genes were tested against the DEG sets using a hypergeometric test. DEGs with a q-value <0.30 were included for analysis.

Gene-level analyses of ET GWAS data

The summary statistics of the ET GWAS was used (N=7154). Imputation was done using Imp-G summary with the 1000 Genomes dataset. A genome-wide gene association study (GWGAS) was done using MAGMA using the European UK Biobank as a reference LD panel. Genome-wide gene association studies considers the

combined association effect of all SNPs in a gene to aggregate into a combined gene-level P-value. A Bonferroni threshold of 2.744E-06 was used to reduce false positives. Genes with a suggestive Bonferroni-corrected p-value (P<0.10) were further queried in downstream functional analyses. Gene expression analysis was done using MAGMA for GTEx 53 v7 and BrainSpan. In the 30-general tissue GTEx v7 and then looked at expression of all brain-relevant regions in GTEx 53 v7. Gene clustering was done using the GeneNetwork v2.0 RNA sequencing database (N=31,499)[21]. Pathway enrichment and gene ontology analyses were also done for GWGAS data. Additionally, all ET-associated GWAS loci (P<1E-06) were queried in GTEx53 to determine if any were eQTLs for the differentially expressed genes.

Phenome-wide association of differentially expressed genes

To understand which phenotypes may be associated with the differentially expressed genes, a pheWAS was done using GWASAtlas, which uses public genome-wide association study (GWAS) data[22]. Bonferroni-corrected cut off of 1.68E-05 (0.05/# of unique traits) was used. At the time, there were 2977 traits.

Transcriptome-wide association study

To identify novel loci and putative genes for ET, a TWAS was done using expression data of 1,245 unrelated controls from the Netherlands Twin Registry (NTR), 1,264 controls from the Young Finns Study (YFS), and relevant GTEx 53 v7 tissues—cerebellum (N=154), cerebellar hemisphere (N=125) and BA9 frontal cortex (N=118).

A TWAS was performed using each panel using the program FUSION for summary-level data from the most recent ET GWAS and the 1000 Genomes LD reference panel. In brief, this method takes expression data from reference panels, an LD panel, and GWAS summary statistics to identify genes associated with a phenotype. Each panel was Bonferroni-corrected for the number of genes.

Cell Culture and drug treatment

DAOY cells were cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% fetal bovine serum (FBS) and Glico Pen-Strep-Glutamine cocktail at 1X. Cells were maintained at 37°C and 5% CO_2 and passaged all at once every 2 days when they reached 80-90% confluence. Cells were treated in triplicate with propranolol at 50 μM for a week. RNA was extracted and RT-qPCR was done using the same method as described above.

Results

Differentially expressed genes identify potentially disease relevant pathways

Six genes were differentially expressed in the cerebellar cortex and two genes were differentially expressed for the dentate nucleus for an FDR of 0.05 (Table 1).

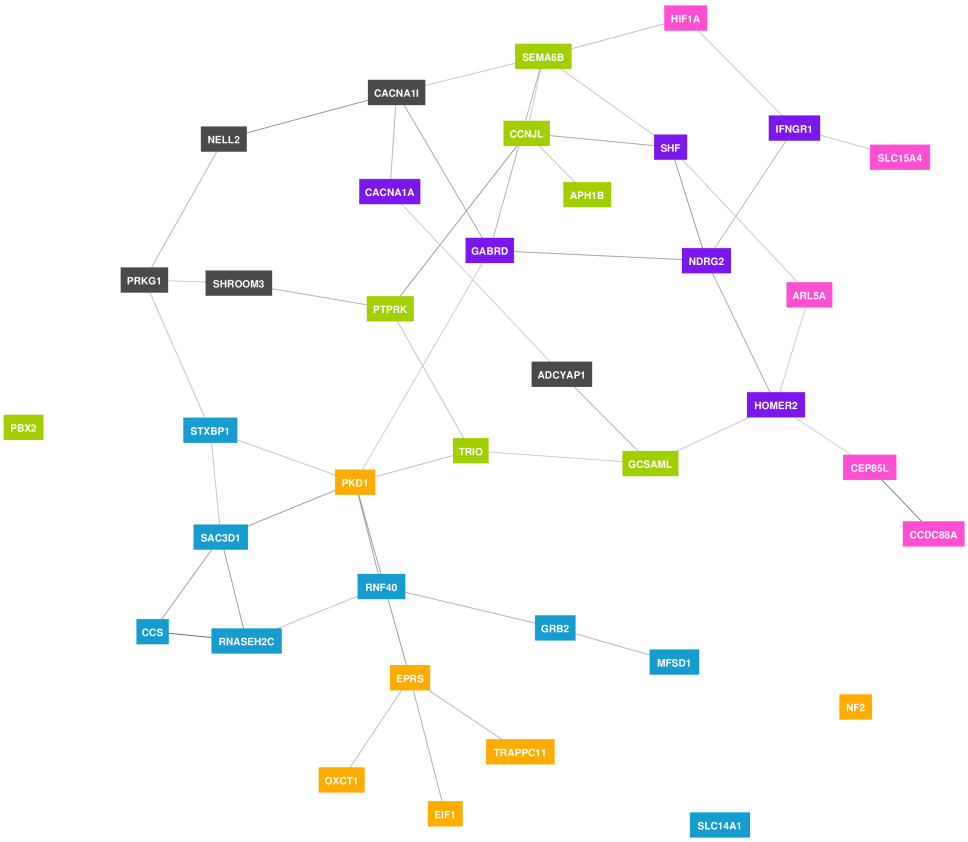
Table 1. Differentially expressed transcripts of the cerebellum in ET patients compared to controls.

Gene	Ensembl ID	Function	P-value	Q-value	Beta	Tissue
PRKG1	ENST00000373980.9	Kinase	1.08 E-08	0.00068	-11.89647	Cerebellar Cortex
SAC3D1	ENST00000398846.5	Mitotic function	2.84 E-07	0.00903	+26.09570	
SHF	ENST00000458022.6	Apoptosis	1.22 E-06	0.02089	-17.69694	
TRAPPC11	ENST00000512476.1	Protein trafficking	1.31 E-06	0.02089	-16.590866	
NELL2	ENST00000547751.5	Neuronal survival	2.28 E-06	0.02896	-8.60137	
CACNA1A	ENST00000636549.1	Calcium channel	8.89 E-06	0.04421	-15.301447	
PLCG2	ENST00000359376.7	Phospholipase	2.39 E-07	0.04381	-9.73396	Dentate Nucleus

ALDH3A2	ENST00000395575.6	Dehydrogenase	1.81E-06	0.04491	-21.87150

The QQ-plot did not show any large stratification of the data in cerebellar cortex or dentate nucleus (Supplementary Figure 1, 2). Interestingly, the top dentate nucleus differentially expressed genes differed compared to the cerebellar cortex. The top non-coding RNA differentially expressed was *LINC00599* in the cerebellar cortex, which was highly associated with calcium ion-regulated exocytosis of neurotransmitter (P = 3.3E-06), ionotropic glutamate receptor signaling pathway (P = 9.2E-10), neurotransmitter secretion (P = 1.6E-09) and synaptic vesicle exocytosis (P=1.0E-07) in the GO database based on GeneNetwork's co-expression database. Pathway analyses of the DEGs based on co-expression identified six clusters for the differentially expressed genes (Figure 1A). Several novel pathways possibly implicated in ET were identified including axon guidance, olfactory receptor activity, and voltage-gated calcium channel activity for the cerebellar cortex (Supplementary Table 2). Two gene clusters were identified for the dentate nucleus (Figure 1B).

A.


HIF1A
SEMA6B
CACNA1I
NELL2
CCNJL
SHF
IFNGR1
SLC15A4
CACNA1A
APH1B
GABRD
NDRG2
PRKG1
SHROOM3
PTPRK
ARL5A
ADCYAP1
HOMER2
PBX2
STXBP1
TRIO
GCSAML
CEP85L
PKD1
SAC3D1
CCDC88A
RNF40
CCS
RNASEH2C
GRB2
MFSD1
EPRS
NF2
TRAPPC11
OXCT1
EIF1
SLC14A1

B.

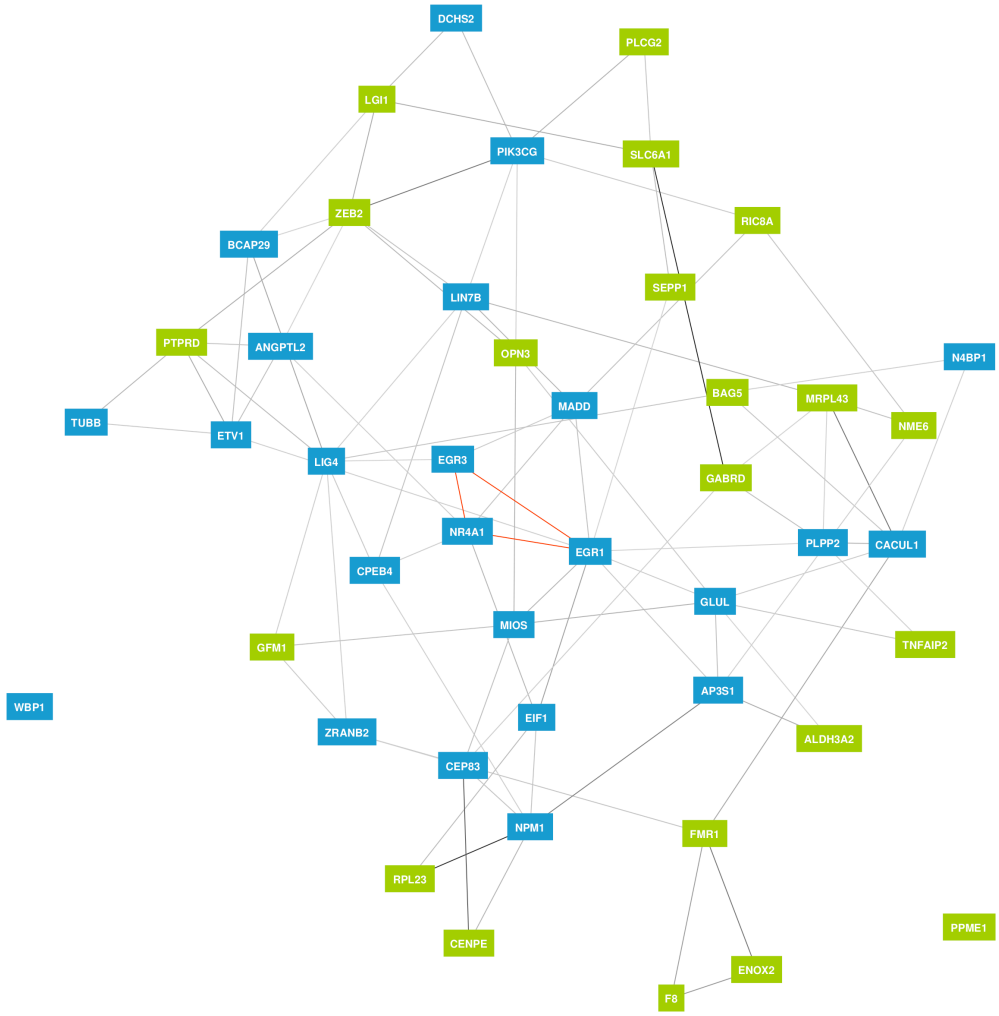


Figure 1. Gene clustering of differentially expressed genes for the cerebellar cortex based on gene co-expression. (A) Public RNA sequencing data (N=31,499) was used to determine co-expression profiles. Gene cluster 1 identified in blue. Gene cluster 2 identified in green. Gene cluster 3 identified in purple. Gene cluster 4 identified in orange. Gene cluster 5 identified in pink. Gene cluster 6 identified in black. (B) Gene clustering of differentially expressed genes for the dentate nucleus based on gene co-expression.

Public RNA sequencing data (N=31,499) was used to determine co-expression profiles. Gene cluster 1 identified in blue. Gene cluster 2 identified in green.

Many pathways were enriched in the dentate nucleus, including olfactory transduction, olfactory signaling pathway and MAPK signaling (Supplementary Table 3). Splicing analysis with rMATs and SUPPA2 did not find any significant retained introns, mutually exclusive exons, alternative 3' splice sites, alternative 5' splice sites or skipped exons between cases and controls. Reverse transcriptase qPCR (RT-qPCR) of the DEGs supported the direction as the Wald test statistic (Supplementary Table 1).

Expression levels across different tissue types and brain developmental stages

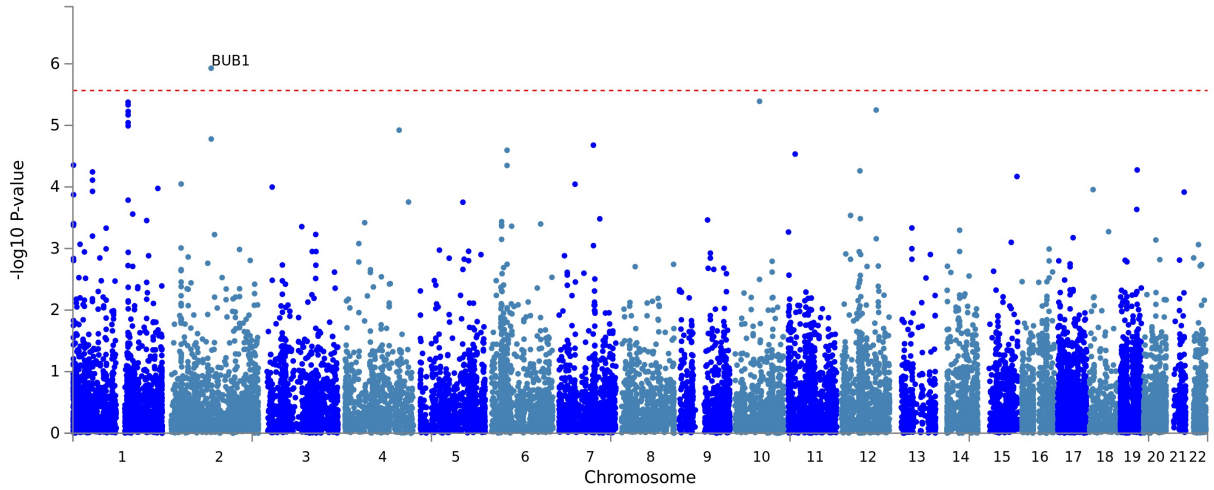
Expression levels in GTEx53 show that most of the DEGs are highly expressed in the brain cerebellar hemisphere and brain cerebellum (Supplementary Figure 3). Additionally, different regions of the brain have different expression levels for these genes. Expression levels across 29 different ages of brain samples from BrainSpan show that most of the DEGs are stably expressed during development (Supplementary Figure 4).

Genome-wide gene association study of previous ET GWAS data identifies calcium-relevant pathways

To narrow down relevant pathways, a genome-wide gene association study (GWGAS) was done. The input SNPs were mapped to 18,220 protein coding genes. The genome-wide gene association study (GWGAS) identified *BUB1* reaching Bonferroni genome-wide significance and several genes reaching suggestive significance (Figure 2A).

Clustering of the genes based on co-expression identified four distinct clusters (Supplementary Figure 5). From the pathway analyses, calcium ion-regulated exocytosis of neurotransmitters in GO was significantly enriched in the pathway in cluster three (P=9.4E-03). Gene-level enrichment analyses in GTEx 30 v7 found the brain to be significantly associated (P=0.0012). After, the enrichment was queried in the brain-relevant regions of GTEx 53 v7 we found the cerebellar cortex and frontal cortex to pass Bonferroni-corrected significance (Figure 2B). No ET GWAS SNPs were found to be eQTLs for the dysregulated genes based on the GTEx53 database (Supplementary Figure 6-12).

A.



B.

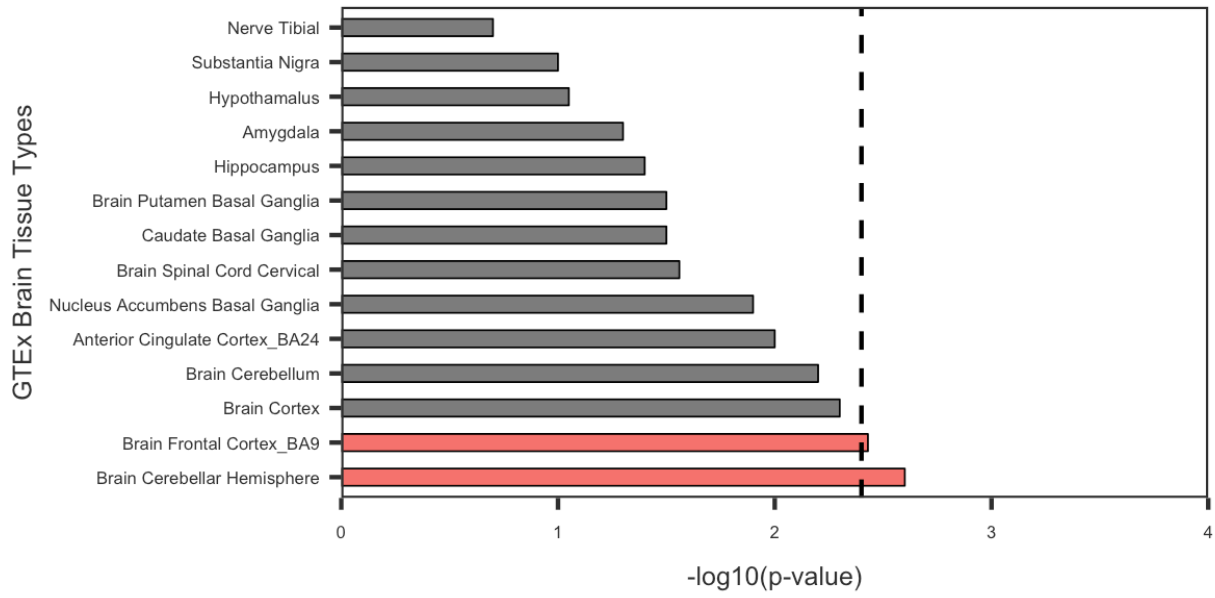


Figure 2. Genome-wide gene Manhattan plot and brain-relevant tissue enrichment profile. (A) Manhattan plot of gene-level associations (N=7,154). Bonferroni significance threshold at 2.744E-06 shown with the red dashed line in the plot. **(B)** Gene-level

enrichment analysis of GWGAS genes in brain-relevant tissue of GTEx53. Bonferroni-corrected significance set at 0.0036, indicated by the dashed line.

Phenome-wide association of DEGs and drug screening

The phenome-wide association study (pheWAS) showed that *SHF* was significantly associated with blood pressure medication (P=9.30E-08) and body mass index (BMI) (P=1.5E-07) (Figure 3). No other DEGs had associations that passed a Bonferroni threshold of 1.68E-05 (0.05/# of unique traits).

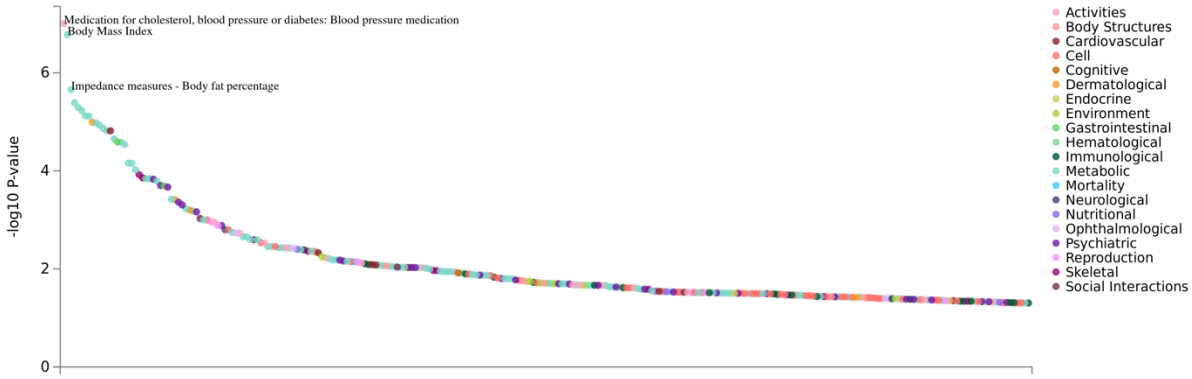


Figure 3. PheWAS manhattan plot of *SHF* for different domains. Colours indicate respective domains. Only p-values less than 0.05 were plotted.

To understand the effects of beta-blockers on *SHF*, DAOY cells were treated with propranolol compared to untreated controls. A significant increase in *SHF* was seen in treated cells when comparing RNA expression levels (P=0.008926) (Figure 4).

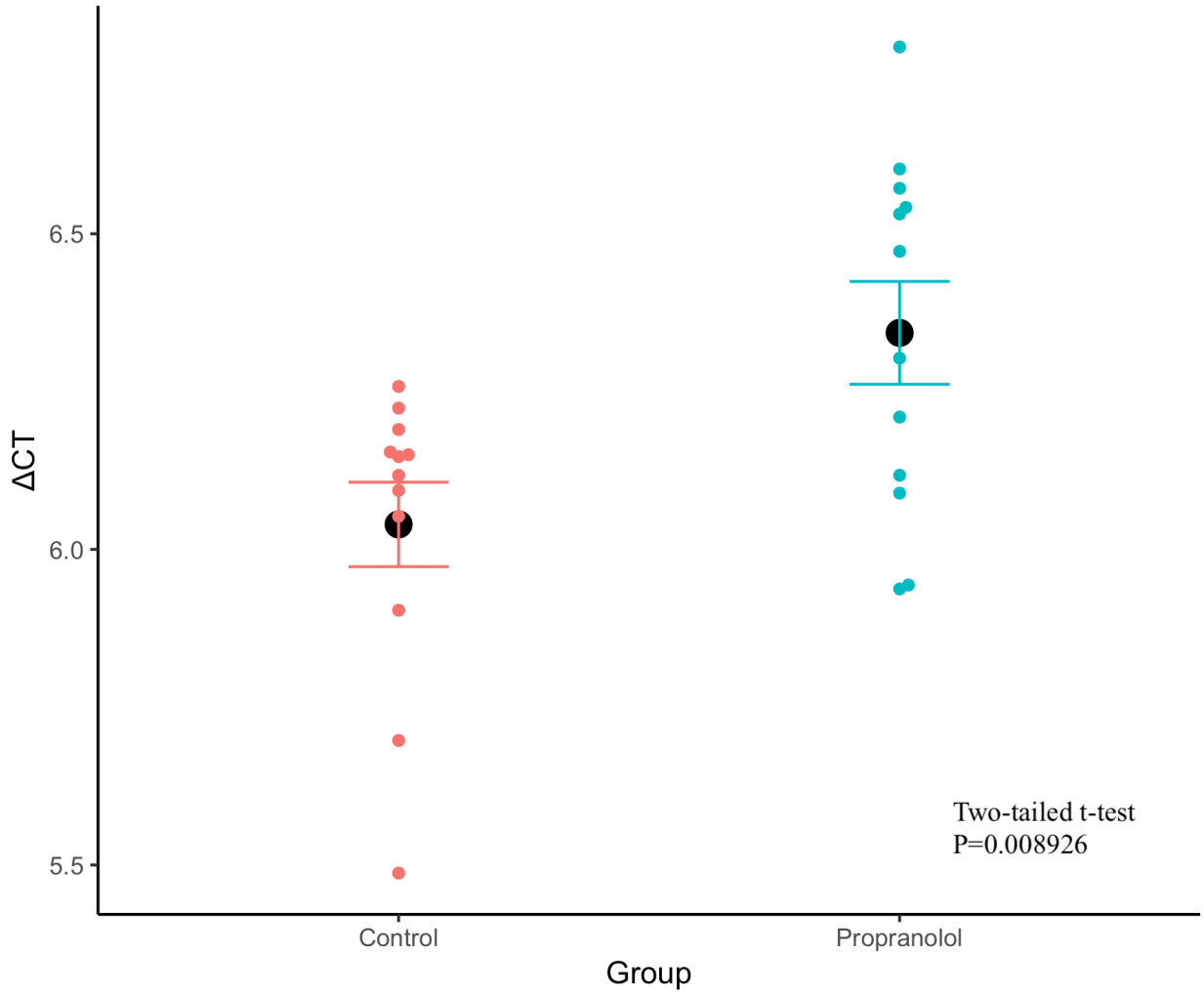


Figure 4. RT-qPCR expression levels of *SHF.* Larger blackdot represents the mean. Error bars are ±SE.

Transcriptome-wide association study

The TWAS identified several significantly associated genes for ET (Supplementary Figure 13). After, pathway enrichment validated several pathways that were previously implicated by the above transcriptomic data including: olfactory signaling pathway (P=4.0E-05), neurotransmitter receptor activity (P=6.4E-05) and positive regulation of calcium ion-dependent exocytosis (P=7.4E-03).

Discussion

Interestingly, calcium pathways and relevant genes were significant for differential expression and pathway analyses. In a paper by Topaktas *et al.* (1987), the use of calcium blockers led to intensified tremors in ET patients[23]. Interestingly, the calcium channel gene, *CACNA1A*, had lower levels of expression in ET patients, suggesting that cellular calcium may be relevant to the ET phenotype. In mice, knockout *CACNA1A* lines shown a tremor phenotype according to The Jackson Laboratory mice database. Furthermore, *CACNA1A* has been shown to be highly expressed in Purkinje cells, a relevant ET cell type[24]. The translated protein of *PRKG1*, PKG, has been reported to increase opening of calcium-activated potassium channels, further indicative of the relevance of calcium in ET[25]. Furthermore, irregular GABA-A receptor function has been previously shown to be affected by *CACNA1A*[26]. It is hypothesized that defective GABA receptors contribute towards the ET phenotype by disinhibition of cerebellar pacemaker output[27]. Additionally, the gene has been shown to be highly co-expressed with *GABRA4* based on the Gene Network database (P=1.6E-13), reinforcing *CACNA1A* as a gene of interest for ET.

The top DEG, *PRKG1*, has been shown to regulate cardiovascular and neuronal health[25]. Specifically, the RNA isoform two (PRKG1B) was most significantly differentially expressed at the transcript level. Currently, there has yet to be any study to link *PRKG1* and ET. However, the gene is highly expressed in Purkinje cells, which is a relevant cerebellar cell type in ET[28]. Also, the ET brain staining did not show significant Purkinje cell loss, suggesting transcriptomic dysregulation of Purkinje cells may be more relevant to ET than degeneration. Interestingly, *PRKG1* has been associated with alcohol misuse

and many ET patients report reduced tremor intensity with alcohol, however, the pheWAS data did not show any associations[29].

Pathway analyses additionally found several potentially relevant pathways such as axon guidance and neuromuscular junction. Interestingly, a possibly deleterious variant in *TENM4*, a regulator of axon guidance, was shown to segregate in ET families and cause tremor in knockout mice, reinforcing the relevance of this pathway in ET[30].

The GWGAS identified *BUB1* as a significantly enriched gene for ET. *BUB1* is transcribed and translated into a serine/threonine kinase, similar to *STK32B*, which was a significant ET locus previously identified[16]. Amongst the significantly enriched pathways in the GWGAS and DEGs, calcium ion-regulated exocytosis of neurotransmitter was found to be in common between the two. This suggests that dysregulation in calcium homeostasis may affect relevant neurotransmitter exocytosis in ET patients.

The relationship of *SHF* with blood pressure medication from the pheWAS may suggest that beta-blockers interact with *SHF*. Beta-blockers such as propranolol can reduce kinetic tremor in certain ET patients[31]. The gene was clustered with the calcium-related genes such as *CACNA1A*, suggesting that beta-blockers may influence pathways identified from that cluster such as calcium channel activity. The addition of propranolol to the media of DAOY cells was observed to increase the expression level of *SHF*. Considering that the ET cerebellar transcriptomes showed a decrease in the *SHF* RNA levels, it is interesting to observe that propranolol has an action that could restore levels

of *SHF* in patients. Furthermore, with the associated BMI trait, it could suggest that it is a potential risk factor for ET. Future studies should investigate the relationship between this gene and those phenotypes and determine whether *SHF* may be a biomarker for beta-blocker responsive ET patients.

Interestingly, examination of the dentate nucleus and cerebellar cortex from the same individuals revealed them to have distinct transcriptome profiles—the top DEGs were different between the two tissues. Based on the GTEx53 expression profiles of different tissue types, the differential expression across tissues reinforces the notion that the dentate nucleus and cerebellar cortex would have different DEGs. The BrainSpan database showed that the DEGs are similarly expressed across different ages and that adulthood has moderate to high expression of the DEGs.

Olfactory transduction and signaling were pathways enriched in both the dentate nucleus and cerebellar cortex. Past studies have had conflicting views on whether olfactory loss is an endophenotype for ET. However, the transcriptomic data objectively supports that a subset of ET patients likely have dysregulated olfactory phenotypes. Furthermore, *MAPK* pathways were enriched in the dentate nucleus. This is interesting because beta-blockers have been shown to have downstream effects on MAPK-relevant pathways.

The TWAS identified several putative genes associated with ET. Interestingly, the pathway enrichment of the top significant genes identified pathways that overlapped with those derived from the transcriptomic, phenomic, and GWGAS analyses. The TWAS

successfully reinforced the relevance of olfactory signalling and calcium exocytosis pathways, suggesting that the molecular mechanism of ET may be relevant to genes in those pathways.

Here, we report the first study of ET that integrates transcriptome, phenome, and genome data. We identified several dysregulated genes and relevant pathways, including calcium, axon guidance and olfactory pathways, and showed relevance of them in multiple independent datasets. However, we acknowledge that bulk RNA sequencing cannot thoroughly distinguish which cell types may be driving the differentially expressed signals. Further replication studies investigating the transcriptome should be done for ET as the disease is highly heterogeneous. We additionally were unable to include PMI as a covariate due to missing information from 1 sample that was listed as <24 Hr. However, we did not find any significant differences between PMI for the two groups with the available data, despite this. Future studies could investigate the spatial transcriptomics or single-cell sequencing of ET relevant tissue and integrate also large datasets to further refine and validate relevant genes and pathways.

Data Availability

Any data produced from this study will be provided by contacting the corresponding author.

Author Contribution

C.L. conducted the experiments, analyses, conceptualized the project and drafted the manuscript. F.S., F.R., G.H., F.A., Q.H. helped with molecular and/or bioinformatic analyses. A.D.L, D.S. helped with bioinformatics. A.R. dissected the brains. P.A.D. and G.A.R. oversaw the experiments and helped draft the manuscript.

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Conflicts of Interest

Dr. Deuschl reports personal fees from Boston Scientific, Cavion, Functional Neuromodulation, Thieme publishers, grants from Medtronic, outside the submitted work. All other authors report no conflicts of interest.

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Bridging statement to Chapter three

In chapter 2, we performed RNA sequencing of post-mortem brain tissue to interrogate the transcriptome and leveraged multi-omics data to prioritize differentially expressed genes between ET cases and controls. In chapter 3, we seek to complement the transcriptome by assessing the genome and identifying common variants that increase risk for ET through a genome-wide association study.

CHAPTER 3: GENOME-WIDE ASSOCIATION STUDY OF ESSENTIAL TREMOR IDENTIFIES SEVERAL NOVEL RISK LOCI

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Key Points

Question: Can we identify common genetic variants associated with essential tremor (ET)?

Findings: By leveraging the genetic data of 483,054 individuals, we identified five genome-wide significant loci associated with ET risk and identified that common variants explain approximately 18% of ET heritability.

Meaning: Overall, we demonstrate that common variants contribute towards ET risk and identify several new associated loci.

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Abstract

Importance: Essential tremor (ET) is one of the most common movement disorders, affecting 5% of the population above the age of 65. Common variants are thought to contribute toward the susceptibility to ET, but no variants have been robustly identified.

Objective: To identify common genetic factors associated with ET risk.

Design: A case-control genome-wide association study where an inverse-variance meta-analysis was used to combine cohorts. Samples were collected since January 2010 until September 2019 as part of an ongoing study. There was no follow-up necessary for this study.

Setting: Multicenter samples collected from European populations.

Participants: Case samples included patients that were clinically diagnosed or reported to have essential tremor. Controls were participants that were not diagnosed or reported to have essential tremor. Of 485,250 individuals, 483,054 samples passed data quality control and were used.

Intervention or exposures: N/A

Main outcome and Measures: Genotypes of common variants associated with ET risk.

Results: Of the 483,054 samples included, there were 7,177 ET cases (3693 [51.46%] female; mean [SD] age of 62.66 [15.12]), and 475,877 controls (253,785 [53.33%] female; mean [SD] age of 56.40 [17.6] years). We identified five independent genome-wide significant loci and demonstrated that common variants explain approximately 18% of ET heritability. Functional analyses found significant enrichment in the cerebellar hemisphere, cerebellum, and axonogenesis pathways. Interestingly, genetic correlation (r_g), which measures the degree of genetic overlap, revealed significant common variant overlap with Parkinson's disease (PD) (r_g=0.28, P=2.38 x 10^{-8}) and depression (r_g=0.12, P=9.78 x 10^{-4}). A separate fine-mapping of transcriptome-wide association hits identified genes such as *BACE2*, *LRRN2*, *DHRS13*, and *LINC00323* in disease-relevant brain regions, such as the cerebellum.

Conclusions and relevance: Through this study we demonstrated that a portion of ET heritability can be explained by common genetic variation and identify new common genetic risk factors.

Introduction

Essential tremor (ET) is a complex neurological disorder affecting 1% of the overall population and up to 5% of individuals above the age of 65[1,2]. It is clinically characterized as a bilateral, largely symmetric kinetic or postural tremor[3]. This can greatly decrease the quality of life and debilitate daily function. Previous studies have implicated the cerebellum as a putative region of interest for ET[4,5]. Specifically, abnormalities of Purkinje cells have been observed in post-mortem brain tissue obtained from individuals with ET[6]. Furthermore, several transcriptomic studies and imaging studies have also highlighted the importance of the cerebellum in ET[4,7,8].

Moreover, the genetic etiology of ET remains elusive despite twin studies that demonstrated the trait to be heritable[9–11]. For instance, one twin study suggested ET to have a concordance of 69–93% in monozygotic twins, and of 27–29% in dizygotic twins[9]. Studies in which familial ET cases were sequenced have also implicated specific genes[12–14]. For instance, rare variants in *FUS* and *TENM4* were found to segregate in large families but the lack of replication suggests they are potentially private variants[15,16]. Finally, past genome-wide association studies (GWAS) also identified putative ET loci, but none of these loci were statistically significant at a genome-wide level, likely due to the size of the cohorts examined[17,18]. The loci from these GWAS implicated nearby genes, such as *STK32B* and *LINGO1*, for which subsequent replication studies were conducted[19–23]. However, most of these GWAS loci had conflicting replication results and were done in smaller cohorts.

Here, we present a genome-wide meta-analysis identifying the first genome-wide significant loci for ET using a cohort of 7,177 ET cases and 475,877 control individuals. We additionally identified novel loci, implicated tissue-relevant genes, and found a significant genetic overlap between PD and ET. Overall, this report further demonstrated the heritable nature of ET and implicated new disease relevant loci and genes.

Methods and Statistical Analyses

Sample description

The meta-analysis comprised of cohorts collected in North America and Europe, which totalled 7,177 cases and 475,877 controls. This was further grouped into cohorts based on study cohort, chip and time of genotyping as described in the Supplementary Note. Individual clinical diagnoses are described previously or in the Supplementary Note. The review board at the McGill University Health Center Research Ethics Board (MUHC) approved the study protocols (reference number: IRB00010120).

Genotyping and quality control

The cohorts were genotyped and followed standardized quality control (QC), imputation, and post-imputation QC. Briefly, samples were removed if >2% missingness, autosomal heterozygous deviation (Fhet <0.2), and failed sex check. Low quality SNPs were removed based on Hardy-Weinberg Equilibrium (P>1E-6), SNP missingness < 0.02 after sample removal. Samples were mapped against the 1000 Genomes Project phase 3 reference panel after pruning and removing SNPs from high-LD regions, and only individuals of inferred- European ancestry were retained. No relatedness filter was done

because a linear-mixed model was used subsequently to account for relatedness. Imputation was done using the Sanger Imputation Server with Eagle v2.3.5 and the Haplotype Reference Consortium Reference Panel v1.1[24]. Further details on cohort and quality control are described in the Supplementary Note for the UK BioBank and 23andMe dataset.

Genome-wide association

A Bayesian linear-mixed model was done using BOLT-LMM 2.3.4 including 20 principal components (PCs) and sex as covariates to accelerate convergence[25]. The non-infinitesimal model was used if there was an increase in power. Subsequently, the data were meta-analyzed using an inverse-variance weighted fixed effects model with METAL[26]. Only markers with an effective sample size $N_{eff} = 4/(1/N_{cases} + 1/N_{controls}) > 70\%$ were retained, leaving a total of 6,892,661 variants[27].

SNP heritability and partitioning of the heritability

To determine the SNP heritability on the liability scale, the slope of the LD Score Regression (LDSC) was calculated with European-ancestry samples from the 1000 Genomes Project[28]. The effects of confounding factors were determined by assessing the deviation of the LDSC intercept from 1. Specifically, the ratio between the (intercept – 1) divided by the (mean χ^2 −1) ascribes confounding other than polygenicity[28]. The heritability was partitioned by different tissue, cell and functional sets using LDSC[29]. The same previously described European 1KG cohort was used for LD and allele frequencies.

Genetic correlation

The genetic correlation was calculated for ET and other GWAS traits using LDHub[30]. This platform uses LDSC to broadly assess multiple traits with publicly available GWAS. Traits with an updated GWAS were replaced, as defined by a larger sample size and/or a more recently published GWAS. Only traits with European ancestry were retained and data with low relative Z-scores (as reported by LDHub) were excluded. SNPs from the MHC region were removed for traits. One of any duplicate traits were remained, prioritizing the most recent study or largest sample size.

Conditional analysis

To determine whether there were any genome-wide significant loci with multiple independent signals, Genome-wide complex trait analysis (GCTA)-COJO was used[31]. Briefly, the program takes the ET summary statistics and conditions genome-wide significant lead SNPs while using the LD of a reference panel. Here, the raw genotyping data from control samples and the CARTaGENE cohort were used as the reference panel[32]. A stepwise approach was used to condition the top independent SNPs ($P<5 \times 10^{-8}$) and a minor allele frequency > 0.01.

Click or tap here to enter text.

Multi-trait analysis

To increase power, we did a multi-trait analysis of GWAS (MTAG), using phenotypes with significant positive correlation (Parkinson's disease and depression)[34–36]. The program MTAG was used to conduct the analysis. MTAG jointly meta-analyzed summary statistics from Parkinson's disease, depression with ET to increase power to

identify ET-specific associations. Increase in power was defined as ($GWAS_{MTAG}$ mean $\chi^2 - 1$) / ($GWAS_{non\text{-}MTAG}$ mean $\chi^2 - 1$) * 100%.

Multi-trait conditional analysis

To assess the relationship between Parkinson's disease and ET, a multi-trait conditional analysis was done using mtCOJO—adjusting ET by Parkinson's disease. Summary statistics from Nalls et al. (2019) were used[35]. Briefly, mtCOJO removed pleiotropic signal with PD from the ET GWAS. Typically, most pleiotropic loci should have reduced conditional effect sizes, but trait-specific effects would have larger conditional effects.

Gene-based, gene-set, and tissue-set enrichment analyses

P-values that quantify the genic associations and gene-set enrichment for ET were calculated using MAGMA v1.08 as implemented in FUMA (https://fuma.ctglab.nl)[37,38]. A Bonferroni-correction was applied for the number of genes (N=18,517) tested with a threshold of $P=2.70 \times 10^{-6}$. Enrichment amongst GTEx V8 was also done using FUMA, with a significance threshold at $P=9.26 \times 10^{-4}$).

Transcriptome-wide association

To identify genes influenced by cis-eQTLs, a transcriptome-wide association study (TWAS) was done using FUSION. Brain imputation panels were used from the Genotype-Tissue Expression (GTEx) project and CommonMind Consortium (CMC)[39,40]. The 1KG v3 LD panel was used for TWAS. Bonferroni-adjusted p-values <0.05 were considered

transcriptome-wide significant. A brain omnibus test was done to test for effect across reference panels, which accounts for pairwise correlation between features. A Bonferroni-threshold was used for the omnibus (0.05/7,221) (number of genes tested).

To address co-regulation in TWAS, FOCUS (Fine-mapping of causal gene sets) was used for genome-wide significant loci to model predicted expression correlations and assign a posterior probability for causality in the previously mentioned imputation panels[41]. In brief, FOCUS will identify genes for each TWAS signal to be included in a 90% credible set.

Gene-set analyses were done using GeneNetwork v2.0 (https://genenetwork.nl), which leverages RNA-sequencing data (n=31,499) to provide co-regulated genes within each pathway[42]. Genes meeting a Bonferroni-adjusted p-value <0.05 were used. Agnostic analyses of pathways in databases such as Reactome, and GO were used.

To assess potential colocalization between the top significant loci with eQTLs, FUMA was used to map eQTLs with the top significant loci. Data from GTEx 53 v8 for brain tissue and the CommonMind Consortium were used. All SNP-gene pairs of cis-eQTLs that were nominally significant were included (P<0.05). The sign of the original eQTL data indicates the direction of effect for the tested allele. The lead SNPs from the five genome-wide significant loci were compared against the eQTL data.

Phenome-wide association

To investigate whether any top loci were associated with other phenotypes, the SNPs were assessed on the genetics Open Targets Platform (genetics.opentargets.org) and a PheWeb for the UK BioBank imaging data (https://open.win.ox.ac.uk/ukbiobank/big40/pheweb/).

Bivariate gaussian mixture modelling of polygenicity

To determine the univariate estimate of non-null SNPs (polygenicity) and shared polygenicity with PD, MiXeR v1.3 was used on the summary statistics of ET and PD[43]. In the cross-trait analysis, MiXeR modelled additive genetic effects as a combination of the following four components: 1) SNPs not influencing either ET or PD, 2 and 3) SNPs influencing only one of two traits, 4) SNPs influencing both traits. After fitting the model, the dice coefficient (a parameter that estimates the proportion of overlapping variants), is calculated.

Results

Genome-wide significant ET risk loci

For the GWAS, samples from 14 different clinical centers and two biobanks were included (see Supplementary Note for detailed descriptions). The cohorts were divided by genotyping array, leading to a total of four genotyping cohorts. The first clinical cohort was genotyped on the Axiom Genome-wide CEU 1 Array (Affymetrix). The second clinical cohort was genotyped on the Illumina GSA array. The two biobank cohorts were from 23andMe, Inc. and the UK BioBank. Prior to analysis, stringent quality control was performed on the data and ancestrally predicted Europeans were retained based on the

1000 Genomes Project Phase 3 reference panel (Methods). Independent cohorts were meta-analyzed using an inverse-variance weighted fixed effects model. Of the 483,054 samples included, there were 7,177 ET cases (3693 [51.46%] female; mean [SD] age of 62.66 [15.12]), and 475,877 controls (253,785 [53.33%] female; mean [SD] age of 56.40 [17.6] years). Subsequently, variants with an effective sample size >70% of the full meta-analysis were retained, leaving 6,892,661 markers.

From the meta-analyzed GWAS, the h^2_{SNP}, the heritability explained by SNPs, was estimated to be 0.1829 (SE=0. 0141) using Linkage Disequilibrium Score Regression (LDSC). The LDSC intercept, which indicates the degree of inflation due to confounding, was 1.051 (SE=0.00074), which suggests low levels of confounding. The attenuation ratio, which assesses the degree of inflation due to polygenicity instead of confounding, was 0.14 (SE=0.036), suggesting most inflation was due to polygenicity (Supplementary Figure 1) [28]. The genomic inflation factor (λ_{1000}) was also determined to be 1.01. Genetic correlation, which captures the degree of genetic overlap with another cohort or trait, was calculated between cohorts using LDSC. Across the cohorts, the correlations were significant and positive, providing evidence that effects were consistent across cohort designs (Supplementary Table 1). The clinical cohorts had a genetic correlation of 0.88 ± 0.20 ($P=1.86 \times 10^{-5}$), and these clinical cohorts respectively had a genetic correlation of 0.96 ± 0.148 ($P= 9.34 \times 10^{-11}$), and 0.52 ± 0.14 ($P=1.21 \times 10^{-4}$) with the 23andMe cohort (Supplementary Table 1). The lower genetic correlation of 0.52 was between the clinical samples genotyped on the Illumina Array and 23andMe. Due to the small effective

sample size of the UK Biobank cohort, pairwise genetic correlation was not calculated for it.

Table 1. Lead SNPs for genome-wide significant loci in 7,177 cases and 475,877 controls.

CHR	POS (hg19)	rsID	Alleles (Eff/Ref)	Direction	Odds Ratio [95% CI]	Z	P
1	117532790	rs1127215	C/T	+++-	1.09 [1.06 – 1.13]	5.55	2.756e-08
4	24362541	rs17590046	C/T	----	0.89 [0.85 – 0.93]	-5.70	1.180e-08
5	67827456	rs28562175	C/T	----	0.92 [0.88 – 0.95]	-5.51	3.483e-08
18	37207175	rs1945016	G/T	++++	1.10 [1.06 – 1.13]	5.69	1.255e-08
21	42520134	rs9980363	C/T	++++	1.16 [1.12 – 1.20]	7.52	4.921e-14

A total of five genome-wide significant loci ($P<5x10^{-8}$) were identified (Figure 1, Table 1). None of the of the top loci were found to be significantly heterogeneous (Supplementary Table 2). Furthermore, there were no additional independent secondary genome-wide significant signals found after conditioning on the lead SNPs iteratively.

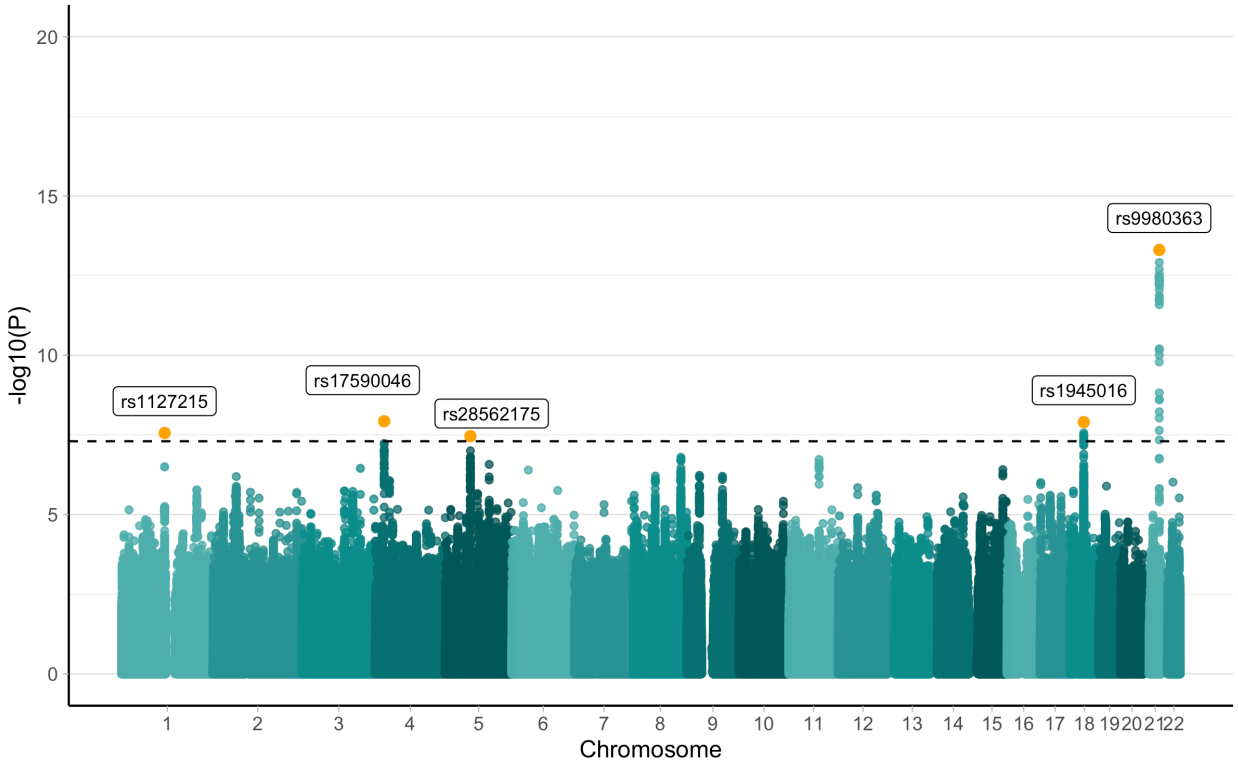

Figure 1. Manhattan plot of genome-wide association study of 7,177 essential tremor cases and 475,877 controls. The x-axis shows chromosome number (chromosomes 1-22) and points are ordered by genomic position. The y-axis shows the statistical significance of each locus, represented as -log10(P). The black-dashed line indicates the genome-wide significance threshold $P=5 \times 10^{-8}$). Lead SNPs are highlighted in orange.

Transcriptome-wide associations

A transcriptome-wide association study (TWAS) was conducted using FUSION by leveraging brain data from the Genotype-Tissue Expression Project (GTEx) and the CommonMind Consortium (CMC)[112,113,117]. The TWAS identifies genes that are predicted to have altered expression due to ET-associated common variants. The Bonferroni-significant hits were *BACE2* and *LINC00323* (Figure 2, Supplementary Table 3). A brain omnibus test, which assesses the degree of shared signal across brain tissues, showed that *BACE2*, *LINC00323,* and *ANGEL2* were significant after Bonferroni correction, suggesting effect across multiple brain tissue types (Supplementary Table 4).

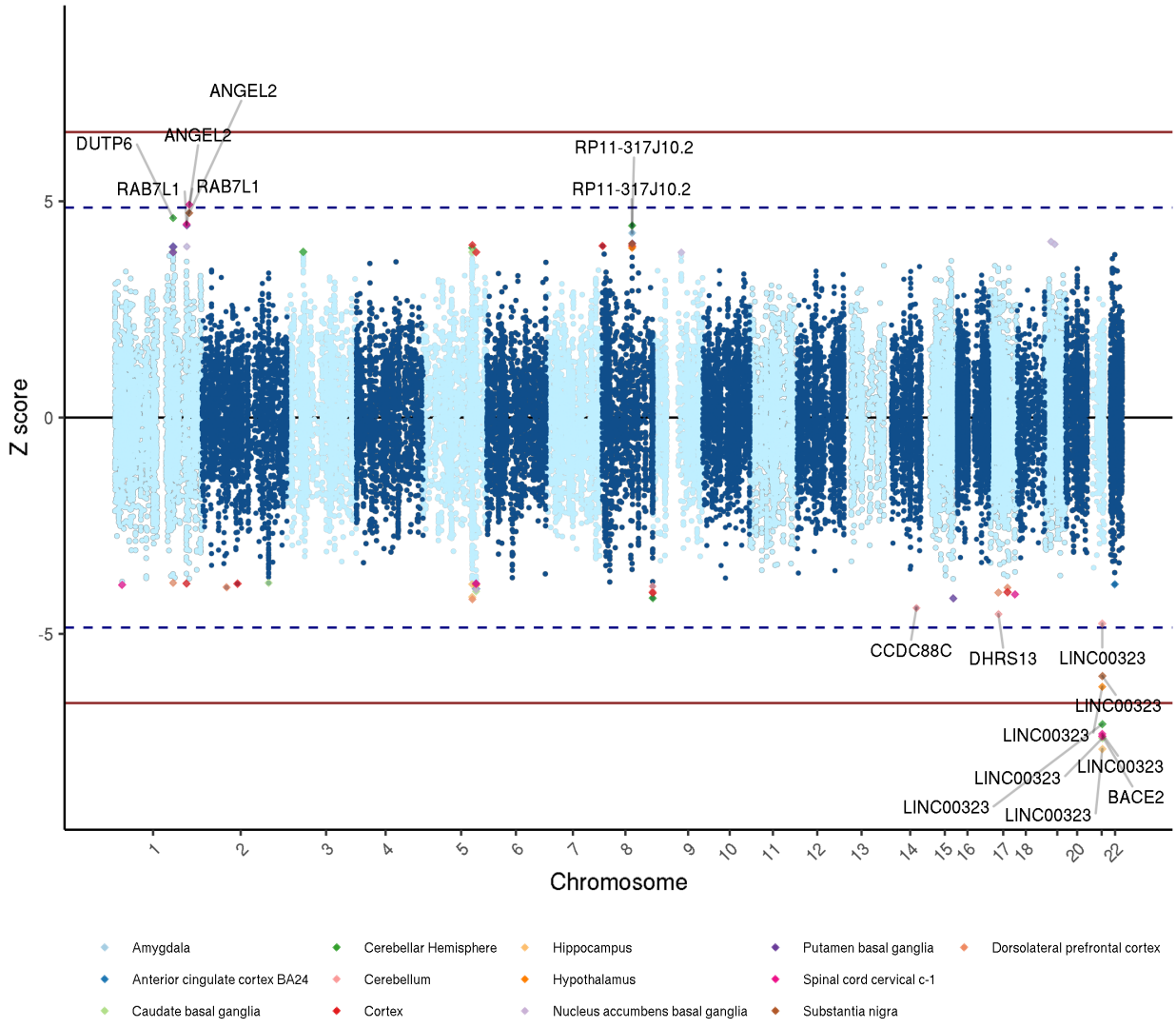

Figure 2. Mirrored Manhattan plot of transcriptome-wide associations for essential tremor. The red line indicates Bonferroni-significance threshold. The dashed blue line indicates the false-discovery rate threshold.

To prioritize the most genes, TWAS fine-mapping was done using FOCUS across the set of 90%-credible genes[41]. The program FOCUS models the correlation amongst TWAS signals so that the likely causal gene(s) at genome-wide significant loci are prioritized. Across the genome-wide significant loci, *BACE2* was prioritized with a posterior inclusion probability (PIP) of 0.80 (Z=-7.09) (Supplementary Table 5).

Additionally, the top significant loci were mapped to eQTLs derived from the GTEx 53 brain tissues and the CommonMind Consortium. The colocalization pointed towards three genes, *PTGFRN*, *LINC00323*, and *BACE2*. The *PTGFRN* gene was only significant in the cerebellum, whereas the latter two genes were implicated across multiple brain tissues (Supplementary Table 6).

Functional enrichment of genomic regions

To identify patterns of heritability from the GWAS data, the heritability was partitioned by function annotations using partitioned LD score regression[29]. This analysis indicated that there were significant enrichments from SNPs in H3K9ac peaks, H3K27ac, and conserved regions (Supplementary Table 7).

Moreover, genome-wide analyses were done using MAGMA, which aggregates significance across loci into gene-level significance[37]. The top gene set identified after enrichment was axonogenesis (P_{bon}=0.047) (Supplementary Table 8, Supplementary Figure 2-3). There was significant enrichment in the cerebellum (P=6.3 x 10^{-5}) and cerebellar hemisphere (P=7.9 x 10^{-5}) in GTEx 53, and overall enrichment in the brain

(P=0.0012) (Supplementary Figure 4)[38]. There was no significant gene enrichment across the 29 different ages in the BrainSpan cohort (Supplementary Figure 5)[45].

Phenome-wide associations of top loci

For the genome-wide significant loci, a phenome-wide association study (pheWAS) was done to identify putatively relevant phenotypes. For the top *BACE2* locus associated with ET (rs9980363), the pheWAS of brain imaging data showed it was a top significant association for white matter intracellular volume fraction (ICVF) in the left and right inferior cerebellar peduncle (β=0.08, SE: 0.012, P=1.2 x 10^{-12}).

Genetic correlation

To determine whether ET had a significant genome-wide genetic correlation with other diseases and traits, LD Hub and LDSC were used[28,30]. After correcting for multiple testing, ET was shown to have a significant genetic correlation with Parkinson's disease (PD) rg=0.28 ± 0.051 (P=6.44 x 10^{-8}) and depression rg=0.12 ± 0.04 (P=9.78 x 10^{-4}) (Supplementary Table 9).

Dissecting genetic relationship between ET and PD

Considering the epidemiological implications and positive genetic correlation that have been reported between ET and PD, we sought to further dissect their genetic relationship. MiXer, a bivariate causal mixture model was used to estimate the number of causally shared SNPs. It was found that there were 500 causally shared SNPs between PD and ET, with a total of 700 variants that influence ET and 4800 that influence PD.

To determine whether the genome-wide significant signals were pleiotropic for ET and PD, mtCOJO (multi-trait-based conditional & joint analysis using GWAS summary data) was used to estimate the SNP effect size of the outcome trait (ET) after conditioning on exposure trait (PD)[31]. To do this, mtCOJO takes the PD genome-wide significant loci to estimate the effect of exposure on ET, and then undergoes a genome-wide conditioning with the estimated effect for the outcome trait[31]. All the genome-wide significant ET loci remained significant after conditioning suggesting no pleiotropy with PD for these loci (Supplementary Table 10).

To assess whether novel ET loci could be identified given the genetic correlation with PD, a multi-trait association analysis was done using MTAG (multi-trait analysis of genome-wide association studies)[34]. This method leverages the genetic correlation between traits to increase power for each respective phenotype. An additional genome-wide significant locus was identified through MTAG on chromosome 3 (lead SNP: rs703174), with up to an increase in power of 8.5% (Supplementary Table 11).

Discussion

Here, we identified five genome-wide significant loci for ET, demonstrating the importance of common variants. One of the signals on chromosome 4, had nominal significance in a previous GWAS, and was found to be consistent across the other cohorts included in this meta-analysis[17]. The previous largest GWAS did not find any genome-

wide significant loci that met the multiple testing significance threshold but found three suggestive loci[24].

For the chromosome 1 locus (rs1127215), the UK Biobank cohort did not have a consistent direction with the other cohorts. This may be due to bias from population biobanks, batch effects, and smaller case count. Interestingly, the UK Biobank cohort also had a low prevalence (~0.06%) despite the expected prevalence of 1-5%. This may suggest an underreporting of ET in biobank surveys or a bias where there was decreased participation of ET patients in the UK Biobank. Our study revealed multiple characteristics about the genetic architecture of ET. The SNP-based heritability was found to be 18.29% on the liability scale, suggesting that a considerably large portion of heritability is explained by common variants. This is comparable with a variety of other brain-relevant disorders such as bipolar disorder, intracranial aneurysms, and PD[35,46,47]. We also found this heritability to be enriched in histone markers such as H3K9ac and H3K27ac, which suggest future studies could investigate the importance of epigenetics for ET. We found an additional novel locus by leveraging the genetic overlap with PD with MTAG, which may suggest this locus is pleiotropic for the two phenotypes. Through gene-set enrichment analysis, we identified axonogenesis as an important cellular process for the disease, consistent with previous studies that implicate the importance of axons[20,48–50]. Furthermore, we found significant associations between ET and the cerebellum, providing further evidence that ET may be a cerebellar disorder or reflective of neurons driving the signal due to high proportion of neurons in the cerebellum[51–54].

A transcriptome-wide association study using brain cis-eQTL data from GTEx and CMC found *BACE2*, *LINC00323*, and *ANGEL2* to be transcriptome-wide significant for ET. Probabilistic fine mapping further prioritized *BACE2* amongst the TWAS signals. The eQTL mapping also found converging evidence and implicated *BACE2*. The gene *BACE2* encodes for a β-secretase homolog that is capable of cleaving amyloid beta precursor protein (APP) resulting in the formation of amyloid-β protein (Aβ), a major component in the pathogenesis of Alzheimer's disease[55–57]. Interestingly, a post-mortem study has found increased levels of insoluble and soluble Aβ protein in the cerebellar and parietal cortex of ET patients by comparison to control and PD patients[58].

Moreover, ET was found to be significantly genetically correlated with depression and PD, suggesting common variant overlap. Previous studies have found that ET is associated with both self-reported depression and antidepressant medication use, concordant with the genetic correlation results[59]. We conditioned ET by PD and found an attenuation of genome-wide association strength but found that the top ET loci were still genome-wide significant. This result suggests that these loci are likely to be robustly associated with ET and not PD.

Phenome-wide association analysis of these loci revealed that the *BACE2* loci was associated with increased intracellular volume fraction, a marker of neuronal density, in the inferior cerebellar peduncles (ICP). The ICP harbours the main afferent fibers of the cerebellum, channeling proprioceptive information from the spinal cord and brain stem nuclei to the cerebellar cortex[60]. A recent volumetric analysis of MRI brain scans found

that the middle and inferior peduncles of the cerebellum of ET patients displayed significant atrophy by comparison to healthy controls[61]. In addition, stimulation of these afferent proprioceptive fibers was shown to be effective at reducing tremors in ET patients[62]. Interestingly, another study showed increased radial diffusivity, a parameter strongly associated with myelin abnormalities, in the inferior cerebellar peduncles of patients with ET[63]. These results, paired with our findings of enrichment of genes in this region of the cerebellum and axonogenesis, highlight the potential implication of ICP afferent fibers in ET pathophysiology.

In summary, we identified five genome-wide significant loci for ET in a meta-analysis of 7,177 cases and 475,877 controls. We demonstrated that approximately 18% of ET heritability can be explained by common variation. The meta-analysis implicates genes such as *BACE2* and reinforce the importance of the cerebellum for the etiology of ET. The results also point towards approximately 30% shared common variant overlap with PD, and no genetic evidence for ET as a risk factor for PD.

Our work has several limitations, such as the lack of diverse ancestry. We did not have a large number of non-European samples which prevented us from assessing multi-ancestral analyses. Moreover, there is a lack of deep phenotyping information for the population-based biobanks such as 23andMe. This may lead to an increased number of diagnostic inaccuracies at the expense of increased power. However, we emphasize that there was still a high genetic correlation with the clinical cohorts, suggesting that the

population-based biobanks are still capturing genetic signal. Overall, ET risk can partly be explained by common genetic variation.

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Data and code availability

The summary statistics for the 10,000 most informative SNPs will be made available, in accordance with the 23andMe policy. The following dataset access instructions (or some variation of it): The full GWAS summary statistics for the 23andMe discovery data set will be made available through 23andMe to qualified researchers under an agreement with 23andMe that protects the privacy of the 23andMe participants. Please visit https://research.23andme.com/collaborate/#dataset-access/ for more information and to apply to access the data.

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Supplementary Methods

Detailed description of clinical samples

The two clinical cohort as described in the methods are partitioned by genotyping array. The first clinical cohort was ascertained and genotyped on the Axiom Genome-wide CEU 1 Array by Affymetrix as described in Müller et al. (2016)[1]. Briefly, samples from Kiel, Tuebingen, Innsbruck, Baylor, Columbia/Yale, Montreal, VCU, NIH, Mayo Clinic Florida, and the University of Saskatchewan were part of clinical cohort one (genotyped on Axiom array). The second clinical cohort was ascertained and genotyped on the Illumina GSA array. Samples were from Kiel, Montreal, Mayo Clinic, University of Saskatchewan, University of Navarra, CARTaGENE, Hopsital Universitario del Sureste (Arganda del Rey) / University of Extremadura, and University of Genoa. Both clinical cohorts are characterized by the genotyping array (clinical cohort 1 – Axiom Array, clinical cohort 2 – Illumina Array). Briefly, the contributing institutions diagnoses' have been summarized below.

Kiel

Patients were recruited within the "Population Based Assessment of Genetic Risk Factors for Essential Tremor (PopGen ET)" cross-sectional study from the Department of Neurology of Kiel University hospital and from newspaper calls for patients with tremor disorders from 2001 to 2013. Participants were all of German origin. Patients were diagnosed with essential tremor (ET) according to consensus criteria from the Movement Disorder Society (MDS) by one of five movement disorder experts. Only patients with definite or probable ET were genotyped in order to increase diagnostic certainty. Patients with a history of other neurological symptoms were excluded, including symptoms of

Parkinson's disease. Informed consent was obtained for all recruited patients and study approval was given by the local ethics committee.

Tuebingen

Patients were recruited by referral to either a specialised outpatient clinic or the neurological ward for movement disorders of the department of neurodegeneration in Tuebingen. Blood samples were collected after obtaining signed informed consent. Familial history of tremor prompted examination and collection of samples from other family members. Only 'definite' or 'probable' cases of ET were retained based on criteria from the Tremor Research Investigational Group (TRIG), whilst excluding secondary causes of tremor. Sample collection was approved by the local ethics committee of Tuebingen.

Innsbruck

Diagnosis and recruitment of ET patients was based on the MDS Consensus Criteria for Classical Essential Tremor. Patients were recruited at the movement disorder clinical in the Department of Neurology, Medical University Innsbruck. All patients underwent clinical examination, recording of demographic and clinical information, as well as recording of treatments received, family history and data on race and ethnic group. Written informed consent of all patients was obtained. The clinical-epidemiological study received approval by the Ethics Committee. Excluded were patients with abnormal neurologic signs, enhanced physiological tremor, recent exposure to tremorogenic drugs or drug withdrawal, direct or indirect injury to the nervous system within 3 months preceding tremor onset, or clinical history of psychogenic tremor, sudden onset or stepwise deterioration of tremor.

Baylor College of Medicine (BCM)

TRIG criteria for 'definite' or 'probable' ET were used for diagnosis of referred patients to the movement disorders clinic. Exclusion criteria were abnormal neurologic signs, enhanced physiological tremor, recent exposure to tremorogenic drugs or drug withdrawal states, direct or indirect trauma to the nervous system within 3 months

preceding tremor onset, historic or clinical evidence of psychogenic tremor and evidence of sudden onset or stepwise deterioration of tremor.

Columbia/Yale

Patients were recruited at the Neurological Institute of New York, Columbia University as part of a clinical-epidemiological study. The study was approved by the Institutional Review Board at Columbia University. Signed informed consent was obtained from all participants.

Patient assessment included a demographic and medical history questionnaire, information on first- and second-degree relatives with nonspecific tremor, ET or Parkinson's disease, self-reported tremor onset age, videotaped neurological examination, as well as self-reported data on race and ethnicity. Diagnosis was reconfirmed following the viewing of the videotaped neurological examination by a senior neurologist specializing in movement disorders (E.D.L). Exclusionary criteria for ET included bradykinesia or other signs of parkinsonism, except isolated rest tremor. No patients or controls had signs of amyotrophic lateral sclerosis (ALS) or a history of ALS.

Montreal

Ethical approval for the recruitment of ET patients was obtained from multiple institutes: Centre de recherche du Centre hospitalier de l'Université de Montréal (CIRHUM; project no: ND043076), Centre hospitalier affilié universitaire de Québec (CHA; project no: PEJ-280) and the Sainte Justine University Hospital Center (CHSJ; project no: 2352). Blood samples used for DNA extraction and cell line establishment were collected from ET patients after obtaining written consent. ET diagnoses were reviewed by a senior neurologist. Exclusion criteria were exaggerated physiological tremor, other neurological deficits (parkinsonism, polyneuritis, others), and presence of orthostatic or psychogenic-like tremors.

Virginia Commonwealth University (VCU)

ET patients were given a research diagnosis based on published research criteria following examination by a movement disorder neurologist or review of longitudinal movement disorder clinical notes with examination, medical response data, handwriting and research interview data. All patients included in the study gave their written consent. Self-identified ethnicity was also obtained from all participants. No known related samples between or within groups were present in the study. Patients with diagnoses of both ET and Parkinson's disease were excluded, as well as patients with signs of dystonia or other significant neurological diagnoses (history of or evidence upon examination). Parkinson's diagnoses were based on UK Brain Bank criteria. Control subjects were recruited based on availability of clinical research control samples and fulfilment of previously mentioned criteria (see above). Exclusion of control subjects were based on reported personal or family history of tremor, ET, Parkinson's, dystonia and other significant neurological diagnoses.

National Institute of Health (NIH)

ET patients that met the MDS Consensus Criteria for Classical Essential Tremor were recruited within the National Institutes of Neurological Disorders and Stroke, Intramural Research Program under a genotype-phenotype protocol. This protocol was approved by the NIH Combined Neurosciences IRB. Written consent was obtained for all participant prior to recruitment.

Mayo Clinic Florida

Patients and control subjects were recruited under the Mayo Clinic IRB Committee approved protocol, IRB#: 1087-98, P.I. Zbigniew K. Wszolek, M.D. entitled: "Clinical and Genetic Studies of Neurodegenerative Syndromes, Dystonia, and Restless Leg Syndrome". All patients and controls signed the consent form. Material Transfer Agreement specifying the legal conditions of collaboration between Mayo Clinic Florida (MCF) and McGill University was developed prior to transfer of de-identified data and DNA samples from MCF to McGill University. The patients were diagnosed with essential tremor (ET) by movement disorders specialists according to the Movement Disorders Society ET diagnostic criteria. Both sporadic and familial cases were collected. The

patients with other than ET neuroglial symptoms and signs were excluded from this study. Also the patients in which ET was part of a broader phenotype and the patients who first presented with ET and later develop additional clinical/neurological features such as rest tremor, rigidity, bradykinesia, dystonia, tremor, chorea,and others were excluded from this study. The controls were mainly opportunistic (not specifically sought for this study), and mainly included spouses, other family members, and care-givers. A subset of controls was recruited through electrodiagnostic laboratory and included the patients who were electro-diagnostically studied for the median neuropathy at the wrist, ulnar neuropathy at the elbow, peroneal neuropathy at the fibular head, or minor complains consistent with peripheral neuropathy.

University of British Columbia (UBC) / University of Saskatchewan

Patients were recruited from the Movement Disorder Clinic Saskatchewan (MDCS). Diagnosis was made based on postural and/or kinetic tremor of upper limbs or head tremor with no other known neurological cause. In certain cases, brain pathological studies were performed following autopsy to identify known tremor causes.

University of Navarra (Spain)

The study was approved by the University of Navarra's Ethical Committee and all informed consent was obtained to patients prior to their inclusion in the study. Patients were diagnosed by a movement disorder neurologist with either 'definite' or 'probable' ET based on current criteria used by the Department of Neurology from the Clinica Universidad de Navarra, Pamplona, Spain. Only one affected subject from each family with the familial form of the disease were included in the study. Controls were unrelated healthy subjects or spouses from PD cases, recruited within the same university center or from the community. Exclusion criteria were patients with other neurological disorders or a positive family of neurodegenerative disease.

Hopsital Universitario del Sureste (Arganda del Rey) and University of Extremadura

Participants were recruited from the University Hospitals of Sureste (Arganda del Rey, Madrid) and *Principe de Asturias* (Alcalá de Henares, Madrid). Diagnosis of ET patients

were based on ‘definite’ ET criteria from MDS. Only patients without other neurological disorders and without thyroid dysfunction were included. All ET patients had at least 1 first-degree relative with ET and only 1 family member per family was included. Control particpiants were recruited from the Infanta Cristina University Hospital (Badajoz) and from the Clinica Universitaria de Navarra (Pamplona). These were healthy, gender-matched, unrelated participants without tremor. Informed consent was obtained before inclusion in the study. Ethical approval was given by the Ethics Committees of the Infanta Cristina University Hospital and the *Principe de Asturias* University Hospital.

University of Genoa (Genova, Italy)

ET patients with ‘definite’ or ‘probable’ ET based on criteria from the Tremor Investigation Group[2,3] were recruited at the collaborating Neurological Centres (Naples and Bologna) by specialized neurologists. Assessment of patients involved neurological examination, identification of tremor type and its topography as well as recording of associated signs or symptoms, drugs in use, videotaping and other paraclinical investigations. Self-reported age of onset was recorded. Samples from patients and relatives (when possible) were obtained after written informed consent was given. Age- and gender-matched controls were recruited among the spouses of Italian ET patients or other subjects without neurological dysfunctions (as assessed by the same neurologists and protocols previously mentioned).

CARTaGENE

The CARTaGENE cohort was used as additional controls for the study. CARTaGENE is population-based biobank of individuals from Quebec, Canada[4]. It is a long-term cohort of over 20,000 participants that consent to visiting assessment sites to provide health and sociodemographic information. Individuals without neurological or psychiatric disorders were used as controls. Further details about CARTaGENE are described in Awadalla et al (2013)[4].

Quality control and association analyses for clinical cohorts

The samples from both clinical cohorts 1 and 2 that were genotyped on the Axiom CEU Array and Illumina GSA array respectively. and followed standardized quality control (QC), imputation, and post-imputation QC. Briefly, samples were removed if >2% missingness, autosomal heterozygous deviation (Fhet <0.2), and failed sex check[5]. Low quality SNPs were removed based on Hardy-Weinberg Equilibrium (P>1E-6), SNP missingness < 0.02 after sample removal. Samples were mapped against the 1000 Genomes Project phase 3 reference panel after pruning and removing SNPs from high-LD regions, and only individuals of inferred- European ancestry were retained[6]. No relatedness filter was done because a linear-mixed model was used subsequently to account for relatedness. Imputation was done using the Sanger Imputation Server with Eagle v2.3.5 and the Haplotype Reference Consortium Reference Panel v1.1[7,8]. A Bayesian linear-mixed model was done using BOLT-LMM 2.3.4 including 20 principal components (PCs) and sex as covariates to accelerate convergence[9]. The non-infinitesimal model was used if there was an increase in power.

Description of population-level cohorts

Two separate biobanks, 23andMe and the UK BioBank were used for the study.

23andMe

The 23andMe samples consisted of individuals who sent saliva samples to 23andMe Inc. and agreed to partake in research and answered questions related to ET. Participants involved provided informed consent and answered questions in accordance to their human subjects' protocol, which was approved and reviewed by Ethical and Independent Review services, an AAHRPP-accredited institutional review board. 23andMe provided summary statistics of their ET GWAS that included cases that responded "yes" to the question "Have you ever been diagnosed with a neurological condition?" and later indicated that they had been diagnosed with ET or indicated that they had been diagnosed with Parkinson's disease but were later re-diagnosed with ET. Cases were excluding individuals genotyped on the 23andMe v1 genotyping chip and individuals younger than 30 years old.

Controls were recruited from all eligible participants. The age, sex, genotyping platform and survey response time were matched against the cases that met the same criteria but excluded cases that were genotyped on the 23andMe v1 and subjects under 30 years old. Controls were iteratively matched by age, gender and genotyping platform. This was done by splitting individuals into 20 age bins, each containing an approximately equal number of ET cases, date of entry to the 23andMe research cohort (by splitting individuals into 20 bins, each containing an approximately equal number of individuals), genotyping platform, and sex.

Participants were restricted to a set of individuals with European ancestry through an analysis of local ancestry. Briefly, the algorithm partitions phased genomic data into intervals of 300 SNPs. Iteratively for each window, a support vector machine (SVM) was used to characterize each haplotype into reference populations (1000 Genomes, HapMap, Human Genome Diversity Project, 23andMe customers reporting four grandparents from same country). To account for relatedness, a segmental identity-by-descent (IBD) estimation algorithm was used. Individuals that shared roughly 20% of the genome were considered related. When selecting for case/control, cases were preferentially retained. Imputation was done with an imputation panel combining the 1000 Genomes Phase 3 haplotypes with the UK10K reference panel[10]. Participants from the v5 platform and prior, the tool Finch was used to phase participant data. For samples from the v5 array, Eagle2 was used.

Association tests for genotyped data was done using a logistic regression assuming additive effects. For imputed data, the dosage was used over hard-call genotypes. Quality control was done independently for dosage and imputed data. The directly genotyped SNPs were flagged for failed QC if they were only genotyped on v1 or v2 platforms due to smaller sample size. SNPs with a Hardy-Weinberg ($P<10E-20$) or a call rate of <90% were flagged. Any SNPs that were flagged by ANOVA of genotypes against a factor dividing genotyping date into 20 roughly equal bins were additionally flagged. SNPs with large sex-effects were additionally flagged (ANOVA of genotypes, $r2 >0.1$). Finally, SNPs with probes that match multiple genomic positions in the hg19

reference genome were flagged. For the imputed data, any SNPs with an rsq < 0.30 or evidence of batch effect were flagged based on an ANOVA F-test of SNP dosage against a factor of v4 and v5 platform (P<10E-50). Across all data, SNPs that do not have a sample size >20% of the total GWAS were flagged. Furthermore, SNPs that did not converge during logistic regression.

UK BioBank

Cases were individuals that answered "yes" to have benign / essential tremor in the UK Biobank. Controls were individuals that answered "no" to the question. A total of 216 cases and 395,209 controls were included. Summary statistics were ascertained from Zhou et al. (2018) (https://www.leelabsg.org/resources), which were publicly available summary statistics of UKBiobank phecode binary phenotypes that were ran using SAIGE (Scalable and Accurate Implementation of Generalized mixed model). SAIGE was used due to the large case-control imbalance. Data analysis can be found in detail in Zhou et al. (2018)[11].

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DISCUSSION

The focus of this work is on essential tremor (ET), a common movement disorder characterized by action tremor with high heritability. The age of onset is typically bimodal and occurs during the second and sixth decade of life, with severity commonly increasing with age[3]. It can be comorbid with other disorders such as Parkinson's disease (PD) and depression[129]. Moreover, it is considered a major burden towards quality of life. There currently are not any approved medications developed specifically for ET, and current pharmacotherapies consist of trial-and-error with diminishing benefit with prolonged use[14,15].

Given that ET has a large burden on society and affected individuals, it gives motivation to research the disorder and which molecular and genetic risk factors are associated, in order to improve detection and putative treatment. The work within this thesis and completed as part of my doctorate requirement has the goal of characterizing genetic and molecular susceptibility factors for ET by using a variety of different agnostic approaches. The overarching aim of this thesis sought to agnostically identify novel genes and common variant risk factors that drive ET etiology and to leverage these genetic findings to inform on downstream molecular consequences and pathways in ET. The combination of studies in this thesis represents several first and major contributions towards understanding ET genetics and biology.

The findings and experimental design are largely divided into two different approaches to understanding ET. Specifically, chapter 2 elucidates on the first aim, to investigate the transcriptomic differences in the cerebellum of ET patients compared to controls and leverage multi-omics data to identify susceptibility factors for ET. One of the main results was demonstrating that the dysregulated gene, *SHF*, converged on a transcriptome- and phenome- wide level, making it a strong candidate or marker gene for ET. Chapter 3 of this thesis follows an agnostic genome-wide approach to identify ET genetic risk factors and identified several cerebellar genes of interest. One of the main results was identifying several significant loci and identifying the proportion of ET heritability explained by common variants.

The first objective was done using high-throughput next-generation sequencing of the RNA. Chapter 2 investigates ET risk factors through the following:

1. Post-mortem cerebellum tissue from ET cases and matched controls were carefully selected and underwent RNA sequencing. The majority of studies have solely focused on genetic differences but have not considered the transcriptome. Given the lack of replicability across ET genetic studies, we sought to look at transcriptional consequences to better understand ET risk factors. We identified several dysregulated genes of interest such as *SHF* and *CACNA1A*. The *SHF* gene was significantly associated with blood pressure medication usage, which is commonly used to treat ET. After, we showed that treatment of cerebellar cells with beta-blockers led to an increase in *SHF* expression.

2. We found that several of the differentially expressed genes pointed towards the importance of calcium pathways and axon guidance. After, we saw convergence across pathways with genetic data. We also demonstrated that the dysregulated genes tended to be enriched in the cerebellum.

Based on the findings from chapter 2, we suggest that genes such as *SHF* and *CACNA1A* may be important towards understanding ET. Given that *SHF* is associated with ET medication usage and treatment of cells leads to an increase in *SHF* expression, dysregulation of the gene may either be a marker for ET or a risk factor. Currently, there is not enough data to tease apart the direct effects *SHF* may have on ET risk.

Future studies that leverage whole-genome or whole-exome sequencing could assess whether there is an increased burden of loss of function or missense variants in this gene compared to controls. This could provide more evidence on whether this gene is directly implicated in ET risk or is a potential marker for ET.

Given the results, we hypothesize that dysregulation of calcium in the cerebellum may lead to the onset of ET. Prior studies have shown that calcium channel blockers such as nifedipine and verapamil, lead to a 71.4% (standard deviation of 22.6%) increase in tremor intensity for ET patients[80]. Furthermore, Nifedipine also increased tremor in healthy controls by 56% (standard deviation of 21.9%). This further provides evidence that dysregulation of calcium may be associated with tremor manifestation.

There are currently an increasing number of studies that are starting to implement the importance of calcium for ET risk. Future studies could investigate whether calcium is dysregulated in induced pluripotent stem cells of ET patients. This data could also be linked with transcriptomic data to identify gene expression and phenotypic correlates.

The second objective was to identify common variants associated with ET risk in a larger cohort than previous studies. Chapter 3 investigates these risk factors through the following:

1. A genome-wide association study of several genotyped cohorts was done using an inverse-variance meta-analysis. This was a multi-center effort of 17 different cohorts, consisting of 7,177 cases and 475,877 controls after quality control. From this, we identified five genome-wide significant loci. We used linkage disequilibrium score regression to estimate the heritability and found that approximately 18% of ET heritability can be explained by common variation in European ancestry individuals. We demonstrated that there is strong common variant genetic overlap with neurological and psychiatric traits such as Parkinson's disease and depression.

2. After, we had sought to functionally characterize the effects of these variants. We conducted a transcriptome-wide association study, which found that genes such as *BACE2* and *LINC00323* were associated with ET risk. We also found significant

enrichment in the cerebellar hemisphere, cerebellum, and axonogenesis pathways.

Based on the findings of chapter 3, we demonstrate that ET is a heritable disorder and there is a significant contribution of common variants towards ET heritability. We provide further evidence that the cerebellum is relevant to ET risk and hypothesize that amyloid protein may have a putative role in the disease, given that *BACE2* was one of the top TWAS genes. There has been previous literature that has shown that beta-amyloid levels have been higher in ET cases compared to controls and potential neurodegeneration in ET. Given that beta-amyloid tends to be a marker for neurodegeneration, this may provide further evidence that neurodegenerative processes occur in ET[124].

Interestingly, we also found a strong genetic correlation (~30%) between ET and Parkinson's disease, suggesting that there may be a high degree of common variant overlap. In the literature, there are epidemiological studies that suggest ET may be a risk factor for Parkinson's disease, but bidirectionality is often not assessed. For instance, Parkinson's patients may later develop ET and the comorbidity goes unnoticed or is masked by Parkinson's disease[9–11]. The latter would support the idea of pleiotropy and explain the high degree of genetic overlap.

However, there could be alternative reasons for this large overlap. First, it may also be due to heterogeneous phenotyping, where Parkinson's disease and ET are

misdiagnosed. This would lead to inclusion of Parkinson's samples in the ET GWAS or inclusion of ET samples in the Parkinson's GWAS. Previous research has shown that there is a high level of misdiagnosis.

To assess whether we could identify ET specific markers that are not pleiotropic with ET, we conducted a multi-trait conditional analysis. In brief, we conditioned the ET GWAS by the Parkinson's disease GWAS. This would theoretically remove any significant ET loci that are pleiotropic with Parkinson's disease. We found that the top five loci were still significant after conditioning, suggesting that these may be robust markers that differentiate ET from Parkinson's disease.

Future studies could also conduct a case-case GWAS of Parkinson's disease compared to ET. This could identify loci that have opposite directional effects, which can be quantified into a risk score to differentiate ET and Parkinson's disease.

Given that there are no quantitative markers for ET, there may be opportunity for the genetics of ET to define the phenotype. We hypothesize that genetic profiles will eventually identify novel ET subtypes and differentiate ET from other movement disorders. This will require much larger sample sizes, but with the advent of large genetic datasets such as All of Us, this becomes more feasible.

Across both chapters, we saw that there was convergence for cerebellar and axon pathways. Given that both have been previously implicated in ET, it provides further

evidence on the relevance to the disease. Moreover, we demonstrate the importance of using multiple different methods and independent datasets in order to identify potential biological insights. The findings presented in this thesis and shared with the research community represent important contributions to our understanding of susceptibility factors for essential tremor. However, given the heterogeneity and complexity of the disorder, there is still much work to be done towards characterizing and understanding the disease. With the recent initiatives towards an essential tremor genetics consortium, we expect many larger studies that will help elucidate the genetics of ET.

Conclusions and Future Directions

Essential tremor (ET) is a movement disorder that is driven by both environmental and genetic susceptibility risk factors. Currently, the majority of the literature points towards cerebellar and Purkinje dysfunction for ET, however, the genetic findings for ET have been inconsistent and there are issues with replication. To better understand genetic risk factors for ET, the work in this thesis leverages several genome-wide and transcriptome-wide methods to identify genes that may be associated with ET. Several major findings include the first transcriptomic study for ET, where we identified differentially expressed genes in ET post-mortem brain tissue, that are associated with beta-blocker medication. Furthermore, we conducted the current largest genome-wide association study and identified 5 genome-wide risk loci. We also determined that 18% of ET heritability can be explained by common variants. These contributions ultimately help with filling in large gaps of knowledge on ET genetics and molecular pathways.

With the development of new approaches and availability of larger datasets such as All of Us, there will be opportunity to further dissect the biology of ET. Methods such as whole-genome sequencing and long-read sequencing will be quintessential to continue identifying genetic risk factors of ET. Furthermore, a push towards more open science practices and data sharing will allow for aggregation of all existing ET data. Through this, we will hopefully expand our understanding of ET genetics.

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APPENDICES

Appendix 1: Significant contributions by the author of this thesis towards other projects

First-author publications not included in the thesis:

Liao, C., Laporte, A., Spiegelman, D., Akçimen, F., Joober, R., Dion, P.A., Rouleau, G.A. (2019) Transcriptome-wide association study of attention deficit disorder identifies associated genes and phenotypes. Nature Communications, 10, 4450

Liao, C., Akçimen, F., Diez-Fairen, M., Houle, G., Ross, J., Schmilovich, Z., Spiegelman, D., Vuokila, V., Catoire, H., Meijer, I., Pastor, P., Rajput, A., Dion, P.A.,

Rouleau, G.A. (2020) Assessing the NOTCH2NLC GGC expansion in European essential tremor patients. Brain, 143(11), e89-e89

Liao, C., Vuokila, V., Laporte, A., Spiegelman, D., Dion, P.A., Rouleau, G.A. (2019) Probabilistic fine- mapping of transcriptome-wide association study for miserableness. BioRxiv.

Liao, C., Vuokila, V., Catoire, H., Akçimen, F., Ross, J.P., Bourassa, C.V., Dion, P.A., Meijer, I.A., Rouleau, G.A. (2019) Increased expression of genetically-regulated *FLT3* implicated in Tourette Syndrome. BioRxiv.

Liao, C., Sarayloo, F., Rochefort, D., Akçimen, F., Spiegelman, D., Laporte, A., He, Q., Dion, P.A., Rouleau, G.A. (2019). Transcriptomic changes resulting from *STK32B* overexpression identifies pathways potentially relevant to essential tremor. Frontiers in Genetics 11, 813

Liao, C., Houle, G., He, Q., Laporte, A., Dion, P.A., Girard, S.L., Rouleau, G.A. (2018). Investigating the association and causal relationship between restless legs syndrome and essential tremor. *Parkinsonism & Related Disorders*. 61, 238-240.

Contributions to other projects not directly relevant for the thesis topic:

Mullins, N., …**Liao, C.** as part of the International Suicide Genetics Consortium.. et al. (2020) Dissecting the shared genetic architecture of suicide attempt, psychiatric disorders and known risk factors. Biological Psychiatry.

Mullins, N., …**Liao,C.** as part of the Bipolar Disorder Working Group of the Psychiatric Genomics Consortium (PGC).. et al. (2020) Genome-wide association study of over 40,000 bipolar disorder cases provides novel biological insights. Nature Genetics, 53, 817-829.

Akçimen, A., Sarayloo, F., **Liao, C.,** Ross, J., Oliviera, R.D.B., Dion, P.A., Rouleau, G.A. (2020) Transcriptome-wide association study for restless legs syndrome identifies new susceptibility genes. Communications Biology, 3 (1), 1-15

Khaayachi, A., Ase, A., **Liao, C.,** Kamesh, A., Kuhlmann, N., Schorova, L., Chaumette, B., Dion, P.A., Alda, M., Séguéla, P., Rouleau, G.A., Milnerwood, A. (2020) Chronic lithium treatment alters the excitatory/inhibitory balance of synaptic networks and reduces mGluR5-PKC signaling. Journal of Psychiatry & Neuroscience. [Accepted]

Demontis, D., ...**Liao.C.** as part of the ADHD Working Group of the Psychiatric Genomics Consortium (PGC).. et al. (2021) Identification of risk variants and characterization of the polygenic architecture of disruptive behavior disorders in the context of ADHD. Nature Communications, 12 (1), 1-12.

Alfradeique-Dunham, I., Al-Ouran, R., Voelln, R.V., Blauwendraat, c., Hill, E., Luo, L., Stillwell, A., Young, E., Tan, M., **Liao, C.**, Brockmann, K., Schulte, C., Gasser, T., Pihlstrom, L., Marinus, J., Van Hilten, J.J., Grosset, D., Heutink ,P., Shulman L.M., Liu, Z., Rouleau G.A., International Parkinson's Disease Genomics Consortium (IPDGC), Nalls, M.A., Singleton, A.B., Morris, H., Jankovic, J., Shulman, J.M. (2020) Genome-Wide Association Study Meta-Analysis for Parkinson Disease Motor Subtypes. Neurology Genetics, 7, 2

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Castonguay, C.E., **Liao, C.**, Khayachi, A., Houle, G., Ross, J.P, Dion, P.A., Rouleau, G.A. Convergent transcriptomic targets of propranolol and primidone identify potential biomarkers for essential tremor. (2021) BioRxiv.

Appendix 2. Ethics certificate for the RouBank.

MUHC REB – NEUPSY / CÉR CUSM – NEUPSY
3801, rue University, # 686
Montréal (Québec) H3A 2B4
Tel. 514.398.1046
reb.neuro@mcgill.ca
www.leneuro.ca

Annual renewal submission

Submit date: **2021-02-10 10:11**
Project's REB approbation date: **2015-03-20**
Project number(s): **2015-164, MP-CUSM-14-051, MP-37-2015-164**
Form status: **Approved**

Submitted by: **Zaharieva, Vessela**
Nagano identifier: **ROU BANK**
Form: **F9-70479**

Administration

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

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

3. **Comments on the decision:**

   The renewal for ethics approval applies for the following centres:
   - McGill University Health Centre
   - Centre Hospitalier de l'Université de Montréal
   - CHU - de - Quebec
   - CHU - Sainte-Justine
   - CIUSSS de l'Ouest-de-l'Ile-de-Montréal

4. **Renewal Period Granted:**
   From 2021-03-20 Until 2022-03-19

5. **Date of the REB final decision & signature**

2021-02-12

Signature

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

6. FWA 00000840 - FWA 00004545

7. **Local REB number**

IRB00010120

8. **Note:**

In order to be in compliance with Good Clinical Practices, the MUHC REB (when acting as the Reviewing REB), and the PM of the MUHC does not directly communicate with sponsors. The communication channels existing between the PIs and the sponsors will continue to ensure the transmission of documents.

A. General information

1. **Indicate the full title of the research study**

Rou Bank.

2. **If relevant, indicate the full study title in French**

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

Rouleau, Guy

4. **Are there local co-investigators & collaborators involved in this project?**

No

5. **For each participating centre part of the Québec health and social services network (RSSS), indicate the name of the external investigator**

Sylvain Chouinard

What is the name of the participating center(s)?

CHU-Montréal

Nicolas Dupré

What is the name of the participating center(s)?

CHU-de-Québec

Jacques Michaud

What is the name of the participating center(s)?

CHU-SJ

Gustavo Turecki

What is the name of the participating center(s)?

CIUSSS-OMTL

Ridha Joober

What is the name of the participating center(s)?

CIUSSS-OMTL

6. **Indicate the name and the affiliation of the external collaborator(s),(if any)**

Voir la liste des sections 5 & 9

7. **Identify the study coordinator(s)**

Zaharieva, Vessela

Indicate the role of the collaborator(s)

Administrative agent

Mirarchi, Cathy

Indicate the role of the collaborator(s)

Administrative agent

B. Project development

1. **Study start date:**

2006-09-21

2. **Expected ending date of the study:**

- ☐ Determined date
- ☑ Undetermined date

3. **Date of recruitment of the first participant?**

- ☑ 1st enrollment date is...
- ☐ No participant enrolled

1st participant enrollment date:

2006-09-22

4. **Indicate the current study status at MUHC.**

Study and recruitment in progress

5. **Add a brief statement on the study status**

Study is still in progress

6. **Information about the participants at this institution, since the beginning of the project**

Number of participants who have been recruited

17071

Number of participants who have not yet completed the study (still in progress)

0

Number of participants who've completed the study

17071

Number of participants who were recruited to the study, but who were then excluded or withdrawn:

0

Number of participants who dropped out (voluntary withdrawal):

0

Number of participants who died during the study

0

7. **Information about the participants at this institution (MUHC) since the previous REB approval**

Number of participants who have been recruited

100

Number of participants who have not yet completed the study (still in progress)

0

Number of participants who've completed the study

100

Number of participants who dropped out (voluntary withdrawal):

0

Number of participants who died during the study

0

8. **Since the previous REB approval (annual renewal or initial approval):**

Were there any changes to the protocol (or to the databank management framework) ?

No

Specify the current version/date:

version 1, March 14, 2016

Date approved by the REB:

2016-03-22

Were there any changes to the information and consent form?

Yes

Specify the current version/date:

version 2, December 2020

REB approval date:

2021-01-29

Were there any reportable adverse events at this site (or, for multi-center projects, at an institution under the jurisdiction of our REB) that should be reported to the REB under section 5.2.1 of " SOP- REB-404001 " ?

https://muhc.ca/cae/page/standard-operating-procedures-sops

No

Has there has been any new information likely to affect the ethics of the project or influence the decision of a participant as to their continued participation in the project ?

No

Were there any deviations / major violations protocol (life -threatening or not meeting the inclusion / exclusion criteria)?

No

Was there a temporary interruption of the project?

No

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

No

Has the REB been notified of a conflict of interest - (apparent , potential or actual), of one or more members of the research team - that was not known when it was last approved project?

No

Do you want to bring any other info to the REB's attention?

No

9. **For all external participating institutions, please answer the following questions:**

Please select the name of the institution concerned and attach the "Formulaire de renouvellement annuel pour les projets sites externes - Projets multicentriques":

CHU-de-Québec

Please print a copy of the "Formulaire de renouvellement annuel pour les sites externes" (see link below), have it completed by other institutions and attach it here.

Formulaire de renouvellement pour les sites externes (MP project)

Submit da ibmie: : Pnmbr oPij iPcjPd eoidPnPed uPdnsiRoei EdBPr ipenP(

Demande de renouvellement annuel pour les sites externes-CHUdu Quebec 2021 (002)N. Dupre.pdf

CHU-SJ

Please print a copy of the "Formulaire de renouvellement annuel pour les sites externes" (see link below), have it completed by other institutions and attach it here.

Formulaire de renouvellement pour les sites externes (MP project)

Submit da ibmie: : Pnmbr oPij iPcjPd eoidPnPed uPdnsiRoei EdBPr ipenP(

Demande de renouvellement annuel pour les sites externes-CHUSJ 2021 (003)-signed.pdf

CHU-Montréal

Please print a copy of the "Formulaire de renouvellement annuel pour les sites externes" (see link below), have it completed by other institutions and attach it here.

Formulaire de renouvellement pour les sites externes (MP project)

Submit da ibmie: : Pnmbr oPij iPcjPd eoidPnPed uPdnsiRoei EdBPr ipenP(

document03-02-2021-090642 CHUM SC.pdf

CIUSSS-OMTL

Please print a copy of the "Formulaire de renouvellement annuel pour les sites externes" (see link below), have it completed by other institutions and attach it here.

Formulaire de renouvellement pour les sites externes (MP project)

Submit da ibmie: : Pnmbr oPij iPcjPd eoidPnPed uPdnsiRoei EdBPr ipenP(

20210209 Demande de renouvellement annuel pour les sites externes-CIUSS-OMTL 2021 GT.pdf

Is there any institution's info (pdf form) missing?

No

10. **Is there a data safety monitoring committee analyzing data on the safety and efficacy of the treatment?**

No

C. Signature

1. **I confirm that all information is complete & accurate.**

First & last name of person who completed the submission

Vessela Zaharieva
2021-02-10 10:11