LINGO1 and clinical characteristics of essential tremor

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Ritgerð til meistaragráðu
Háskóli Íslands
Læknadeild
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HÁSKÓLI ÍSLANDS

LINGO1 og einkenni eðlislægs handskjálfta

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Ritgerð til meistaragráðu í líf- og læknisfræði

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Ágrip

Ritgerðin lýsir sambandsrannsókn sem spannaði erfðamengið (*genome wide association study*, GWAS) í leit að erfðabreytileika sem eykur líkurnar á eðlislægum handskjálfta (*essential tremor*, ET). Í rannsókninni, sem er fyrsta GWAS rannsóknin á eðlislægum handskjálfta á heimsvísu, er sýnt fram á að allel G í einkirnisbreytileikanum (*single nucleotide polymorphism*, SNP) rs9652490 í innröð 3 í geninu LINGO1 er í sambandi við hættuna á að fá handskjálfta. LINGO1 er gen sem m.a. tekur þátt í stjórnun uppbyggingar á taugasímum og þroskun oligodendrocyta. Einnig eru í ritgerðinni metin áhrif breytileikans á ýmis einkenni handskjálfta.

Í sambandsrannsókn fannst erfðamark í LINGO1 sem stóð í erfðamengismarktæku (*genome wide significant*) sambandi við eðlislægan handskjálfta með áhættuhlutfall (*odds ratio*, OR) upp á 1.55 og P-gildið (*P*) 1.2×10^{-9}. Þegar upphaflega sýnasafnið var útilokað frá rannsókninni og hún endurtekin eingöngu með viðbótarefniviði var sambandið enn marktækt (OR = 1.44; *P* = 0.001).

Til að varpa frekara ljósi á orsakasamband LINGO1 við handskjálfta var einnig litið á áhrif erfðamarksins rs9652490 á ýmis einkenni handskjálfta: kyn, aldur við fyrstu einkenni, fjölskyldusögu, höfuðskjálfta, svörun við propranolol lyfjagjöf og inntöku áfengis, auk niðurstaðna fingur-nef-prófa og spíralteiknunar-prófa (Arkímedesarspíralar).

Arfgerð fyrir erfðamarkið rs9652490 var greind í einstaklingum sem höfðu eðlislægan handskjálfta og viðbótarupplýsingar um einkenni hans, frá fjórum löndum (fjöldi einstaklinga með arfgerðargreiningu í sviga): Íslandi (451), Þýskalandi (282), Bandaríkjunum (135) og Austurríki (123). Einnig var arfgerð viðmiðunareinstaklinga úr sömu þýðum greind fyrir rs9652490 (N = 32.811, 333, 387 og 241, í sömu röð og áður).

Einstaklingum með eðlislægan handskjálfta var skipt í fjóra hópa eftir aldri við upphaf einkenna: snemmbúið upphaf einkenna (*early onset*: 0-19 ára, N = 220), miðaldursbúið upphaf (*intermediate onset*: 20-39 ára, N = 158), síðbúið upphaf (*late onset*: 40-59 ára, N = 158) og mjög síðbúið upphaf einkenna (*very late onset*: 60 ára eða eldri, N = 107). Í öllum hópum var marktækt samband milli rs9652490 og eðlislægs handskjálfta, og munurinn á áhættuhlutfallinu milli hópa var ekki marktækur. Þetta bendir til að rs9652490 miðli áhættu á upphafi einkenna eðlislægs handskjálfta á öllum aldursskeiðum.

Marktækt samband var á milli allels G í rs9652490 og eðlislægs handskjálfta, óháð því hvort fjölskyldusaga var til staðar (OR = 1.74, $P = 1.0\times10^{-10}$) eða ekki (OR 1.36, *P* = 0.0057); hvort sjúklingur var með höfuðskjálfta (OR = 1.68, *P* = 0.00048) eða ekki (OR = 1.62, *P* = 0.00011); og því hvort alkóhól dró úr skjálftanum (OR = 1.85, $P = 8.2\times10^{-8}$) eða ekki (OR = 1.49, *P* = 0.012). Sambandið milli allels G í rs9652490 og eðlislægs handskjálfta var mun sterkara í hópi þeirra sem svöruðu lyfjagjöf með propranololi (OR = 1.17, *P* = 0.00076) en meðal þeirra sem gerðu það ekki (OR = 1.17, *P* = 0.49), en munurinn á hópunum var þó ekki marktækur. Einnig var marktækt samband milli allels G í rs9652490 og eðlislægs handskjálfta, óháð því hvort sjúklingar þóttu hafa mjög mikinn/frekar mikinn skjálfta eða lítinn/engan skjálfta, bæði á fingur-nef-prófum (OR = 2.03 og P = 1.3×10^{-8} fyrir mjög mikinn/frekar mikinn skjálfta; OR = 1.54 og P = 0.0047 fyrir lítinn/engan skjálfta) og spíralprófum (OR =

1.90 og P = 2.6×10^{-8} fyrir mjög mikinn/frekar mikinn skjálfta; OR = 1.41 og P = 0.016 fyrir lítinn/engan skjálfta).

Breytileikinn í LINGO1 eykur ekki áhættuna á neinum sérstökum einkennum eðlislægs handskjálfta umfram önnur. Það getur verið að annar erfðabreytileiki leggi meira til þróunar tiltekinna einkenna. Því má vera að allel G í erfðamarkinu rs9652490 miðli áhættunni með því að ýta undir hættu á eðlislægum handskjálfta í samspili við erfðamörk sem segja fyrir um hvaða einkennum eðlislægs handskjálfta hverjum einstaklingi fyrir sig er hætt við að þróa með sér.

Abstract

This thesis reports on the first genome wide association study (GWAS) for genetic variants conferring risk for essential tremor (ET). It shows that the single nucleotide polymorphism (SNP) rs9652490 in intron 3 of LINGO1, a gene important in regulating axon regeneration and oligodendrocyte maturation, is associated with essential tremor (ET). It furthermore explores the effect of the variant on a range of clinical characteristics related to essential tremor.

A marker in LINGO1 was identified through a GWAS approach, showing genome wide significant association with essential tremor, with an odds ratio (OR) of 1.55 and P-value (*P*) of 1.2×10^{-9}. When the discovery dataset was excluded and the analysis done on only the follow-up material, the odds ratio was 1.44, with a P-value of 0.001.

To further clarify the role of LINGO1 in the aetiology of ET, this study also looked at the effect of the SNP rs9652490 on a range of ET characteristics: sex, age at onset, presence or absence of family history and head tremor, response to propranolol medication and alcohol, and the outcome of finger-nose and spirography tests (Archimedes spirals).

ET subjects with information on clinical characteristics from four countries, Iceland (N = 230), Germany (N = 225), USA (N = 111) and Austria (N = 81) were genotyped for marker rs9652490. Controls from the same populations were also genotyped for marker rs9652490 (N = 32,811; 333; 387; and 241, respectively).

The ET individuals were divided into four groups depending on the age at onset: early onset (0-19 years old, N = 220), intermediate onset (20-39 years old, N = 158), late onset (40-59 years old, N = 158) and very late onset (60 years or older, N = 107). All four groups showed significant association of rs9652490 to ET, and the odds ratio was not significantly different in the four age groups. Thus, rs9652490 confers risk of ET for all ages of onset.

There was significant association of allele G of rs9652490 to ET irrespective of presence or absence of family history (ORs 1.74 and 1.36, P-values 1.0×10^{-10} and 0.0057, respectively), presence or absence of head tremor (ORs 1.68 and 1.62, P-values 0.00048 and 0.00011, respectively), and irrespective of whether alcohol response reduced the tremors or not (ORs 1.85 and 1.49, P-values 8.2×10^{-8} and 0.012, respectively). While the association of allele G of rs9652490 is stronger for those who respond positively to medication with propranolol than for those who do not (ORs 1.94 and 1.17, P-values 0.00076 and 0.49, respectively), the difference between the two groups was not significant. There was also significant association of allele G of rs9652490 to ET irrespective of whether patients scored as having a severe/moderate tremor or mild/no tremor, both on finger nose tests (ORs 2.03 and 1.54, P-values 1.3×10^{-8} and 0.0047, for severe/moderate and mild/negative, respectively) and spirography tests (ORs 1.90 and 1.41, P-values 2.6×10^{-8} and 0.016, for severe/moderate and mild/negative, respectively).

The LINGO1 variant does not confer significantly greater risk to any particular clinical characteristic of ET. Thus, it is possible that other variants, conferring greater risk of ET, contribute more to the distinct characteristics. Allele G on marker rs962490 may therefore confer its risk by exacerbating the risk of ET in combination with other markers that dictate which characteristics of ET each subject may develop.

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Abbreviations

ANOVA: Analysis of variance

BTBD9: BTB (POZ) domain containing 9, a gene encoding for a BTB/POZ domain-containing protein.

CNS: Central nervous system

CNV: Copy number variation

CRIN: Clinical Research In Neurology

DNA: Deoxyribonucleic acid

DRD3: Dopamine D3 receptor, the ETM1 gene.

ET: Essential tremor

ETM1: Essential tremor 1, a gene associated essential tremor. See DRD3.

ETM2: Essential tremor 2, a gene associated essential tremor. See HS1BP3.

ETM3: Essential tremor 3, a genetic locus linked to essential tremor.

FET: Familial essential tremor

FHM: Familial hemiplegic migraine

G: A maximum likelihood based chi-square test statistic

GJB1: Gap junction beta-1 protein

GWAS: Genome wide association study

HCLS1: Hematopoietic cell-specific Lyn substrate 1

HS1BP3: HCSL1-binding protein 3, the ETM2 gene.

Hz: Hertz, a measure of frequency. In the context of the thesis, it measures tremors per second.

Ig: Immunoglobulin

IRB: Institutional Review Board

LBVET: Lewy body variant essential tremor

LD: Linkage disequilibrium. Among measurements of LD are D' and r^2.

LINGO1: Leucine-rich repeat- and Ig domain containing Nogo receptor-interacting protein 1

MAP2K5: Mitogen-activated protein kinase kinase 5

MDS: Movement Disorder Society

MEIS1: Meis homeobox 1

N: Sample size

Nogo: A protein. Also known as reticulon 4.

P: P-value (the probability of making a type I error)

PAR: Population attributable risk

PD: Parkinson's disease

PMP22: Peripheral myelin protein 22

PTPRD: Protein tyrosine phosphatase, receptor type, D

OR: Odds ratio

QQ-plot: Quantile-Quantile plot

RLS: Restless legs syndrome

Rtn4r: Reticulon 4 receptor. See Nogo.

Ser9Gly: Serine → Glycine amino acid change at position 9

SLC1A2: Solute carrier family 1 (glial high affinity glutamate transporter), member 2

SNP: Single nucleotide polymorphism

TRIG: Tremor Investigation Group

U.S., USA: United States of America

y: years

1 Introduction

1.1 Essential tremor

The term *essential tremor* has since late 19th century been used to describe an ailment of which tremor of the hands during movement (action tremor, kinetic tremor) is an essential component (1,2,3). It is often familial (2) and occurs without other neurologic signs (1). Essential tremor is typically characterized by such kinetic tremor of the arms and hands, and by a tremor that is present when the patient holds the arms in an outstretched position for an extended period of time (postural tremor) (4,5). Characteristically, it gets more pronounced at the end of a purposeful, intended movement (intention tremor, terminal tremor), as when touching a finger to one's nose (6), whereas in contrast to *e.g.* Parkinson's disease (PD), it is typically absent during rest (rest tremor) (2). When present, rest tremor has been associated with a more severe form of the disease (7).

ET is generally addressed as a benign ailment (6), sometimes even called 'benign essential tremor' (8), and may not always be easily distinguishable from normal, physiological tremor (9). Even so, it has a wide range of symptoms which may have a debilitating effect on the quality of life of the affected individual (5), and it has been suggested that ET imposes an increased risk of incident dementia (10), and an increased risk of mortality (11).

Generally, ET appears first in the hand and forearm. It can manifest in both arms simultaneously, but is more commonly mildly asymmetrical (2,12). The tremor is usually more severe in the non-dominant arm (12). Tremor of the head and voice may occur along with the upper extremity tremor, but the legs are rarely affected (13,14). ET typically has a frequency of 4-12 Hz (2,15,16), with lower frequency related to larger amplitude tremor (8). Tremor frequency decreases with age (15), and there is an inverse correlation between the amplitude and the frequency of the tremor (17). ET often impairs writing, drinking, eating and various other activities of daily living (18), and circumstances of stress, embarrassment and tiredness frequently make the symptoms worse (15).

Diagnosis of ET is typically done via an examination by a movement disorder specialist, involving tests for the presence of kinetic tremor and postural tremor and questionnaires for reporting difficulties with various activities of daily living, such as difficulty holding a tea/coffee cup, doing up buttons, writing a letter, etc. (5). The examination may include the subject being asked to provide a sample of their handwriting; a test where the subject touches their nose with their finger to test for signs of intention tremor (finger nose test); and a test where the subject is instructed to draw an Archimedes spiral from inside to out with at least five turns in it (spirography test; Figure 1) (19). Bain et al. (5) introduced a clinical rating scale for measuring the severity of tremor (Bain's scale), which has in recent years become standard in measuring severity in postural, kinetic and intention tremor, as observed through such tests and physical examination.

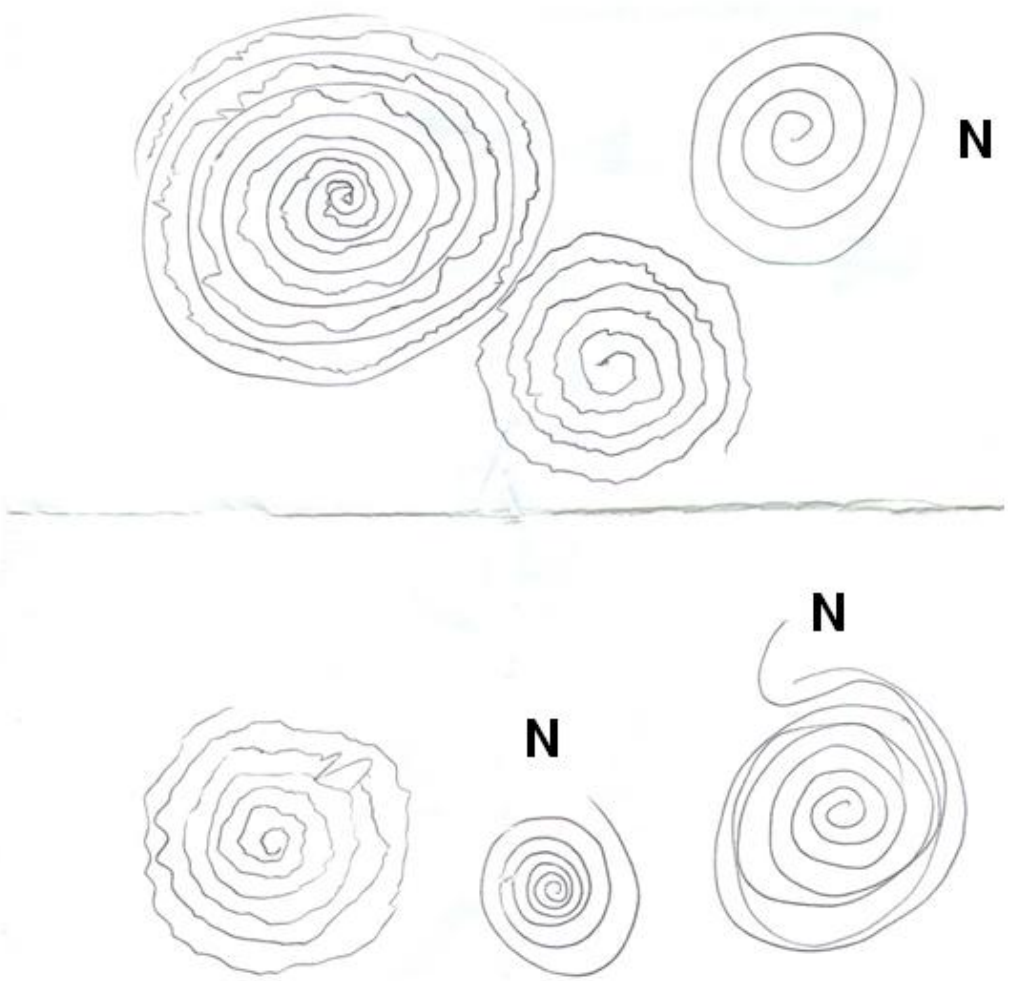

Figure 1: Examples of drawing of Archimedes spirals, exhibiting tremors of varying severity. N is normal, done by the observer to illustrate how the test is performed and for comparasion. Printed with permission from Dr. Finnbogi Jakobsson.

Since there are no histological or biochemical markers that can be used to establish the diagnosis of essential tremor, prevalence estimates of essential tremor vary widely, depending on the diagnostic criteria used and the ascertainment approach (20). As only a small fraction of individuals with essential tremor seek medical attention, studies based on clinical records are likely to underestimate the true prevalence for ET, one of the most common neurological disorders (20). Recent studies estimate the prevalence of ET in the range of 4%-6% for individuals over the age of 65 (20), and population based studies suggest the prevalence might be even higher (21,20). The annual incidence rate may be as high as 600 per 100,000 (22).

The age of onset of ET is extremely variable (2,23,24,13,25). The onset of essential tremor can be in childhood and throughout adulthood, and its rate of progression increases with a higher age at onset (23,26). Population-based studies show an onset peak for essential tremor in later life, with a barely discernible young-onset peak (23,25), whereas clinical series indicate that age of onset is bimodal (23,25). Additionally, having a family history of tremor is correlated with younger reported age

of tremor onset in ET (24). Thus, the bimodal appearance of the age at onset distribution may be due to the preferential referral to tertiary centres of individuals with young-onset, familial essential tremor (25). Familial cases seem to account for approximately 50% of young-onset cases from a population-based study (25), whereas more than 80% of young-onset cases from clinical series seem to be familial (25).

For an ailment with as wide a range of age of onset as essential tremor, it is of use to fractionate the material with respect to age groups for the purposes of analysis. In the literature, clear guidelines on how to fractionate by age of onset have not been defined, but while there are notable exceptions (15), a number of studies present data on age of onset based on 10-year age strata (27,13,21,25,14) or, in cases where data is sparse, 20 year age strata (27), following life decade of onset.

In general, males and females are affected equally by ET (2,21), although it has been noted that pediatric cases of ET are three times more likely to be males than females (28). Also, females are at a four- to six-fold increased risk of head tremor, when compared to males (29,30).

While environmental factors have been proposed to play a role in ET (31,32), including harmane, lead, and pesticides (32) and a protective effect of cigarette smoking (33,34), the disorder runs strongly in families, and genetic factors are believed to contribute to its onset. This is supported by the observation that pairwise concordance is two times higher (35) in monozygotic twins than in dizygotic twins (60% vs. 30% respectively) (31). An analysis restricted to cases of probable and definite ET estimated the concordance rate for monozygotic twins as 93% (vs. 29% for dizygotic twins) (35), suggesting ET as a good candidate for a phenotype to be used for linkage and association studies.

1.2 Neuropathology of essential tremor

While ET has traditionally been viewed as a monosymptomatic disease (36,37), a view of ET as a heterogeneous disorder has recently gained appreciation (38,39,37,40,41).

Since the 1st half of the 20th century, it has been known that ET is associated with a cellular reduction in the Purkinje layer of the cerebellum (2). In neuropathological studies, ET brains typically show cerebellar degenerative changes, ranging from Purkinje cell loss and cerebellar sclerosis, to increase in the numbers of Bergmann glia (40,41). A pattern of Lewy bodies in the brainstem has also been noted (40,41), suggesting a possible Lewy body variant form of essential tremor (LBVET) in patients with brainstem Lewy bodies and a relatively preserved cerebellum (42). Even so, pathological findings in recent studies on brains of affected individuals support the notion of the cerebellum as a potential hotspot of morphologic changes (43).

The findings of loss of Purkinje cell density (44), Purkinje cell heterotopias (45), cerebellar gliosis (40,41), and axonal swellings of Purkinje cells (torpedoes) (46,47) give rise to the speculation that essential tremor should be considered as a neurodegenerative disease (48). This cerebellar pathology is well in line with the clinical observation of cerebellar dysfunction in essential tremor (43).

It has long been known that in many cases, consumption of alcohol in small doses temporarily reduces the symptoms of ET (1,2,15). Positron emission tomography studies of affected individuals

have demonstrated increased cerebellar blood flow both during tremor and at rest; this increase in blood flow, as well as the tremor, is suppressed with alcohol (18). However, since long term alcohol consumption in moderate doses results in Purkinje cell loss, higher levels of chronic alcohol use may increase the risk of developing ET (49).

It has been suggested that ET is basically physiological tremor, but occurs at a higher amplitude than usual (15), and that it is the “normal” mode of functioning of certain cerebral mechanism, which is kept in check by other, genetic mechanisms (2).

Propranolol is a sympatholytic non-selective beta blocker, which has been known to reduce symptoms of ET since the early 1970s (50). Response to treatment with propranolol varies, and has been linked to variation in electromyographic tremor pattern and the occurrence of abnormal reflex responses (51). Current treatments of essential tremor using beta blockers or primidone have only limited efficacy (18). Surgery is a treatment option for highly disabled individuals with medication resistant tremor, with the ventral intermediate nucleus of the thalamus being an effective target for ablative surgical treatment of the tremor (52).

1.3 Genetics of essential tremor

In some families, essential tremor seems to be caused by an autosomal dominant variant with high but not full penetrance (13). This, along with incomplete pairwise concordance in twin studies (approximately 60% in monozygotic twins, compared with 30-40% in dizygotic twins) (31,35), suggests that environmental factors are also likely to have a role in the aetiology of essential tremor, in addition to genetic factors (18).

Through methods of genetic linkage, three genetic loci have been located in patients and families with the disorder (53,54,55). In 1997, a genome wide scan with 75 individuals in 16 Icelandic families mapped a familial ET gene to a 10 cM region on chromosome 3q13 (ETM1) (53). Further attempts to identify sequence variants associating with essential tremor have led to the proposal of the Ser9Gly polymorphism in the Dopamine D3 receptor (DRD3) as a causative gene in familial ET (ETM1) (56,57,58), although its role in the pathogenesis of ET has been disputed (59,60,61,62).

In 1997, a linkage analysis in a large American family of Czech descent mapped the ETM2 gene to a 15 cM region 2p24.1 (54). A variant in the gene for HS1-binding protein 3 (HS1BP3) has been associated with familial essential tremor (63,64), although the role of the variant as a causative gene at the ETM2 locus is putative and may simply represent a polymorphism in the HS1BP3 gene (65).

In 2005, a linkage analysis in seven large North American families that included 65 patients diagnosed as definite ET mapped an ET gene to a region on chromosome 6p23 (ETM3) (55).

However, until recently, no genetic variants had been identified and unequivocally confirmed. One purpose of the study was to identify SNPs affecting genes and pathways conferring risk of ET, using a genome wide association scan approach (GWAS).

In 2009, during the initial stages of this study, the author of this thesis led the statistical analysis in a team that found a SNP in the gene for the leucine rich repeat and Ig domain containing, Nogo

receptor interacting protein 1 (LINGO1), that associated with essential tremor in a dataset of Icelandic tremor patients (66). This was then replicated based on further Icelandic, German and Austrian data, and published in the paper *Variant in the sequence of the LINGO1 gene confers risk of essential tremor*, of which the author of this thesis was one of three equally contributing first authors (66). In recent years, the results of this study have already been replicated for both Caucasian and Asian populations in several studies (67,68,69,70,71,72). Even though there have also been reports of failure to replicate the signal (73,74,75), and a couple of studies using patients of Caucasian origin from North America show an anomalous association in the opposite direction to what would have been expected (76,77), the association of LINGO1 to essential tremor has been further corroborated by meta-analyses (69,78).

LINGO1 is a type I transmembrane protein with 11 leucine rich repeats, an immunoglobulin (Ig) domain in the extracellular portion, a transmembrane domain, and a short cytoplasmic tail containing a canonical epidermal growth factor receptor-like tyrosine phosphorylation site. Its amino acid sequence is extremely well conserved among vertebrates, with human and mouse orthologue sharing 99.5% identity (79,80). LINGO1 is exclusively expressed in the central nervous system (81). It negatively regulates axon regeneration (81), oligodendrocyte differentiation and myelination (82,81), as well as neuron survival (81).

Most recently, a genome wide association scan found polymorphisms in the glial glutamate transporter SLC1A2 to be associated with essential tremor, pointing towards the role of glial high-affinity glutamate reuptake in the pathology of ET (72). There may be genetic variants associated with ET that are yet to be discovered, be that with linkage, candidate-based or genome wide association, or genome wide sequencing approaches.

2 Aim of present study

The aim of the study was to discover new SNPs relevant to the aetiology and progression of Essential Tremor, using a GWAS approach.

Following the discovery of the associating polymorphism in LINGO1, a further aim was the genotype-phenotype correlation of the LINGO1 polymorphism to ET characteristics available for the study, stratifying according to the following criteria:

1. Sex (male vs. female)
2. Age at onset
3. Family history
4. Head tremor
5. Response to propranolol medication
6. Effect of alcohol on tremor symptoms
7. Results of finger-nose tests
8. Results of spirography tests.

3 Materials and methods

3.1 Genome Wide Association Scan of Essential Tremor

A detailed description of the materials and methods can be found in the Supplementary Methods section of Appendix B, which was published as a supplement to the paper *Variant in the sequence of the LINGO1 gene confers risk of essential tremor*, by Stefánsson, Steinberg, Pétursson et al. (66). In short, to search for sequence variants that confer risk of essential tremor, we conducted a genome-wide association study on Icelandic subjects with essential tremor, using the Illumina HumanHap300 and HumanCNV370 chips. After quality filtering, 305,624 SNPs were tested for association with essential tremor in a sample of 452 Icelandic essential tremor cases and 14,394 population controls. The results were adjusted for relatedness between individuals and potential population stratification by the method of genomic control (83).

3.2 LINGO1 and characteristics of Essential Tremor

3.2.1 Icelandic subjects and selection of clinical characteristics

The collection of the 487 Icelandic ET subjects who had previously been included in the genome wide association scan of essential tremor has already been described (see Appendix B and Stefánsson, Steinberg, Pétursson et al. (66)). For 415 subjects, additional information on a range of characteristics was available. Those characteristics are listed as follows (unless otherwise stated, the total number of subjects (including ungenotyped individuals) who had available information on each of the characteristics is presented in brackets):

1. Sex was available for all individuals in the study (N = 487). Of those, 451 individuals were successfully genotyped for the SNP marker rs9652490 and were used for the analysis on the effect of sex.

2. Age of onset was available for 248 individuals. Of those, 230 individuals had available rs9652490 genotypes and were used for the age at onset analysis. Individuals with ET were divided into 4 groups depending on the age at onset: Early onset (0-19y), intermediate onset (20-39y), late onset (40-59y) and very late onset (60y or later).

3. Information on family history was available both through self-reporting, and also through use of the genealogy database at deCODE Genetics (Book of Icelanders; deCODE Genetics (84)) If individuals clustered together in ancestral pedigrees at 2 meiotic events (i.e. were 1st cousins or more related), they were positive for family history (N = 319). Those who neither clustered in ancestral pedigrees nor reported family history were negative for family history (N = 33). Others were excluded for this variable. Of these 352 individuals, 343 were successfully genotyped for rs9652490 and were used for the family history analysis.

4. Information on the presence of head tremor was available for 205 individuals. Of those, 199 had genotypes for rs9652490.

5. Information on the effect of medication with propranolol was available for 80 individuals. Of those, 55 had genotypes for rs9652490. The study included one individual with prescription for atenolol, a selective β_1 receptor antagonist, whereas in all other cases the medication was described as propranolol.

6. Information on the effect of ethanol was available for 199 individuals. Of those, 169 had genotypes for rs9652490.

7. Results of tests during examination where the neurologist had the subject touch their nose with their finger while monitoring for irregularity in timing and control of the movement (finger nose tests) were available for 185 individuals. Of those, 179 had been successfully genotyped for rs9652490.

8. Results of tremor assessments from drawings of Archimedes spirals (spirography tests) were available for 170 individuals. Of those, 167 had available genotypes for rs9652490. In the finger nose test and spiral test, the subjects had not been scored on Bain's scale (5), but instead were directly assigned a severe, moderate, mild or negative tremor status.

9. Information on whether the subject had tried propranolol medication (N = 192).

10. Information on whether the subject had shaky hands at examination (N = 256).

11. Information on whether the subject claimed the tremor was worse under stress (N = 254).

12. Information on whether subject claimed the tremor was made worse by tiredness (N = 182).

13. Information on whether the subject claimed to experience difficulty writing (N = 233).

14. Information on whether the subject claimed to have difficulties holding a coffee cup (N = 248).

15. Information on whether the subject claimed to have difficulties with precision work, e.g. threading a needle (N = 190).

16. Information on presence of postural tremor at examination (N = 200).

17. Information on whether the tremor was more prominent in the right hand or the left hand (N = 26).

18. Information on whether the tremor was progressive or regressive (N = 13).

19. Information on presence of voice tremor (N = 8).

After preliminary analysis, the first eight characteristics were chosen for analysis in the German dataset. The other characteristics were dropped from further analysis, either due to their not being of clinical interest (item 9), almost all subjects analysed had the same classification (items 10 and 16), too few subjects were available for analysis (items 17-19), or the characteristic was not available for analysis for the German subjects (items 11-15).

The 32,811 Icelandic controls included in the study on characteristics of essential tremor came from the same source as the controls included in the genome wide association study that led to the discovery of the association of LINGO1 to essential tremor (Supplementary methods in Appendix B, (66)). They were recruited as part of various genetic programs at deCODE and were not screened for ET. The controls came from genetic programs in a range of diseases studied at deCODE Genetics, including addiction, Alzheimer's disease, anxiety, asthma, attention deficit hyperactivity disorder, benign prostatic hyperplasia, breast cancer, chronic obstructive pulmonary disease, colorectal cancer, coronary artery disease, dyslexia, endometriosis, enuresis, hypertension, infectious diseases, lung cancer, melanoma, migraine, osteoporosis, peripheral artery disease, polycystic ovary syndrome, pre-eclampsia, prostate cancer, psoriasis, restless legs syndrome, schizophrenia, sleep apnea, stroke, type II diabetes, a set of longevous individuals, and population controls.

All work was approved by the relevant data protection and ethics committees in Iceland.

3.2.2 German subjects

The German subjects were collected as part of the study published in the paper *LINGO1 polymorphisms are associated with essential tremor in Europeans* by Thier et al. (68), and a detailed description can be found in the methods section of that paper. In short, 284 German ET patients were examined by movement disorder specialists. ET was diagnosed on three levels of certainty as defined by the consensus criteria of the Tremor Investigation Group (TRIG) (16). Only patients with definite ET were analyzed. 62% of patients in the German dataset had a positive family history for ET. 334 controls were screened to be negative for ET either by a validated screening procedure for ET (85) or by clinical observation. Available information supplied for the purposes of this study included sex, family history, age at onset, head tremor, effect of propranolol, effect of alcohol, finger nose test and spirography test. Control individuals were matched for sex and geographical origin to the patients and were older than 50 years (mean age of 69.2 $\pm$ 5.5 years, ranging from 59 to 93 years) to minimize the probability that they would still develop ET. All participants gave their informed consent. The study was approved by the German Ethical Committee (reference no. A143/00).

3.2.3 American subjects

A detailed description of the American subjects can be found in the Supplementary Methods section of Appendix B, which was published as a supplement to the paper *Variant in the sequence of the LINGO1 gene confers risk of essential tremor*, by Stefánsson, Steinberg, Pétursson et al. (66). All work was approved by the Emory Institutional Review Board (IRB). Informed consent was obtained from all subjects using IRB-approved consent forms and protocols. All enrolment work was done under the Clinical Research in Neurology (CRIN) protocol held up by the Department of Neurology, Emory University School of Medicine. ET genotyping work was done under specific IRB protocols. Immediate and future research involving the use of DNA were specifically covered in the consent, as was contact for future research connected to the same study or different studies.

3.2.4 Austrian subjects

A detailed description of the Austrian subjects can be found in the Supplementary Methods section of Appendix B, which was published as a supplement to the paper *Variant in the sequence of the LINGO1 gene confers risk of essential tremor*, by Stefánsson, Steinberg, Pétursson et al. (66). The 123 Austrian ET patients (up from 77 due to later genotyping) were recruited through the movement disorders clinic at the Department of Neurology, Medical University of Vienna on a consecutive basis. Diagnosis of ET was made based on established Movement Disorder Society (MDS) Consensus Criteria (16) (established to reduce diagnostic ambiguity) and all subjects were examined by a movement disorders neurologist. Information on sex, family history, age at onset, and effect of alcohol was supplied for the purposes of the study. Control individuals (N = 342) were healthy blood donors from the same geographical region as the ET cases. All work was approved by the local ethics committee of the Medical University of Vienna and the Vienna General Hospital.

3.2.5 Statistical Analysis

For the single marker association, a maximum likelihood based chi-square test was used to calculate two-sided P-values for the at-risk allele, based on the standard likelihood ratio statistic G, which is asymptotically chi-square distributed (86). When testing for single marker association, and under the scenario of complete information in general, it is identical to the maximum likelihood chi-square test statistic used in Stefánsson, Steinberg, Pétursson et al. (66).

For the Icelandic, American and Austrian datasets, a decrease in effective sample size had to be taken into account, due to the fact that genotypes of relatives are not independent. P-values were adjusted for relatedness and possible population stratification by dividing the chi-square statistics by an estimated inflation factor. For the Icelandic dataset, the correction for relatedness was done by using genomic control correction (87). In the case of the genome-wide typed material, the inflation factor was estimated from the observed median chi-square statistic divided by 0.675^2.

The American and Austrian material was also in part based on families. Since genome wide SNP data was not available for those individuals and genomic control correction therefore not possible, Mendelian drop-down simulations based on pedigrees were used to calculate the inflation factor for the American and Austrian P-values. The code for doing the Mendelian drop-down simulations was written in R and is supplied in Appendix C.

The German dataset was based on a set of unrelated individuals, and therefore the uncorrected P-values are used for that dataset.

Since the multiplicative model had been found to give an adequate fit (P = 0.26 for a test of the full model against the null hypothesis of the multiplicative model) (66), it was chosen as the model of choice over models of dominant or recessive penetrance. In the multiplicative model, the risks of the two alleles an individual carries are independent. Therefore, they can be multiplied for the risk of the individual, which means, *e.g.*, that an individual homozygous for a disease associated allele with an OR of 1.7 would have a 2.9-fold increase in risk of having the disease.

Odds Ratios were calculated assuming a multiplicative model for risk. The odds ratio (OR) of the allelic frequency was calculated as

$$\mathrm{OR} = \frac{p_A/1 - p_A}{p_C/1 - p_C}$$

where p_A and p_C are the allelic frequencies for the affecteds and the controls, respectively.

Population attributable risk (PAR) is defined as the fraction of cases that would be reduced from the population if the risks of all individuals could be made, e.g. through a treatment, to be the same as non-carriers of the at-risk variant(s). The general form of the formula has been previously described; see *e.g.* a good description in the Supplementary material to Stefánsson et al. (87). Due to its definition, the general form of the formula is a bit convoluted. However, under the multiplicative model, for the purposes of this thesis, it simplifies to the much simpler and elegant

$$\mathrm{PAR} = 1 - \frac{(1 - p_A)^2}{(1 - p_C)^2}$$

where p_A and p_C are the same as above.

The Mantel-Haenszel model (88) was used to combine results from the Icelandic, German, Austrian and U.S. material, and for the calculation of confidence intervals.

Significance of difference between characteristics is derived from overlap of confidence intervals, based on the Mantel-Haentzsel method.

4 Results

4.1 Genome wide association scan of essential tremor

Stefánsson, Steinberg, Pétursson et al. (66) found association of the SNP rs9652490 to essential tremor in Icelandic and American individuals. The paper, titled *Variant in the sequence of the LINGO1 gene confers risk of essential tremor*, of which Stefánsson, Steinberg and Pétursson were joint 1st authors, is part of the current thesis and can be found in Appendix A, along with its supplement in Appendix B. To summarize, a marker in LINGO1 was identified through a GWAS approach using 305,624 SNP markers, showing genome-wide significant association with essential tremor (OR = 1.55; $P = 1.2\times10^{-9}$). When the discovery dataset was excluded and the analysis done on only the follow-up material, the OR was 1.44, with a P-value of 0.001 (see Appendix A, Table 1).

None of the 305,624 markers reached the genome-wide significance level ($P = 1.6\times10^{-7}$) and a quantile-quantile (QQ) plot (see Supplementary Fig. 1 in Appendix B) showed only a slight excess of signal. Two markers, rs9652490 and rs11856808, both located in intron 3 of the LINGO1 gene on 15q24.3, had P values below 1×10^{-5} in the discovery dataset (Supplementary Table 1 in Appendix B). After testing for association in follow-up material from Austria, Germany, the United States and Iceland (Supplementary Methods and Supplementary Table 1 in Appendix B), significant association was found with allele G of marker rs9652490 in the follow-up material (P = 0.0010, OR = 1.44). ORs in the Austrian, German, American and Icelandic follow-up datasets were 1.73, 1.39, 1.32 and 1.29, respectively, compared to 1.63 in the discovery set. In the combined discovery and follow-up material, the association was genome-wide significant ($P = 1.2\times10^{-9}$, OR = 1.55) and the population attributable risk was approximately 20%. Allele T of marker rs11856808 was also associated with essential tremor in the follow-up material (Supplementary Table 1 in Appendix B), although not significantly associated after adjustment for the effect of rs9652490 (Supplementary Table 2 in Appendix B). The multiplicative model gave an adequate fit (P = 0.26 for a test of the full model against the null hypothesis of the multiplicative model), resulting in an estimated OR of 2.40 for those homozygous for the risk allele, who are approximately 5% of the population.

The associated SNP is located in intron 3 of the LINGO1 gene (Fig. 1 in Appendix A) in a block of markers in strong linkage disequilibrium (LD). Follow-up genotyping on markers with $r^2 > 0.5$ in the HapMap CEU did not uncover markers associating more strongly with essential tremor than marker rs9652490. In addition, none of the markers were significantly associated with essential tremor after adjustment for the effect of rs9652490 (Supplementary Table 2 in Appendix B).

All five exons of the LINGO1 gene as well as exons of one transcript, BC042092, located within the same LD block as marker rs9652490 (Supplementary Fig. 2 in Appendix B), were sequenced (Supplementary Table 3 in Appendix B). None of the SNPs identified in exons of the LINGO1 gene or the transcript could account for the effect of allele G of marker rs9652490 (Supplementary Table 4 in Appendix B).

4.2 Clinical characteristics of ET and association of rs9652490

To follow up on the discovery of the association of rs9652490 to essential tremor, data on the clinical characteristics of essential tremor that had already been collected was used to further characterize the effect of the LINGO1 polymorphism on essential tremor. Data from four data sources was available (nationality of dataset in brackets): deCODE Genetics Inc. (Icelandic), Emory (American), Kiel University (German) and Vienna University (Austrian). The clinical characteristics under study were sex (data from all four sources), age at onset (data from all four sources), family history (data from all four sources), presence or absence of head tremor (data from Iceland and Germany), positive or negative response to propranolol medication (data from Iceland and Germany), alleviation of tremor symptoms following ingestion of alcohol (data from Iceland and Germany), and results from finger-nose tests (data from Iceland and Germany) and spirography tests (data from Iceland and Germany).

Total number of rs9652490 genotypes available for the clinical characteristics study can be seen in Table 1. There was significant association to essential tremor in the Icelandic, German and Austrian material (ORs 1.61, 1.61 and 1.99; and P-values 3.4×10^{-7}, 0.00093 and 0.0016; respectively), but not in the American dataset (OR = 1.25 and P-value = 0.23). When analyzing the combined material, there was strong association of rs9652490 to essential tremor, with an OR of 1.59 ($P = 1.5\times10^{-11}$).

Table 1: Association of allele G of rs9652490 with essential tremor, fractionated by sex. The table includes the combined results from all four populations (Combined), and the results for each of the four populations separately. Frequencies of allele G in the four populations are included. Total stands for the total of males and females. Numbers and frequencies for controls are included. Since population differences in baseline frequencies may cause problems when interpreting differences in allelic frequencies in affecteds and controls between populations, frequencies for the combined material were not included. P-values for the Icelandic, American and Austrian datasets have been corrected with genomic controls (Iceland) and Mendelian drop-down simulations (USA, Austria) to take into account relatedness of participants. The German subjects were unrelated.

Combined					
	N		**OR**	**95% CI**	***P***
Males	507		1,68	1.43-1.97	3.4×10^{-10}
Females	484		1,52	1.27-1.81	4.2×10^{-6}
Total	991		1,59	1.39-1.81	1.5×10^{-11}
Controls	33.772				
Iceland					
	N	**Frequency**	**OR**	**95% CI**	***P***
Males	203	0,3670	1,90	1.50-2.42	1.4×10^{-7}
Females	248	0,2984	1,40	1.11-1.75	0,004
Total	451	0,3293	1,61	1.34-1.93	3.4×10^{-7}
Controls	32.811	0,2336			
Germany					
	N	**Frequency**	**OR**	**95% CI**	***P***
Males	179	0,2318	1,56	1.13-2.15	0,007
Females	103	0,2476	1,70	1.16-2.50	0,0069
Total	282	0,2376	1,61	1.21-2.13	0,00093
Controls	333	0,1622			
USA					
	N	**Frequency**	**OR**	**95% CI**	***P***
Males	62	0,2581	1,15	0.69-1.91	0,59
Females	73	0,2877	1,33	0.87-2.04	0,19
Total	135	0,2741	1,25	0.87-1.79	0,23
Controls	387	0,2326			
Austria					
	N	**Frequency**	**OR**	**95% CI**	***P***
Males	63	0,3175	2,11	1.27-3.52	0,0041
Females	60	0,2917	1,87	1.11-3.14	0,018
Total	123	0,3049	1,99	1.30-3.05	0,0016
Controls	241	0,1805			

4.2.1 Sex: Males vs. females

How the numbers and results of association of genotyped individuals available for the study are broken up into males and females may be observed in Table 1.

In the Icelandic, German and Austrian material, both sexes showed significant association between rs9652490 and essential tremor. In the American dataset, the signal went in the same direction as in the other studies, but was neither significant for males nor females (Table 1).

For the combined material, there was significant association of rs9652490 to ET for both males and females (OR = 1.68 and 1.52, $P = 3\times10^{-10}$ and 4×10^{-6} for males and females, respectively; Table 1). The difference in the association of rs9652490 to ET between males and females was not significant ($P = 0.57$).

4.2.2 Age at Onset

The numbers for individuals who had available age at onset data were as follows (the number of individuals successfully genotyped for the SNP rs9652490 in brackets): Iceland – 248 (230); Germany – 225 (223); USA – 115 (111); Austria – 80 (79). The age at onset distributions for the data from each of the 4 populations can be seen in Figure 2. In this study, a majority of cases had age at onset before 40 in the Icelandic (74%) and German (57%) datasets; whereas the same fraction was lower in the American (39%) and Austrian (46%) material.

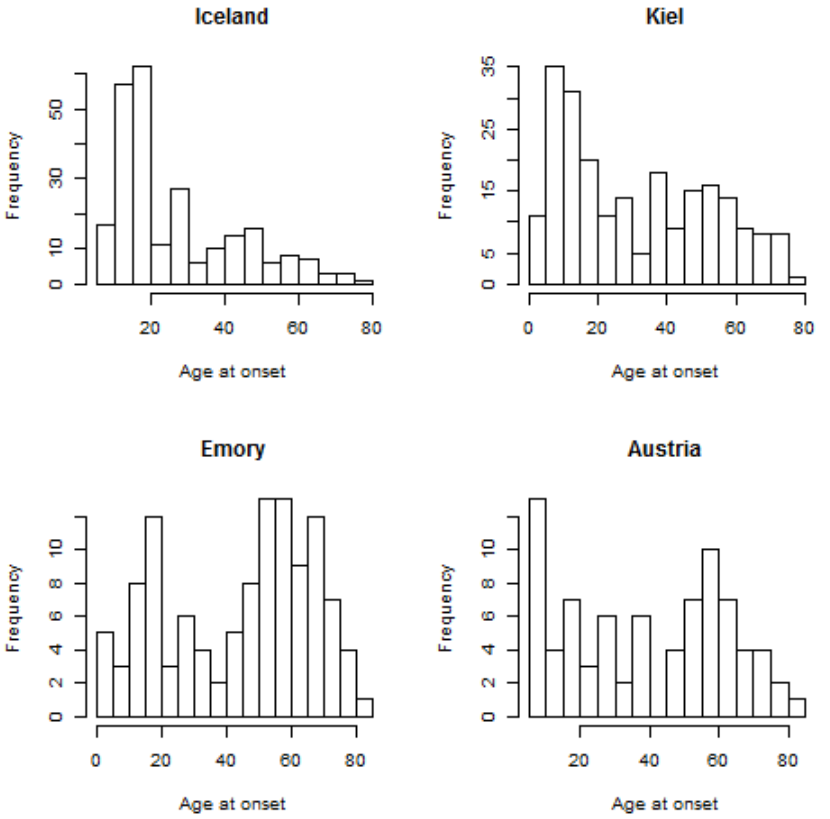

Figure 2: Age at onset of essential tremor: Age at onset distributions for the Icelandic, German (Kiel), American (Emory), and Austrian material.

To study the effect of rs9652490 on age at onset, the data was split into age at onset categories. Whereas no clear guidelines can be seen in the literature on how to fractionate by age of onset, a number of studies present data on age of onset based on 10-year age strata (27,13,21,25) or, in cases where data is sparse, 20 year age strata (27), following life decade of onset. Rather than splitting the material into 10 year age strata, with the aim of increasing sample sizes for tests of statistical significance, the dataset was split into four groups of two life decades of onset each; early onset (age at onset between 0 and 19 years), intermediate onset (age at onset between 20 and 39 years), late onset (age at onset between 40 and 59 years), and very late onset (age at onset 60 years or older, including four individuals who had age at onset 80 years or higher). Table 2 shows the number of genotyped individuals with essential tremor in each of the age categories.

Table 2: Number of genotyped individuals with essential tremor, fractionated by age at onset.

Group	0-19	20-39	40-59	60+	Total
Iceland	97	75	42	16	230
Germany	80	47	61	35	223
USA	24	19	32	36	111
Austria	19	17	23	20	79
Total	220	158	158	107	643

The results for the association of rs9652490 to essential tremor when fractionated by age at onset can be seen in Table 3 and Figure 3. The allelic status of rs9652490 showed significant association to ET, irrespective of age at onset. The association was strongest for early and late onset ET (OR = 1.81 and 1.79, $P = 3\times10^{-7}$ and 0.0003, respectively), but there was also significant association between rs9652490 and essential tremor for the intermediate and very late onset groups (OR = 1.42 and 1.53, $P = 0.03$ and 0.01, respectively).

Table 3: Association of allele G of rs9652490 with essential tremor, fractionated by age at onset in years. The table includes the combined results from all four populations (Combined), and the results for each of the four populations separately. For numbers of affecteds, see Table 2. Controls were the same as in Table 1. In the USA dataset, the P-value for the intermediate onset (20-39 years) and late onset (40-59 years) was equal to one and therefore a confidence interval could not be calculated. As in Table 1, frequencies for the combined data were not included. P-values for the Icelandic, American and Austrian datasets have been corrected with genomic controls (Iceland) and Mendelian drop-down simulations (USA, Austria) to take into account relatedness of participants. The German subjects were unrelated.

Combined				
AAO (y)		**OR**	**95% CI**	***P***
0-19		1,81	1.44-2.28	3.2×10^{-7}
20-39		1,42	1.04-1.95	0,029
40-59		1,79	1.31-2.46	0,00028
60+		1,53	1.09-2.16	0,015
Iceland				
AAO (y)	**Frequency**	**OR**	**95% CI**	***P***
0-19	0,3505	1,77	1.28-2.45	0,00056
20-39	0,2867	1,32	0.91-1.92	0,15
40-59	0,3690	1,92	1.21-3.06	0,0060
60+	0,3125	1,49	0.70-3.17	0,30
Germany				
AAO (y)	**Frequency**	**OR**	**95% CI**	***P***
0-19	0,2750	1,96	1.29-2.97	0,0015
20-39	0,2021	1,31	0.75-2.28	0,34
40-59	0,2213	1,47	0.90-2.39	0,12
60+	0,2286	1,53	0.83-2.83	0,17
USA				
AAO (y)	**Frequency**	**OR**	**95% CI**	***P***
0-19	0,3333	1,65	0.84-3.24	0,15
20-39	0,2368	1,02	NaN	1,00
40-59	0,2344	1,01	NaN	1,00
60+	0,2639	1,18	0.66-2.11	0,57
Austria				
AAO (y)	**Frequency**	**OR**	**95% CI**	***P***
0-19	0,2895	1,85	0.82-4.17	0,14
20-39	0,2941	1,89	0.86-4.18	0,11
40-59	0,3261	2,20	1.04-4.65	0,040
60+	0,3250	2,19	1.05-4.55	0,036

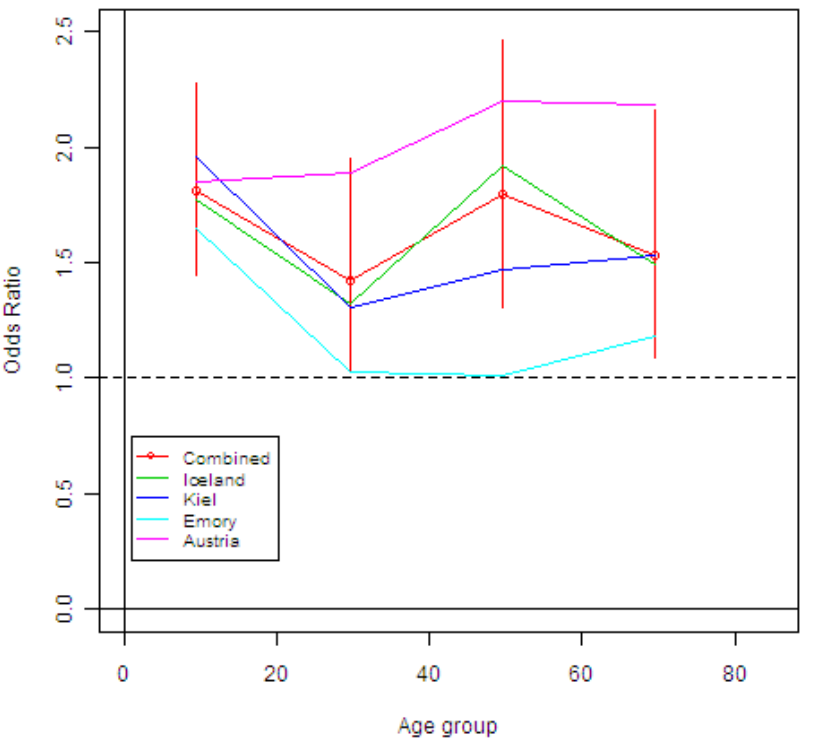

Figure 3: Association of allele G of rs9652490 to essential tremor, fractionated by age at onset. Vertical red bars correspond to confidence intervals for the combined data. Numbers are from Table 3.

Age at onset was approximately 4 years lower for males than for females, and consistently lower for males than for females in all the four studies (Table 4). An analysis of variance (ANOVA) of the age at onset when conditioning on sex and the origin of the dataset showed, in addition to a strongly significant difference in age at onset between the centres ($P = 3\times10^{-14}$, Table 5), a significant difference in age at onset between males and females ($P = 0.005$, Table 5).

Table 4: Age at onset for males and females. Numbers include both genotyped and ungenotyped individuals.

Males			
Sample	**Mean**	**Std.dev.**	**N**
Iceland	26,2	16,30	114
Germany	31,7	20,78	139
USA	44,1	21,57	50
Austria	34,3	24,18	44
Total	32,0	20,75	347

Females			
Sample	**Mean**	**Std.dev.**	**N**
Iceland	29,0	17,44	134
Germany	35,6	21,70	86
USA	46,4	23,13	65
Austria	47,5	19,88	36
Total	36,3	21,43	321

Table 5: Analysis of variance of the age at onset for males and females. Numbers include both genotyped and ungenotyped individuals.

Factor	Df	Sum of sq	Mean sq	F	P-value
Sex	1	3154	3154	7,80	0,005
Population	3	27977	9325,6	23,07	3×10^{-14}
Residuals	663	267974	404,2		

4.2.3 Family History

The numbers for individuals genotyped for the SNP rs9652490 who had available data on family history were as follows: Iceland – 343; Germany – 266; USA – 135; Austria – 123. How these numbers split up into familial and sporadic cases of essential tremor can be seen in Table 6.

Table 6: Association of allele G of rs9652490 with essential tremor, fractionated by family history. The table includes the combined results from all four populations (Combined), and the results for each of the four populations separately. Controls were the same as in Table 1. As in Table 1, frequencies for the combined data were not included. P-values for the Icelandic, American and Austrian datasets have been corrected with genomic controls (Iceland) and Mendelian drop-down simulations (USA, Austria) to take into account relatedness of participants. The German subjects were unrelated.

Combined					
	N		**OR**	**95% CI**	***P***
Familial	598		1,74	1.47-2.06	1.0×10^{-10}
Sporadic	269		1,36	1.09-1.69	0,0057
Iceland					
	N	**Frequency**	**OR**	**95% CI**	***P***
Familial	316	0,3497	1,76	1.41-2.21	7.8×10^{-7}
Sporadic	27	0,2407	1,04	0.64-1.69	0,87
Germany					
	N	**Frequency**	**OR**	**95% CI**	***P***
Familial	177	0,2514	1,74	1.26-2.39	0,0007
Sporadic	89	0,2303	1,55	1.02-2.34	0,039
USA					
	N	**Frequency**	**OR**	**95% CI**	***P***
Familial	41	0,2683	1,21	0.58-2.52	0,61
Sporadic	94	0,2766	1,26	0.87-1.83	0,22
Austria					
	N	**Frequency**	**OR**	**95% CI**	***P***
Familial	64	0,3359	2,30	1.26-4.19	0,0067
Sporadic	59	0,2712	1,69	1.03-2.78	0,039

The results of association of allele G at rs9652490 to ET after fractionating the material according to whether the cases were familial and sporadic can be seen in Table 6. There was significant association of rs9652490 to ET in the familial material from Iceland, Germany and Austria. Only a small fraction (<10%) of the Icelandic dataset was sporadic. For the Icelandic sporadic group the association was not significant, whereas the German and Austrian studies showed significant association of rs9652490 to ET, irrespective of whether the material was familial or sporadic. In the American dataset, neither familial nor sporadic cases showed significant association of rs9652490 to essential tremor.

For the combined material, there was significant association of rs9652490 to ET after fractionating by family history, both for the familial and sporadic groups (OR = 1.74 and 1.36, $P = 1\times10^{-10}$ and 0.006 for familial and sporadic ET, respectively; Table 6). Although the association was stronger in the

familial group than the sporadic group for the combined material, the difference was not significant (*P* = 0.21).

4.2.4 Head Tremor

Data on head tremor were only available for the Icelandic and German datasets. For those who had been successfully genotyped for the SNP rs9652490, data on the presence or absence of head tremor were available for 199 individuals from Iceland, and 97 individuals from Germany. The breakdown of these numbers for the presence or absence of head tremor is included in Table 7.

Table 7: Association of allele G of rs9652490 with essential tremor, fractionated by presence or absence of head tremor. The table includes the combined results from the Icelandic and German studies (Combined), as well as the separate results for Iceland and Germany. Controls were the same as in Table 1. As in Table 1, frequencies for the combined data were not included. P-values for the Icelandic dataset have been corrected with genomic controls to take into account relatedness of participants. The German subjects were unrelated.

Combined					
	N		**OR**	**95% CI**	***P***
Present	132		1,68	1.26-2.25	0,00048
Absent	164		1,62	1.27-2.07	0,00011
Iceland					
	N	**Frequency**	**OR**	**95% CI**	***P***
Present	71	0,3380	1,68	1.16-2.42	0,006
Absent	128	0,3594	1,84	1.36-2.49	7.7×10^{-5}
Germany					
	N	**Frequency**	**OR**	**95% CI**	***P***
Present	61	0,2459	1,68	1.05-2.71	0,031
Absent	36	0,1944	1,25	0.66-2.34	0,49

In the combined analysis, rs9652490 showed strong association to essential tremor ($P < 0.001$), irrespective of the presence or absence of head tremor, with odds ratios of 1.68 and 1.62, respectively (Table 7).

Head tremor was significantly more common in the ET material among females than among males: The odds ratio for the presence of head tremor for females vs. males was 2.32 ($P = 0.00056$; CI = [1.44-3.74]). Due to the different risk of head tremor between the sexes, it was of particular interest to see if the LINGO1 polymorphism might play a role in that pattern. However, the sample sizes were too small to clarify whether there was a difference in the effect of rs9652490 on the presence or absence of head tremor when fractionated by sex.

4.2.5 Propranolol Response

Data on whether individuals with essential tremor responded positively to propranolol medication were only available for the Icelandic and German datasets. In the Icelandic study, 55 individuals who were genotyped for the SNP rs9652490 had available information on the response to propranolol medication. The corresponding number for the German dataset was 66. The breakdown of these numbers for the positive or negative response to propranolol medication is included in Table 8.

Table 8: Association of allele G of rs9652490 with essential tremor, fractionated by positive or negative effect of propranolol medication on tremor. The table includes the combined results from the Icelandic and German studies (Combined), as well as the separate results for Iceland and Germany. Controls were the same as in Table 1. As in Table 1, frequencies for the combined data were not included. P-values for the Icelandic dataset have been corrected with genomic controls to take into account relatedness of participants. The German subjects were unrelated.

Combined					
	N		**OR**	**95% CI**	***P***
Positive	69		1,94	1.32-2.86	0,00076
Negative	52		1,17	0.75-1.83	0,49
Iceland					
	N	**Frequency**	**OR**	**95% CI**	***P***
Positive	32	0,3750	1,97	1.17-3.32	0,011
Negative	23	0,2391	1,03	0.73-1.46	0,86
Germany					
	N	**Frequency**	**OR**	**95% CI**	***P***
Positive	37	0,2703	1,91	1.08-3.40	0,027
Negative	29	0,2241	1,49	0.76-2.92	0,24

There was significant association of rs9652490 to ET in individuals who responded positively to propranolol medication (OR = 1.94, *P* = 0.0008; Table 8), whereas no association was detected to ET in the negative group (OR = 1.17, *P* = 0.49; Table 8). The difference in association when fractionating by propranolol response was not significant (*P* = 0.23). Sample sizes for responses to propranolol medication were small, and considerably smaller than for other factors that were included in this study. To seriously address the question of whether allele G at rs9652490 is more strongly associated to ET in positive responders to propranolol than in negative responders, a larger study would be needed.

4.2.6 Alcohol response

Data on whether individuals with essential tremor showed temporary alleviation of symptoms after ingestion of alcohol were available from Iceland, Germany and Austria. When the genotyped Austrian material was split up into two groups, depending on whether there was available information about

response to alcohol response on ET or not, there was a significant difference between the two groups. This raised questions about the quality of this variable in the Austrian dataset. Therefore, the Austrian material was excluded from the following analysis.

In the Icelandic dataset, 169 individuals who were genotyped for the SNP rs9652490 had available information on the effect of alcohol on the temporary alleviation of tremor symptoms. The corresponding number for the German material was 212.

When looking at the association of rs9652490, fractionating with respect to whether alcohol reduced tremor (positive response) or not (negative response), there was significant association irrespective of whether the subjects reported a positive response to alcohol or not (OR = 1.85 and 1.49, $P = 8.2\times10^{-8}$ and 0.012 for positive and negative response, respectively; Table 9). Even though the association was stronger in the responder group than for the non-responders, the difference in association when fractionating by alcohol response was not significant ($P = 0.42$).

Table 9: Association of allele G of rs9652490 with essential tremor, fractionated by positive or negative effect of alcohol ingestion on the reduction of tremor. The table includes the combined results from the Icelandic and German studies (Combined), as well as the separate results for Iceland and Germany. Controls were the same as in Table 1. As in Table 1, frequencies for the combined data were not included. P-values for the Icelandic dataset have been corrected with genomic controls to take into account relatedness of participants. The German subjects were unrelated.

Combined					
	N		**OR**	**95% CI**	***P***
Positive	263		1,85	1.48-2.32	8.2×10^{-8}
Negative	118		1,49	1.09-2.03	0,012
Iceland					
	N	**Frequency**	**OR**	**95% CI**	***P***
Positive	116	0,3448	1,73	1.27-2.34	0,00043
Negative	53	0,3208	1,55	1.02-2.35	0,039
Germany					
	N	**Frequency**	**OR**	**95% CI**	***P***
Positive	147	0,2789	2,00	1.43-2.78	4.3×10^{-5}
Negative	65	0,2154	1,42	0.88-2.28	0,15

4.2.7 Finger nose test

Data on results from finger nose tests were only available for the Icelandic and German datasets. In the Icelandic material, there was a total of 179 individuals who were genotyped for the SNP rs9652490, thereof 131 individuals who were recorded as having a severe or moderate positive result from a finger nose test, whereas 48 individuals were recorded as having a mild or negative result. For the German dataset, the individuals were scored on Bain's scale and then given a severe / moderate /

mild / negative tremor status based on the grading scheme given in Bain et al. (5). There were a total of 177 German individuals with finger nose test outcomes genotyped for rs9652490, thereof 78 with a severe or moderate tremor, and 99 individuals with a mild or negative result (Table 10).

Table 10: Association of allele G of rs9652490 with essential tremor, fractionated by outcomes of a finger nose test. Responders were split into two groups, depending on whether their score on the test was indicative of a severe or moderate tremor for one group, or mild tremor or absence of tremor (negative) for the other. The table includes the combined results from the Icelandic and German studies (Combined), as well as the separate results for Iceland and Germany. Controls were the same as in Table 1. As in Table 1, frequencies for the combined data were not included. P-values for the Icelandic dataset have been corrected with genomic controls to take into account relatedness of participants. The German subjects were unrelated.

Combined					
	N		**OR**	**95% CI**	***P***
Severe/Moderate	209		2,03	1.59-2.59	1.3×10^{-8}
Mild/ Negative	147		1,54	1.14-2.08	0,0047
Iceland					
	N	**Frequency**	**OR**	**95% CI**	***P***
Severe/Moderate	131	0,3664	1,90	1.41-2.55	2.1×10^{-5}
Mild/Negative	48	0,3438	1,72	1.11-2.66	0,015
Germany					
	N	**Frequency**	**OR**	**95% CI**	***P***
Severe/Moderate	78	0,3013	2,23	1.48-3.36	0,00013
Mild/Negative	99	0,2121	1,39	0.93-2.08	0,11

There was very strong association of rs9652490 to ET in individuals who had either severe or moderate tremor in finger nose tests (OR = 2.03, $P = 1.3\times10^{-8}$; Table 10), and a weaker yet still significant association was detected to ET in the individuals who scored as either mild or negative for tremor (OR = 1.54, $P = 0.0047$; Table 10). The difference in association between the two groups was not significant ($P = 0.32$).

4.2.8 Spirography test

Data on results from spirography tests were only available for the Icelandic and German datasets. In the Icelandic dataset, there was a total of 167 individuals who were genotyped for the SNP rs9652490, thereof 114 individuals who were recorded as having a severe or moderate positive result from a spirography test, while 53 individuals were recorded as having a mild or negative result. For the German dataset, as in the case of the finger nose tests, the individuals were scored on Bain's scale and then given a severe / moderate / mild / negative tremor status based on the projection given in Bain et al. (5). There were a total of 186 German individuals with spirography test outcomes

genotyped for rs9652490, thereof 145 with a severe or moderate tremor, and 41 individuals with a mild or negative result (Table 11).

Table 11: Association of allele G of rs9652490 with essential tremor, fractionated by outcomes of a spirography test. Responders were split into two groups, depending on whether their score on the test was indicative of a severe or moderate tremor for one group, or mild tremor or absence of tremor (negative) for the other. The table includes the combined results from the Icelandic and German studies (Combined), as well as the separate results for Iceland and Germany. Controls were the same as in Table 1. As in Table 1, frequencies for the combined data were not included. P-values for the Icelandic dataset have been corrected with genomic controls to take into account relatedness of participants. The German subjects were unrelated.

Combined					
	N		**OR**	**95% CI**	***P***
Severe/Moderate	259		1,90	1.52-2.39	2.6×10^{-8}
Mild/ Negative	94		1,41	1.07-1.88	0,016
Iceland					
	N	**Frequency**	**OR**	**95% CI**	***P***
Severe/Moderate	114	0,3640	1,88	1.38-2.56	6.0×10^{-5}
Mild/Negative	53	0,3679	1,91	1.27-2.88	0,0020
Germany					
	N	**Frequency**	**OR**	**95% CI**	***P***
Severe/Moderate	145	0,2724	1,93	1.38-2.70	0,00011
Mild/Negative	41	0,1463	0,89	0.47-1.68	0,71

There was very strong association of rs9652490 to ET in individuals who had either severe or moderate tremor in finger nose tests (OR = 1.90, $P = 2.6\times10^{-8}$; Table 11). For the individuals who scored as either mild or negative for tremor, the effect was considerably weaker (OR = 1.41, P = 0.016; Table 11). The difference in association between the two groups was not significant (P = 0.25).

There was a clear difference between the Icelandic and German spirography test datasets with respect to the association of rs9652490 to ET in the Mild/Negative groups. In the Icelandic dataset, the OR for the Mild/Negative group was of a similar magnitude to (and even slightly higher than) the OR in the Severe/Moderate group (1.91 vs. 1.88, respectively; Table 11). In the German dataset, there was no sign of association in the Mild/Negative group (OR = 0.89; Table 11), while in the Severe/Moderate group, the results were comparable to the results for the Icelandic dataset (OR = 1.93; Table 11).

5 Discussion

Essential Tremor is one of the most common movement disorders in humans (21,20). A traditional view of ET is of a kinetic or postural tremor of the arms with a frequency of 8-12 tremors per second (21,20), which is often combined with head, voice or tongue tremors. However, people affected with ET may also have other neuropathological manifestations, such as dementia (10,89). There is considerable variability in manifestation, e.g. in the character and frequency of the tremors, which body parts are affected (and in which order), and its age of onset can be anywhere between pediatric and geriatric (28).

During the design stage of this study, not much was known about the genetics of ET. Approaches were mainly based on neuropathological evidence, focusing on genes related to the cerebellum, and Purkinje cells in particular. Positional cloning approaches using familial ET material had recovered the loci ETM1, located at 3q13 (53); and ETM2, located at 2p25-p22 (54). Fine mapping of the ETM2 locus further showed that a variant in the gene encoding for the HCLS1 binding protein 3 (HS1BP3) was associated with FET (63). Furthermore, the Ser9Gly polymorphism in the gene coding for the dopamine receptor D3 protein (DRD3) may be associated with susceptibility to ET (56).

The paper *Variant in the sequence of the LINGO1 gene confers risk of essential tremor* by Stefánsson, Steinberg, Pétursson et al. (66), which is included in this thesis (see Appendices A and B), was the first study using a GWAS approach to look for genetic variants that associated with ET. In that study, allele G in the SNP rs9652490, in intron 3 of the leucine rich repeat and Ig domain containing Nogo receptor interacting protein-1 (LINGO1), was shown to be associated with essential tremor (66). All five exons of the LINGO1 gene as well as exons of one transcript, BC042092, located within the same LD block as marker rs9652490, were sequenced (Supplementary Figure 2 and Supplementary Table 3 in Appendix B). None of the SNPs identified in exons of the LINGO1 gene or the transcript could account for the effect of allele G of marker rs9652490 (Supplementary Table 4 in Appendix B).

Stefánsson, Steinberg, Pétursson et al. (66) was the first paper to implicate the gene encoding for the LINGO1 protein in the pathology of ET. Since then, during the time the clinical characteristics part of this study was carried out, a range of papers has been published, claiming either replication (76,77,67,68,69,70,71) or non-replication (73,75,74) of the association of SNPs in LINGO1 to ET. Recently, a meta-analysis has been published, suggesting a relationship between the LINGO1 rs11856808 polymorphism and the risk for both familial ET and ET in general, but could only show association of the rs9652490 polymorphism with familial ET (78).

Although many ET cases appear to be idiopathic, ET appears to a large extent to run in families. In view of the large ET families that through linkage analysis helped uncover the three previously known linkage loci (53,54,55), and in the wake of recent successes in using GWAS methods to find multiple high risk variants contributing to the pathology of other diseases of the CNS, e.g. BTBD9, MEIS1, MAP2K5 and PTPRD in Restless Legs Syndrome (RLS) (87,90,91); and CACNA1A, ATP1A2 and SCN1A in Familial Hemiplegic Migraine (FHM) (92), it is interesting that success has not been as great with respect to association analyses of Essential Tremor. Is ET a common ailment with many rare

disease associated variants, each of them too rare to be picked up on its own? Do the large ET families represent such rare variants cosegregating within a family? It will be interesting to see whether more recent advances in the field of genome wide sequencing methods will be fruitful for further clarifying the genetics of essential tremor.

5.1 Clinical characteristics

In order to further characterize the effect of the LINGO1 polymorphism on essential tremor, data on clinical characteristics of ET were gathered and analyzed further. Four different research facilities provided material from four different populations: deCODE Genetics Inc. (Iceland) (66), Kiel University (Germany) (68), Emory General Clinical Research Center (USA) (66), and the Medical University of Vienna (Austria) (66). The clinical characterization was done separately for each dataset, and it is worth noting that the classification criteria for ET varied in their methods of patient assessment. While the clinical classifications for the Icelandic dataset were of a positive/negative nature, the classification for the German material was based on the Bain's scale (5).

Since the publication of the first paper showing association between polymorphisms in LINGO1 and ET (66), it has been debated which polymorphism best captures the effect of LINGO1 on ET. At the time when this study was designed, rs9652490 was considered to be a marginally better SNP than rs11856808 for tagging the underlying effect, based on the evidence at hand. Judging from a recent meta-analysis of data that has been produced since then in an effort to replicate the initial signal, that issue may not be resolved fully as of yet, and rs11856808 may perform at least as well as rs9652490 in tagging an underlying causative polymorphism linking LINGO1 with the pathology of ET (78). Since rs9652490 and rs11856808 are in linkage disequilibrium (D' = 0.84, r^2 = 0.11; Supplementary Table 4 in Appendix B) this should not come as a surprise, and given more data, it may even turn out that a haplotype over one or both of these SNPs would produce an even better tag for the causative polymorphism. For the purposes of this study, applying rs9652490 as a tagging polymorphism to study the effect of the LINGO1 polymorphism on various clinical characteristics of ET is a valid approach, irrespective of whether other tagging polymorphisms may be in stronger LD with the underlying polymorphism that is a causative factor in the pathology of ET.

5.1.1 Sex

In the Icelandic, German and Austrian material, both sexes showed significant association between rs9652490 and essential tremor. In the American dataset, as in the other studies, allele G of rs9652490 showed a mild risk for ET in both males and females. However, for neither sex was the American signal significant (Table 1). That was expected, considering the results for the U.S. material in the initial genome wide study where it was initially used for follow-up (see Table 1 in Appendix A (66)).

When looking at the results for the combined material, the strongly significant association of rs9652490 to ET in both males and females points to an effect of LINGO1 on ET that is independent

of sex. That fits well with the observation that the male:female sex ratio in ET patients is close to 1:1, both in this study (where the male:female ratio is 1.04 for the combined material), and in the published literature (cite). It should be pointed out that there was a high proportion of males in the German dataset (male:female ratio = 1.74, P = 0.001).

5.1.2 Age at onset

Essential tremor may manifest at any age and no single age interval can be considered its peak age of onset (2). Previous studies have observed a bimodal distribution for the age at onset of essential tremor, with an early onset peak in the 2nd life decade, and a late onset peak in the 5th life decade (13,23,25). Furthermore, studies have noted a difference in age at onset distribution between family studies and population based studies, with an early onset peak being more prominent in family studies and a late onset peak more prominent in population based studies (13,23,25).

In this study, a majority of cases had age at onset before 40 in the Icelandic (74%) and German (57%) material; whereas the same fraction was lower in the American (39%) and Austrian (46%) studies. Interestingly, the case collection in all cases was based on ascertainment through medical centres, and the collection was in no way more population based in the case of the American and Austrian studies, as opposed to Iceland and Germany.

To study the effect of rs9652490 on age at onset, the data was split into age at onset categories. Whereas no clear guidelines can be seen in the literature on how to fractionate by age of onset, a number of studies present data on age of onset based on 10-year age strata (27,13,21,25) or, in cases where data is sparse, 20 year age strata (27), following life decade of onset. Rather than splitting the material into 10 year age strata, with the aim of increasing sample sizes for tests of statistical significance, the dataset was split into four groups of two life decades of onset each; early onset (age at onset between 0 and 19 years), intermediate onset (age at onset between 20 and 39 years), late onset (age at onset between 40 and 59 years), and very late onset (age at onset 60 years or older, including four individuals who had age at onset 80 years or higher).

The allelic status of rs9652490 showed significant association to ET, irrespective of age at onset. The association was strongest for early and late onset ET, but there was also significant association between rs9652490 and essential tremor for the intermediate and very late onset groups.

In this study, age at onset is approximately 4 years lower for males than for females, and consistently lower for males than for females in all four datasets. Analysis of variance showed a significant difference in age at onset between males and females (P = 0.005). This is contrary to what has previously been observed, that age at onset of ET is similar in men and women, both according to population-based studies as well as studies based on ascertainment through medical centres (27). On the other hand, not only has it been suggested that paediatric ET is more common among males than among females (28), but also that the incidence of ET in the first 2 decades of life is 42% higher in males than in females (27,28).

The effect of LINGO1 on ET is present irrespective of when the disease manifests. This points towards a universal mechanism and LINGO1 as a key player in all forms of ET, be it a familial, early onset form, or a late onset form of senile tremor.

5.1.3 Family history

There was significant association of rs9652490 to ET in the familial material from Iceland, Germany and Austria. Only a small fraction of the Icelandic dataset was sporadic (<10%), and for that group, the association was not significant, whereas the German and Austrian studies showed significant association of rs9652490 to ET, irrespective of whether the material was familial or sporadic. In the American dataset, neither familial nor sporadic cases showed significant association of rs9652490 to essential tremor.

For the combined material, there was significant association of rs9652490 to ET after fractionating by family history, both for the familial and sporadic groups. Although the association was stronger in the familial group than the sporadic group, the difference was not significant.

5.1.4 Head tremor

Head tremor was significantly more common in the ET material among females than among males. This is consistent with what had previously been observed in the literature, that females with ET have a higher risk of head tremor than males with ET (23,29,30). Still, the observed increase in risk for females (OR = 2.3; CI = [1.44-3.74]) is somewhat lower than the four-to sixfold risk that has been previously reported.

In the combined analysis, rs9652490 showed strong association to essential tremor, irrespective of the presence or absence of head tremor. Due to the different risk of head tremor between the sexes, it was of particular interest to see if the LINGO1 polymorphism might play a role in that pattern. However, there was no clear difference in the effect of rs9652490 on the presence or absence of head tremor when fractionated by sex. These results suggest that any differences between the sexes in the distribution of disease pathology within the brain are independent of the effect that LINGO1 has on ET.

5.1.5 Response to propranolol

When looking at the effect of the LINGO1 polymorphism on ET with respect to a positive or negative effect to propranolol medication, material was available for only two of the four populations (Iceland and Germany). For the Icelandic dataset, the data on medication were qualitative, noting the name of the drug and what response the individual had had to the medication (i.e. positive vs. negative). When processing the data, for the purposes of obtaining a larger sample, the data for the Icelandic individuals was used, not only if they had data available on response to propranolol medication, but also if they had been prescribed other beta blockers for the purpose of reducing tremor. This added one individual with prescription for atenolol, a selective β_1 receptor antagonist.

There was significant association of rs9652490 to ET in individuals who responded positively to propranolol medication, whereas no association was detected to ET in the negative group. The difference in association when fractionating by propranolol response was not significant. The lack of significance in the difference between the two groups may well be attributed to small sample size.

The mode of action of propranolol in reducing essential tremor is probably due in part to peripheral blockage of beta adrenergic receptors, possibly with a second mode of action through its function as a central nervous depressant. These results suggest that polymorphisms in LINGO1 are possible candidates for genetic diagnostics for ET, with a potential for improvement in drug treatment based on the genetic profile of the ET patient.

5.1.6 Alcohol response

When looking at the association of rs9652490, fractionating with respect to whether alcohol reduced tremor (positive response) or not (negative response), there was significant association to ET, not only in the group that responded positively to alcohol, but also in the group that did not report any reduction in tremor after ingestion of alcohol.

The effect of ethanol on the central oscillator in ET and its possible damaging effect on cells in the cerebellum has been a subject of some controversy (49). LINGO1 has a stronger effect (if not significantly so) on essential tremor cases that report a positive effect of alcohol on the reduction of tremor than on the cases that report no such effect. This might point towards the possible existence of an ET subgroup of patients for which LINGO1 might play a stronger role.

5.1.7 Finger nose test and spirography test

There was very strong association of allele G at rs9652490 to ET in individuals who had either severe or moderate tremor, both in finger nose tests and spirography tests. The association in individuals who either had a mild tremor or were negative was weaker in both the finger nose tests and the spirography tests, yet still significant.

The difference between the Icelandic and German datasets with respect to the association of rs9652490 to ET in the Mild/Negative groups in spirography tests was striking. In the Icelandic dataset, the OR for the Mild/Negative group was of a similar magnitude to (and even slightly higher than) the OR in the Severe/Moderate group, whereas in the German dataset, there was no sign of association in the Mild/Negative group. Here it is necessary to bear in mind that the two datasets were not graded in an entirely comparable fashion with respect to tremor severity. Whereas in the German dataset the severity of the tremor was scored according to Bain's scale (5), the Icelandic dataset was collected between September 1996 and February 1998, before the publication of the TRIG consensus statement (16). Also, since Bain's scale had not yet received universal acceptance as a grading tool for tremor severity at that time, the Icelandic dataset was graded using more qualitative measures. The greater difference between the two groups in the German dataset could be an indication of a

greater objectivity in the Bain's scale approach, recommending it as a grading scheme over other methods, which may be more vulnerable to the subjectivity of the examiner performing the test.

5.2 LINGO1 and Essential Tremor

A number of different adhesion and cell–cell interaction molecules such as polysialic acid neural cell adhesion molecule, Notch, neuregulin, LINGO1, integrins and extracellullar matrix proteins provide negative and positive signals that coordinate the formation of the myelin membrane (93). The genome-wide significant association with markers in the LINGO1 gene suggests that one pathogenic mechanism of essential tremor may be related to impaired axonal function caused by LINGO1 defects altering either neurite outgrowth, myelination or neuronal survival (Figure 4).

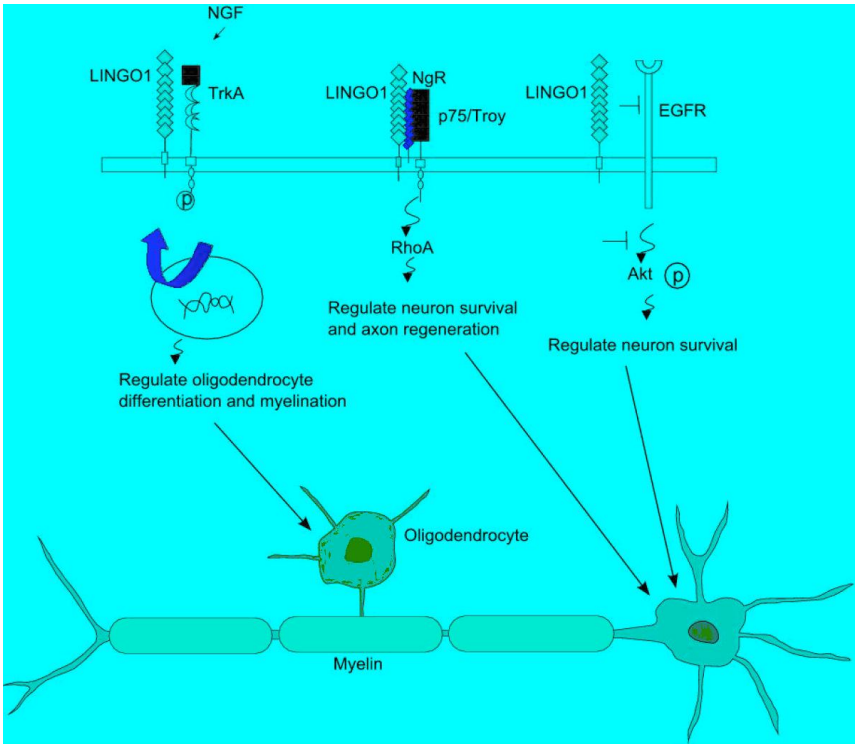

Figure 4: Signaling pathways for LINGO1. It has been shown that the gene is exclusively expressed in the central nervous system. It regulates axon generation, oligodendrocyte differentiation and myelination as well as neuron survival. Adapted and re-drawn with permission from Mi S, et al. (81).

Inhibitors of LINGO1 activity have been shown to protect dopamine neurons against degeneration (81), and it has been shown that LINGO1 inactivation of glycogen synthase kinase-3b enhances survival of granular neurons of the cerebellum (94). Essential tremor-like tremor can be a part of the phenotypic spectrum of some peripheral neuropathies, and genes such as GJB1 (gap junction beta-1 protein (connexin-32)) and PMP22 (peripheral myelin protein-22) have been considered candidate

genes for essential tremor (95). Additionally, some animal models with myelin defects are known to have tremor (96).

In mice, Lingo1 is expressed at early developmental stages without Rtn4r (reticulon-4 receptor precursor (Nogo-66 receptor)), which supports the notion that LINGO1 may participate in activities in developing neurons apart from the oligodendrocyte maturation or axon extension inhibition it influences in the adult. It has been proposed that the intracellular domain of LINGO1 may interact with the postmitotic neuronal-specific zinc-finger protein myelin transcription factor 1-like and regulate its activity by affecting its subcellular localization (97).

Treatments that reduce neuronal degeneration and maintain or restore neuronal pathways and physiological circuits are likely to be of therapeutic benefit in some neurodegenerative diseases. For this purpose, LINGO1 is a particularly compelling target because of its potent and negative regulatory influences on axonal extension, neuronal survival and oligodendrocyte differentiation. Pleiotropic roles of LINGO1 coincide with the deficits seen in some myelin diseases. Indeed, the upregulation of LINGO1 in conditions such as rat models of both spinal cord injury and glaucoma, as well as human multiple sclerosis and Parkinson's disease (81) suggests that LINGO1 and associated pathways may inhibit repair in these conditions. In vitro and in vivo experiments with LINGO1 antagonists provide support for the hypothesis that antagonism of LINGO1 may be a worthwhile approach to the treatment of some diseases of the central nervous system (81). The association with essential tremor described here places essential tremor on the top of the list of diseases to be assessed.

This study describes the genome-wide significant association of a sequence variant with ET, implicating axon regeneration, central-nervous-system myelination and regulation of neuronal survival in the pathophysiology of essential tremor. Axonal swellings of Purkinje cell are considered to be one of the pathological findings in essential tremor (48). Increased axon integrity observed in LINGO1 mouse knockout models (82) highlights the potential role of LINGO1 in the pathophysiology of essential tremor and opens up a new field in the research into essential tremor. One may even hope that LINGO1 will point the way to new treatments for severe cases of the disease.

6 Conclusions

The SNP rs9652490 in LINGO1 is associated with ET. This association does not depend on the age at onset, nor family history of ET, and points towards a common disease mechanism for the familial early onset and more sporadic late onset forms. An interesting and unexpected observation is a lower age at onset in males than in females.

The association of LINGO1 to ET does not appear to vary with presence or absence of head tremor, nor with results of finger nose tests or tremor observed in the drawing of Archimedes spirals (spirography). The universal effect of LINGO1 indicated by its association to ET irrespective of sex, age at onset, family history or head tremor points towards the possibility that other variants may contribute more to the distinct characteristics. A causative variant in LD with allele G on marker rs962490 may therefore confer its risk by increasing the risk of ET in combination with other variants that dictate which characteristics of ET each subject may develop, as well as environmental factors.

The results suggest that LINGO1 status may be associated with the response of essential tremor patients to propranolol medication and alcohol response. The association of allele G of rs9652490 when fractionating based on the response to propranolol medication points towards LINGO1 as possibly playing a role in the therapeutics of ET, and indicates that a better understanding of LINGO1 and its role in the biochemical pathways of ET might help better understand the mechanisms of beta-blocker response in ET medication.

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Appendices

Appendix A

The paper *Variant in the sequence of the LINGO1 gene confers risk of essential tremor*, by Stefánsson, Steinberg, Pétursson et al. Reprinted with permission.

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Variant in the sequence of the *LINGO1* gene confers risk of essential tremor

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We identified a marker in *LINGO1* showing genome-wide significant association ($P = 1.2 \times 10^{-9}$, odds ratio = 1.55) with essential tremor. LINGO1 has potent, negative regulatory influences on neuronal survival and is also important in regulating both central-nervous-system axon regeneration and oligodendrocyte maturation. Increased axon integrity observed in *Lingo1* mouse knockout models highlights the potential role of LINGO1 in the pathophysiology of essential tremor.

Essential tremor can present in childhood and throughout adulthood. A recent population-based study showed an onset peak for essential tremor in later life, but a young-onset peak was barely discernable. By contrast, clinical series indicate that age of onset is bimodal[1]. Thus, bimodal onset may be due to the preferential referral to tertiary centers of individuals with young-onset, familial essential tremor[1]. Familial cases seem to account for approximately 50% of young-onset cases from a population-based sample[1], whereas more than 80% of young-onset cases from clinical series seem to be familial[1].

In some families, essential tremor seems to be caused by an autosomal dominant variant with high but not full penetrance[2]. Thus, environmental factors are also likely to have a role in the etiology of essential tremor, in addition to genetic factors[3]. Genetic linkage studies of essential tremor have identified susceptibility loci on chromosomes 3q13 (*ETM1*)[3] and 2p24.1 (*ETM2*)[3], but fine mapping of these loci has not led to the identification of sequence variants associating with essential tremor. There are no histologic or biochemical markers that can be used to establish the diagnosis of essential tremor. Prevalence estimates of essential tremor, therefore, vary widely, depending on the diagnostic criteria used and the ascertainment approach. As only a small fraction of individuals with essential tremor seek medical attention, studies based on clinical records are likely to underestimate the true prevalence that may be as high as 13% in people older than 65 (ref. 4).

Little is known about the pathophysiology of essential tremor, but positron emission tomography studies of affected individuals have demonstrated increased cerebellar blood flow both during tremor and at rest; this increase in blood flow, as well as the tremor, are suppressed with alcohol[3]. Current treatments of essential tremor using beta blockers or primidone have only limited efficacy[3], and surgery is the only treatment option for affected individuals with medication-resistant tremor, yielding significant disability. The ventral intermediate nucleus of the thalamus has been found to be the most effective target for ablative surgical treatment of the tremor. Postmortem studies may reveal underlying brain changes in individuals with essential tremor. Although heterogenous, pathological findings in recent studies on brains of affected individuals support the notion of the cerebellum as a potential hot spot of morphologic changes[5]. The findings of loss of Purkinje cell density, Purkinje cell heterotopias, cerebellar gliosis and axonal swellings of Purkinje cells (torpedoes) give rise to the speculation that essential tremor should be considered as a neurodegenerative disease[6]. This cerebellar pathology, which is well in line with the clinical observation of cerebellar dysfunction in essential tremor[5], was recently confirmed with an independent series showing cerebellar atrophy and Purkinje cell loss[7].

Essential tremor is most frequently characterized by action and postural tremor of the arms and hands. Tremor of the head and voice may occur along with the upper extremity tremor, but the legs are rarely affected[2]. Although tremor frequency decreases with age, tremor amplitude tends to increase. Action tremor often impairs writing, drinking, eating and various other activities of daily living[3].

To search for sequence variants that confer risk of essential tremor, we conducted a genome-wide association study on Icelandic subjects with essential tremor, using the Illumina HumanHap300 and HumanCNV370 chips. After quality filtering, 305,624 SNPs were tested for association with essential tremor in a sample of 452 Icelandic essential tremor cases and 14,394 population controls (**Supplementary Methods** online). The results were adjusted for

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Table 1 Genome-wide significant association with rs9652490[G], located in intron 3 of the *LINGO1* gene on chromosome 15q24.3

	Controls		Cases			
	N	Frequency	*N*	Frequency	OR (95% CI)	*P* value
Discovery						
Iceland	14,378	0.230	452	0.329	1.63 (1.35, 1.97)	3.0×10^{-7}
Follow-up						
Austria	342	0.193	77	0.292	1.73 (1.15, 2.59)	0.0082
Germany	176	0.233	69	0.297	1.39 (0.89, 2.17)	0.15
US	611	0.222	119	0.273	1.32 (0.92, 1.90)	0.14
Iceland	290	0.224	35	0.271	1.29 (0.71, 2.36)	0.41
All follow-up	1,419	–	300	–	1.44 (1.16, 1.78)	0.0010
All combined	15,797	–	752	–	1.55 (1.35, 1.79)	1.2×10^{-9}

Combined OR and *P* values were calculated using the Mantel-Haenszel model[15].

relatedness between individuals and potential population stratification by the method of genomic control[8]. Specifically, the χ^2 statistics were divided by an adjustment factor of 1.66. The large size of the adjustment factor is the result of the familial nature of the sample collection. It is worth mentioning that all cases in this study are clinical series and represent probably a more familial form of the disease, with possibly lower age of onset than population-based datasets.

None of the 305,624 markers reached the genome-wide significance level ($P < 1.6 \times 10^{-7}$) and a quantile-quantile (QQ) plot (**Supplementary Fig. 1** online) showed only a slight excess of signal. Two markers, rs9652490 and rs11856808, both located in intron 3 of the *LINGO1* gene on 15q24.3, had *P* values below 1×10^{-5} in the discovery sample (**Supplementary Table 1** online). These markers were tested for association in follow-up samples from Austria, Germany, the United States and Iceland (**Supplementary Methods** and **Supplementary Table 1**). Significant association was found with allele G of marker rs9652490 in the follow-up samples ($P = 0.0010$, OR = 1.44). ORs in the Austrian, German, American and Icelandic follow-up datasets were 1.73, 1.39, 1.32 and 1.29, respectively, compared to 1.63 in the discovery set. In the combined discovery and follow-up sample, the association is genome-wide significant ($P = 1.2 \times 10^{-9}$, OR = 1.55, **Table 1**) and the population attributable risk is approximately 20%. Allele T of marker rs11856808 was also associated with essential tremor in the follow-up sample (**Supplementary Table 1**), although not significantly associated after adjustment for the effect of rs9652490 (**Supplementary Table 2** online). The multiplicative model gives an adequate fit ($P = 0.26$ for a test of the full model against the null hypothesis of the multiplicative model), resulting in an estimated OR of 2.40 for those homozygous for the risk allele, who are approximately 5% of the population. The associated SNP is located in intron 3 of the *LINGO1* gene (**Fig. 1**) in a block of markers in strong linkage disequilibrium (LD). Follow-up genotyping on markers with $r^2 > 0.5$ in the HapMap CEU did not uncover markers associating more strongly with essential tremor than marker rs9652490. In addition, none of the markers was significantly associated with essential tremor after adjustment for the effect of rs9652490 (**Supplementary Table 2**).

All five exons of the *LINGO1* gene as well as exons of one transcript, BC042092, located within the same LD block as marker rs9652490 (**Supplementary Fig. 2** online), were sequenced (**Supplementary Table 3** online). None of the SNPs identified in exons of the *LINGO1* gene or the transcript could account for the effect of allele G of marker rs9652490 (**Supplementary Table 4** online).

A number of different adhesion and cell–cell interaction molecules such as polysialic acid neural cell adhesion molecule, Notch, neuregulin, LINGO1, integrins and extracellular matrix proteins provide negative and positive signals that coordinate the formation of the myelin membrane[9]. The genome-wide significant association with markers in the *LINGO1* gene suggests that one pathogenic mechanism of essential tremor may be related to impaired axonal function caused by LINGO1 defects altering either neurite outgrowth, myelination or neuronal survival (**Supplementary Fig. 3** online). Inhibitors of LINGO1 activity have been shown to protect dopamine neurons against degeneration[10], and it has been shown that LINGO1 inactivation of glycogen synthase kinase-3β enhances survival of granular neurons of the cerebellum[11]. Essential tremor–like tremor can be a part of the phenotypic spectrum of some peripheral neuropathies, and genes such as *GJB1* (gap junction beta-1 protein (connexin-32)) and *PMP22* (peripheral myelin protein-22) have been considered candidate genes for essential tremor[12]. Additionally, some animal models with myelin defects are known to have tremor[13].

In mice, *Lingo1* is expressed at early developmental stages without *Rtn4r* (reticulon-4 receptor precursor (Nogo-66 receptor)), which supports the notion that *LINGO1* may participate in activities in developing neurons apart from the oligodendrocyte maturation or axon extension inhibition it influences in the adult. It has been proposed that the intracellular domain of *LINGO1* may interact with the postmitotic neuronal-specific zinc-finger protein myelin transcription factor 1-like and regulate its activity by affecting its subcellular localization[14].

Treatments that reduce neuronal degeneration and maintain or restore neuronal pathways and physiological circuits are likely to be of therapeutic benefit in some neurodegenerative diseases. For this purpose, LINGO1 is a particularly compelling target because of its

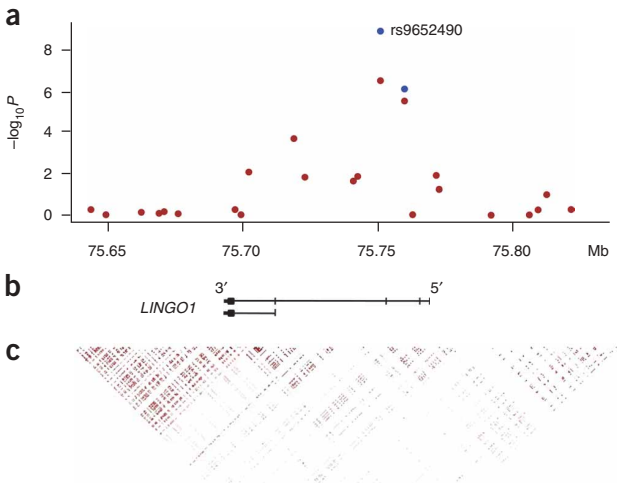

Figure 1 Overview of the 185-kb region around rs9652490. (**a**) Single marker association results from both the genome-wide scan of Icelandic subjects with essential tremor (red circles) and the combined analysis including four follow-up datasets (blue circles). *P* values are corrected for relatedness and potential population stratification. (**b**) Exon structure of the *LINGO1* gene. The associated marker is located in intron 3 of the gene. (**c**) Pairwise correlation coefficient (r^2) from the CEU HapMap population.

potent and negative regulatory influences on axonal extension, neuronal survival and oligodendrocyte differentiation. Pleiotropic roles of LINGO1 coincide with the deficits seen in some myelin diseases. Indeed, the upregulation of LINGO1 in conditions such as rat models of both spinal cord injury[10] and glaucoma[10] and human multiple sclerosis and Parkinson's disease[10] suggests that LINGO1 and associated pathways may inhibit repair in these conditions. *In vitro* and *in vivo* experiments with LINGO1 antagonists provide support for the hypothesis that antagonism of LINGO1 may be a worthwhile approach to the treatment of some diseases of the central nervous system[10]. The association with essential tremor described here places essential tremor on the top of the list of diseases to be assessed.

This is the first report showing genome-wide significant association of a sequence variant with essential tremor and also the first implicating axon regeneration, central-nervous-system myelination and regulation of neuronal survival in the pathophysiology of essential tremor. Axonal swellings of Purkinje cell are considered to be one of the pathological findings in essential tremor[6]. Increased axon integrity observed in LINGO1 mouse knockout models[16] highlights the potential role of LINGO1 in the pathophysiology of essential tremor and opens up a new field in the research into essential tremor. It is our hope that LINGO1 will point the way to new treatments for severe cases of the disease.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

The study was designed and results were interpreted by H.S., K.S., A.K., S.S., T.J., H.P., D.H. and J.G. Subject ascertainment and recruitment was carried out by K.S., J.B., J.G., F.J., K.K., M.D., L.S., F.A., A.R.R., L.A.M., C.M.T., C.H., E.A., A.Z., D.R., G.B., D.H. and G.A.J. Genotyping and laboratory experiments were performed by T.T., J.S., S.T.P., G.A.J., O.G., U.T., Y.B. and I.H.G. Authors H.S., K.S., S.S., O.G. and Y.B. drafted the manuscript. All authors contributed to the final version of the paper.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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Corrigendum: Genome-wide association study identifies *ANXA11* as a new susceptibility locus for sarcoidosis

Sylvia Hofmann, Andre Franke, Annegret Fischer, Gunnar Jacobs, Michael Nothnagel, Karoline I Gaede, Manfred Schürmann, Joachim Müller-Quernheim, Michael Krawczak, Philip Rosenstiel & Stefan Schreiber
***Nat. Genet*, 40, 1103–1106 (2008), published online 10 August 2008; corrected after print 24 March 2009**

In the version of this article initially published, the SNP rs1049550 listed in the abstract was indicated incorrectly as a T>C change. It is a C>T change. On the third page of the article, the haplotype containing rs1049550 was incorrectly listed as TATACC. It should be AGCATT. These errors have been corrected in the HTML and PDF versions of the article.

Corrigendum: Variant in the sequence of the *LINGO1* gene confers risk of essential tremor

Hreinn Stefansson, Stacy Steinberg, Hjorvar Petursson, Omar Gustafsson, Iris H Gudjonsdottir, Gudrun A Jonsdottir, Stefan T Palsson, Thorlakur Jonsson, Jona Saemundsdottir, Gyda Bjornsdottir, Yvonne Böttcher, Theodora Thorlacius, Dietrich Haubenberger, Alexander Zimprich, Eduard Auff, Christoph Hotzy, Claudia M Testa, Lisa A Miyatake, Ami R Rosen, Kristleifur Kristleifsson, David Rye, Friedrich Asmus, Ludger Schöls, Martin Dichgans, Finnbogi Jakobsson, John Benedikz, Unnur Thorsteinsdottir, Jeffrey Gulcher, Augustine Kong & Kari Stefansson
***Nat. Genet*, 41, 277–279 (2009), published online 1 February 2009; corrected after print 24 March 2009**

In the version of this article initially published, one sentence in the abstract and two sentences in the final paragraph of the paper were in error and have been modified.
The final sentence in the abstract should read:
Increased axon integrity observed in *Lingo1* mouse knockout models highlights the potential role of LINGO1 in the pathophysiology of ET.
The final three sentences of the paper should read:
Axonal swellings of Purkinje cells are considered to be one of the pathological findings in essential tremor[6]. Increased axon integrity observed in LINGO1 mouse knockout models[16] highlights the potential role of LINGO1 in the pathophysiology of ET and opens up a new field in the research into essential tremor. It is our hope that LINGO1 will point the way to new treatments for severe cases of the disease.
Finally, one reference has been added:
16. Mi, S. *et al. Nat. Med.* **13**, 1228–1233 (2007).
These errors have been corrected in the HTML and PDF versions of the article.

Appendix B

Supplement to the paper *Variant in the sequence of the LINGO1 gene confers risk of essential tremor*, by Stefánsson, Steinberg, Pétursson et al. Reprinted with permission.

SUPPLEMENTARY MATERIAL FOR:

Variant in the sequence of the *LINGO1* gene confers risk of essential tremor

Hreinn Stefansson, Stacy Steinberg, Hjorvar Petursson, Omar Gustafsson, Iris H. Gudjonsdottir, Gudrun A. Jonsdottir, Stefan T. Palsson, Thorlakur Jonsson, Jona Saemundsdottir, Gyda Bjornsdottir, Yvonne Böttcher, Theodora Thorlacius, Dietrich Haubenberger, Alexander Zimprich, Eduard Auff, Christoph Hotzy, Claudia M. Testa, Lisa A Miyatake, Ami R. Rosen, Kristleifur Kristleifsson, David Rye, Friedrich Asmus, Ludger Schöls, Martin Dichgans, Finnbogi Jakobsson, John Benedikz, Unnur Thorsteinsdottir, Jeffrey Gulcher, Augustine Kong, Kari Stefansson.

This document includes Supplementary Methods, Supplementary Tables 1-4 and Supplementary Figures 1-3.

Nature Genetics: doi:10.1038/ng.299

Supplementary Methods

Icelandic subjects.

In total, 487 familial and sporadic ET cases from Iceland were included in the study. Diagnosis was confirmed by history (patterned after Bain et al.[1]) and examination. Neurologists performed a physical examination on each patient with the aid of the TRIG criteria for definite tremor: bilateral postural tremor, with or without kinetic tremor, of hands or forearms, visible and persistent and lasting for at least five years. The exclusion criteria used were 1) the presence of other abnormal neurological signs, including dystonia and Parkinson's disease; 2) recent exposure to tremorogenic drugs or presence of drug or alcohol withdrawal; 3) neurological trauma within three months of the tremor; 4) clinical evidence for psychogenic origin of the tremor; 5) a history of dramatic onset of the tremor. Of the Icelandic cases, 338 had a relative with ET within three meiotic events. The remaining 149 cases were sporadic or missing ET diagnosis information for their relatives. The cases had a median age at onset of 20, a median age at study of 52, and 47% were male.

The 14,394 Icelandic controls included in the study were recruited as part of various genetic programs at deCODE and were not screened for ET. The controls came from genetic programs in the following diseases (approximate number of participants in brackets): addiction (1700), Alzheimer's disease (200), anxiety (500), asthma (700), attention deficit hyperactivity disorder (300), benign prostatic hyperplasia (300), breast cancer (600), chronic obstructive pulmonary disease (500), colorectal cancer (400), coronary artery disease (1400), dyslexia (400), endometriosis (100), enuresis (300), hypertension (900), infectious diseases (1300), longevity (400), lung cancer (100), melanoma (300), migraine (500), osteoporosis (1000), peripheral artery disease (500), polycystic ovary syndrome (300), pre-eclampsia (400), prostate cancer (500), psoriasis (300), restless legs syndrome (200), schizophrenia (300), sleep apnea (200), stroke (600), type II diabetes (500) and a set of population controls (300). Because some of the individuals used as controls were participants in more than one program, the numbers of participants in individual programs sum to more than 14,394. The controls had a median age at study of 55 and were 44% male. All work was approved by the relevant data protection and ethics committees in Iceland.

Austrian subjects.

The 77 Austrian ET patients were recruited through the movement disorders clinic at the Department of Neurology, Medical University of Vienna on a consecutive basis. The clinic of the department acts as a tertiary referral center with specialty clinics such as movement disorders, as well as a primary care facility for general neurology. All work was approved by the local ethics committee of the Medical University of Vienna and the Vienna General Hospital (AKH).

Diagnosis of ET was made based on established Movement Disorder Society (MDS) Consensus Criteria[2] (established to reduce diagnostic ambiguity) and all subjects were examined by a movement disorders neurologist. The diagnosis was made clinically; however, in cases of uncertainty, additional studies were performed to confirm the diagnosis (e.g. dopamine-transporter SPECT to rule out Parkinson's disease, electrophysiological testing including accelerometry and surface EMG to rule out enhanced physiological or psychogenic tremor). Patients included in the study were 18 years of age and older. Patients with the co-occurrence of

PD and ET were excluded, as were cases with a history of dystonia or any other secondary cause of tremor. The patients' median age at onset was 40, the median age at time of examination was 66.5, and 54% were male.

Control individuals (N = 342) were healthy blood donors from the same geographical region as the ET cases. They had a median age at study of 41 and 60% were male.

German subjects.

German ET patients (N = 69) were recruited from the movement disorder clinics at the Center of Neurology, University of Tuebingen, and at the Department of Neurology, Klinikum Grosshadern, Ludwig-Maximilians-University. Patients were systematically examined and diagnosed by movement disorder specialists (F.A, M.D and L.S.). All patients were followed at least five years after the initial diagnosis. Patients who showed at referral or during follow-up clinical signs or imaging findings questioning the diagnosis of ET, such as progressive cerebellar dysfunction, parkinsonism, dystonia or MR abnormalities, were excluded. ET was therefore diagnosed according to established criteria. The German patients had a median age at onset of tremor of 50, a median age at study entry of 69 and were 45% male.

Controls were 176, all of European descent originating from the south of Germany. Neurological disorders were excluded by neurological examination at the time of blood sampling. The controls had a median age at study of 48 and were 48% male.

Informed consent of all participants was obtained and the genetic studies were approved by the local ethics committees.

American subjects.

All work was approved by the Emory Institutional Review Board (IRB). Informed consent was obtained from all subjects using IRB-approved consent forms and protocols. All enrollment work was done under the Clinical Research in Neurology (CRIN) protocol and consent. ET genotyping work was done under specific IRB protocols. Immediate and future research involving the use of DNA were specifically covered in the consent, as was contact for future research connected to the same study or different studies.

Case determination

Samples were drawn from either review of previously enrolled subjects in the CRIN database, or prospective enrollment of ET subjects into CRIN/ET observational and genetics work. CRIN provides an umbrella structure for subject enrollment in observational and genetic studies in Neurology, consent-approved data sharing across studies and disorders, and consistent sample processing. All subjects underwent a basic structured interview for demographics and family history. A Folstein Mini Mental Status Exam (MMSE)[3,4] was administered to all CRIN subjects by trained CRIN personnel supervised by a neuropsychologist per published guidelines.

All CRIN database subjects enrolled prior to January 2007 with a reported diagnosis of 333.1 (any tremor) were reviewed, as specifically approved in the consent. Detailed review was then done for all subjects consenting to future use of DNA samples. A research diagnosis was made by CMT using review of Emory movement disorders clinic visits, documented exams and medication responses, any outside medical records and the CRIN intake forms (providing a

handwriting sample as well as patient and family reporting of disease). ET subjects were then called in for full in-person assessments as below whenever possible.

ET subjects mid-2006 onward were recruited through IRB-approved ads in the Emory Movement Disorders and Neurosurgery deep brain stimulation group clinics, and ET community education events. ET subjects and family members were examined directly by at least one movement disorders specialist; two independent exams were obtained whenever possible. Exams included a tremor rating scale (TRS) derived from the Fahn-Tolosa-Marin scale and Tremor Research Group (TRG) scale items, the motor United Parkinson Disease Rating Scale (UPDRS), Tinetti gait and balance scales[5], tandem gait[6], and assessment for dystonia. Semi-structured interviews included ET specific questions derived from the Fahn-Tolosa-Marin scale and WHIGET studies[7].

CRIN review and new enrollment subjects were given a research diagnosis of ET using Movement Disorders Society (MDS) and TRG criteria[2,8,9]. ET cases were assigned possible, probable (80-99% certainty) or definite (>99% certainty) given the quality of data, disease duration, and tremor severity[2,10].

All individuals with definite or probable ET were included, both unrelated individuals and members from family groups, with specific pre-defined exceptions: we excluded cases carrying, in addition in ET diagnoses, either PD or dystonia diagnoses [2]. Subjects were excluded if an in-person exam and re-interview determined a different diagnosis, if movement disorders clinical notes listed an uncertain or different final diagnosis (i.e. medication induced tremor), or if there was a lack of sufficient exam, medication response, and other data to clearly establish an ET research diagnosis.

Finally, individuals who were estimated to have less than 90% European ancestry using the program STRUCTURE (see **Statistical Analysis** for additional information) were excluded. Altogether, 122 subjects were included. These patients had a median age at onset of 52, a median age at study of 70 and consisted of 43% males.

Included controls (N = 614) were also recruited at Emory University. They were self-reported European descent; in addition, individuals estimated to have less than 90% European ancestry (see **Statistical Analysis**) were excluded. The controls had a median age of 66 years and were 52% male.

Genotyping

Genome-wide arrays

The genome-wide study was carried out using the HumanHap300, HumanHap300-Duo and HumanCNV370-Duo BeadChips (Illumina). These three chips contained 314,125 SNPs in common. Prior to analysis, SNPs that were monomorphic, had yield less than 95% in either cases or controls, deviated from Hardy-Weinberg equilibrium or showed divergent allele frequencies between the chips were removed. This resulted in the exclusion of 8501 SNPs; thus, our final analysis was based on 305,624 SNPs. All samples included had a call-rate of greater than 98%.

Follow up SNP Genotyping – Iceland and Emory

Single SNP genotyping for the replication 798 samples from Emory as well as the 1,250 Icelandic samples used to type follow-up markers was carried out at deCODE genetics with the Centaurus (Nanogen, Bothell, WA, USA) platform. The quality of each Centaurus SNP assay was evaluated by genotyping each assay on the CEU samples and comparing the results with the

HapMap data. Assays with >1.5% mismatch rate were excluded and a linkage disequilibrium (LD) test was used for markers known to be in LD. Key markers from the genome-wide analysis were re-genotyped on more than 10% of samples and a mismatch was observed in less than 0.5% of samples.

Follow up SNP Genotyping Austria and Germany
Genotyping was performed in Austria using commercially available Taq Man-based allelic discrimination assays (Applied Biosystems) Standard procedures were used based on Applied Biosystems reagents and 20 ul reaction volumes. Allelic discrimination was assessed using an Applied Biosystems 7900 detection system.

Statistical Analysis.

Association analysis was carried out using a likelihood procedure described previously[11] ORs were calculated assuming a multiplicative model for risk. Association results from the various study groups were combined using the Mantel-Haenszel model. *P*-values were adjusted for relatedness and possible population stratification by dividing the chi-square statistics by an estimated inflation factor. In the case of the genome-wide typed samples, this inflation factor was estimated from the observed median chi-square statistic divided by 0.675[2]. For the Icelandic sample used for follow-up genotyping as well as the American sample from Emory, this factor was determined by a previously described simulation procedure[12].

For the American sample, we also used the program STRUCTURE[13] to estimate ancestry. Data from 30 microsatellite markers chosen for their informativeness of Caucasian, Asian and African ancestry were used and the program was run with the HapMap European (CEU), Asian (CHB+JPT) and African (YRI) individuals defined as training samples for three assumed populations.

Sequencing of *LINGO1* and *BC042092*.

All five exons of the *LINGO1* gene (OMIM: 609791, Ref Seq gene variants NM_032808.5 and *BC068558*, see exon structure in **Supplementary Figure 3**) and 3 exons from predicted gene *BC042092* (UCSC ID: uc002bcv.1) were sequenced in 93 Icelandic individuals. PCR amplifications and sequencing reactions were set up on SciClone ALH3000 robotic workstations (Caliper Life Sciences, Hopkinton, MA, USA) and amplified on MJR Tetrads (Bio-Rad Laboratories, Hercules, CA, USA).

Total reaction volume was 5 µL per well and contained 15 ng genomic DNA, 0.35 µM of each primer, 1 M Betaine (Sigma-Aldrich, St. Louis MO, USA), 2.5 mM MgCl2, 0.35 U of Taq DNA Polymerase and 0.5 µl of 10x buffer with $(NH_4)_2SO_4$ (Fermentas Inc, Ontario, Canada). PCR was performed under the following conditions: 94°C for 2 minute, followed by 40 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 1 minute at 72°C and a final single step of 8 minutes at 72°C. PCR products were verified for correct length by agarose gel electrophoresis and purified with AMPure (Agencourt Bioscience, Beverly, MA, USA). Purified products were sequenced with an ABI PRISM Fluorescent Dye Terminator system (Applied Biosystems, Foster City, CA, USA) with a total reaction volume per well of 4 µL containing: 1 µL purified PCR product, 3 µL cycle sequencing mix containing 10X Buffer (800 mM TRIS-HCl pH: 9.0 and 20 mM MgCl2), either primer (0.35 µM) and BigDye version 3.0 at 1/32 strength. The cycle

sequencing reaction was run under the following conditions: 96°C for 1 minute, followed by 30 cycles of 96°C for 10 seconds, 50°C for 4 seconds and 60°C for 4 minutes. Dye terminator removal was set up on a SciClone ALH3000 liquid handling station on the entire cycle sequencing reaction volume, using 5 µL CleanSEQR (Agencourt), and resolved on Applied Biosystems 3730 capillary sequencers. SNP calling from primary sequence data was carried out with deCODE Genetics Sequence Miner software (deCODE Genetics, Reykjavik, Iceland). All variants identified by the automated systems were confirmed by manual inspection of primary signal traces. Primer sequences are listed in below.

Exon	Forward	Reverse
LINGO1.e1.a	AGCCTGGCTGGAGTGTCC	TGCCTGTGACCTTGACTGC
LINGO1.e1.b	CATCATTGCAGTCAAGGTCAC	GGAGGGAGTAAGAGGAGAGTCC
LINGO1.e1.c	GATCTGTCGGCTTCTATTAGGC	TGAGGATGAGGAGGATAGGG
LINGO1.e1.d	TAATAATACCCGCCCGAAGG	TGATGGTCCAGCTATTACCC
LINGO1.e1.e	CCCTCTTCATCTTCCCAACC	CCATCATCTGACACACGTACAG
LINGO1.e2	AAAGGGATCCTGACCGATAC	TTCCAGGGATCTTCCTTTGGTC
LINGO1.e3	GGGCTCATTCGCTAAACG	TGCTGGGAAGTGGGTAGG
LINGO1.e4	CGGAATTTGTTGGGGTTGG	GGCGGACAGACGGACAGC
LINGO1.e5.a	TCAGGATCCCCTTCAGGTCTA	GGACAGGTCTGCCGATGG
LINGO1.e5.b	CTCCAGCCCCTGGCTCTC	ACACGCGGCAGAGTCAAT
LINGO1.e5.c	GTGGGTGGGGCTGGAACC	CGCAAGTTCAACATGAAGATGATATG
LINGO1.e5.d	TGACTCTGCCGCGTGTCG	CAGCTACTCGCCCGACTG
LINGO1.e5.e	TGATGAGGGTCTTGATGTCG	AAGGACTTCCCTGATGTGCTAC
LINGO1.e5.f	TGAGACCAGGTGCTTTCG	TCTATCTCCGCTTCCTCAACC
LINGO1.e5.g	TTGCCAGAGACATTGAGCAC	GCGACAATGACCTCGTCTAC
LINGO1.e5.h	TTGAAGGAGTAGTCCCGGATG	AAGCGCTTTGTGGCAGTC
LINGO1.e5.i	GCTCACGATGTTCTCGTTG	AAGACTCCAGGCAGGGTAAG
BC042092.e1	AGGAGTGGGCGTGGTAGG	TAAACAGCCAGGCAGACG
BC042092.e2	CATTGTGACCTGTAAGGGACTC	GGGATGGAGATGAGCGAATA
BC042092.e3.a	ATGCCCTTCTTGTCCCTAAC	GGTGCTCCTTCAATGGTAAC
BC042092.e3.b	GGAAAGGAAAGGGCAGTC	CAGTCAACATGGGAGTAGAGC
BC042092.e3.c	GCAATGTCCACTCACACAACC	CCTTGGGAAATGGGTATGC
BC042092.e3.d	CCAAATGGGACAGAGATCC	GTCTGAAAGGCACTTCCCTATG
BC042092.e3.e	CCTGCTGCCCTGCTAAAC	AAACAGGCATTCCCACTGAC

Accession numbers and information on the SNPs identified through sequencing are provided in **Supplementary Table 3**.

Online databases:
Online Mendelian Inheritance in Man: LINGO1 (OMIM: 609791),
NCBI Reference Sequences (RefSeq): NM_032808.5
GenBank accession numbers: BC042092, BC068558
dbSNP accession numbers: ss105111441, ss105111440, rs10851895, ss105111439, ss105111442, rs11633842, ss105111438, ss105111437, ss105111436, ss105111443, ss105111435, ss105111434, rs2271398, rs2271397, rs2271396, rs3743481, rs11853396, rs3144, rs12438314, rs1058129.

Supplementary Table 1. Genome-wide association analysis for 452 ET cases and 14,394 controls. Two markers with *P*-values lower than 1×10^{-5} were tested for association in follow-up samples from Austria, Germany, the United States and Iceland. Allele T of marker rs11856808 was also associated with ET in the follow-up sample, although it was not significantly associated after adjusting for the effect of rs9652490 (**Supplementary Table 2**).

		Frequency			
Study Group [*N* cases/*N* controls]	**Marker[allele]**	**Cases**	**Controls**	**OR [95% CI]**	***P* value**
Discovery					
Iceland [452/14,378]	rs9652490[G]	0.329	0.230	1.63 [1.35,1.97]	3.0×10^{-7}
Iceland [451/14,385]	rs11856808[T]	0.451	0.352	1.51 [1.27,1.80]	3.0×10^{-6}
Follow-up					
Austria [77/342]	rs9652490[G]	0.292	0.193	1.73 [1.15,2.59]	0.0082
Austria [77/341]	rs11856808[T]	0.422	0.334	1.45 [1.01,2.08]	0.041
Germany [69/176]	rs9652490[G]	0.297	0.233	1.39 [0.89,2.17]	0.15
Germany [69/173]	rs11856808[T]	0.370	0.335	1.16 [0.77,1.76]	0.47
U.S. [119/611]	rs9652490[G]	0.273	0.222	1.32 [0.92,1.90]	0.14
U.S. [120/611]	rs11856808[T]	0.371	0.358	1.06 [0.76,1.46]	0.75
Iceland [35/290]	rs9652490[G]	0.271	0.224	1.29 [0.71,2.36]	0.41
Iceland [35/283]	rs11856808[T]	0.400	0.314	1.45 [0.84,2.51]	0.18
All follow-up [300/1,419]	rs9652490[G]	-	-	1.44 [1.16,1.78]	0.0010
All follow-up [301/1,408]	rs11856808[T]	-	-	1.23 [1.02,1.50]	0.035
Combined					
All combined [752/15,797]	rs9652490[G]	-	-	1.55 [1.35,1.79]	1.2×10^{-9}
All combined [752/15,793]	rs11856808[T]	-	-	1.39 [1.22,1.58]	7.7×10^{-7}

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Supplementary Table 2. Association results for marker rs9652490 and seven neighboring SNPs with $r^2 > 0.5$ with rs9652490 in the HapMap CEU. Results are shown for 487 ET cases and 649 controls from Iceland. *P*-values adjusted for the association of rs9652490 are given in the last column (*P*-adj). Four-hundred fifty two of the ET samples from the discovery sample are included in this Icelandic sample.

		Frequency				
Marker	**Allele**	**Cases**	**Controls**	**OR [95%CI]**	***P***	***P*-adj**
rs9652490	G	0.324	0.223	1.67 [1.34,2.09]	6.3×10^{-6}	1.0
rs13313467	A	0.324	0.228	1.63 [1.30,2.03]	1.8×10^{-5}	1.0
rs7177008	G	0.324	0.234	1.57 [1.26,1.96]	6.4×10^{-5}	1.0
rs11856808	T	0.447	0.350	1.50 [1.22,1.83]	0.00010	0.34
rs7176315	G	0.448	0.352	1.49 [1.22,1.83]	0.00011	0.38
rs11856876	C	0.447	0.355	1.47 [1.20,1.80]	0.00022	0.52
rs8028808	A	0.213	0.151	1.52 [1.17,1.98]	0.0018	0.49
rs11631120	G	0.218	0.166	1.40 [1.10,1.79]	0.0062	0.081

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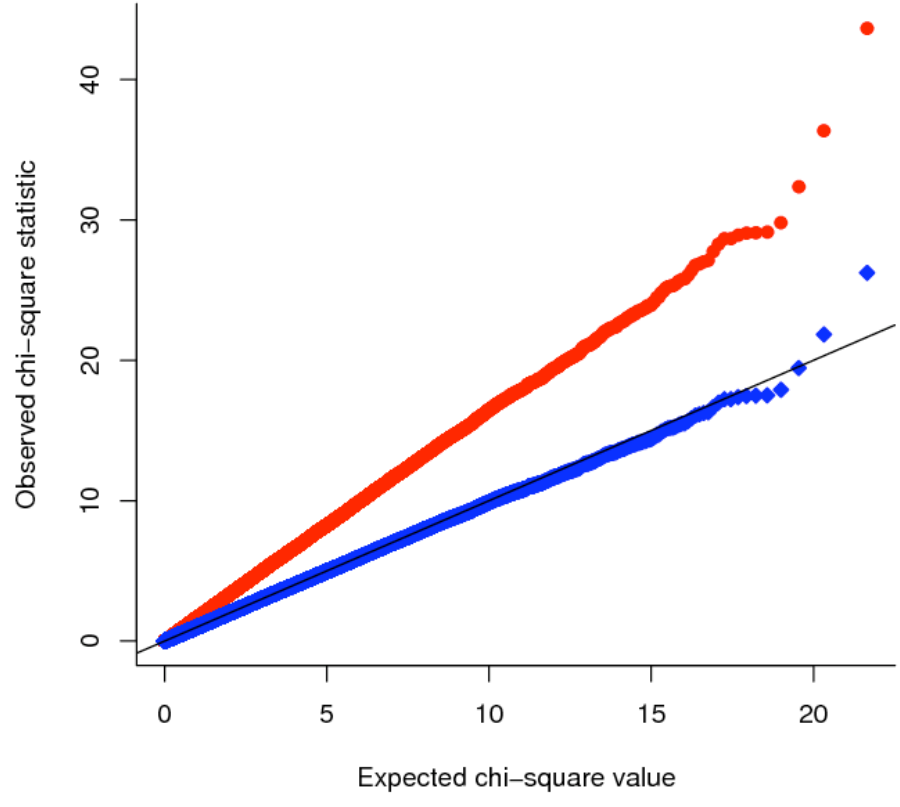
Supplementary Table 3. SNPs and in/dels identified through sequencing exons of the *LINGO1* gene. For highlighted SNPs with rs names, HapMap (CEU) minor allele frequency (MAF) is given. For the remaining markers, which include both new markers with ss names and rs SNPs without frequency in the HapMap CEU, MAF from 93 Icelandic tremor patients is given. Genomic position is taken from NCBI Build 36.

Marker	Variation	Minor allele	MAF	Genomic position	Location in gene	Change
ss105111441	G/A	A	0.01	75776529	5´untranslated	-
ss105111440	G/C	C	0.13	75776023	5´untranslated	-
rs10851895	C/G	G	0.35	75770175	5´untranslated	-
ss105111439	A/T	T	0.02	75770099	5´untranslated	-
ss105111442	-/C	C	0.08	75756682	5´untranslated	-
rs11633842	G/A	A	0.008	75728395	5´untranslated	-
ss105111438	T/C	C	0.01	75727810	5´untranslated	-
ss105111437	C/T	T	0.09	75727390	5´untranslated	-
ss105111436	G/A	A	0.04	75725908	5´untranslated	-
ss105111443	-/14bp ins	14bp ins	0.12	75725786	5´untranslated	-
ss105111435	T/C	C	0.01	75721442	5´untranslated	-
ss105111434	C/T	T	0.01	75721143	5´untranslated	-
rs2271398	G/A	G	0.33	75694839	exon	synonymous
rs2271397	T/C	T	0.429	75694830	exon	synonymous
rs2271396	C/G	C	0.442	75694590	exon	synonymous
rs3743481	G/A	A	0.328	75694200	exon	synonymous
rs11853396	C/G	G	0.22	75693286	3´untranslated	-
rs3144	T/C	C	0.292	75693259	3´untranslated	-
rs12438314	G/A	A	0.33	75692741	3´untranslated	-
rs1058129	A/G	A	0.33	75692716	3´untranslated	-

Supplementary Table 4. Linkage disequilibrium (LD) between rs9652490 and markers identified by sequencing exons of the *LINGO1* gene. For all markers, LD results from 93 Icelandic tremor patients are shown. Position is taken from NCBI Build 36.

Marker	Position	D'	r^2
ss105111441	75705293	1.00	0.003
ss105111440	75704787	0.82	0.184
rs10851895	75698939	0.37	0.137
ss105111439	75698863	0.42	0.002
ss105111442	75685446	1.00	0.164
rs11633842	75657159	1.00	0.072
ss105111438	75656574	1.00	0.010
ss105111437	75656154	0.46	0.011
ss105111436	75654672	1.00	0.017
ss105111443	75654551	0.91	0.216
ss105111435	75650206	1.00	0.007
ss105111434	75649907	1.00	0.003
rs2271398	75623603	0.20	0.010
rs2271397	75623594	0.20	0.010
rs2271396	75623354	0.15	0.006
rs3743481	75622964	0.06	0.003
rs11853396	75622050	0.84	0.110
rs3144	75622023	0.23	0.038
rs12438314	75621505	0.05	0.003
rs1058129	75621480	0.16	0.007



Supplementary Figure 1. Quantile-quantile plot of the 305,624 chi-square statistics from a genome-wide association analysis of 452 ET cases versus 14,394 controls. Both unadjusted chi-square statistics (red circles) and genomic-control adjusted chi-square statistics (blue squares) are shown.

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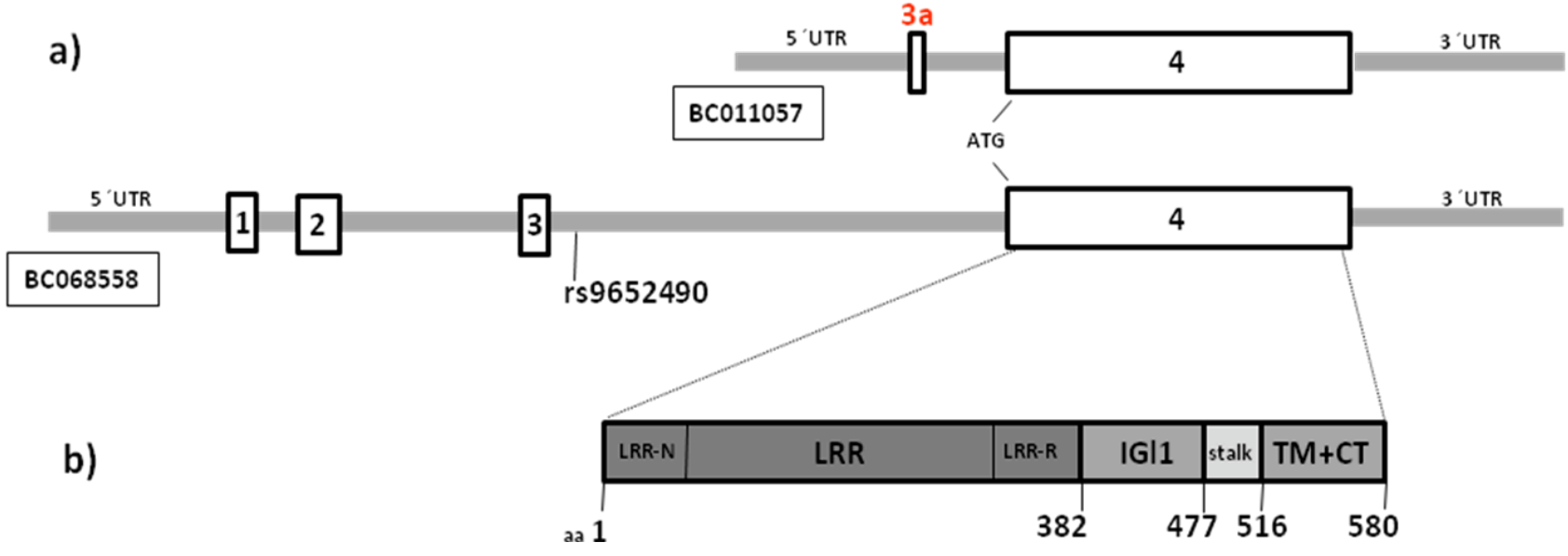
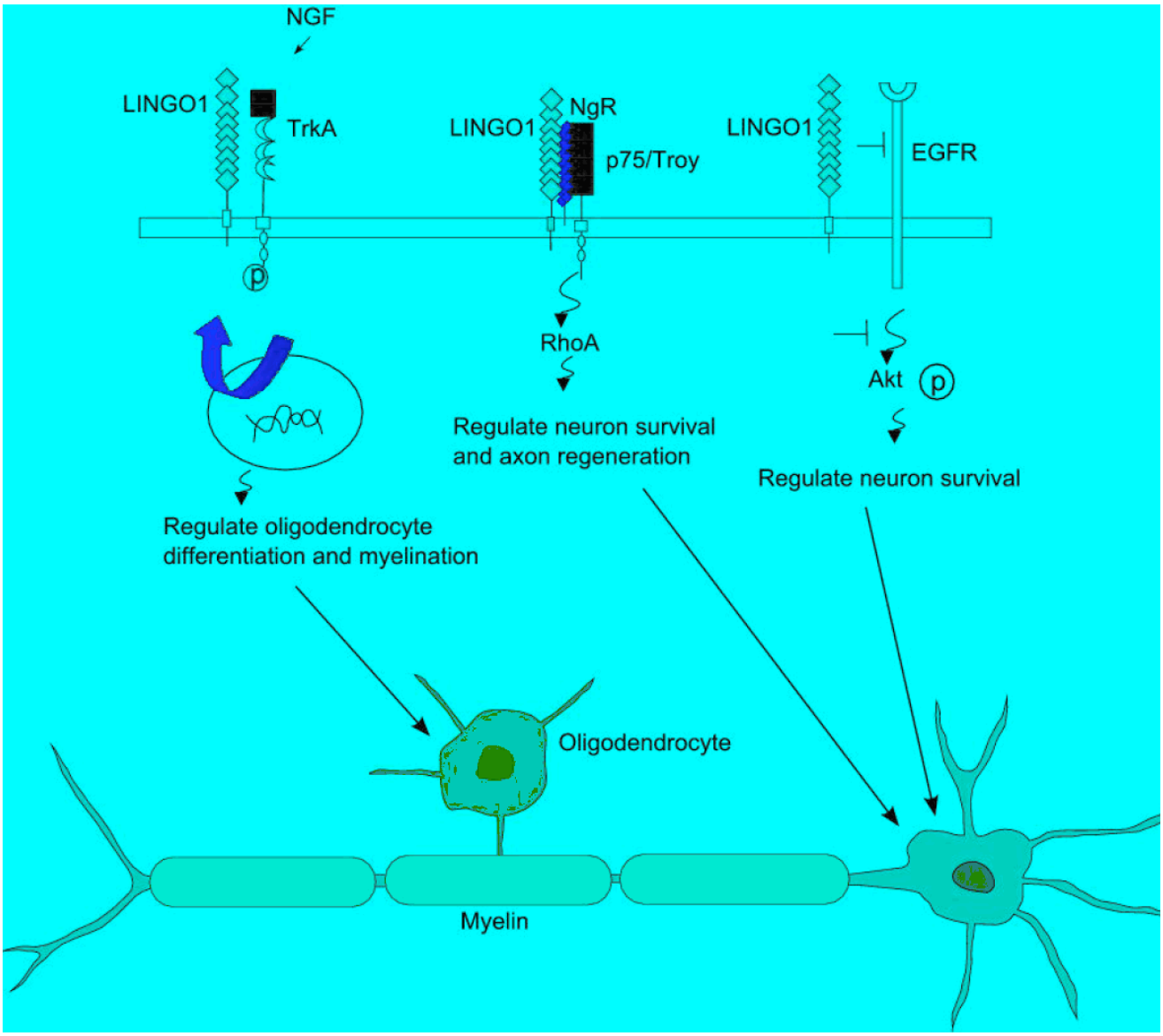


Supplementary Figure 2. Genetic structure **a)** and protein domains **b)** of the leucine-rich repeat neuronal 6A gene (*LINGO1*). Boxes represent exons. The single nucleotide polymorphism rs9652490, positioned in intron 3 is significantly associated with ET. This figure is not scaled. Domain organization of LINGO1 was re-drawn from Mosyak L et al.[14]: LRR (leucine-rich repeat); IGl1 (immuno-Globulin-like domain); stalk-region; TM (trans-membrane region); CT (cyto-plasmic tail).



Supplementary Figure 3. Signaling pathways have been described for LINGO1**.** It has been shown that the gene is exclusively expressed in the central nervous system. It regulates axon generation, oligodendrocyte differentiation and myelination as well as neuron survival. Adapted and re-drawn from Mi S, et al.[15] with permission from the publisher.

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Appendix C

R code for the functions simulation(), simul() and G(), used for Mendelian drop-down simulations. Written in R version 2.13.2.

```
### Funcions written in R for Mendelian drop-down simulations:
### 1. simulation()
### 2. simul()
### 3. G()
### Hjorvar Petursson, 2012


### 1. simulation()
simulation <- function(pre, allfreq, number=100) {
     # Wrapper around simul, gathering Gs and Zs
     # Set up in a table with frqs, Gscore, Zscore, OR and Pval
     frq1 <- frq2 <- Gscore <- Zscore <- numeric(number)
     nsim <- 1:number
     for(x in nsim) {
          tmp <- simul(pre=pre, allfreq=allfreq)
          frq1[x] <- tmp$af
          frq2[x] <- tmp$cf
          Gscore[x] <- tmp$Gstat
          Zscore[x] <- tmp$Zstat
     }
     r <- (frq1/(1-frq1))/(frq2/(1-frq2))
     Pval <- pnorm(abs(Zscore),lower=F)*2
     outframe <-
data.frame(cbind(nsim,frq1,frq2,r,Gscore,Zscore,Pval))
     names(outframe)[7] <- "p-val_2sd"
     return(outframe)
}


### 2. simul()
simul <- function(pre, allfreq=0.3) {
# Runs one simulation with following output:
# Allelic frequenciess of affecteds and controls, G and signed
squareroot of G.
# WARNING: If the prefile is not absolutely flawless,
# then this function will not work the way it should.
# Uses function G()
# Input: Prefile style matrix and allelic frequency of at-risk
allele
     N <- dim(pre)[1]
     probs <- c(1-allfreq,allfreq)
     simpre <- matrix(nrow=nrow(pre),ncol=8)
     simpre[,1:6] <- pre
     samples <- prod(dim(simpre[simpre[,3]==0,7:8]))
```

```
     simpre[simpre[,3]==0,7:8] <-
sample(0:1,samples,replace=T,prob=probs)
     haves <- simpre[!is.na(simpre[,7]),2]
     havenots <- simpre[is.na(simpre[,7]),2]
     while(any(is.na(simpre[,7]))) {
          # collect offspring of founders, use for 2nd round
          checkthese <- is.na(simpre[,7])*1:N
          checkthese <- checkthese[checkthese!=0]
          for(i in checkthese) {
               if(any(simpre[i,3]==haves) &
any(simpre[i,4]==haves)) {
                    father <- haves[haves==simpre[i,3]]
                    mother <- haves[haves==simpre[i,4]]
                    fgts <- simpre[simpre[,2]==father,7:8]
                    mgts <- simpre[simpre[,2]==mother,7:8]
                    simpre[i,7] <- sample(fgts,1)
                    simpre[i,8] <- sample(mgts,1)
               }
          haves <- simpre[!is.na(simpre[,7]),2]
          havenots <- simpre[is.na(simpre[,7]),2]
          }
     }

### 2 by 2 tables and G test:
     ftable1 <- table(c(simpre[simpre[,3]==0 &
simpre[,6]!=1,7:8],c(0,1)))-1 # non-controls
     ftable2 <- table(c(simpre[simpre[,3]==0 &
simpre[,6]==1,7:8],c(0,1)))-1 # controls
     afftable <- table(c(simpre[simpre[,6]==2,7:8],c(0,1)))-1
     contable <- table(c(simpre[simpre[,6]==1,7:8],c(0,1)))-1
     # Preparing sign:
     cf <- contable[2]/sum(contable)
     af <- afftable[2]/sum(afftable)
     Gstat <- round(G(c(afftable,contable)),11)
     Zstat <- sign(af-cf)*sqrt(Gstat)
     outlist <- list(af,cf,Gstat,Zstat)
     names(outlist) <- c("af","cf","Gstat","Zstat")
     return(outlist)
}


### 3. G()
G <- function(n1,n2=NULL,n3=NULL,n4=NULL) {
### The likelihood based G statistic for 2x2 tables
```

```
if(length(n1)==4) {
     n4 <- n1[4]
     n3 <- n1[3]
     n2 <- n1[2]
     n1 <- n1[1]
     }
aa <- n1*log(n1)+n2*log(n2)+n3*log(n3)+n4*log(n4)
bb <- (n1+n2)*log(n1+n2)+(n3+n4)*log(n3+n4)
cc <- (n1+n3)*log(n1+n3)+(n2+n4)*log(n2+n4)
dd <- (n1+n2+n3+n4)*log(n1+n2+n3+n4)
2*(aa-bb-cc+dd)
}
```