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

Hreinn Stefansson^{1,11}, Stacy Steinberg^{1,11}, Hjorvar Petursson^{1,11}, Omar Gustafsson¹, Iris H Gudjonsdottir¹, Gudrun A Jonsdottir¹, Stefan T Palsson¹, Thorlakur Jonsson¹, Jona Saemundsdottir¹, Gyda Bjornsdottir¹, Yvonne Böttcher¹, Theodora Thorlacius¹, Dietrich Haubenberger², Alexander Zimprich², Eduard Auff², Christoph Hotzy², Claudia M Testa³, Lisa A Miyatake⁴, Ami R Rosen⁴, Kristleifur Kristleifsson¹, David Rye⁴, Friedrich Asmus⁵, Ludger Schöls⁶, Martin Dichgans⁷, Finnbogi Jakobsson⁸, John Benedikz⁹, Unnur Thorsteinsdottir^{1,10}, Jeffrey Gulcher¹, Augustine Kong¹, and Kari Stefansson^{1,10}

¹deCODE genetics, Sturlugata 8, IS-101 Reykjavík, Iceland. ²Department of Neurology, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria. ³Emory Department of Neurology and Center for Neurodegenerative Diseases, Whitehead Biomedical Research Building, 505F, 615 Michael Street, Atlanta, Georgia 30322, USA. ⁴Emory Department of Neurology, Wesley Woods Health Center, 1841 Clifton Road, 3rd Floor, Atlanta, Georgia 30329, USA. ⁵Dystonia Genetics Unit, Hertie-Institute for Clinical Brain Research, Center of Neurology, University of Tuebingen, Tuebingen, Germany. ⁶Hertie-Institute for Clinical Brain Research, Center of Neurology, University of Tuebingen, Tuebingen, Germany. ⁷Department of Neurology, Klinikum Grosshadern, University of Munich, Munich, Germany. ⁸Department of Neurology, Landspítaliinn University Hospital, Fossvogur, IS-108 Reykjavik, Iceland. ⁹Department of Neurology, Landspítaliinn University Hospital, Landakot, IS-101 Reykjavik, Iceland. ¹⁰University of Iceland, School of Medicine, Sturlugata 8, IS-101 Reykjavik, Iceland.

Abstract

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. An increase in the number of fusiform swellings of Purkinje cell axons in *LINGO1* knockout models highlights the potential role of *LINGO1* in essential tremor pathophysiology.

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

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Correspondence should be addressed to K.S. (kstefans@decode.is).

¹¹These authors contributed equally to this work.

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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

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was barely discernable. By contrast, clinical series indicate that age of onset is bimodal¹. Thus, bimodal onset may be due to the preferential referral to tertiary centers of individuals with young-onset, familial essential tremor¹. Familial cases seem to account for approximately 50% of young-onset cases from a population-based sample¹, whereas more than 80% of young-onset cases from clinical series seem to be familial¹.

In some families, essential tremor seems to be caused by an autosomal dominant variant with high but not full penetrance². Thus, environmental factors are also likely to have a role in the etiology of essential tremor, in addition to genetic factors³. 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 alcohol³. Current treatments of essential tremor using beta blockers or primidone have only limited efficacy³, 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 heterogeneous, pathological findings in recent studies on brains of affected individuals support the notion of the cerebellum as a potential hot spot of morphologic changes⁵. 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 disease⁶. This cerebellar pathology, which is well in line with the clinical observation of cerebellar dysfunction in essential tremor⁵, was recently confirmed with an independent series showing cerebellar atrophy and Purkinje cell loss⁷.

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 affected². Although tremor frequency decreases with age, tremor amplitude tends to increase. Action tremor often impairs writing, drinking, eating and various other activities of daily living³.

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 relatedness between individuals and potential population stratification by the method of genomic control⁸. 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 membrane⁹. 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 degeneration¹⁰, and it has been shown that *LINGO1* inactivation of glycogen synthase kinase-3 β enhances survival of granular neurons of the cerebellum¹¹. 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 tremor¹². Additionally, some animal models with myelin defects are known to have tremor¹³.

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 localization¹⁴.

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 injury¹⁰ and glaucoma¹⁰ and human multiple sclerosis and Parkinson's disease¹⁰ 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 system¹⁰. 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 cells, considered to be one of the pathological findings in essential tremor⁶, could represent a morphological correlate of the impaired axonal integrity observed in LINGO1 knockout models, highlighting the potential role of LINGO1 in the pathophysiology of essential tremor. Association of essential tremor with *LINGO1* opens up a new field in the research into essential tremor, and it is our hope that LINGO1 will point the way to new treatments for severe cases of the disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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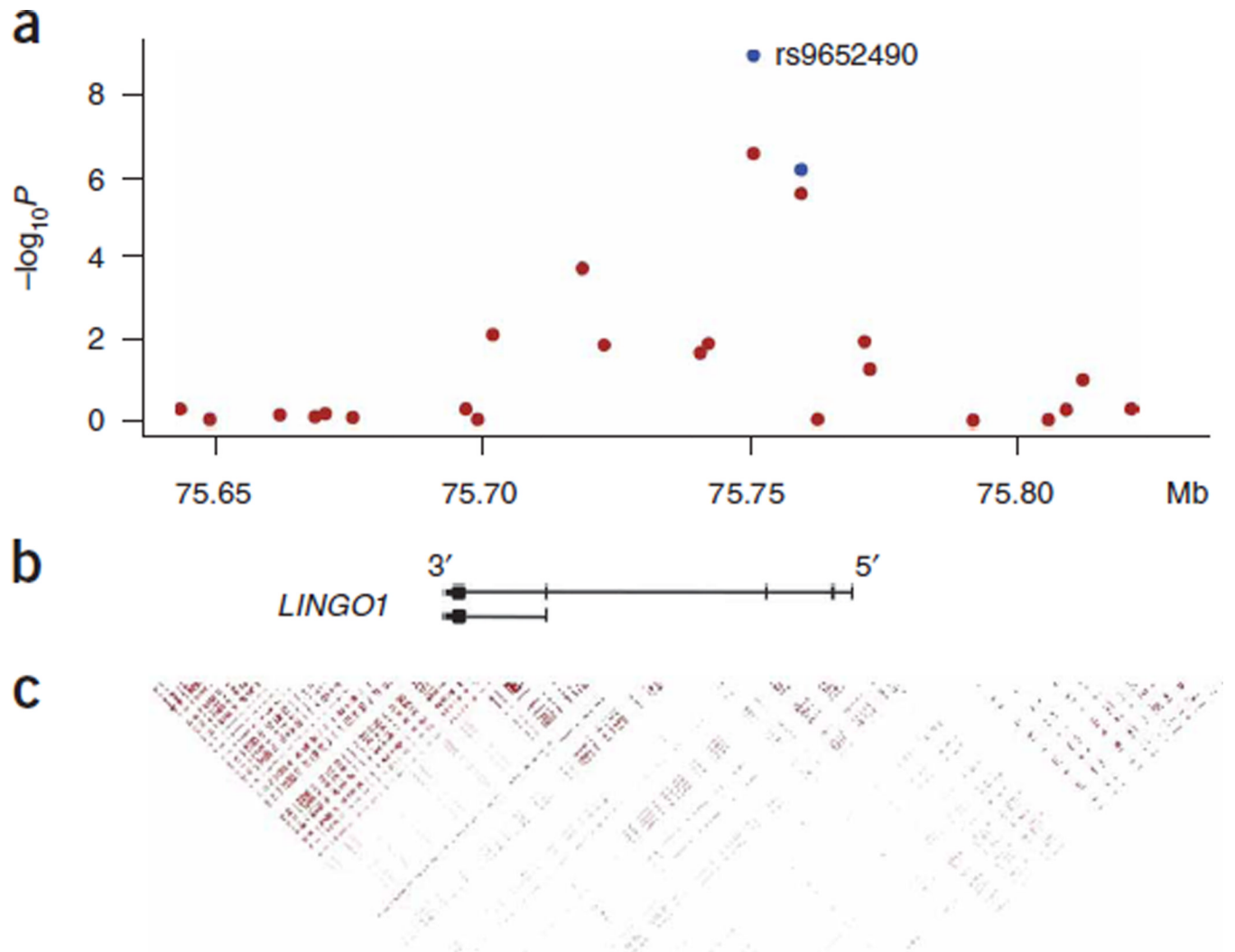


Figure 1. Overview of the 185-kb region around rs9652490. **(a)** Single marker association results from both the genome-wide scan of Icelandic subjects with essential tremor (red circles) and the combined analysis including four follow-up datasets (blue circles). P values are corrected for relatedness 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.

Table 1

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

	Controls		Cases		OR (95% CI)	P value
	N	Frequency	N	Frequency		
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 model¹⁵.