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SHORT REPORT

Replication of the *LINGO1* gene association with essential tremor in a North American population

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A marker in the *LINGO1* gene, rs9652490, showing significant genome-wide association with essential tremor (ET), was recently reported in an Icelandic population. To replicate this association in an independent population from North America, we genotyped 15 SNPs in the *LINGO1* gene in 257 Caucasian ET cases ('definite,' 'probable' or 'possible') and 265 controls enrolled in an epidemiological study at Columbia University. We observed a marginally significant association with allele G of the marker rs9652490 (P=0.0569, odds ratio (OR)=1.33). However, for 'definite' or 'probable' ET, rs9652490 was significantly associated with ET (P=0.03, OR=1.41). Our subsequent analysis of early-onset ET (age at onset <40 years) revealed that three SNPs, rs177008, rs13313467 and rs8028808, were significantly associated with ET (P=0.028, OR=1.52; P=0.0238, OR=1.54; and P=0.0391, OR=1.55, respectively). These three SNPs represent a 2.3 kb haplotype. Finally, a meta-analysis of three published studies confirms allelic association with rs9652490 and two adjacent SNPs. Our study independently confirms that the *LINGO1* gene is a risk factor for ET in a Caucasian population in North America, and further shows that those with early-onset ET are likely to be at high risk.
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INTRODUCTION

Essential tremor (ET) is one of the most common neurological diseases,[1] with a prevalence (age ≥40 years) estimated to be 4.0% and prevalence in the oldest old exceeding 20.0%.[2] The underlying etiological factors and disease mechanisms are not well understood and little is known about the contribution of genetic risk factors to ET. Linkage studies in families with ET have identified three gene loci, namely, ETM1 (OMIM 190300), ETM2 (OMIM 602134) and ETM3 (OMIM 611456);[3–5] however, the causal genes have yet to be identified and candidate gene studies in ET case–control populations have also failed to replicate associations. Several clinical and post-mortem studies suggest an association between ET and Parkinson's disease (PD)[6–8] and studies have investigated whether genetic risk factors for PD may also contribute to the genetic etiology of ET. Our own and other published studies suggest that two of the most significant risk factors for PD, *LRRK2* and *GBA*, do not contribute to ET[9–13] Recently, a genome-wide association study of ET in an Icelandic population identified a marker in the *LINGO1* gene to be significantly associated (P=1.2×10^{-9}; odds ratio (OR)=1.55), which was replicated in the same report in follow-up samples from Austria (P=0.0082; OR=1.73), Germany (P=0.15; OR=1.39) and the United States (P=0.14; OR=1.32).[14]

In this study, we further examined the *LINGO1* gene using detailed clinical and ethnic background information obtained from a case–control (257 ET cases *vs* 265 controls) study conducted in northern Manhattan. The study had four aims. First, using our sample, we performed a case–control association analysis to evaluate the association of the marker rs9652490 and further characterized its relation with clinical subtype. Second, we performed a haplotype analysis using seven SNPs flanking rs9652490. Third, we performed a meta-analysis of three published studies[14–16] in addition to our own study. Finally, we evaluated the frequency in our sample of seven variants that were previously identified through sequencing of *LINGO1* exons in Icelandic ET cases; these variants included four 'synonymous' coding SNPs, a variant located in the 5′ UTR, and two SNPs located in the 3′ UTR.

MATERIALS AND METHODS

Subjects

ET cases and controls were enrolled in an epidemiological study at the Neurological Institute, Columbia University, beginning in 2000. Each signed a written informed consent approved by the University Human Ethics Committee. ET cases were recruited from the Neurological Institute. Controls, ascertained from the same set of zip codes in New York, New Jersey and Connecticut as cases, were recruited using random-digit telephone dialing and were frequency-matched on age (5-year strata), gender and race categories. Each control was initially screened for tremor using a questionnaire and later underwent the same detailed videotaped neurological examination and tremor examination as the cases to ensure that they did not have ET. All participants underwent a demographic and medical history questionnaire, a family history questionnaire (any first- or second-degree relative with tremor (nonspecific),

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ET or PD), and a videotaped neurological examination and tremor examination. Self-reported information on race and ethnic group was obtained. Beginning in 2002, self-reported information on Jewish ancestry was also collected. Data on age of onset of tremor, which we have shown to be reliable,[17] were by self-report. On the basis of previous data on the distribution of age of onset in ET, early age of onset was designated as <40 years of age.[18]

After review of the history and videotaped examinations, the diagnosis of ET was then reconfirmed by a senior neurologist specializing in movement disorders (E.D.L) using published criteria for possible, probable or definite ET,[19] with the latter two categories requiring tremor of greater severity. The presence of bradykinesia or any other sign of parkinsonism (except isolated rest tremor) was an exclusionary criterion for ET.

There were initially 699 participants, of whom 617 (88.3%) were non-Hispanic White (328 ET cases and 289 controls). We included in these analyses 265 non-Hispanic white controls and 257 of 328 non-Hispanic white cases whose ET diagnoses were reconfirmed and who had an available sample (total $n=522$).

Table 1 Demographic and clinical characteristics of genotyped subjects

	ET Cases (N=*257*)	*Controls* (N=*265*)
ET diagnosis: definite/probable/possible	75/118/64	0
% Male (*n*)*	52.1% (134)	42.6% (113)
Mean age at tremor onset (years) (SD)	43.8 (22.7)	Not applicable
Mean years of education (SD)	15.3 (3.8)	15.6 (3.4)
% With family history of PD (*n*)	9.3% (24)	6.0% (16)
% With family history of ET (*n*)**	34.2% (88)	1.1% (3)
% Ashkenazi Jewish ancestry (*n*)**	36.6% (94)	23.4 % (62)

*$P<0.05$, **$P<0.001$.

Molecular genetic analysis

SNP genotyping. *LINGO1* SNPs reported by Stefansson *et al*[14] were genotyped. *LINGO1* SNP-marker genotyping was performed using matrix-assisted laser desorption/ionization time of flight mass spectrometry (Sequenom, San Diego, CA, USA). PCR assays and mass extend reactions were designed using mass array assay design software (Sequenom). SNP details, assays, PCR primers and mass extend primers are provided in Supplementary Table 1. PCR assays were performed using Applied Biosystems (Foster City, CA, USA) Geneamp PCR thermocyclers. Extension products were analyzed using the mass array compact mass spectrometer (Bruker Daltonik, Billerica, MA, USA), and spectra were analyzed using Spectro TYPER 2.0 software (Sequenom). All genotyping was performed in duplicate with separate assays; analyses were performed blind to case–control status.

Statistical analysis

For our association analyses of *LINGO1*, we studied 522 non-Hispanic whites (257 ET cases and 265 controls). Before association analysis, we assessed SNP markers in controls for deviations from Hardy–Weinberg equilibrium using the HAPLOVIEW program (http://www.broadinstitute.org/mpg/haploview; Haploview, Cambridge, MA, USA).[20] The χ^2-test (or the Fisher's exact test when samples fewer than five) was used to assess genotypic and allelic associations between ET and each of the SNP markers. The HAPLOVIEW program was used to perform single point analysis as well as estimation of linkage disequilibrium (LD) structure and haplotype blocks. Haplotype analyses were performed with HAPLO.STATS v1.1.1 for case–control data using the same sliding window of two to three contiguous SNPs.[21] To minimize the risk of a false-positive finding from rare haplotypes, we computed empirical *P*-values by generating the null distribution on the basis of 1000 replicates of haplotype analyses.

We performed meta-analysis to assess whether rs9652490 and flanking SNPs were significantly associated in the three published studies[14–16] with our study. For this purpose, we estimated meta-analysis of *P*-values from four studies as implemented in METAL (http://www.sph.umich.edu/csg/abecasis/metal/), which estimates a single summary *P*-value across studies with varying ethnicity, phenotype distribution, sex. An overall *Z*-statistic and *P*-value are calculated while taking into account the number of individuals studied in each study. To assess the association independent of the discovery set and to evaluate the overall effect, we performed two meta-analyses: one excluding the original study by Stefansson *et al*[14] and one combining all studies.

RESULTS

Clinical characteristics and demographics of genotyped ET cases and controls

For the *LINGO1* analyses, 257 cases and 265 controls were similar in years of education (Table 1). A larger proportion of ET cases were male and Ashkenazi Jewish (AJ), and a higher proportion had a family history of ET (Table 1). Fifteen SNPs in the *LINGO1* gene, including rs9652490, were genotyped in 257 ET cases and 265 controls. Details of the SNPs genotyped in this study are included in Table 2a and Supplementary data.

LINGO1 single point association

Although allele G of rs9652490, identified by Stefansson *et al*[14] in an Icelandic population, was marginally associated with ET ($P=0.0569$, OR=1.33, 95% confidence interval (CI)=0.99–1.80) in this data set (Table 2a), we observed that rs7177008 and rs13313467, located 1.3 and 2.3 kb away from rs9652490, respectively, were significantly associated with ET in the total sample ($P=0.0473$, 0.0393, respectively) (Table 2a). We then restricted the analysis to early-onset (<40 years of age) ET ($n=104$ ET cases) and 255 controls and focused on the haplotype block (See supplementary Figure 1A) that included candidate SNPs to identify a high-risk group. We observed that the magnitude of association strengthened for rs177008 (OR=1.52, 95% CI: 1.04–2.22; $P=0.028$), rs13313467 (OR=1.54, 95% CI: 1.06–2.25; $P=0.0238$) and rs8028808 (OR=1.55, 95% CI: 1.02–2.35; $P=0.039$) (Table 2b). When we compared the cases with a family history of ET *vs* controls, rs8028808 remained significant (OR=1.58, 95% CI: 1.02, 2.45; $P=0.038$), but flanking SNPs no longer reached a *P*-value of <0.05 (Table 2c).

To assess whether the association was driven by a group of clinically homogeneous cases, we restricted analysis to ET cases that had received a diagnosis of 'definite' or 'probable' ET ($n=193$ ET cases) and 265 controls, and found the same three SNPs (rs177008, $P=0.0257$; rs13313467, $P=0.0213$; and rs8028808, $P=0.0329$) as well as rs9652490 ($P=0.0328$) to be significantly associated with disease (data not shown). However, we did not extend this restricted analysis to early onset and to family history of ET because of small numbers of cases in these subgroups.

To determine whether the observed allelic associations were the same in Ashkenazi Jews as in the overall Caucasian samples, we restricted the analysis to 94 ET cases and 62 controls who reported AJ ancestry. This analysis revealed that rs9652490 was no longer associated, but an SNP located in the 3′UTR of *LINGO1*, rs11853396, showed evidence of association ($P=0.0317$, OR=1.65) (data not shown).

Linkage disequilibrium and haplotype analysis

Our 3-mer sliding window haplotype analyses of SNPs 8–15 suggested a risk haplotype of 'GGGGA' with allele 'G' at rs9652490 in *LINGO1* for ET (Table 3). As shown in Table 3, the strong and consistent association was observed in early-onset ET, centering around SNPs 10–12 (empirical $P=0.02$). Similarly, haplotypic analysis of the cases

Table 2a Association between ET and SNPs in the *LINGO1* gene for all ET cases vs controls

SNP no.	Marker	Physical location (bp)[a]	Gene location	Associated allele	Frequency Cases (N=257)	Frequency Controls (N=265)	OR (95% CI)	χ^2	P-value
1	rs3144	75 693 259	3′UTR	T	0.745	0.725	1.1 (0.84,1.46)	0.514	0.4735
2	rs11853396	75 693 286	3′UTR	C	0.631	0.595	1.17 (0.91,1.50)	1.439	0.2302
3	rs3743481	75 694 200	Exon2/synon	C	0.565	0.547	1.08 (0.84,1.38)	0.343	0.5579
4	rs2271396	75 694 590	Exon2/synon	C	0.374	0.352	1.10 (0.85,1.42)	0.529	0.4669
5	rs2271397	75 694 830	Exon2/synon	T	0.374	0.354	1.09 (0.85,1.41)	0.465	0.4953
6	rs2271398	75 694 839	Exon2/synon	G	0.381	0.354	1.12 (0.87,1.45)	0.796	0.3722
7	rs11633842	75 728 395	5′UTR	A	0.01	0.004	2.62 (0.51,13.59)	1.427	0.2323
8	**rs9652490**	**75 750 942**	**Intron**	**G**	**0.242**	**0.193**	**1.33 (0.99,1.80)**	**3.626**	**0.0569**
9	rs11631120	75 751 161	Intron	G	0.176	0.133	1.40 (0.99,1.96)	3.733	0.0533
10	rs7176315	75 751 966	intron	G	0.407	0.371	1.16 (0.91,1.50)	1.433	0.2313
11	**rs7177008**	**75 752 255**	**Intron**	**G**	**0.244**	**0.193**	**1.35 (1.00,1.81)**	**3.935**	**0.0473**
12	**rs13313467**	**75 753 223**	**Intron**	**A**	**0.244**	**0.191**	**1.37 (1.01,1.84)**	**4.246**	**0.0393**
13	rs8028808	75 754 471	Intron	A	0.185	0.144	1.35 (0.97,1.88)	3.188	0.0742
14	rs11856808	75 759 825	Intron	T	0.406	0.367	1.17 (0.91,1.51)	1.585	0.2081
15	rs11856876	75 760 067	Intron	C	0.399	0.366	1.15 (0.90,1.48)	1.241	0.2652

SNPs 1–7 were previously identified by Stefansson *et al*[14] by sequencing of the *LINGO1* gene in Icelandic ET cases.
SNPs with $P<0.05$ and the SNP identified in the Icelandic study (rs9652490) are in bold.
[a]Genomic position based on NCBI genome build 36.3.

Table 2b Single point association for early-onset ET cases

SNP no.	Marker	Associated allele	Physical location (bp)[a]	Frequency Early-onset cases[b] (N=104)	Frequency Controls (N=255)	OR (95% CI)	χ^2	P-value
8	**rs9652490**	**G**	**75 750 942**	**0.265**	**0.197**	**1.47 (1.00,2.15)**	**3.954**	**0.0468**
9	**rs11631120**	**G**	**75 751 161**	**0.194**	**0.134**	**1.56 (1.01,2.40)**	**4.153**	**0.0416**
10	rs7176315	G	75 751 966	0.422	0.372	1.23 (0.89,1.72)	1.563	0.2113
11	**rs7177008**	**G**	**75 752 255**	**0.272**	**0.197**	**1.52 (1.04,2.22)**	**4.828**	**0.028**
12	**rs13313467**	**A**	**75 753 223**	**0.272**	**0.195**	**1.54 (1.06,2.25)**	**5.108**	**0.0238**
13	**rs8028808**	**A**	**75 754** 471	0.209	0.146	1.55 (1.02,2.35)	4.255	0.0391
14	rs11856808	T	75 759 825	0.417	0.268	1.23 (0.88,1.71)	1.512	0.2188
15	rs11856876	C	75 760 067	0.412	0.366	1.21 (0.87,1.69)	1.287	0.2566

SNPs with $P<0.05$ are in bold.
[a]Genomic position based on NCBI genome build 36.3.
[b]Early onset defined as those who have definite, probable or possible ET at 40 years of age or younger. For this analysis, we included controls who were >40 years of age to serve as 'super' controls.

Table 2c Single point association for ET cases with a family history of ET

SNP no.	Marker	Associated allele	Physical location (bp)[a]	Frequency Cases with a family history of ET[b] (N=88)	Frequency Controls (N=265)	OR (95% CI)	χ^2	P-value
8	rs9652490	G	75 750 942	0.256	0.193	1.43 (0.96,2.14)	3.121	0.077
9	rs11631120	G	75 751 161	0.184	0.133	1.47 (0.93,2.32)	2.714	0.100
10	rs7176315	G	75 751 966	0.432	0.371	1.29 (0.91,1.82)	2.045	0.153
11	rs7177008	G	75 752 255	0.256	0.193	1.43 (0.96,2.14)	3.121	0.077
12	rs13313467	A	75 753 223	0.256	0.191	1.45 (0.97,2.17)	3.330	0.068
13	**rs8028808**	**A**	**75 754 471**	**0.210**	**0.144**	**1.58 (1.02,2.45)**	**4.304**	**0.038**
14	rs11856808	T	75 759 825	0.426	0.367	1.28 (0.90,1.81)	1.927	0.165
15	rs11856876	C	75 760 067	0.426	0.366	1.29 (0.91,1.82)	2.057	0.152

SNPs with $P<0.05$ are in bold.
[a]Genomic position based on NCBI genome build 36.3.
[b]Self-report of a family history in first-degree relatives.

Table 3 Haplotype analysis of LINGO1 using SNPs 7–15

						All ET cases vs controls (257:265)						*Early-onset ET cases vs controls (104:255)*						*Cases with family history vs controls (88:265)*					
SNP no.			*HAPLO*			*Freq*	*Z*	P-*value*	*Emp P*	*Global P*	*Global Emp P*	*Freq*	*Z*	P-*value*	*Emp P*	*Global P*	*Global Emp P*	*Freq*	*Z*	P-*value*	*Emp P*	*Global P*	*Global Emp P*
7	8	9	G	A	A	0.782	−2.00	0.045	0.041	0.142	0.142	0.782	−2.12	0.034	0.036	0.088	0.085	0.792	−1.79	0.074	0.067	0.333	0.336
7	8	9	G	G	A	0.058	0.16	0.877	0.842			0.060	0.19	0.847	0.796			0.060	0.51	0.607	0.686		
7	8	9	A	G	A	0.007	1.19	0.234	0.171			0.007	1.55	0.121	0.054								
7	8	9	**G**	**G**	**G**	0.154	1.96	0.050	0.060			**0.150**	**2.03**	**0.042**	**0.044**			0.144	1.67	0.094	0.078		
8	9	10	A	A	A	0.611	−1.20	0.232	0.242	0.219	0.237	0.614	−1.21	0.224	0.239	0.172	0.178	0.614	−1.43	0.154	0.149	0.316	0.325
8	9	10	A	A	G	0.171	−0.64	0.523	0.560			0.168	−0.81	0.417	0.434			0.178	−0.06	0.952	0.917		
8	9	10	G	A	G	0.065	0.50	0.618	0.573			0.067	0.65	0.514	0.494			0.064	0.59	0.558	0.608		
8	9	10	**G**	**G**	**G**	0.154	1.96	0.050	0.061			**0.150**	**2.03**	**0.042**	**0.044**			0.144	1.67	0.094	0.078		
9	10	11	A	A	C	0.611	−1.20	0.232	0.242	0.219	0.237	0.614	−1.21	0.224	0.239	0.172	0.178	0.614	−1.43	0.154	0.149	0.316	0.325
9	10	11	A	G	C	0.171	−0.64	0.523	0.560			0.168	−0.81	0.417	0.434			0.178	−0.06	0.952	0.919		
9	10	11	A	G	G	0.065	0.50	0.618	0.574			0.067	0.65	0.514	0.475			0.064	0.59	0.558	0.608		
9	10	11	**G**	**G**	**G**	0.154	1.96	0.050	0.059			**0.150**	**2.03**	**0.042**	**0.044**			0.144	1.67	0.094	0.078		
10	11	12	A	C	C	0.611	−1.17	0.244	0.249	0.150	0.135	0.613	−1.23	0.218	0.257	0.132	0.102	0.614	−1.42	0.156	0.157	0.264	0.258
10	11	12	G	C	C	0.171	−0.63	0.531	0.578			0.168	−0.82	0.414	0.434			0.178	−0.06	0.955	0.915		
10	11	12	**G**	**G**	**A**	**0.217**	**2.03**	**0.042**	**0.044**			**0.217**	**2.22**	**0.026**	**0.020**			0.207	1.84	0.065	0.056		
11	12	13	C	C	G	0.782	−1.95	0.052	0.052	0.152	0.117	0.782	−2.15	0.032	0.034	0.135	0.101	0.791	−1.77	0.076	0.067	0.190	0.191
11	12	13	G	A	G	0.053	0.85	0.397	0.405			0.053	0.77	0.440	0.430			0.047	−0.11	0.916	0.923		
11	12	13	**G**	**A**	**A**	0.164	1.76	0.078	0.077			**0.164**	**2.01**	**0.044**	**0.038**			**0.161**	**2.05**	**0.040**	**0.033**		
12	13	14	C	G	C	0.613	−1.17	0.243	0.255	0.253	0.240	0.616	−1.17	0.244	0.261	0.210	0.194	0.616	−1.34	0.181	0.173	0.313	0.327
12	13	14	C	G	T	0.170	−0.71	0.478	0.514			0.167	−0.98	0.329	0.358			0.176	−0.22	0.824	0.883		
12	13	14	A	G	T	0.053	0.85	0.397	0.405			0.053	0.77	0.440	0.416			0.047	−0.11	0.916	0.874		
12	13	14	**A**	**A**	**T**	0.163	1.84	0.065	0.069			**0.162**	**2.08**	**0.038**	**0.039**			**0.159**	**2.11**	**0.034**	**0.037**		
13	14	15	G	C	A	0.611	−1.17	0.244	0.249	0.470	0.501	0.613	−1.23	0.218	0.241	0.369	0.353	0.614	−1.42	0.156	0.149	0.358	0.346
13	14	15	G	T	C	0.224	−0.15	0.885	0.896			0.221	−0.39	0.696	0.740			0.224	−0.17	0.868	0.856		
13	14	15	A	T	A	0.006	0.08	0.938	0.692			0.006	−0.12	0.908	0.989			0.006	0.00	0.999	0.701		
13	14	15	**A**	**T**	**C**	0.157	1.81	0.071	0.071			**0.156**	**2.05**	**0.040**	**0.034**			**0.152**	**2.03**	**0.042**	**0.040**		

Three haplotypic analyses are shown: all ET cases *vs* controls; early-onset ET *vs* controls; and cases with a family history of ET *vs* controls. A sliding window haplotype analysis on a haplotype block containing SNPs 7–15. Haplotypes with empirical (emp) *P*-value <0.05 are highlighted in bold.

with a family history of ET localized to SNPs 11–15. Among early-onset ET, this 5-mer haplotype (SNPs 8–12: rs9652490, rs11631120, rs7176315, rs7177008 and rs13313467) spans 2281 bp, and was found to be significantly associated with ET in a separate analysis (empirical P=0.044). However, the strength of haplotypic association did not improve when we restricted our analysis to definite and probable ET, suggesting that it is a subset of early-onset ET that is driving the association. The inferred haplotypes for SNPs 7–14 and LD patterns in controls and cases are shown in Table 3 and Supplementary Figure 1A, respectively. LD across these SNPs for Ashkenazi Jewish samples are provided in Supplementary Figure 1A.

Evaluation of *LINGO1* sequence variants

We evaluated the frequency of sequence variants previously identified through sequencing of *LINGO1* exons in Icelandic ET cases. No significant association was observed for any of the variants analyzed.

Meta-analysis of SNPs in *LINGO1*

The overall meta-analysis confirmed the strong allelic association between rs9652490 and ET (P=1.55E-11). Given the large number of subjects (n=19 854) studied by Stefansson *et al*,[14] it is not unexpected that the overall observed association remains strong, largely driven by the original finding. Thus, we performed a meta-analysis excluding the subjects from the original study (Table 4). When three replication studies are examined together, the G allele was significantly associated with ET in all three studies (P=0.0007). In addition, the same alleles for the two adjacent SNPs, rs3144 and rs8028808, were associated (P=0.02421, 0.00148, respectively) with ET in two of the three studies.

DISCUSSION

This is one of the first studies to replicate the association of the SNP marker rs9652490 in the *LINGO1* gene in an independent population from North America. To confirm the earlier association and to further characterize genotype–phenotype relations, we studied a well-characterized set of ET cases and controls that were enrolled in an epidemiological study at Columbia University and that underwent a demographic and medical history questionnaire, a family history questionnaire (any first- or second-degree relative with tremor (nonspecific), ET or PD) and a videotaped neurological examination and tremor examination. We then performed allelic and haplotypic association based on diagnostic criteria, early-onset ET, family history of ET and AJ ancestry to identify high-risk group(s). Finally, no significant association was observed with sequence variants previously identified through sequencing of *LINGO1* exons in Icelandic ET cases.

We extended the Icelandic study to show that SNP rs9652490 was significantly associated with definite or probable ET, and the magnitude of association was strongest for those with early-onset ET. Those who have a family history of ET had the same haplotypes associated with ET, whereas those with AJ ancestry did not demonstrate excess risk. Given the small sample size, further studies are needed. The 2.3 kb haplotype associated with ET in our study includes the associated SNP, rs9652490, within a 9 kb block of SNPs in strong LD in intron 3 of *LINGO1*. We did not observe association of sequence variants previously identified through sequencing of *LINGO1* exons in Icelandic ET cases consistent with reported findings. Our overall meta-analysis including three published studies[14–16] and our own study confirmed the strong allelic association between rs9652490 and ET (P=1.55E-11), and the association remained significant (P=0.0007) when we performed a meta-analysis excluding the subjects from the original study from Stefansson *et al*.[14] In addition, two adjacent intronic SNPs, rs3144 and rs8028808, were associated with ET in two of the three studies (P=0.02421, 0.00148, respectively). Thus, the most likely explanation is that rs9652490 is in LD with putative genetic variant(s).

We have shown previously that the neuropathology of ET, in the majority of cases, is characterized by a marked increase in torpedoes (axonal swelling of Purkinje cells) and Purkinje cell loss compared with their occurrence in normal aging.[7,22,23] The normal function of *LINGO1* in axon regeneration, central nervous system myelination and regulation of neuronal survival, together with the neuropathological features of ET, suggests a possible role for *LINGO1* in the pathophysiology of this disease.[14] In animal CNS disease models that have targeted *LINGO1* inhibition, neuron and oligodendrocyte survival, axon regeneration, oligodendrocyte differentiation, remyelination and improved functional recovery were promoted.[24] Although the mechanism by which rs9652490 and/or additional genetic variants in *LINGO1* lead to disease is unknown, functional studies of the 'normal' *LINGO1* gene suggest that disease-associated risk factors may

Table 4 Summary of allelic association

SNP[a]	Studies[b]	Allele 1	Allele 2	Freq Allele 1	Freq SE Allele 1	Min Freq	Max Freq	Weight[c]	Z score[d]	P-value
(a) Three replication studies combined										
rs3743481	1,4	T	C	0.408	0.024	0.395	0.453	2376	0.75	0.4534
rs9652490	**1,3,4**	**G**	**A**	**0.242**	**0.014**	**0.231**	**0.263**	**3305**	**3.39**	**0.0007**
rs3144	1,4	C	T	0.312	0.030	0.255	0.328	2374	2.25	0.0242
rs8028808	1,4	A	G	0.168	0.012	0.144	0.174	2382	3.18	0.0015
(b) All four studies combined										
rs3743481	1,4	T	C	0.408	0.024	0.395	0.453	2376	0.75	0.4534
rs9652490	1,2,3,4	**G**	**A**	**0.310**	**0.034**	**0.231**	**0.329**	**19854**	**6.74**	**1.55E-11**
rs3144	1,4	C	T	0.312	0.030	0.255	0.328	2374	2.25	0.02421
rs8028808	1,4	A	G	0.168	0.012	0.144	0.174	2382	3.18	0.00148

Only rs9652490 was genotyped in all studies. For each of the remaining SNPs, studies that had the genotype are indicated:
[a]In this study.
[b]Stefansson *et al*.[14]
[c]Tan *et al*.[16]
[d]Vilariño-Güell *et al*.[15] When alleles were called differently, alleles from this study were used. Weight represents the total number of subjects genotyped. The direction of the *Z* score represents allelic association with allele 1.

lead to 'a gain-of-function' or 'overexpression' of *LINGO1*. Future studies, including 'deep' sequencing and gene expression studies, will be needed to clarify the genetic contribution of rs9652490 and additional sequence variants in *LINGO1* to disease risk and functional studies to determine the disease mechanism.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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