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Mutations in the Parkinson's disease genes, Leucine Rich Repeat Kinase 2 (LRRK2) and Glucocerebrosidase (GBA), are not associated with essential tremor

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Abstract

We evaluated an association between essential tremor (ET) and the Parkinson's disease (PD) genes, Leucine Rich Repeat Kinase 2 (LRRK2) and Glucocerebrosidase (GBA). Clinical studies demonstrate an association between ET and PD, suggesting possible shared pathophysiologies, yet LRRK2 has rarely been studied in ET, and GBA, not at all. ET cases (n = 275, including 42 with rest tremor) and controls (n = 289) were enrolled in an epidemiological study (Columbia University). Post-mortem brain tissue samples were obtained on 24 additional ET cases, including 3 with brainstem Lewy bodies. We performed a comprehensive analysis of the LRRK2 gene by genotyping 4 LRRK2 mutations (G2019S, I2020T, R1441C and Y1699C), 2 rare LRRK2 variants (L1114L and I1122V) and 19 LRRK2 SNPs. All GBA exons were sequenced in a subset of 93 Ashkenazi Jewish (AJ) cases, 62 AJ controls and 24 ET brains. LRRK2 mutations were not found in any ET cases or ET brains and none of the LRRK2 SNPs was associated with ET. GBA mutations were found in 7.5% (7/93) of AJ ET cases and 4.8% (3/62) of AJ controls (p = 0.75). 8.3% (2/24) of ET brains carried a GBA mutation. Four different heterozygous mutations were identified, including 3 previously reported mutations (N370S, R496H, and E326K) and 1 new missense variant (R44C). As suggested by several smaller prior reports, the known mutations for the LRRK2 gene are not risk factors for ET. Furthermore, a similar frequency of GBA mutations in AJ ET cases and controls suggests that GBA is not a common cause of ET either.

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Keywords

Essential tremor; LRRK2; GBA mutation; Association; Risk factor

1. Introduction

Essential tremor (ET) is one of the most common movement disorders, with a prevalence (age ≥40 years) estimated to be 4.0%. The underlying etiologies and disease mechanisms are not well understood. Several clinical studies suggest an association between ET and Parkinson's disease (PD) [1] and recent post-mortem studies have reported brainstem Lewy bodies (mainly in the locus ceruleus) in some ET cases [2]. These clinical and post-mortem data suggest that ET may, in some cases, share similar disease mechanisms with PD. These observations provide a rationale to evaluate association between PD genetic risk factors and ET, particularly with rest tremor.

Recent studies have investigated whether genetic risk factors that are common in PD may also contribute to the genetic etiology of ET. Mutations in the Leucine Rich Repeat Kinase 2 (LRRK2) gene are associated with both familial and sporadic PD [3,4] with G2019S, one the most frequently reported of these mutations (2–5% of Caucasian PD cases). To date, four studies have evaluated an association with ET by genotyping specific LRRK2 mutations or variants (G2019S, I2012T, I2020T, G2385R and P755L) in patients with ET [5–8]. We and others have shown that mutations in the beta-Glucocerebrosidase (GBA) gene are associated with PD and Dementia with Lewy bodies and that GBA mutation status is significantly associated with the presence of cortical Lewy bodies [9,10]. The GBA gene has not previously been evaluated as a candidate susceptibility gene for ET.

Our rationale for studying LRRK2 and GBA rather than other PD linked genes (a-synuclein, parkin, DJ-1 and PINK1) is that mutations in these genes represent two of the most significant susceptibility genes/risk factors identified to date in PD populations worldwide and a comprehensive analysis of these genes has not previously been performed in ET.

One motivation for the current study was that, in addition to the availability of a large sample of several hundred ET case as well as control bloods, we capitalized on the availability of a large sample of recently-collected ET brains in the Essential Tremor Centralized Brain Repository, which represents the largest collection of ET brains. These post-mortem analyses provided several unique opportunities. First, approximately 30–50% of "ET" cases are misdiagnosed, with most of these cases having PD. The demonstrated absence of Lewy bodies in the substantia nigra pars compacta in our autopsy ET cases minimized the possibility of such diagnostic misclassification. Second, a number of these ET brains had Lewy bodies restricted to the locus ceruleus. Whether these individuals are at increased risk for developing clinical–pathological PD is an open question. Therefore, it was added interest to examine PD-associated genetic markers in this sub-sample of ET cases.

We conducted three types of analyses. First, in 564 living subjects (275 ET cases [including 42 [15.3%] with rest tremor on examination and 233 [84.7%] without rest tremor] and 289 controls) and 24 ET brains (including 3 [12.5%] with brainstem Lewy bodies and 21 [87.5%] without Lewy bodies), we evaluated the frequency of 4 LRRK2 mutations (G2019S, I2020T, R1441C and Y1699C) and 2 rare LRRK2 variants (I1122V, L1114L) that have been reported in PD. Second, in the same living subjects, we performed a case–control association analysis to evaluate the frequency of 19 LRRK2 SNPs that span the entire LRRK2 gene. Third, in a subset of living subjects (93 AJ cases and 62 controls) and 24 ET brains, we also sequenced all GBA exons to evaluate genetic variation and frequency of GBA mutations in ET. Unique

features of the study included (1) the evaluation of GBA mutation status, (2) case–control association analysis of LRRK2 SNPs in addition to evaluation of the frequency of LRRK2 mutations located in exons encoding the kinase, leucine rich repeat and Ras domains, (3) stratification of ET cases based on presence of rest tremor on examination (a parkin-sonian feature), and (4) analysis of ET brains, including several that were known to have Lewy bodies on post-mortem examination.

2. Methods

These analyses made use of blood samples from living ET cases and controls as well as post-mortem brain tissue samples from deceased ET cases (autopsy samples).

2.1. Living ET cases and controls

ET cases and controls were enrolled in an epidemiological study at the Neurological Institute, Columbia University, beginning in 2000. Each signed an informed consent approved by our Human Ethics Committee. ET cases were patients at the Institute who carried clinical diagnoses of ET. 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 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, and a videotaped neurological examination and tremor examination. Race was by self-report. Beginning in June 2002, data on Jewish ancestry were also collected; ancestry as AJ was by self-report.

The videotaped examination included detailed assessment of arm tremor during six tests (five kinetic, one postural) and an assessment of parkinsonian features. Each videotaped examination was reviewed by a senior neurologist (E.D.L). Tremor in each arm was rated during each of six tests using a 0–3 scale, resulting in a total arm tremor score (range = 0–36). Rest tremor was coded as present if visualized during the videotaped examination while the subject was seated, standing, or walking. After review of the history and videotaped examination, the diagnosis of ET was then reassessed by a senior neurologist specializing in movement disorders (E.D.L) using published criteria for ET (moderate or greater amplitude kinetic tremor of the arms during at least three tests or head tremor, in the absence of PD, dystonia, or another neurological disorder) [11]. The presence of bradykinesia or any other sign of parkinsonism (except isolated rest tremor) was an exclusionary criterion for ET.

There were 699 participants of whom 617 (88.3%) were non-Hispanic White (328 ET cases and 289 controls). We included in these analyses all 289 non-Hispanic white controls and the 275 of 328 non-Hispanic white cases who met the aforementioned published criteria for ET (total $n = 564$).

2.2. Autopsy samples

Twenty-four post-mortem brain tissue samples (mean age at death = 85.3 ± 7.6 years, 9 [37.5%] male, 23 [95.8%] non-Hispanic white) were obtained from the Essential Tremor Centralized Brain Repository, New York Brain Bank, and included brains of all patients diagnosed with ET during life whose clinical diagnoses were re-confirmed using brain bank criteria [2]. None of these had participated in the epidemiological study. Lewy body pathology was assessed according to the 3rd report of the DLB consortium, and utilized α-synuclein immunohistochemistry. Brains also received ratings of Braak stage, CERAD and NIA-RI for

Alzheimer tangle and plaque pathologies. Data on AJ status were not routinely available on these ET cases. Three of the 24 ET brains had brainstem Lewy bodies; 21 did not.

2.3. Molecular genetic analysis

2.3.1. LRRK2—LRRK2 genotyping was performed by MALDI-TOF mass spectrometry on the Sequenom platform as described previously [12]. A total of 275 ET cases and 289 controls in addition to 24 autopsy ET brain samples were genotyped for 4 mutations (G2019S, I2020T, R1441C and Y1699C), 2 rare LRRK2 variants (L1114L and I1122V) and 19 LRRK2 SNPs.

2.3.2. GBA—All GBA exons were sequenced as described previously [10] in a subset of AJ samples (93 ET cases and 62 controls) and 24 autopsy ET brain samples.

2.4. Statistical analysis

For our association analyses of LRRK2, we studied 564 non-Hispanic whites (275 ET cases and 289 controls). In addition, we sequenced all GBA exons using 93 AJ ET cases and 62 AJ controls (a subset of the total 564) as well as 24 autopsy ET brain samples, because the carrier frequency of GBA mutations is known to be high in the AJ population.

We assessed SNP markers in controls for deviations from Hardy–Weinberg equilibrium using the HAPLOVIEW program. The χ^2 test (or the Fisher's exact test when samples fewer than 5) 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. To minimize the risk of a false positive finding from rare haplo-types, we computed empirical *p*-values by generating the null distribution based on 1000 replicates of the haplotype analyses.

3. Results

3.1. Clinical characteristics and demographics of ET patients and controls

For the LRRK2 analyses, cases and controls were similar in their age and education (Table 1). A larger proportion of cases were male and Ashkenazi Jewish (AJ) than controls, and a marginally higher proportion ($p = 0.08$) of cases had a family history of PD (Table 1). For the GBA analyses, cases and controls were similar in age, gender and education (Table 1). As expected, in both sets of analyses, a larger proportion of cases than controls had a family history of ET and a larger proportion had rest tremor on examination.

4. LRRK2

LRRK2 mutations were not identified in any of the 275 living ET cases, 24 ET brains or 289 living controls. Single point and haplotype association analyses were performed between all LRRK2 mutations and coding SNPs and disease; however, we found no evidence for association for any SNPs or with the G2019S mutation and the previously reported haplotype.

5. GBA

GBA mutations were observed in a similar proportion of ET cases and controls: 7.5% (7/93) living AJ ET cases and 4.8% (3/62) living AJ controls ($p = 0.74$) carried a heterozygous GBA mutation. Of the 15 living AJ cases with rest tremor on examination, 2 (13.3%) had GBA mutations vs. 5 (6.4%) of 78 living AJ cases without rest tremor ($p = 0.63$). Overall, 8.3% (2/24) of ET brains carried a heterozygous GBA mutation, neither of whom had brainstem

Lewy bodies on post-mortem examination. None of the three ET brains with brainstem Lewy bodies on post-mortem examination carried a heterozygous GBA mutation.

Four different mutations were identified, including 3 previously reported mutations (N370S, R496H, and E326K) and 1 new missense variant (R44C) (Table 2). All the mutations identified have been previously classified as mild or of unknown effect based on the expected clinical phenotype (Table 2). The clinical characteristics of mutation carriers are shown in Table 2.

6. Discussion

This is the first study to evaluate the GBA gene as a susceptibility gene in ET. In our study we did not find evidence for an association with ET, even though a strong genetic association between GBA and PD has been well established. These results occurred despite the fact that our sample included 15 living AJ ET cases with rest tremor on examination as well as 3 ET cases with Lewy bodies on post-mortem examination, each of whom one might argue could have a forme–fruste of PD.

We also performed a comprehensive analysis of the LRRK2 gene in a large sample of ET cases (n = 299; 275 living ET cases and 24 autopsy ET cases), which included 42 living ET cases with rest tremor on examination and 3 ET cases with Lewy bodies on post-mortem examination. Published studies that have analysed LRRK2 in ET have genotyped either specific mutations located in exon 41 (G2019S, I2012T, I2020T) which encodes the kinase domain [5,8] or rare variants found to be associated with PD in specific ethnicities (Asian; P755L and G2385R) [6,7]. In our study, in addition to evaluate the frequency of mutations in the kinase domain (exon 41; G2019S, I2020T), we also analysed mutations and rare variants located in regions encoding other functional domains of the LRRK2 protein including the Leucine rich repeat domains (exon 24: L1114L; exon 25 I1122V) and Ras GTPase (exon 31: R1441C; exon 35: Y1699C) and performed a case–control association analysis to evaluate the frequency of 19 LRRK2 SNPs that span the entire LRRK2 gene. Consistent with previously published studies, we did not identify any of the ‘common’ LRRK2 mutations, including G2019S, nor did we observe an association of LRRK2 SNPs or haplotypes with ET. Moreover, the current study has >80% power at a p-value of 0.05 to detect allelic association under an additive model where the risk ratio associated with a single copy of the variant in LRRK2 is 1.75 and disease allele frequency is 0.2. Thus, although we cannot rule out with certainty, inadequate power is unlikely to be the explanation for these results.

There is a possibility that the mutations and SNPs that we and others have analysed do not represent risk factors for ET and that genetic variation elsewhere in the gene is associated with the disease. Thus, further analysis of the LRRK2 gene including complete sequence analysis of the entire gene (exons 1–51) may be warranted in ET.

A contribution of genetic factors to ET has been suggested based on evidence from twin and family studies. Candidate gene studies in ET have assessed a number of genes shown to be associated with the etiology of other neurological disorders including PD (alpha-Synuclein, parkin, LRRK2, CYP2D6), idiopathic torsion dystonia (DYT1), X-linked spinal bulbar and muscular atrophy (SMAX1), spinocerebellar ataxia type 12 (SCA-12), and Fragile X-associated tremor/ataxia syndrome (FMR1). In most studies, however, sample size as well as gene analysis were limited, and the direction and magnitude of association were conflicting in different populations. Genome wide association studies have been successful in identifying susceptibility loci for a number of diseases and this approach may be useful in identifying the genetic variation that contributes to ET.

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Table 1

Clinical and demographic characteristics of ET patients and controls.

	LRRK2 Analyses		GBA analyses	
	Cases (*n* = 275)	**Controls (*n* = 289)**	**Cases (*n* = 93)**	**Controls (*n* = 62)**
% male (*n*)	51.6 (142)*	41.9 (121)	44.1 (41)	41.9 (26)
Age (years) (sd)	67.3 (15.0)	67.7 (11.1)	68.4 (15.8)	70.2 (11.9)
% Ashkenazi Jewish (*n*)	53.3 (104)*,[a]	40.9 (70)[a]	100.0 (93)	100.0 (62)
Years of education (sd)	15.2 (3.8)	15.6 (3.3)	16.0 (3.2)	16.3 (2.7)
% With family history of PD[b] (*n*)	9.8 (27)	5.9 (17)	8.6 (8)	4.8 (3)
% With family history of ET[c] (*n*)	62.2 (171)**	11.8 (34)	68.8 (64)**	4.8 (3)
% With rest tremor on examination (*n*)	15.3 (42)**	0.0 (0)	16.1 (15)**	0.0 (0)

* $p < 0.05$

** $p < 0.001$ when comparing cases to controls.

Values are mean (sd) or percentages (numbers).

[a] Data available on 195 ET cases and 171 controls.

[b] Report of one or more first- or second-degree relative with PD.

[c] Report of one or more first- or second-degree relative with ET.

Table 2

GBA mutations identified in living cases and controls and autopsy samples.

Subject	GBA mutation/severity	cDNA nt. Substitution	Exon	Zygosity	Clinical		Lewy body pathology on post-mortem examination
					Age	Rest tremor on examination	
	Mild						
Control 1	N370S	c.1226A > G	9	Heterozygous	72	No	NA
Case 1	N370S	c.1226A > G	9	Heterozygous	72	No	NA
Control 2	N370S	c.1226A > G	9	Heterozygous	62	No	NA
Autopsy Case 1	N370S	c.1226A > G	9	Heterozygous	90	Unknown	No
Case 2	N370S	c.1226A > G	9	Heterozygous	85	No	NA
Case 3	N370S	c.1226A > G	9	Heterozygous	76	Yes	NA
Case 4	R496H	c.1604G > A	11	Heterozygous	89	No	NA
	Unknown						
Control 3	E326K	c.1093G > A	8	Heterozygous	60	No	NA
Case 5	E326K	c.1093G > A	8	Heterozygous	77	Yes	NA
Case 6	E326K	c.1093G > A	8	Heterozygous	83	No	NA
Autopsy Case 2	T369M	c.1223C > T	8	Heterozygous	94	No	No
Case 7	R44C	c.247 C > T	3	Heterozygous	79	No	NA