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Glucocerebrosidase Gene Mutations:

A Risk Factor for Lewy Body Disorders

Ignacio F. Mata, PhD, **Ali Samii, MD**, **Seth H. Schneer**, **John W. Roberts, MD**, **Alida Griffith, MD**, **Berta C. Leis, PhD, RN**, **Gerard D. Schellenberg, PhD**, **Ellen Sidransky, MD**, **Thomas D. Bird, MD**, **James B. Leverenz, MD**, **Debby Tsuang, MD, MSc**, and **Cyrus P. Zabetian, MD, MS**
Department of Neurology, University of Washington School of Medicine (Drs Mata, Samii, Schellenberg, Bird, Leverenz, and Zabetian), Geriatric Research Education and Clinical Center (Drs Mata, Schellenberg, Bird, and Zabetian), Mental Illness Research Education and Clinical Center (Drs Leverenz, Tsuang, and Zabetian), and Parkinson's Disease Research Education and Clinical Center (Drs Samii, Leverenz, and Zabetian), VA Puget Sound Health Care System, Virginia Mason Medical Center (Dr Roberts), and Departments of Medicine (Drs Schellenberg and Bird), Pharmacology (Dr Schellenberg), and Psychiatry and Behavioral Sciences (Drs Leverenz and Tsuang), University of Washington, Seattle; Department of Biology, Oberlin College, Oberlin, Ohio (Mr Schneer); Booth Gardner Parkinson's Care Center, Evergreen Hospital Medical Center, Kirkland, Washington (Drs Griffith and Leis); and Section on Molecular Neurogenetics, Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland (Dr Sidransky).

Abstract

Background—Mutations in the glucocerebrosidase (*GBA*) gene have been reported to modify risk for Parkinson disease (PD) and dementia with Lewy bodies (DLB). However, these findings have not been consistently replicated, and most studies have had substantial methodological shortcomings.

Objective—To better assess the role of *GBA* variants in altering risk for Lewy body disorders.

Design—Case-control study.

Setting—Four movement disorder clinics in the Seattle, Washington, area.

Participants—Seven hundred twenty-one patients with PD, 554 healthy control subjects, and 57 patients with DLB.

Main Outcome Measures—Disease status and presence or absence of the 2 most common *GBA* mutations (N370S and L444P).

Correspondence: Cyrus P. Zabetian, MD, MS, Geriatric Research Education and Clinical Center S-182, VA Puget Sound Health Care System, 1660 South Columbian Way, Seattle, WA 98108 (zabetian@u.washington.edu)..

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Results—We observed a significantly higher heterozygote frequency for the 2 mutations in patients with PD (2.9%; *P*<.001) and those with DLB (3.5%; *P*=.045) compared with control subjects (0.4%).

Conclusion—Our findings suggest that *GBA* mutations exert a large effect on susceptibility for Lewy body disorders at the individual level but are associated with a modest (approximately 3%) population-attributable risk in individuals of European ancestry.

GAUCHER DISEASE, THE MOST common glycolipid storage disorder, results from a recessively inherited deficiency of the lysosomal enzyme glucocerebrosidase. Patients with Gaucher disease present with a broad range of phenotypes, but the disease is classified into 3 subtypes based on the absence (type 1) or presence (types 2 and 3) of neurologic manifestations. Although type 1 disease is traditionally considered nonneuronopathic, a small subset of patients develop parkinsonism with brainstem or diffuse Lewy body pathology.[1] Furthermore, an increased incidence of parkinsonism has been reported in relatives of patients with Gaucher disease.[2,3] These observations suggested that mutations in the *GBA* gene, which encodes glucocerebrosidase, might represent a risk factor for Lewy body disorders. Nine case-control analyses[4-12] of Parkinson disease (PD) and 1 of dementia with Lewy bodies (DLB) have been undertaken to test this hypothesis and, although nearly all have reported a higher frequency of *GBA* mutations among cases, the difference has failed to reach significance (or been of marginal significance) in most studies.

Although these case-control data are intriguing, interpretation has been difficult and several criticisms have been raised. Most of the studies had adequate power to detect only large effects at the expected allele frequencies, 2 failed to include an independent control group, and in some race/ethnicity was incompletely characterized. The number of mutations assessed varied greatly, from only the 2 most common (N370S and L444P) to comprehensive screenings of the entire coding region. Finally, mutation frequencies in patients have varied several-fold among studies, even within individuals of similar ancestry. With these issues in mind, we sought to further assess the role of *GBA* in Lewy body disorders by examining the frequency of the N370S and L444P mutations in a large PD case-control sample of European origin and in a cohort of patients with DLB.

METHODS

STUDY PARTICIPANTS

The study population included 721 patients with PD, 554 control subjects, and 57 patients with DLB. The PD group was primarily composed of a cohort of patients (n=706) consecutively recruited at 4 movement disorder clinics in the Seattle, Washington, area. All patients with PD met clinical diagnostic criteria for PD[13] as determined by a movement disorder specialist, and neuro-pathological confirmation was available for 1 patient. Control subjects had no history of parkinsonism or dementia (by structured interview) and were either spouses of patients with PD (n=310) or volunteers from the local community (n=244). Only patients with PD and control subjects of European origin were included in the study.

The DLB group was composed of 3 living patients who met revised clinical diagnostic criteria for probable DLB[14] and 54 patients with dementia who met pathologic criteria for high- (n=21) or intermediate- (n=33) likelihood DLB.[14] Patients with Lewy-related pathology confined to the amygdala were excluded from the study. Only patients with DLB of self-defined white ancestry were included in the analysis. Insufficient information was available to differentiate between patients with DLB of European vs Middle Eastern–North African origin (eg, Ashkenazi Jews).

All study participants had previously been screened for pathogenic *LRRK2* mutations, and those who carried 1 or more of these variants were excluded from the study. The institutional

review boards at each participating site approved the study, and all participants gave informed consent.

GENOTYPING AND DATA ANALYSIS

We genotyped N370S by TaqMan Assay (Applied Bio-systems, Foster City, California) using primers 5′-GCCTTTGTCTCTTTGCCTTTGTC-3′ (forward) and 5′-GGGGTTCAGGGCAAGGTT-3′ (reverse) and probes 5′-VIC-TACCCTAGAaCCTCCTG-3′ and 5′-6FAM-TACCCTAGAgCCTCCT-3′. The L444P mutation was genotyped by sequencing a polymerase chain reaction template that spanned the 3′ half of exon 9 and the full length of exon 10 using primers 5′-CCAATTGGGTGCGTAACTTT-3′ (forward) and 5′-TAGGGAGCAGGGAGGAGAAG-3′ (reverse). L444P occurs either as an individual mutation or *in cis* with other variants (eg, A456P and V460V) as a recombinant allele formed by recombination between *GBA* and a nearby pseudogene.[15]

Genotype frequencies in patients and control subjects were compared by means of the Fisher exact test. Population-attributable risk was calculated using the following equation:

$$P(OR-1)/1+P(OR-1),$$

where P is the prevalence of mutation carriers among control subjects and OR is the odds ratio for disease (PD or DLB).

RESULTS

Twenty-one of the 721 patients with PD (2.9%), 2 of the 57 patients with DLB (3.5%), and 2 of the 554 control subjects (0.4%) were heterozygous for *GBA* mutations N370S or L444P (Table 1). A significantly higher frequency of mutation carriers was found in the PD sample compared with the control group (odds ratio, 8.3; 95% confidence interval, 2.0-73.1; $P<.001$). A marginally significant overrepresentation of mutation carriers was observed among patients with DLB (odds ratio, 10.0; 95% confidence interval, 0.7-139.8; $P=.045$).

The clinical characteristics of the 21 patients with PD heterozygous for *GBA* mutations are given in Table 2. Most of these patients had late-onset disease (14 of 21) and reported no family history of PD (17 of 21). Five of the patients had developed substantial cognitive impairment more than 1 year after onset of parkinsonism. No significant difference was found in mean age at onset, disease duration, or sex distribution between patients with PD with and without mutations (Table 3).

The 2 patients with DLB who carried *GBA* mutations both had diffuse neocortical Lewy-related pathology. One carried a recombinant allele (Rec 1) that contained L444P and had a high level of concomitant Alzheimer-type pathology (Braak stage V, Consortium to Establish a Registry for Alzheimer's Disease plaque score C; intermediate-likelihood DLB). The other was heterozygous for N370S and had a low degree of Alzheimer pathology (Braak stage II, Consortium to Establish a Registry for Alzheimer's Disease plaque score A; high-likelihood DLB).

In sequencing the region flanking L444P, we identified 2 novel variants (H422T and T410T) and several intronic polymorphisms of unknown functional significance. However, we did not detect any other mutations that have been reported as pathogenic for Gaucher disease.

COMMENT

Our data suggest that *GBA* mutations might represent a significant risk factor for Lewy body disorders. However, although the effect sizes observed in our case-control sample were large (odds ratios in the 8-10 range), the frequency of mutation carriers among both the PD and DLB groups was low (Table 1). Thus, we estimate that the population-attributable risk for *GBA* mutations in Lewy body disorders is only approximately 3% in individuals of European ancestry (Table 1).

Most patients with PD heterozygous for *GBA* mutations in our cohort had sporadic, late-onset disease that was responsive to levodopa, consistent with previously published data (Table 2).[4,11,12] This finding is in contrast to some parkinsonian patients with Gaucher disease in whom parkinsonism was of early onset and refractory to treatment.[1]

Our work has 3 major strengths compared with 6 previously published studies[6-11] on *GBA* mutations in PD populations of primarily European origin. First, our study had a large sample size. A frequent observation among genetic association analyses is the initial report of a large effect in a small sample followed by more powerful studies that typically fail to reproduce the initial results or occasionally validate the effect, but at a more modest level.[16] Of the 6 studies on *GBA* previously mentioned, the first study[9] reported a mutation frequency of 14% among 57 patients with PD and 0% among 44 control samples derived from US brain banks. The 5 subsequent studies[6-8,10,11] have observed effects of marginal or no significance, but 4 of these have included a PD cohort of fewer than 100 patients and were thus underpowered. Our study addressed this issue by using a PD cohort that exceeded the combined sample size of patients with PD across all 6 studies and suggests a potentially bona fide but more modest effect than originally reported.[9]

Second, our study limited the sample to individuals of European ancestry. The N370 mutation has a much higher prevalence among Ashkenazi Jews than in individuals of European origin.[4,5,9-11] Thus, spurious associations might arise if cases and control subjects are drawn differentially from these populations.[16] We collected detailed information on ancestry from patients with PD and control subjects at the time of enrollment and were thus able to account for this important confounder. In contrast, such data were largely lacking in previous studies.

Third, we included a matched control group. Some studies have failed to include a control group and have instead relied on previous estimates of *GBA* mutation frequencies derived indirectly from epidemiologic studies on Gaucher disease.[7,8] Another derived control subjects from brain banks with minimal data available on ancestry.[9] These approaches are subject to substantial bias and confounding. We used a control group screened for parkinsonism and matched closely for age, ancestry, and area of residence.

Our study also had several limitations. Although more than 200 pathogenic *GBA* mutations have been reported,[17] we genotyped only the 2 most common ones, which together account for approximately 70% of the disease alleles in white patients with Gaucher disease (excluding Ashkenazi Jews; International Collaborative Gaucher Group Gaucher Registry, unpublished data, September 2006). Thus, we might have underestimated the true mutation frequency. The sample size of the DLB group was small, and there was insufficient information to separate individuals of European ancestry from those of other white populations. Thus, findings from our analysis of the DLB group must be interpreted with caution, but these data suggest that the remarkably high mutation frequency (23%) observed in a previous DLB sample (n=35) might be an overestimate.[8]

Common variants in many genes have been nominated as risk factors for PD in populations of European origin, but arguably all but 2 (*SNCA* and *MAPT*) have later failed validation.[18-20]

This phenomenon has engendered a healthy skepticism in evaluating newly nominated susceptibility genes, and *GBA* is no exception. Given the large burden of proof incumbent on candidate gene studies, our findings should not be considered definitive replication but indicate that the role of *GBA* in Lewy body disorders merits intensive study. This will require large-scale collaborative efforts and well-designed studies on thousands of individuals.

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Table 1

Frequency of *GBA* Mutation Carriers Among Patients and Control Subjects

Mutation	Patients With PD, No. (%) (n=721)	Patients With DLB, No. (%) (n=57)	Control Subjects, No. (%) (n=554)
N370S	11 (1.5)	1 (1.8)	2 (0.4)
L444P	10 (1.4)	1 (1.8)	0
N370S or L444P	21 (2.9)[a]	2 (3.5)[b]	2 (0.4)
Wild type	700 (97.1)	55 (96.5)	552 (99.6)

Abbreviations: DLB, dementia with Lewy bodies; PD, Parkinson disease.

[a] Odds ratio vs control subjects, 8.3; 95% confidence interval, 2.0-73.1; P <.001; population-attributable risk, 2.6.

[b] Odds ratio vs control subjects, 10.0; 95% confidence interval, 0.7-139.8; P=.045; population-attributable risk, 3.2.

Table 2

Clinical Characteristics of Patients With PD Heterozygous for *GBA* Mutations

Patient No.	Age at Last Assessment, y/Sex/Age at Onset, y	Family History of PD	Resting Tremor	Rigidity	Bradykinesia	Asymmetric Onset	Hoehn and Yahr Stage	Response to Levodopa	Dementia
N370S									
IPD238	66/F/47	−	+	+	+	+	3	+	+
IPD260	82/M/65	−	−	+	+	+	3	+	+
IPD348	58/F/54	−	+	+	+	+	2	+	−
IPD365	54/M/52	+	+	+	+	+	2	+	−
IPD419	64/M/58	−	+	+	+	+	3	+	−
IPD428	84/M/73	+	−	+	+	+	5	+	+
IPD461	50/F/43	−	−	+	+	−	2.5	+	−
IPD468	52/F/48	−	+	+	+	+	2	I	−
IPD648	61/F/50	−	+	+	+	+	2.5	+	−
IPD722	66/M/60	+	+	+	+	+	2	+	−
IPD763	84/M/82	−	+	+	+	+	2.5	+	−
L444P									
IPD254	57/M/48	−	−	+	+	+	3	+	−
IPD359[a]	62/F/60	−	+	+	+	+	2	+	−
IPD471	75/M/72	−	−	+	+	+	3	+	−
IPD495	62/M/57	−	−	+	+	−	2.5	+	−
IPD507	61/F/51	−	+	+	+	+	2	+	−
IPD632[a]	75/M/64	−	+	+	+	+	2	+	−
IPD769	54/M/42	−	+	+	+	−	3	+	−
IPD815	62/M/61	−	+	+	+	+	2.5	I	−
IPD816[a]	66/M/64	−	+	+	+	+	2.5	+	+
PD24602	68/M/36	+	+	+	+	+	4	+	+

Abbreviations: I, inadequate trial; PD, Parkinson disease; +, positive; −, negative.

[a]Rec 1 allele carrier (L444P, A456P, and V460V).

Table 3

Comparison of Clinical Features by Carrier Status

	PD Cases		DLB Cases		Control Subjects	
Feature	**Carriers (n=21)**	**Noncarriers (n=700)**	**Carriers (n=2)**	**Noncarriers (n=55)**	**Carriers (n=2)**	**Noncarriers (n=552)**
Age, mean (SD), y[a]	64.9 (10.1)[b]	67.2 (11.0)[b]	65.5 (10.6)	77.8 (7.7)	66.0 (7.1)	65.3 (12.5)
Age at onset, mean (SD), y[c]	56.5 (11.3)[b]	58.3 (12.1)[b]	61.0 (9.9)	67.9 (9.4)	NA	NA
Disease duration, mean (SD), y	8.4 (7.4)[b]	9.1 (7.5)[b]	4.5 (0.7)	9.9 (4.7)	NA	NA
Male, No (%)	14 (66.7)[d]	526 (75.1)[d]	2 (100.0)	33 (60.0)	2 (100.0)	199 (36.1)

Abbreviations: DLB, dementia with Lewy bodies; NA, not applicable; PD, Parkinson disease.

[a] Age at last assessment for patients with PD and control subjects and age at death or last assessment for patients with DLB.

[b] No significant difference between PD carriers and noncarriers (*t* test, *P*=.34 for age; *P*=.50 for age at onset; *P*=.67 for disease duration).

[c] Defined as age at which first cardinal feature of parkinsonism was reported for patients with PD and age at onset of dementia for patients with DLB.

[d] No significant difference between PD carriers and noncarriers (Fisher exact test, *P*=.44).