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Glucosidase-beta variations and Lewy Body Disorders

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Abstract

It has been proposed that there is an increased frequency of *glucosidase-β* mutations in Lewy body disorders. Our comprehensive DNA sequencing approach found a small number of *glucosidase-β* mutations in 101 neuropathologically defined Lewy body disease cases (3%) compared to 99 healthy post-mortem controls (1%); odds ratio 3.0 (95% CI: 0.3 – 29, p=0.3). All three affected carriers were classified as diffuse Lewy body disease (n=3/50; 6%). Our study suggests *glucosidase-β* variants have a limited role in susceptibility to Lewy body disease in North America.

Keywords

Gaucher; Parkinson; Genetic; Lewy body

Introduction

The identification of different monogenic forms of familial Parkinson's disease (PD) reflects the heterogeneity of this complex syndrome [1]. Even individuals within the same family who harbor the same mutation can present with different clinical and pathological symptoms. These observations demonstrate the significant contribution of disease modifiers whether genetic, environmental or stochastic.

Autosomal recessively inherited mutations of the *glucosidase-β gene (GBA)*, notably N370S and L444P, result in the lysosomal storage disorder Gaucher's disease (OMIM *606463). Heterozygous inheritance of these mutations has been postulated to increase susceptibility to forms of parkinsonism, ranging from classic levodopa-responsive PD to subjects with rapidly progressive dementia with Lewy Bodies (DLB) consistent with *post-mortem* diffuse Lewy body disease (DLBD) [2].

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In 2004, two studies reported an increased frequency of *GBA* mutations in patients with Parkinson's disease (PD). Lwin and colleagues examined 57 Caucasian patients with PD and 44 healthy control subjects and reported eight heterozygous *GBA* mutations (14%) among cases and none in controls [3]. Aharon-Peretz and colleagues reported a large series of Ashkenazi Jewish subjects in which 31 *GBA* mutation carriers were identified in 99 patients with PD (31%), whereas only 95 heterozygous carriers were found among 1543 control subjects (6%) [4]. *GBA* mutations are relatively frequent in the Ashkenazi population, and association with PD was primarily driven by the glucosidase- β N370S substitution (26% in PD versus 6% in controls). Since these original reports a number of studies have tried to replicate these findings [5–11]. However, most studies screen for a limited number of mutations in clinical samples from specific populations. Complete sequencing the *GBA* gene is seldom performed and most PCR-based studies are confounded by the presence of a highly homologous pseudogene situated 16Kb downstream of the functional copy [2].

Herein we examine the frequency of *GBA* mutations in a sample of neuropathologically confirmed Lewy body disease (LBD) and a series of autopsy confirmed control subjects. We employ long-range PCR with primers specific for amplification of the functional *GBA* gene, which was then sequenced in each case.

Subjects and methods

Cases were selected based on pathologic features consistent with Lewy body disorders. Half had a prior clinical diagnosis of PD and included both brainstem LBD (n=34) or transitional LBD (n=17). Half had a prior clinical diagnosis of DLB, with autopsy confirmed DLBD (n=50). For patients with PD, the mean age of onset was 65.7 ± 8.3 (n=47), but for the majority of DLB patients age at symptom onset was not available. The average age at death in our sample was 76.7 ± 7.2 for PD patients (n= 49) and 78.3 ± 7.8 for DLB patients (n=50). The control group consisted of brains from neurologically normal, aged individuals with a mean age at death of 77.3 ± 12.5 (n=97). All 101 cases and 99 control subjects were obtained from the Mayo Clinic Jacksonville brain bank, University of Saskatoon and University of Miami/NPF Brain Endowment Bank™. Post-mortem referrals were primarily from these localities but a subject's final place of residence may not reflect their place of birth. All subjects were Caucasian from North America and of European descent. For cultural and religious reasons brain donation from Ashkenazi subjects is rare, even in Miami where this religious group is relatively common. However, additional demographic data on the ethnic and religious denomination of study subjects is not available. Research was approved by Institutional Review Boards of participating centers and Mayo Foundation.

DNA was obtained from frozen cerebellum for all subjects using standard protocols. Long-range PCR was employed to specifically amplify the functional *GBA* gene and not the *GBA* pseudogene, in three fragments ranging from 1.7 kb to 3 kb in length encompassing exons 1–5, 5–7 and 8–11 [12]. PCR products were purified from unincorporated nucleotides using a Biomek FX (Beckman Coulter) and Agencourt bead technology. Products were directly sequenced with internal primers adjacent to all exon and exon-intron boundaries, using 8–45 ng of each purified amplicon and 1.6 pM of oligonucleotide primer. Electropherograms were analyzed with SeqScape v2.5 (ABI, Applied Biosystems, Foster City, CA, USA), and independently viewed by two people.

Results

Direct DNA sequencing of the 101 neuropathologically confirmed LBD cases revealed three heterozygous IVS2 +1G>A, 882 T>G (His255Gln) and 1263-1317del *GBA* mutation carriers (3%) (Table). These were found in DLB patients with a diagnosis of DLBD on autopsy.

However, detailed clinical data was only available for one individual who suffered from depression and mixed personality disorder, and who developed dementia at age 56 and died aged 66. Her mother also suffered from an unspecified neurodegenerative disease. Only one heterozygous pathogenic mutation carrier, an 84G>GG insertion, was identified in the 99 control subjects (1%).

Two *GBA* polymorphisms were also observed in the study, E326K and T369M. The E326K substitution was identified in 5% of cases (5/101; three DLB and two PD) and in one control subject, which may reflect a trend towards an association with disease. Functional analysis suggests glucosidase- β E326K may be a disease modifier variant rather than a neutral single nucleotide polymorphism, although homozygosity of the polymorphism does not lead to Gaucher's disease [13]. In affected E326K carriers the mean age of death was 72.5 ± 5.4 , whereas the one control subject died at 86 years. Within controls there were also two carriers of the neutral T369M polymorphism.

Overall, there was marginal albeit non-significant association of heterozygous *GBA* pathogenic mutations in LBD (odds ratio 3.0, 95% CI: 0.3 – 29, $p=0.3$). Considering all variants except the neutral T369M polymorphism, evidence for association with LBD approaches significance (odds ratio 4.2, 95% CI: 0.9 – 20, $p=0.06$).

Discussion

Overall this study identified few pathogenic *GBA* mutations in patients and control subjects (3% vs 1%). The most common pathogenic glucosidase- β variants, N370S or L444P, were not observed and carrier frequencies of pathogenic mutations, modifier variants and neutral polymorphisms were lower than in previous studies. In this sample of autopsy confirmed LBD from North America the contribution of heterozygous *GBA* mutations to disease appears to be marginal.

Strengths of our study include long-range PCR followed by complete sequencing of the *GBA* gene in autopsy confirmed LBD cases. Cases consisted of an equal proportion of clinically diagnosed patients with PD and DLB. All pathologic diagnoses were made by one pathologist (DWD). Complete sequencing was also performed in a similar autopsy-confirmed series of control subject, free of neurologic symptoms and Lewy body pathology. Long-range PCR ensured all variants reported were in the functional *GBA* gene rather than the homologous *GBA* pseudogene, which is non-functional and may accumulate mutations. In many previous studies that genotype specific *GBA* mutations within PCR amplified exons, the frequency of variants reported must potentially reflect the sum of both the *GBA* gene and the pseudogene.

However, our study also had weaknesses as ascertainment bias in *post-mortem* series is often problematic. Patients with clinical PD or DLB may not present at autopsy unless they have a unique or unusual presentation. Clinical data was also limited as the study was retrospective with patients derived from several centers. All subjects were Caucasians from North America but their religious denomination was not available. Rare recombination events between the functional and pseudogenes may also have been missed, but are unlikely given the low frequency of mutations observed [14].

Goker-Alpan and colleagues previously assessed 75 autopsied patients of mixed ethnicity with DLB, PD or multiple system atrophy, and reported *GBA* mutations in eight patients with DLB (23%, $n=35$) and in one patient with classical PD (4%, $n=28$) [8]. Given the frequency of *GBA* mutations observed, and as no control group was assessed, we reasoned a study of 200 subjects including 101 with LBD should be sufficient for replication. Our effort was limited given the cost of high-fidelity long-range PCR across the *GBA* locus. However, our findings

now suggest complete sequencing of the *GBA* locus may only show a significant difference if ≥ 620 cases and a similar series of control subjects are assessed (80% power, $\alpha = 0.05$).

Conflicting results between this and past studies may reflect different populations, small samples, biased ascertainment and the method of mutation screening. A meta-analysis of this and prior studies may provide a consensus on the contribution of heterozygous *GBA* variants to susceptibility of PD and LBD in different populations. PDgene presently cites *GBA* N370S as the most compelling genetic association for PD (odds ratio 3.2, 95% CI: 2.08 – 4.91; <http://www.pdgene.org/meta.asp?geneID=148>). However, the positive association is driven by three studies in which subjects were exclusively or predominantly from the Ashkenazi Jewish population [4,5,7]. The other eight studies (nine, if this study is included) are equivocal (as they include an odds ratio of 1.0 in the confidence interval) and largely report non-Ashkenazi Jewish subjects.

GBA mutations are rare in the North America in which $<1.8\%$ of subjects claim Jewish ethnicity (<http://www.jpippi.org.il/>). Based on this re-sequencing study and current meta-analysis, *GBA* variants are unlikely to play a significant role in susceptibility to LBD. Nevertheless studies in Ashkenazi subjects suggest heterozygous *GBA* mutations are associated with parkinsonism. In parents and grandparents of patients with Gaucher's disease it will be important to know whether *GBA* heterozygosity is associated with an increase prevalence of PD. Segregation of parkinsonism and *GBA* mutations may also be evident in Ashkenazi Jewish families. While genetic association is not causation, data from population-based surveys may yet provide the rationale for functional studies of *GBA* mutations in PD susceptibility.

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Table 1

GBA mutations identified in Lewy body disease cases and control subjects.

Exon	Mutation [†]	Protein	Designation	Diagnosis
Cases (n=101)				
2	IVS2 +1G>A	Splice Variant	Mutation	DLBD
7	882 T>G	H255Q	Mutation	DLBD
8	1093 G>A	E326K	Modifier SNP	PD
8	1093 G>A	E326K	Modifier SNP	PD
8	1093 G>A	E326K	Modifier SNP	DLBD
8	1093 G>A	E326K	Modifier SNP	DLBD
8	1093 G>A	E326K	Modifier SNP	DLBD
9	1263–1317 del	Frameshift	Mutation	DLBD
Controls (n=99)				
2	84 G>GG	Frameshift	Mutation	
8	1093 G>A	E326K	Modifier SNP	
8	1223 C>T	T369M	Neutral SNP	
8	1223 C>T	T369M	Neutral SNP	

[†] *GBA* mutations are electronically referenced by Online Mendelian Inheritance in Man, OMIM *606463, <http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=606463>. *GBA* mutation frequencies in Gaucher disease and in Ashkenazi Jewish subjects are referenced by Tel Aviv University http://www.tau.ac.il/lifesci/bioinfo/genedis/gaucher/gaucher.html#point_300.