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Glucocerebrosidase mutations in diffuse Lewy body disease☆

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Abstract

Clinicogenetic and pathological studies have shown that mutations of the glucocerebrosidase gene (*GBA*) are a risk factor for Parkinson's disease and Lewy body disorders. In the present study, we have identified *GBA* mutations in 6.8% (4/59) of cases with a pathological diagnosis of diffuse Lewy body disease. Taken with previous studies, it appears that *GBA* mutations are associated with a more diffuse pattern of Lewy body distribution involving the cerebral cortex than the brainstem/limbic distribution observed in typical Parkinson's disease.

Keywords

DLBD; Gaucher; disease; GBA; genetics

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Conflict of interest

The authors declare no financial or other conflicts of interest.

1. Introduction

Lewy body disease (LBD) is an umbrella term that includes disorders with a range of α-synuclein immunopositive pathologies such as Lewy bodies and Lewy neurites. LBD was initially classified as brainstem predominant, transitional and diffuse (DLBD) by Kosaka [1] and this classification was adopted in consensus criteria for dementia with Lewy bodies (DLB) [2]. More recently, Braak and colleagues have proposed a 6 stage scheme for Lewy-type pathologies in Parkinson's disease (PD) [3], with the later 3 stages involving cortical areas and roughly corresponding to transitional LBD for stage 4 and DLBD for stages 5 and 6. DLBD is often accompanied by varying degrees of Alzheimer-type pathology, and is the second most frequent pathological finding in some autopsy series of dementia in the elderly [4]. It is also the most common pathological finding in cases with a prospective clinical diagnosis of DLB [5]. DLB is a disorder with characteristic clinical features, including spontaneous parkinsonism, rapid eye movement sleep behavior disorder and visual hallucination with cognitive impairment sufficient to cause functional impairment [6]. Although some patients with autosomal dominant forms of PD, including *SNCA* and *LRRK2* mutation carriers, have clinical features consistent with DLB [7,8], no major genetic determinant for DLB has yet been identified [9].

Gaucher disease (GD) is a recessively inherited lysosomal storage disease caused by mutations and rearrangements in the glucocerebrosidase gene (*GBA*) (MIM# 606463) [10]. In addition to causing GD, *GBA* mutations are now considered to be one of the most frequent genetic risk factors for PD [11–13]. Interestingly, Parkinson-associated pathology in GD shares some features with DLBD such as selective involvement of the CA2/3 sector of the hippocampus [14,15]. Recently, Neumann and colleagues reported the pathological findings of heterozygous *GBA* mutation carriers, including 15 cases with an initial clinical diagnosis of PD and 2 with an initial clinical diagnosis of multiple system atrophy (MSA); all had cortical Lewy bodies at autopsy, with Braak PD stages 5 and 6 [16]. Although detailed clinical information was not available on all cases, 9 (53%) had cognitive impairment or dementia and 12 (71%) had hallucinations, one of the cardinal clinical features of DLB [2]. These findings are reminiscent of earlier studies that also showed that *GBA* mutations were more frequent in patients with clinical diagnoses of DLB than in PD [17,18]. The present study set out to determine the frequency of *GBA* variants in clinically diagnosed and pathologically-confirmed cases of DLBD.

2. Subjects and methods

The 59 cases (44 males) were selected according to a pathological diagnosis of DLBD. Mean age at death was 78.3 ± 5.4 years. All cases were Caucasians and the majority of the cases had dementia and parkinsonism; only 13.8% (n = 8) did not report dementia. The cases were obtained from Brain Banks for neurodegenerative diseases at Mayo Clinic in Jacksonville, FL (n = 17), Manchester University in Salford, UK (n = 22), Royal University Hospital in Saskatoon, Canada (n = 11) and the National Parkinson Foundation brain bank at the University of Miami, Miami, FL (n = 9). All cases had diffuse cortical Lewy bodies based upon immunohistochemistry for α-synuclein [19]. Ethical approval was obtained from Institutional review boards and all cases or legal next-of-kin provided informed consent.

DNA was extracted from frozen cerebellar tissue by standard protocols. All cases were sequenced for the 11 exons and exon-intron boundaries for the *GBA* gene and had not been examined in our previous study [18]. Primer sequences are provided in Supplemental Table 1. PCR products were purified from un-incorporated nucleotides using Agencourt bead technology (Beverly, MA) with Biomek FX automation (Beckman Coulter, Fullerton, CA). Electropherograms were analyzed with SeqScape v2.1.1 using 3730 DNA Analyzer (ABI, Applied Biosystems, Foster City, CA, USA). Genotype frequencies in patients and control subjects were compared with a Fisher's exact test and chi-square using StatsDirect statistical software version 2.6.7 (StatsDirect Ltd, UK). All identified variants are referred to using the traditional nomenclature including the 39-residue signal peptide.

3. Results

In this series of 59 DLBD cases, we identified four *GBA* mutations; one homozygote (p.A292T) and three heterozygote (p.N370S, p.E388K and p.L444P), giving a total carrier frequency of 6.8% (4/59). The p.A292T was not found in 101 pathologically-confirmed controls from our previous study and was not observed in an additional screening of 363 clinical control samples from the US Caucasian population (data not shown). All carriers had clinical parkinsonism and dementia. Employing the US Caucasian control group (n = 99) from our previous study, the frequency of *GBA* mutations in DLBD was higher than that of normal controls (1.0%), albeit not statistically significant (p = 0.07; odds ratio = 7.3; 95% confidence interval = 0.8–65.4). It should be noted that the small sample size of our series restricts the power to detect association. When combining these data with that from our previous report, a *GBA* mutation frequency of 6.4% (7/109) in DLBD cases is derived. As the same trend is observed in both studies; statistical analysis of the frequency of *GBA* mutations in the combined set was significantly different from that observed in controls (p = 0.04; odds ratio = 6.7; 95% confidence interval = 0.8–55.7). Table 1 summarizes all entire *GBA* gene sequencing studies to date where the frequency of *GBA* mutations in pathologically-confirmed LBD cases has been examined. Overall analysis suggests *GBA* mutations increase risk of LBD ($p < 0.001$; odds ratio = 7.2 confidence interval = 2.9–18.0), with an even stronger association with the subset of cases defined as DLBD (or Braak stages 5 and 6) ($p < 0.001$; odds ratio = 21.0 confidence interval = 8.3–53.5).

4. Discussion

Our comprehensive screening of *GBA* mutations in DLBD indicates a carrier mutation frequency of 6.8%. One novel variant was identified p.A292T and three previously reported p.N370S, p.E388K [21] and p.L444P. Interestingly, the homozygous carrier of GBA p.A292T showed no evidence of GD, suggesting that this variant is not highly pathogenic, though it may represent a less penetrant mutation, acting in a recessive manner to increase risk of DLBD, or simply be a rare benign variant. The prediction program PolyPhen-2 was used to assess the possible affect of this variant on protein function and predicted the substitution to be potentially damaging, however the SIFT prediction program did not [22,23]. There was no knowledge of consanguinity in this case which might explain the homozygosity of this rare variant.

Given the pathological basis of the present study, the carrier of p.A292T was retained for comparison; however, removing this case still results in a *GBA* mutation frequency of 5.1%. This figure is slightly higher than that recently reported by Mata and colleagues for clinical DLB patients of European Caucasian ancestry (3.7%); although this latter study only screened for the two common substitutions p.N370S and p.L444P [20]. A much higher mutation frequency was reported by Goker-Alpan and co-workers who noted 22.9% (8/35) of pathologically-confirmed DLBD cases and 3.6% (1/28) of pathologically-confirmed PD cases harbored *GBA* mutations [17]. However, this study included Ashkenazi Jewish patients who have a high frequency of *GBA* mutations, and may have introduced a prevalent carrier bias. Recently, Clark and colleagues reported a frequency of 28.4% (27/95) of *GBA* mutations in a series of LBD cases with the majority of carriers having DLBD pathology [21]. However, as some SNPs of unknown pathogenicity (e.g., p.E326K and p.T369M) were included in the analysis the true frequency of mutation carriers is unclear.

Neumann and co-workers showed a more modest increase in frequency of *GBA* mutations in British DLBD patients [16], identifying 17/380 (4.5%) carriers of *GBA* mutations, thirteen of which displayed DLBD pathology. Similarly, our previous study reported a 3.0% frequency of *GBA* mutation in 101 cases of LBD in which all patients with *GBA* mutations also had DLBD pathology (6.0%; 3/50) [18]. Glucocerebrosidase has recently been reported in Lewy body inclusions in patients with *GBA* mutations and may become a distinctive neuropathologic feature [26].

If *GBA* mutations exacerbate Lewy body pathology in PD, it may be postulated that they might also affect other α-synucleinopathies. However, sequencing of *GBA* in 27 multiple system atrophy (MSA) cases did not identify any pathogenic variants (data not shown). Similarly, Goker-Alpan and co-workers did not identify *GBA* mutations among 12 MSA patients [17]. Comparable results were recently reported in a British MSA-control series, with the frequency of *GBA* mutations in this cohort being low and similar between cases (0.9%) and controls (1.2%) [16,24]. These data suggest that the patho-mechanism by which *GBA* mutations increase the risk of DLBD is different to the etiology of α-synuclein aggregation in MSA. GBA may be a factor favouring aggregation of α-synuclein in neurons in LBD, whereas in MSA it is largely found in oligodendroglia [25].

Given the present findings, we speculate that *GBA* mutations drive or facilitate a more widespread brain distribution of Lewy bodies, resulting in a more severe pathological phenotype in DLBD than that seen in patients with parkinsonism without *GBA* mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi: 10.1016/j.parkreldis.2010.09.009.

Table 1

Full gene sequencing studies of *GBA* mutations in pathologically-confirmed LBD.

Study	LBD	*GBA* mutations (%)	DLBD	*GBA* mutations (%)	control	*GBA* mutations (%)
Current study	59	4 (7)	59	4 (7)		
Clark et al 2009	95	27 (28)	85	21 (25)	32	1 (3)
Farrer et al 2009	101	3 (3)	50	3 (6)	99	1 (1)
Neumann et al 2009	380	17 (4)	17	17 (4)	257[a]	3 (1)
Goker-Alpan et al 2006	63	9 (12)	35	8 (23)		
Eblan et al 2005	26	2 (8)				
Total counts	724	62 (9)[b]	246	53 (18)[c]	388	5 (1)

GBA mutation studies including pathologic cases of Lewy Body Disease. These studies have examined the frequency of *GBA* mutations in pathologic Lewy body disease cases by full gene sequencing.

[a] Clinical controls.

[b] Chi-square = 22.3 with 1 degrees of freedom. ($P < 0.001$) OR 7.2 (2.9, 18).

[c] Chi-square = 71.9 with 1 degrees of freedom. ($P < 0.001$) OR 21 (8.3, 53.5).

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