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ORIGINAL PAPER

Role of *GBA* variants in Lewy body disease neuropathology

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Abstract

Rare and common *GBA* variants are risk factors for both Parkinson's disease (PD) and dementia with Lewy bodies (DLB). However, the degree to which *GBA* variants are associated with neuropathological features in Lewy body disease (LBD) is unknown. Herein, we assessed 943 LBD cases and examined associations of 15 different neuropathological outcomes with common and rare *GBA* variants. Neuropathological outcomes included LBD subtype, presence of a high likelihood of clinical DLB (per consensus guidelines), LB counts in five cortical regions, tyrosine hydroxylase immunoreactivity in the dorsolateral and ventromedial putamen, ventrolateral substantia nigra neuronal loss, Braak neurofibrillary tangle (NFT) stage, Thal amyloid phase, phospho-ubiquitin (pS65-Ub) level, TDP-43 pathology, and vascular disease. Sequencing of *GBA* exons revealed a total of 42 different variants (4 common [MAF > 0.5%], 38 rare [MAF < 0.5%]) in our series, and 165 cases (17.5%) had a copy of the minor allele for ≥ 1 variant. In analysis of common variants, p.L483P was associated with a lower Braak NFT stage (OR = 0.10, $P < 0.001$). In gene-burden analysis, presence of the minor allele for any *GBA* variant was associated with increased odds of a high likelihood of DLB (OR = 2.00, $P < 0.001$), a lower Braak NFT stage (OR = 0.48, $P < 0.001$), a lower Thal amyloid phase (OR = 0.55, $P < 0.001$), and a lower pS65-Ub level (β: −0.37, $P < 0.001$). Subgroup analysis revealed that *GBA* variants were most common in LBD cases with a combination of transitional/diffuse LBD and Braak NFT stage 0-II or Thal amyloid phase 0–1, and correspondingly that the aforementioned associations of *GBA* gene-burden with a decreased Braak NFT stage and Thal amyloid phase were observed only in transitional or diffuse LBD cases. Our results indicate that in LBD, *GBA* variants occur most frequently in cases with greater LB pathology and low AD pathology, further informing disease–risk associations of *GBA* in PD, PD dementia, and DLB.

Keywords Lewy body disease · *GBA* · Genetics · Neuropathology

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Abbreviations

LBD	Lewy body disease
LB	Lewy body
PD	Parkinson's disease
DLB	Dementia with Lewy bodies
AD	Alzheimer's disease
GWAS	Genome-wide association study
TH-ir	Tyrosine hydroxylase immunoreactivity
SN	Substantia nigra
NFT	Neurofibrillary tangle
pS65-Ub	Phospho-ubiquitin
TDP-43	TAR DNA-binding protein-43
VaD	Vascular disease
H & E	Hematoxylin and eosin
MF	Middle frontal
ST	Superior temporal
IP	Inferior parietal
CG	Cingulate
PH	Parahippocampal
SN	Substantia nigra
PC	Principal component
MAF	Minor allele frequency
OR	Odds ratio
CI	Confidence interval

Introduction

Lewy body disease (LBD) is a neuropathologically defined disorder that is characterized by occurrence of Lewy bodies (LBs) and Lewy neurites in the brainstem (brainstem-predominant LBD) that can extend to the limbic (transitional LBD) and neocortical (diffuse LBD) regions [21]. Common clinical presentations of LBD include Parkinson's disease (PD), PD with dementia (PDD), and dementia with Lewy bodies (DLB); the presence of Lewy body (LB) pathology is considered a pathological hallmark of these α-synucleinopathies [21]. Additionally, Alzheimer's disease (AD) neuropathological changes are commonly observed in LBD [21], and conversely up to 50% of AD patients display LB pathology [8, 9]. Unlike PD, PDD, and DLB, LB pathology is not thought to play a major role in the pathogenesis of AD [9].

The *GBA* gene encodes glucocerebrosidase, a lysosomal enzyme involved in the metabolism of glucosylceramide [35]. Importantly, impaired activity of glucocerebrosidase is associated with an accumulation of α-synuclein [35], which is the main component of LBs [21]. Although initially discovered to cause Gaucher's disease in a recessive manner [35], *GBA* variants also play an important role in LBD on several levels. First, presence of rare exonic *GBA* variants is associated with a markedly increased risk of both PD (odds ratio [OR] ~ 5) [37] and DLB (OR ~ 8) [7, 31], where disease risk is predominantly driven by the p.L483P and p.N409S variants. Second, common exonic *GBA* variants, including p.E365K and p.T408M, are risk factors for PD (both variants) and DLB (p.E365K) [31, 41]. Third, *GBA* is a susceptibility locus for both PD and DLB in unbiased genome-wide association studies (GWAS) [13, 30], where evidence suggests that the GWAS signal is driven by the p.E365K variant [4, 13]. On the other hand, *GBA* variation has not been linked with susceptibility to AD [40, 42].

Presence of rare *GBA* variants has also been associated with specific clinical features in individuals with LB disorders, including (but not limited to) a younger age at onset, shorter survival, and more frequent cognitive impairment/dementia [12, 36]. However, studies assessing whether *GBA* variants may also influence neuropathology in individuals who have developed LBD have been limited by small sample sizes [1, 32, 34]. With this in mind, in the current study we evaluated associations of *GBA* variants with a variety of neuropathological features in a large series of LBD cases.

Materials and methods

Study patients

A total of 943 neuropathologically confirmed LBD cases from the Mayo Clinic Florida brain bank for neurodegenerative disorders were included in this study. All LBD cases were evaluated by a single neuropathologist (D.W.D). LBD cases were excluded if they had significant coexisting non-AD neurodegenerative conditions (e.g., progressive supranuclear palsy, corticobasal degeneration, multiple system atrophy, amyotrophic lateral sclerosis, etc.), had amygdala-predominant LBD in the setting of advanced AD, or did not have information available for any of the neuropathological outcome measures examined in this study. Demographic and clinical information was collected regarding age at death, sex, and presence of dementia, with age at disease onset and disease duration collected in a subset of 384 LBD cases. Fifteen different neuropathological outcomes were assessed and included LBD subtype ($N = 943$), presence of high likelihood of clinical DLB [27] ($N = 943$), LB counts in five different regions ($N = 753$ to 762), dorsolateral and ventromedial putaminal tyrosine hydroxylase immunoreactivity (TH-ir) ($N = 443$), neuronal loss in the ventrolateral part of the substantia nigra (SN) ($N = 486$), Braak neurofibrillary tangle (NFT) stage ($N = 943$), Thal amyloid phase ($N = 785$), phospho-ubiquitin (pS65-Ub) level ($N = 723$), TDP-43 pathology ($N = 751$), and vascular disease (VaD) ($N = 942$). Characteristics of LBD cases are shown in Table 1.

Table 1 Characteristics and outcomes of LBD cases

Variable	*N*	Median (minimum, maximum) or No. (%) of cases
Age at death (years)	943	79 (48, 103)
Sex (Male)	943	545 (57.8%)
Age at disease onset (years)	384	71 (35, 94)
Disease duration (years)	384	8 (0, 36)
LBD subtype		
Brainstem	943	124 (13.1%)
Transitional		336 (35.6%)
Diffuse		483 (51.2%)
High likelihood of clinical DLB	943	402 (42.6%)
Lewy body counts		
Middle frontal gyrus	762	2 (0, 35)
Superior temporal gyrus	759	7 (0, 50)
Inferior parietal gyrus	759	2 (0, 30)
Cingulate gyrus	753	8 (0, 45)
Parahippocampal gyrus	761	12 (0, 45)
Putaminal TH-ir		
Dorsolateral	443	4.09 (0.26, 42.18)
Ventromedial	443	9.98 (0.26, 33.63)
Ventrolateral substantia nigra neuronal loss score		
0 = none	486	2 (0.4%)
0.5 = none/mild		12 (2.5%)
1 = mild		85 (17.5%)
1.5 = mild/moderate		46 (9.5%)
2 = moderate		166 (34.2%)
2.5 = moderate/severe		65 (13.4%)
3 = severe		110 (22.6%)
Braak NFT stage		
0	943	19 (2.0%)
I		34 (3.6%)
II		168 (17.8%)
III		231 (24.5%)
IV		153 (16.2%)
V		147 (15.6%)
VI		191 (20.3%)
Thal amyloid phase		
0	785	103 (13.1%)
1		70 (8.9%)
2		45 (5.7%)
3		153 (19.5%)
4		84 (10.7%)
5		330 (42.0%)
pS65-Ub	723	3.14 (0.06, 27.35)
TDP-43 pathology	751	369 (49.1%)
VaD	942	238 (25.3%)
Dementia	876	779 (88.9%)

LBD Lewy body disease, *TH-ir* tyrosine hydroxylase immunoreactivity, *VaD* vascular disease

Neuropathological assessment

Formalin-fixed brains underwent systematic and standardized sampling with neuropathologic evaluation. Regions sampled for histopathologic assessment included six regions of the neocortex, two levels of the hippocampus, a basal forebrain section that includes the amygdala, lentiform nucleus and hypothalamus, anterior corpus striatum, thalamus at the level of the subthalamic nucleus, midbrain, pons, medulla, and two sections of the cerebellum, one including the deep nuclei. Paraffin-embedded 5-µm thick sections mounted on glass slides were stained with hematoxylin and eosin (H&E) and thioflavin S (Sigma-Aldrich, St. Louis, MO). Sections of the cortex, hippocampus, and basal forebrain, and brainstem were immunostained with anti-α-synuclein antibody (NACP; rabbit polyclonal; 1:3000; a gift from Dr. Petrucelli, Mayo Clinic, Jacksonville, Florida; formic acid pretreatment) [10] to establish a neuropathological diagnosis of LBD [27].

LBD subtype was classified as brainstem, transitional, or diffuse as described by McKeith et al. [23, 28] Presence of a high likelihood of clinical DLB (Braak NFT stage 0–II and transitional/diffuse LBD, or Braak NFT stage III–IV and diffuse LBD) was assessed according to the criteria of the fourth report of the DLB consortium [27]. LBs were manually counted in the middle frontal (MF), superior temporal (ST), inferior parietal (IP), cingulate (CG), and para-hippocampal (PH) gyrus in the field of highest density at x200 magnification. The TH-ir in the dorsolateral and ventromedial putamen was assessed using the antibody against TH (rabbit polyclonal, 1:600; Affinity Bioreagents, Golden, Colorado), as previously described [18]; a lower TH-ir value corresponds to a greater degree of putaminal dopaminergic degeneration The neuronal loss in the ventrolateral part of the substantia nigra (SN) was semi-quantitatively scored on H&E-stained sections as follows: 0 = none, 0.5 = minimal, 1 = mild, 1.5 = mild-to-moderate, 2 = moderate, 2.5 = moderate-to-severe, 3 = severe.

Braak NFT stage and Thal amyloid phase were assessed with thioflavin S fluorescence microscopy, a method validated in our prior work and recognized by the National Institute on Aging-Alzheimer's Association guidelines for its consistency with tau immunohistochemistry results, particularly in large-scale studies [5, 22, 25, 29, 38]. The mitophagy alteration was assessed in the hippocampus on immunostained slides of the mitophagy marker phosphorylated ubiquitin on serine 65 (pS65-Ub, in-house rabbit polyclonal antibody, 1:650), and pS65-Ub positive cell density was quantified using established algorithm via digital pathology (Aperio ImageScope v12) [11, 15–17]. TDP-43 pathology, such as neuronal or glial cytoplasmic inclusions, dystrophic neurites, neuronal intranuclear inclusion, spheroid, or perivascular inclusion, was screened in

the amygdala based on immunostained slides of phospho-TDP-43 (pS409/410, mouse monoclonal, 1:5000, Cosmo Bio, Tokyo, Japan) [19]. VaD included lacunar infarcts, microscopic infarcts, hemorrhages, cerebral amyloid angiopathy, and leukoencephalopathy, which were assessed on H&E-stained sections or thioflavin S fluorescent microscopy [20]. All immunohistochemistry was done using IHC Autostainer 480S (Thermo Fisher Scientific Inc., Waltham, MA) and DAKO EnVision™ + reagents (Dako, Carpinteria, CA).

Genetic analysis

All cases underwent automated DNA extraction from frozen cerebellar tissue using the Autogen 245 T and Autogen FlexStar + (Holliston, MA). Sanger sequencing of *GBA* (NM_001005742) was performed. *GBA* was amplified in 3 amplicons (exons 1–5, exons 5–7, exons 8–11; primer sequences available on request) to avoid amplification of the pseudogene (*GBAP*). All PCR products were cleaned using Agencourt AMPure XP magnetic beads and the Biomek FXP (Beckman Coulter). The individual exons were then bidirectionally cycle-sequenced using internal primers and ABI BigDye Terminator chemistry. The cycle sequence products were cleaned with Agencout CleanSEQ magnetic beads and analyzed on an ABI 3730xl DNA Sequencer. Sequences were analyzed using Seqscape v3.0 Software. All coding and flanking regions within 25 bp of *GBA* were analyzed, and call rates for each variant were > 98%. LBD cases with call rates < 95% were excluded.

Additionally, GWAS data were available from other studies performed involving these LBD cases (see the Supplemental Information for methodological details). We utilized this GWAS data in the current study in order to exclude subjects with non-European ancestry, and also to extract principal components (PCs) in order to adjust for population stratification in regression analyses.

Statistical analysis

For common *GBA* variants with a minor allele frequency (MAF) of > 0.5%, associations of presence of the minor allele with neuropathological outcomes were evaluated using regression models appropriate for the nature of the given outcome (continuous, binary, ordinal, or count) that were adjusted for age at death, sex, and the top five PCs of available GWAS data. Specifically, proportional odds logistic regression models were used for ordinal outcomes (LBD subtype, ventrolateral SN neuronal loss score, Braak NFT stage, and Thal amyloid phase), linear regression models were used for continuous outcomes (dorsolateral putaminal TH-ir, ventromedial putaminal TH-ir, and pS65-Ub), binary logistic regression models were used for binary outcomes (high likelihood of clinical DLB, TDP-43 pathology, VaD), and negative binomial regression models were used for count outcomes (MF, ST, IP, CG, and PH LB counts). Associations of presence of the minor allele of common *GBA* variants with dementia and male sex were assessed using the aforementioned binary logistic regression models; the associations with male sex were assessed due to recent reports of differences in the frequency of *GBA* mutations according to sex [33, 39]. Owing to their skewed distributions, in linear regression analysis, dorsolateral putaminal TH-ir and pS65-Ub were examined on the natural logarithm scale, while ventromedial putaminal TH-ir was examined on the square root scale.

For LBD subtype, ventrolateral SN neuronal loss score, Braak NFT stage, and Thal amyloid phase, ORs and 95% confidence intervals (CIs) are interpreted as the multiplicative increase in the odds of a more severe category for cases with presence of the minor allele for the given *GBA* variant. For dorsolateral putaminal TH-ir, ventromedial putaminal TH-ir, and pS65-Ub, regression coefficients (denoted as β), and 95% CIs are interpreted as the additive increase in the mean value of the given outcome measure (on the natural logarithm scale or square root scale) for cases with presence of the minor allele for the given *GBA* variant. For high likelihood of DLB, TDP-43 pathology, VaD, dementia, and male sex, ORs, and 95% CIs are interpreted as the multiplicative increase in the odds of occurrence of the given outcome for cases with presence of the minor allele for the given *GBA* variant. For MF, ST, IP, CG, and PH LB counts, multiplicative effects and 95% CIs are interpreted as the multiplicative increase in the mean outcome measure for cases with presence of the minor allele for the given *GBA* variant.

For rare variants with a MAF < 0.5%, single-variant analysis was not performed due to the low power such analysis would have to detect associations with neuropathological outcomes. Instead, gene-burden tests were performed by collapsing across all rare variants and evaluating the associations of presence of the minor allele for any rare variant with neuropathological outcomes using the previously described regression models that were adjusted for age at death, sex, and the top five PCs [24]. A similar gene-burden analysis was also employed when considering all *GBA* variants, regardless of MAF. P-values < 0.0029 are considered as statistically significant after applying a Bonferroni correction for the 17 different outcome measures that were examined. All statistical tests were two-sided. Statistical analyses were performed using SAS (version 9.4; SAS Institute, Inc., Cary, North Carolina).

Results

A summary of *GBA* variants that were observed in our series of 943 LBD cases is provided in Table 2. Of the 42 different variants that were observed, four (rs140335079 x7−18 bp [MAF 1.49%]; rs2230288 p.E365K [MAF 3.19%]; rs75548401 p.T408M [MAF 1.38%]; rs76763715 p.N409S [MAF 0.69%]) were common variants with a MAF > 0.5%, and the remaining 38 were rare. Also of note, the MAF of rs421016 p.L483P was 0.48%. Of the 943 LBD cases, 165 (17.5%) had a copy of the minor allele

Table 2 Summary of *GBA* variants

Variant	Amino acid	*N*	Major allele	Minor allele	Minor allele frequency	No. (%) of carriers of the minor allele
rs41264927	x1−15 bp	936	A	G	0.21%	4 (0.43%)
rs150466109	K13R/K13T	939	A	C or G	0.11%	2 (0.21%)
rs104886460	x2 + 1 bp	938	G	A	0.05%	1 (0.11%)
N/A	x3−8 bp	939	C	G	0.05%	1 (0.11%)
rs1141812	R83C	943	C	T	0.05%	1 (0.11%)
N/A	x3 + 5 bp	943	C	A	0.05%	1 (0.11%)
N/A	M124V	943	A	G	0.05%	1 (0.11%)
N/A	S146IfsX5	943	T	A	0.05%	1 (0.11%)
rs147411159	I158I	943	C	T	0.21%	2 (0.21%)
rs439898	R159W	943	C	T	0.05%	1 (0.11%)
N/A	A163T	943	G	A	0.05%	1 (0.11%)
rs398123530	R170C	943	C	T	0.05%	1 (0.11%)
N/A	Y174C	943	A	G	0.05%	1 (0.11%)
rs147138516	D179H	943	G	C	0.11%	2 (0.21%)
N/A	S212X	942	C	–	0.00%	0 (0.00%)
rs774539868	G234G	942	G	T	0.05%	1 (0.11%)
rs398123534	G241R	943	G	A	0.05%	1 (0.11%)
rs140335079	x7−18 bp	942	T	A	1.49%	22 (2.34%)
N/A	F290Y	943	T	A	0.05%	1 (0.11%)
rs367968666	H294Q	943	T	G	0.11%	2 (0.21%)
rs78973108	R296Q	943	G	A	0.05%	1 (0.11%)
N/A	F298L	943	C	A	0.05%	1 (0.11%)
rs140955685	R301H	943	G	A	0.11%	2 (0.21%)
rs199628072	T306I	943	C	T	0.05%	1 (0.11%)
rs753890133	L325L	943	G	A	0.05%	1 (0.11%)
rs2230288	E365K	940	G	A	3.19%	57 (6.06%)
rs75548401	T408M	942	C	T	1.38%	25 (2.66%)
rs111417507	x9−22 bp	943	T	C	0.05%	1 (0.11%)
rs377143075	x9−3 bp	943	T	C	0.05%	1 (0.11%)
rs76763715	N409S	943	A	G	0.69%	13 (1.38%)
rs121908311	G416S	943	G	A	0.05%	1 (0.11%)
rs80356768	L422PfsX3	943	T	C	0.16%	3 (0.32%)
rs75243000	F436S	943	T	–	0.00%	0 (0.00%)
rs1064651	D448H	942	G	C	0.11%	2 (0.21%)
rs75671029	D482N	943	G	-	0.00%	0 (0.00%)
rs421016	L483P	943	T	C	0.48%	9 (0.95%)
rs368060	A495P	943	G	C	0.21%	4 (0.42%)
rs1135675	V499V	943	G	C	0.21%	4 (0.42%)
rs80356771	R502C	942	C	T	0.27%	5 (0.53%)
rs368832292	x11−12 bp	943	C	T	0.11%	2 (0.21%)
rs1354387236	K505X	943	A	–	0.00%	0 (0.00%)
N/A	R535C	942	C	T	0.05%	1 (0.11%)

for at least one *GBA* variant, while 79 (8.4%) had a copy of the minor allele for at least one *GBA* variant with a MAF < 0.5%.

Associations of the four common (MAF > 0.5%) *GBA* variants with neuropathological outcome measures (as well as dementia and male sex) are displayed in Table 3 for outcomes where a significant ($P < 0.0029$ after multiple testing adjustment) or nominally significant ($P < 0.05$) association was observed, with results for the remaining neuropathological outcomes shown in Supplemental Table 1. Additionally, we also assessed associations with outcomes for the rs421016 p.L483P PD risk variant as its MAF was only slightly below the > 0.5% threshold used to identify common variants. After adjusting for multiple testing, a significant association was observed between rs421016 p.L483P and lower Braak NFT stage (≥ 4: 11.1% vs. 52.5%, OR = 0.10, $P < 0.001$), with nominally significant associations observed toward a less severe LBD subtype (Diffuse: 33.3% vs. 51.4%, OR = 0.23, $P = 0.021$), a lower Thal amyloid phase (≥ 3: 22.2% vs. 72.8%, OR = 0.19, $P = 0.008$), and a lower pS65-Ub level (Median: 0.83 vs. 3.23, β: −0.98, $P = 0.009$). Additional nominally significant associations were observed of rs2230288 p.E365K with an increased odds of a high likelihood of clinical DLB (57.9% vs. 41.8%, OR = 1.88, $P = 0.025$) and greater likelihood of male sex (70.2% vs. 57.0%, OR = 1.85, $P = 0.042$), and of rs7673715 p.N409S with a decreased Braak NFT stage (≥ 4: 15.4% vs. 52.6%, OR = 0.23, $P = 0.005$).

Table 4 shows gene-burden analysis assessing associations with neuropathological outcomes for presence of the minor allele for any *GBA* variant, and presence of the minor allele for any *GBA* variant with a MAF < 0.5%; only outcomes where at least a nominally significant association are displayed, with results for other outcomes provided in Supplemental Table 2. After correcting for multiple testing, presence of any *GBA* variant was associated with a greater odds of a high likelihood of clinical DLB (58.8% vs. 39.2%, OR = 2.00, $P < 0.001$), a lower Braak NFT stage (≥ 4: 35.2% vs. 55.7%, OR = 0.48, $P < 0.001$), a lower Thal amyloid phase (≥ 3: 58.5% vs. 75.1%, OR = 0.55, $P < 0.001$), and a lower pS65-Ub level (Median: 1.88 vs. 3.56, β: −0.37, $P < 0.001$), with similar findings observed when considering presence of any *GBA* variant with a MAF < 0.5% (all P ≤ 0.001, Table 4). These results are further illustrated in Fig. 1 (presence of any *GBA* variant) and Supplemental Fig. 1 (presence of any *GBA* variant with a MAF < 0.5%). Of note, results of rare-variant (MAF < 0.5%) gene-burden analyses were relatively similar when excluding rs421016 p.L483P (Table 4), indicating that the aforementioned findings were not driven by this rare variant. Additionally, although only nominally significant, presence of any *GBA* variant was associated with a lower dorsolateral putaminal TH-ir (Median: 2.83 vs. 4.39, β: −0.28, $P = 0.014$). A similar association with dorsolateral putaminal TH-ir was noted when examining presence of any *GBA* variant with a MAF < 0.5% (Median: 2.63 vs. 4.37, β: −0.41, $P = 0.007$).

Given the significant association between *GBA* gene-burden measures and an increased odds of a high likelihood of clinical DLB, in Table 5, we further explored this finding by assessing the frequencies of gene-burden measures in nine different subgroups defined by a combination of LBD subtype (brainstem, transitional, or diffuse) and Braak NFT stage (0–II, III–IV, or V–VI), as these same subgroups are used to define likelihood of DLB [27]. Subgroups defined by a combination of LBD subtype and Thal amyloid phase (0–1, 2–3, or 4–5) were also examined (Table 5). When considering presence of any *GBA* variant, observed frequencies were the highest in LBD subgroups with Braak NFT stage 0–II and either transitional LBD (37.8%) or diffuse LBD (28.4%); in comparison to a reference group of brainstem LBD and Braak NFT stage 0–II patients (in analysis adjusted for age at death, sex, and the top 5 PCs), the likelihood of presence of any *GBA* variant was higher for transitional LBD and Braak NFT stage 0–II (OR = 3.24, $P = 0.007$), with a slightly weaker finding for diffuse LBD and Braak NFT stage 0–II (OR = 2.20, $P = 0.077$). Findings were similar when assessing LBD subtype and Thal amyloid phase, with the highest frequencies of *GBA* gene-burden noted for the transitional LBD and Thal amyloid phase 0–1 (OR [vs. brainstem LBD/Thal 0–1] = 4.52, $P = 0.001$) and diffuse LBD and Thal amyloid phase 0–1 (OR = 5.14, $P = 0.005$) subgroups. Results were consistent when examining the presence of any *GBA* variant with a MAF < 0.5% (Supplemental Table 3).

The results shown in Table 5 suggest the presence of interactions between *GBA* variants and LBD subtype with regard to association with AD-related neuropathology. Therefore, we assessed this possibility in Table 6, where associations of *GBA* gene burden measures with Braak NFT stage, Thal amyloid phase, and pS65-Ub were assessed separately for each LBD subtype. There were significant interactions between presence of any *GBA* variant and LBD subtype regarding the association with Braak NFT stage (interaction $P < 0.001$) and Thal amyloid phase (interaction $P < 0.001$), where presence of *GBA* variants was only associated with a lower Braak NFT stage and Thal amyloid phase for cases with transitional or diffuse LBD. No significant interaction between presence of any *GBA* variant and LBD subtype in relation to pS65-Ub level (interaction $P = 0.21$) was noted. These results were relatively consistent when assessing presence of any *GBA* variant with a MAF < 0.5% (Table 6). Of note, the results of all of the aforementioned regression analyses were similar when adjusting regression models for age at disease onset instead of age at death in the 384 LBD cases who had information available for both measures (data not shown), which is to be expected given the very strong

Table 3 Associations between common *GBA* variants and neuropathological outcomes; only outcomes where a significant ($P<0.0029$) or nominally significant ($P<0.05$) association was observed are displayed

Outcome	Association measure	Association with rs140335079 x7-18 bp		Association with rs2230288 E365K		Association with rs75548401 T408M		Association with rs7673715 N409S		Association with rs421016 L483P	
		Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value
LBD subtype	Odds ratio	1.55 (0.65, 3.66)	0.32	0.97 (0.58, 1.62)	0.90	1.35 (0.61, 2.98)	0.46	0.53 (0.18, 1.54)	0.24	0.23 (0.07, 0.80)	0.021
High likelihood of DLB	Odds ratio	1.28 (0.54, 3.02)	0.58	1.88 (1.08, 3.26)	0.025	1.36 (0.60, 3.08)	0.46	2.47 (0.71, 8.57)	0.15	1.25 (0.32, 4.92)	0.75
Braak NFT stage	Odds ratio	0.90 (0.42, 1.90)	0.78	0.79 (0.49, 1.27)	0.32	0.81 (0.40, 1.65)	0.56	0.23 (0.08, 0.65)	0.005	0.10 (0.03, 0.35)	<0.001
Thal amyloid phase	Odds ratio	0.82 (0.33, 2.01)	0.66	1.15 (0.67, 1.98)	0.61	0.58 (0.26, 1.30)	0.19	0.43 (0.14, 1.31)	0.14	0.19 (0.05, 0.64)	0.008
pS65-Ub	β coefficient	0.09 (−0.48, 0.66)	0.76	−0.20 (−0.54, 0.13)	0.23	−0.09 (−0.61, 0.43)	0.74	−0.62 (−1.34, 0.10)	0.092	−0.98 (−1.72, −0.25)	0.009
Sex (Male)	Odds ratio	1.26 (0.51, 3.09)	0.62	1.85 (1.02, 3.35)	0.042	1.81 (0.74, 4.48)	0.20	1.64 (0.42, 6.37)	0.48	0.30 (0.08, 1.18)	0.085

LBD Lewy body disease, *DLB* dementia with Lewy bodies, *NFT* neurofibrillary tangle, *pS65-Ub* phospho-ubiquitin, β = regression coefficient, *CI* confidence interval

Odds ratios, multiplicative effects, and β coefficients from proportional odds logistic regression models (LBD subtype, Braak NFT stage, and Thal amyloid phase), linear regression models (pS65-Ub), and binary logistic regression models (high likelihood of DLB and male sex) that were adjusted for age at death, sex, and the top 5 principal components of genome-wide genetic data. For LBD subtype, Braak NFT stage, and Thal amyloid phase, odds ratios are interpreted as the multiplicative increase in the odds of a more severe category for cases with presence of the minor allele for the given *GBA* variant. For pS65-Ub, β coefficients are interpreted as the additive increase in the mean value (on the natural logarithm scale) for cases with presence of the minor allele for the given *GBA* variant. For high likelihood of DLB and male sex, odds ratios are interpreted as the multiplicative increase in the odds of occurrence of the given outcome for cases with presence of the minor allele for the given *GBA* variant. P-values < 0.0029 are considered as statistically significant after applying a Bonferroni correction for the 17 different outcome measures that were examined

Table 4 Gene-burden analysis of associations between rare *GBA* variants and neuropathological outcomes; only outcomes where a significant ($P < 0.0029$) or nominally significant ($P < 0.05$) association was observed are displayed

Outcome	Association measure	Association with presence of any *GBA* variant		Association with presence of any *GBA* variant with a MAF < 0.5%		Association with presence of any *GBA* variant with a MAF < 0.5% (excluding rs421016 L483P)	
		Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value
High likelihood of DLB	Odds ratio	2.00 (1.41, 2.84)	< 0.001	2.22 (1.36, 3.63)	0.001	2.15 (1.29, 3.57)	0.0031
Dorsolateral putaminal TH-ir	β coefficient	−0.28 (−0.50, −0.06)	0.014	−0.41 (−0.70, −0.11)	0.007	−0.39 (−0.70, −0.08)	0.013
Braak NFT stage	Odds ratio	0.48 (0.35, 0.65)	< 0.001	0.35 (0.23, 0.53)	< 0.001	0.43 (0.28, 0.66)	< 0.001
Thal amyloid phase	Odds ratio	0.55 (0.39, 0.77)	< 0.001	0.33 (0.21, 0.53)	< 0.001	0.39 (0.24, 0.63)	< 0.001
pS65-Ub	β coefficient	−0.37 (−0.58, −0.15)	< 0.001	−0.51 (−0.81, −0.22)	< 0.001	−0.39 (−0.70, −0.09)	0.012

DLB dementia with Lewy bodies, *NFT* neurofibrillary tangle, β regression coefficient, *CI* confidence interval

Odds ratios, multiplicative effects, and β coefficients from proportional odds logistic regression models (Braak NFT stage and Thal amyloid phase), linear regression models (pS65-Ub), and binary logistic regression models (high likelihood of DLB and male sex) that were adjusted for age at death, sex, and the top 5 principal components of genome-wide genetic data. For Braak NFT stage and Thal amyloid phase, odds ratios are interpreted as the multiplicative increase in the odds of a more severe category for cases with presence of the minor allele for any of the *GBA* variants of interest for the given *GBA* gene-burden variable. For pS65-Ub, β coefficients are interpreted as the additive increase in the mean value (on the natural logarithm scale) for cases with presence of the minor allele for any of the *GBA* variants of interest for the given *GBA* gene-burden variable. For high likelihood of DLB and male sex, odds ratios are interpreted as the multiplicative increase in the odds of occurrence of the given outcome for cases with presence of the minor allele for any of the *GBA* variants of interest for the given *GBA* gene-burden variable. *P*-values < 0.0029 are considered as statistically significant after applying a Bonferroni correction for the 17 different outcome measures that were examined

degree of correlation between these two age-related variables (Spearman's $r = 0.85$, $P < 0.001$).

Discussion

GBA variants are well-known risk factors for both PD and DLB [7, 13, 30, 31, 37, 41]. However, little is known regarding whether *GBA* variation may modify neuropathology in LBD cases. Herein, we assessed associations between *GBA* variants and 15 different neuropathological features in a series of 943 LBD cases. One significant association was observed when examining common variants after correcting for multiple testing, which was between p.L483P and a lower Braak NFT stage. In gene-burden analysis, we observed significant associations of presence of the minor allele for any *GBA* variant (rare variants only, and also all variants regardless of MAF) with a greater odds of a high likelihood of clinical DLB, a less severe Braak NFT stage and Thal amyloid phase, and a lower pS65-Ub level in the overall series of LBD cases. In subgroup analysis, *GBA* gene-burden measures were most common in cases with either transitional or diffuse LBD and low levels of AD pathology (Braak NFT stage and Thal amyloid phase), and correspondingly the aforementioned associations of presence of any *GBA* variant with lower Braak NFT stage and Thal amyloid phase were observed predominantly for transitional and diffuse LBD cases. Importantly, all findings were independent of age at death, sex, and top PCs of genome-wide genetic data.

Some of the strongest findings of our study were the aforementioned protective associations that we observed in gene-burden analysis of the overall LBD series for the AD-related measures of Braak NFT stage, Thal amyloid phase, and pS65-Ub level. Previous studies of PD patients have failed to identify associations between presence of *GBA* mutations and AD-related neuropathological outcomes [1, 32, 34]. However, sample sizes have been very small. It is also worth noting that *GBA* variants are not associated with a decreased risk of AD from large population case–control GWAS and sequencing studies [3, 14]. Thus, our findings likely reflect a higher frequency of *GBA* mutation carriers in PD and DLB (where Braak, Thal, and pS65-Ub level are lower) compared to clinical AD (where Braak, Thal, and pS65-Ub level are higher). To further investigate this, we also assessed gene-burden associations vs. the outcome of clinically assessed dementia. The fact that no notable associations with dementia were observed suggests that our significant findings regarding Braak NFT stage, Thal amyloid phase, and S65-Ub level are indeed indicative of the disease–risk associations for PD and DLB but not AD, rather than a general independent association between *GBA* variants and dementia-related measures in LBD.

Interestingly, we also identified a strong association between presence of *GBA* mutations and increased risk of a high likelihood of DLB. This initially seems to reflect

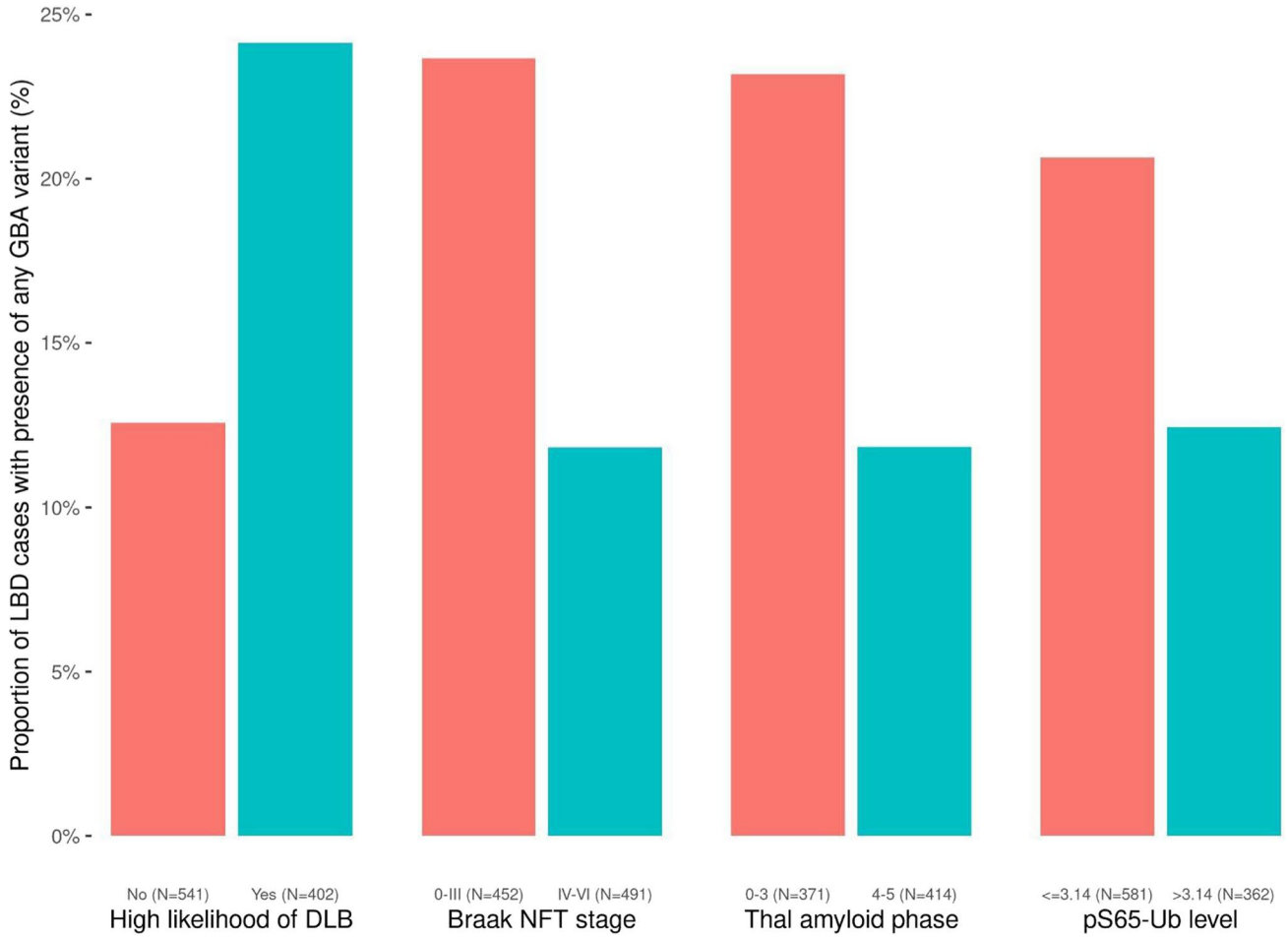

Fig. 1 Proportion of LBD cases with presence of any *GBA* variant according to high likelihood of clinical DLB, Braak NFT stage, Thal amyloid phase, and pS65-Ub level. pS65-Ub level was dichotomized based on the sample median

the aforementioned associations between presence of *GBA* variants and a lower Braak NFT stage, lower Thal amyloid phase, and lower pS65-Ub level, as a high likelihood of DLB is defined in part by presence of low AD pathology (Braak NFT stage 0–II). However, subsequent examination of the frequency of *GBA* variants according to specific subgroups defined by combinations of LBD subtype and either Braak NFT stage or Thal amyloid phase reveals important insights into all of these findings, in that LBD subtype appears to interact with severity of AD pathology in regard to association with *GBA* gene-burden measures. Specifically, *GBA* gene-burden measures were only associated with Braak NFT stage and Thal amyloid phase in LBD cases with transitional or diffuse LBD, with no notable association observed in the brainstem LBD subgroup. The aforementioned associations of presence of *GBA* variants with a less severe Braak NFT stage and Thal amyloid phase in the overall series appear to have been driven mostly by the very high frequency of such variants in LBD cases with transitional or diffuse LBD and low levels of AD pathology, who of course comprise part of the high likelihood of DLB subgroup. These findings are in agreement with those of Tsuang et al., who noted a higher frequency of *GBA* mutations in 80 cases with "pure DLB" (dementia, transitional or diffuse LBD, no/low levels of AD pathology) compared to 231 cases with "LBD-AD" (dementia, transitional or diffuse LBD, high levels of AD pathology) [40].

In our LBD series, *GBA* variants were not strongly associated with severity of LB pathology (as measured by LB counts and LBD subtype), with striatal dopaminergic degeneration (as measured by putaminal TH-ir), or with substantia nigra neuronal loss. Though again limited by small sample size, previous studies have mostly observed a similar lack of association [1, 34], with the exception of one report of a more severe LBD subtype for 17 PD *GBA* mutation carriers compared to 16 PD non-carriers [32]. The negative findings of our large study of LBD cases could indicate that while *GBA* variants are strong risk factors for developing the LB disorders of PD and DLB, they do not act as disease modifiers for PD/DLB-related neuropathological outcomes

Table 5 Proportion of LBD cases who are carriers of any *GBA* variant according to combinations of LBD subtype with Braak NFT stage and Thal amyloid phase

	Braak NFT stage 0-II	Braak NFT stage III-IV	Braak NFT stage V-VI
Brainstem LBD			
Likelihood of DLB according to the fourth report of the DLB consortium	Low	Low	Low
Fraction (%) with any *GBA* variant	9/58 (15.5%)	5/45 (11.1%)	3/21 (14.3%)
OR (95% CI)	1.00 (reference)	0.96 (0.29, 3.19)	1.16 (0.27, 4.90)
P-value	N/A	0.95	0.84
Transitional LBD			
Likelihood of DLB according to the fourth report of the DLB consortium	High	Intermediate	Low
Fraction (%) with any *GBA* variant	31/82 (37.8%)	14/100 (14.0%)	15/154 (9.7%)
OR (95% CI)	3.24 (1.38, 7.61)	1.15 (0.45, 2.92)	0.74 (0.30, 1.82)
P-value	0.007	0.77	0.51
Diffuse LBD			
Likelihood of DLB according to the fourth report of the DLB consortium	High	High	Intermediate
Fraction (%) with any *GBA* variant	23/81 (28.4%)	43/239 (18.0%)	22/163 (13.5%)
OR (95% CI)	2.20 (0.92, 5.26)	1.38 (0.62, 3.07)	0.90 (0.38, 2.11)
P-value	0.077	0.43	0.81
	Thal amyloid phase 0–1	Thal amyloid phase 2–3	Thal amyloid phase 4–5
Brainstem LBD			
Fraction (%) with any *GBA* variant	7/64 (10.9%)	3/20 (15.0%)	4/23 (17.4%)
OR (95% CI)	1.00 (reference)	1.59 (0.36, 7.03)	2.11 (0.54, 8.24)
P-value	N/A	0.54	0.28
Transitional LBD			
Fraction (%) with any *GBA* variant	30/83 (36.1%)	6/41 (14.6%)	12/141 (8.5%)
OR (95% CI)	4.52 (1.80, 11.32)	1.73 (0.53, 5.66)	0.84 (0.31, 2.28)
P-value	0.001	0.37	0.73
Diffuse LBD			
Fraction (%) with any *GBA* variant	11/26 (42.3%)	29/137 (21.2%)	33/250 (13.2%)
OR (95% CI)	5.14 (1.66, 15.89)	2.41 (0.98, 5.93)	1.28 (0.53, 3.07)
P-value	0.005	0.055	0.59

LBD Lewy body disease, *DLB* dementia with Lewy bodies, *OR* odds ratio, *CI* confidence interval, *MAF* minor allele frequency

Odds ratios, 95% CIs, and p-values results from binary logistic regression models that were adjusted for age at death, sex, and the top 5 principal components of genome-wide genetic data, where the outcome was presence of any GBA variant, and the predictor variable of interest was the LBD subtype/Braak NFT stage subgroup with a reference group of brainstem LBD/Braak NFT stage 0-II

in individuals who have already developed LBD. Alternatively, these results could suggest that *GBA* variation may influence specific aspects of disease that may occur earlier in the disease course, rather than severity of disease at the later stages of disease as measured by our neuropathological outcomes. For example, it may be that *GBA* mutations play a role in the initial accumulation, seeding and aggregation of α-synuclein, or the spread of pathology to the cortical regions; further mechanistic studies are needed to tease out the interplay of *GBA* and α-synuclein [6].

Our detailed neuropathologic characterization of the frequency of *GBA* mutations across the spectrum of both LB and AD pathology in LBD also further informs disease–risk associations involving PD, PDD, and DLB. For example, OR estimates regarding *GBA* mutations have typically been higher for DLB than for PD [31, 37] (with DLB more commonly having severe LB pathology), and also *GBA* mutations are associated with an increased likelihood of dementia in PD [12, 26] (with PDD also more frequently presenting with greater LB pathology compared to PD without dementia). Of note, recent reports have suggested a possible sex-specific difference in the frequency of *GBA* mutations in PD [33, 39]; with the exception of the p.E365K mutation that was nominally more common in males, we were unable to demonstrate such differences in our LBD series.

Table 6 Assessment of interactions with LBD subtype for gene-burden analysis of associations of rare *GBA* variants with Braak NFT stage, Thal amyloid phase, and pS65-Ub

Outcome	Association measure	Brainstem LBD ($N=124$)		Transitional LBD ($N=336$)		Diffuse LBD ($N=483$)		Interaction *P*-value
		Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value	
Association with presence of any *GBA* variant								
Braak NFT stage	Odds ratio	0.97 (0.38, 2.50)	0.96	0.25 (0.14, 0.43)	< 0.001	0.51 (0.34, 0.78)	0.002	< 0.001
Thal amyloid phase	Odds ratio	1.75 (0.60, 5.05)	0.30	0.26 (0.14, 0.48)	< 0.001	0.53 (0.33, 0.85)	0.008	< 0.001
pS65-Ub	β coefficient	−0.11 (−0.78, 0.57)	0.76	−0.68 (−1.05, −0.31)	< 0.001	−0.32 (−0.57, −0.06)	0.015	0.21
Association with presence of any *GBA* variant with a MAF < 0.5%								
Braak NFT stage	Odds ratio	0.29 (0.06, 1.35)	0.11	0.17 (0.08, 0.36)	< 0.001	0.44 (0.25, 0.78)	0.004	0.004
Thal amyloid phase	Odds ratio	0.90 (0.18, 4.46)	0.90	0.16 (0.07, 0.39)	< 0.001	0.29 (0.15, 0.55)	< 0.001	0.018
pS65-Ub	β coefficient	−0.55 (−1.53, 0.43)	0.27	−0.70 (−1.21, −0.19)	0.007	−0.47 (−0.81, −0.13)	0.007	0.76

LBD Lewy body disease, β regression coefficient, *CI* confidence interval

Odds ratios and β coefficients result from proportional odds logistic regression models (Braak NFT stage and Thal amyloid phase) and linear regression models (pS65-Ub) that were adjusted for age at death, sex, and the top 5 principal components of genome-wide genetic data. Odds ratios are interpreted as the multiplicative increase in the odds of a more severe category for cases with presence of the minor allele for any of the *GBA* variants of interest for the given *GBA* gene-burden variable. β coefficients are interpreted as the additive increase in the mean pS65-Ub level (on the natural logarithm scale) for cases with presence of the minor allele for any of the *GBA* variants of interest for the given *GBA* gene-burden variable. For tests of interaction, p-values result from the aforementioned regression models, with the covariates for LBD subtype and the interaction between LBD subtype and the given *GBA* variant measure

Strengths of our study include the large number of neuropathological features that were assessed, the relatively large number of LBD cases that were included, and the fact that we were able to adjust for population stratification using top PCs of genome-wide data. However, although the sample size is relatively large for an LBD series, it is still somewhat limited for a genetic association study. Therefore, the possibility of a type II error (i.e., a false-negative finding) is important to consider, particularly for the dichotomous outcomes of TDP-43 pathology and VaD, for outcomes with a greater amount of missing data, and when examining individual common *GBA* variants, as power would have been lowest in these scenarios. Additionally, our study examined only individuals of Caucasian ancestry; future studies evaluating associations between *GBA* variants and neuropathology of LBD in other racial/ethnic groups will be important.

Several other limitations of our study are important to draw attention to. The possibility of ascertainment bias in our autopsy-based cohort must be acknowledged; our LBD series is likely over-represented with AD patients, as evidenced by the high proportion of cases with a Thal amyloid phase equal to 5 (42.0%). However, as we directly analyzed AD-related neuropathological outcomes in our study, we do not feel that this would have had any noticeable impact on our results. Another limitation to highlight is the presence of missing data for a number of our neuropathological outcomes, as well as for disease duration and age at disease onset. Regarding age at onset, it is noteworthy to mention that although we could not directly account for the potential confounding influence of this variable in our regression analyses due to the extent of missing data, we did adjust for age at death in all of our models. Though these two age-related variables capture different components of disease, due to their very high degree of correlation, the choice of which age-related variable is adjusted for in multivariable analysis has a minimal impact on the results involving individual *GBA* variants and *GBA* gene-burden measures. Furthermore, TDP-43 pathology was assessed only in the amygdala; evaluation of other regions, such as hippocampus, midbrain, and frontal lobe, could have impacted our findings regarding this neuropathological outcome. Finally, though the guidelines utilized in our study for evaluation of Lewy pathology are well-established and widely used, other guidelines that have recently been proposed would also have been a reasonable alternative [2], noting that this would have only changed the analyses regarding LBD subtype.

In conclusion, the results of our study shed light on the role of *GBA* variation in determining severity of neuropathology in LBD and also aid in our understanding of the role of *GBA* mutations in susceptibility to specific LB disorders, such as PD, PDD, and DLB. Specifically, the presence of *GBA* mutations is associated with a lower severity of AD-related neuropathological features in LBD, with associations involving Braak NFT stage and Thal amyloid phase

observed solely in cases with transitional or diffuse LBD. The latter observation is reflective of the high frequencies of *GBA* gene-burden measures in LBD cases with either transitional or diffuse LBD and also low levels of AD pathology (Braak NFT stage 0–II, or Thal amyloid phase 0–1). Interestingly, *GBA* variation was not strongly associated with neuropathological features that are more specific to PD and DLB. Unbiased GWAS or whole-genome sequencing studies will be important in order to more fully characterize genetic drivers of neuropathological variation in LBD cases.

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Authors contribution RLW performed genotyping and quality control assessments on all samples, and assisted in drafting the manuscript. SK, KK, and DWD provided brain tissue samples for all cases and provided manuscript improvements. DWD also performed all neuropathological assessments of LBD cases. AIB, TG, MEM, XH, FCF, WS, RJU, JAF, HB, VKR, KK, VJL, CRJ, NE, RS, JG, RCP, JEP, RRR, NRG, TJF, BFB, and ZKW provided manuscript improvements. LJW performed the statistical analysis. OAR led the study, oversaw all methodological developments, and approved the final manuscript. MGH performed the statistical analysis and drafted the manuscript.

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Data availability The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflicts of interest All authors declare that they have no competing interests.

Ethical approval This study was approved by the Mayo Clinic Institutional Review Board. All subjects or legal next of kin provided written informed consent.

Consent for publication Not applicable.

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