Parkinsonism and Related Disorders 124 (2024) 107023

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Increased glucosylsphingosine levels and Gaucher disease in *GBA1*-associated Parkinson's disease

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https://doi.org/10.1016/j.parkreldis.2024.107023
Received 5 March 2024; Received in revised form 9 May 2024; Accepted 31 May 2024
Available online 1 June 2024

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ARTICLE INFO

Keywords:
Parkinson's disease
Gaucher disease
Genetic
Glucocerebrosidase
Glycosphingolipids

ABSTRACT

Introduction: Gaucher's disease (GD) is caused by biallelic mutations in the *GBA1* gene, leading to reduced glucocerebrosidase (GCase) activity and substrate (glucosylceramide and glucosylsphingosine, GlcSph) accumulation. *GBA1* variant carriers are at risk of Parkinson's disease (PD), but only those with biallelic mutations cross the threshold of GCase reduction, leading to substrate accumulation and GD. The link between *GBA1* mutations, GD and PD is not fully understood. Here we aimed at reporting the results of a large PD population screening with dried blood spot tests for GD.

Methods: We measured GCase activity and GlcSph levels in 1344 PD patients with dried blood spot tests, and performed *GBA1* genetic sequencing.

Results: While the GCase activity was reduced in GBA1-PD carriers compared to wild type PD, GlcSph was increased in GBA1-PD compared to GBA1-controls, regardless of the underlying type of *GBA1* variant. 13.6 % and 0.4 % of PD patients had mono- or biallelic *GBA1* mutations respectively. GCase deficiency, lipid accumulation and clinical manifestations of GD was detected in five PD patients with biallelic *GBA1* mutations, of whom four had a risk combined with a GD causing variant.

Conclusions: GlcSph appearing higher in PD may represent a reliable biomarker of the disease and deserves to be further investigated. This study highlights the importance of screening PD patients for possible underlying GD, which is a treatable condition that should not be missed. We diagnosed GD cases carrying a "risk" variant in one allele, which is an unprecedented finding deserving further investigation.

1. Background

Biallelic mutations of the *GBA1* gene can result in Gaucher's disease (GD), the most common autosomal recessive lysosomal storage disorder in humans. The *GBA1* gene encodes glucocerebrosidase (GCase), the enzyme converting the glucosylceramide (GlcCer) to glucose and ceramide in the lysosomes [1]. Defective GCase activity leads to the accumulation of GlcCer which is converted to glucosylsphingosine (GlcSph) through a process of deacylation performed by the ceramidase enzyme [2]. GCase activity and GlcSph dosage are established biomarkers for the diagnosis of GD [2], with GlcSph being also used for enzyme replacement therapy (ERT) monitoring.

The association between *GBA1* variants and GD is primarily linked to the impact of the mutations on the residual enzymatic activity (REA) of GCase, yet other contributing factors remain unclear [1]. For instance, variants such as N370S, the most prevalent variant in GD1 patients, whether in homozygosity or combined with other *GBA1* mutations, do not always result in disease manifestation. Individuals carrying two copies of common GD-associated variants, like N370S, can remain asymptomatic throughout their lives. Moreover, *GBA1* variants with a more pronounced effect on enzymatic activity, such as L444P, exhibit significant phenotypic variability, ranging from severe acute neuronopathic forms to those resembling GD1 [3]. Lastly, certain *GBA1* variants (e.g., E326K, T369M, E388K), despite having a modest impact on GCase activity, are generally not considered diagnostic for GD, as individuals carrying such variants in homozygous state have been reported not to display pathological GCase levels and clinical features of GD so far [4–6]. Based on the estimated REA and the most likely associated phenotype, *GBA1* mutations are classified as "mild" (e.g., N370S) or "severe" (e.g., L444P). A broader categorization also includes mutations being part of "complex" alleles (e.g., derived by insertion into *GBA1* of sequences coming from the close pseudogene *GBA1p*), "synonymous" variants and variants of "unknown" pathogenicity. The "risk" variants (e.g., E326K, T369M, E388K) are those that still lack evidence of association with the development of GD, even though they increase the chance of developing Parkinson's disease (PD) [6,7].

To date, nearly 400 mutations/polymorphisms have been linked to PD and Dementia with Lewy Bodies [8]. However, the classification based on the association with the GD type is informative only for 20–30 % of the variants. Indeed, if a not yet classified *GBA1* variant is reported in a GD patient in compound heterozygosity with the common N370S, the resulting phenotype will be GD1, due to the presence of at least one mild mutation. Such genotypes preclude the possibility of assigning a clear severity score to the not yet classified variants as the phenotype would not change either in the case of mild or severe mutation. Differently, if an unclassified variant occurs in subjects with neuronopathic GD then it is reasonable to classify the variant as severe.

Given the high frequency of *GBA1* variants in PD patients (5–20 %, with the highest prevalence observed in the Ashkenazi Jewish, AJ, population) [9,10], the risk of encountering GD in these subjects is expected to be higher than in the general population.

The aim of the present study was to (i) report the clinical and molecular features of a series of PD patients screened for GD through dried blood spot tests (DBS) tests, and (ii) to compare the biochemical profiles in patients with mono and biallelic *GBA1* variants as compared to non-mutated PD patients and in subjects without PD.

2. Methods

Twenty-five tertiary Italian PD centers participated in a nationalwide screening for GD in PD patients in the period included between March 2022 and December 2023. Participating neurologists entered an educational program on GD (May 2022–May 2023) and were asked to screen subjects for GD using DBS tests (May 2022–December 2023), evaluating signs or symptoms potentially suggestive of GD (Supplementary Table 1) among consecutive PD cases. A total of 1344 patients were screened, the mean age was 65.3 ± 10.2, 37.9 % (n = 510) were females and 62.3 % (n = 838) were males. The number of cases collected by single centers is reported in Supplementary Table 2.

All samples were analyzed by a single center (CNR IRIB, Palermo, Italy) for (i) GCase enzymatic activity values and (ii) *GBA1* genetic screening. GCase assay was determined by the Dried Blood Filter Paper test (DBFP, or just DBS test) [11], with modifications. Long Polymerase Chain Reaction (Long PCR) approach was applied to identify specific mutations of *GBA1* and *GBAPs* recombinant. We carried out Long-Range PCR of the gene, using two pairs of primers that amplify two macro-regions, from exon 1 to intron 5 and from intron 5 to exon 11 using the Biotech-Rabbit high-fidelity Long-Range DNA Polymerase. Sequencing primers have been designed to sequence the exons of *GBA1* and large flanking intronic regions. The PCR protocol and primers are reported in Supplementary Table 3. Cases with low (≤2.5 nMol/h/ml) or borderline (≤3 nMol/h/ml) GCase activity were also tested through Multiplex Ligation-dependent Probe Amplification (MLPA) P338-X1 GBA kit (MRC-Holland). Samples carrying heterozygous

(GBA1$^{mut/wt}$-PD), compound heterozygous or homozygous mutations (GBA1$^{mut/mut}$-PD), and/or low or borderline GCase activity were selected for GlcSph dosage (normal range ≤6.8 ng/ml). Detection of GlcSph was performed in dried blood spots by LC-MS/MS assay [12]. In patients with more than one *GBA1* variant, the phase was ascertained through segregation analysis in family members. GBA1$^{mut/mut}$-PD cases with altered GCase activity were selected for a further clinical description. Hence, clinical information on the presence of visceral, skeletal, and hematologic symptoms of GD was retrospectively collected in patients with biallelic *GBA1* mutations with and without abnormal GlcSph levels.

GCase activity and GlcSph values of PD patients with and without *GBA1* mutations were compared with a cohort of Italian control subjects (n = 113) with (GBA1$^{mut/wt}$-CTR, n = 39) and without (GBA1$^{wt/wt}$-CTR, n = 74) *GBA1* variants, referred to CNR IRIB due to the suspicion of GD (supplementary materials). Even in this case patients with unknown phase (n = 1) were excluded from further analysis.

The same analysis was repeated after manually matching cases according to the prevalence and type of *GBA1* variants (supplementary materials).

GBA1 mutations were classified according to the Clinvar database (www.clinicalgenome.org) and to HGMD (www.hgmd.cf.ac.uk). The severity of *GBA1* mutations was determined based on their impact on GCase activity and on GD phenotype [13–17]. Briefly, if the variants occurred in GD1 either in homozygosity, or in combination with a known *GBA1* severe variant, then they were classified as mild, while if they occurred in neuronopathic GD, as severe. Variants not clearly associated with GD, but increasing the risk of PD, were classified as "risk" [7,8]. Recombinant alleles were classified as "complex", while mutations with uncertain significance were classified as "unknown". We also included "synonymous" in our classification when mutation coded for the same original amino acid.

2.1. Statistical analysis

Data were retrospectively collected. Continuous variable distributions were tested through the Shapiro Wilks test. Differences across groups on parametric or non-parametric variables were tested through respective tests (*t*-test, Wilcoxon) and ANOVA or Kruskall-Wallis if multiple groups were tested, with post-hoc corrections for multiple comparisons. Categorical variables were tested through the chi-squared test. Association through groups and variables were investigated in correlation studies and further confirmed with univariate and multivariate logistic regression models. A $p < 0.05$ was adopted for statistical significance. Statistics were performed through the JMP software 17.0 (SAS Inc.).

The study was conducted in accordance with the declaration of Helsinki and has been approved by the local ethic committee. The data used in the preparation of this manuscript are available upon reasonable request.

3. Results

3.1. Molecular findings in the Parkinson's disease cohort

One thousand three hundred forty-four (n = 1344) DBS samples were collected from patients with PD. Thirty-seven (n = 37, 2.75 %) of these samples were deemed insufficient for screening enzymatic activity, but *GBA1* sequencing was conducted in all cases. The mean GCase activity of the cohort was 5.8 ± 2.6 nMol/h/ml (range 0.8–22). A total of 15.3 % (n = 206) subjects exhibited borderline or low GCase activity, and 14.6 % (n = 196) presented with at least one *GBA1* mutation; 206 PD were tested for GlcSph, the mean value was 6.5 ± 17 ng/ml (1.3–250). Patients with unknown phase at *GBA1* gene testing (n = 7 PD and n = 1 CTR) were excluded from further analysis.

Seven (n = 7) PD patients were found to have two *GBA1* mutations, six of whom were confirmed to be biallelic through segregation studies (n = 6 GBA1$^{mut/mut}$-PD, 0.4 %, as shown in Fig. 1). Specifically, five patients had a GD-causing variant in combination with a risk variant (E326K or T369M), while one PD patient carried two GD-causing variants (N370S/RecNcil) (Table 1). GBA1$^{mut/mut}$-PD patients (n = 6) exhibited lower GCase activity (1.7 ± 0.6, range 0.8–2.6) compared to both GBA1$^{mut/wt}$-PD (4.7 ± 2.2, range 2–14.7*)* and wild type PD subjects (GBA1$^{wt/wt}$-PD; 6.2 ± 2.4, range 1.5–22) ($p < 0.001$) (Fig. 1B). Similarly, GBA1$^{mut/mut}$-PD had higher GlcSph values (48 ± 99, range 4.7–250) than GBA1$^{mut/wt}$-PD (5.4 ± 1.6, range 1.3–9.7; $p = 0.026$) and GBA1$^{wt/wt}$-PD (4.8 ± 1.3, range 2–8.3; $p = 0.008$) (Fig. 1B). A trend ($p = 0.056$) towards higher values of GlcSph was observed evaluating GBA1$^{mut/wt}$-PD versus GBA1$^{wt/wt}$-PD, at post-hoc analyses. The patient carrying the N370S/RecNcil genotype displayed a very low REA and high GlcSph value (0.8 nMol/h/ml and 250 ng/ml) (Table 1).

After excluding GBA1$^{mut/mut}$-PD patients (n = 6), we compared GBA1$^{mut/wt}$-PD (n = 183, 13.6 % of PD) versus GBA1$^{mut/wt}$-CTR (n = 39, 34.5 % of CTR).

GBA1$^{mut/wt}$-PD were older (62.5 ± 9.7 vs 51.7 ± 19.8, $p = 0.002$), and showed higher GCase activity (4.7 ± 2.2 vs 3.75 ± 1.8, $p < 0.001$) and GlcSph values (5.4 ± 1.6 vs 2.7 ± 1.1, $p = 0.016$) than GBA1$^{mut/wt}$-CTR. The sex distribution did not differ across groups (38 % vs 55 % of women, $p = 0.063$).

Groups significantly differed in GBA1 mutation classes distribution ($p = 0.004$) with GBA1$^{mut/wt}$-PD reporting a higher number of cases with severe (27.8 vs 5.1 %), risk (34.9 vs 30.7 %), unknown (6.5 vs 5.1 %) and synonymous (4.5 vs 0 %) variants, and lower number of patients with mild mutations (20.2 vs 41 %) than GBA1$^{mut/wt}$-CTR (Fig. 1; Supplementary Tables 4–6).

The differences in GCase and GlcSph levels between patients and controls, and GBA1 patients and GBA1 controls, maintained even after manually matching groups by GBA1 prevalence, the type of variants and age (Supplementary Table 7).

3.2. Influence of sex, age, GBA1 type of variant on glucocerebrosidase activity and glucosylsphingosine levels

We evaluated the effect of different types of *GBA1* variant on GCase activity and GlcSph levels. PD patients carrying a risk, or a synonymous variant had a significantly higher GCase activity than those carrying mild or severe mutations (5.3 ± 2.6 in risk vs 5.8 ± 2.56 in synonymous vs 4.27 ± 1.87 in mild vs 3.77 ± 1.48 in severe; risk vs mild $p = 0.002$, risk vs severe $p = 0.0002$, synonymous vs mild $p = 0.017$, synonymous vs severe $p = 0.007$). No differences were observed in the GCase activity in patients with mild versus severe variants ($p = 0.164$). GlcSph levels did not differ between patients with different *GBA1* mutation classes ($p = 0.318$) (Fig. 2).

To further investigate the impact of *GBA1* mutation class (e.g., mild, severe, risk, complex, synonymous, unknown), of the clinical diagnosis of PD, and of the age at sampling on GCase activity and GlcSph levels, we performed correlation analysis with univariate and multivariate models in all subjects (GBA1$^{mut/wt}$-PD, GBA1$^{wt/wt}$-PD, GBA1$^{mut/wt}$-CTR and GBA1$^{wt/wt}$-CTR) (Supplementary Table 8).

Subjects affected by PD showed a higher GCase activity than controls regardless of the *GBA1* genotype (5.8 ± 2.6 vs 3 ± 1.6, $p < 0.001$). Similarly, GBA1$^{mut/wt}$-PD showed an increased GCase activity than GBA1$^{mut/wt}$-CTR (4.7 ± 2.2 vs 3.7 ± 1.8, $p = 0.001$) (Fig. 2). In the healthy control group, the GCase and GlcSph were significantly higher in GBA1$^{mut/wt}$-CTR than GBA1$^{wt/wt}$-CTR. (3.7 ± 1.8 vs 2.6 ± 1.3, $p < 0.001$ and 4.6 ± 1.8 vs 2.8 ± 1.1, $p < 0.001$, respectively).

Collectively, the GCase activity was influenced by the *GBA1* mutation class including mild, severe, risk, unknown and synonymous variants at a multivariate analysis (Supplementary Table 7). Differently, GlcSph levels were higher in PD vs CTR (5.2 ± 1.6 vs 3.4 ± 1.6, $p = 0.001$), and in GBA1$^{mut/wt}$-PD vs GBA1$^{wt/wt}$-PD (5.4 ± 1.6 vs 4.8 ± 1.3, $p = 0.05$) (Fig. 2). Age was not related to GCase and GlcSph levels in

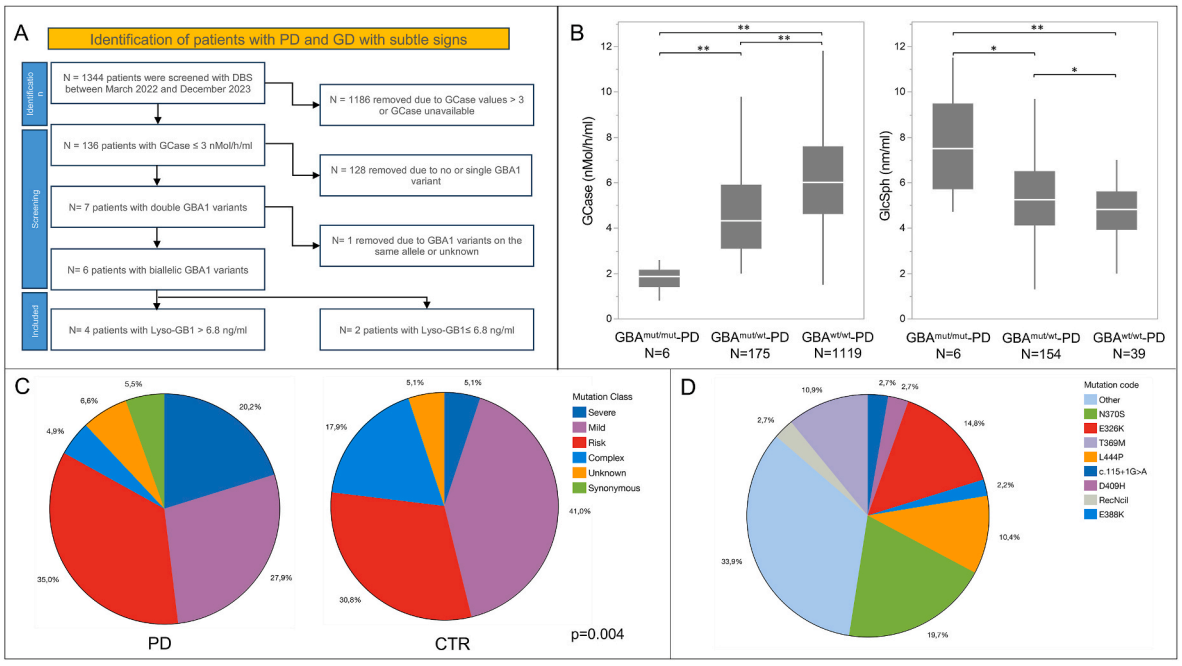

Fig. 1. A) Identification of patients with subtle GD signs among PD patients
B) distribution of GCase (left) and GlcSph (right) levels in PD patients according to the genetic status. An outlier case of the GBA1$^{mut/mut}$-PD with a GlcSph value of 250 ng/ml (N370S/RecNcil) was removed. *p ≤ 0.05, **p < 0.01. C) Mutation class distribution of GBA1$^{mut/wt}$-PD (left) and GBA1$^{mut/wt}$-CTR (right); D) *GBA1* variant prevalence in the PD cohort.

Table 1
Non-neurological features and biochemical profile of patients with biallelic *GBA1* variants.

Case	Sex	Age	GCase (nMol/h/ml)	GlcSph (ng/ml)	Allele 1 (class)	Allele 2 (class)	Visceral	Skeletal	Hematologic
1	M	51	0.8	250	N370S (mild)	RecNcil (complex)	Splenomegaly	–	Anaemia, low platelet count, hyperferritinaemia
2	M	55	1.8	11.5	E326K (risk)	C342F (unknown)	Hepatomegaly	Bone frailty	Lymphadenopathy, anaemia, hyperferritinaemia, MGUS
3	F	64	1.9	7.5	E326K (risk)	S345F (unknown)	Hepatomegaly	Young onset osteoporosis	
4	M	64	1.6	6.7	T369M (risk)	N370S (mild)	–	–	MGUS
5	F	58	2.0	7.5	T369M (risk)	N370S (mild)	Hepatomegaly	Bone pain	–
6	F	75	2.6	4.7	T369M (risk)	RecNcil (complex)	–	–	–
GD1 patients without PD (N = 44) analyzed in the same laboratory		0.2–2.5	14.6–720						

GCase, glucocerebrosidase; GlcSph, glucosylsphingosine.

either group (p = 0.374, p = 0.197 for the PD group and p = 0.261, p = 0.730 for the non-PD group, respectively), and it was not associated with any of the two dependent variables at multivariate models (Supplementary Table 7). A trend towards higher levels of GlcSph was observed in GBA1$^{mut/wt}$-PD men compared to women (5.56 ± 1.57 vs 5.06 ± 1.66, p = 0.051). However, this difference was not evident in wild-type subjects or controls, and no such trend was observed for GCase activity (4.8 ± 2.09 vs 4.72 ± 2.46, p = 0.460). Finally, sex was not found to be influential in the multivariate analysis.

3.3. Features of PD patients with biallelic GBA1 variants and defective GCase activity

One case of GBA1$^{mut/mut}$-PD carrying N370S/RecNcil mutation had defective GCase, increased GlcSph and signs and symptoms suggestive of GD (Table 1). He was then diagnosed as GD1. The GBA1$^{mut/mut}$-PD patients carrying a risk variant in combination with a GD causing variant unexpectedly presented a GCase activity below the threshold used for the diagnosis of GD (2.5 nMol/h/ml) in 4 out of 5 cases, although the values were slightly higher than those found in GD1 patients (range 1.8–2 vs 0.2–2.5, Table 1). Hence, a total of 5 patients (collectively 1 out of 270 PD cases) were furtherly evaluated by GD expert physicians to characterize the visceral, skeletal, and hematologic involvement of GD1 (Table 1). One patient (case 2) started ERT (imiglucerase), others are under evaluation. Nine months after initiation of ERT, the ferritin levels were reduced, lymphadenopathy and hepatomegaly were not detected, and the patient reported a marked improvement of fatigue and quality of life. A detailed description of the clinical and molecular findings of these patients required a separate report.

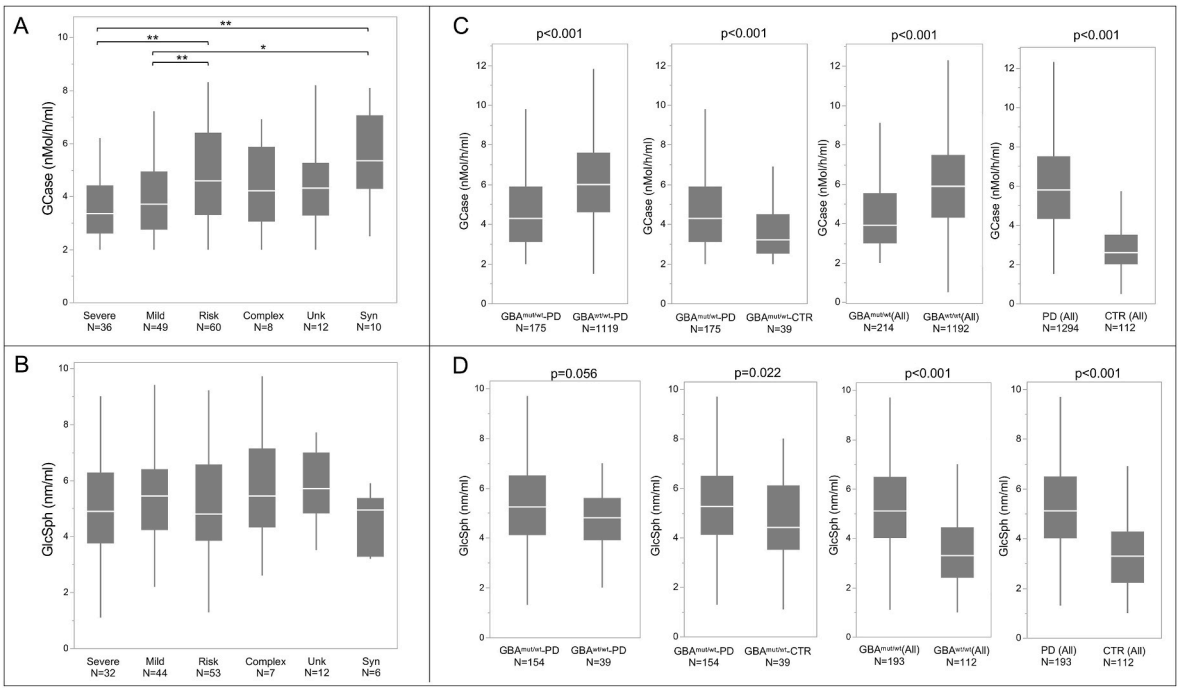

Fig. 2. Difference in (A) GCase and (B) GlcSph across mutation classes in PD carriers of heterozygous GBA1 mutations; values of (C) GCase and (D) GlcSph across groups stratified according to the *GBA1* status and the diagnosis of PD or CTR.

4. Discussion

The understanding of how *GBA1* mutations lead to Gaucher and PD represents an invaluable opportunity to unravel the pathogenesis of PD. *GBA1* gene mutations lead to two major consequences: (i) the production of an aberrant GCase, which may be partially retained in the ER or removed through autophagy mechanisms, and (ii) substrates (i.e., GlcCer and GlcSph) accumulation [18]. Misfolded aberrant GCase may, in turn, promote impaired lysosomal function, leading to autophagy failure and aggregation of alpha-synuclein fibrils in Lewy Bodies [19,20]. Also, it may impair mitochondrial complex I, resulting in oxidative damage and energy failure [21]. However, the contribution of the toxic function of misfolded GCase, its defective enzymatic activity, and the role of substrate accumulation in PD are still not well defined.

The genetic architecture encompassing the *GBA1* locus in PD is unraveling population specific risk variants [22], the GCase activity and GlcSph profiles has been less explored in large PD cohorts, thus precluding a full understanding of the impact of *GBA1* gene variants on the pathogenesis of PD.

In this study, the dried blood spot screening allowed us to examine the GCase activity and GlcSph in a large cohort of PD patients and their *GBA1* gene status.

The multivariate analysis clearly indicated an independent influence of the *GBA1* mutation type on GCase activity (Fig. 2A and Supplementary Table 7) in each cohort. Interestingly, the comparison among cohorts showed a higher GCase activity and GlcSph levels in PD patients than controls, also after correcting for age, GCase variant distribution and number of carriers. This observation strongly suggests that being affected by PD is a major determinant of the altered enzymatic and lipidic profile, regardless of the genotype.

The finding of a higher GCase activity in PD, including PD-GBA1, compared to controls was somewhat unexpected. An intriguing hypothesis underlying this observation could be that the higher activity measured in PD-GBA1 may be influenced by the amount of GCase retained in the endoplasmic reticulum (ER), which would not be present in CTR carrying *GBA1* variants. Indeed, the blood spot assay reflects the enzymatic activity measured in the extracted protein lysates, which include the cytosolic and lysosomal GCase, while in living cells the functional GCase is only that present in the lysosome. This possibility requires to be thoroughly investigated with studies aimed at clarifying the impact of *GBA1* variants on lysosomal GCase activity versus the one measured on total protein lysate.

The finding of a higher GlcSph level in PD patients regardless of their genetic status may support a potential leading role for these lipids in the pathophysiology of the disease [23]. GlcCer and GlcSph can interfere with lysosomal function and with mechanisms of chaperone-mediated autophagy [23]. This interference may promote the accumulation and aggregation of fibrillar alpha-synuclein in the cytoplasm, ultimately contributing to the formation of Lewy bodies in patients with GBA1-PD and PD-GD [24] (Fig. 3).

The role of GlcSph as a potential biomarker for GBA1-PD has been extensively debated. The increased levels of GlcSph observed in GBA1-PD and in carriers without PD compared to PD patients without *GBA1* mutations [25] may still be influenced by the impact of the *GBA1* type of variant, as outlined by Gleason and colleagues [26]. Here we show that the major determinant of GlcSph levels is being affected by PD, rather than the consequence of the different types of *GBA1* variants.

Downstream metabolites of GlcCer, such as GlcSph, are potential mediators of alpha-synuclein toxicity in GBA1-PD [27]. GlcSph has been shown to induce the formation of cytosolic oligomeric alpha-synuclein species capable of templating endogenous alpha-synuclein into aggregates in mammalian culture and human neurons [27]. Furthermore, acknowledging the influence of sex biology on lysosomal metabolism and lipids [28], the association of GBA1-PD with the male sex observed in some cohorts [29], and considering the potential elevation in GlcSph levels among male GBA1-PD patients of our study, we advocate for further prospective studies to elucidate the relationship between sex, GlcSph, and the risk of PD.

The second outcome of this study is the finding of GD patients among PD cohorts (5/1344, 0.4 % of the PD cohort). Remarkably, aside from one case carrying the N370S/RecNCil mutation, the others harbored *GBA1* variants typically considered non-GD causing (E326K, T369M), in conjunction with the known GD-causing mutation N370S, or other *GBA1* likely pathogenic variants.

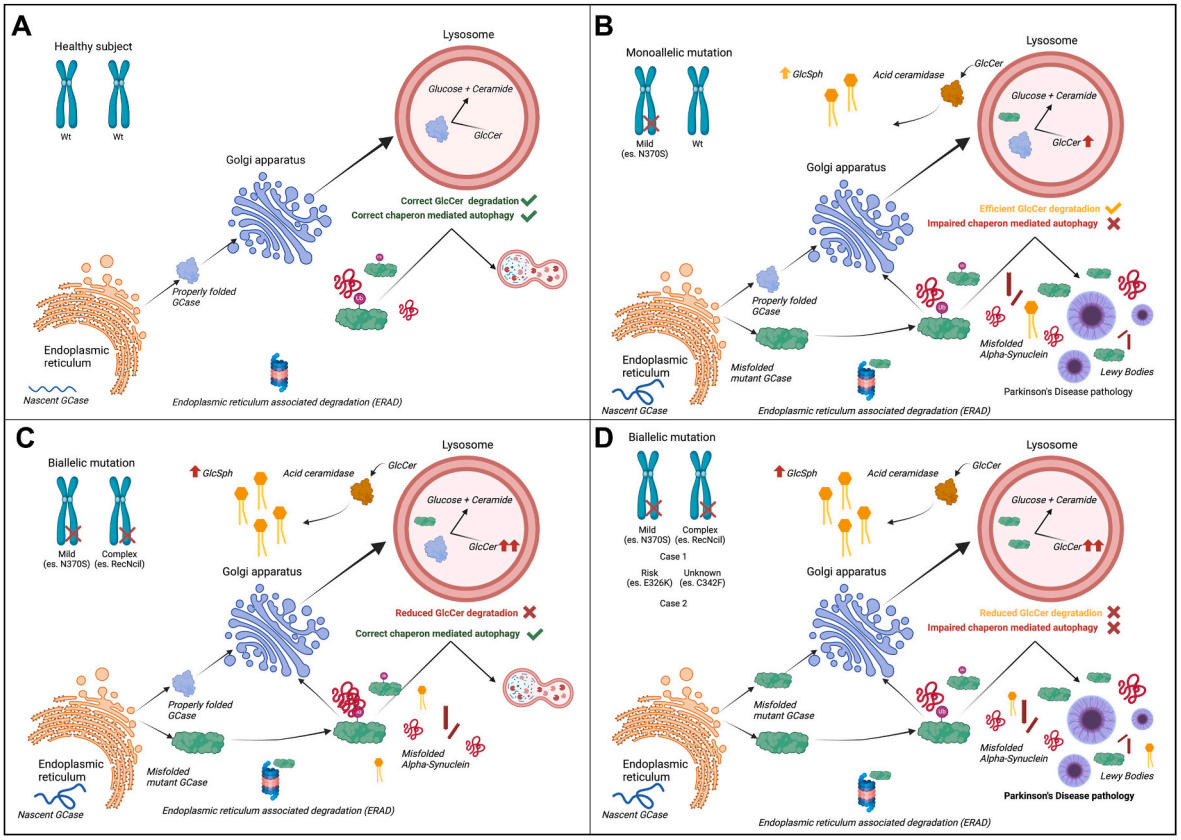

Fig. 3. Possible pathologic scenarios in GBA1-PD and GD-PD. A) healthy subjects; C) carriers of heterozygous *GBA1* mutations with PD; C) patients with biallelic *GBA1* mutations and GD without PD; C) patients with biallelic *GBA1* mutations with PD. This figure has been generated on biorender (www.biorender.com).

Despite the ongoing debate regarding the role of *GBA1* E326K mutations in GD [30,31], in vitro studies indicate that E326K leads to enzymatic activity depletion comparable in magnitude to that of N370S, supporting its possible role as a pathogenic for GD [30]. Furthermore, carriers of a T369M allele, with or without PD, exhibited lower REA than subjects without *GBA1* mutations. Notably, in the same study, the activity of *GBA1*-T369M PD carriers was even lower than that observed in patients carrying an E326K mutation [32].

The combination of alleles observed in these patients resulted in very mild substrate accumulation (6.7–11.5 ng/ml) compared to "classic" GD1 (14.6–720) (Table 1), manifesting with subtle visceral, skeletal, and even hematological manifestations of GD.

This restricted cohort of subjects may gain epidemiologic importance within the Western European PD community, given the higher prevalence of risk alleles compared to any other PD population (e.g. Asian, AJ) [8]. In our study, E326K, T369M, and E388K represented 15 %, 11 %, and 2 % of our GBA1-PD population, respectively - contributing to almost one third (28 %) of cases. This is a new scenario where neurologists must collaborate closely with GD experts to ascertain whether PD and even atypical parkinsonism have an underlying GD that necessitates appropriate treatment. This collaboration can facilitate comprehensive evaluation and management, ensuring optimal care for affected individuals.

The retrospective cross-sectional nature of our study prevents us from drawing any conclusions about the role of GCase and GlcSph in PD progression and in the phenoconversion of at-risk carriers (e.g., control group). While an additional study on the clinical profiling of our cohort is ongoing, analysis on blood samples from GD biobanks would possibly shed the light on the role of GCase and GlcSph in prodromal patients (e.g., GD patients without PD who will eventually develop the disease in time versus who will not) [33].

The impact of possible other modifiers that may have influenced proper GCase function in the lysosome (e.g., *PSAP*, *SCARB2*) and lysosomal activity in PD patients (e.g., *LRRK2*, LSD genes) deserve to be explored in further studies [34,35].

In conclusion, the present study underscores the potential for integrating robust biomarkers and therapeutic targets from GD, such as GlcSph, into PD research. This opportunity merits further validation through rigorous approaches. Additionally, we emphasize the possibility of identifying mild GD cases within our PD population, which should not be overlooked.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data statement

Data will be made available upon request to the corresponding author.

Ethics approval

The present study has been approved by the Ethical Committee of the Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Italy (RetroGBA, 2023.067).

CRediT authorship contribution statement

Massimo Marano: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Carmela Zizzo:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data

curation, Conceptualization. **Maria Chiara Malaguti:** Investigation. **Ruggero Bacchin:** Investigation. **Francesco Cavallieri:** Investigation. **Rosa De Micco:** Investigation. **Francesca Spagnolo:** Writing – review & editing, Investigation. **Anna Rita Bentivoglio:** Investigation. **Tommaso Schirinzi:** Investigation. **Roberta Bovenzi:** Investigation. **Silvia Ramat:** Investigation. **Roberto Erro:** Writing – review & editing, Investigation. **Cristiano Sorrentino:** Investigation. **Patrizia Sucapane:** Investigation. **Andrea Pilotto:** Writing – review & editing, Investigation. **Alessandro Lupini:** Investigation. **Alessandro Magliozzi:** Investigation. **Ilaria Di Vico:** Investigation. **Miryam Carecchio:** Writing – review & editing, Investigation. **Giulia Bonato:** Investigation. **Roberto Cilia:** Investigation. **Fabiana Colucci:** Investigation. **Filippo Tamma:** Investigation. **Elena Caputo:** Investigation. **Giovanni Mostile:** Investigation. **Gennarina Arabia:** Investigation. **Nicola Modugno:** Investigation. **Maurizio Zibetti:** Investigation. **Maria Gabriella Ceravolo:** Investigation. **Nicola Tambasco:** Investigation. **Giovanni Cossu:** Investigation. **Franco Valzania:** Investigation. **Paolo Manganotti:** Investigation. **Vincenzo Di Lazzaro:** Investigation. **Mario Zappia:** Investigation. **Giovanni Fabbrini:** Investigation. **Michele Tinazzi:** Investigation. **Alessandro Tessitore:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Giovanni Duro:** Writing – review & editing, Investigation. **Alessio Di Fonzo:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2024.107023.

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