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Beyond Parkinson's disease: expanding the neurodegenerative spectrum of GBA1- associated disorders

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Abstract

Heterozygous variants in the *GBA1* gene represent the most significant genetic risk factor for Parkinson's disease (PD). Among a larger cohort of 110 individuals with atypical parkinsonism and multisystem phenotypes, we identified nine patients carrying monoallelic pathogenic *GBA1* variants. Clinical and biochemical phenotyping revealed marked heterogeneity, and a high prevalence of non-motor comorbidities.

Exome sequencing of the entire cohort highlighted substantial *GBA1* allelic complexity, with four patients carrying multiple variants *in cis*. Additional likely pathogenic variants were identified in five patients. These variants were heterogeneously distributed, with each individual carrying unique mutations in one or two genes associated with neurodegeneration or epilepsy. These findings suggest that a synergistic genetic architecture may drive these complex phenotypes.

Our results expand the *GBA1*-related phenotypic spectrum beyond classical PD and underscore the necessity of recognizing genetic complexity to improve diagnostic accuracy, disease stratification, and the development of targeted therapies in neurodegenerative conditions.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting over 6 million individuals worldwide[1]. While traditionally considered sporadic, a growing body of evidence has revealed a major contribution of genetic factors to PD pathogenesis. Among these, variants in the *GBA1* gene, encoding the lysosomal enzyme β-glucocerebrosidase (GCase), have emerged as the most frequent genetic risk factor for PD, present in approximately 5–15% of patients depending on population ancestry[2].

The association between *GBA1* variants and PD was first recognized through the observation of Parkinsonian features in patients with Gaucher disease, caused by biallelic *GBA1* pathogenetic variants[3]. The multicenter study by Sidransky et al. (2009) showed that heterozygous *GBA1* variants confer a 5–10-fold increased risk of PD, often associated with earlier onset and more severe disease progression [4].

In a large Italian cohort of 874 PD patients, *GBA1* mutations were identified in 14.3%, with carriers of complex variants showing the lowest enzymatic activity, and carriers of severe or complex variants had the highest risk of hallucinations and dementia[4]. These findings are consistent with earlier reports from Mediterranean populations supporting a variant-severity gradient whereby severe *GBA1* variants (e.g., L444P, or p.Leu483Pro as new nomenclature) are associated with earlier onset, faster progression, and increased cognitive decline compared to mild variants (e.g., N370S, or p.Asn409Ser, new nomenclature) [5].

At the pathophysiological level, *GBA1*-associated PD reflects a convergence between lysosomal dysfunction and α-synuclein pathology. Reduced GCase activity leads to accumulation of glucosylceramide and related sphingolipids, disrupting lysosomal homeostasis[6]. This impairs autophagy-mediated clearance of α-synuclein, promoting its aggregation into Lewy bodies, a pathological hallmark of PD. Moreover, α-synuclein accumulation can in turn inhibit GCase activity, establishing a self-reinforcing pathogenic loop that exacerbates proteostatic stress and neurodegeneration[7,8].

Beyond PD, *GBA1* variants confer susceptibility to a broader neurodegenerative spectrum, including dementia with Lewy bodies (DLB), frontotemporal dementia (FTD), and multiple system atrophy[9,10]. The clinical expression among carriers is highly variable, likely modulated by additional genetic and environmental factors[11]. Recent genome-wide and longitudinal studies further confirm that the variant-severity gradient influences not only age at onset but also the rate of cognitive decline, with severe-variant carriers facing a substantially higher risk of dementia than patients with idiopathic PD[12].

The advent of next-generation sequencing (NGS) has expanded diagnostic potential, enabling the detection of rare or private variants across lysosomal, mitochondrial, and synaptic genes[13]. Building on our prior experience with lysosomal storage disorders[14], we focused our study on patients exhibiting phenotypes bridging common neurodegenerative disorders and lysosomal dysfunction. Within this cohort of 110 patients, we identified nine individuals carrying distinct *GBA1* pathogenic variants, each associated with a severe neurodegenerative phenotype. In addition, data from exome analyses suggested that additional rare variants in neurodegeneration-related genes may act as genetic modifiers.

RESULTS

GBA1 molecular analyses

We characterised nine patients with pathogenic variants in the *GBA1* gene in a cohort of 110 unrelated patients presenting with unclassified neurodegenerative phenotypes[14].

The most frequent variants were **c.1448T>C, p.Leu483Pro** (formerly L444P), and **c.882T>G, p.His294Gln** (formerly H255Q), detected in three individuals, respectively. Additional alleles included *p.Asn227Ser*, *p.Thr408Met*, and *p.Asp448His*, previously reported as N188S, T369M, and D409H (Table 1).

Pts 3, 4, 6, and 8 carried two *GBA1* variants (Table 1, 2). Although parental analyses were not possible, we are confident that the two pathogenic variants identified in these patients are *in cis*, as indicated by GCase enzyme activity that, although reduced in some cases, remained above the range typical of Gaucher disease (Table 2). Three of the *GBA1* variants detected *in cis* are new (c.1389-5T>A and c.1506-10_*7dup), or previously reported as a variant of uncertain significance: p.(Pro12Ser) as a possible genetic modifier in PD[15]. Predictive algorithms suggest that this amino acid variant has a likely benign

effect on the resulting protein. However, the first 39 amino acids of GCase constitute a critical leader sequence required for its translocation to the endoplasmic reticulum (ER)[16]. Deletion of this N-terminal signal peptide reduces ER localization, underscoring its essential role in the enzyme's intracellular trafficking[17]. Specifically, the p.(Pro12Ser) substitution likely decreases the hydrophobicity of the signal peptide core, potentially impairing the efficient ER targeting of GCase (Figure 2).

In Pt4, the second pathogenic variant identified, c.1389-5T>A, is an intronic variant that is likely to affect the consensus acceptor splice sites, with an estimated probability of approximately 98.5% (gathered using Alamut Visual plus integrated predictions) (Figure 3).

Interestingly, we identified a new duplication in the *GBA1* gene of Pt8. This rearrangement includes the exon 12 of the gene. The duplication was identified in the Copy Number Variation (CNV) report of the NGS analysis and validated by TaqMan assay as shown in Figure 1. This rearrangement was not further investigated in order to identify the breakpoint regions, but it has not been previously reported in the literature, as confirmed by HGMD professional and Clin Var databases.

Exome findings

Exome sequencing revealed additional rare and likely pathogenic variants in five of the nine patients included in this study, involving genes previously implicated in neurodegenerative disorders, epilepsy, and lysosomal dysfunction (Table 3).

In particular, Pt2, who presented with indeterminate tremor and vascular leukoencephalopathy, carried a heterozygous likely pathogenic variant in *CHMP2B* (c.85A>G; p.Ile29Val), a gene associated with FTD, amyotrophic lateral sclerosis (ALS), and DLB[18–20]. Similarly, Pt4 harbored, in addition to the *GBA1* variants p.Leu483Pro and c.1389-5T>A, a heterozygous likely pathogenic variant in *SCN1A* (c.379C>G; p.His127Asp), a gene known to play a key role in neuronal excitability and in epilepsy syndromes[21,22].

A heterozygous pathogenic variant in *SMPD1* (c.340G>A; p.Val114Met) was identified in Pt5, who exhibited clinical features suggestive of DLB. This gene encodes acid sphingomyelinase, a lysosomal enzyme involved in Niemann–Pick disease and associated with an increased risk of Parkinson's disease[23,24].

A truncating heterozygous variant in *TOR1A* (c.862C>T; p.Arg288Ter), previously linked to neurodevelopmental disorders[25,26], was identified in Pt6, who was characterized by Parkinsonism, cognitive decline, and vascular leukoencephalopathy.

Finally, Pt9 harbored two additional heterozygous variants in genes associated with epilepsy and Parkinsonism: *TBC1D24* (c.116C>T; p.Ala39Val)[27,28] and *PINK1* (c.709A>G; p.Met237Val)[29] , both classified as likely pathogenic.

Genotype- Phenotype Correlations

In our cohort, subjects carrying the p.Leu483Pro variant (Pts 1, 4, and 8), classified as a severe GCase variant[30], showed a relatively homogeneous phenotype characterized by bradykinesia and rigidity in all cases, frequent axial symptoms, and a high burden of cognitive impairment, which was severe in two subjects (Pts 1 and 4). In Pt 4, the clinical picture was further complicated by the presence of a likely pathogenic heterozygous variant in *SCN1A*, a gene implicated in modulating neuronal excitability and associated with epilepsy syndromes[22]. Non-motor symptoms, including psychiatric manifestations (3/ 3) and sleep disturbances (2/ 3), were also observed. Pts 1 and 4 have a very similar PD phenotype, with early onset, and rapid disease progression. Both underwent deep-brain stimulation of the subthalamic nuclei. After a few years, they both developed progressive cognitive impairment, which wasn't present before surgery, thus supporting the hypothesis that *GBA1* variants may affect postoperative cognitive trajectories[31–33]. Overall, this subgroup displayed a PD phenotype with early and prevalent non-motor or systemic involvement (Table 2- 3).

Carriers of the p.His294Gln (Pts 2, 6, and 9) showed a diverse range of motor and non-motor symptoms. These included autonomic dysfunction and vascular comorbidities, ranging from orthostatic hypotension (Pt6) to vascular leukoencephalopathy (Pts 2 and 9). Interestingly, although the clinical presentation of Pts 2 and 9 was severe, this did not lead to a diagnosis of PD, as the onset was atypical and typical Parkinsonian symptoms were absent. Pt6 underwent deep brain stimulation of subthalamic nuclei, and several years after surgery he developed dementia. The phenotypic complexity in these patients appears to be influenced by a significant multi-genic burden identified through exome sequencing: Pt2 carries a likely pathogenic variant in *CHMP2B*, Pt6 in *TOR1A*, and Pt9 in *TBC1D24* and *PINK1* (see Table 3).

Pts 3 and 5, who carry the p.Asn227Ser variant in the GCase, exhibited a phenotype characterized by non-motor cognitive dysfunction, rather than the classic Parkinsonian motor features. One case suggested atypical Parkinsonism, resembling progressive supranuclear palsy (Pt3), while the other presented features compatible with DLB (Pt5). In Pt5, the identification of a concomitant heterozygous variant in *SMPD1* (p.Val114Met) establishes a double hit in the lysosomal pathway (Table 2- 3).

Pt7, who carries the p.Thr408Met variant in the GCase, presented a complex phenotype with very early- onset symptoms, multisystem involvement including marfanoid phenotype, palpebral ptosis, and the absence of typical Parkinsonian features. As previously underscored for Pt 2 and 9, also Pt7 was initially classified as having a complex neurodegenerative disorder without PD or Parkinsonism (Table 2).

Notably, four subjects (Pts 3, 4, 6, and 8) carried two *GBA1* variants on the same allele (*in cis*). They exhibited complex phenotypes, including severe cognitive impairment (Pts 3, 4, and 6) and, in Pt3, atypical neurodegenerative features, i.e., cerebral palsy and diffused neuronal hyperexcitability.

Autoimmune symptoms (Pts 3 and 4) and cerebrovascular abnormalities (Pts 6 and 8) complicated the phenotypes of this subgroup of patients (see Table 2).

DISCUSSION

The phenotypic spectrum associated with monoallelic *GBA1* variants is increasingly recognized as extending beyond classical Parkinson's disease[2,34].

In our study, patients carrying pathogenic *GBA1* variants exhibited heterogeneous neurological presentations characterized by prominent cognitive, psychiatric, autonomic, and multisystem manifestations, often occurring in the absence of isolated Parkinsonian features. This clinical variability is consistent with growing evidence that *GBA1*-associated disorders are characterized by earlier onset, faster progression, and a greater burden of cognitive and neuropsychiatric manifestations compared with idiopathic PD[34,35]. In addition, when stratified according to mutation severity, distinct phenotypic patterns emerged. Patients with the p.Leu483Pro GCase variant showed non-classical PD with an early onset and a more severe neurological phenotype. These findings are consistent with previous studies showing that severe *GBA1* mutations are associated with earlier disease onset, an increased risk of dementia, and more pronounced non-motor involvement[4,11].

Systemic comorbidities were consistently observed across the cohort and range from autonomic and sleep dysfunction, immune and inflammatory dysregulation, cerebrovascular burden, gastrointestinal features, marfanoid traits, and myopia. The most common *GBA1* mutations typically associated with PD, specifically p.(Glu365Lys) and p.(Asn409Ser) (formerly E326K and N370S), which account for approximately 80% of PD cases carrying a *GBA1* pathogenic variant [36,37], were notably absent in our cohort. This absence suggests that distinct genetic patterns may drive the observed phenotypic complexity and multisystemic involvement in these patients.

Interestingly, four of nine patients carried multiple *GBA1* variants *in cis*. Although complex *GBA1* alleles have previously been described in GD and PD cohorts[4,38,39], their contribution to phenotypic variability remains incompletely understood. In our series, all patients carrying complex alleles exhibited severe cognitive impairment, atypical neurodegenerative manifestations, or marked multisystem involvement. Existing biochemical evidence supports a dose-dependent effect of GCase dysfunction on lysosomal homeostasis and proteostasis[6,17,40]. Variant-specific alterations in protein folding, ER retention, and lysosomal trafficking may cumulatively reduce effective GCase activity, even when variants are located on the same allele. In this context, the identification of two previously unreported variants, including the c.1389-5T>A intronic change and the c.1506-10_*7dup rearrangement, further suggests that complex *GBA1* alleles may be under-recognized contributors to phenotypic heterogeneity.

A further relevant finding is the identification of a previously undescribed *GBA1* rearrangement involving exon 12. Structural variants affecting *GBA1* remain relatively underreported, partly because the locus is complicated by the presence of the highly homologous *GBAP1* pseudogene, which can interfere with sequencing, alignment, and variant interpretation[2,34]. This rearrangement may contribute to the allelic complexity observed in Pt 8 and further expand the mutational spectrum associated with *GBA1*-related neurodegeneration. The independent validation of the duplication by TaqMan copy number analysis strengthens the reliability of this finding and highlights the importance of incorporating CNV detection strategies into routine genetic investigations of neurodegenerative disorders.

An additional relevant finding of our study is the identification of rare, likely pathogenic variants in other neurodegeneration-related genes in five of nine patients. These include variants in *CHMP2B, SCN1A, SMPD1, TOR1A, TBC1D24*, and *PINK1*, all genes previously implicated in dementia, epilepsy, lysosomal storage disorders, or monogenic forms of Parkinsonism[18,21,24,25,27,29]. The coexistence of *GBA1* variants with additional rare variants supports the hypothesis of an oligogenic architecture underlying complex neurodegenerative phenotypes[41,42]. Rather than acting in isolation, *GBA1* pathogenic variants may lower the threshold for neurodegeneration. At the same time, additional hits in genes involved in lysosomal function (*SMPD1*), mitochondrial quality control (*PINK1*), membrane trafficking (*CHMP2B, TOR1A*), or neuronal excitability (*SCN1A, TBC1D24*) may modulate disease onset, progression, and cognitive involvement[10,43].

In particular, the convergence between *GBA1* and *SMPD1* variants in lysosomal pathways (as seen in Pt5) and between *GBA1* and *PINK1* in mitochondrial-lysosomal crosstalk (as seen in Pt9) reinforces the concept of interconnected cellular stress mechanisms contributing to neurodegeneration[13,24,29]. Similarly, variants in *CHMP2B* (Pt2) and *TOR1A* (Pt6), both involved in endosomal and membrane trafficking pathways, may exacerbate proteostatic imbalance[20,26]. Rather than representing independent disease mechanisms, these variants may collectively increase cellular vulnerability by disrupting interconnected components of the autophagy–lysosomal network[10,13,42].

Taken together, our findings suggest that the enrichment of rare variants affecting interconnected neurodegenerative pathways may contribute to the phenotypic complexity observed in this cohort. The predominance of cognitive, psychiatric, autonomic, and multisystem manifestations in our cohort further supports the emerging view that *GBA1*-related disorders should not be considered solely as a subtype of PD.

Importantly, these patients were identified through a stepwise selection process within a substantially larger neurological population, ultimately representing a small subgroup of individuals with atypical parkinsonian and multisystem presentations. Their identification highlights the potential under-recognition of *GBA1*-associated disorders outside the classical PD spectrum. *GBA1* dysfunction may define a broader neurodegenerative syndrome characterized by variable combinations of synucleinopathy, cognitive impairment, lysosomal dysfunction, and systemic involvement[2,34,35]. Genome-wide association studies, which enable the prioritization of risk loci for PD, already represent a crucial tool for advancing precision medicine and optimizing

personalized therapeutic strategies[44,45]. In addition, established monogenic genes such as *GBA1*, *LRRK2*, and *SNCA* are already the focus of ongoing clinical trials[46].

The combination of severe *GBA1* variants, complex *cis* configurations, novel variants, and additional rare mutations affecting neurodegeneration-associated genes supports a continuum model in which the broader genetic background influences both disease susceptibility and phenotypic expression[11,47]. Future studies integrating genome sequencing, transcriptomic analyses, and functional validation approaches will be essential to clarify the contribution of these genetic interactions and to identify patients most likely to benefit from emerging GCase-targeted therapies and precision medicine strategies.

METHODS AND MATERIALS

Criteria for patients' inclusion

The study included patients of Italian origin. Patients were included if they had at least one major neurological symptom including ataxia, cognitive impairment, parkinsonism with the following red flags: early falls, rapidly progressive dementia, and early perceptual disturbances, dysarthria, epilepsy, myoclonus, tremor, neuropathy, and behavioral problems such as hyperactivity, and two or more minor non-neurological manifestations such as coarse facies, cerebrovascular dysfunctions, hearing and visual loss, organomegaly, multiplex dysostosis, cardiomyopathy, and immunological symptoms. Exclusion criteria were brain injury or tumors, stroke, post-infectious or prenatal brain abnormalities, and known genetic conditions.

Sequencing and *in silico* analysis

We isolated DNA from peripheral blood leukocytes using a QiaSymphony SP robot (Qiagen, Hilden, Germany) according to the manufacturer's protocol. We performed exome analyses of all patients by next-generation sequencing (NGS) using a custom-designed panel (Illumina, San Diego, CA). Libraries were prepared using a Paired-End 150 bp protocol and sequenced on the NextSeq 500 platform (Illumina), according to the manufacturer's instructions. We annotated variants both with the BaseSpace Variant Interpreter platform of Illumina (https://platform.login.illumina.com/) and by the Franklin by Genoox platform (https://franklin.genoox.com/clinical-db/home).

The CoNVaDING tool (Copy Number Variation Detection In Next-generation sequencing Gene panels by Johansson et al. 2016) was used to detect gross rearrangements. The GBA1 nomenclature used predominantly throughout the text, figures, and tables refers to NM_001005741.3, as reported in the HGMD Professional database (https://my.qiagendigitalinsights.com/bbp/view/hgmd/pro/start.php). For clarity purposes, the legacy nomenclature (e.g., L444P and N370S together with the current p.Leu483Pro and p.Asn409Ser) is also cited in conjunction with the updated terms in some cases. As segregation analysis within trios was not feasible, we selected only known disease-causing mutations previously reported in the HGMD Professional database and classified likely pathogenic or pathogenic according to ACMG guidelines[48]. Additionally, based on the observed clinical phenotypes, we specifically reported variants in genes associated with Parkinsonism and neurodegeneration, referencing the relevant HPO terms (i.e., HP:0002511; HP:0002548, HP:0002145, and HP:0002180).

Alamut Visual Plus (version 1.7; including SpliceSiteFinder-like, MaxEntScan, NNSPLICE, and GeneSplicer) was used to evaluate the effect of the c.1389-5T>A *GBA1* variant.

Bioinformatic Sweeties (https://bioinformaticsweeties.biocomp.unibo.it/) [49] and Alamut visual Plus were used to evaluate the impact of the p.(Pro12Ser) variant of unknown significance according to ACMG guidelines.

TaqMan assay

GBA1 assays to quantify exon 12 in Pt8 were performed combining TaqMan® MGB probe chemistry with Real Time PCR instruments (Applied Biosystems® 7500 Real-Time PCR) (Life Technologies Italia, Monza, Italy).

To specifically validate the duplication of exon 12, a custom Human TaqMan® probe (FAM dye-labeled) was designed specifically for *GBA1* exon 12 (target) sequence (6-FAM-TCTCACCTGGCT ACTC) paired with forward and reverse primers 5'- TGTGGGCTTCCTGGAGACA-3' and 5'-ACGCCACAGGTAGGTGTGAAT-3', respectively. A Human TaqMan® probe specific for *GBA1* intron 4 was used as an internal control (Hs00116386_cn, Catalog #4400291).

Assays were run in triplicate and normalized against the human RNase P TaqMan® probe (VIC dye-labeled, cat. 4403326) as an endogenous reference gene. Normalisations were performed by relative quantifications, determined by the ΔΔCt [(FAM Ct- VIC Ct) sample - (FAM Ct – VIC Ct) calibrator] method as previously reported[50] (figure 1).

Enzyme assays by spectrofluorometric analysis

Control and patient samples were anonymized and used only for research purposes. Isolated leukocytes were disrupted by sonication, and a Pierce™ BCA Protein Assay Kits (#A55864, Thermo Fisher Scientific) was used to determine the total protein amount for the enzymatic assay, according to the manufacturer's instructions. GCase activity in cell lysates of leukocytes was measured by using a previously described fluorigenic method[51]. Plasma chitotriosidase was also measured as described[52] using commercially available fluorogenic substrates (Koch-Light Laboratories, Ltd., Colnbrook, UK).

Declarations

Ethics declarations

We conducted this study in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. It was also approved by the ethics board review of the Tuscany Region, “Bando Ricerca Salute 2018” for the study "LYSOLATE" (Ref CEAVC 16774, approved on May 5th, 2020, Comitato Etico Regionale per la Sperimentazione Clinica della Regione Toscana, Area Vasta Centro, Florence, Italy). All study participants were adults (over 18 years of age) at the time of enrolment. According to ethical guidelines, all blood samples for storage and analysis were obtained only after legally authorized representatives gave written informed consent. The patients' informed consent contained permission to share scientific results.

DATA AVAILABILITY

The datasets generated and analyzed during the current study are not publicly available due to ethical and privacy restrictions related to due to privacy or ethical restrictions but are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

F.F: investigation, methodology, writing – review and editing, project administration, data curation. S.R.: supervision, visualization, writing – review and editing, resources, funding acquisition. L.T.: data curation, visualization, writing – review and editing, resources, funding acquisition. A.G.: investigation, writing – review and editing, validation, project administration. L.C.: methodology, formal analysis, data curation. G.G.: methodology, formal analysis, visualization, project administration. D.M.: methodology, software, formal analysis, data curation. S.F.: investigation, methodology, data curation, visualization. F.M.: investigation, methodology, data curation, validation, software. A.P.: investigation, visualization, supervision, project administration. A.M.: funding acquisition, writing – review and editing, project administration, supervision, data curation, resources. A.C.: conceptualization, investigation, writing – review and editing, supervision, writing – original draft.

COMPETING INTERESTS

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Tables

Table 1. Classification of the *GBA1* variants identified in our cohort.

***GBA1* variants**[a]	**Gaucher disease**		**PD**	
	Type	**First report**	**Type**	**First Report**
c.1448T>C; p.Leu483Pro, ex L444P	2	Tsuji (1987) N Engl J Med 316, 570	Early onset	Clark (2007) Neurology 69: 1270
c.882T>G; p.His294Gln, ex H255Q	2	Stone (2000) Hum Mutat 15: 181	Early onset	Moraitou (2011) Mol Genet Metab 104: 149
c.34C>T, p.Pro12Ser, ex P28S	-	-	VUS	Davis (2016) JAMA Neurol 73: 1217
c.680A>G, p.Asn227Ser, ex N188S	3	Kim (1996) Hum Mutat 7: 214	Early onset	Choi (2012) Neurosci Lett 514: 12
c.1342G>C; p.Asp448His, ex D409H	2	Eyal (1990) Gene 96: 277	Early onset	Dos Santos (2010) Neurosci Lett 485: 121
c.1223C>T; p.Thr408Met, ex T369M	VUS	Beutler (1996) Proc Assoc Am Physicians 108: 179	Early onset	Nichols (2009) Neurology 72: 310
c.1506-10_*7dup	-	-	-	Present study
c.1389-5T>A	-	-	-	Present study

Abbreviations: PD= Parkinson's disease; a HGMD Professional 2023.4 accession no. NM_001005741.3

Table 2. Clinical, biochemical and *GBA1* molecular analysis of the cohort patients.

Pt	Age at Onset (years)	Onset	Motor Symptoms	Cognitive and Psychiatric Features	Systemic Comorbidities	*GBA1* variants, zygosity	GCase assay (n.v. 4.8-14nmoli/mg/h)	Chitotriosidase assay (n-v. 4.0-80.0 nmol/ml/h)
1	36	Bradykinesia, Rigidity	Parkinsonism < 65y, Axial symptoms, Tremor	Severe cognitive impairment, Hallucinations, mood disturbance	Sleep disturbances, dysarthria	c.1448T>C, p.Leu483Pro, het	4.2	22.7
2	64	Indeterminate tremor	Indeterminate tremor	Mood disturbance	Hypotension, frequent faints, vascular leukoencephalopathy, diabetes mellitus, allergies	c.882T>G; p.His294Gln, het	5.9	19.0
3	64	Cognitive impairment	Parkinsonism < 65y, Cerebral palsy, Diffuse neuronal hyperexcitability	Severe cognitive impairment, depression	Hyperactivity, sleep disturbances, autoimmune symptoms (thyroiditis and monoclonal gammopathy)	c.680A>G, p.Asn227Ser, het/ c.34C>T, p.Pro12Ser, het	4.6	217.4
4	43	Bradykinesia, Rigidity	Parkinsonism < 65, Axial symptoms	Severe cognitive impairment, anxiety	Sleep disturbances, hyposmia	c.1448T>C, p.Leu483Pro het; c.1389-5T>A, het	4.5	39.7
5	61	Axial tremor and cognitive impairment	Parkinsonism < 65y	DLB features, dyskinesia	Constipation hypertension, hyperlipidemia	c.680A>G, p.Asn227Ser, het	4.5	36.7
6	42	Axial tremor	Parkinsonism < 65y	Cognitive decline, Behavioral disturbances	Orthostatic hypotension, anemia, vascular leukoencephalopathy	c.882T>G, p.His294Gln het/ c.1342G>C, p.Asp448His, het	2.8	32.3
7	8	Indeterminate tremors	Proximal myopathy	Developmental delay	Mild dysphagia, marfanoid phenotype, ptosis, immunological abnormalities, severe myopia	c.1223C>T, p.Thr408Met, het	5	12.5
8	64	Bradykinesia and depression	Parkinsonism < 65y, Axial symptoms	DLB features, Cognitive impairment, major depression	Severe sleep disturbances, urinary incontinence, gastro enteric symptoms	c.1448T>C, p.Leu483Pro, het/ c.1506-10_*7dup, het	2.9	50
9	60	Indeterminate tremor	Indeterminate tremor, Axial symptoms, Ataxia	Dementia, Bipolar disturbance	Sleep disturbance, epilepsy, Vascular leukoencephalopathy, Hyposmia	c.882T>G, p.(His294Gln), het	6.4	64.5

Table 3. Pathogenic variants identified in patients' cohort in addition to *GBA1* variants.

Pt	Gene	OMIM	Associated conditions/ inheritance (OMIM database)*	Amino acid and nucleotide change/ Zygosity	HGMD database#	ACMG classification ^	Reference of variant
2	*CHMP2B*	NM_014043.4	Frontotemporal dementia and/or amyotrophic lateral sclerosis 7/ AD	p.(Ile29Val), c.85A>G/ het	Path	Likely path	**Keogh (2016); Grassano (2022); Bartoletti-Stella (2018)**
4	*SCN1A*	NM_001165963.4	Developmental and epileptic encephalopathy 6B, non-Dravet/ AD; Dravet syndrome/ AD; Febrile seizures, familial, 3A/ AD Generalized epilepsy with febrile seizures plus, type 2/ AD; Migraine, familial hemiplegic/ AD	p.(His127Asp), c.379C>G/ het	Path	Likely path	**Zuberi (2011)**
5	*SMPD1*	NM_000543.5	Niemann-Pick disease, type A and B/ AR	p.(Val114Met), c.340G>A/ het	Path	Likely path	**Gucev (2013)**
6	*TOR1A*	NM_000113.3	Arthrogryposis multiplex congenita 5/ AR; Dystonia-1, torsion/ AD	p.(Arg288Ter), c.862C>T/ het	Path	Path	**Tran Mau-Them (2020); Saffari (2023)**
9	*TBC1D24*	NM_001199107.2	Deafness, autosomal dominant 65/ AD; Deafness, autosomal recessive 86/ AR; Developmental and epileptic encephalopathy 16/ AR; DOORS syndrome/ AR; Epilepsy, rolandic, with paroxysmal exercise-induce dystonia and writer's cramp/ AR; Myoclonic epilepsy, infantile, familial/ AR	p.(Ala39Val), c.116C>T/ het	Path	Likely path	**Ragona (2017); Zhao (2022)**
	PINK1	NM_032409.3	Parkinson disease 6, early onset/ AR	p.(Met237Val), c.709A>G/ het	Path	Likely path	**Sim (2012)**

Path= Pathogenic; Pt= Patient; het= heterozygous state; AD= Autosomal dominant inheritance; AR= autosomal recessive inheritance.
*https://www.ncbi.nlm.nih.gov/omim; # https://my.qiagendigitalinsights.com/bbp/view/hgmd/pro/start.php; ^ https://wintervar.wglab.org/.

Figures

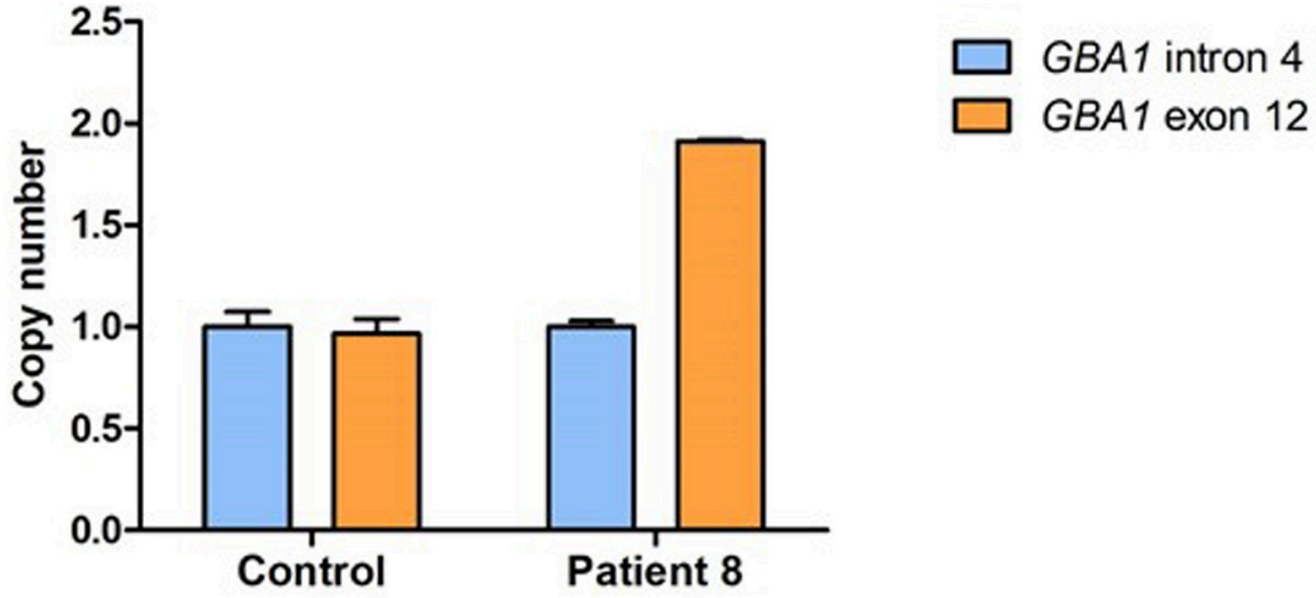


Figure 1

Identification of the novel GBA1 c.1506-10_*7dup variant. TaqMan copy number assays targeting exon 12 and intron 4 were performed to assess the newly identified GBA1 (NM_001005741.3): c.1506-10_*7dup variant. Pt8 showed an increased copy number of exon 12 compared to control sample, consistent with a duplication, whereas intron 4, used as an internal reference region, displayed a normal copy number comparable to control. bars represent the mean calculated copy number relative to control sample, and error bars indicate the standard deviation of three replicates. Data were plotted using GraphPad Prism (version 5.02, GraphPad Software, , San Diego, CA, USA).

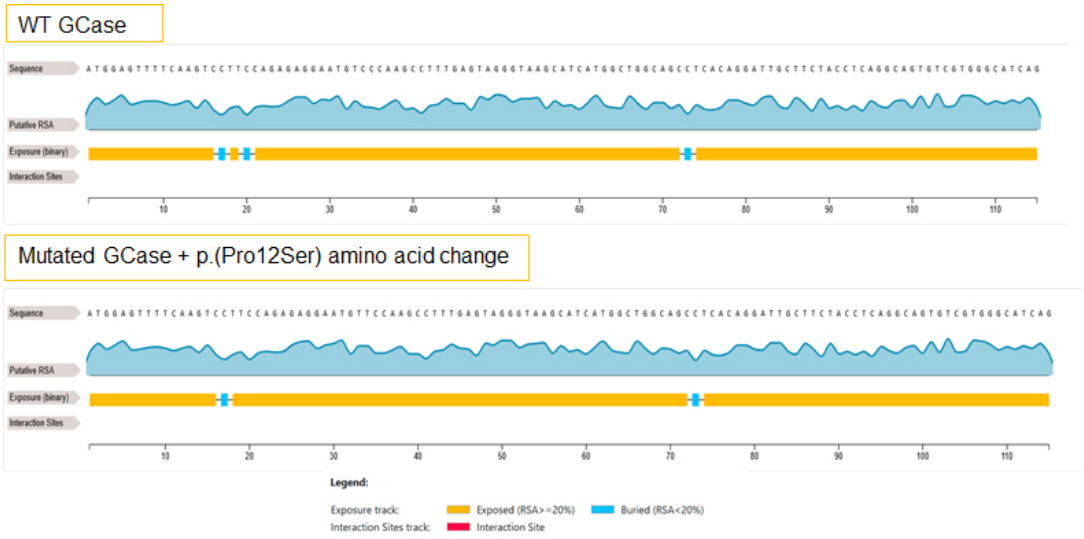


Figure 2

Prediction of residue Relative Solvent Accessibility (RSA) in the GBA1 signal peptide. The wild-type (WT) and mutated exon 1 *GBA1* sequences (first 115 bp), including the p.(Pro12Ser) substitution, were used as inputs in the E-pRSA tool[53] to evaluate and compare changes in residue RSA. Figure adapted from E-pRSA web server outputs (Bioinformatics Sweeties, https://bioinformaticsweeties.biocomp.unibo.it/).

Alamut Visual Plus Splicing Predictions

Gene: **GBA1** - Transcript: **NM_001005741.3** - Variant: **c.1389-5T>A**
Analysis range: **c.1388+125 (Intron 10) - c.1530 (Exon 12) [481 bps]**

Acceptor Sites

	SSF [0-100]	MaxEnt [0-16]	NNSPLICE [0-1]	GeneSplicer [0-21]	SPiP [0-1]
Threshold	*≥ 70*	*≥ 0*	*≥ 0.4*	*≥ 0*	*No threshold*
Intron 10 - c.1388+150	= 75.01				
Intron 10 - c.1389-57		= 2.25			
Exon 11 - c.1389 N	76.75 ⇒ 70.25 (-8.5%)	7.66 ⇒ 5.55 (-27.6%)		7.70 ⇒ 5.00 (-35.1%)	

Figure 3

Effect of the c.1389-5T>A variant on the consensus acceptor splice site of GBA1 exon 11. *In silico* splicing prediction of the intronic variant c.1389-5T>A in the *GBA1*gene, performed via Alamut Visual Plus, was used to assess cryptic/alternative donor sites and the natural acceptor splice site of exon 11. Scores generated by the SpliceSiteFinder-like (SSF), MaxEntScan, NNSPLICE, and GeneSplicer algorithms demonstrated a significant reduction in the strength of the natural acceptor site. Figure adapted from Alamut™ Visual Plus.