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GBA1 variants and Parkinson's Disease: A dual approach combining clinical data and literature review

GBA1 varyantları ve Parkinson Hastalığı: Klinik veriler ve literatür incelemesini birleştiren çift yaklaşım

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ABSTRACT

Aim: The *GBA1 (ENSG00000177628)* gene, a key susceptibility gene in Parkinson's disease (PD) and closely linked to Gaucher disease, has garnered significant research attention over the past two decades. Mutations in *GBA1* are associated with an elevated risk of developing PD. This study aims to elucidate genetic mutations related to PD in a cohort of patients undergoing clinical exome analysis. We aimed to investigate the clinical and genetic profiles of patients with Parkinson's disease, focusing on *GBA1* gene mutations and additional neurodegenerative-related genetic alterations.

Materials and Methods: Fourteen patients with a preliminary diagnosis of Parkinson's disease underwent clinical exome analysis. The research included genetic testing to identify variants of uncertain significance, likely pathogenic mutations, and pathogenic mutations in the *GBA1* gene. Further genetic alterations were evaluated in patients with positive *GBA1* mutation results.

Results: 14 out of 177 diagnosed individuals had *GBA1* mutations. The group had 8 men and 6 women with a mean onset age of 53.7 years. Tremor, stiffness, bradykinesia, hypomimia, and postural instability predominated. Dopamine agonists were often administered for symptoms. Disorders like depression, anxiety, hypercholesterolemia, and diabetes were frequent. *GBA1* gene study found many missense variants, with the p.Asn409Ser (N370S) mutation being the most common. Four individuals also had mutations in *EIF4G1*, *APP*, and *SLC20A2*, demonstrating a complex genetic landscape affecting PD.

Conclusion: Our research highlights PD's genetic diversity, particularly *GBA1* mutations' role in disease onset and progression. Additional genetic variants may worsen PD symptoms. Further research is needed on these mutations' pathogenicity and consequences on patient care.

Keywords: Parkinson's disease, *GBA1* gene, glucocerebrosidase, clinical exome analysis, motor symptoms.

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ÖZ

Amaç: *Parkinson hastalığında (PH) önemli bir duyarlılık geni olan ve Gaucher hastalığıyla yakından ilişkili olan GBA1 (ENSG00000177628) geni, son yirmi yılda önemli bir araştırma ilgisi topladı. GBA1'daki mutasyonlar, PH geliştirme riskinin artmasıyla ilişkilidir. Bu çalışma, klinik ekzom analizi geçiren bir hasta kohortunda PH ile ilişkili genetik mutasyonları açıklamayı amaçlamaktadır. GBA1 gen mutasyonlarına ve ek nörodejeneratif ilişkili genetik değişikliklere odaklanarak Parkinson hastalığı olan hastaların klinik ve genetik profillerini araştırmak amaçlanmıştır.*

Gereç ve Yöntem: *Parkinson hastalığı ön tanısı olan on dört hasta klinik ekzom analizinden geçti. Araştırma, belirsiz öneme sahip varyantları, muhtemel patojenik mutasyonları ve GBA1 genindeki patojenik mutasyonları belirlemek için genetik test içeriyordu. Pozitif GBA1 mutasyon sonuçları olan hastalarda daha fazla genetik değişiklik değerlendirildi.*

Bulgular: *Araştırmamız PH'nin genetik çeşitliliğini, özellikle GBA1 mutasyonlarının hastalığın başlangıcında ve ilerlemesindeki rolünü vurgulamaktadır. Ek genetik varyantlar PH semptomlarını kötüleştirebilir. Bu mutasyonların patojenitesi ve hasta bakımı üzerindeki sonuçları hakkında daha fazla araştırmaya ihtiyaç vardır.*

Sonuç: *Teşhis konulan 177 kişiden 14'ünde GBA1 mutasyonu vardı. Grupta ortalama başlangıç yaşı 53,7 yıl olan 8 erkek ve 6 kadın vardı. Titreme, sertlik, bradikinezi, hipomimi ve duruş bozukluğu baskındı. Semptomlar için genellikle dopamin agonistleri uygulandı. Depresyon, anksiyete, hiperkolesterolemi ve diyabet gibi bozukluklar sıktı. GBA1 gen çalışması, p.Asn409Ser (N370S) mutasyonunun en yaygın olduğu birçok anlamsız varyant buldu. Dört kişide ayrıca EIF4G1, APP ve SLC20A2 mutasyonları vardı ve bu da PH'yi etkileyen karmaşık bir genetik manzarayı göstermektedir.*

Anahtar sözcükler: *Parkinson hastalığı, GBA1 geni, glukoserebrosidaz, klinik ekzom analizi, motor semptomlar*

INTRODUCTION

Parkinson's disease (PD) is the second-most common neurodegenerative disorder. Defining features of PD include bradykinesia with tremor, rigidity, or postural instability. On the basis of the pathology, dopaminergic neuron loss in the substantia nigra (SN), alpha synuclein accumulation, and Lewy body formation are evident (1). Although the majority of cases occur at random, about 15% of individuals carry a gene that causes the condition (OMIM; 168600). Familial and early-onset types are more likely to be genetic. Disease incidence ranges from 8 to 18 cases per 100,000 people-years. Less than one percent of those over the age of 60 have PD, and the prevalence rises with age (1, 2).

There are 33 current PD phenotypes (PS168600) in OMIM: one third is inherited as an autosomal recessive form, one form is X-linked, and the remainder, which constitutes the majority, is inherited as an autosomal dominant form. Inheritance associated with the *GBA1* gene, identified as the late-onset PD susceptibility gene with PMIM number 168600, has been described as autosomal dominant.

Parkinson's disease and other comparable illnesses are often linked to glucocerebrosidase (*GBA1*) gene mutations, which encode the lysosomal enzyme defective in Gaucher disease. Rare cases of Gaucher disease and their obligatory carriers showed parkinsonism in the clinic, revealing this relationship. Findings from extensive studies indicate that individuals with PD and Lewy body-related disorders exhibit a higher prevalence of *GBA1* mutations when compared to control subjects (3).

The incidence of the *GBA1* mutation in PD varies between populations. The *GBA1* mutation has been detected in approximately 10–15% of patients, followed by a diagnosis of PD. The penetrance of *GBA1* mutations and the lifetime risk of developing PD vary with age. In one study, the frequency of *GBA1* mutation in PD patients over the age of 80 was approximately 30%, and in another study, the frequency of mutation in the *GBA1* gene in the PD population was around 3% (4-6).

The risk of PD is increased 20-fold in people with Gaucher disease. The risk of PD at age 65 is 1%, so 20% of people with Gaucher will have PD by age 65. However, 80% of people with Gaucher's

disease will not have Parkinson's disease at age 65. People with a *GBA1* mutation have a 5-8 times higher risk of PD. 5–8% of people with a *GBA1* mutation will have PD. However, 92–95% of patients will not have PD by age 65 (4, 7).

GBA1 Gene

A gene-dense area located on chromosome 1q21, measuring 7.6 kb, encompasses the *GBA1* gene, in addition to nine other genes and two pseudogenes within a 100-kb segment. Though the GGCGGG motif is absent from the *GBA1* promoter region, around 250 base pairs upstream of the ATG start site, there are potential TATA and CAAT-like boxes.

Within the intergenic space that separates the *GBA1* and MTX1 genes are two pseudogenes, *GBA1*P and MTX1P. It seems that a duplication event, about 27–40 million years ago on average, gave rise to these pseudogenes.

Other animals do not possess these pseudogenes, with the exception of humans and primates.
Intronic Alu sequences are found in *GBA1*, yet they are absent from the *GBA1*P gene sequence. These sequences represent a type of transposon that has the potential to relocate within DNA during the course of evolutionary processes. *GBA1*P exhibits a 96% sequence similarity with *GBA1*; however, the lengths of these sequences are limited to just 5 kb.

Additionally, the pseudogene is delineated from the *GBA1* gene by a deletion of 55 base pairs, which is flanked by a brief inverted repeat within exon 9. GCase, a lysosomal hydrolase composed of 497 amino acids, is the functional protein synthesized by *GBA1* through the process of transcription.

The primary role of GCase involves the hydrolysis of glucosylceramide into ceramide and glucose, while also exhibiting the capability to cleave glucosylsphingosine and possibly other β-glucosides. (Human genome assembly hg38, chromosome 1, positions 155234452 to 155241249, UCSC Genome Browser version 462)

Effects of *GBA1* Gene Mutations on Glucocerebrosidase Structure and Function

In 2018, over 300 *GBA1* gene mutations and rearrangements were discovered. These mutations and rearrangements were classified into three types: type I, type II, and type III Gaucher disease (GD). The term "severe *GBA1* mutations" refers to genetic abnormalities that, when inherited from both progenitors, result in profound signs of Gaucher disease, more precisely types II or III. Subtle genetic differences, on the other hand, lead to a manifestation of the disorder that is less severe when it is passed down in either a homozygous or compound heterozygous form, which ultimately results in Gaucher disease type I (8, 9).

Furthermore, certain variants exhibit ambiguous functions in GD while distinctly serving as risk factors for PD, with E326K being the most prominent example. The prevalent mutations associated with *GBA1*-related PD include N370S, L444P, D409H, R120W, V394L, 84insGG, IVS2+1 G→A, IVS10-4 C→T, IVS10-1 G>A, V460V, and R496H (8).

Different *GBA1* mutations affect GCase's enzymatic activity in their own unique ways. There are cases when the range of enzymatic activity seen in moderate and severe *GBA1* mutations overlaps, and there are other cases where the degree of enzymatic activity does not correlate with the severity of GD. Assessing GCase activity exclusively within the lysosomal environment could yield a more precise understanding of GCase's true biological role. Furthermore, factors related to genetics, biology, or the environment, aside from residual GCase activity, could influence the severity of the disease (7, 10).

GBA1 Gene and Potential Mechanisms of Parkinson's Disease

GBA1 causes neurodegeneration via many mechanisms. Mazzulli et al. found that glucosylceramide, a GCase substrate, may increase α-synuclein and decrease GCase activity. Regardless of whether they are carriers of the *GBA1* mutation or not, studies show that α-synuclein overexpression reduces GCase activity in cell models and animals. PD patients, on the other hand, have lower GCase levels in brain tissue, cerebrospinal fluid, and peripheral blood than controls (10).

GCase inhibitors promote α-synuclein aggregation in human iPSn neural models. Recent research suggests a neurotoxic cycle between α-synuclein and GCase, which may partly explain *GBA1* PD, although the sensitivity of particular neuron types remains unclear. Studies have not found elevated

glucosylceramide concentrations in *GBA1* heterozygotes (7, 10, 11).

Another mechanism by which *GBA1* mutations may lead to PD is through the disorder of ER-associated degradation and the cell death associated with ER stress. The buildup of α-synuclein can lead to endoplasmic reticulum stress, hinder the degradation of ER-associated degradation substrates, and disrupt the trafficking between the endoplasmic reticulum and Golgi apparatus. Certain genes associated with PD, including PARK2, are involved in the endoplasmic reticulum-associated degradation process, which corroborates this finding (12).

It is highly likely that ER stress and ERAD contribute significantly to the development of PD. In this context, the detection of ER involvement in experiments with certain mutated forms of GCase indicates that ER stress may contribute to the pathogenesis of PD in individuals carrying specific *GBA1* mutations. Mutated GCase has been observed to interact with parkin, facilitating the accumulation of GCase (12). The proposed processes are called into question by the fact that certain mutations, such as 84GG, IVS2+1, R359X, and others, increase the likelihood of developing PD yet do not produce a protein product. The ER stress noted in models exhibiting *GBA1* mutations may stem from the buildup of α-synuclein, rather than from GCase itself. *GBA1* mutations may enhance susceptibility to PD through multiple pathways, with each mechanism potentially playing a role in the progression of the disease (7, 10, 13). The precise mechanism underlying this relationship remains ambiguous. In conclusion, it is proposed that there are interactions involving α-synuclein, lysosomal dysfunction, endoplasmic reticulum stress, and ceramide metabolism.

Synucleinopathies and the *GBA1* Gene

GBA1-REM Sleep Disorder

The majority of individuals with REM Sleep Behavior Disorder (RBD) are predisposed to developing synucleinopathies, including PD, Dementia with Lewy Bodies (DLB), or Multiple System Atrophy (MSA). Numerous similarities exist between *GBA1*-associated PD and RBD-associated PD (14-16).

Patients with PD who possess *GBA1* mutations, as well as those with REM sleep behavior disorder (RBD), exhibit accelerated advancement of nonmotor symptoms, including autonomic dysfunction, cognitive deterioration, and dementia. Research consistently corroborates the association between *GBA1* and RBD (14, 17).

GBA1-Lewy Body Dementia

Research indicates that the prevalence of *GBA1* mutation carriers is elevated in individuals with DLB, and there exists an inverse correlation between *GBA1* mutations and the severity of Alzheimer's pathology. While it indicates that *GBA1* mutations may play a role in the emergence of synucleinopathies, the underlying mechanism of this association is still not well understood. Nonetheless, it is probable that lysosomal dysfunction plays a significant role, given that multiple lysosomal genes such as SCARB2, SMPD1, and MCOLN1 have been linked to DLB (14, 18, 19).

GBA1-Multiple System Atrophy

Most likely because MSA is so uncommon, the association between *GBA1* mutations and the disease is less solid. In six of the eight investigations done, no correlation was seen between *GBA1* variations and MSA. The most extensive worldwide MSA clinical investigation to date, undertaken at Columbia University with Ashkenazi Jews, identified a correlation between *GBA1* and MSA (8, 17); (20-23).

Methodology in *GBA1* Gene Mutations

In 2022, studies indicated around 500 mutations associated with Gaucher disease, with a predominant occurrence of missense variants arising from single nucleotide variants (SNVs) (24, 25). The identification of *GBA1* mutations occurs 6.9 kb upstream, exhibiting an overall homology of 96% to *GBA1*. The presence of a pseudogene, specifically *GBA1*P1, located downstream, presents a significant challenge. The percentage rises to 98% within the area extending from intron 8 to the 3'-UTR, characterized by five identical segments, each exceeding 200 base pairs. The presence of high homology facilitates non-allelic homologous recombination events between *GBA1* and *GBA1*P1, resulting in a diverse array of structural variants (SVs) (26, 27).

GBA1-MTX1 and its Pseudogenes

Gauchian, a novel *GBA1* browser for Illumina WGS data that can discover SVs and SNVs inside *GBA1*, performed differently from BWA-

GATK analysis utilizing ONT-targeted sequencing in 2022, proving its confirmatory validity (28). BWA-GATK: Burrows-Wheeler Aligner (BWA) read-mapper with Broad Institute Genome Analysis Toolkit (GATK). A revised ONT amplicon sequencing workflow can identify reciprocal recombinants, indels, and homopolymer alterations in coding exons. Illumina WGS data are now reliably analyzable, and *GBA1* analysis can make ONT-targeted sequencing cost-effective (28).

GBA1 Meta Analysis

A meta-analysis indicated that the *GBA1* variant frequency was markedly elevated in the DLB group compared to the control group, as were the frequencies of L444P (p. Leu483Pro), N370S (p. Asn409Ser), and E326K, but the variant frequency of T369M was Research indicates no significant difference between the groups; however, in patients with DLB, those with the *GBA1* variant exhibit an earlier onset age and a lower Montreal Cognitive Assessment score compared to the non-*GBA1* variant group, with no gender differences observed among *GBA1* variant-positive DLB patients (8, 19, 25).

Clinical Applications—Diagnosis, Counseling and Treatment

Despite the extensive research evidence available in recent years, *GBA1* PD has not significantly impacted patient care. Genetic testing is not now the normal procedure in PD clinical follow-up (4, 7, 29).

After the initiation of precision medicine clinical trials aimed at *GBA1* mutation carriers, patients want to ascertain their genotypes. Numerous studies are now being conducted for the treatment of *GBA1*-PD in patients (Clinicaltrials.gov).

At present, ambroxol hydrochloride, a small molecular chaperone demonstrated to augment brain GCase activity in murine and primate models, is under evaluation as a potential therapeutic option (30, 31).

Ambroxol, first discovered via a library screening of FDA-approved chemicals, is now undergoing clinical studies for PD (clinicaltrials.gov identifier: NCT02941822-completed-April-2020) and PD with dementia (clinicaltrials.gov identifier: NCT02914366-Active-August 2022).

Despite indicating a potentially favorable future, the efficacy of these medicines in alleviating symptoms has not yet been conclusively shown (32). Although *GBA1*'s limited penetrance makes it difficult to advise *GBA1* mutant carriers, the vast majority of these individuals will not develop synucleinopathy (33).

Although a definitive correlation exists between *GBA1* and PD, there are no established genetic counseling guidelines that specifically outline how to handle this matter with *GBA1* mutation carriers, particularly the relatives of those diagnosed with *GBA1* PD. Some individuals who carry *GBA1* mutations can determine their genetic status through prenatal testing or during the screening of their infants for Gaucher disease (34).

MATERIALS AND METHODS

Patient Selection

For this study, we recruited a cohort of fourteen patients who had received a preliminary diagnosis of PD. Neurologists evaluated the clinical features consistent with PD to select the patients. The Ümraniye Research and Training Hospital Ethics Committee (Approval No. 257254374) granted ethical approval for the study, and all patients provided informed consent for genetic testing.

Demographic, Clinical and Laboratory Profile of Patients

In the study, various demographic, clinical and laboratory parameters of Parkinson's patients with GBA1 gene mutation were examined, including gender, age of disease onset, presence of mutation in another gene, presence of concomitant diseases such as diabetes-cholesterol, neurological findings and symptoms (tremor, hypomimia, bradykinesia, etc.). These parameters contributed to systematic evaluation to understand their relationship with outcomes.

Genetic Testing

We extracted DNA from peripheral blood samples using standard procedures (e.g., QIAamp DNA Blood Mini Kit, Qiagen).

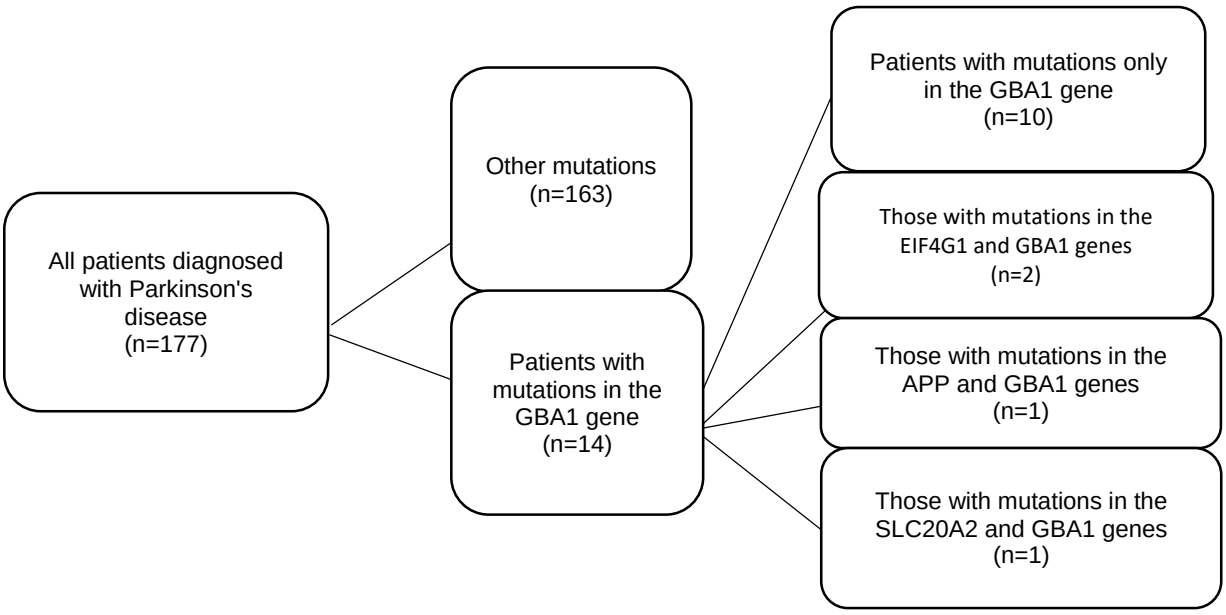

Figure-1: Categorization of Parkinson's disease patients. n: Number of patients.

We performed clinical exome sequencing (CES) to identify genetic variants associated with PD. We carried out CES using Illumina NextSeq, following the manufacturer's protocol. The Sophia DDM platform aligned the sequencing data to the human genome reference (GRCh38) for variant calling and annotation.

Next-generation sequencing was performed using the prepared DNA library and capture probes hybridized to a Celemics Target Enrichment Kit (Celemics, Korea) in a buffer to capture the retina panel of interest. After capture and washing, the captured library was amplified using post-PCR. Finally, the PCR products were sequenced using the NextSeq platform from Illumina Inc. The average depth of coverage of the panel was more than 200-fold.

Single nucleotide variations and small insertion-deletion variants were identified using the Genome Analysis Toolkit (GATK) 3.0 best practice and annotated using ANNOVAR. Variants with a variant allele fraction of less than 0.35 for heterozygous and 0.85 for homozygous were filtered. Copy number variation analysis was also performed for all target genes using the Celemics pipeline. We used several public databases including the Genome Assembly Database (gnomAD), the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project, and the Korean Reference Genome Database to identify common variants.

Variants in genes that cause disease in an autosomal dominant manner were filtered using an allele frequency filter threshold of <0.01%. The filter threshold for autosomal recessive variants was set at <0.05% in the East Asian population. In addition, variants previously reported to cause disease were included as disease-causing variants even if they had higher allele frequencies. Using the ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and HGMD (https://www.hgmd.cf.ac.uk/ac/index.php) databases, variants in genes reported to cause PD were analyzed.

Variants were excluded if there was insufficient clinical correlation with the identified genes. To classify the clinical significance of each variant, we followed the latest recommendations of the American College of Medical Genetics and Genomics (ACMG) standards for the interpretation and reporting of sequence variations: Variants were classified as pathogenic, likely pathogenic, variants of uncertain significance; benign or probably benign.

Cases with two causative variants in genes with recessive inheritance patterns, with more than one pathogenic or likely pathogenic variant, were included. Cases with pathogenic or likely pathogenic variants were included in genes with

dominant inheritance patterns. To assess whether a pair of variants in a gene occurred in cis (same copy of the gene) or in trans (different copies of the gene), we used variant co-occurrence information in the gnomAD browser (https://gnomad.broadinstitute.org/variant-cooccurrence).

GBA1 Gene Mutation Analysis

We focused particularly on identifying mutations in the *GBA1* gene, given its established role as a risk factor for PD. We analyzed both homozygous and heterozygous alterations in the *GBA1* gene. Patients carrying *GBA1* variants underwent further evaluation to assess potential additional genetic alterations. We used Sanger sequencing to confirm the presence of *GBA1* mutations identified by exome sequencing. To find out what the identified variants meant for patients, we used the Sophia program and databases like ClinVar, HGMD, and gnomAD to annotate and interpret the variants. We conducted a further assessment of potential pathogenicity using silico prediction tools such as PolyPhen-2 and SIFT.

RESULTS

As a result of the evaluation of 177 patients diagnosed with PD, 14 patients with *GBA1* mutation were examined. Alongside *GBA1* mutations, clinical exome sequencing identified mutations in other genes in 4 of the 14 patients. Two of the four patients had mutations in the EIF4G1 gene, which are believed to contribute to autosomal dominant PD; nevertheless, the gene's function in neurodegeneration linked to PD remains inadequately understood (35). Furthermore, one patient exhibited an APP gene mutation linked to early-onset autosomal dominant Alzheimer's disease, while another patient presented an SLC20A2 gene mutation responsible for familial brain calcification.

Within our group, Table-1 provides a comprehensive collection of information on the patients, including the mean age of onset (AO) of the illness, the gender ratio, Parkinson's symptoms, and other disorders. In accordance with what is shown in Table 1, the research concentrated mostly on the non-autonomic (motor) symptoms of the condition. The majority of the patients had classic Parkinson's symptoms, which include tremor (shaking), rigidity (muscle stiffness), bradykinesia (slowing down of movements), hypomimia (decreased facial expression), and postural instability (balance issues). These symptoms were noticed in the majority of patients. In order to ease the motor symptoms that the patients were experiencing, dopamine agonists were often administered. Furthermore, depression and anxiety, which are commonly noted independently of motor symptoms and have a substantial impact on the quality of life of the patients, were detected in two individuals in addition to these characteristic symptoms. During the same time period, 66.6% of the patients were found to have excessive cholesterol levels as well as diabetes. On the other hand, this high prevalence might be attributed to the short cohort size or the high average age of patients.

Table-1. Demographic data of patients with mutations detected in the *GBA1* gene.

Patient Characteristics	Percentage
Sex	8 ♂ , 6 ♀
Mean AOO + SD	53,7 ± 9,9
Tremor	33,30%
Postural Instability	16,60%
Hypomimia	50,00%
Bradykinesia	50,00%
Rigidity	33,30%
Psychiatric Symptoms	33,30%
Cholesterol	66,60%
Diabetes	66,60%
Benign Prostatic Hyperplasia	50%

(AOO: Age of onset of disease, SD: Standard deviation)

The results of the investigation and categorization of mutations on the *GBA1* gene in PD patients are shown in Table 2. It can be seen from the table that patients have a variety of mutations that are situated in different exons. The most frequent sort of mutation is known as a missense mutation, which means that it results in the production of an erroneous amino acid in the structure of the protein by the modification of a single nucleotide. Out of the fourteen patients, only one of them had a frameshift mutation. One such mutation that was found in a large number of patients was the p.Asn409Ser mutation. It is estimated that the majority of patients possess the mutation in a heterozygous (HET) form, which indicates that the mutation is only present on one chromosome rather than both. One

patient was classed as "LP" (Likely Pathological), and one patient was classified as "PAT" (Pathological). In terms of categorization, numerous mutations were rated as "VUS" (Variant of Unknown Clinical Significance), and one patient was described as "Pathological." The fact that this is the case suggests that there are varying degrees of ambiguity about the possible pathogenic implications of the mutations. Additionally, it was shown that the mutation was inherited in an autosomal dominant (OD) manner in each of the fourteen individuals who were included in the cohort.

Table-2. Analysis and Classification of Mutations in the GBA1 Gene in PD Patients

Patient Number	Coordinate	Nucleotide Change	Exon	c.DNA	Protein	Mutation Type	HET/HOM	Classification	Heredity
P1	Chr1: 155205634	T>C	Exon 10	c.1226A>G	p.(Asn409Ser)	missens	-	-	OD
P2	Chr1: 155205634	T>C	Exon 9	c.1226A>G	p.(Asn409Ser)	missens	HET	VUS	OD
P3	Chr1: 155205634	T>C	Exon9	c.1226A>G	p.(Asn409Ser)	missens	HET	VUS	OD
P4	Chr1: 155205634	T>C	Exon 9	c.1226A>G	p.(Asn409Ser)	missens	HET	MP	OD
P5	Chr1: 155207203	T >C	Exon 7	c.928A>G	p.(Ser310Gly)	missens	HET	VUS	OD
P6	Chr1: 155205043	A>G	Exon 10	c.1448T>C	p.(Leu483Pro)	missens	HET	VUS	OD
P7	Chr1: 155207249	A>C	7	c.882T>G	p.(His294Gln)	missens	HET	VUS	OD
P8	Chr1: 155208388	-	exon 5	c.508C>T	p.(Arg170Cys)	missens	HET	PAT	OD
P9	Chr1: 155206193	AG>A	Exon8	c.1066del	p.(Leu356Trpfs*8)	frameshift	HET	-	OD
P10	-	-	-	c.1448T>C	p.(Leu483Pro)	missens	HET	VUS	OD
P11	Chr1: 155205518	C>G	Exon 9	c.1342G>C	p.(Asp448His)	missens	HET	VUS	OD
P12	Chr1: 155205634	T >C	Exon 9	c.1226A>G	p.(Asn409Ser)	missens	HET	VUS	OD
P13	Chr1: 155205634	T>C	Exon 9	c.1226A>G	p.(Asn409Ser)	missens	HET	VUS	OD
P14	Chr1: 155207925	-	-	c.761A>C	p.(Lys254Thr)	missense	HET	VUS	OD

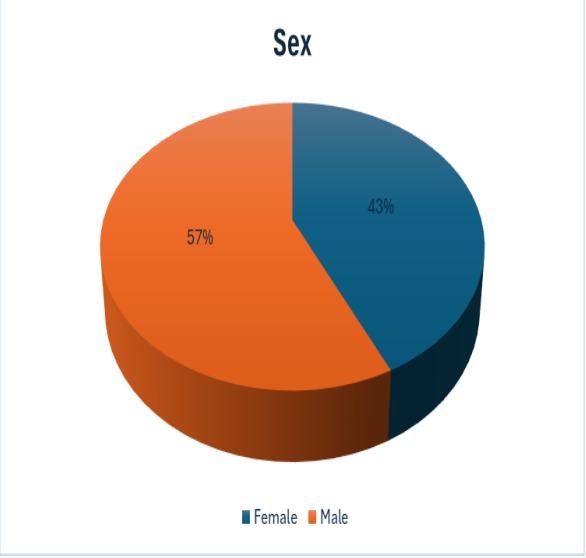

Figure-2. Percentage of women and men in the cohort

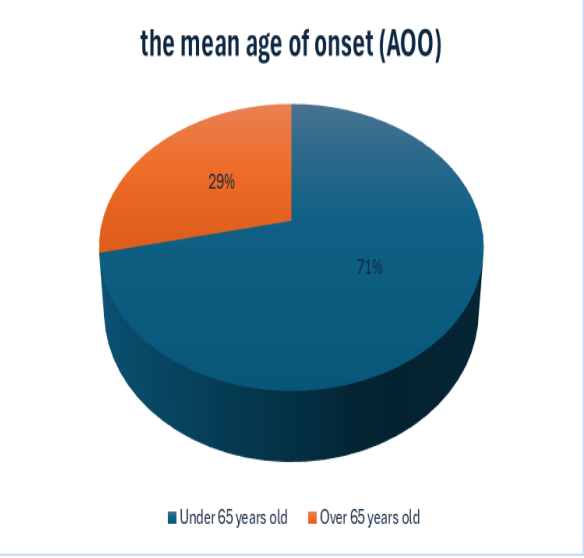

Figure-3. Percentage of age at onset of PD

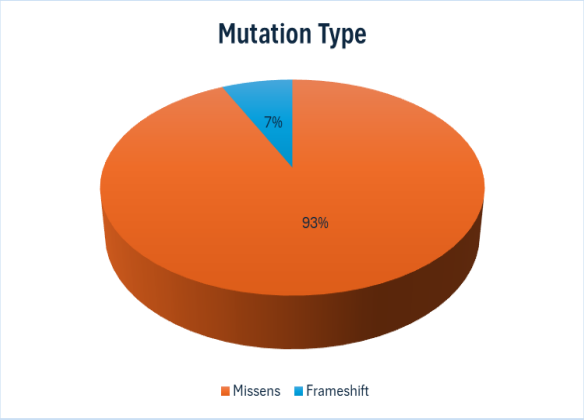

Figure-4. Mutation type percentages of PD patients in the cohort

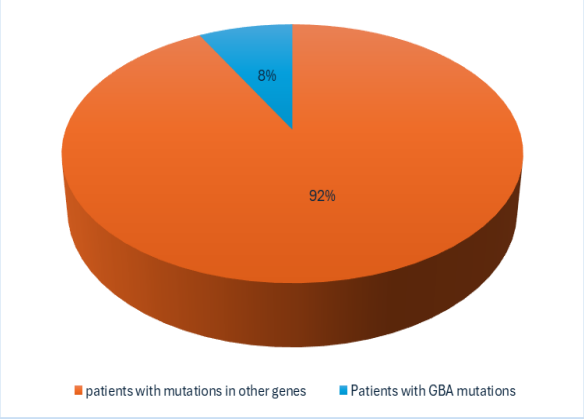

Figure-5. Percentage of PD patients with mutations in the *GBA1* gene and other genes

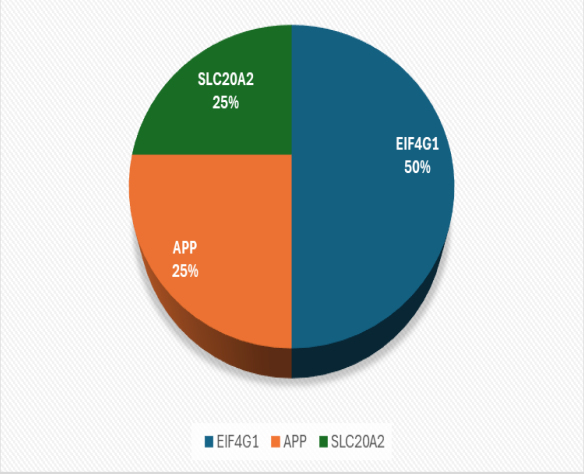

Figure- 6. Percentage of mutations in genes other than *GBA1* in PD patients.

DISCUSSION

Many studies on the correlation between *GBA1* gene mutations and PD have been conducted and are still being conducted worldwide. This study contributes to the existing evidence by identifying *GBA1* mutations including pathogenic, likely pathogenic and variants of uncertain significance (VUS) in 14 patients diagnosed with PD. The findings attempt to highlight the complex role of *GBA1* mutations in the pathogenesis of PD and demonstrate the clinical and genetic diversity observed in *GBA1*-associated PD.

The prevalence of the p.Asn409Ser (N370S) mutation in heterozygous form is a significant observation in our cohort, affecting the majority of patients. This variant is linked to a mild form of Gaucher disease (GD) and an elevated risk of PD, as noted in prior literature. The role of this factor in PD is ambiguous, despite its classification as pathogenic in Gaucher disease (GD), particularly since heterozygous carriers exhibit a relatively early age of onset for PD. The mean age of onset in our cohort was 53.7 years, partially aligned with prior studies indicating that *GBA1*-associated PD manifests earlier than idiopathic PD (36). The variability in age of onset and clinical presentation among *GBA1* mutation carriers indicates the impact of additional genetic and environmental factors on disease progression.

In our study, in addition to motor symptoms, psychiatric findings such as depression and anxiety, which affected approximately one-third of the patients, were also observed in *GBA1* mutation carriers. The findings align with earlier research indicating that non-motor symptoms, including cognitive decline and psychiatric issues, are prevalent among *GBA1*-PD patients (37,38). The prevalence of metabolic comorbidities, such as hypercholesterolemia and diabetes, in two-thirds of patients indicates that *GBA1* mutations exert a broader systemic impact. Prior research indicates that lysosomal dysfunction in *GBA1*-PD could influence metabolic processes; however, the precise mechanisms remain unclear (39).

Our investigation also discovered mutations in additional genes, including EIF4G1, APP, and SLC20A2, which are associated with various neurological illnesses. The existence of these mutations indicates a complex etiology in certain patients, wherein *GBA1* mutations may interact with other genetic variations to affect PD

progression (40). Importantly, APP, which is linked to Alzheimer's disease, and EIF4G1, which is linked to autosomal dominant PD, suggest possible shared mechanisms between PD and other neurodegenerative disorders (41).

The established association between *GBA1* mutations and PD indicates a need for further investigation into the underlying pathogenic mechanisms, which are not yet fully understood. A well-known hypothesis proposes that decreased glucocerebrosidase (GCase) activity leads to lysosomal dysfunction and the resulting accumulation of α-synuclein in vital organelles, a characteristic feature of PD. This accumulation may create a feedback loop, in which α-synuclein further inhibits GCase activity, worsening neurodegeneration (42). Further research is necessary to clarify these mechanisms and to investigate whether *GBA1*-PD constitutes a distinct subtype of the disease or exists on a continuum with idiopathic forms.

Clinically, finding PD-related *GBA1* mutations could pave the way for more effective precision treatment. Ambroxol hydrochloride and other small molecule chaperones show promise in improving GCase function and potentially reducing PD progression (43). Ongoing clinical trials are evaluating treatments targeting GCase activity. One obstacle, though, is that genetic counseling does not yet have established standards. Due to partial penetrance, not all carriers of *GBA1* mutations will get PD, which makes genetic counseling and risk assessments more challenging (44).

CONCLUSION

This work emphasizes the critical significance of *GBA1* mutations in the development of PD, underscoring the necessity for thorough clinical care techniques that encompass both motor and non-motor symptoms in afflicted patients. Our findings indicate the extensive genetic complexity of *GBA1*-associated PD, highlighting the necessity for further study to elucidate the molecular processes underlying this association, especially the link between glucocerebrosidase malfunction and α-synuclein buildup.

Despite significant advancements in understanding the genetics of PD over the last twenty years, the precise mechanisms connecting *GBA1* mutations to neurodegeneration are still unclear. The interactions between *GBA1* mutations and other genetic and environmental modifiers require additional investigation. Further research is necessary to investigate the development of genotype-specific therapies targeting glucocerebrosidase activity, which may provide tailored treatments for the varied clinical manifestations of the disease.

The increasing availability of genetic testing necessitates the establishment of standardized guidelines for genetic counseling, particularly due to the incomplete penetrance associated with *GBA1* mutations. The integration of new precision medicine approaches, including GCase-enhancing therapies, into clinical practice will be essential. Future studies should clarify the relationship between *GBA1* mutations and other synucleinopathies, such as REM sleep behavior disorder (RBD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA), to enhance understanding of the full spectrum of *GBA1*-related neurodegenerative diseases.

Expanding our understanding of *GBA1*-associated PD and its broader clinical and genetic context is critical to developing promising targeted therapies for patients and their families.

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References

1. Savitt JM, Dawson VL, Dawson TM. Diagnosis and treatment of Parkinson disease: molecules to medicine. J Clin Invest. 2006;116(7):1744-54.
2. Lesage S, Brice A. Parkinson's disease: from monogenic forms to genetic susceptibility factors. Hum Mol Genet. 2009;18(R1):R48-59.
3. Halperin A, Elstein D, Zimran A. Increased incidence of Parkinson disease among relatives of patients with Gaucher disease. Blood Cells Mol Dis. 2006;36(3):426-8.
4. Alcalay RN, Levy OA, Waters CC, Fahn S, Ford B, Kuo SH, et al. Glucocerebrosidase activity in Parkinson's disease with and without GBA mutations. Brain. 2015;138(Pt 9):2648-58.
5. Beavan M, Schapira AH. Glucocerebrosidase Gene Mutation and Preclinical Markers of Parkinson Disease-Reply. JAMA Neurol. 2015;72(6):724.

6. Macerollo A. Glucocerebrosidase Gene Mutation and Preclinical Markers of Parkinson Disease. JAMA Neurol. 2015;72(6):723-4.
7. Chatterjee D, Krainc D. Mechanisms of Glucocerebrosidase Dysfunction in Parkinson's Disease. J Mol Biol. 2023;435(12):168023.
8. Huang Y, Yu F, Feng L, Wang X. The Association between E326K of GBA and the Risk of Parkinson's Disease. Parkinsons Dis. 2018;2018:1048084.
9. Wang DX, Shi Q, Wang Z, Zhang Y. (GBA mutations and Parkinson's disease). Sheng Li Xue Bao. 2018;70(3):294-300.
10. Brooker SM, Krainc D. Glucocerebrosidase dysfunction in neurodegenerative disease. Essays Biochem. 2021;65(7):873-83.
11. Dos Santos JCC, Dos Santos LR, Ferreira RR, Costa RB. The Molecular Impact of Glucosylceramidase Beta 1 (GBA1) in Parkinson's Disease: a New Genetic State of the Art. Mol Neurobiol. 2024.
12. Wang D, Wang L, Wang Z. Strategies targeting endoplasmic reticulum stress to improve Parkinson's disease. Front Pharmacol. 2023;14:1288894.
13. Gabbert C, Lu J, Ennis K, Zhang X. GBA1 in Parkinson's disease: variant detection and pathogenicity scoring matters. BMC Genomics. 2023;24(1):322.
14. Hatano T, Okuzumi A, Aoyama M, Hattori N. Alpha-Synuclein: A Promising Biomarker for Parkinson's Disease and Related Disorders. J Mov Disord. 2024.
15. Sobreira-Neto MA, Fukusaki C, Angelim A, Sobreira E. REM sleep behavior disorder: update on diagnosis and management. Arq Neuropsiquiatr. 2023;81(12):1179-94.
16. Boeve BF. REM sleep behavior disorder: Updated review of the core features, the REM sleep behavior disorder-neurodegenerative disease association, evolving concepts, controversies, and future directions. Ann N Y Acad Sci. 2010;1184:15-54.
17. Iftikhar IH, Smith MC, Neubauer DN, Kasper JL. Alpha-synuclein pathology in isolated rapid eye movement sleep behaviour disorder: a meta-analysis. J Sleep Res. 2024;e14204.
18. Adler CH, Hentz JG, Shill HA, Caviness JN. Conjugal Synucleinopathies: A Clinicopathologic Study. Mov Disord. 2024.
19. Liu L, Xue L, Huang X, Chen S. Effect of GBA gene variants on clinical characteristics of dementia with Lewy bodies: a review and meta-analyses. Neurol Sci. 2022;43(6):3541-50.
20. Wernick AI, Aasly J, Blaeser CD, Mathias C. GBA variation and susceptibility to multiple system atrophy. Parkinsonism Relat Disord. 2020;77:64-9.
21. Usenko TS, Prokhorova EA, Chernykh AV, Krylova TD. Impaired Sphingolipid Hydrolase Activities in Dementia with Lewy Bodies and Multiple System Atrophy. Mol Neurobiol. 2022;59(4):2277-87.
22. Matsukawa T, Ito M, Inoue Y, Okada Y. Clinical and Genetic Features of Multiplex Families with Multiple System Atrophy and Parkinson's Disease. Cerebellum. 2024;23(1):22-30.
23. Saffie Awad P, Guerrero-Jaramillo Y, Campos-Arriola D, Rivas D. Frequency of Hereditary and GBA1-Related Parkinsonism in Latin America: A Systematic Review and Meta-Analysis. Mov Disord. 2024;39(1):6-16.
24. Zhang X, Yin Z, Yu X, Li J. Clinical Characteristics and GBA Gene Mutation Analysis of Gaucher Disease Type I. Clin Lab. 2023;69(4).
25. Parlar SC, Guven MM, Ciftci B, Akcan C. Classification of GBA1 Variants in Parkinson's Disease: The GBA1-PD Browser. Mov Disord. 2023;38(3):489-95.
26. Senkevich K, Rudakova A, Sudarkina O, Gureev A. Genetic mechanism vs genetic subtypes: The example of GBA. Handb Clin Neurol. 2023;193:155-70.
27. Long GL, Winichagoon P, Fucharoen S, Fucharoen G. Structure and organization of the human metaxin gene (MTX) and pseudogene. Genomics. 1996;33(2):177-84.
28. Toffoli M, Cenci S, Piacentini M, Tagliaferri L. Comprehensive short and long read sequencing analysis for the Gaucher and Parkinson's disease-associated GBA gene. Commun Biol. 2022;5(1):670.
29. Gan-Or Z, Bar-Shira A, Mirelman A, Gurevich T. Genotype-phenotype correlations between GBA mutations and Parkinson disease risk and onset. Neurology. 2008;70(24):2277-83.
30. Colucci F, Ali M, Silva D, Massaroni S. Ambroxol as a disease-modifying treatment to reduce the risk of cognitive impairment in GBA-associated Parkinson's disease: a multicentre, randomised, double-blind, placebo-controlled, phase II trial. BMJ Neurol Open. 2023;5(2):e000535.

31. Kopytova AE, Proskurina SE, Kruglikov IS, Podlesnykh SI. Ambroxol increases glucocerebrosidase (GCase) activity and restores GCase translocation in primary patient-derived macrophages in Gaucher disease and Parkinsonism. Parkinsonism Relat Disord. 2021;84:112-21.
32. Crotty GF, Schwarzschild MA. What to Test in Parkinson Disease Prevention Trials? Repurposed, Low-Risk, and Gene-Targeted Drugs. Neurology. 2022;99(7 Suppl 1):34-41.
33. Zhao X, Chen X, Huang J, Wang Z. PGRN deficiency exacerbates, whereas a brain penetrant PGRN derivative protects, GBA1 mutation-associated pathologies and diseases. Proc Natl Acad Sci U S A. 2023;120(1):e2210442120.
34. Jones TA, Trinh J, Tagliati M, Schuler M. A cross-sectional survey of genetic counselors providing carrier screening regarding GBA variants and Parkinson disease susceptibility. J Assist Reprod Genet. 2022;39(3):747-55.
35. Deng H, Wu Y, Jankovic J. The EIF4G1 gene and Parkinson's disease. Acta Neurol Scand. 2015;132(2):73-8.
36. Sidransky E, Nalls MA, Aasly JO, Nichols WC. Glucocerebrosidase mutations in Parkinson's disease. N Engl J Med. 2009;361(17):1651-61.
37. Neumann J, Bohnen NI, Müller U, Schulz JB. Non-motor symptoms of Parkinson's disease: Depression, anxiety, and apathy in Parkinson's disease. Mov Disord. 2009;24(9):1212-9.
38. Brockmann K, GBA-associated Parkinson's disease: The effects of GBA mutations on the development of non-motor symptoms. Neurology. 2011;77(13):1221-6.
39. Alcalay RN, Glucocerebrosidase mutations and risk of Parkinson’s disease in Ashkenazi Jews. N Engl J Med. 2012;367(24):2332-9.
40. Singleton A, Gene expression in Parkinson’s disease. Lancet Neurol. 2013;12(11):1140-50.
41. Davis MY, Mutations in GBA1, EIF4G1, and PD risk: Exploring genetic overlap in neurodegenerative diseases. J Parkinsons Dis. 2016;6(4):759-73.
42. Mazzulli JR, α-Synuclein causes lysosomal dysfunction and impaired autophagic clearance in GBA mutation carriers. Science. 2011;333(6048):90-3.
43. Mullin S, Ambroxol for the treatment of patients with Parkinson disease and GBA1 mutations: A nonrandomized, noncontrolled trial. JAMA Neurol. 2020;77(4):427-34.
44. Gan-Or Z, The penetrance of GBA mutations in Parkinson’s disease: The importance of genetic counseling and individualized risk assessment. Mov Disord. 2015;30(1):14-7.