Screening of the glucocerebrosidase (*GBA*) gene in South Africans of African ancestry with Parkinson's disease

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Highlights

- We screened the GBA gene in 30 Parkinson's disease patients of African ancestry.
- In total, 8 variants were found in 17 individuals.
- We found a p.R120W variant in one patient with an age at onset (AAO) of 35 years.
- p.R131L was found in another patient (AAO 3O years) and her sibling (AAO 45 years).
- Two novel variants (p.F216L and p.G478R) were found.

ABSTRACT

Sequence variants in glucocerebrosidase (*GBA*) are a major genetic risk factor for Parkinson's disease (PD), and display ethnic-dependent frequencies, e.g. variants such as p.N370S and 84insGG are common in Ashkenazi Jewish patients. Notably, there are limited studies on Black patients from the African continent; hence, we conducted a study on 30 South African Black PD patients. All 11 exons of *GBA* were screened using a nested PCR approach to avoid pseudogene contamination. We identified previously described Gaucher's disease-associated variants, p.R120W in one patient [age-at-onset (AAO) of 35 years], and p.R131L in another patient (AAO 3O years) and in her affected sibling (AAO 45 years). Also, we found three previously-identified [p.K(-27)R, p.T36del, and p.Q497*], and two novel (p.F216L and p.G478R) variants. Screening of ethnic-matched controls for the novel variants revealed that the allele frequency of p.F216L was 9.9%, whereas p.G478R was not found in the controls. Studies such as these are important and necessary to reveal the genetic architecture underlying PD in the understudied patients of African ancestry.

Keywords: Glucocerebrosidase; *GBA* variants; Parkinson's disease; South African; African ancestry

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder resulting in various motor and non-motor related symptoms. Although the clinical and neuropathological features of PD have been extensively described, the etiology remains unclear. The *GBA* gene, which encodes the lysosomal enzyme glucocerebrosidase, has been consistently shown through various multicenter and genome-wide association studies to be a major risk factor for developing PD (Chang et al., 2017; Sidransky et al., 2009). *GBA* variants are also known to cause Gaucher's disease (GD), an autosomal recessive lysosomal storage disorder, initially described by Phillipe Gaucher in the 1800s (Gaucher, 1882). *GBA* (OMIM 606463) is located on chromosome 1q22 and consists of 11 coding exons. Mutation screening of this gene is hampered by the presence of a highly homologous (97%) pseudogene (*GBAP1*), located 16kb downstream (Horowitz et al., 1989). This complication has led to many researchers conducting screening of only selected regions of the gene.

GBA variants are prevalent within Ashkenazi Jewish PD patients; in one study 19% of the 1,000 PD patients screened harbored variants (Gan-Or et al., 2015). The most common pathogenic variants in this population are p.N370S, p.R496H, 84insGG, IVS2+1G-A, pV394L, p.L444P and 370Rec (Gan-Or et al., 2015). These have been shown to be relatively rare in non-Jewish ethnicities (Zhang et al., 2018). It is hypothesized that diverse populations may harbor unique *GBA* variants, which emphasizes the necessity for screening of the entire coding region in these individuals. *GBA*-associated PD risk in populations on the African continent have been understudied, with only three studies conducted thus far. One was in individuals of North African ancestry, mostly from Algeria (Lesage et al., 2011) and the other study was in North African Arab-Berber individuals from Tunisia (Nishioka et al., 2010). The third study was performed in South African individuals of European ancestry (Barkhuizen et al., 2017). This study identified known and putative pathogenic variants (including p.N370S and p.I368T), as well as risk factors (including p.E326K and p.T369M) in *GBA* which were found more commonly in patients than in controls (12.4% vs. 5.0%). To date, no studies have investigated *GBA* variants in sub-Saharan African PD patients of non-European ancestry. Hence, the aim of the present study was to screen the entire coding region of *GBA* (all 11 exons) in a group of

30 South African Black PD patients to determine whether the variants identified in other populations are also found in African populations.

2. Methods

Study participants: The study was approved by the Health Research Ethics Committee of Stellenbosch University, South Africa (2002/C059). All individuals provided written informed consent. Thirty Black South African PD patients (**Supplementary Table S1**) were recruited from the Movement Disorders clinic at Tygerberg Hospital in Cape Town as well as from various neurology clinics around the country. The mean age-at-onset (AAO) was 47.7 ± 8.4 years (range 30 - 57) and 53% were male. Two individuals reported a family history of the disorder: with both having an affected sibling and an affected parent (**Supplementary Table S1**). Participants were assessed by neurologists and met the UK Parkinson's Disease Society Brain Bank diagnostic criteria for PD diagnosis (Gibb and Lees, 1988). Parkinsonism was present in all patients, and no absolute exclusion criteria were present (Postuma et al., 2015).

Genetic analysis: For all 30 individuals, the 11 exons of *GBA* were PCR amplified using previously described primers (Stone et al., 2000a). Firstly, the exons were amplified in three large fragments, consisting of exons 1-5 (2,972bp), exons 5-7 (2,049bp) and exons 8-11 (1,682bp) (**Supplementary Table S2**). This was followed by a nested PCR step to amplify individual exons from the three large fragments for each individual (Stone et al., 2000a). Thereafter, Sanger sequencing was performed using the BigDye Terminator V3.1 sequencing kit (Applied Biosystems, San Francisco, CA, USA), following the manufacturer's protocol, at the Central Analytical Facilities (Stellenbosch, South Africa). The electropherograms were evaluated using BioEdit version 7.0.5.3 (Hall, 1999). The pathogenicity of non-synonymous variants was assessed with various *in-silico* tools (MutationTaster-2, CADD, polyphen-2, SIFT, MutPred, and FATHMM) using Ensembl Variant Effect Predictor (VEP) (McLaren et al., 2016). Indels were assessed with SIFT-indel and PROVEAN (Choi & Chan, 2015; Hu & Ng, 2013). The Genome Aggregation Database (gnomAD) was used to assess the frequency of selected variants (Karczewski et al., 2019). In addition, the two novel variants were screened in ethnic-matched controls [n=110 individuals; average current age = 89.1 ± 5.2 years (range 81-105) and 48% were male].

3. Results

In total, five missense, one premature stop, one indel and one synonymous *GBA* variant were identified in 17 individuals (**Table 1**). All of the variants were heterozygous. We identified two novel [p.F216L (p.F255L) and p.G478R (p.G517R); **Supplementary Figure S1**] as well as five known [p.K(-27)R (p.K13R), p.T36del (p.T75del), p.R120W (p.R159W), p.R131L (p.R170L), and p.Q497* (p.Q536*)] variants. The two novel variants were absent from the gnomAD, however, when we screened ethnic-matched non-PD controls, we found the allele frequency of p.F216L to be 9.9% (21/212 chromosomes). The p.G478R was not found in any the controls (0/220 chromosomes).

Notably, the p.R120W (p.R159W) variant was present in one individual. Also, a p.R131L (p.R170L) variant, previously associated with GD, was found in one individual. This individual has an affected father and sister, and Sanger sequencing of the sister (AAO 45 years) revealed that she is also a carrier of this variant. The *in-silico* pathogenicity predictions of the variants show that all except p.K(-27)R (p.K13R) are predicted to be deleterious (**Table 2**).

4. Discussion

Given the importance of *GBA* variants in PD risk, we set out to establish if Black South African patients harbored common variants, such as those observed in the Ashkenazi Jewish PD patients. We did not find the common variants associated with PD risk [p.E326K (p.E365K) or p.T369M (p.T408M)]. Also, we did not identify any of the putative pathogenic variants identified in the previous study performed in South African individuals of European ancestry (Barkhuizen et al., 2017). We did, however, find the p.K(-27)R (p.K13R) variant in six individuals. In a previous study, this variant was found in two individuals with PD of North African Arabic ancestry, however, it was found to occur at similar frequencies (approximately 4%) in cases and controls (Nishioka et al., 2010). Another study reported the p.K(-27)R (p.K13R) variant in two individuals, who also harbored the LRRK2 p.G2019S pathogenic mutation, which is common in North African and Ashkenazi Jewish PD patients (Lesage et al., 2011a). According to the gnomAD database, this variant is common in individuals of African ancestry (MAF = 0.07687). Five out of the six *in-silico* tools used predicted this variant to be benign (Table 2).

In addition, two individuals were found to harbor a three-base pair (TAC) in-frame deletion [p.T36del (p.T75del)] in exon 3 of *GBA*, which is reported in dbSNP as a variant of unknown pathogenicity. However, it was predicted to be damaging in our analysis using SIFT-indel and PROVEAN. Interestingly, p.T36del was found in 17/38 chromosomes (all heterozygous) from type 1 GD patients of African ancestry, thereby suggesting that this allele may be relatively frequent in this population (Arndt et al., 2009) but further studies on its frequency are necessary. The other two known variants p.R120W [p.R159W] and p.R131L [p.R170L]) were identified in one individual each. p.R120W is a known cause of type 2 GD but is also found in type 1 GD cases, whereas p.R131L has been found in type 3 GD cases (Pastores and Hughes, 1993; Stone et al., 2000; Wan et al., 2006). Both participants have young-onset PD (35 and 30 years) and one has a family history of PD. p.R120W (p.R159W) has been associated with an increased risk of developing PD, especially in non-Ashkenazi Jewish populations (Zhang et al., 2018). To our knowledge, p.R131L (p.R170L) has not previously been associated with PD. However, pathogenic *GBA* variants have been shown to increase the risk of PD when in a heterozygous form. These two variants were predicted to be pathogenic by all of the *in-silico* tools (Table 2).

Interestingly, we identified two novel variants that are not reversions to the pseudogene. The p.F216L (p.F255L) variant was found in six individuals (with AAO's of 30, 45, 53, 55, 55 and 57 years) and it is predicted to be pathogenic by all six *in-silico* tools (**Table 2**). However, this variant was found in 9.9% of control chromosomes indicating that it is a common polymorphism. One of the individuals (AAO 53 years) is a compound heterozygote, carrying both the p.F216L (p.F255L) variant and a known stop gain variant p.Q497* (p.Q536*) which is predicted to be pathogenic by three of the *in-silico* tools (**Table 2**). p.Q497* (p.Q536*) is a change from glutamine at position 536 to a premature stop codon i.e. the protein is one amino acid shorter than the wild type. This has been reported in dbSNP as a variant of unknown pathogenicity. The other novel variant, p.G478R (p.G517R), was detected in one individual with an AAO of 37 years and was predicted to be pathogenic by all six *in-silico* tools (**Table 2**). It was not found in ethnic-matched controls and therefore further follow-up studies on this variant are necessary. Interestingly, the variant is at the same codon as a known pathogenic variant (p.G478S [p.G517S]) previously found in a compound

heterozygous state (other variant was p.N370S) in a non-Jewish European type 1 GD patient (Beutler et al., 1993).

In conclusion, our study has shown that screening of the entire coding region of *GBA* in diverse populations is important, as unique variants may be identified. Our study is limited by a small sample size, and future studies on individuals of African ancestry should include larger sample sizes. Another limitation is that the majority of our study participants do not know if they have a family history of PD, limiting co-segregation analysis. Despite these limitations, this study is important since it is the first report on *GBA*-associated PD risk in individuals of African ancestry. As new therapeutic targets for *GBA*-associated PD are being developed, including microRNAs and substrate reduction therapy, identification of *GBA*-variant carriers who may benefit from participating in clinical trials, is essential (Ryan et al., 2019).

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Conflict of interest

The authors declare no conflicts of interest.

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Author contributions

ACM conducted the experiments, performed the analysis and wrote the first draft of the manuscript. DGA, ACR, RvC and JC were responsible for patient recruitment and performed clinical assessments on the study participants. OAR provided laboratory reagents and critically reviewed the manuscript. SB conceptualised the study, supervised the first author and obtained funding. All authors reviewed and approved the final version of the manuscript.

Table 1

Table 1 Heterozygous coding variants identified in *GBA* in Parkinson's disease patients of African ancestry

Participant ID no.	Exon	[1] Variant (traditional nomenclature)	[2] Variant (HGVS nomenclature)	cDNA position	Codon change	rs number (dbSNP)	[3] MAF from gnomAD (all)	MAF from gnomAD (African ancestry)	MAF from gnomAD (European ancestry; non-Finnish)
Nonsynonymous variants and indels									
43.59	2	p.K(-27)R	p.K13R	c.38A>G	A**A**G/A**G**G	rs150466109	0.007478	0.07687	0.0001629
12.170	2	p.K(-27)R	p.K13R						
10.313	2	p.K(-27)R	p.K13R						
10.314	2	p.K(-27)R	p.K13R						
11.895	2	p.K(-27)R	p.K13R						
11.910	2	p.K(-27)R	p.K13R						
11.835	3	p.T36del	p.T75del	c.222_224delTAC	GG**TAC**C/GG-C	rs761621516	0.00006367	0.0004409	0.000
11.962	3	p.T36del	p.T75del						
12.486	5	p.R120W	p.R159W	c.475C>T	**C**GG/**T**GG	rs439898	0.00002124	0.0001203	0.00001552
94.69	5	p.R131L	p.R170L	c.509G>T	C**G**C/C**T**T	rs80356763	0.000003981	0.000	0.000
60.39	7	p.F216L	p.F255L	c.765C>T	TT**C**/TT**A**	Novel			
84.52	7	p.F216L	p.F255L						
12.172	7	p.F216L	p.F255L						
12.177	7	p.F216L	p.F255L						
12.179	7	p.F216L	p.F255L						
12.178	7	p.F216L and	p.F255L and						
	11	p.Q497*	p.Q536*	c.1606C>T	**C**AG/**T**AG	rs758806595	0.00002510	0.0002309	0.000
96.87	11	p.G478R	p.G517R	c.1549G>C	**G**GC/**C**GC	Novel			
Synonymous variants									

84.52	10	p.Q432Q	p.Q471Q	c.1413G>A	CA**G**/CA**A**	rs12747811	No data

[1] Amino acid numbering according to the traditional nomenclature used to name variants associated with Gaucher's Disease. This system uses the 40th amino acid as the first codon, as the first 39 amino acids are cleaved off in the active form of the protein (Hruska et al., 2008).

[2] Amino acid numbering according to the reference sequence NP_000148.2 and Human Genome Variation Society nomenclature (HGVS; http://varnomen.hgvs.org/).

[3] MAF (minor allele frequency) is taken from the Genome Aggregation Database of exome and genome sequences of various populations (gnomAD; https://gnomad.broadinstitute.org/).

Table 2 *In-silico* pathogenicity predictions of the variants identified in *GBA*

[1] **Variant**	**Scaled CADD v1.3 score (>15=deleterious)**	**MutPred score (>0.5=deleterious)**	**MutationTaster-2 prediction**	**FATHMM**	**PolyPhen-2 prediction (>0.5= deleterious)**	**SIFT Prediction (<0.05= deleterious)**
p.K13R	9.543	0.243	Polymorphism	Disease causing	Benign (0)	Tolerated (0.53)
[†] p.T75del						
p.R159W	28.6	0.827	Disease causing	Disease causing	Probably damaging (0.934)	Deleterious (0.02)
p.R170L	27.5	0.860	Disease causing	Disease causing	Possibly damaging (0.906)	Deleterious (0.01)
p.F255L	23.9	0.872	Disease causing	Disease causing	Probably damaging (1)	Deleterious (0)
p.G517R	24.8	0.679	Disease causing	Disease causing	Probably damaging (0.944)	Deleterious (0.03)
p.Q536*	36	Not scored	Disease causing	Disease causing	Not scored	Not scored

[1] Amino acid numbering according to the reference sequence NP_000148.2

[†] As this is an indel we used other tools such as SIFT-indel and PROVEAN to assess its pathogenicity