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GBA1 variants in Brazilian Gaucher disease patients

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ABSTRACT

Gaucher disease (GD) is an autosomal recessive lysosomal disorder caused by pathogenic variants in *GBA1* which result in the deficient activity of glucocerebrosidase (GCase). There are few data on the genetic characterization of Brazilian GD patients. This study aimed at characterizing the genotype of 72 unrelated Brazilian GD patients (type I = 63, type II = 4, type III = 5; male = 31). Forty patients were from South Brazil (SB), and 32 were from other regions of Brazil (Others). The exons and exon/intron junctions of *GBA1* were analyzed by Sanger sequencing in 8 patients, or by massive parallel sequencing followed by Sanger of exons 9 and 10 in 64 patients. In total, 31 pathogenic variants were identified. The most frequent allele found was N370S (p.(Asn409Ser)) (41.0%), and the most frequent genotype was N370S/Rec*Nci*I p.[Asn409Ser];[Leu483Pro;Ala495Pro;Val499=] (23.6%). Three variants (N370S – in exon 9, and Rec*Nci*I and L444P (p.(Leu483Pro), in exon 10) correspond to 76.3% of total alleles in SB and 59.4% in Others. Two novel variants were described: c.326del(p.(Gln109Argfs*9)) and c.690G>A (p.(?)). Although sequencing all the exons of *GBA1* is the gold-standard method for the genetic analysis of GD patients, a step analysis can be proposed for Brazilian patients, starting with analysis of exons 9 and 10. The N370S allele is the most frequently associated with GD in Brazil.

1. Introduction

Gaucher disease (GD) is an inborn error of metabolism caused by the deficient activity of the glucocerebrosidase enzyme (GCase; E.C. 3.2.1.45). This deficiency leads to an accumulation of glucocerebroside inside the lysosomes, especially in the reticuloendothelial system. The excessive storage of this substrate is found in the liver, spleen, bone, and bone marrow of GD patients [1].

GD is an autosomal recessive disorder, resulting from pathogenic variants in *GBA1*. This gene is located on chromosome 1q21 and comprises 11 exons. Almost 700 different variants have already been described according to the Human Gene Mutation Database [2]. The most commonly reported *GBA1* variants worldwide are N370S and L444P, but the prevalence of these variants differs among populations; for instance, N370S represents 83.2% of alleles in Ashkenazi-Jews with GD [3,4]. Besides that, the data available is biased because of the underrepresentation of some populations (such as the Latin American and African descendents).

Brazil is a country composed by several interethnic ancestral crossings among Amerindians, Europeans, and African descendents derived from immigration waves that occurred since the XV century, that were different depending on Brazilian regions. For instance, individuals from the Northeast region have strong African ancestry due to the slavery period, whereas the North region had a large influence of Amerindians,

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and the South region was mostly colonized by European immigrants [5,6]. A few studies have described the *GBA1* allele frequencies in Brazilian GD patients; however, it is not possible to confirm the exact number of patients analyzed since some studies included the same individuals (Table 1).

Many protocols for genetic analysis of GD patients included only the investigation of the most frequent variants; this prevents the differentiation between L444P and those resulting from recombination events. Therefore, the main objective of this study was to describe the *GBA1* variants' frequencies in a cohort of unrelated Brazilian GD patients who had the complete exon/intron-exon junctions of *GBA1* analysis performed.

2. Materials and methods

It is an observational study with convenient sampling assessment, which was approved by the local Ethics Committee. Patients from the Brazilian state of Rio Grande do Sul ($n = 34$) are followed at the local Gaucher Reference Center, linked to the research laboratory where the genetic analysis for this study was performed. Others ($n = 38$) were referred for genetic analysis and inclusion in this study after contacting geneticists and hematologists from all Brazilian regions.

2.1. Subjects

A total of 72 unrelated patients with biochemical diagnosis of GD (type I = 63, type II = 4, type III = 5; male = 31) were included in this study. Forty patients (55.6%) were from South Brazil (SB), and 32 (44.4%) were from other Brazilian regions (Southeast = 13/32, Northeast = 12/32, Center-West = 6/32, and North = 1/32) (classified as Others) (Fig. 1, Table 2). Patient 19 had been previously described by Siebert et al., 2013 [9]; and patient 25 had been previously described by Paskulin et al., 2019 [11] (Table 2).

2.2. Genotype analysis

All patients had their genetic diagnosis performed at a research laboratory located at the Reference Center for Rare Diseases of Porto Alegre – Rio Grande do Sul (Hospital de Clínicas de Porto Alegre, South Brazil). Genomic DNA was extracted from peripheral blood collected into EDTA-containing tubes using Easy-DNA™ kit (Thermo Fisher Scientific™) according to the manufacturer's instructions. Eight patients (patients 1, 4, 7, 8, 14, 16, 18 and 29) had their *GBA1* gene (exons and exon-intron junctions) analyzed by Sanger sequencing using the ABI 3500 Genetic Analyzer (Thermo Fisher Scientific™) following Stone et al. experiment designs [12]. All primers used for Sanger were obtained from Stone et al. due to their specificity for the functional gene. For 64 patients, massive parallel sequencing using the Ion Torrent PGM™ platform (Thermo Fisher Scientific™) comprising all exons and exon/intron junctions using a customized AmpliSeq Panel (IAD97194_197; Thermo Fisher Scientific™) was performed, followed by Sanger sequencing of exons 9 and 10. The results were analyzed using Chromas (Technelysium), Ion Reporter™ (Thermo Fisher Scientific™) and Enlis Genome (Enlis, LCC) software.

Since DNA from parents were available for analysis only for patient 19, the cis and trans status of variants found in number > 2 per patient was inferred according to the literature: for instance, the cis status of variants L444P and E326K (p.[Leu483Pro;Glu365Lys]) have already been reported elsewhere [13–17].

2.3. In silico functionality prediction analysis

SpliceAI and NMDEscPredictor were used to predict functional effects of sequence variations [18,19].

2.4. Statistical analysis

Comparison of *GBA1* allele frequencies between individuals from SB and Others was performed by χ^2-test.

3. Results

The demographic information and genotype of GD patients included in the study are shown in Table 2. Thirty-one different pathogenic variants were found, being 6 found both in SB and in Others. Of the 25 remaining variants, 12 were found only in patients from SB, and 13 variants were identified exclusively detected in patients from other regions (Fig. 2). Patients from SB carry 18/80 (22.5%) different alleles, and Others, 19/64 (29.7%). The genotype N370S/Rec*Nci*I (p.[Asn409Ser];[Leu483Pro;Ala495Pro;Val499=]) was the most frequent in our sample (total = 23.6%; SB = 11/40, 27.5%; Others = 6/32, 18.8%).

N370S was the most frequent allele in our cohort (59/144; 41.0%) and in both SB (32/80; 40.0%) or others (27/64; 42.2%). The overall prevalence of the Rec*Nci*I allele was 14.6% (SB = 15/80, 18.8%; others = 6/64, 9.4%) and for L444P, 13.2% (SB = 14/80, 17.5%; others = 5/64, 7.8%). There was no difference in the prevalence of the N370S, L444P, and Rec*Nci*I alleles between SB and Others ($p = 0.909$; $p = 0.087$; $p = 0.161$, respectively). The most frequent alleles (N370S, L444P, and Rec*Nci*I) corresponded to 76.3% (61/80) of total alleles in SB and 59.4% (38/64) in Others.

Two novel alleles were found in Others. One is a single nucleotide deletion located on exon 4, c.326del - p.(Gln109Argfs*9). This deletion determines a frameshift alteration and is subject to degradation by nonsense-mediated decay according to the NMDEscPredictor (https://nmdprediction.shinyapps.io/nmdescpredictor/). This variant was found in a type I GD patient of 29 years old from the Northeast, which carries p.[Gln109Argfs*9];[Arg535His] (patient 54). This variant was classified as pathogenic by the American College of Genetics and Genomics (ACMG) guidelines [20].

The other variant c.690G > A would cause a synonymous change, p.(?). This patient from the Southeast was a type I GD with genotype p.[?];

Table 1
Studies evaluating allelic frequencies in Brazilian GD patients.

Reference	N (types I, II, III)	Region of Brazil	Methodology	Most frequent allele	Observations
Rozenberg et al. 2006[7]*	40 (all I)	N/A	RFLP, dHPLC and Sanger sequencing	N370S (35%)	All exons of *GBA1* analyzed
Rozenberg et al. 2006[8]*	262 (247, 3, 12)	N/A	RFLP	N370S (47%)	Screening of 9 variants
Sobreira et al. 2007[4]*	221 (all I)	N/A	ICGG Gaucher Registry evaluation	N370S (48.2%)	Methodology not described
Siebert et al. 2013[9]	48 (N/A)	N/A	TaqMan PCR, ARMS-PCR and Sanger sequencing	N370S (44.1%)	All exons of *GBA1* analyzed
Chaves et al. 2015[10]	5 (all GD type I)	NE	Sanger sequencing	G377S (100%)	Screening of 4 variants

Abbreviations: I, GD type I; II, GD type II; III, GD type III; N/A, not available; NE: Northeast region; ICGG, International Collaborative Gaucher Group; RFLP, restriction fragment length polymorphism; dHPLC, denaturing high performance liquid chromatography; ARMS-PCR, Amplification Refractory Mutation System-PCR; *The three studies shared patients.

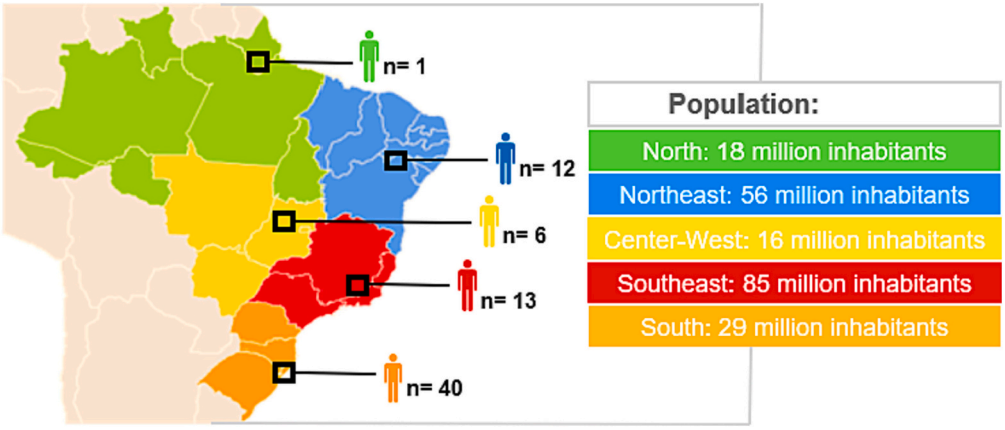

Fig. 1. Brazilian regions. In this study, Others include patients from four regions: Southeast, Northeast, North, and Center-West. N = patients included in this study.

[Asn409Ser] (patient 48). In silico analysis showed that this variant is predicted pathogenic to create a splice site at 3 bp downstream (donor gain score = 0.66) according to the SpliceAI tool. This variant was classified as of uncertain significance by ACMG.

The location of *GBA1* variants found in our cohort of GD patients is demonstrated in Fig. 3, showing they are distributed along the whole gene, with exception of exons 1 and 2.

4. Discussion

This study highlights the allelic heterogeneity of GD in Brazil and suggests that N370S is the most frequent *GBA1* variant in patients from the regions analyzed, followed by the Rec*Nci*I allele, and L444P. It should be highlighted that the number of GD patients from the SB region comprises more than half of the study's patient cohort, which could potentially introduce a bias within this population. The South region is overrepresented because it is the region where our research laboratory is located. N370S is located at exon 9 and L444P at exon 10 of *GBA1*. Rec*Nci*I is the most frequent recombinant allele, and includes the variants L444P, A456P and V460V (p.[Leu483Pro;Ala495Pro;Val499=]). Similarly, in a cohort of 126 Argentinean GD patients [21], the most frequent allele was N370S followed by Rec*Nci*I (but with a higher allele frequency: 86.3% and 52.7%, respectively), and the most frequent genotype was N370S/Rec*Nci*I (46.6%, approximately twice the frequency found in Brazil). The differences between the Brazilian and Argentinean cohorts reflects the higher genetic homogeneity of the Argentinean population.

Sequencing of all exons and exon/intron boundaries of *GBA1* is the main strategy for accurate genotyping of GD patients; however, massive parallel short-read sequencing might include homologous regions such as of exon 10 from *GBAP1* (pseudogene). The amplification of pseudogene can be avoided by using specific primers targeting *GBA1* followed by Sanger sequencing. Of note, genotyping based on PCR followed by Sanger sequencing screening for only common pathogenic variants fails to detect rare alleles and recombinant alleles comprised of multiple single nucleotide variants which is relevant for the differentiation of L444P and recombinant complex alleles [22]. If sequencing of all exons of *GBA1* is not available, our data suggest a stepwise approach for Brazilian patients, starting with Sanger sequencing of exons 9 and 10.

The L444P allele (standalone or as part of recombinant alleles) is strongly associated with neuronopathic GD: a combination of L444P and Rec*Nci*I leads to type II, suggesting that complex alleles increase the severity of the GD, and homozygosity for L444P is found in GD types II and III [22]. L444P and Rec*Nci*I alleles are responsible for 27.8% of the variants identified in Brazilian patients, which could explain the relatively high prevalence of GD II/III in our cohort (9/72, or 12.5%). It is important to emphasize that, depending on the established genotype, reassessment would be appropriate, as milder neuronopathic patients may manifest later in childhood or adolescence (this would be the case for patient 40, genotype R463C/R463C (p.[Arg502Cys];[Arg502Cys]), reported as having GD I at 3 years). All L444P/L444P patients included in this study (patients 9, 30 and 68) presented GD III, and the L444P/Rec*Nci*I patients, GD II (patients 1 and 34). On the other hand, the N370S variant is considered neuroprotective, and there are no reported cases of neuronopathic GD in individuals with this variant. Although the prevalence of N370S in our sample is 41.0%, only 7 patients (all with GD I) were found to be homozygous for this variant, which may suggest an underdiagnosis of GD type I patients in the country.

Our findings are in accordance with previous studies by Rozenberg et al. [7,8], Sobreira et al. [4], and Siebert et al. [9], which also reported N370S as the most frequent variant in Brazil. Chaves et al. [10] evaluated the founder effect for the G377S (p.(Gly416Ser)) variant among GD patients in a population from Northeastern Brazil; all GD patients were homozygous for G377S, suggesting that the high prevalence of GD in this population may be due to a combination of consanguinity and founder effect [10].

Siebert et al. [9] identified the G377S (11.1%) allele as the third most frequent variant among 48 Brazilian GD patients. The authors suggested that G377S variant must be included in preliminary screens of Brazilian GD patients. G377S was the 4th most prevalent variant in our sample and it is located in the exon 9 of *GBA1*.

SB may have a slightly different profile of *GBA1* pathogenic alleles compared to other regions. Three alleles (N370S, Rec*Nci*I, and L444P) were overrepresented in SB, with a combined frequency of 76%, however, those represent only 59% in Others. This could be explained by the different immigration process in the different Brazilian regions [6]. Furthermore, the frequency of the alleles associated with GD types II and III (L444P and Rec*Nci*I), appears to be higher in SB when compared to the rest of the country. We must acknowledge the presence of regional disparities in Brazil based on geographical locations – as the access to health in better in the South and Southeast regions, for instance, this could explain the higher rate of diagnosis of GD II and III in the South

Table 2
Characterization of Gaucher disease patients from different regions of Brazil and genotype findings ($n = 72$).

N	Gender	GD type	Age (y)	*GBA1* genotype	cDNA [allele 1; allele 2] (NM_001005742.2)	protein [allele 1; allele 2] (NP_001005742.1)	Origin
1	M	II	**	L444P/Rec*Nci*I***	c.[1448T>C]; [1448T>C;1483G>C;1497G>C]	p.[Leu483Pro];[Leu483Pro;Ala495Pro; Val499=]	South
2	M	I	10	N370S/IVS9+1G>A	c.[1226A>G];[1328+1G>A]	p.[Asn409Ser];[?]	South
3	F	I	59	N370S/L444R	c.[1226A>G];[1448T>G]	p.[Asn409Ser];[Leu483Arg]	South
4	F	I	7	N370S/Rec*Nci*I***	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	South
5	F	I	42	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	South
6	F	I	10	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	South
7	F	I	26	N370S/L444P***	c.[1226A>G];[1448T>C]	p.[Asn409Ser];[Leu483Pro]	South
8	F	I	16	N370S/L444P***	c.[1226A>G];[1448T>C]	p.[Asn409Ser];[Leu483Pro]	South
9	M	III	1	L444P/L444P	c.[1448T>C];[1448T>C]	p.[Leu483Pro];[Leu483Pro]	South
10	F	I	57	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	South
11	M	I	22	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	South
12	M	I	7	N370S/L444P+A456P	c.[1226A>G];[1448T>C;1483G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro]	South
13	M	I	14	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	South
14	F	I	15	N370S/L444P***	c.[1226A>G];[1448T>C]	p.[Asn409Ser];[Leu483Pro]	South
15	M	I	26	N370S/L444P	c.[1226A>G];[1448T>C]	p.[Asn409Ser];[Leu483Pro]	South
16	F	I	13	N370S/L444P***	c.[1226A>G];[1448T>C]	p.[Asn409Ser];[Leu483Pro]	South
17	M	I	49	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	South
18	F	I	11	N370S/L444P***	c.[1226A>G];[1448T>C]	p.[Asn409Ser];[Leu483Pro]	South
19	F	I	4	N370S/ L461P+IVS10+1G>T[a]	c.[1226A>G];[1499T>C;1505+1G>T]	p.[Asn409Ser];[Leu500Pro;?]	South
20	F	I	12	N370S/R163*	c.[1226A>G];[604C>T]	p.[Asn409Ser];[Arg202*]	South
21	M	I	8	N370S/G202R	c.[1226A>G];[721G>A]	p.[Asn409Ser];[Gly241Arg]	South
22	M	I	2	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	South
23	M	I	10	N370S/R120W	c.[1226A>G];[475C>T]	p.[Asn409Ser];[Arg159Trp]	South
24	F	I	29	N370S/L444P	c.[1226A>G];[1448T>C]	p.[Asn409Ser];[Leu483Pro]	South
25	F	I	42	E349K/S366N[b]	c.[1162G>A];[1214G>A]	p.[Glu388Lys];[Ser405Asn]	South
26	M	I	54	N370S/N370S	c.[1226A>G];[1226A>G]	p.[Asn409Ser];[Asn409Ser]	South
27	F	I	8	N370S/N370S	c.[1226A>G];[1226A>G]	p.[Asn409Ser];[Asn409Ser]	South
28	F	I	13	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	South
29	M	I	5	N370S/L444R***	c.[1226A>G];[1448T>G]	p.[Asn409Ser];[Leu483Arg]	South
30	M	III	2	L444P/L444P	c.[1448T>C];[1448T>C]	p.[Leu483Pro];[Leu483Pro]	South
31	F	I	35	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	South
32	M	I	62	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	South
33	F	I	20	N370S/L444P	c.[1226A>G];[1448T>C]	p.[Asn409Ser];[Leu483Pro]	South
34	M	II	0.03	L444P/Rec*Nci*I	c.[1448T>C]; [1448T>C;1483G>C;1497G>C]	p.[Leu483Pro];[Leu483Pro;Ala495Pro; Val499=]	South
35	M	I	56	G377S/G377S	c.[1246G>A];[1246G>A]	p.[Gly416Ser];[Gly416Ser]	South
36	F	I	11	R48W/R48W	c.[259C>T];[259C>T]	p.[Arg87Trp];[Arg87Trp]	South
37	F	I	30	N370S/S366G	c.[1226A>G];[1213A>G]	p.[Asn409Ser];[Ser405Gly]	South
38	M	*	2	N396T/Rec*Nci*I	c.[1304A>C]; [1448T>C;1483G>C;1497G>C]	p.[Asn435Thr];[Leu483Pro;Ala495Pro; Val499=]	South
39	F	II	1	Rec*Nci*I/F213I	c.[1448T>C;1483G>C;1497G>C]; [754T>A]	p.[Leu483Pro;Ala495Pro;Val499=]; [Phe252Ile]	South
40	F	I	3	R463C/ R463C	c.[1504C>T];[1504C>T]	p.[Arg502Cys];[Arg502Cys]	South
41	M	III	11	P245T/del55	c.[850C>A];[1263–317]	p.[Pro284Thr];[Leu422Profs]	Southeast
42	F	I	55	L444P+E326K/R496H	c.[1448T>C;1093G>A];[1604G>A]	p.[Leu483Pro;Glu365Lys];[Arg535His]	Southeast
43	F	I	**	N370S/L444P	c.[1226A>G];[1448T>C]	p.[Asn409Ser];[Leu483Pro]	Southeast
44	M	I	38	N370S/L444P	c.[1226A>G];[1448T>C]	p.[Asn409Ser];[Leu483Pro]	Southeast
45	M	I	32	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	Southeast
46	M	II	0.04	L444P+E326K/H311R	c.[1448T>C;1093G>A];[1049A>G]	p.[Leu483Pro;Glu365Lys];[His350Arg]	Southeast
47	F	I	54	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	Southeast
48	M	I	25	N370S/**V191V**	c.[1226A>G];[**690G>A**]	p.[Asn409Ser];[?]	Southeast
49	F	I	26	N370S/R120Q	c.[1226A>G];[476G>A]	p.[Asn409Ser];[Arg159Gln]	Southeast
50	M	I	23	N370S/W184R	c.[1226A>G];[667T>C]	p.[Asn409Ser];[Trp223Arg]	Southeast
51	M	I	15	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	Southeast
52	F	I	12	N370S/N370S	c.[1226A>G];[1226A>G]	p.[Asn409Ser];[Asn409Ser]	Southeast
53	F	I	31	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	Southeast

(*continued on next page*)

Table 2 (*continued*)

N	Gender	GD type	Age (y)	*GBA1* genotype	cDNA [allele 1; allele 2] (NM_001005742.2)	protein [allele 1; allele 2] (NP_001005742.1)	Origin
54	F	I	29	R496H/**p.Gln109Argfs*9**	c.[1604G>A];[**326del**]	p.[Arg535His];[**Gln109Argfs*9**]	Northeast
55	F	I	22	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	Northeast
56	M	I	4	N370S/W378C	c.[1226A>G];[1251G>C]	p.[Asn409Ser];[Trp417Cys]	Northeast
57	F	I	21	N370S/N370S	c.[1226A>G];[1226A>G]	p.[Asn409Ser];[Asn409Ser]	Northeast
58	M	I	27	N370S/L444P+E326K	c.[1226A>G];[1448T>C;1093G>A]	p.[Asn409Ser];[Leu483Pro;Glu365Lys]	Northeast
59	M	I	10	N370S/L444P+E326K	c.[1226A>G];[1448T>C;1093G>A]	p.[Asn409Ser];[Leu483Pro;Glu365Lys]	Northeast
60	M	I	62	N370S/N370S	c.[1226A>G];[1226A>G]	p.[Asn409Ser];[Asn409Ser]	Northeast
61	F	I	0.3	R48W/R48W	c.[259C>T];[259C>T]	p.[Arg87Trp];[Arg87Trp]	Northeast
62	F	I	46	N370S/N370S	c.[1226A>G];[1226A>G]	p.[Asn409Ser];[Asn409Ser]	Northeast
63	M	I	55	N370S/W378C	c.[1226A>G];[1251G>C]	p.[Asn409Ser];[Trp417Cys]	Northeast
64	M	I	6	N396T/L444P+E326K	c.[1304A>C];[1448T>C;1093G>A]	p.[Asn435Thr];[Leu483Pro;Glu365Lys]	Northeast
65	F	III	13	G377S/W378C	c.[1246G>A];[1251G>C]	p.[Gly416Ser];[Trp417Cys]	Northeast
66	F	I	7	N370S/L444P	c.[1226A>G];[1448T>C]	p.[Asn409Ser];[Leu483Pro]	Center-West
67	F	I	30	N370S/N370S	c.[1226A>G];[1226A>G]	p.[Asn409Ser];[Asn409Ser]	Center-West
68	F	III	**	L444P/L444P	c.[1448T>C];[1448T>C]	p.[Leu483Pro];[Leu483Pro]	Center-West
69	F	I	**	R353W/RecTL	c.[1174C>T]; [1342G>C;1448T>C;1483G>C;1497G>C]	p.[Arg392Trp];[Asp448His;Leu483Pro; Ala495Pro;Val499=]	Center-West
70	M	I	13	N370S/Rec*Nci*I	c.[1226A>G]; [1448T>C;1483G>C;1497G>C]	p.[Asn409Ser];[Leu483Pro;Ala495Pro; Val499=]	Center-West
71	F	I	**	G377S/G377S	c.[1246G>A];[1246G>A]	p.[Gly416Ser];[Gly416Ser]	Center-West
72	F	I	29	N370S/V398I	c.[1226A>G];[1309G>A]	p.[Asn409Ser];[Val437Ile]	North

Abbreviations: N, patients; M, male; F, female; GD, Gaucher disease; Age (y), age in years at diagnosis; *unknown GD type; **unknown age at diagnosis; ***Sanger sequencing; [a]Patient described by Siebert et al., 2013; [b]Patient described by Paskulin et al., 2019; Novel variants are set in bold.

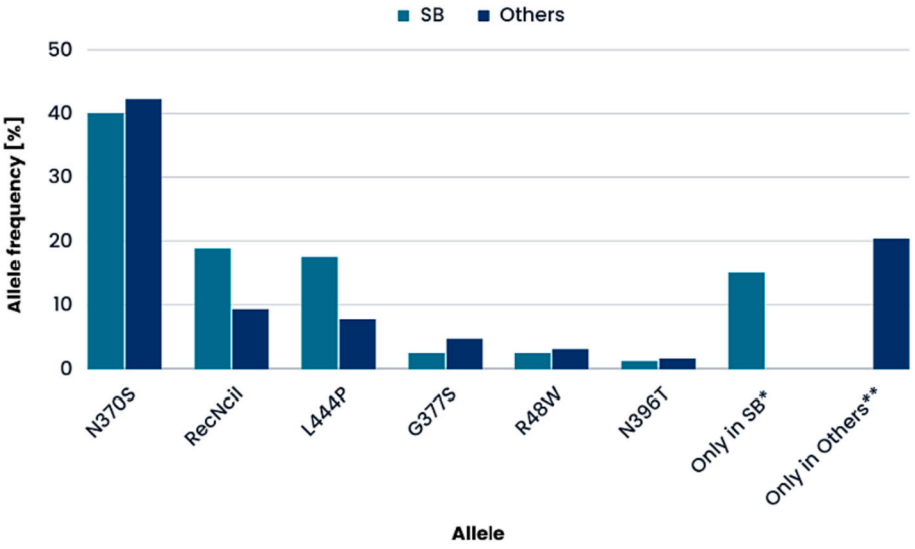

Fig. 2. Allele frequencies found in South Brazil (SB) vs. other regions (Others) using the usual nomenclature of the six common variants in the *GBA1* gene identified in these regions. *Variants: p.(Leu483Arg), p.(Arg159Trp), p.(Arg202*), p.(Gly241Arg), p.(Phe252Ile), p.(Glu388Lys), p.(Ser405Gly), p.(Ser405Asn), c.(1328 + 1G > A), p.(Leu483Pro;Ala495Pro), c.(1499 T > C;1505 + 1G > T), p.(Arg502Cys) **Variants: p.(Trp417Cys), p.(Arg535His), p.(Gln109Argfs*9), p.(Arg159Gln), p.(Trp223Arg), p.(?), p.(Pro284Thr), p.(His350Arg), p.(Arg392Trp), p.(Val437Ile), p.(Leu422Profs), p.(Asp448His;Leu483Pro;Ala495Pro;Val499=), p.(Leu483Pro;Glu365Lys).

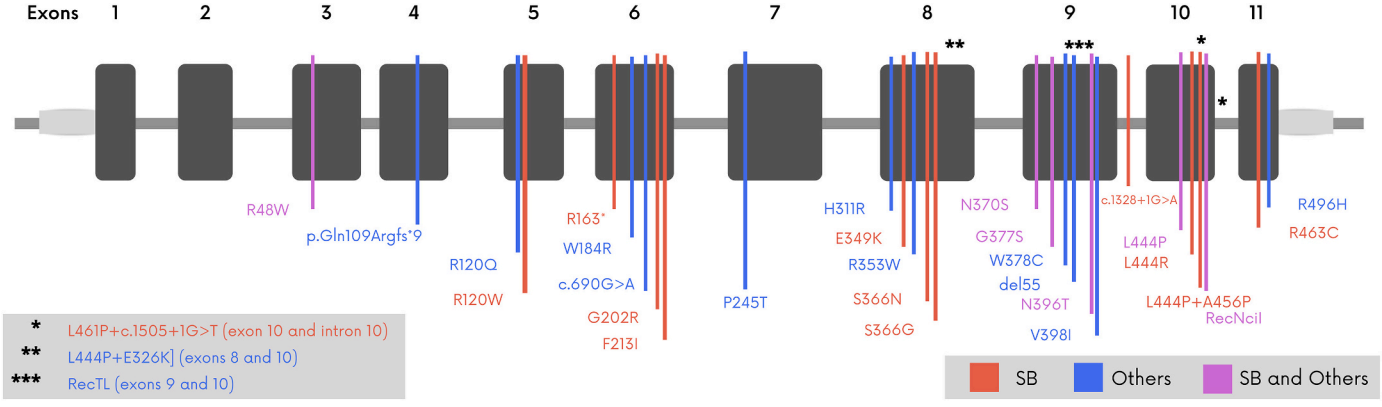

Fig. 3. Location of *GBA1* variants found in Gaucher Disease patients from South Brazil (SB), Others and both SB and Others.

region. Unfortunately, we do not have enough phenotypic information from patients referred for genetic testing at our laboratory to confirm if this genetic difference translates into phenotypic differences. Haplotypes studies are ongoing to try to clarify this issue.

5. Conclusion

The N370S appears to be the most frequent variant associated with GD type I in Brazil. If the complete sequencing of all exons of *GBA1* is not available, a stepwise approach can be proposed for Brazilian patients, starting with Sanger sequencing of exons 9 and 10. However, this strategy can miss both other possibly novel variants as well as other alleles with multiple variants.

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Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre (protocol code 2019–0219/CAAE 10903019800005327 and 10/27/2021).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

CRediT authorship contribution statement

Suelen Porto Basgalupp: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Vivian Altmann:** Formal analysis, Investigation, Writing – review & editing. **Filippo Pinto e Vairo:** Conceptualization, Formal analysis, Writing – review & editing. **Ida Vanessa Doederlein Schwartz:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. **Marina Siebert:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

All supporting data is available upon request.

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