



## Population analysis of the *GLB1* gene in South Brazil

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### Abstract

Infantile GM1 gangliosidosis is caused by the absence or reduction of lysosomal beta-galactosidase activity. Studies conducted in Brazil have indicated that it is one of the most frequent lysosomal storage disorders in the southern part of the country. To assess the incidence of this disorder, 390 blood donors were tested for the presence of two common mutations (1622-1627insG and R59H) in the *GLB1* gene. Another group, consisting of 26 GM1 patients, and the blood donors were tested for the presence of two polymorphisms (R521C and S532G), in an attempt to elucidate whether there is a founder effect. The frequencies of the R59H and 1622-1627insG mutations among the GM1 patients studied were 19.2% and 38.5%, respectively. The frequency of polymorphism S532G was 16.7%, whereas R521C was not found in the patients. The overall frequency of either R59H or 1622-1627insG was 57.7% of the disease-causing alleles. This epidemiological study suggested a carrier frequency of 1:58. Seven different haplotypes were found. The 1622-1627insG mutation was not found to be linked to any polymorphism, whereas linkage disequilibrium was found for haplotype 2 (R59H, S532G) ( $p < 0.001$ ). These data confirm the high incidence of GM1 gangliosidosis and the high frequency of two common mutations in southern Brazil.

**Key words:** GM1 gangliosidosis, beta-galactosidase, *GLB1* gene, founder effect, linkage disequilibrium.

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GM1 gangliosidosis and mucopolisaccharidosis IV-B (Morquio B) are lysosomal storage disorders caused by a  $\beta$ -galactosidase (*GLB1*; EC 3.2.1.23) deficiency. This deficiency occurs due to mutations in the *GLB1* gene, but the differences that lead either to GM1 gangliosidosis or to Morquio B are not known so far. *GLB1* is located on chromosome 3 at 3p21.33 (Takano and Yamanouchi, 1993) and is composed of 62.5 kb divided into 16 exons (Morreau *et al.*, 1989). Hypotheses have been raised proposing that more than 100 different mutations could be responsible for the Morquio or GM1 gangliosidosis phenotypes (Callahan, 1999; Brunetti-Pierri and Scaglia, 2008). However, patients with intermediate phenotypes have also been described (Giugliani *et al.*, 1987; Mayer *et al.*, 2009).

The incidence of type 1 GM1 gangliosidosis is considered to be between 1:100,000 and 1:200,000 live births (Sinigerska *et al.*, 2006). However, higher frequencies have been described for specific regions, such as Malta (1:3,700

(Lenicker *et al.*, 1997). The incidence in Brazil has been estimated at 1:17,000 live births, and the carrier frequency was found to be 1:67 (Severini *et al.*, 1999). Mutation analysis performed in 19 Brazilian patients detected the presence of mutations 1622-1627insG (Silva *et al.*, 1999) and/or R59H (Morrone *et al.*, 1997) in about 60% of the alleles. These results stand out when compared to mutation frequencies found by other groups in different parts of the world.

Here, we report a screen to detect and confirm the incidence of type 1 GM1 gangliosidosis in southern Brazil by analyzing 390 anonymous blood donor samples for the presence of the two common mutations. In addition, these control samples and a group of 26 GM1 patients were tested for the presence of two polymorphisms located within the *GLB1* gene, in an attempt to elucidate whether there is a founder effect, which could explain the high frequency of this disorder in the studied region.

Mutation analysis was performed on samples from 26 unrelated patients with GM1 gangliosidosis and their relatives (whenever possible) to determine the linkage phase of the alleles.

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Patients were diagnosed at the Laboratory of Inborn Errors of Metabolism of the Hospital de Clínicas de Porto Alegre (LREIM/HCPA), Brazil. The diagnosis was confirmed by low levels of  $\beta$ -galactosidase enzyme activity in leukocytes. Anonymous control samples were collected from blood donors of the HCPA Blood Bank, for a total of 390 samples.

Genomic DNA was extracted from peripheral blood leukocytes, using the salting-out procedure (Miller *et al.*, 1988). Molecular analysis was performed using PCR for exons 2 and 15, in order to detect the R59H, 1622-1627insG, R521C and S532G mutations. Primers and conditions were as described by Silva *et al.* (1999). The PCR fragments of exon 2 were digested with *Nla*III (New England Biolabs) and visualized on a 3% agarose gel, to assess for the presence of the R59H mutation. Gels for SSCP (single-strand conformation polymorphism) analysis of exon 15 contained 25% MDE (Mutation Detection Enhancement – FMC) and 5% glycerol, and were run for 14-15 h at 250V/4W at room temperature. The gels were silver-stained as described by Orita *et al.* (1989). Alterations were identified by comparison with known sequenced samples. Nucleotide sequences were aligned using the Alignment Explorer/ClustalW Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0 (Tamura *et al.*, 2007). Linkage disequilibrium was calculated using the DNA Sequence Polymorphism 5 (DNASP) program (Rozas and Rozas, 2000) and the haplotype construction.

We investigated 26 patients with GM1 gangliosidosis for mutations R59H and 1622-1627 and for polymorphisms R521C and S532G. Twenty-two patients had one or both mutations. The frequencies of R59H and 1622-1627insG were 19.2% and 38.5%, respectively (Table 1). The frequency of the S532G polymorphism was 15.4%, while that of the R521C polymorphism was 0 (zero); the latter polymorphism was however present in one of the parents of four patients. Four patients presented none of the aforementioned mutations.

The frequencies of these two disease-causing mutations and of the two polymorphisms in 390 control subjects

**Table 1** - Allele frequencies in patients and controls.

Alleles	Patients		Controls	
R59H	10/52 =	0.192	1/780 =	0.001
1622-1627insG	20/52 =	0.385	3/780 =	0.004
S532G	8/52 =	0.154	58/780 =	0.074
R521C	0/52 =	0.0	21/780 =	0.027

are also shown in Table 1. The R59H mutation was in heterozygosis in one subject, and the 1622-1627insG was in heterozygosis in another three. The estimated overall frequency of these two mutations is 0.5% in the normal population of Porto Alegre. The higher frequency of polymorphism S532G in patients (0.154) than in controls (0.074) was marginally significant ( $p = 0.046$ , Fisher's Exact Test), whereas R521C showed no difference between the two groups.

Because R59H and 1622-1627insG correspond to 57.7% of the disease-causing alleles, it was possible to estimate the frequency of mutant alleles in the population of Porto Alegre at 0.0007509 and the carrier proportion at 1:58. These figures would lead to a disease incidence of 1:13,317 live births.

Polymorphisms R521C and S532G were used in haplotype analysis to identify a founder effect in the Brazilian population. Seven different haplotypes were found. Table 2 shows the haplotype frequencies in patients and in controls. In about 10% of the patients and 2% of the controls it was not possible to determine the haplotypes.

In the patient group, haplotype 2 was the most frequent (36.5%), but close to haplotype 1 (34.6%). Haplotypes 3 and 7 were found only in the control group, in which haplotype 1 was the most frequent (89.1%). The 1622-1627insG mutation was not found to be in linkage disequilibrium with any polymorphism, whereas R59H was found to be in linkage disequilibrium with S532G (haplotype 5) in the patient group ( $p < 0.001$ ).

Severini *et al.* (1999) reviewed 63 GM1 type I cases diagnosed at the LREIM/HCPA and found that 68% were

**Table 2** - Haplotype frequencies (as to the presence of R59H and 1622-1627insG mutations and R521C and S532G polymorphisms) in patients and controls.

Haplotype	R59H;1622-1627insG; R521C;S532G*	Patients (n = 52)	Controls (n = 780)
1	1-1-1-1	0.346	0.891
2	1-2-1-1	0.365	0.003
3	1-2-1-2	0.0	0.001
4	2-1-1-1	0.058	0.001
5	2-1-1-2	0.097	0.0
6	1-1-1-2	0.019	0.068
7	1-1-2-1	0.0	0.019
8	ND	0.115	0.016

\* 1 = wild-type allele; 2 = mutant allele; ND = not determined.

from the State of Rio Grande do Sul. The epidemiological study suggested a carrier frequency of 1:67. This observation was confirmed by the molecular analysis of the 390 control subjects of the present work. The presence of four heterozygous individuals led to the determination of a carrier frequency of 1:58 in Porto Alegre. This number is similar to the phenylketonuria carrier frequency in the same area (1:56) (Santana da Silva *et al.*, 2003) and similar to that of Gaucher and Tay-Sachs disease carriers among Ashkenazi Jews (Risch *et al.*, 2003).

Using these figures, we were able to estimate the incidence of GM1 type 1 in Porto Alegre at 1:13,317 live births, slightly higher than that of 1:17,000 live births reported by Severini *et al.* (1999). These frequencies are extremely high when compared to those found in other studies, reported to be 1:100,000-1:200,000 (Beattie and Harvey, 1992; Sinigerska *et al.*, 2006) and 1:320,000 (Meikle *et al.*, 1999). The small proportion of consanguineous couples in our sample (a single case out of 26) is also consistent with the high incidence of this disorder in Brazil, especially considering that 27% of the patients were found to be homozygous for one of the two analyzed mutations.

The two mutations (R59H and 1622-1627insG) were detected in 57.7% of the alleles. This number is quite high for a heterogeneous genetic disorder like GM1 gangliosidosis, for which about 100 mutations were described (Brunetti-Pierri and Scaglia, 2008). The R59H mutation was first described by Morrone *et al.* (1997) in an Italian patient. Silva *et al.* (1999) found this mutation in 23.8% of the alleles of 20 Brazilian patients. These patients presented cardiac involvement similar to that described by Morrone *et al.* (2000). Santamaria *et al.* (2006) and Sinigerska *et al.* (2006) suggested that the R59H mutation is of Gypsy origin and that the presence of this mutation in South America is a reflection of their diaspora.

The 1622-1627insG mutation has only been reported in Brazilian patients so far, except for one patient from Uruguay described by Santamaria *et al.* (2007a). Silva *et al.* (1999) found a frequency of 42.8% for this mutation. Taken together, these two mutations showed a frequency of 62.5% in the patients studied by Silva *et al.* (1999), which is similar to the one found in the present work (57.7%).

Polymorphism S532G was significantly more frequent in patients than in controls. It is not clear if this alteration has an effect on protein activity, because expression data showed contradictory results, with values ranging from 41% (Hilson *et al.*, 1995) to 86% (Zhang *et al.*, 2000) of normal enzyme activity.

The R521C polymorphism was found only in controls and in four parents of patients, but not in any of our patients. This polymorphism was first described by Silva *et al.* (1999) and was present in 10% of patients' alleles. However, in that sample, this polymorphism was found in patients bearing other mutations than the ones studied in this work. Caciotti *et al.* (2005) described a patient who was ho-

mozygous for R521C and assumed that it was likely to be the disease-causing mutation. In the work of Santamaria *et al.* (2007b), the R521C polymorphism resulted in 33.2% enzyme activity. This observation could explain the apparent paradox of 4% of the general population of Porto Alegre carrying the R521C mutation, since it could have a low penetrance rate (Santamaria *et al.*, 2007b).

The lower frequency of haplotypes 6 (S532G only) and 4 (R59H only) in relation to haplotype 5 (R59H and S532G) suggests that each of these DNA changes took place independently and that recombination events were responsible for their grouping in haplotype 5. Moreover, the statistical analysis indicated the occurrence of at least one recombination event.

It was not possible to precisely define the existence of a founder effect, despite the high frequency of 1622-1677insG among patients, because this mutation was not linked to any of the polymorphisms studied. As this mutation is an insertion of one G into a string of six Gs, multiple origins cannot be ruled out. However, if this were the case, this mutation would be expected to have also been found in other populations. The fact that this mutation was never reported in any other region is strongly suggestive of a founder effect in the southern part of Brazil and in Uruguay. Supporting this hypothesis, there is the high proportion of homozygous patients with a low consanguinity rate found in this work and by Silva *et al.* (1999) in the same population.

The data obtained in this study confirm the high incidence of Type 1 GM1 gangliosidosis and the high frequency of two common mutations (R59H and 1622-1727insG) in southern Brazil. It was, however, not possible to prove that the high frequency of the latter mutation is due to a founder effect.

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