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Short Communication

Genotypic and phenotypic characterization of Brazilian patients with GM1 gangliosidosis

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ABSTRACT

GM1 gangliosidosis is a lysosomal disorder caused by β-galactosidase deficiency due to mutations in the *GLB1* gene. It is a rare neurodegenerative disorder with an incidence of about 1:100,000–1:200,000 live births worldwide. Here we review *GLB1* mutations and clinical features from 65 Brazilian GM1 gangliosidosis patients. Molecular analysis showed 17 different mutations and c.1622–1627insG was the most frequent, accounting for 50% of the alleles. Cognitive impairment was the main clinical sign, observed in 82% of patients, followed by hepatosplenomegaly observed in 56% of patients. It was possible to establish a significant correlation between age at onset of symptoms preceding the first year of life and the presence of the mutation c.1622–1627insG ($p = 0.03$). Overall our findings differ from literature and represent the exclusive genotypic profile found in Brazilian GM1 gangliosidosis patients.

1. Introduction

Hereditary deficiency of lysosomal acid β-galactosidase (EC 3.2.1.23) is expressed clinically as two different diseases: GM1 gangliosidosis and mucopolisaccharidosis IV B (Morquio B) (Suzuki et al., 2001). This deficiency occurs due to mutations in the *GLB1* gene, although the differences that lead to GM1 gangliosidosis or Morquio B are not completely understood. *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). It has been shown that more than 130 different mutations are responsible for Morquio B or GM1 gangliosidosis phenotypes (Brunetti-Pierri and Scaglia, 2008; Callahan, 1999; Hofer et al., 2009). However, patients with intermediate phenotype have also been described (Giugliani et al., 1987; Mayer et al., 2009).

GM1 gangliosidosis is a neurodegenerative condition for which three main clinical forms have been identified: type I (infantile, OMIM# 230500), type II (late infantile/juvenile, OMIM# 23060), and type III (adult, OMIM# 23650) (Brunetti-Pierri and Scaglia, 2008). The severe infantile phenotype (type I) is characterized by psychomotor regression by the age of 6 months, visceromegaly, cherry red spot, facial and skeletal abnormalities. GM1 gangliosidosis type II usually starts between 7 months and 3 years of age with slowly progressive neurological signs including early motor problems, strabismus, muscle weakness, seizures, lethargy, and susceptibility to infections. Dysmorphisms and skeletal changes are less severe than seen in type I. The adult form (type III), the attenuated phenotype, with onset between 3 and 30 years, is characterized by cerebellar dysfunction, dystonia, slurred speech, short stature and mild vertebral deformities (Callahan, 1999; Suzuki et al., 2001). The incidence of GM1 gangliosidosis is considered to be between 1:100,000 and 1:200,000 live births (Sinigerska et al., 2006), although higher frequencies have been described for specific regions, such as Malta (1:3,700) (Lenicker et al., 1997). The estimated incidence in Brazil has been calculated to be 1:13,317 live births, and the carrier frequency was found to be 1:58 (Baiotto et al., 2011). Mutation analysis performed in Brazilian patients detected the presence of mutations c. 1622–1627insG and/or p. R59H in 62.5% and 57.7% of the alleles (Baiotto et al., 2011; Silva *et al.*, 1999). Here, we report the results of *GLB1* gene mutation screening in 65 patients with GM1 gangliosidosis and clinical data in 32 of them.

2. Materials and methods

2.1. Patients and ethics

The present work has been approved by the Ethics Research Committee of Hospital de Clinicas de Porto Alegre, followed the Declaration of Helsinki, and the standards established by the author's Institutional Review Board and granting agency.

Abbreviation: LREIM, Laboratory of Inborn Errors of Metabolism; HCPA, Hospital de Clínicas de Porto Alegre; **DNA**, Deoxyribonucleic acid; **SSCP**, Single-stranded conformational polymorphism; **PAGE**, Polyacrilamide gel electrophoresis; SIFT, Sorting Intolerant from tolerant; **cDNA**, Complementary deoxyribonucleic acid.

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Patients were referred from different regions from Brazil and were diagnosed at the Laboratory of Inborn Errors of Metabolism from Hospital de Clínicas de Porto Alegre (LREIM/HCPA), Brazil. Clinical suspicion was confirmed by low enzyme activity of β-galactosidase in leukocytes or fibroblasts. Residual β-galactosidase activity from patients was measured using the artificial 4-methylumbelliferyl β-galactopyranoside substrate (Sigma).

2.2. Molecular analysis

Molecular data were obtained from patient's records and most of the mutations have been published elsewhere (Baiotto et al., 2011; Silva et al., 1999; Vieira, 2006). For the 27 out of 65 patients not previously analyzed, genomic DNA was extracted from peripheral blood leukocytes using the Easy-DNA Kit (Invitrogen). Molecular analysis was performed by PCR using primers and conditions described by Silva et al. (1999). All exons were screened by Single Strand Conformational Polymorphism (SSCP) analysis followed by automated DNA sequencing to establish the specific mutation in each patient. The conditions used for SSCP varied according to the exons that were being analyzed: 8% or 12% polyacrylamide-agarose gel electrophoresis (PAGE) at room temperature. Amplicons with mobility shift were purified with Exo-SAP (GE Healthcare) and submitted to automated sequencing on ABI 3100 Genetic Analyzer using BigDye v3.0 (Life Technologies).

Novel mutations were analyzed *in silico* using computer programs to predict the effect on protein. PolyPhen2 was accessed at http://genetics.bwh.harvard.edu/pph2/ using protein sequence NP_000395.2 (Adzhubei et al., 2010). SIFT (Sorting Intolerant from Tolerant) was accessed at http://sift.jcvi.org using protein sequence ENSP00000306920 (Kumar et al., 2009). In addition, novel mutations were searched at the 1000 genomes project (Durbin et al., 2010) (www.1000genomes.org) that contains data from different population groups.

2.3. Clinical features

Clinical information was obtained from patient's records. Correlation between age at onset of symptoms and the presence of mutation was analyzed using chi-square test with Fisher's exact test.

3. Results

3.1. Molecular analysis

From 1982 to 2012, 65 patients were diagnosed with GM1 gangliosidosis at LREIM/HCPA and had their DNA analyzed. The molecular and clinical characteristics of some of these patients have been previously reported (Baiotto et al., 2011; Silva et al., 1999; Vieira, 2006). For the 27 previously unreported patients, sequencing of exons with mobility shift detected by SSCP, revealed three novel mutations. All new mutations are missense (p.Q580R, p.R590S and p.C626R), and located in exons 15 and 16. Taken together, the 65 patients present 17 different mutations: 13 missense mutations, 3 frameshift mutations and one splice mutation (Table 1 and Suppl. Table 1).

The three novel mutations were analyzed *in silico* by SIFT and PolyPhen. PolyPhen predicted that p.R590S and p.C626R would be probably damaging and p.580R would be possibly benign. On the other hand, SIFT predicted that p.Q580R and p.R590S would be damaging, whereas p.C626R would be tolerated. In addition, these changes were searched at the 1000 genomes (www.1000genomes.org) and none of them was found (for instance, p.S532G has a frequency of 5.8%).

The c.1622–1627insG was the most frequent mutation, found in 67 out of 130 mutated alleles, accounting for 51.5% of the alleles found. It is followed by p.R59H mutation, which showed a frequency of 19.2% (25/130). The other mutations were found in only one or two alleles (Table 1). In 16 alleles the disease-causing mutation was not determined, in part due to the lack of enough biological material. However, the possibility of gross deletions may not be excluded, as these were not investigated by whole gene sequencing at genomic and cDNA level.

Table 1
Allele frequencies in 65 Brazilian GM1 gangliosidosis patients ($n = 130$ chromosomes).

Alleles	Frequency	Number of alleles	Reference
c.1622–1627insG	0.51	67	Silva et al., 1999
p.R59H	0.19	25	Morrone et al., 1997
c.638-641insT	0.02	3	Silva et al., 1999
p.R201H	0.01	2	Kaye et al., 1997
p.R208C	0.01	2	Boustany et al., 1993
p.Y36S	0.01	2	Vieira, 2006
p.V240M	0.01	2	Silva et al., 1999
p.Q580R	0.01	2	New
p.R38G	0.007	1	Vieira, 2006
p.F63Y	0.007	1	Vieira, 2006
p.Y64F	0.007	1	Vieira, 2006
p.R121S	0.007	1	Silva et al., 1999
c.895–896insC	0.007	1	Silva et al., 1999
c.2(+1)G>A	0.007	1	Georgiou et al., 2005*
p.D491N	0.007	1	Silva et al., 1999
p.R590S	0.007	1	New
p.C626R	0.007	1	New
ND	0.12	16	–

ND = not determined.

3.2. Clinical and biochemical features

This is a retrospective study in which clinical information was obtained from patients' records, thus, we only had access to clinical information of 32 of these patients. For 32 of these patients, biochemical reports were available to obtain the levels of enzyme activity for β-galactosidase (Table 2). Residual activity in leucocytes or fibroblasts from patients, measured using 4-methylumbelliferyl-β-galactopyranoside artificial substrate, varied from 1.74 to 14 nmol/h/mg protein on leukocytes and 5–33 nmol/h/mg protein on fibroblasts. The average enzyme activity of β-galactosidase was 6.5 nmol/h/mg protein on leukocytes (normal reference value: 78–280 nmol/h/mg protein); and 19 nmol/h/mg protein on fibroblasts (normal reference value: 394–1440 nmol/h/mg protein). Even though neuraminidase assay has not been performed to exclude the diagnosis of galactosialidosis, all patients had at least one mutated allele in *GLB1*, thus confirming the diagnostics of GM1 gangliosidosis.

Five patients had the infantile form of the disease, 15 patients had the juvenile form and 9 had the adult form. Cognitive impairment was the main disease manifestation, observed in 78% of patients; followed by hepatosplenomegaly that was observed in 59% of the patients analyzed (Table 2). Other frequent clinical signs were multiple dysostosis (37%), hypotonia (34%), coarse facies (31%), macular cherry-red spots (21%), seizures (18%), ataxia (12%), and dystonia (12%).

Ophthalmologic findings observed included nystagmus, squint, hypertelorism and amaurosis. Other involvements of the central nervous system were characterized by macrocephaly, hydrocephalus, cerebellar atrophy, dysarthria, speech delay, and leukodystrophy. Finally, clinical findings involving the bone structure were kyphosis, espondilo-epiphyseal dysplasia, kyphoscoliosis, skull thickness, and generalized skeletal deformities.

3.3. Genotype–phenotype correlations

No clear genotype–phenotype relationship could be established for most patients. The most frequent mutation, c.1622–1627insG, was associated with cognitive delay and hypertonia in homozygous patients and ophthalmic findings in compound heterozygous patients (Table 2). Enzyme activity was also highly variable, even for patients with the same