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Original Article

Molecular Analysis of 9 Unrelated Families Presenting With Juvenile and Chronic GM1 Gangliosidosis

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Abstract

GM1 gangliosidosis is a rare autosomal recessive lysosomal storage disorder with high prevalence in Brazil (1:17 000). In the present study, we genotyped 10 individuals of 9 unrelated families from the States of São Paulo and Minas Gerais diagnosed with the juvenile and chronic forms of the disease. We found the previously described p.Thr500Ala mutation in 8 alleles; c.1622-1627insG and p.Arg59His in 2 alleles (the latter also segregating with c.1233+8T>C); and p.Phe107Leu, p.Leu173Pro, p.Arg201His, and p.Gly311Arg in 1 allele each. Two mutations (p.Ile354Ser and p.Thr384Ser) and 1 neutral alteration (p.Pro152=) are described for the first time. All patients presented as compound heterozygotes. A discussion on genotype–phenotype correlation is also presented.

Keywords

GLB1 gene, β-galactosidase, mutation, Brazilian population, sequencing

Introduction

GM1 gangliosidosis (MIM# 230500) is an autosomal recessive lysosomal storage disorder caused by β-galactosidase (βGal) enzyme deficiency (E.C. 3.2.1.23) which is coded by *GLB1* gene and involved in the degradation of GM1 ganglioside, oligosaccharides carrying terminal β-linked galactose and glycosaminoglycans (keratan sulfate). The reduction in or absence of βGal leads to the excessive accumulation of GM1 ganglioside within lysosomes, particularly in the neuronal tissues.[1] The incidence of GM1 gangliosidosis has been estimated in about 1 in 100 000 to 200 000 live births with pan-ethnic distribution.

The disease has been classified into 3 clinical forms based on the age of onset and severity of symptoms: infantile (type I), juvenile (type II), and chronic/adult (type III). They all present with variable degrees of progressive bone dysplasia (dysostosis multiplex), visceromegaly, and neurologic deterioration with or without cherry-red spot. The infantile form of the disease is described more often than the others. However, in Japan, adult patients have most been reported.[1] There is an overlap with the so-called Morquio B disease, which presents a massive dysostosis multiplex with storage of keratan sulfate but without proven involvement of primary central nervous system.[2]

The enzyme activity varies from 0.07% to 1.3% in patients with the infantile form, from 0.3% to 4.8% in patients with the juvenile form, and up to 9% in the adult chronic form of the disease. The severity of each type is inversely related to the residual activity of the mutant βGal enzyme.[3]

The βGal gene (*GLB1*) is located on chromosome 3p21.33 and contains 16 exons spanning approximately 62.5 kb. The complementary DNA encodes 677 amino acids, and up to date more than 140 mutations have been identified in this gene.[4] Brunetti-Pierri and Scaglia[5] reviewed clinical, molecular, and therapeutic aspects in 209 patients with all types of GM1 gangliosidosis, comprising 130 infantile, 23 juvenile, and 56 adult patients. Among them, a total of 102 mutations in the *GLB1*

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gene have also been reviewed by these authors, showing extensive molecular heterogeneity and hindering a clear genotype–phenotype correlation.

The *GLB1* gene encodes 2 alternatively spliced products, GLB1 and elastin-binding protein (EBP). The GLB1 associates with protective protein/cathepsin A (PPCA) and neuraminidase to form a complex that is essential for its stabilization and for its normal posttranslational processing to the mature form of the enzyme complex. It was already demonstrated that PPCA is essential for intralysosomal activation and stabilization of the other 2 enzymes.[6,7] Caciotti et al[3] described 3 patients with the infantile form of the disease, who had a markedly reduced amount of PPCA in Western blot analysis of the fibroblasts, suggesting that some GLB1 regions might be regulation sites in the interaction or stabilization of GLB1 and PPCA in the lysosomal complex.

The alternative splice product, EBP, results from deletion of exons 3, 4, and 6 and a frameshift in the translation of exon 5 encoding the unique elastin-binding domain of EBP. The EBP is a major component of the non-integrin cell surface elastin receptor complex. Although the involvement of EBP in GM1-gangliosidosis and Morquio B has yet to be fully elucidated, some key aspects have been identified, such as a relationship between mutations either affecting EBP or impaired elastic–fiber assembly.[3,7]

Large case series with clinical descriptions of GM1 gangliosidosis are rare, particularly in non-Japanese individuals,[8] and only a few focuses specifically on types II and III GM1 gangliosidosis. In this article, we report the results of a mutational screening in 9 families from Brazil with GM1 gangliosidosis. We identified 9 different well-known pathogenic mutations and present 3 undescribed alterations.

Patients and Methods

Patients

Mutation analysis was performed on DNA extracted from lymphocytes in 10 patients (including a pair of siblings) belonging to 9 unrelated families presenting with juvenile or chronic GM1 gangliosidosis. Their relatives, when available, went through the same procedure. All patients were from the region of Campinas, São Paulo, and the southern neighboring state of Minas Gerais and have been evaluated by the same clinical geneticist. Genetic studies were carried out according to institutional guidelines and after obtaining informed consent in accordance with the Helsinki declaration of 1975 (as revised in 1983). The study was approved by the Research Ethics Committee, FCM/University of Campinas, under process number 236/2011 (CAAE: 0179.0.146.000-1).

Initial symptoms in most cases consisted of short trunk dwarfism (individuals 1, 2, 3b, 4, 5, 6, and 8) with radiological aspect of dysostosis multiplex and laboratorial finding of chondroitin sulfaturia in routine metabolic screening. Afterward, they all developed variable degrees of dystonia, dysarthria, and dysphagia. Individuals 3a, 7, and 9 initially presented with neurological symptoms such as gait disorder, learning disability, or cognitive regression. Clinical follow-up revealed dystonia, dysarthria, and dysphagia, in addition to bone changes compatible with dysostosis multiplex. The diagnosis was confirmed in all patients by the low level of βGal enzyme activity in leukocytes. Patient's clinical data and biochemical findings are summarized in Table 1, and a more detailed clinical description was provided by Kannebley et al.[9]

Methods

All patients were diagnosed with GM1 gangliosiosis confirmed by low enzyme activity of βGal measurement in leukocytes or fibroblasts using artificial 4-methylumbeliferyl β-galactosidase.

Genomic DNA was amplified by polymerase chain reaction (PCR) for the 16 exons and their flanking regions of *GLB1* gene. Gene nucleotide numbering is according to sequence NM_000404.2, and the genomic reference is NC_000003.11.

Direct DNA sequencing was performed with Kit BigDye Terminator Cycle Sequencing Standard Version 3.1 in the ABI 3500 xL Genetic Analyzer (Applied Biosystems, Austin, TX, USA), and the products used according to the manufacturer recommendations. The same primers used to amplify DNA were used for sequencing the amplified PCR product.

The new missense alterations found were analyzed in silico by prediction softwares (MutPred,[10] Polyphen,[11] and Phd-SNP[12]), screened in 50 controlss, and the polymorphic effect was excluded by the analysis of the frequencies in the 1000 Genomes Project database.[13]

Results

Molecular testing of *GLB1* gene identified 5 previously known missense mutations: p.Arg59His (rs72555392; c.176G>A; g.33114105C>T),[14] p.Phe107Leu (rs397515616; c.319T>C; g.33110389A>G),[15] p.Leu173Pro (rs397515617; c.518T>C; g.33106989A>G),[16] p.Arg201His (rs189115557; c.602G>A; g.33099712C>T),[17] p.Gly311Arg (rs368568171; c.1048G>A; g.33093274C>T),[18] and p.Thr500Ala (rs72555368; c.1498A>G; g.33055784T>C).[2] The other mutations comprise 1 known polymorphism, c.1233+8T>C (rs13093698; g.33063050A>G); 1 new neutral mutation, p.Pro152= (rs397515618; c.456A>T, g.33109723T>A); the known insertion, c.1622-1627insG (g.33055710_33055705insG)[19]; and 2 previously undescribed missense mutations, p.Ile354Ser (rs397515613; c.1061T>G; g.33087619A>C) and p.Thr384Ser (rs397515614; c.1150A>T, g.33063141T>A). All patients are compound heterozygotes. Results are also shown in Table 1.

Discussion

The p.Thr500Ala was the most common mutation in this group found in 40% of the identified alleles. This codon has been previously described as crucial for Morquio B disease[15] which was the main clinical presentation at referral in this sample. This frequency differs from another sample of the Brazilian

Table 1. Clinical Synopsis, Biochemical, and Mutations Found in This Sample.[a]

Patient	Diagnosis at Referral	Current Clinical Phenotype	Age of onset (Current age)	β-Galactosidase Activity Level	Simultaneous Control Enzyme	Neuraminidase Activity	Mutation 1 Origin	Mutation 2 Origin	Polymorphism Origin
1	"Morquio B"	GM1 gangliosidosis type 3	3 years (31 years)	15 (RV: 394-1440) Fibroblasts 38%	α-Iduronidase 77 (RV: 74-148) Fibroblasts	17 (RV: 30-38) Fibroblasts	[b]p.Thr384Ser NP	p.Thr500Ala Maternal	–
2	Spondyloepiphyseal dysplasia	GM1 gangliosidosis type 2	2 ½ years (32 years)	6 (RV: 78-280) Leukocytes 7.7%	β-glucosidase 12.2 (RV: 10-45) Leukocytes	NP	p.Arg59His Maternal	p.Thr500Ala NP	c.411+8T>C Maternal
3a	Neurologic regression + bone dysplasia	GM1 gangliosidosis type 3	7 years (29 years)	3.3 (RV: 78-280) Leukocytes 4.2%	α-Iduronidase 204 (RV: 74-148) Fibroblasts	4.9 (RV: 1.02-5.9) Fibroblasts	pArg59His Maternal	p.Arg201Hih Paternal	c.411+8T>C Maternal
3b	Neurologic regression + "Morquio B"	GM1 gangliosidosis type 3	7 years (26 years)	4 (RV: 78-280) Leukocytes 5.1%	β-Glucosidase 9.8 (RV: 10-45) Leukocytes	NP	p.Arg59His Maternal	p.Arg201His Paternal	c.411+8T>C Maternal
4	"Morquio B"	GM1 gangliosidosis type 2	14 months (27 years)	7.8 (RV: 78-280) Leukocytes 10%	β-Glucosidase 8.6 (RV: 10-45) Leukocytes	NP	p.Thr500Ala Maternal	c.1622_1627insG NP	–
5	"Morquio B"	GM1 gangliosidosis type 2	12 months (23 years)	21 (RV: 394-1440) Fibroblasts 5.3%	α-Iduronidase 48 (RV: 32-56) Leukocytes	43 (RV: 30-38) Fibroblasts	p.Thr500Ala Maternal	–	–
6	"Morquio B" + speech disorder	GM1 gangliosidosis type 3	3 ½ years (23 years)	9 (RV: 78-280) Leukocytes 11.5%	α-Iduronidase 130 (RV: 74-148) Fibroblasts	54 (RV: 30-38) Fibroblasts	p.Gly311Arg Maternal	p.Thr500Ala Paternal	–
7[c]	Neurologic regression	GM1 gangliosidosis type 3	3 years (18 years)	11.7 (RV: 394-1440) Fibroblasts 2.9%	Arllsulfatase A 9.1 (RV: 5-20) Leukocytes	19 (RV: 30-38) Fibroblasts	p.Phe107Leu Maternal	p.Leu173Pro-	–
8	"Morquio B"	GM1 gangliosidosis type 3	5 years (21 years)	9.3 (RV: 78-280) Leukocytes 11.9%	Galactose 6-sulfate sulfatase 0.72 (RV: 0.53-1.03) Fibroblasts	NP	[b]p.Ile354Ser NP	p.Thr500Ala Maternal	–
9	"Morquio B"	GM1 gangliosidosis type 3	12 years (21 years)	36 (RV: 394-1440) Fibroblasts 9.1%	α-Iduronidase 114 (RV: 74-148) Fibroblasts	33 (RV: 30-38) Fibroblasts	p.Thr500Ala Maternal	c.1622_1627insG Paternal	–

Abbreviations: NP, not performed; RV, reference value.
[a]Values in nmol/h/mg protein; RefSeq: NM_000404.2.
[b]New mutation.
[c]In this patient we also found a new neutral alteration p.Pro152= (paternal origin).

population collected in Rio Grande do Sul, for which the most common alteration was the insertion c.1622-1627insG.[19,20] This difference could be explained by variation in genetic backgrounds in these geographic regions and/or the fact that the present sample included only individuals presenting with the juvenile and the chronic forms of GM1 gangliosidosis, while the group in Rio Grande do Sul studied patients with mainly the infantile form.

A genotype–phenotype correlation was initially discussed by Bagshaw et al[2] when this mutation was first described and followed by Santamaria et al.[16] These authors investigated patients with the clinical diagnosis of Morquio B disease. Due to initial skeletal findings, all the patients presenting the p.Thr500Ala alteration were referenced to our service by pediatricians or orthopedists with a clinical diagnosis of Morquio disease. However, after several years, all of them developed a combination of skeletal and neurologic symptoms.

Recently, Bidchol et al[4] reported a large cohort of patients with GM1 gangliosidosis in India, most of them presenting the infantile form. The p.T500A mutation was not found in this population, and only c.1622_1627insG (p.W527Lfs*5) was common in both series. Thus, besides differences in clinical presentation (mainly age of onset) and allele frequency, ethnic background variation is seen in both populations.

The second most frequent alterations were c.1622-1627insG and p.Arg59His presented in 2 families each. While the former was described only in the Brazilian population,[19,20] the latter was associated with the gipsy population from India[21] but was also reported in the southern Brazilian population.[19,20] The p.Arg59His mutation is known to cause the infantile form of GM1 gangliosidosis. This codon was almost totally buried inside the protein core, forming hydrogen bonds with the codons 56 (histidine), 313 (asparagine), 128 (alanine), and 129 (glutamic acid). These interactions stabilized the structure of the ligand-binding pocket. When mutated to histidine, these stabilizing interactions were disrupted, and the shape of the ligand-binding pocket was changed.[22]

In the present sample, mutation p.Arg59His (c.176G>A) also segregates with c.1233+8T>C, which represents a previously unreported association. We consider it as a rare haplotype that could indicate a founder effect in our population, since it is a previously undescribed association found in 2 apparently unrelated families from the same geographic area.

Other mutations were described in 1 family each. Hofer et al[15] described the p.Phe107Leu mutation in 1 individual of Greek origin diagnosed with juvenile onset; in our sample, it was found in a patient presenting with onset at the age of 3 years, thus diagnosed as chronic type. This same individual presents the mutation p.Leu173Pro and the previously undescribed neutral alteration p.Pro152=. The p.Leu173Pro was described for the first time by Santamaria et al[16] in 2 caucasian patients. According to them, this mutation could be associated with less severe phenotypes because in EBP frame, it is a synonymous alteration that does not affect the EBP formation.

In our sample, individuals 3a and 3b presented with p.Arg201His as compound heterozygous with p.Arg59His/c.1233+8T>C. Curiously, both siblings presented with a late-onset and severe neurological degeneration, including bone disease, but only the boy showed a Morquio B phenotype. A homozygous patient for p.Arg201His was classified by Santamaria et al[16] as having Morquio B disease, while several other reports on heterozygous patients carrying p.Arg201His described more severe, juvenile, or adult GM1 phenotypes.[23,24] Our data support the causal relationship between the p.Arg201His and the juvenile or adult GM1 phenotype. The p.Arg201His mutation is located in the protein surface, far from the ligand-binding pocket. This codon is completely exposed to the solvent and formed a salt bridge to the 198 codon (aspartic acid). No large structural rearrangement occurs in either mutation except for loss of salt bridge.[22]

The p.Gly311Arg was described in a single case report of an individual presenting with developmental delay and Morquio B phenotype.[18] A similar presentation was seen in patient 6 of the present sample who also exhibited borderline achievement of the motor skills and speech delay.

The 2 new missense alterations presently described had the polymorphic effect excluded by the analysis of their frequencies in the 1000 genomes Project Database, as no alterations were found in the regions of p.Ile354Ser (g.33087619) and p.Thr384Ser (g.33063141) in the population samples studied. By in silico analysis, the p.Ile354Ser was classified as pathogenic mutation for all softwares due to a disorder caused by the substituting isoleucine for serine. According to MutPred ([http://mutpred.mutdb.org/] is a web application tool developed to classify an amino acid substitution as disease-associated or neutral in human. In addition, it predicts the molecular cause of disease/deleterious alterations through a code that calculates the evolutionary conservation), the substitution p.Thr384Ser presents 40% chance of being associated with the disease.

Finally, although p.Thr500Ala has been the most frequent mutation in this sample, a great diversity of mutations and the fact that all patients were compound heterozygotes suggest that *GLB1* is a gene with a great diversity of mutations. All these findings were possible because the study was conducted on gene sequencing. This approach allowed not only the identification of deleterious mutations but also the description of a neutral alteration and the previously unreported association of a known mutation with a polymorphism (p.Arg59His-c.1233+8T>C).

Conclusion

Despite the limited number of patients, our sample showed a diversity of alterations. Mutation p.Thr500Ala was the most frequent in this group and differs from another Brazilian study not only in the geographic and genetic background but in clinical subtype as well. In addition, this work pioneers in describing 2 missense mutations and 1 neutral alteration that add new information to GM1 gangliosidosis types II (juvenile) and III (chronic/adult) databases. Genotype–phenotype correlation is

still unclear. All individuals in the present sample and most in the literature present as compound heterozygotes.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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