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Identification and Clinical Characterization of a Novel Alpha-Galactosidase A Mutation

Nilton Salles Rosa Neto, MD, PhD[1]

Abstract

Fabry disease (FD) is an inborn error of metabolism characterized by deficient/absent activity of lysosomal enzyme alpha-galactosidase A, which results in systemic accumulation of glycosphingolipids and progression to renal failure, heart and cerebrovascular disease, and small-fiber peripheral neuropathy. This article describes a Brazilian family affected by FD caused by a novel mutation in exon 6 of the alpha-galactosidase A (*GLA*) gene (c.812G>C). Signs and symptoms identified were pain crisis, acroparesthesia, hypohidrosis, abdominal cramps and diarrhea, chronic kidney disease, cornea verticillata, left ventricular hypertrophy, and complete heart block. Headache was a common complaint and 1 of the patients presented with aseptic meningitis. The novel missense mutation in the *GLA* gene identified in this Brazilian family is consistent with the classic FD phenotype.

Keywords

Fabry disease, missense mutation, alpha-galactosidase A, aseptic meningitis, small-fiber neuropathy

Introduction

Fabry disease (FD; Online Mendelian Inheritance in Man [OMIM]301500) is an inborn error of glycosphingolipid catabolism characterized by deficient/absent activity of lysosomal enzyme alpha-galactosidase A (GLA).[1-3] The result of impaired enzyme activity is accumulation of glycosphingolipids, mainly globotriaoslyceramide (Gb3), in plasma and cellular lysosomes.[2,3] To date, more than 600 mutations responsible for FD have been identified.[4] Fabry disease should not be considered an X-linked "recessive" disorder since heterozygous women with FD may experience life-threatening conditions requiring treatment.[5,6]

Symptoms arise at childhood and complications begin at adolescence/early adulthood. Clinical manifestations and course of the disease vary even among members of the same family.[2] For all patients, this difference may depend on enzyme residual activity, which is related to the type of mutation,[1] and, in female patients, nonrandom X-chromosome inactivation may not fully explain phenotypic expression in all cases.[5,6] Accumulation of Gb3 leads to renal failure, heart and cerebrovascular disease, and small-fiber peripheral neuropathy among other signs and symptoms.[2]

Enzyme replacement therapy (ERT) is the available therapy and consists of intravenous infusion of recombinant enzyme, which reduces Gb3 accumulation and may halt progression of disease according to the time of diagnosis and initiation of treatment.[7] Early identification of patients should be a target for health care providers. This article describes a Brazilian family affected by FD and introduces a previously unreported mutation of the *GLA* gene.

Case Report

The index patient is a 15-year-old boy (III-5) who presented with relapsing bouts of pain on hands and feet and fever as high as 39.5°C. Episodes started at age 12 years, recurred every 5 months, and lasted approximately 3 days but were increasing in frequency. Events were induced by exercise or temperature changes and were self-limited. Patient also presented with acroparesthesia and hypohidrosis. There were no upper limbs, skin, gastrointestinal, or urinary symptoms. Past medical history was positive for allergic rhinitis. Physical examination was unremarkable. Family history revealed an aunt with fibromyalgia and a grandfather who died at age 33 years due to renal failure, both on the maternal side.

[1] Centro de Dor e Neurocirurgia Funcional, Hospital Nove de Julho, São Paulo, Brazil

Corresponding Author:
Nilton Salles Rosa Neto, Centro de Dor e Neurocirurgia Funcional, Hospital Nove de Julho, Rua Peixoto Gomide, 527, São Paulo, SP, Brazil – 01409-902.
Email: nsalles@yahoo.com

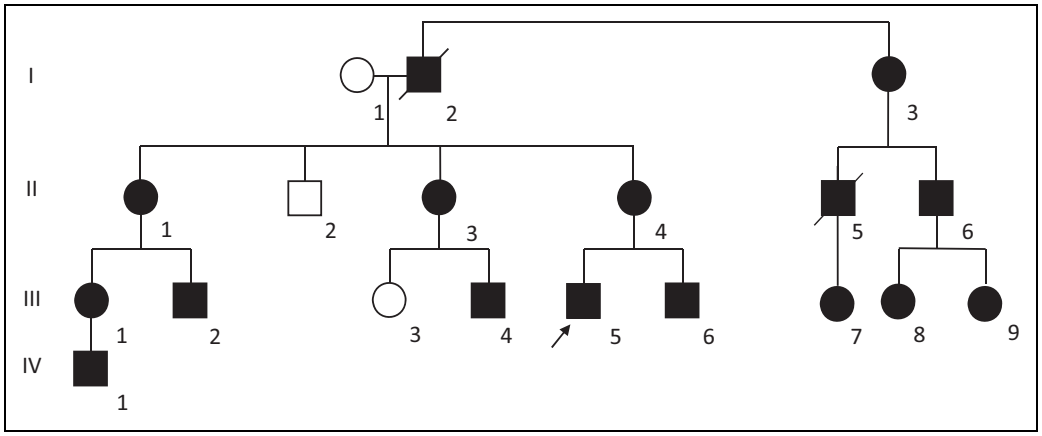

Figure 1. Family pedigree.

Based on history and examination results, a hypothesis of FD was made. Activity of GLA enzyme in dried blood spots was measured by tandem mass spectrometry (Centogene AG, Germany) resulting in 1.0 μmol/L/h (ref: ≥2.5μmol/L/h). The *GLA* gene was analyzed by polymerase chain reaction and sequencing of both DNA strands of the entire coding region and the highly conserved exon–intron splice junctions (Centogene AG). A previously unreported hemizygous variant in exon 6 (c.812g>c p.G271A) was found. Software analyses by Polymorphism Phenotyping (PolyPhen),[8] Sorts Intolerant From Tolerant substitutions (SIFT),[9-13] and Grantham Variation–Grantham Deviation (Align-GVGD)[14,15] predicted the variant as probably pathologic. Concentration of lyso-Gb3 measured by high-performance liquid chromatography/tandem mass spectrometry (Centogene AG) was 9.7 ng/mL (ref: 1.3 ± 0.9 ng/mL; 95% percentile: 3.2 ng/mL), within pathologic range.

Additional examinations included 24-hour urine protein collection within normal range, plus normal brain and angio magnetic resonance imaging (MRI), echocardiogram, renal ultrasound, lung function tests and audiometry. Ophthalmologic evaluation revealed cornea verticillata. Agalsidase-alpha 0.2 mg/kg every 2 weeks and gabapentin 300 mg/d were prescribed.

Eighteen relatives were evaluated according to the pedigree depicted in Figure 1. Fourteen family members were found to bear the same mutation on the *GLA* gene. Demographic and laboratory data are shown in Table 1. Signs and symptoms of affected/carrier patients are shown in Table 2.

Patient II-3 had a 19-year history of bouts of pain and edema on hands and feet and repeated hospital admissions. The patient did not have fever or did not report weakness. She had been extensively studied for autoimmune/rheumatic diseases and was eventually diagnosed with fibromyalgia. A striking feature was lack of response to opioid/nonopioid analgesics, steroidal/nonsteroidal anti-inflammatory drugs, and antidepressants/anticonvulsants. After FD diagnosis, cornea verticillata and left ventricular hypertrophy were identified.

Pain control was achieved with carbamazepine 1200 mg/d and chlorpromazine 50 mg/d. Agalsidase-alpha 0.2 mg/kg every 2 weeks was started. After 3 months of ERT, she developed refractory headache. Previous history of headache was consistent with infrequent migraine attacks and frequent tension-type headache complaints. Neurologic evaluation revealed hypoesthesia on both hands and neck stiffness. Fever or infectious signs were not observed. Fundoscopic examination was normal. A lumbar puncture disclosed lymphomonocytic pleocytosis with normal protein levels and absence of pathogens (Table 3). Brain angiogram MRI was normal. A diagnosis of aseptic meningitis (AM) was made, and continuous chlorpromazine and high-dose steroids were given for better pain control, and the medication eventually tapered. A month later headache relapsed, and cerebrospinal fluid (CSF) analysis 2 months later showed the recurrence of milder lymphomonocytic pleocytosis (Table 3). Limb pain had increased and a new autoimmune panel revealed positive antinuclear antibody (ANA) and antihistone. Diagnosis of drug-induced lupus (DIL) was made and carbamazepine and chlorpromazine were interrupted. The ERT continued and the patient still complained of worsening symptoms of limb pain alongside elevated lyso-Gb3 values. A repeated antihistone analysis and antiagalsidase-alpha antibodies immunoglobulin (Ig) E and IgG (enzyme-linked immunosorbent assay) were negative. Change in medication was proposed and agalsidase-beta started 1.0 mg/kg every 2 weeks with azathioprine 50 mg/d for AM. Headache and pain crises persisted. Gabapentin 3600 mg/d was introduced and azathioprine increased to 100 mg/d but still unsuccessful. Anticonvulsant medication was changed to lamotrigine 100 mg/d, and during hospital admission the patient received intravenous lidocaine 2% for 5 days with adequate control of pain. Two further CSF analyses revealed normal values (Table 3).

Patients I-3 and II-5 began agalsidase-alpha 0.2 mg/kg every 2 weeks. After 6 months of ERT, patient II-5 presented with septic shock because of pulmonary infection and died. The remaining family members are still being evaluated for target organ damage, refused treatment (II-4; II-6), or are under surveillance.

Table 1. Demographic and Laboratory Data of Family Members.

Family Member	Sex	Age, y	GLA Enzyme Activity (ref: ≥2.5 μmol/L/h)	GLA gene mutation	Lyso-Gb3 at diagnosis (ref: 1.3 ± 0.9 ng/mL)	Symptoms	Status
I-1	♀	63	NA	Absent	NA	None	Not carrier
I-2	♂	–	NA	NA	NA	Yes	Died
I-3	♀	70	NA	**Present**	3.2	Yes	Affected
II-1	♀	40	NA	**Present**	2.2	No	Carrier
II-2	♂	38	2.5	Absent	NA	None	Not carrier
II-3	♀	37	NA	**Present**	**11**	Yes	Affected
II-4	♀	32	NA	**Present**	2.0	Yes	Affected
II-5	♂	50	**0.4**	**Present**	**10.2**	Yes	Affected/died
II-6	♂	49	**0.3**	**Present**	**22.9**	Yes	Affected
III-1	♀	23	NA	**Present**	**3.9**	No	Carrier
III-2	♂	22	**0.1**	**Present**	**11.9**	No	Affected
III-3	♀	9	NA	Absent	NA	None	Not carrier
III-4	♂	3	**0.3**	**Present**	**11.2**	Yes	Affected
III-5	♂	15	**1.0**	**Present**	**9.7**	Yes	Affected
III-6	♂	3	**0.4**	**Present**	**10.2**	Yes	Affected
III-7	♀	4	NA	**Present**	1.1	No	Affected
III-8	♀	22	NA	**Present**	1.8	No	Carrier?
III-9	♀	18	NA	**Present**	2.4	No	Carrier?
IV-1	♂	1	**0.2**	**Present**	**3.7**	No	Affected

Abbreviations: NA, not available; GLA, alpha-galactosidase A; Gb3, globotriaosylceramide; Bold text denotes values or findings within pathological range.

Table 2. Signs and Symptoms of *GLA* Gene Mutation Carriers.

Family Member	Signs and Symptoms
I-3	Acroparesthesia, heart disease and pacemaker, chronic kidney disease
II-1	None
II-3	Pain crises, acroparesthesia, hypohidrosis, heat intolerance, abdominal cramps and diarrhea, migraine, aseptic meningitis, cornea verticillata, myocardial hypertrophy, elevated CRP
II-4	Migraine, acroparesthesia, parapelvic kidney cyst, elevated CRP
II-5	Acroparesthesia, chronic kidney disease on hemodialysis, heart disease and pacemaker
II-6	Pain crises, acroparesthesia, heart disease, renal transplantation because of chronic kidney disease
III-1	None
III-2	NA
III-4	Abdominal cramps and diarrhea, headache
III-5	Pain crises with fever, acroparesthesia, heat intolerance, hypohidrosis, cornea verticillata
III-6	Abdominal cramps and diarrhea, headache, pain crises with fever
III-7	Abdominal cramps, headache
III-8	NA
III-9	NA
IV-1	None

Abbreviations: CRP, C-reactive protein; GLA, alpha-galactosidase A; NA, information not available.

Discussion

Fabry disease is associated with dysfunction of several cell types and results in systemic vasculopathy. Mutations in the *GLA* gene responsible for FD are located in Xq22. Despite the fact that deletion and insertion mutations were found to cause frameshifts, most mutations are point mutations, including missense, nonsense, and splicing mutations.[2]

In this article, a Brazilian family with classical FD is depicted. Sequence analysis of the *GLA* gene revealed a single-nucleotide point mutation at nucleotide c.812G>C in exon 6. This results in the replacement of glycine by alanine at codon 271, probably influencing the folding of the protein, altering normal GLA activity. Amino acid changes at this position have already been described as causing disease, in 2002 (c.811G>T p.G271C)[16] and 2006 (c.812G>T p.G271V and c.811G>A p.G271S).[17] All are missense mutations of buried residues and associated with classical FD.[4]

Heterogeneity of FD manifestations causes high variability in its clinical symptoms, in both hemizygous and heterozygous patients.[2,5] Manifestations often start in childhood or adolescence and include acroparesthesias and heat and exercise intolerance.[2] Additional signs and symptoms may include recurrent pain crises, angiokeratomas, hypohidrosis, cornea verticillata, and abdominal cramps. Progressive Gb3 accumulation in vascular endothelium eventually leads to chronic renal disease and/or vascular disease of heart and brain.[2]

In this family, 3 male patients and 1 female patient developed chronic kidney disease. Two died (I-2 and II-5) and patient II-6 had a renal transplantation. Patients I-3 and II-5 had pacemakers implanted, and patients II-3 and II-6 are under surveillance for cardiomyopathy.

Diagnosis of Fabry disease in male patients with classic/variant phenotypes can be achieved by demonstration of deficient GLA activity in plasma, isolated leukocytes, and/or cultured cells.[2] However, female patients often have mildly reduced

Table 3. Cerebrospinal Fluid Analyses for Patient II-3.

Variable	Results			
Date	April 10, 2013	June 22, 2013	February 27, 2014	June 30, 2014
Initial pressure, cm H_2O	29	–	>20	15
Final pressure, cm H_2O	18	–	–	10
Protein, mg/dL	29	44	18	19
Glucose, mg/dL	75	74	51	56
Lactate, mg/dL	12.4	14.4	10	10
Erythrocytes, cells/mm^3	0	0	0	0
Leucocytes, cells/mm^3	14	4	1	3
Neutrophils	1%	0	–	–
Eosinophils	0	0	–	–
Lymphocytes	83%	96%	–	–
Monocytes	16%	4%	–	–

or normal enzyme activity. Therefore, finding of a mutated *GLA* gene is needed for confirmation of FD in female patients.[5]

Neurological features presented in this family are remarkable. Extremity pain and headaches were common complaints. Of note, patient II-3 had significant neurological symptoms, with drug refractory extremity pain, recurrent pain crises, and AM.

In FD, neurological symptoms reflect damage to small fibers of peripheral and autonomic systems.[18] Heterozygotes have higher rates of peripheral neuropathy.[3] The term refractory pain used to address patient III-2 should be viewed with discretion since ERT studies evaluated improvement in pain outcomes with agalsidase-alpha at 6 to 36 months of therapy[2,19] and agalsidase-beta at 18 to 23 months of therapy,[2,18,19] and patient III-2 did not reach such length of ERT use.

Headache and AM had already been described in FD. Aseptic meningitis was associated with intracranial hypertension and multiple/recurrent strokes.[20-23] No such changes were found in patient II-3. The use of carbamazepine has been implicated with AM, but reported cases in the literature presented with fever and skin rash, features not reported in these cases.[24,25] A possible diagnosis of syndrome of transient headache and neurological deficits with CSF lymphocytosis cannot be excluded, but considering the lack of definite neurological deficit and the number of family members affected with headache, assigning AM to FD seems more reasonable.[26,27] Azathioprine therapy was described in FD with AM alongside ERT with reduction in the CSF cell count.[23] In patient II-3, improved pain control may have derived from multiple approaches such as suppression of carbamazepine and chlorpromazine; introduction of azathioprine, lamotrigine, and lidocaine; and change in ERT from agalsidase-alpha to agalsidase-beta.

To date, regarding adverse events related to treatment, there is no description of DIL related to ERT. Aseptic meningitis is neither an expected feature of DIL. The patient received drugs classically related to DIL (carbamazepine and chlorpromazine) alongside agalsidase-alpha and many other pain medications. Antihistone antibody became negative after withdrawal of suspected drugs.

Although antibody formation can be related to ERT infusion reactions[28] and the patient experienced reactions to agalsidase-alpha treatment, no IgG or IgE antibodies were found.

Conclusion

In summary, this article reported the phenotypic characterization of a Brazilian family with classic FD caused by a novel missense mutation in the *GLA* gene.

Declaration of Conflicting Interests

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