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Case Report

Novel alpha-galactosidase A mutation in a female with recurrent strokes

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ABSTRACT

Anderson–Fabry disease (AFD) is an X-linked inborn error of glycosphingolipid catabolism resulting from the deficient activity of the lysosomal exoglycohydrolase, a-galactosidase A. The complete genomic and cDNA sequences of the human alpha-galactosidase A gene have been determined and to date, several disease-causing alpha-galactosidase A mutations have been identified, including missense mutations, small deletions/insertions, splice mutations, and large gene rearrangements We report a case of a 56-year-old woman with recurrent cryptogenic strokes. Ophthalmological examination revealed whorled opacities of the cornea (cornea verticillata) and dilated tortuous conjunctival vessels. She did not show other typical signs of Fabry disease such as acroparesthesias and angiokeratoma. The patient's alpha-galactosidase A activity was 4.13 nmol/mL/h in whole blood.

Alpha-galactosidase A gene sequence analysis revealed a heterozygous single nucleotide point mutation at nucleotide c.550 T>A in exon 4 in this woman, leading to the p.Tyr184Asn amino acid substitution.

Introduction

Anderson–Fabry disease (AFD) is an X-linked inborn error of glycosphingolipid catabolism resulting from the deficient activity of the lysosomal exoglycohydrolase, alpha-galactosidase A. The enzymatic defect results in the progressive accumulation of neutral glycosphingolipids with terminal a-linked galactosyl moieties (primarily globotriaosylceramide [Gb3]) in the plasma and in the lysosomes of cells throughout the body [1].

The complete genomic and cDNA sequences of the human alpha-galactosidase A gene have been determined [2].

This gene is unique among eukaryotic genes, because it lacks a 3′ UTR, except for rare variant mRNAs having short 3′ UTRs of 6 or 7 nt. The polyadenylation signal (pAS) is in the coding sequence, and the termination signal is in the last codon [3].

Several disease-causing alpha-galactosidase A mutations have been identified, including missense mutations, small deletions/insertions, splice mutations, and large gene rearrangements [4].

In heterozygous women, alpha-galactosidase A activity in blood can lie within the reference range, in accordance with the Lyon hypothesis of random X chromosome inactivation [5].

There is a high frequency of AFD manifestations also in female patients and within these manifestations are also ischemic cerebrovascular events [6,7].

Strokes are an important cause of morbidity and mortality in young adults. However, in most cases the cause of the stroke remains unclear. AFD causes an endothelial vasculopathy followed by cerebral ischemia. Rolfs et al. [8] showed a high frequency of AFD in a cohort of patients with cryptogenic stroke, which corresponds to about 1.2% in young stroke patients. On this basis AFD must be considered in all cases of unexplained stroke in young patients. We report a case of a middle aged woman with recurrent cryptogenic strokes, a family history of ischemic cerebrovascular events and a novel mutation of human alpha-galactosidase A gene.

A 56-year-old female presented with clinical history of three previous ischemic strokes involving the brainstem over a 15 year period. She has no clinical history of hypertension and diabetes. She was well

Abbreviations: AFD, Anderson–Fabry disease; GL-3, globotriaosylceramide; cDNA, complementary DNA; 3′ UTR, three prime untranslated region; pAS, polyadenylation signal; CT, computed tomography; magnetic resonance imaging, MRI; DBFP, Dried Blood Filter Paper; K,EDTA, Potassium Ethylenediamine Tetraacetic Acid; α-Gal A, alpha-Galactosidase A; CPB, Citrate Phosphate Buffer; PCR, polymerase chain reaction; TIAs, transient ischemic attacks; CNS, central nervous system; FOS, Fabry Outcome Survey; Gb3, glycotriaosylceramide.

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until age 40 when she suffered a first episode of ischemic stroke, a second episode at age 42 and a third episode at age 54. Her family clinical history revealed a high frequency of ischemic cerebrovascular events: her parents died at age 61 and age 78 of ischemic stroke, four brothers died respectively at age 49, 52, 51 and 57 years of ischemic stroke, whereas of two sisters, one died at age 61 of ischemic stroke and one is alive but with a history of hypertension, diabetes and two previous ischemic strokes at age 55 and 57 (family pedigree of case shown in Fig. 1).

Her first ischemic stroke presented with dysarthria and right arm paresis. A computed tomography (CT) of the brain revealed two acute punctuate infarcts on the basis pontis. Two years later the patient presented with dysarthria; she was placed on aspirin and discharged after returning to baseline function. At age 54 she had a new episode of dysarthria and right arm paresis and a brain magnetic resonance imaging (MRI) revealed a new ischemic lesion in the region of the left basis pontis. She has been asymptomatic for 12 months since she started a treatment with tiklopidine and losartan therapy. She did not experience acroparesthesias, joint pains, and she did not have hypohydrosis. There was no known family history of skin lesions. The patient was referred for a dermatologic examination to detect any sign of angiokeratoma with no clinical evidence of skin lesions.

We detected no other sign of organ damage (kidney, heart) linked to Fabry disease on a clinical, laboratory (proteinuria) and instrumental (mono and bi-dimensional Echocardiography) evaluation of our patient.

Ophthalmological examination revealed whorled opacities of the cornea (cornea verticillata). Our patient was not taking any drug such as cloroquine or amiodarone that causes cornea verticilata.

The patient's alpha-galactosidase A activity in whole blood was 4.13 nmol/mL/h (see Results) which is compatible with a heterozygote subject for Fabry disease or healthy control. alpha-galactosidase A gene sequence analysis (see Results) revealed a heterozygous single nucleotide point mutation; she started enzyme replacement therapy (ERT) with recombinant human α-galactosidase A. In October 2008 she started enzyme replacement therapy (ERT) with agalsidase alfa (Replagal) (0.2 mg/kg of body weight biweekly).

Methods

Measuring alpha-galactosidase A activity by Dried Blood Filter Paper (DBFP).

Refrigerated K, EDTA whole blood samples, arrived at our laboratory (max 24 h) from different hospitals via a prepaid shipping agency (TNT). Samples were listed and spitted for biochemical and genetic study.

Reaction mix

Reaction mix was prepared in Citrate Phosphate Buffer (CPB) 0.15 M pH 4.5 (P4809-50 TAB Sigma, a tab dissolved in 33 mL H_2O to obtain the wanted molarity). 4-methylumbelliferyla-D-galactopyranoside 5 mM (Sigma M763) strained by 0.45 nm syringe filter, and N-acetyl-D-galactosamine (Sigma A2795) 0.25 M, were mixed in a ratio of 2.5:1. 70 μl of this mix was used for each well.

αlpha-Galactosidase A inhibitor

A 2 mg/mL of Deoxygalactonojirimycin-hydrochloride (Sigma D9641) was prepared in distilled water. Prepared solution afterwards diluted in a ratio of 1:3 with CPB (Citrate Phoshate Buffer). Using 3 μl of prepared dilution, the final concentration in the assay was 1.3×10^{-7} M [9–11].

Sample analysis

Analysis was performed using a Wallac Microtiter Fluorometer plate reader (excitation 355 nm, emission 460 nm) from Perkin Elmer using a

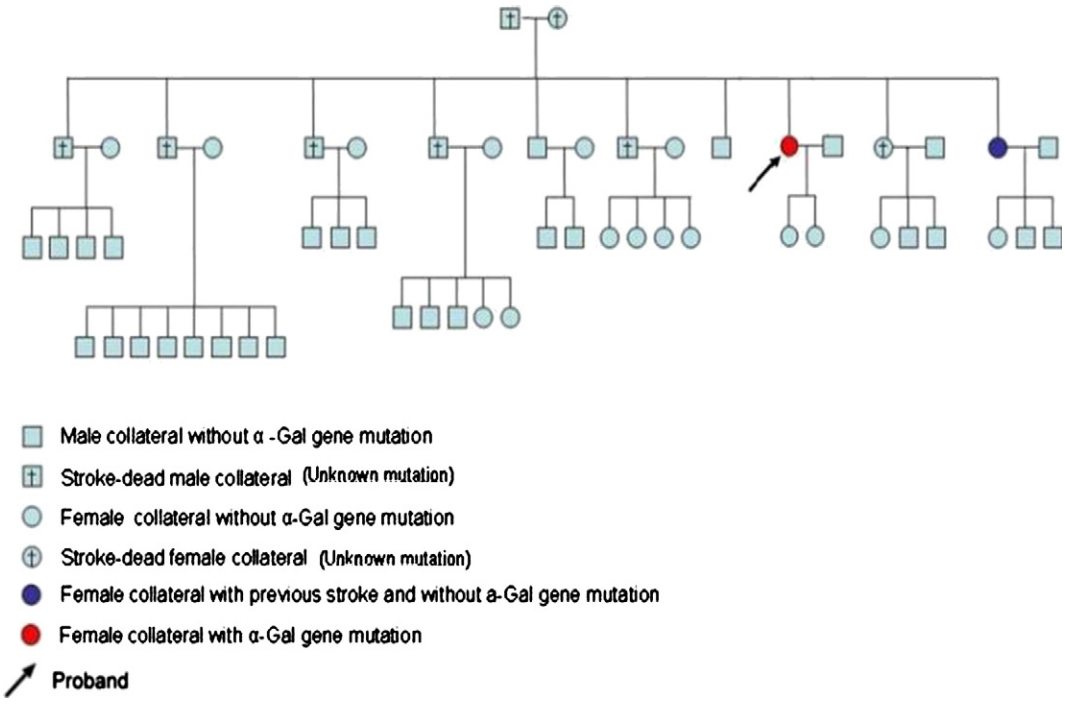

Fig. 1. Pedigree from family case.