Molecular Genetics and Metabolism Reports 4 (2015) 19–24

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: http://www.journals.elsevier.com/molecular-genetics-and-metabolism-reports/

Late onset variants in Fabry disease: Results in high risk population screenings in Argentina

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ARTICLE INFO

Article history:
Received 6 April 2015
Received in revised form 29 May 2015
Accepted 29 May 2015
Available online 7 June 2015

Keywords:
Fabry disease
GLA variants
Late onset variants
Lyso-Gb3

ABSTRACT

Background: Screening for Fabry disease (FD) in high risk populations yields a significant number of individuals with novel, ultra rare genetic variants in the *GLA* gene, largely without classic manifestations of FD. These variants often have significant residual α-galactosidase A activity. The establishment of the pathogenic character of previously unknown or rare variants is challenging but necessary to guide therapeutic decisions.
Objectives: To present 2 cases of non-classical presentations of FD with renal involvement as well as to discuss the importance of high risk population screenings for FD.
Results: Our patients with non-classical variants were diagnosed through FD screenings in dialysis units. However, organ damage was not limited to kidneys, since LVH, vertebrobasilar dolichoectasia and cornea verticillata were also present. Lyso-Gb3 concentrations in plasma were in the pathologic range, compatible with late onset FD. Structural studies and in silico analysis of p.(Cys174Gly) and p.(Arg363His), employing different tools, suggest that enzyme destabilization and possibly aggregation could play a role in organ damage.
Conclusions: Screening programs for FD in high risk populations are important as FD is a treatable multisystemic disease which is frequently overlooked in patients who present without classical manifestations.

1. Introduction

Fabry disease (FD, MIM #301500) is an inherited metabolic disorder that results from the deficient activity of the lysosomal enzyme α-galactosidase A (α-Gal A, EC 3.2.1.22) [1]. This lysosomal hydrolase, encoded by the *GLA* gene (locus Xq22.1), catalyzes the removal of terminal α-linked galactosyl residues from neutral glycosphingolipids, the most prominent being globotriaosylceramide (Gb3) [2]. Patients with FD show progressive accumulation of Gb3 and related glycosphingolipids in neurons, podocytes, cardiomyocytes, endothelial, perithelial and vascular smooth muscle cells. Recently, the deacylated soluble derivative globotriaosylsphingosine (lyso-Gb3) and analogs, were found to be increased in plasma and urine of FD patients [3,4]. FD has an estimated birth incidence of 1:40.000 newborns, although recent systematic screenings in different low and high risk cohorts indicate a higher prevalence [5–7]. The phenotypic spectrum of the disease comprises the classic or early onset form and the non-classical form, diagnosed later in life time, formerly referred as heart or renal variants [1]. In the classic form, the first disease manifestations may become evident during childhood or adolescence as neuropathic distal pain in limbs, hypohydrosis, angiokeratoma, gastrointestinal symptoms, cornea verticillata, dysautonomia, fatigue and auditive impairment [2]. During adulthood, most males develop left ventricular hypertrophy (LVH) and/or arrhythmia, renal insufficiency and/or stroke [1,2]. The concept of late onset or non-classical variants arose in 1995 after enzymatic screening in patients with idiopathic LVH [8,9]. Recent increase of screenings in high risk populations, due to treatment availability, allowed the identification of variants causative of non-classical disease. A third category of genetic alterations in *GLA*, genetic variants of unclear significance (GVUS), also called fringe alleles due to their unclear pathological consequences, further adds complexity to FD diagnosis and management [10,11].

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http://dx.doi.org/10.1016/j.ymgmr.2015.05.006

2. Patients materials and methods

Patients

Individuals carrying the GLA variants p.(Cys174Gly) and p.(Arg363His) alleles were identified on systematic screenings at renal units, using dried blood spots (DBS) and leukocyte α-Gal A levels as screening methods.

Structural analysis

To evaluate the location of an amino acid residue involved in substitution, the solvent-accessible surface area (ASA) value of an amino acid in the wild type *GLA* was calculated using Stride (http://webclu.bio.wzw.tum.de/stride/). Structural models of mutant AGAL_HUMAN proteins with p.(Cys174Gly) and p.(Arg363His) were built using TINKER (http://dasher.wustl.edu/tinker/). As a template, the crystal structure of human AGAL_HUMAN (PDB: 1R46) was used and energy minimization was performed. The root-mean-square gradient value was set at 0.05 kcal/mol · Å. Then, each mutant model was superimposed on the wild type *GLA* structure based on the Cα atoms by the least-square-mean fitting algorithm, in which the optimal rotations and translations are found by minimizing the sum of the squared distances among all structures in the superposition. We defined that the atom was affected by an amino acid substitution when the position of the atom in a mutant differed from that in the wild type structure by more than 0.15 Å. For evaluation of the structural changes caused by amino acid substitutions, we calculated the root-mean-square distance (RMSD) values, as described previously [12]. Furthermore, coloring of the influenced atoms in the three-dimensional structure of the enzyme was performed to determine the influence of the amino acid substitutions geographically and semi-quantitatively. The colors of affected atoms were shown on the basis of the distance between the wild type and mutant one.

In silico mutations evaluation

The mutations in the *GLA* coding region c.520C > G and c.1088G > A, that changes the translation of codons 174 and 363 to p.(Cys174Gly) and p.(Arg363His), were analyzed using several web-based tools. MutationTaster [13], SIFT [14] PolyPhen-2 [15], PhD-SNP [16], V7 and V8 Methods [17], SNPeffect 4.0 [18] I-Mutant Suite 3.0 [19] and SDM [20], in order to assess their potential pathogenicity. Biochemical studies: Lyso-Gb3 quantitation in plasma was performed as previously described [21].

3. Results

3.1. Clinical data

Case 1

A 46 year old male, with no family history of renal disease, sought care for lower limb edema. The patient was normotensive and physical exam was unremarkable. Laboratory evaluation showed 1.3 g/24hs of proteinuria (with normal urinary sediment). Work up for his proteinuria ruled out diabetes and autoimmune nephropathies. Renal ultrasound was also normal. He initiated treatment with an angiotensin converting enzyme inhibitors (ACEi). Two years later, proteinuria had increased to 2.8 g/24 h, lower limb edema worsened and serum creatinine was 1.07 mg/dl (95.5 μmol/l). Three years after his initial presentation, he was found to have increased proteinuria at 4 g/24h and a serum creatinine of 1.29 mg/dl (114 μmol/l). Renal biopsy was performed (electron microscopy was not done) but did not yield a definitive diagnosis. At age 50, serum creatinine continued to rise to 2.35 mg/dl (207 μmol/l) and proteinuria reached 8.5 g/24 h. A subsequent renal biopsy, employing light microscopy, revealed segmental sclerosis in two glomeruli and cytoplasmic vacuolization in podocytes, tubulointerstitial fibrosis and moderate tubular atrophy. Electron microscopy (EM) showed zebra bodies and cytoplasmic vacuolization in podocytes, with prominent foot process effacement [22], specific findings of FD. Zebra bodies were notably absent in endothelial, mesangial and tubular cells. Leukocyte α-Gal A activity was 2.8 nmol/h/mg (Normal Values: 30.5–57.7 nmol/h/mg) and *GLA* sequencing identified the hemizygous missense variant c.520C > G; p.(Cys174Gly) in exon 3. Lyso-Gb3 measurement in plasma was 2.9 ng/ml or 3.6 nM (Normal Range: ≤0.9 ng/ml or 1.1 nM). Brain magnetic resonance images (MRI) showed severe vertebrobasilar dolichoectasia (VBD), without evidence of white matter involvement. Echocardiographic studies revealed mild LVH. Upon further review, the patient did not manifest hypohidrosis, neuropathic pain, or angiokeratomas. The patient was started on enzyme replacement therapy (ERT), however, despite this he progressed to end stage renal disease (ESRD), requiring renal replacement therapy (RRT) five years after diagnosis. Maternal inheritance of the missense variant c.520C > G was confirmed by genotyping the proband's sister and niece, both showing gastrointestinal symptoms, mild neuropathic pain and depression. The 22 year old heterozygote proband's daughter, had neuropathic pain, heat intolerance and gastrointestinal discomfort. She recently underwent kidney biopsy due to mild proteinuria. On light microscopy, glomeruli showed enlarged podocytes with foamy appearing microvacuoles and mild interstitial fibrosis, suggestive of early Fabry nephropathy. Four hemizygote relatives of the index case, related by a common grand-grand mother, two adult males in their sixties and two young males aged 20 and 21 year old, presenting with LVH, bradycardia, mitral valvular disease, proteinuria and depression in the former and episodic distal pain, heat intolerance and microalbuminuria in the later, are under ERT. Remarkably, in both branches of the family, four patients bearing the p.(Cys174Gly) mutation, two untreated females and two males under treatment show severe psychiatric manifestations.

Case 2

A kidney biopsy was performed in a 40 year old male with an 8 year history of proteinuria and progressive decrease of glomerular filtration rate. Light microscopy revealed focal and segmental glomerulosclerosis. Electron microscopy evaluation was not performed. The patient had no family history of renal disease. His mother and father died of coronary arterial disease at ages 65 and 68 years old respectively. The patient continued to progress to ESRD requiring RRT. At the age of 55, after 5 years of RRT, during a screen for FD in a hemodialysis unit, leukocyte α-Gal A activity was found to be 2.9 nmol/h/mg. Sequencing analysis of the *GLA* gene detected the c.1088G > A; p.(Arg363His) missense variant in exon 7. Plasma lyso-Gb3 value was 1.8 ng/ml or 2.3 nM (Normal Range: ≤0.9 ng/ml or 1.1 nM). Brain MRI did not show alterations. Echocardiographic studies showed mild LVH and ocular examination on slit lamp, revealed corneal deposits in the lower external quadrant of right eye. The patient notably did not manifest neuropathic pain, hypohidrosis nor angiokeratoma. His two obligate carrier daughters remain asymptomatic at the ages of 9 and 11 years. After diagnosis in the index case, the patient's elder brother (68 year old), also in dialysis for 3 years in another renal unit, was tested and found positive for p.(Arg363His). The patient's only sister refused testing, but she referred to have LVH and bradyarrhythmia. She underwent pacemaker implantation before FD diagnosis in the index case. Genotyping was extended to his brother's children. The obligate carrier 30 year old niece harbored the heterozygous mutation and underwent thorough evaluation for skin, renal, cardiac, ophthalmologic and neurologic alterations. She showed impaired perception of temperature changes, indicative of small fibers compromise, assessed by quantitative sensory testing (QST) as the only alteration that could be related to FD. A 65 year old cousin from the maternal branch of the index case, hemizygous for p.(Arg363His) is under dialysis treatment for 2 years and is a candidate for renal transplantion. He showed minimal QST alterations with no other organ systems involved. The index case and his male relatives are under ERT with agalsidase beta, 1 mg/kg, every other week.