Previously Unreported in Women *Galactosidase Alpha* Pro409Ser Variant Is Associated With Fabry Disease

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Fabry disease is a rare X-linked lysosomal storage disorder involving a deficiency in α- galactosidase A. This condition results in an impaired ability to metabolize globotriaosylceramide in the glycosphingolipid metabolic pathway, which accumulates within tissues throughout the body. Fabry disease affects 1 in 40 000 to 117 000 men with an unknown prevalence in women.[1] Clinical presentations can be variable, ultimately resulting in potentially severe end-organ damage. In light of the variability in clinical presentation and rarity of the disease, initial misdiagnosis is common with a mean delay to diagnosis of between 13.7 and 16.3 years from symptom onset.[1] Typical manifestations can include cutaneous lesions (angiokeratoma corporis), peripheral neuropathy, cerebrovascular accidents, proteinuria, renal insufficiency, and cardiac dysfunction.[2,3] Cardiac manifestations include increased ventricular wall thickness, heart failure, valvular thickening and dysfunction, and coronary artery disease.[2,3] Accurate and early diagnosis is imperative because early treatment with agalsidase β had been demonstrated to reduce the incidence of major adverse outcomes, including renal failure, stroke, cardiac events, and death.[2]

Clinical Case

A 50-year-old woman presented to our institution with a recent onset of worsening exertional shortness of breath, fatigue, and chest tightness on a background of a presumptive diagnosis of hypertrophic cardiomyopathy made 10 years before. Her family history was significant for ischemic heart disease in her father and brother and valvular disease in her sister, but there was no known family history of hypertrophic cardiomyopathy. There were no other systemic symptoms, and clinical examination revealed a holosystolic murmur without other features of systemic disease, including cornea verticillata. Baseline renal function was normal with a creatinine of 0.8 mg/dL. Echocardiography (Figure 1A and 1B) demonstrated severe concentric increase in left ventricular wall thickness with systolic anterior motion of the mitral valve leaflets resulting in severe left ventricular outflow tract (maximal instantaneous gradient, 34 mm Hg at rest rising to 121 mm Hg with amyl nitrite) and mid-cavitary obstruction (maximal instantaneous gradient, 46 mm Hg). A left ventricular apical pouch was identified without apical thrombus, and there was evidence of right ventricular free wall thickening. Coronary angiography demonstrated subtotal occlusion of the ostial left anterior descending artery and ostial ramus intermedius. Cardiac magnetic resonance imaging (Figure 1C and 1D) demonstrated severe concentric left ventricular hypertrophy with and apical pouch and transmural delayed gadolinium enhancement involving the inferolateral wall.

She had been referred for and underwent an extended septal myectomy and concomitant coronary artery bypass grafting. Histopathologic evaluation of the septal myectomy specimen demonstrated striking sarcoplasmic vacuolization (Figure 2). Transmission electron microscopy was subsequently performed, to evaluate to the possibility of an underlying storage disease and revealed numerous sarcoplasmic myeloid bodies suggestive of Fabry disease.

Genetic and Biochemical Analysis

Leukocyte α-galactosidase A enzyme activity was reduced, measuring 14.7 nmol·h·mg (normal >23.1 nmol·h·mg) suggesting that the patient may be a carrier for Fabry disease. Plasma acid β-glucosidase, acid α-glucosidase, sphingomyelinase, galactocerebrosidase, and α-L-iduronidase enzyme activities were normal, suggesting that deficiencies in these enzymes were not a cause for her lysosomal storage disorder.

Bi-directional sequence analysis was performed to test for the presence of a mutation in all coding regions and intron–exon boundaries of the *GLA* gene (GenBank accession number, NM_000169; build GRCh37 [hg19]). Additional sequence analysis was performed for the c.640-859C>T and c.640-801G>A genetic variants. Genetic analysis revealed the patient to be heterozygous for a nonsynonymous single-nucleotide mutation, c.1225C>T corresponding to a p.Pro409Ser change in the amino acid sequence. There is a single report in ClinVar of this variant occurring in a man with Fabry disease.[4] The amino acid is highly conserved throughout evolution, and in-silico models predict that the alteration may affect protein function. Nevertheless, functional data and information on how the variant segregates with disease was lacking. Therefore, the variant was ultimately classified as a variant of undetermined significance.

Discussion

Fabry disease is an X-linked disease caused by mutations in the *GLA* gene that is located on the X chromosome (Xq22.1) and encodes the lysosomal enzyme α-galactosidase A.[5] The

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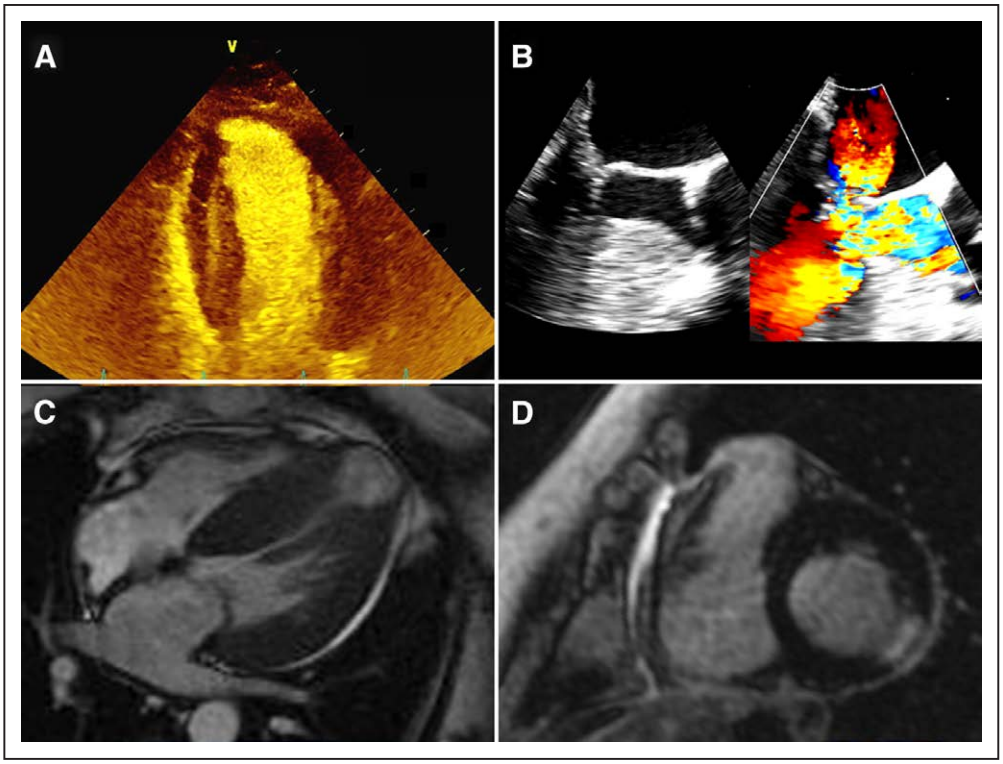

Figure 1. **A**, Microbubble contrast-enhanced transthoracic echocardiographic apical 4-chamber view demonstrating severely increased left ventricular wall thickness with a ventricular septal thickness of 20 mm. **B**, Transesophageal echocardiographic mid-esophageal long-axis image showing severe hypertrophy involving the basal septum with systolic anterior motion of the anterior mitral valve leaflet. The corresponding color Doppler is demonstrated on the right of demonstrating flow turbulence in the left ventricular outflow tract consistent with obstruction. **C**, Cardiac magnetic resonance imaging in the apical 4-chamber view demonstrating severe concentrically increased left ventricular wall thickness with a thinned left ventricular apex consistent with an apical pouch. **D**, Cardiac magnetic resonance delayed gadolinium enhancement imaging at mid-ventricular level demonstrating transmural enhancement involving the inferolateral wall.

resulting α-galactosidase A deficiency results in accumulation of the substrate globotriaosylceramide, which accumulates within lysosomes resulting in the myeloid bodies that can be identified ultrastructurally. It has been suggested that the accumulation of globotriaosylceramide within cardiac myocytes triggers intracellular signaling pathways, which results in hypertrophy, fibrosis, apoptosis, and necrosis.[5] Additionally, accumulation of globotriaosylceramide within the vascular endothelium may result in microvascular ischemia.[3] Over 431 (mostly missense) mutations have been identified within the 7 exons of this gene, with most such mutations being unique to individual families.[5–7]

The disease has a male predilection owing to the fact that it is X linked. However, female carriers also seem to be affected albeit with a later age of onset and milder disease. It is hypothesized that lyonization, where 1 X chromosome in each cell in inactivated, may play a role in the disease expression seen in female carriers.[5] Although random inactivation of the normal allele more than the diseased allele may be one possible explanation for variable disease expression in women, it fails to explain the fact that a majority of female carriers will have symptoms/manifestations of the disease.[3,5,8] However, such reports may be biased because unaffected women may escape medical attention.

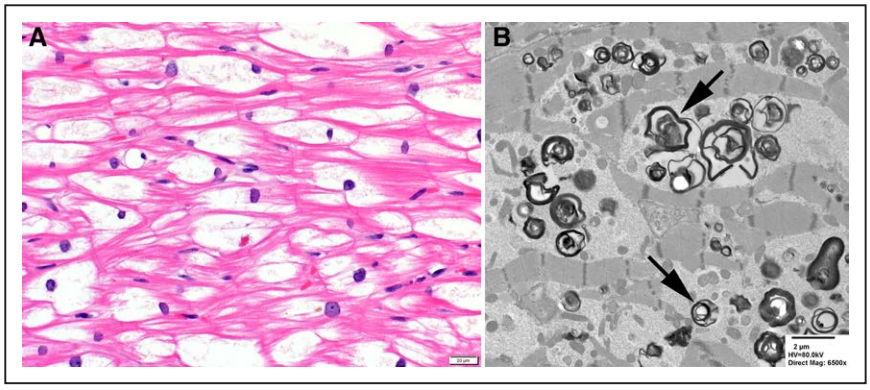

Figure 2. **A**, Photomicrograph showing the extensive sarcoplasmic vacuolization on hematoxylin–eosin staining. **B**, Ultrastructural studies reveal numerous sarcoplasmic myelin figures (arrows).

Testing for Fabry disease should include a combination of biochemical analysis of plasma and leukocyte α-galactosidase A enzyme levels and genetic testing.[7] Although cardiac or renal biopsy is not required to make the diagnosis of Fabry disease, the presence of known disease may help in defining the pathological nature of identified polymorphisms. With the exception of men with normal α-galactosidase levels, all other patients should undergo genetic testing to evaluate for known *GLA* mutations to confirm biochemical testing results or exclude disease.

Genetic testing involves sequencing the entire *GLA* gene and 5′ and 3′ flanking regions to identify known mutations.[7] Additional microarray-based duplication and deletion testing of the *GLA* gene may be useful to identify variants when traditional sequencing fails to identify such variants.[7] Because of the frequency of novel variants that are specific to individual families, new variants may first be identified in the individual proband and could subsequently be used to identify affected family members.

Current guidelines advocate screening of all first-degree family members of affected patients, with both genetic and biochemical testing.[8] In the presence of a known genetic variant, such as that found in our patient, genetic testing can be restricted to identification of the known familial mutation. In accord with current guidelines, familial screening was recommended to our patient with testing for the familial P409S missense variant, in addition to biochemical analysis of plasma and leukocyte α-galactosidase A enzyme levels.

The P409S missense variant identified in this patient has not been reported previously to be associated with development of Fabry disease in women. Previously, because of the paucity of evidence to support this variant's pathogenicity, it was reported as a variant of unknown significance. Although the patient clearly has Fabry disease (by enzymatic assay, histopathology, and clinical history) with predominantly cardiac involvement, care should be taken when drawing a causal association with the reported P409S variant.[9] The P409S variant in the context of this case using American College of Medical Genetics Criteria[10] should be reported as likely pathogenic for the following reasons: a functional study was performed that has been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting that supports the damaging effect of the gene product (the reduced leukocyte α-galactosidase A enzyme activity in this patient) with the caveat that other promoters or intronic variants or indels that could have affected gene expression may be present, and the effect of these variants if any cannot be ruled out. The P409S variant is absent in controls in publically available sequencing databases; it is a missense variant in a gene that has a low rate of benign missense variants and in which missense variants are a common mechanism of disease and computational evidence supports a deleterious effect on the gene product. In addition, this missense change occurs at an amino acid residue (P409S) where a different missense change determined to be pathogenic has been reported before (P409A and P409T).[11]

Conclusion

Fabry disease is frequently misdiagnosed at initial presentation and should be considered within the differential diagnosis of patients presenting with thickened ventricular walls or hypertrophic cardiomyopathy. Testing should include a combination of biochemical, histological, and genetic testing to identify affected individuals. Multiple genetic variants in the *GLA* gene have been identified and disease-causing variants may be specific to individual families. The significance of novel Fabry disease genetic variants should be made in the context of histopathologically proven disease, abnormal leukocyte, or plasma α-galactosidase A enzyme activity, reported frequency of such variants in controls and computational predictions of the effect of the variants on protein expression. Genetic variants that are identified as likely pathogenic or pathogenic can then be useful to screen family members.

Disclosures

None.

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