Is it Fabry disease?

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Fabry disease is caused by mutations in the *GLA* gene that lower α -galactosidase A activity to less than 25–30% of the mean normal level. Several *GLA* variants have been identified that are associated with relatively elevated residual α -galactosidase A. The challenge is to determine which *GLA* variants can cause clinical manifestations related to Fabry disease. Here, we review the various types of *GLA* variants and recommend that pathogenicity be considered only when associated with elevated globotriaosylceramide

in disease-relevant organs and tissues as analyzed by mass spectrometry. This criterion is necessary to ensure that very costly and specific therapy is provided only when appropriate.

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Advances in the past decade have led to the elucidation of the genetic and metabolic basis of an extensive array of rare and often devastating diseases, which has been crucial for the development of therapeutics for those diseases. Genotype–phenotype correlative studies in these rare disorders and the identification of common allelic variants have provided interesting insights into the potential role that rare disease gene variants may play in the pathogenesis of more common disease symptoms within the general population.

Fabry disease is caused by mutations of the X-linked *GLA* gene that produce a deficiency of the lysosomal enzyme α -galactosidase A.¹ Deficiency of α -galactosidase A results in the inability of cells to catabolize glycosphingolipids with terminal α -D-galactosyl residues.² These glycosphingolipids, particularly globotriaosylceramide (Gb₃), progressively accumulate in virtually all organs, resulting in a progressive multisystem disease. The cardinal features of the disease are acroparesthesias, progressive proteinuric renal insufficiency, cardiac disease consisting of rhythm and conduction disturbances and progressive hypertrophic cardiomyopathy, as well as cerebrovascular stroke. Patients with the classic form of Fabry disease have very low, if any, residual enzyme activity.³ Over the years, attenuated forms of the disease have been increasingly recognized. Importantly, patients with attenuated disease have clinically significant disease, with cardiac involvement being the most common manifestation. In addition, patients with attenuated disease tend to have higher enzyme activity and a more protracted disease natural history than patients with classic disease.⁴

The incidence of Fabry disease is approximately 1 in 117,000 live births for males,⁵ although recent newborn screening surveys suggest that the incidence may be much higher (up

to 1:3,100).^{6–11} In Taiwan, the incidence of the relatively mild IVS4+919G>A mutation is 1:875 male live births and 1:399 female live births.¹¹

Screening of various at-risk patient populations, i.e., patients with cardiomyopathy,^{12,13} stroke,¹⁴ or renal failure,^{15,16} has identified a low, but not negligible, prevalence of Fabry disease in the general symptomatic population. Some of these variants have normal or almost normal enzyme activity and, in other cases, mildly to moderately reduced α -galactosidase A activity. These observations pose two fundamental questions: (i) do patients identified in this manner (many of whom have common symptoms, i.e., stroke, cardiomyopathy, proteinuria, or renal failure) have Fabry disease? and (ii) should α -galactosidase enzyme replacement or other Fabry-specific therapies be considered in their management?

Because of the recognition of frequent *GLA* variants, it has become important to establish criteria by which a patient with or without symptoms and a specific variant would be considered to have or to be at risk for Fabry-related complications. Here, we discuss problems related to the interpretation of *GLA* variants and propose criteria for designating pathogenicity in the clinical context. Determination of the true significance of a *GLA* variant is critical to apply specific therapy only when indicated.

DIAGNOSIS OF FABRY DISEASE

The diagnosis of Fabry disease requires the demonstration of enzyme deficiency and a resultant increase in cell/organ globotriaosylceramide, typically in lysosomes.¹⁷ It is the accumulation of substrate that initiates pathological cascades, which ultimately are responsible for patient symptoms rather than the enzyme deficiency per se.² Therefore, ensuring that the increase

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of this glycosphingolipid is present in a relevant organ system is central to dealing with whether the patient has Fabry disease or is at risk for Fabry disease-related complications.

WHAT IS THE THRESHOLD FOR CLINICALLY SIGNIFICANT α -GALACTOSIDASE A DEFICIENCY?

Patients with the classic form of Fabry disease have no residual enzyme activity.^{4,18} However, the enzyme activity level above which α -galactosidase A deficiency does not play a role in human disease is not known. It has been estimated that residual activity of 30–35% of mean normal α -galactosidase A activity is the cutoff for diagnosing Fabry disease.^{11,13,19,20} Enzymatic activity is being routinely measured in isolated peripheral blood white cells or in dry blood spots. However, α -galactosidase A activity in peripheral blood may not reflect the activity in affected organs such as the kidney, heart, and vascular system. The true threshold of pathogenicity for Fabry disease is not known. Although such a threshold has been claimed for other diseases,²¹ it may vary from organ to organ and from one patient to another. The frequent observation of exclusive cardiac involvement in Fabry disease suggests that the heart is the most susceptible organ to α -galactosidase A deficiency.^{22,23} It is important to note that although clinically significant renal disease is often not detected in Fabry patients with exclusive cardiac disease, it is not known whether renal storage of glycolipids is present in these patients. Exclusive kidney disease has very rarely been described. Clinical observation suggests that patients with the classic form of Fabry disease are those who are at the highest risk for stroke. Therefore, a heart-kidney-cerebrovascular system susceptibility gradient is likely.

INTERACTION OF α -GALACTOSIDASE DEFICIENCY WITH OTHER FACTORS

The clinical heterogeneity noted in Fabry individuals carrying the same pathogenic mutation, even within the same family, is clear evidence that an individual's risk for complications of Fabry disease depends on interactions between α -galactosidase A deficiency and other factors. The additional factors could include genetic, epigenetic, and environmental factors. In a threshold model of symptom emergence, the higher the residual enzyme activity, the greater the influence these other factors probably have on the likelihood of developing Fabry-related clinical complications. Little work has been done to identify genetic modifiers in Fabry disease. Genotypes of polymorphisms G-174C of interleukin-6, G894T of endothelial nitric oxide synthase, factor V G1691A mutation (factor V Leiden), and the A-13G and G79A of protein Z were all significantly associated with the presence of presumably ischemic cerebral lesions on brain magnetic resonance imaging (MRI).²⁴ Using the mouse model for Fabry disease, it was shown that the absence of α -galactosidase A activity combined with the factor V Leiden mutation significantly increases the number of vascular thrombi compared to either mouse model alone.²⁵ The role of factor V Leiden mutation in increasing the likelihood of thromboembolic events was subsequently confirmed.²⁶

GLA VARIANTS AND CLINICAL EXPRESSION

One can divide *GLA* variants into three categories of α -galactosidase A residual activity (**Figure 1**). Nonsense and certain missense variants that are virtually always associated with Fabry disease have a level of enzyme activity of 0 to approximately 10% of normal in males.^{27,28} In this case, non-*GLA* modifiers (genetic, epigenetic, or other) have relatively little effect on the likelihood of having Fabry-related clinical complications. Such complications are less common in those with missense and certain splice *GLA* variants, with residual enzyme activity in the range of 15–30% of normal in males.³ The clinical expression of this second group likely depends, to a large extent, on putative genetic and epigenetic modifiers. The third group consists of *GLA* variants that are not associated with substantially reduced enzyme activity, i.e., activity above 35–40% of mean normal controls in males. In this group of *GLA* variants, clinical complications similar to those that occur in Fabry disease are more likely to be unrelated to the below average activity level of α -galactosidase A. The most controversial group of variants seems to be the nonpathogenic variants. A few examples illustrate the pitfalls associated with identification of such variants in patients. A 7-year-old boy with neuropathic pain was found to have the S126G variant; therefore, he was diagnosed as having Fabry disease. This variant was identified in a screening of females only and was considered pathogenic.²⁹ However, enzyme activity in this hemizygous male patient was found to be normal by two national laboratories (A. Basinger, unpublished data) and is considered benign or tolerated by prediction tools such as Sorting Intolerant From Tolerant and PolyPhen. D313Y was initially described as a disease-causing mutation²⁷ and has been associated with stroke³⁰ and white matter damage.³¹ However, previous publications found lower than normal plasma activity due to reduced activity in neutral pH, whereas activity in white blood cells was normal or minimally reduced.^{32,33} Normal lyso-Gb₃ in patients was considered proof that this variant is not pathogenic.³⁴ The R118C is considered a pathogenic mutation in a number of publications.^{14,35} Male patients with the R118C variant have mean α -galactosidase levels of 32% of normal.¹⁹ No zebra bodies (lysosomal inclusions) were found in a cardiac biopsy specimen of a male patient with this variant and no tissue Gb₃.

Figure 1 The relationship between the various types of *GLA* gene variants and the residual α -galactosidase A activity.

levels were measured.¹⁹ As can be gleaned from Figure 4 in the work by Ferreira *et al.*¹⁹, it is possible that the lowered residual enzyme activity may be sufficient to contribute to cardiovascular or renal disease in some patients with other risk factors. This concept has sometimes been called synergistic heterozygosity.^{36,37} A number of intronic variants have been described in male and female patients with stroke, cerebral white matter abnormality, and small-fiber neuropathy.³⁸ In addition, -10C>T is associated with reduced *GLA* expression and, consequently, mildly reduced α -galactosidase A activity (reduced by approximately 15–20% but not in disease range).³⁹ Therefore, -10C>T is likely to be a disease modifier when associated with a pathogenic variant. Of note, symptomatic -10C>T variant patients have been administered ERT, resulting in apparent reduction in neuropathic pain and increased physical activity³⁸.

The association of clinical manifestations in the presence of the exonic variants such as D313Y or intronic variants cannot be explained solely based on deficiency of α -galactosidase A. Studies that do show such associations³¹ have not demonstrated increased Gb₃ in tissues; therefore, the clinical findings are more likely due to other genetic or environmental factors and may or may not be linked to the variant allele.

HOW TO DETERMINE THE PATHOGENICITY OF A *GLA* VARIANT

It is clear from this discussion that the level of enzyme activity alone is not always sufficient to determine whether a particular *GLA* variant is pathogenic (**Figure 2**). Unfortunately, there are no databases that capture accurate and comprehensive Fabry-related clinical findings in association with *GLA*

variants. Nevertheless, when a *GLA* variant is identified, databases such as ClinVar and the Exome Variant Server (Exome Variant Server, NHLBI GO Exome Sequencing Project, Seattle, WA; <http://evs.gs.washington.edu/EVS/>) should be interrogated to determine the allele frequency and potential phenotypes. If these databases are proven to be not helpful, then other approaches are suggested. The most commonly invoked is the demonstration of substrate accumulation or altered sphingolipid levels, i.e., Gb₃ or lyso-Gb₃ in plasma or urine.^{34,40,41} However, Gb₃ and lyso-Gb₃ have limitations because both may be normal in attenuated forms of the disease or in females.^{40,42} Conversely, lyso-Gb₃ was recently found to be increased in plasma of untreated patients with Gaucher disease type 1 to the range seen in patients with Fabry disease^{43,44} and in heart disease patients who do not have any *GLA* variants and have normal enzyme activity.⁴⁵ Alternatively, identification of typical lysosomal inclusions in tissue biopsy specimens has been suggested as the gold standard of Fabry disease diagnosis.^{46,47} In our view, although its presence suggests Fabry disease, it is not the ideal criterion. First, lysosomal inclusions (sometime referred to as zebra bodies) are not specific to Fabry disease. They may occur in other lysosomal storage diseases such as GM2 gangliosidosis and Niemann-Pick disease and have also been demonstrated in silicon nephropathy.^{48,49} Conversely, Gb₃ level may be increased in the absence of lysosomal inclusions.^{50,51}

The most specific way to assess whether α -galactosidase A deficiency is clinically significant in a particular individual is to demonstrate the presence of an increased tissue level of this enzyme's direct substrate (Gb₃). Ideally, a disease-relevant organ such as the kidney or the heart should be assessed using

Figure 2 An algorithm for evaluating a *GLA* variant of unknown significance.

mass spectrometry, but skin may be adequate in some cases (Figure 2).⁵² Gb₃ elevation can also be demonstrated by immunohistochemistry in a tissue biopsy specimen using anti-Gb₃ antibody.^{50,51}

CONCLUSIONS

The nonspecific nature of the complications of Fabry disease and the relatively high population frequency of Fabry disease are risks for the wrong attribution of pathogenicity to certain GLA variants when they are identified in such patients. This can lead to unnecessary initiation of expensive and invasive therapies such as ERT instead of appropriately addressing the true causes of cerebrovascular, cardiac, renal, and neurological manifestations. Therefore, before concluding that a variant with relatively high α -galactosidase A activity is pathogenic, one must demonstrate evidence of altered sphingolipid homeostasis. Demonstration of elevated Gb₃ using mass spectroscopy is critical. When the specificity of the Gb₃ elevation is in doubt, the presence of a Fabry-specific lipid profile is likely to be helpful.⁵³

DISCLOSURE

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