P696

Case Presentation: Co-segregation of a Rare *GLA* Variant of Uncertain Significance within Two Multiplex Families Facilitates Variant Reclassification to Pathogenic

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Introduction

Fabry disease (OMIM# 310500) is an X-linked lysosomal disease associated with pathogenic variants in the *GLA* gene, which encodes the alpha-galactosidase A (α-Gal A) enzyme. Reduced α-Gal A activity results in progressive lysosomal accumulation of glycosphingolipids within various tissues and organs leading to the diverse clinical manifestations associated with Fabry disease. Due to its rarity and varied clinical presentation, a definitive diagnosis often requires genetic testing. This is especially true in heterozygous females where disease severity is highly variable; some exhibit features of classic Fabry disease, albeit to a lesser extent than hemizygous males, while others remain asymptomatic.

A genetic diagnosis of Fabry disease is critical for disease management as early intervention is paramount for minimizing disease progression and complications. In addition, the variant type assists healthcare professionals and patients in making informed decisions about treatment, expectations, and long-term care planning. Specifically, enzyme replacement therapy requires a genetic diagnosis as does eligibility for clinical trials. Known familial variants also enable informed family planning choices and allow for cost-effective, targeted testing. Moreover, avoiding unnecessary diagnostic procedures, treatments, and/or hospitalizations reduces risk and limits undue financial burden. Thus, accurate *GLA* variant classification is essential for optimizing disease management and patient quality of life. We present two multiplex families with co-segregation of a rare *GLA* variant in individuals with clinical and biochemical features consistent with Fabry disease, providing invaluable information for variant classification.

Case Presentation

Family 1

The proband is a 52-year-old female with a history of abdominal pain, hypertrophic cardiomyopathy, coronary artery disease, and an unspecified psychiatric disorder. Lyso-Gb3, the hallmark biomarker for Fabry disease, from a dried blood spot (DBS) sample was elevated, and targeted next-generation sequencing (NGS) of *GLA* revealed a heterozygous variant of uncertain significance (NM_000169.2: c.1078G>C, p.(Gly360Arg)). The variant, previously identified in two unrelated individuals with Fabry disease, is absent in population databases and predicted to be deleterious by *in silico* meta-analysis. The variant was identified as heterozygous or hemizygous in 12 additional family members. Clinical or biochemical features consistent with Fabry disease were observed in at least six additional family members. Unfortunately, phenotypic information was not included with the test requisition for the remaining individuals. Three unaffected family members negative for the variant were also identified.

Patient 2 is the 56-year-old brother of the proband and has a history of childhood-onset renal insufficiency, acroparesthesia, cardiac hypertrophy, and end-stage renal disease (ESRD) with subsequent renal transplant. Patient 3 is the 40-year-old maternal first cousin of the proband and has a history of nausea, constipation, anemia, Hashimoto's thyroiditis, and headaches. Patients 4 and 5 are the daughters of the proband. Patient 4 is 35 years old and has a history of proteinuria, atrial fibrillation, and multiple episodes of stroke. Patient 5 is 28 years old and has a history of abdominal pain, proteinuria, supraventricular tachycardia, and intralysosomal inclusion bodies on renal histopathology. DBS lyso-Gb3 was elevated in both patients 4 and 5. Patient 6 is the 13-year-old son of patient 4. Although no clinical features of disease were submitted with his sample, biochemical testing showed reduced DBS α-Gal A activity and elevated DBS lyso-Gb3. Patient 7 is the 9-year-old son of patient 5. He has a history of abdominal pain and an elevated DBS lyso-Gb3.

Family 2

The proband is a 47-year-old male with a history of ESRD, hypertrophic cardiomyopathy, acroparesthesia, angiokeratoma, and diarrhea. Patient 2 is the 27-year-old daughter of the proband and has a history of abdominal pain, diarrhea, acroparesthesia, tinnitus, hypohidrosis, and headaches. *GLA* sequencing by NGS identified the c.1078G>C p.(Gly360Arg) variant in both individuals. They are currently receiving enzyme replacement therapy.

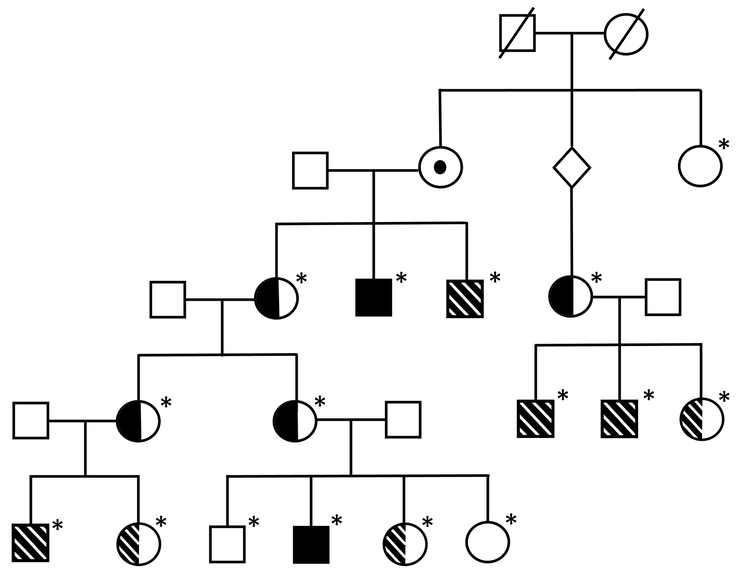

Figure 1. Pedigree of family 1; filled symbols, positive for the variant, clinical history consistent with Fabry disease; hatched symbols, positive for the variant, clinical features not provided; partially filled circle, obligate carrier

Table 1. Clinical and biochemical phenotypes of individuals positive for the *GLA* c.1078G>C p.(Gly360Arg) variant

Individual	Testing Method	Clinical Features	*GLA* c.1078G>C p.(Gly360Arg)	α-Gal A Reference Range ≥ 1.10 µmol/L/hr	Lyso-Gb3 Reference Range ≤ 1.11 ng/ml
Family 1					
Proband	Targeted NGS: *GLA*	Abdominal pain, hypertrophic cardiomyopathy, coronary artery disease, psychiatric disorder	Heterozygous	n.d.	3.978 ng/mL
Patient 2	Familial testing	ESRD s/p renal transplant, acroparesthesia, cardiac hypertrophy	Hemizygous	n.d.	n.d.
Patient 3	Familial testing	Nausea, constipation, anemia, Hashimoto's thyroiditis s/p thyroidectomy, headache	Heterozygous	n.d.	n.d.
Patient 4	Targeted NGS: *GLA*	Proteinuria, atrial fibrillation, multiple episodes of stroke	Heterozygous	n.d.	4.18 ng/mL
Patient 5	Targeted NGS: *GLA*	Proteinuria, supraventricular tachycardia, abdominal pain, zebra bodies present on renal biopsy, psychiatric disorder	Heterozygous	n.d.	11.154 ng/mL
Patient 6	Familial testing	None provided	Hemizygous	0.2 µmol/L/hr	50.24 ng/mL
Patient 7	Familial testing	Abdominal pain	Hemizygous	n.d.	51.19 ng/mL
Family 2					
Proband	Targeted NGS: *GLA*	ESRD awaiting transplant, hypertrophic cardiomyopathy, acroparesthesia, angiokeratoma, diarrhea	Hemizygous	n.d.	n.d.
Patient 2	Targeted NGS: *GLA*	Abdominal pain, diarrhea, tinnitus, acroparesthesia, hypohidrosis, headache	Heterozygous	n.d.	n.d.

Summary

Co-segregation of a variant with disease in a multiplex family provides statistically powerful evidence in support of pathogenicity. These two families provide the Fabry disease community with seven documented segregations of the variant in affected individuals – critical evidence needed for the reclassification of this *GLA* variant to pathogenic per ACMG/AMP guidelines.

The inclusion of patient phenotypes, family history, and/or clinic notes assists in shortening the timeline for VOUS resolution.

Accurate variant classification provides these two families with both a confirmed genetic diagnosis as well as access to specialized medical care and treatments. Equally as important, other individuals found to harbor the c.1078G>C p.(Gly360Arg) *GLA* variant in the future will have immediate access to these resources, which may prevent disease progression and result in a better prognosis.

All patients have consented to the use of their deidentified data for the purpose of research