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RESEARCH REPORT

Elevated Lyso-Gb3 Suggests the R118C GLA Mutation Is a Pathological Fabry Variant

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Abstract *Background*: Fabry disease (FD), an X-linked lysosomal storage disease, results from an α-galactosidase A deficiency and altered sphingolipid metabolism. An accumulation of globotriaosylsphingosine (lyso-Gb3) likely triggers the pathological cascade leading to disease phenotype. The pathogenic significance of several Fabry mutations including the R118C α-galactosidase (GLA) gene variant has been disputed. We describe three members of the same family with the R118C variant, each having documented clinical signs of FD, low residual enzyme levels, and an elevated lyso-Gb3 in one heterozygote.

Determining the clinical significance of each GLA gene variant remains an ongoing challenge, with potential for inadequate treatment if the diagnosis of FD is missed. Elevated lyso-Gb3 has been shown to be the most reliable noninvasive marker of clinically relevant GLA variants. While the R118C variant will likely lead to a milder phenotype, additional genetic, epigenetic, and environmental factors can ameliorate or exacerbate the expression and impact on the resultant phenotype and associated complications. Patients affected with this variant warrant closer review and better management of disease risk factors.

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Abbreviations

αGal	Alpha-galactosidase A
FD	Fabry disease
Gb3	Globotriaosylceramide
GLA	Gene encoding α-galactosidase
Lyso Gb3	Globotriaosylsphingosine

Introduction

Fabry disease (FD) (OMIM 301500) is an X-linked lysosomal storage disease with reduced α-galactosidase A (αGal) activity (Desnick et al. 1973) and disrupted glycosphingolipid homeostasis resulting from mutations in the α-galactosidase (GLA) gene. Intracellular accumulation of the glycosphingolipid globotriaosylceramide (Gb3) triggers inflammation, hypertrophy, and fibrosis and causes widespread organ injury (von Scheidt et al. 1991). More than 700 GLA gene variants have been reported (Smid et al. 2015), 60% being mis-sense mutations, leading to a significant heterogeneity in phenotype, even within families carrying the same variant. Accurate and reliable diagnosis of FD and the potential phenotype–genotype relationship is extremely important in patient management.

In classical FD, patients have very low or no residual αGal activity, with resultant life-threatening end-organ injury including progressive hypertrophic cardiomyopathy, renal impairment, and cerebrovascular disease including strokes (Mehta et al. 2009). Another group of patients have delayed or attenuated forms of FD, presenting clinically with single organ involvement, often cardiac, or with milder phenotypes (Desnick et al. 2003). These variants tend to be associated with higher residual but still subnormal αGal activity. A third group of GLA variants, including D313Y and S126G, have been described as non-organ affecting (Linthorst et al. 2010;

Houge et al. 2011). These variants have been reported to have residual enzyme levels above that likely to cause disease (Schiffmann et al. 2016). The GLA variant R118C has previously been included in this nonpathogenic group based on the absence of end-organ pathology in a large Portuguese family (Ferreira et al. 2015).

The deacylated form of globotriaosylsphingosine (lyso-Gb3) has been suggested as a biomarker of disease. Lyso-Gb3 accumulates in vasoendothelial cells and has been detected at a high level in plasma (Aerts et al. 2008). It correlates with left ventricular hypertrophy (Aerts et al. 2008; Rombach et al. 2010) and has been associated with the development of cerebrovascular accidents and white matter lesions (Rombach et al. 2010). Elevated lyso-Gb3 has therefore been recommended as an accurate determinant and noninvasive indication of clinically significant Fabry disease (Nowak et al. 2017). We therefore challenge the assertion that R118C is nonpathogenic, based on our clinical and biochemical findings in three family members with this mutation.

Methods

All samples were processed by the South Australian Pathology Service, the National referral Laboratory for Metabolic disease analysis, a Nationally Accredited facility. Genetic determination of Fabry sequence variants was performed on blood EDTA samples with restriction enzyme analysis. Alpha-Galctosidase activity was measured in dried blood spot samples. Lyso Gb3 was assayed by LC/MS (Talbot et al. 2017).

Peripheral neuropathy was determined by bedside examination including thermal discrimination testing.

Case Report

A 25-year-old heterozygous female with a family history of cardiovascular disease was identified as carrying the R118C GLA gene variant of FD through screening for familial cardiomyopathy. Plasma αGal activity was 1.5 nmol/h/mL (Normal 2.0–6.9 nmol/h/mL) on dried blood spot and lyso-Gb3 was 5 pmol/mL (Normal <5 pmol/mL). Clinical features included peripheral neuropathy, with temperature analgesia in hands and feet, hypohidrosis, and mild septal hypertrophy, but no proteinuria or cerebrovascular disease (see Table 1). In the absence of altered renal or cardiac function there were no indications for cardiac or renal biopsies. She has not to date been referred for enzyme replacement therapy.

The father of the index case, a 60-year-old homozygous male with hypertension, had reduced αGAL activity at 1.4 nmol/h/mL on dried blood spot, but no elevation in lyso-Gb3. Clinical signs included mild concentric left ventricular hypertrophy, cerebrovascular disease manifesting as white matter lesions, hypohidrosis, and peripheral neuropathy with temperature analgesia in hands and feet. In the absence of altered renal or significant cardiac dysfunction there were no indications for cardiac or renal biopsies. He has not been referred for enzyme replacement therapy at this point.

The sister of the index case, a 28-year-old heterozygous female, had αGal activity at the lower level of normal at 2.0 nmol/h/mL (Normal 2.0–6.9 nmol/h/mL) on dried blood spot, and no elevation in lyso-Gb3. Clinically she had hypohidrosis, temperature analgesia to hands and feet, and gastrointestinal symptoms. Of note she had a long cardiac history with symptomatic heart block requiring a pacemaker and cardiomegaly. A cardiac biopsy performed several years earlier showed occasional small and single lamellar bodies consistent with early Fabry disease. These pathological Fabry findings however were out of keeping with the severity of her cardiac disease suggesting a second pathology exacerbating her presentation.

Discussion

In classical FD phenotypes, diagnosis is established by low αGal levels, most reliably in males, and increased lyso-Gb3 in tissues or plasma (Schiffmann et al. 2016; Desnick et al. 1973). The pathological GLA mutation is then identified by genetic testing. Lyso-Gb3 has been confirmed as a biochemical marker in cases of uncertain diagnosis (Aerts et al. 2008; Nowak et al. 2017). We present clear evidence of the potential pathogenicity of the R118C GLA gene variant. Within our R118C family, three members had clinical phenotype of hypohidrosis, temperature analgesia, and reduced αGal levels, while an elevated plasma lyso-Gb3 was present in one heterozygote, and a cardiac biopsy showed early Fabry related changes in another. While disease severity is mild in this family, a diagnosis of mild but clinically significant FD has been established. This diagnosis has the potential to dramatically alter patient management, including the necessity for close monitoring and provision for prophylactic therapy against disease complications.

This family highlights both the difficulties of definitive causality of some GLA mutations in Fabry disease and the need for accurate biomarkers. Clinical signs were supportive of the diagnosis in each patient but have been debated in the literature. Definitive tissue morphology was limited to a single cardiac biopsy with only early Fabry changes. Indeed it is likely that a second pathology like a viral associated cardiomyopathy exacerbated this patient's presentation.

Table 1 Baseline clinical features of relatives with the R118C GLA mutation

	Patient 1	Patient 2	Patient 3
Age	25	60	28
Gender	Female	Male	Female
α-galactosidase A (normal 2.0–6.9 nmol/h/mL)	1.5	1.4	2.0
Lyso Gb3 (pmol/mL)	5	Not elevated	Not elevated
BMI kg/m^2	24.9	32.5	39
Symptoms			
Hypohidrosis	Yes	Anhidrosis	Yes
Angiokeratoma	Nil	Scattered	Nil
Neuropathic pain	Nil	Nil	Moderate
Gastrointestinal	Nil	Nil	Diarrhoea
Dyspnoea	Nil	Nil	NYHA 2
Cardiac			
ECG	NAD	LVH	Paced/bradycardia
IVSD (mm)	11	11	8
PWT (mm)	7	11	9
E/Ea	–	11	–
Renal			
eGFR (MDRD)	112	129	83
Proteinuria (g/24 h)	0.12	0.18	0.13
Serum creatinine	57	56	72
Neurological			
MRI brain			
WML	Nil	Mild	–
CVA	Nil	Nil	–
Ectasia	Nil	Mild	–
Neuropathy	Temperature analgesia	Temperature analgesia	Temperature analgesia

However, left ventricular hypertrophy has been described previously in a patient with this R118C mutation (Caetano et al. 2014). Recently Lyso-Gb3 has become established as a noninvasive maker for the presence of Fabry disease (Nowak et al. 2017). Where serum lyso-Gb3 is detected it serves as a marker of tissue involvement but its absence does not preclude tissue injury. Given that there has been debate in the literature about the cut-off of pathological lyso-Gb3 levels we have previously published a lyso-Gb3 assay (Talbot et al. 2017) with high cut-off of 5 pmol/mL such that there are no false positive results. However, aside from establishing the diagnosis, the correlation between specific disease mutations and lyso-Gb3 levels (Lukas et al. 2013) has not yet been proven, especially in females. Indeed lyso-Gb3 is not elevated in the late-onset M296I mutation (Mitobe et al. 2012) and 19% of females with the IVS4 + 914G>A mutation have lyso Gb3 within the normal range (Liao et al. 2013). Other studies however suggest that lyso-Gb3 levels can differentiate clinically relevant Fabry mutations (Lukas et al. 2013; Niemann et al. 2014; Nowak et al. 2017) and can separate high-risk affected females with the late-onset IVS4 + 914G>A mutation from unaffected controls (Liao et al. 2013). Furthermore there is currently no plasma lysoGb3 concentration that can predict either the absence or the presence of end-organ damage.

As in many diseases multiple additional genetic and epigenetic factors are likely to ameliorate or exacerbate the impact of each Fabry mutation, and hence underlie the significant heterogeneity of disease. For example, in FD patients with the p.A143T GLA mutation, the 10C>T polymorphism in the 5′ untranslated region of GLA has been shown to reduce alpha-galactosidase activity (Desnick et al. 2015). This could partially explain the pathogenic variability seen in this mutation. Whether a similar polymorphism can explain the variability in the R118C

mutation has not been shown. In any case family members with this mutation require ongoing assessment and end-organ investigation.

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Take Home Message

The Fabry R118C GLA mutation may cause clinically significant disease with reduced α galactosidase level and requires ongoing patient follow-up.

Compliance with Ethics Guidelines

Details of Contributions of Individual Authors

Andrew Talbot was responsible for data interpretation and original manuscript preparation.

Kathy Nicholls was involved in data interpretation and original manuscript preparation.

Conflict of Interest

Nil direct.

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Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000(5). Informed consent was obtained from all patients for data analysis of results included in the study.

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