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Fabry Disease: will markers of early disease enable early treatment and better outcomes?

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Fabry Disease: will markers of early disease enable early treatment and better outcomes?

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Abstract (200 words):

Purpose of review:

This review explores the clinical and pathological features of Fabry disease with particular focus on the cardiac pathology and presentation and the differences between the classical and later onset cardiac predominant forms. New modalities of imaging, biomarkers and long term treatment effects are discussed.

Recent findings:

Fabry disease is clinically heterogeneous and in women the clinical severity has recently been linked to skewing of X-inactivation. Two phenotypes have been described; one with early onset manifestation including pain and one with later onset single organ manifestations; however, the cardiac outcomes in these two groups appears similar. Fibrosis is found in renal and cardiac tissues on biopsy and appears to be a critical point in the pathology of Fabry disease after which response to enzyme replacement therapy is more limited. In vitro studies have suggested that lyso-globotriosylceramide may have an important role in the generation of fibrosis. Imaging including cardiac magnetic resonance imaging may have a role in detection of early stages of the disease. Long term outcomes for patients treated with enzyme replacement therapy are now being described with positive effects on renal function, cardiac architecture and clinical events with some suggestion that patients treated at earlier points in the disease course may have better outcomes.

Summary:

Fabry disease is a heterogeneous condition. Recent advance in understanding pathology, disease processes and treatment effects may enable future rational targeting of treatment to early disease with improved outcomes.

Keywords:

Fabry disease; enzyme; lysosomal storage; hypertrophy; fibrosis

Abbreviations:

Enzyme replacement therapy: ERT

Globotriaosylceramide: Gb3

alpha galactosidase A: GLA

Left Ventricular hypertrophy: LVH

Glomerular Filtration Rate: GFR

Mainz severity score index: MSSI

Chronic Kidney Disease: CKD

New York Heart Association: NYHA

Cardiac magnetic resonance imaging: CMR

Electrocardiogram: ECG

Introduction

Fabry disease (MIM 301500) is a rare X-linked lysosomal storage disorder resulting from deficiency of Alpha Galactosidase A and accumulation of it substrate globotriaosylceramide (Gb3) (1). Clinical features include pain in the extremities (acroparasthesia), sweating abnormalities, a rash (angiokeratoma) and gastrointestinal symptoms. Progression to proteinuric renal failure, cardiac hypertrophy and stroke may occur (2). The presentation is very heterogeneous affecting both males and females of all ages. Broadly, two phenotypes have been recognized an early onset classical form manifesting in childhood and a later onset form which often affects a single organ without the peripheral manifestations. However, the relationship of individual mutations to clinical presentation is not always clear and the impact of other factors on progression is unknown. Treatment is by intravenous infusion of the missing enzyme, (enzyme replacement therapy, ERT) and whilst originally described in terms of effects on surrogates such as substrate clearance on biopsy (3,4,) it has now been available for over 15 years and the long term clinical effects are emerging and new modalities of therapy are being described.

Pathophysiology

Over 600 mutations encompassing the 7 exons of the alpha galactosidase A (GLA) gene have been described with varying effects on alpha galactosidase A enzyme activity depending on type and site of mutation (5). In addition a specific splice-site mutation (IVS4+919>A) has been found to be prevalent within the Chinese Han population (6). While the relationship between GLA mutation, reduction in enzyme activity and substrate accumulation is qualitatively clear the role of individual mutations in generation of quantitatively different levels of substrate and its derivatives in tissues and their impact on organ changes is not well understood. The accumulated sphingolipid substrate Gb3 is converted to lysos Gb3 by acid ceramidase within tissues and is detectable on biopsy of affected organs, in the

circulation and in the urine (7,8). Findings in an in vitro model of increased collagen production after addition of lyso Gb3 point to a critical role in the genesis of fibrosis found in heart and kidney, possibly through notch1 signalling (9). However, little lyso-Gb3 is found in the plasma of patients with some so called late onset mutations associated with relatively preserved enzyme activity despite significant single organ effects such as left ventricular hypertrophy, posterior wall fibrosis, conduction defects and ensuing morbidity (10).

Diagnosis

Diagnosis in males is by confirmation of reduced alpha galactosidase A enzyme activity with confirmatory genotyping (11). However, in females random X-inactivation in the peripheral blood may result in non-informative enzyme activity in the normal range and a requirement for genotyping of the GLA gene to confirm the diagnosis. Measurement of Gb3 substrate and its derivatives such as lyso Gb3 and within organs, plasma and urine may be helpful in classification and assignment of pathogenicity but are not independently discriminatory for diagnosis since elevated levels have been found in patients with cardiac symptoms but without Fabry disease suggesting a possible contributory role to other conditions (12). Screening studies of patients with relatively non-specific symptoms may result in the detection of variants of unknown significance for which the pathogenic impact is unclear (13). Recent consensus initiatives have attempted to define criteria for the diagnosis of Fabry disease when the pathogenicity of a new mutation has not previously been described. Critical attributes include demonstration of low enzyme activity, relevant clinical features and ultimately demonstration of substrate accumulation in the relevant organ by biopsy where possible (14). Whilst classical and later onset forms of the condition often segregate with specific genotypes this is not absolute and each case should be individually assessed. For example while the N215S GLA mutation has been denoted a ‘cardiac variant’ presenting with cardiomyopathy, some patients have been described with proteinuria and renal failure (15).

Prior to diagnostic testing patients require genetic counselling and information in regard to possible future prognosis, therapy and implications of the condition for family making, employment and insurance. Pedigree analysis of an X-linked condition will be informative of genetically at risk individuals and individuals may require support in communicating the possibility of a life-limiting diagnosis to other family members (16). However, genetic screening of younger family members provides an opportunity for early diagnosis assessment and therapy of individuals who might have otherwise only come to medical attention after a detrimental event.

Screening and prevalence

In early studies the prevalence of Fabry disease was reported to be 1 in 117 000 (Australia)(17), 1 in 468 000 (Netherlands) (18) and 1 in 833 000 (Portugal) (19); however new born screening has detected a higher incidence of GLA mutations for example 1 in 3100 newborn Italian males (20) and 1 in 1600 in Taiwan (21). The pathogenicity and natural history of all of all mutations found in newborns is unknown and there remain some ethical implications of such strategies. Screening in Taiwan has uncovered a large population of patients with a single GLA mutation resulting in a later onset cardiac predominant phenotype of Fabry disease with more immediate clinical relevance to parents and grandparents than the newborn index case (22). The screening of targeted high risk populations has informed prevalence in symptomatically enriched populations; a systematic review by Linthorst et al. of screening studies in high risk groups showed a prevalence of 0.33% in male dialysis patients, 0.1% in female dialysis patients and at least 1% for patients with left ventricular hypertrophy (23). However, the prevalence in such screening studies may be artificially inflated due to removal of patients with other diagnoses and possible misattribution of pathogenicity to polymorphisms.

Clinical Features and classification

In the classical early onset form of the condition, males with low enzyme activity present during childhood with pain, gastrointestinal symptoms and sweating abnormalities (24). Intolerance to heat and exertion provokes painful episodes in the extremities and leads to a failure to participate in a sporting activities and pain crises at the time of febrile illnesses may prolong absence from school. Corneal opacities (cornea verticillata) and rash (angiokeratoma) in characteristic regions around the mouth, umbilicus, scrotum and hands are external features of the disease which may suggest the diagnosis (25). Proteinuria occurs in the second decade heralding decline in glomerular filtration rate and progression to end stage renal disease in a proportion of patients whilst concentric left ventricular hypertrophy and conduction abnormalities emerge subsequently. In addition to pain, neurological manifestations include hearing loss, CNS white matter lesions of unknown pathogenesis, TIA and stroke (26). Despite the X-linked inheritance females may manifest any of the clinical features of Fabry disease and whilst often of later onset and milder severity compared with males, significant clinical events including renal failure, arrhythmias and stroke are described. This finding emphasizes the importance of local enzyme activity and substrate accumulation in the tissues compared with other X-linked disorders such as haemophilia where a small percentage of normal enzyme activity may

function effectively within the circulation. The particularly variable severity in females is probably related to the random nature of X-inactivation with recent studies demonstrating a relationship between clinical features, enzyme activity and X-inactivation in the peripheral blood (27). Further work is required to confirm the relevance of X-inactivation in cardiac and renal tissues on local organ manifestations.

More recently case finding studies aimed at patients with single organ manifestations such as renal failure, left ventricular hypertrophy and stroke have facilitated the diagnosis of patients with apparently later onset manifestations and more preserved enzyme activity (non-classical disease). Whilst diagnosis is later due to the absence of childhood suggestive symptoms such as acroparasthaesia and angiokeratoma, organ manifestations may be comparable to the classical form with similar burden of organ-specific morbidity.

Cardiac features

Features of the cardiac involvement of Fabry disease include left ventricular hypertrophy, rarely with left ventricular outflow tract obstruction, impaired myocardial function, and progressive myocardial fibrosis, resulting in chest pain, heart failure and arrhythmias, including, on occasion, sudden cardiac death (28). Perinuclear vacuolation, intracytoplasmic whorled bodies, myocyte hypertrophy and fibrosis have been described on cardiac biopsy and at post mortem with macroscopic findings of left ventricular hypertrophy, posterior wall thinning and mild right ventricular hypertrophy. Coronary arteries are thickened without occlusion of the lumen with little atherosclerotic disease and whilst valvular abnormalities have been described they are rarely clinically significant (29).

ECG abnormalities, such as shortened PR interval, LVH on voltage criteria, and t wave inversion are also early findings and may precede detection of LVH on echocardiography. Pacing for atrioventricular and sinus node disease is common; QRS duration and PR interval were found to be independent predictors of future anti-bradycardia pacing (30).

Risk factors most strongly associated with CV events of myocardial infarction, heart failure and death in both sexes were found to be hypertension and LVH (31). Patients with renal involvement have been found to have more severe cardiac disease. Class 5 chronic kidney disease (CKD5) was associated with worse baseline cardiac parameters and progressive LVH. LVMI increased by 35.4±31.8 g/m$^{2.7}$ in CKD5 *vs* 5.7±7.9 g/m$^{2.7}$ in non-CKD5, p=0.044) and cardiovascular events (including sudden death, arrhythmia and pacing device insertion) occurred in 100% of patients with CKD5 (21 events) compared with 26% in non-CKD5 patients (7 events) (32). However, the type of mutation does not appear to be associated with cardiac outcome. In a study of 207 consecutive patients from one cardiology clinic

(47% male, mean age 44 years) 28% had mutations associated with a cardiac predominant phenotype, 10% developed severe heart failure (NYHA) ≥3) 6% developed atrial fibrillation, 6% received device for bradycardia, and 3% cardiac deaths were recorded. When a composite endpoint of new onset AF, NYHA ≥ 3, device insertion for bradycardia and cardiac death was considered the incidence: 2.64 per 100 person-years (CI 1.78 to 3.77) did not differ between significantly between classical and cardiac variant late onset phenotypes (33). Independent predictors of outcome were the Mainz severity score index (MSSI) and QRS interval but not genotype. (33).

Myocardial fibrosis: cardiac imaging and biomarkers

Myocardial fibrosis, a common finding within the posterior wall at post mortem appears to correspond with posterior wall late gadolinium enhancement on cardiac magnetic resonance (CMR) imaging (34). Subjects with high annual increase in left ventricular fibrosis are at risk for sudden cardiac death. In males, fibrosis is generally detected subsequent to emergence of LVH. One study found late enhancement was not seen with end-diastolic LV wall thickness less than 12 mm (LV mass <99 g/m^2) and was always associated with low systolic strain rate however in females fibrosis was described prior to LVH and the severity of functional impairment was independent of LV wall thickness (35). This finding has possible implications for the timing of initiation of specific therapy since late gadolinium enhancement was associated with increased LV Mass, worse myocardial function and failure of regression with enzyme replacement therapy (36). Similarly, abnormalities on 2D speckle-tracking have been independent of LVH and in patients with normal ejection fraction. Morris et al. found principal factors linked to reduced myocardial function include LV septal wall thickness > 15 mm, RV free wall thickness > 7 mm, and LV longitudinal dysfunction. LV and RV fibrosis were also linked to reduced LV and RV strain and patients with reduced LV, RV, and LA strain had a worse functional class (37).

A recent sophistication of Fabry CMR imaging is non-contrast T-1 mapping where lipid is known to reduce the T1 parameter and was hypothesized to detect early cardiac involvement. T1 in Fabry disease appears to be discriminatory of other disorders causing LVH such as hypertension, hypertrophic cardiomyopathy and amyloid, and also to correlate inversely with left wall thickness. Low T1 may represent sphingolipid infiltration and 'pseudonormalisation' in the posterior, wall fibrosis, however correlative biopsy data is not yet available (38). Low T1 appears to occur in some patients before hypertrophy and may be a marker of early involvement and possible imaging marker of treatment effect.

Potential circulating biomarkers include both substrate and related compounds and downstream markers of pathophysiology including fibrosis. Reports are varied as to the effects of lyso-Gb3 on collagen synthesis (9,39). However, matrix metalloproteinase 9 was significantly higher in Fabry disease and correlated positively with MSSI and negatively with endocardial fraction shortening (40). NT propBNP is a marker of diastolic dysfunction and correlated with age, creatinine, left atrial index, and abnormal ECG (41).

Fabry specific therapy

Current specific therapy for Fabry disease entails intravenous infusion of recombinant (agalsidase beta; Fabrazyme) or gene-activated (agalsidase alfa; Replagal) preparations of the deficient alpha galactosidase A. Both preparations have been available for 15 years and whilst initial demonstration of effect was based on substrate clearance on biopsy, reduction in pain and left ventricular hypertrophy (3,4) longer term clinical endpoints in patients starting early and later in the course of the disease are now being reported. A recent study of severe clinical events, renal function and cardiac structure in patients from original phase 3 clinical trials who had received agalsidase beta for ten years found 81% were free of severe clinical events (chronic dialysis, kidney transplant, myocardial infarction, congestive heart failure, major cardiac procedures, stroke and death) at ten years and survival was 94% (42). Patients classified at the outset as having low or high renal involvement had different slopes in GFR (-1.89 *vs* -6.82 mL/min/1.73m^2/year). Similarly patients who started treatment later (over 40 years) had increases in IVST LPWT compared with those under the age of 40 who had no change in LPWT and under 30 who had no change in IVST; patients commencing therapy ≥30 years to <40 years had small increases in IVST. Further suggestion of the importance of early therapy is provided in a retrospective registry study of patients receiving agalsidase beta for 5 years where the risk of severe renal, cardiovascular and cerebrovascular clinical events during the first six months of therapy was greater in males, and patients > 40 years and in each case decreased with further time receiving enzyme replacement (43). In patients with clinical events prior to therapy the risk of further events during the first 6 months was not different to patient without events but did increase thereafter.

A single centre retrospective study of patients receiving agalsidase alfa for ten years found heart failure classification had improved by at least 1 class in 22/42 patients, and angina was stable or improved in 41/42 patients. During this time no patient without LVH at baseline developed new LVH patients. In males with baseline values ≥50 g/m$^{2.7}$, LVMI was significantly reduced after 10 years although in similar females whilst an early improvement was noted mean LVMI was not significantly

different from baseline at ten years (44). In an attempt to compare long term registry data of ERT treated patients with the natural history of untreated patients data of patients from the Fabry Outcome Survey (a Shire sponsored database) who were had received agalsidase alfa for a median of 5 years were compared with published series of untreated patients. A slower decline in renal function and slower progression of LVH was seen in patients receiving agalsidase alfa was noted and clinical events occurred later in treated patients, (16% risk of a composite morbidity event after 24 months with ERT *vs* 45% without treatment). (45)

Emerging therapies

Recently the utility of active site specific chaperone therapy that bind and stabilise mutant protein facilitating trafficking to the lysosome has been explored. Results of three phase 2 studies of migalastat HCL (AT-1001, GR181413A, 1-deoxygalactonojirimycin), an orally bioavailable imino sugar, have been presented indicating increased activity of alpha galactosidase A and reduction of substrate in relevant tissues in vivo. (46,47) Phase 3 studies are ongoing. The use of substrate reduction therapy by inhibition of glucosyl ceramide synthase is an alternative approach to the development of an oral therapy for Fabry disease that is in early clinical development. Treating Fabry mice with compound Genz-682452 resulted in reduced tissue substrate and a delayed the onset of a deficit in thermosensory responses. The effects were most notable in young mice prior to the development of pathology (48). These products are not yet approved and remain investigational.

Conclusion

After 15 years of the availability of ERT for Fabry disease long term data for the follow up of original trial cohorts and registry studies is demonstrating an effect not only on symptoms and surrogates including left ventricular mass and GFR but also on clinical events and mortality. Risk analyses indicates that patients commencing therapy at older ages and later stages of disease are less likely to have good response to therapy. This new understanding may allow the coherent use of investigations to detect early disease and direct Fabry-specific therapy to the pre-fibrotic stage of disease when outcomes might be optimised.

Key points:

- Fabry disease is a rare X-linked disorder resulting in renal dysfunction, left ventricular hypertrophy and stroke.

- An early onset classical form is characterised by acroparasthaesia, angiokeratoma, sweating abnormalities and corneal whorls; a later onset form with single organ, usually cardiac manifestations is recognised.
- The pathology of cardiac and renal dysfunction appears to involve fibrosis and having occurred this appears to limit the effects of Fabry specific enzyme replacement therapy
- New imaging biomarkers such as t1 mapping may allow the detection of early stages of the disease
- Long term data on patients treated with Enzyme replacement therapy are now becoming available and point to better responses when patients are treated at an earlier stage

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Conflicts of interest

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Structured abstract	Y
Key words (max. 5)	Y
Introduction	Y
Headings in text	Y
Conclusion	Y
Key points box	Y

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