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Review

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Fabry nephropathy: a treatable cause of chronic kidney disease

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Abstract

Fabry disease is a rare X-linked inborn error of metabolism that has a high prevalence of chronic kidney disease (CKD) and renal failure. It is due to the deficiency of the α-galactosidase A (α-Gal) lysosomal enzyme with subsequent accumulation of globotriaosylceramide (Gb3) in lysosomes. In the kidney, the podocyte is the main target of this disease, although all cell types are involved. The podocyte, being terminally differentiated, does not replicate and thus accumulates Gb3 throughout life. Podocytes are injured by Gb3, leading to their detachment from the glomerular basement membrane and subsequent loss in the urine. Albuminuria starts in childhood and progresses to overt proteinuria in the teens and 20 s. CKD ensues with adults starting dialysis at an average age of 42 years. Patients have a high prevalence of stroke and cardiomyopathy with hypertrophic change, heart failure, and dysrhythmias. Patient survival is limited in both genders. Diagnosis is based on the demonstration of a low α-Gal activity and a pathogenic *GLA* mutation. Clinical features are highly variable, which makes recognition of this condition difficult. Treatment with intravenous recombinant human enzyme replacement therapy (ERT) and oral pharmacologic chaperone are available. Control of proteinuria to 0.5 g/day or less is of critical importance to limit progression to end-stage renal disease. Early initiation of treatment gives the best results, but the optimal age to start is uncertain. Fabry nephropathy remains a challenge due to its multisystem nature, difficult diagnosis, and complicated management. It is important as a treatable cause of CKD.

Keywords: Rare disease, enzyme replacement therapy, pharmacologic chaperone, Fabry disease, Gb3, lyso-Gb3

INTRODUCTION

Most lysosomal diseases are characterized by the deficiency of lysosomal enzyme function. In the case of Fabry disease (OMIM 301500), the deficiency is that of alpha-galactosidase A (α-Gal), which cleaves the terminal galactose residue from the glycosphingolipid substrates, the main one being globotriaosylceramide (Gb3). As a result of this deficient enzyme activity, Gb3 accumulates in lysosomes in virtually all cells and tissues throughout the body, leading to organ dysfunction. This results in strokes, renal failure, cardiomyopathy, sensory peripheral neuropathy, hearing loss, altered sweating, corneal dystrophy, and angiokeratomas with disability and early death[1]. It is the most common lysosomal disease that has a major kidney phenotype with a high prevalence of end-stage kidney disease (ESRD).

Kidney involvement in Fabry disease becomes clinically apparent in childhood with microalbuminuria. It is highly prevalent, with most adult patients having renal Gb3 deposits on biopsy and/or proteinuria. However, classically affected male patients and those with renal variant disease are most likely to develop chronic kidney disease (CKD) and progress to ESRD.

While females are twice as likely as males to be involved in this X-linked disorder, mosaicism exhibited in heterozygotes results in milder disease. As a result, females with Fabry disease are far less likely to have progressive CKD or end up needing renal replacement therapy[2].

Overall, Fabry nephropathy is a rare cause of both CKD[3] and ESRD. However, it is important as a treatable cause of CKD. Efforts to make an early diagnosis with genetic counseling and early therapy can be of great benefit to patients.

The diagnosis of Fabry disease is difficult as this rare condition is hard for clinicians to recognize due to its shared clinical features with many other common diseases. Like many genetic disorders, Fabry disease is characterized by great heterogeneity of both genotype and phenotype. There are over 1,000 variants described so far in the *GLA* gene that codes for α-Gal (HGMD, ClinVar). Knowledge of a patient's genotype is helpful as it will often predict the Fabry disease phenotype, either classic early-onset Fabry disease or later-onset cardiac or renal-limited disease. Male hemizygotes with very low α-Gal activity (< 3%) will have a more severe disease phenotype than the female heterozygotes[1].

Management of Fabry disease has advanced greatly since the introduction of the first treatment with recombinant human enzyme replacement therapy (ERT) in 2001, but there remain many unmet patient needs. There are now additional agents available such as pharmacologic chaperone and modified ERT. Investigational approaches include substrate reduction therapy, gene therapy, T and B cell therapies, and mRNA therapies.

We review Fabry nephropathy to provide an update on this challenging condition.

EPIDEMIOLOGY

Fabry disease is the second most common form of lysosomal disease, next to Gaucher disease. It is pan-ethnic and worldwide in distribution. Its prevalence has been estimated to be between 1 in 50,000 to 100,000 people[4].

Newborn screening studies have reported higher frequencies of 1/1,600 males and 1/4,000 females[5-7], with a preponderance of late-onset or attenuated variants and variants of unknown significance (VUS) compared

with classical genotypes. Kermond-Marino *et al.* (2023) estimated that 1 in 3,225 individuals in the general population have undiagnosed Fabry disease, based on an assessment of gnomAD variant pathogenicity[8]. These observations suggest that the true prevalence likely greatly exceeds earlier estimates.

In Canada, the Canadian Fabry Disease Initiative registry (CFDI) has 671 patients enrolled, with 57 reported deceased; ascertainment is over 95%, with a female-to-male ratio of 2:1[9]. There are 30 to 40 new patients entered annually into the CFDI, suggesting that there are still many patients yet to be discovered. The prevalence of Fabry disease in Canada is currently estimated at about 671/38,781,291 or 1 in 57,796. In Nova Scotia, due to a significant founder effect, the prevalence is higher at 1 in 10,083, with 105 patients in a 1,058,694 population, illustrating the marked geographic variability in the prevalence of this condition.

Cross-sectional estimates of prevalence based on the presence of proteinuria/albuminuria and decreased estimated glomerular filtration rate (eGFR) will vary based on age and sex distribution as well as the genotype (classic *vs.* late-onset variant) of each patient population. Age is the most important factor in this progressive disease, as younger patients may exhibit no albuminuria or proteinuria and have normal kidney function. Gender is also important, as female heterozygotes have much less ESRD than male hemizygotes, with a 1:7 ratio[2], in contrast with the 2:1 ratio expected in an X-linked condition such as Fabry disease. Kidney disease is common in classic males with Fabry disease and those males with variant renal phenotypes. In a referral population of 105 men with mainly classical genotypes, prior to ERT, 74% developed CKD, defined as overt proteinuria or decreased eGFR[10].

Ortiz *et al.* (2008) reported on 1,262 untreated adults with Fabry disease from the Fabry Registry[11]. CKD stage distribution by gender is presented in Figure 1A and B. Men had twice the percentage in CKD stages III-V than women (28% *vs.* 13%). These percentages were higher in patients older than 40 years (yr), at 45% for men and 20% for women, reflecting CKD progression. The excess of males with advanced CKD relative to females in this study is probably an overestimate as female enrollment was limited at 48% when 66% would have been expected for this X-linked disease[11].

Patients with late-onset variant genotypes tend to have less and later kidney disease than those with classic genotypes, but there is considerable variability among the late-onset variant genotypes, especially the N215S variant. Germain (2018) reported on 59 men and 66 women, mean ages 51.9 and 41, respectively, with the N215S variant; 17% of men and 3% of women had eGFR < 60 mL/min/1.73 m^2. During follow-up, 1/125 (0.8%) N215S progressed to ESRD *vs.* 17/381 (4.4%) classic genotype[12]. Oder (2017) studied 26 N215S patients, mean age 49 years, 50% males, with Fabry cardiomyopathy. Two men aged 53 and 74 years, respectively, had albuminuria > 1 g/g with CKD stage II and III; the rest were normal[13]. Lavalle (2018) compared N215S patients, 37 men and 47 women, with non-N215S patients[14]. Two men with the N215S variant presented with ESRD and two more progressed to ESRD. CKD prevalence at presentation was 9/78 (11.5%) *vs.* 18/148 (12.2%) in nonN215S patients, although CKD and proteinuria started later in the N215S group.

Data on renal parameters in patients with the IVS4+919G>A intronic late-onset cardiac variant from Taiwan are limited. CKD distribution was Stage I 41%, II 41%, III 14%, and IV/V 5% in 22 adults, with 17 males and 5 females and median age 61 years[15]. Microalbuminuria was reported in 25% of men and 18% of women with this variant[16].

The prevalence of Fabry disease patients on dialysis in the US is reported at 0.02% in the 1995-1998 USRDS cohort, similar to that in Europe[2]. As there are more known patients with Fabry disease than 25 years ago, the prevalence of this disease has likely risen in the dialysis population.

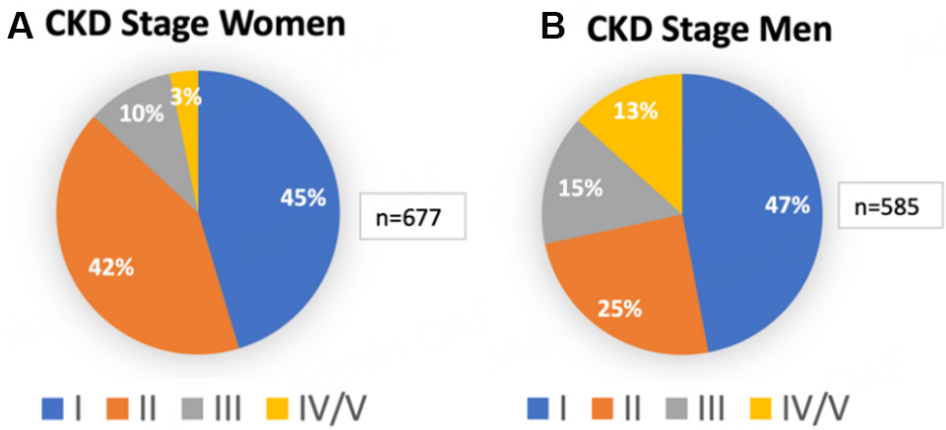

Figure 1. CKD stage distribution in women (A) and men (B). Data from Ortiz *et al.* (2008)[11]. CKD: Chronic kidney disease.

CLINICAL FEATURES

Fabry nephropathy is characterized by significant phenotypic heterogeneity, even within families, affecting both classic and late-onset variants[17]. For example, the age at which dialysis begins can differ by up to 40 years among men with the same classic A143P variant. (unpublished data M. West)

Urine sediment

The urine in Fabry disease will contain glycosphingolipids such as Gb3, globotriaosylsphingosine (lyso-Gb3), and others. These will be present early in childhood and are not associated with albuminuria or proteinuria. These molecules are thought to be too large to be filtered by the normal glomerulus. Their presence in urine is thought to arise from degenerating podocytes and tubular cells. Lamellar bodies can be identified by electron microscopy within the spun urine sediment. Under polarized light, birefringent Maltese crosses may be seen in free lipid droplets, fatty casts, oval fat bodies, and Mulberry cells which are enlarged tubular epithelial cells full of lipid droplets[18].

Podocyturia

Podocytes can be found in the urine of normal individuals but are increased in patients with CKD, reflecting drop-off of the glomerular basement. Podocyturia is increased in Fabry nephropathy and increases with age. It occurs before albuminuria or proteinuria, and the amount of podocyturia correlates positively with proteinuria in both genders and inversely with eGFR in males but not females with Fabry disease[19]. While podocyturia could be a biomarker for glomerulopathies, there is no standardization of the identification or quantitation of urine podocytes and this test is not available outside of a research setting.

Proteinuria

In Fabry disease, albuminuria often starts in childhood and progresses to overt proteinuria with age. Most adults with classical Fabry disease will have some degree of proteinuria. The prevalence and magnitude of proteinuria are higher in males than in females at all ages and at all CKD stages. In a NIH referral population of 105 men, with median age 35 years, overt proteinuria occurred in 85%, starting at age 14 years. Nephrotic-range proteinuria occurred in 18%, with a mean of 5.6 g/day, but nephrotic syndrome with hypoalbuminemia and hypercholesterolemia was rare[10]. In a cross-sectional analysis of 1,262 adults with Fabry disease, there were CKD patients in both genders with eGFR < 60 mL/min/1.73 m^2 who had no

proteinuria (males 11% and females 28%). Median proteinuria was 572 mg/day in males and 180 mg/day in females[11]. In 84 men and women with the N215S late-onset variant, the prevalence of proteinuria was not different from the 167 non-N215S patients, 17.9% *vs.* 26.3%, respectively[14].

Hematuria

Microhematuria has been reported in Fabry disease, but its significance is uncertain[20]. In other podocytopathies, microhematuria has been associated with a worse renal prognosis[21]. Whether this applies to Fabry nephropathy is unknown.

Parapelvic cysts

Parapelvic cysts are common in Fabry nephropathy, and their prevalence increases with age to over 45% by age 50 years in both men and women compared with a prevalence of 1%-6% in the general population[22]. Therefore, the detection of parapelvic cysts during kidney ultrasound should prompt the consideration of Fabry disease. There is no association with decreased eGFR or proteinuria, suggesting that the parapelvic cysts do not contribute to the progression of Fabry nephropathy. Glycosphingolipids have been implicated in polycystic kidney disease, suggesting a role for Gb3 in parapelvic cyst formation in Fabry disease.

Tubular syndromes

Fanconi syndrome and nephrogenic diabetes insipidus are both rare complications of Fabry nephropathy, with involvement of proximal and distal renal tubular epithelium, respectively[23,24]. They are most likely to occur in males with classic Fabry phenotype. As they may be the presenting feature of Fabry disease, nephrologists should be aware of this association.

Progressive CKD

In males with classical genotypes, progressive CKD is the norm, with most patients developing increasing proteinuria and declining eGFR between the ages of 20 and 55 years. They will progress to ESRD unless they die from another Fabry disease complication such as cardiomyopathy or stroke. Females with classical genotypes are far less likely to progress to ESRD due to mosaicism and perhaps skewing of X chromosome. Males with rare late-onset renal variants are also at high risk for ESRD, but there are few data as to this subgroup. Males with the late-onset cardiac genotype face a risk of developing Fabry nephropathy, particularly those with the N215S genotype. Females with late-onset cardiac variant disease probably have minimal risk of developing advanced Fabry nephropathy.

In a referral population of 105 men at the NIH, prior to ERT, 74% developed CKD; only 19% received angiotensin-converting enzyme inhibitors (ACEinh) or angiotensin receptor blockers (ARB)[10]. CKD onset was observed in 37%, at mean age 42 years (ranging from 19-54 years). Fifty percent of patients had decreased GFR by the median age of 42. 23% started dialysis with an onset age range of 21-55 years; there were kidney transplants in 14 patients. All patients who survived to age 55 started dialysis; all patients were dead by age 60 years, with a mean age at death of 50 years.

In men with N215S late-onset variant, mean eGFR was no different from that of men with classic Fabry disease; there was no difference in eGFR between women in these two groups, indicating that some late-onset variants can have significant Fabry kidney disease[12].

eGFR slopes are higher in untreated males than in females, reflecting their different genotypes. There is a range of slopes reflecting the effect of different variants as well as other factors such as hypertension, use of ACEinh or ARB, proteinuria, and possible modifier genes and other factors. In males, mean GFR slopes were reported as -12.2[10], -3.85[25], -7.0[26], and -8.3 mL/min/1.73 m^2/yr[27]. In females who did not reach

ESRD, eGFR slopes were the same as in the general population, at -1.02 and -0.83 mL/min/1.73 m^2/yr[25,28]. Females who did reach ESRD had eGFR slopes similar to males at -3.05 mL/min/1.73 m^2/yr[25]. Few of these patients received ACEinh or ARB therapy.

Increasing proteinuria is associated with a marked decrease in eGFR whether or not patients are receiving ERT[25,26]. Schiffmann (2009) reported worsening eGFR slope in untreated men with Fabry disease from -1.6 to -3.3 to -6.9 mL/min/1.73 m^2/yr as proteinuria increased from < 0.1 to ≥ 0.1- < 1.0 to ≥ 1.0 g/day[25]. Similarly, in women, the eGFR slope worsened from -0.6 to -2.2 to -4.6 mL/min/1.73 m^2/yr over the same range of proteinuria. Men had a much greater decline in eGFR than women at all levels of proteinuria[25]. In 108 ERT-treated men with proteinuria < 1 g/day, eGFR slopes were 1.0-2.0 mL/min/1.73 m^2/yr compared with eGFR slopes > 5 mL/min/1.73 m^2/yr for 38 patients with proteinuria ≥ 1 g/day[26].

Renal events defined as stage V CKD, dialysis, or kidney transplant were studied in 541 Fabry patients. Men with classical phenotype had a much higher renal event rate than men with non-classical disease ($P < 0.01$) and women with classical phenotype ($P < 0.05$)[29].

Risk factors for progressive CKD in Fabry disease include age, gender, classic phenotype, proteinuria, low eGFR, hypertension, high plasma lyso-Gb3, very low α-Gal activity, conservative mutation, and vitamin D deficiency[10,30,31].

Chiorean *et al.* (2023) reported on a retrospective cluster analysis of pre-treatment Fabry disease patients from a large US electronic health record network database. The prevalence of Fabry disease was 1 in 101,729[32]. They divided the 234 Fabry patients, mean age 46 years, into 7 clusters based on eGFR and age patterns. Follow-up duration averaged 9.2 years. Data from 5 clusters were informative, with patients showing eGFR slopes from -1.13 to -3.11 mL/min/1.73 m^2/yr. Prevalence of CKD stages II-V varied from 17% to 77% across the clusters. Fabry complications varied between groups. This study shows the spectrum of phenotypes in Fabry disease.

Cardiorenal syndrome (CRS) type 5 has been identified in Fabry disease, where concurrent diseases in the kidney and heart contribute to pathology in other organs[33]. Siegenthaler (2017) conducted a study on 104 adults with Fabry disease over a median of 105 months. Kaplan Meier analysis revealed that the survival rate was lowest for the 28 patients with CRS, among whom six died[34]. There is bilateral organ crosstalk through various mechanisms that are activated as each organ is damaged by Fabry disease and begins to fail. As heart function is progressively compromised by Fabry cardiomyopathy with both diastolic and systolic dysfunction, this will lead to decreased kidney perfusion, which will, in turn, result in decreased eGFR and worsening Fabry nephropathy. Alternately, as Fabry kidney disease progresses, there will be an accumulation of salt and water, which will contribute to fluid overload and worsening heart failure. Anemia and hypertension from kidney disease will increase left ventricular hypertrophy (LVH); metabolic acidosis and various electrolyte disorders will contribute to decreased cardiac function and arrhythmias. The activation of the renin-angiotensin system occurs in both kidney and heart diseases and is an important avenue for intervention with ACEinh and ARB. Newer agents such as SGLT2 inhibitors will reduce proteinuria, stabilize eGFR, and treat both systolic and diastolic heart failure, making them suitable for managing CRS[35]. Recognition of CRS is important given its poor prognosis, allowing for optimized therapy for both kidney and heart conditions.

Dialysis

In a cohort of 250,352 dialysis patients from the USRDS database, 42 untreated Fabry patients were identified, comprising 37 males and 5 females (12%), with a mean starting age of 42 years. Their survival rate was found to be intermediate compared to age-matched controls with diabetes mellitus or without diabetes mellitus; specifically, the survival rate at 3 years was 63% for the Fabry patients[2].

Renal cell cancer

There is a single-center report of a marginally reduced rate of all cancers but possibly increased rates of melanoma, urological malignancies, and meningioma in Fabry disease patients compared with the age-matched general population[36]. In particular, there is an increased association with renal cell carcinoma[37]. A pathophysiologic link between Gb3 accumulation, oxidative stress, and oncogenesis via the von Hippel-Lindau/hypoxia-inducible factor 1 pathway has been suggested[37].

Extrarenal manifestations

Most patients have multiple organ system involvement in Fabry disease. Common features include cornea verticillata, clustered angiokeratomas, hypohidrosis, and acroparesthesia which is a sensory peripheral neuropathy involving the distal limbs with pain, pins and needle sensation and dysesthesia. Strokes and TIA commonly start in middle age. White matter lesions are frequently observed in brain imaging. Cardiac involvement can include cardiomyopathy with hypertrophic change, diastolic dysfunction, dysrhythmias, elevated hsTroponin, EKG abnormalities (resting sinus bradycardia, LVH, short PR interval), and cardiac MRI with low T1 and late gadolinium enhancement of the left ventricular mesomyocardial area often over the posterolateral wall. Hearing loss, both acute and chronic, is common, along with chronic tinnitus, dizziness, fatigue, and obstructive lung disease often misdiagnosed as asthma. Chronic symptoms of alternating diarrhea and constipation, and abdominal bloating with pain may be misdiagnosed as irritable bowel syndrome[1]. See Figure 2.

DIAGNOSIS

The diagnosis of Fabry disease rests on the demonstration of a pathogenic variant in the DNA of the *GLA* gene on the X chromosome. All males will show a decrease in α-Gal activity in leukocytes, plasma, or whole blood, with classical males having a marked reduction (< 5%). Males with late-onset variant disease and all females will demonstrate a lesser decrease in α-Gal activity and up to a third of females may have normal enzyme activity due to mosaicism[38].

Elevated biomarkers such as Gb3 or its metabolite lyso-Gb3 in urine or plasma are commonly documented to confirm pathogenicity. For patients with a VUS, pathogenicity must also be supported by demonstrating glycosphingolipid deposits in tissues, typically through skin, kidney, or cardiac biopsy, in addition to measurements of α-Gal activity and biomarkers. Some variants are of uncertain pathogenicity as there are conflicting reports in the literature, e.g., R118C[39,40] and A143T[41,42]. Given the marked heterogeneity of Fabry disease phenotype with these variants, a diagnosis of Fabry disease can only be made on a case-by-case basis by demonstrating pathogenicity. This will require further investigations and possibly evaluation by a medical geneticist and a Fabry specialist at a center of excellence for Fabry disease.

Elevated Gb3 or lyso-Gb3 biomarkers or modestly decreased α-Gal activity by themselves are also insufficient for the diagnosis of Fabry nephropathy, as these results may be abnormal in some patients with CKD of other causes. See Table 1. These observations mean that the use of these parameters for diagnosis or screening may not be accurate in CKD patients unless *GLA* analysis is included. *GLA* analysis is also important for determining phenotype and informing treatment decisions regarding chaperone therapy.

Table 1. Pitfalls in the diagnosis of Fabry nephropathy

	Reference
Low α-Gal activity in patients without Fabry disease on hemodialysis	Nakao *et al.*, 2003[43]
Low α-Gal activity reported in 9% of patients with FSGS but without Fabry disease	Hasbal *et al.*, 2020[44]
Abnormal elevation sphingolipids reported in patients with FSGS and diabetic nephropathy	Mersher *et al.*, 2014[45]
Increased urine glycosphingolipids in patients with chronic glomerulonephritis	Townsend *et al.*, 1978[46]
Elevated urine Gb3 in men with nephrotic syndrome without Fabry disease	West *et al.*, 2012[47]
Increased plasma lyso-Gb3 in other lysosomal diseases	Ferraz *et al.*, 2016[48]

α-Gal: Alpha-galactosidase A; FSGS: focal segmental glomerulosclerosis; Gb3: globotriaosylceramide; lyso-Gb3: globotriaosylsphingosine.

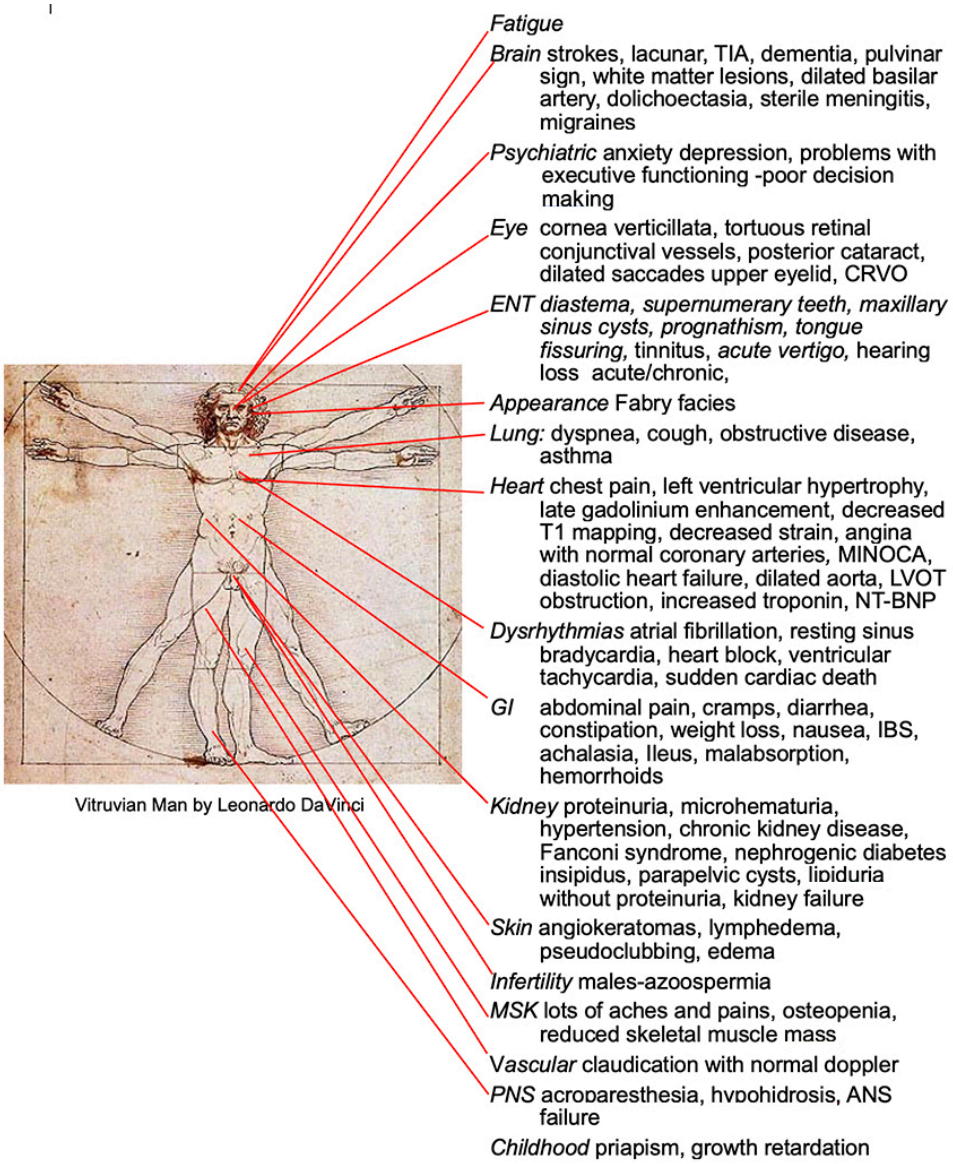

Figure 2. Multisystem clinical features in Fabry disease. TIA: Transient ischemic attack; CRVO: central retinal vein occlusion; MINOCA: myocardial infarction with non-obstructive coronary arteries; LVOT: left ventricular outflow tract obstruction; NT-BNP: N-terminal brain natriuretic peptide; IBS: irritable bowel syndrome; ANS: autonomic nervous system.

A renal biopsy showing lamellar bodies is also insufficient for diagnosis as these are not specific to Fabry disease. The most common mimic of Fabry nephropathy is a drug-induced phospholipidosis (DIP). There are over 50 cationic amphiphilic drugs that inhibit lysosomal enzymes with resultant glycosphingolipid accumulation in lysosomes[49]. The renal pathology is indistinguishable from Fabry nephropathy[50]. Patients have CKD with proteinuria and occasionally low α-Gal activity. The only way to rule out DIP is through the identification of a pathologic *GLA* variant. Choung *et al.* (2022) reported lamellar bodies in 32 [(0.73%) of 4,400 renal biopsies; only 6 (18.8%) had Fabry disease , and 26 (81.2%) had other renal pathology and/or possible DIP][50]. This suggests that DIP might be more prevalent than Fabry nephropathy in some areas and is an argument for *GLA* analysis in all Fabry disease patients.

When examining a patient with unexplained CKD, the presence of Maltese crosses in the urine sediment under polarized light indicating lipiduria raises the possibility of Fabry nephropathy, especially if proteinuria is sub-nephrotic or absent[18]. Urine Mulberry cells will also show Maltese crosses and these are said to be specific to Fabry disease[51].

Another renal clinical clue suggesting Fabry disease is the presence of parapelvic cysts on renal ultrasound. Note that patients do not need to have a positive family history, as 5%-10% of variants are new mutations.

KIDNEY BIOPSY

Renal biopsy may be undertaken in patients with Fabry disease for various reasons. See Table 2. Electron microscopy is essential for identifying the location, size, and number of glycosphingolipid deposits. Advanced CKD in women with Fabry disease is uncommon relative to males with classic disease[2]. A renal biopsy should be considered to confirm the diagnosis and to rule out concomitant renal disease.

There are numerous case reports of patients with Fabry disease and a concurrent second renal disease, usually a type of glomerulonephritis with IgA nephropathy as the most common[52]. Clues to the presence of a second glomerular disease would be the new onset of microhematuria, a sudden and marked increase in proteinuria, or acute kidney injury superimposed on CKD in a previously stable patient.

While there are very rare reports of an immune complex membranous glomerulonephritis with anti-drug antibodies during ERT in Pompe disease, another lysosomal disease, this has yet to be recognized in Fabry disease[53,54].

SCREENING FOR FABRY NEPHROPATHY

Screening for Fabry disease is usually done by a combination of *GLA* analysis, α-Gal activity, and plasma lyso-Gb3. High-risk population screening studies for Fabry nephropathy have been carried out in patients with CKD, focal segmental glomerulosclerosis (FSGS), those undergoing dialysis, or post renal transplantation. In the largest meta-analysis to date, Linares (2023) included 55 studies with 84,062 screened patients[55]. Of these, 251 cases were positive for Fabry disease; 37.8% of the reported *GLA* variants were non-disease causing, 31.7% had classical variants, 15.5% were late-onset, and 14.7% were VUS. The overall prevalence was 0.10% in dialysis patients, 0.28% in kidney transplant patients, and 0.17% in those with CKD[55]. Thus, yields from screening for Fabry nephropathy are quite low, ranging from 1/1,000 to 1/357. Despite recommendations in favour of screening CKD patients for Fabry disease, the cost-effectiveness of such screening is questionable due to high costs[56]. Conversely, family screening shows a much higher yield, up to 50%[57] or more, and may identify additional cases of Fabry kidney disease, making it a significantly more cost-effective option.

Table 2. Indications for a renal biopsy in Fabry disease

To confirm Fabry nephropathy as the cause of chronic kidney disease
Acute kidney injury or active urine sediment
Poor response to therapy
Nephrotic syndrome suggestive of concomitant disease, e.g., MCD, FSGS
Suspect immune complex glomerulonephritis due to anti-drug antibodies

MCD: Minimal change disease; FSGS: focal segmental glomerulosclerosis.

Novel strategies are being developed for genetic screening for CKD patients as there are over 500 single gene disorders in nephrology and an estimated 30% or more of CKD patients with a genetic renal condition. These techniques include the use of gene chips to screen for multiple genetic conditions at the same time, whole-exome sequencing, and whole-genome sequencing. While a causative gene can be identified in up to 37% of patients, Fabry nephropathy has only been identified in very few patients through these studies[58,59].

Machine learning to screen for Fabry disease using an algorithm with clusters of signs and symptoms has been applied to a large electronic medical record system with positive results. Fabry disease patient presence in the riskiest 1% of patients identified by the algorithm was confirmed 1 in 2,100, nearly 24-fold greater than the baseline prevalence. This technique would identify a high-risk population for subsequent genetic screening[60].

PATHOPHYSIOLOGY

The pathophysiology of Fabry nephropathy remains unclear. The accumulation of Gb3, the main substrate of the α-Gal enzyme, occurs in cells throughout the kidney in Fabry nephropathy. Gb3 was initially thought to be solely responsible for cell and tissue injury as Gb3 can trigger inflammation as well as immune reactions as a novel glycolipid[61]. Gb3 can also cause both lysosomal and mitochondrial dysfunction with impaired energy production and increased autophagy, which in turn can lead to apoptosis[62]. Gb3 accumulation can lead to the inhibition of nitrous oxide synthase in endothelial cells with an increase in reactive oxygen species as part of the small vessel vasculopathy. Increased oxidative stress, along with lipid peroxidation and an increase in 3-nitrotyrosine, a marker of protein nitroxidative damage, also occurs[63].

A major metabolite of Gb3 is the deacylated version known as lyso-Gb3[64]. This is created by the action of cellular acid ceramidase in Fabry disease[48]. lyso-Gb3 is normally metabolized by the α-Gal enzyme[64]. When present in high levels, lyso-Gb3 inhibits the α-Gal enzyme, leading to the accumulation of more Gb3. lyso-Gb3 is more soluble than Gb3 and circulates in the plasma. It also plays a major role in the pathogenesis of cell injury in Fabry disease. lyso-Gb3 can cause vascular injury in Fabry disease with the stimulation of smooth muscle proliferation[64]. It can injure podocytes and trigger inflammation and fibrosis in the kidney via increased transforming growth factor-ß1 (TGF- ß1), fibronectin, type IV collagen, and CD74[65,66]. lyso-Gb3 has also been shown to cause increased protein ubiquitination involving chaperone and heat shock proteins, cytoskeletal proteins, and proteins involved in synthesis/translation in the endoplasmic reticulum. In this way, multiple cell processes are altered[67]. Lyso-Gb3 alters human bowel microbiota with decreased butyrate, which may, in turn, increase inflammation. Unlike Gb3, plasma lyso-Gb3 levels have been shown to correlate with the risk of Fabry complications[68], suggesting that this metabolite could play a more important role in cell and tissue injury.

Gb3 burden is responsible for cell injury, which leads to podocyte detachment from the glomerular basement membrane with podocyturia. With podocyte loss, the glomerular basement membrane function is

altered with failure of permselectivity and increasing proteinuria. This ultimately leads to focal and then global glomerulosclerosis with loss of nephron function.

It has been postulated that Gb3 accumulation and uptake into proximal tubular cells can trigger focal tubular atrophy and interstitial fibrosis, in addition to the inflammation and fibrosis that can be induced by increased proteinuria itself[69]. In a study of 15 human Fabry disease kidney biopsies, Rozenfeld (2020) showed increased TGF-β1 production mainly from proximal tubular cells rather than glomerular cells including podocytes[70]. There was also increased production of fibroblast growth factor (FGF-2) and vascular endothelial growth factor (VEGF), the latter expressed in the glomerulus and blood vessels; this is consistent with the fact that TGF-β1 activates FGF-2 expression in endothelial cells which then promotes VEGF production. VEGF is known to cause thickening of the glomerular basement membrane, glomerular enlargement, and mesangial proliferation, in addition to foot process effacement in other glomerular diseases. Caspase-3 positive staining was also observed to be consistent with apoptosis since caspase activates this process, which is known to be caused by the combination of TGF-β1 and VEGF. Myofibroblasts, which produce protein components of the fibrillar matrix that contributes to fibrosis, and are stimulated by TGF-β1, were identified on pericytes surrounding peritubular capillaries, mesangial cells, and glomeruli. All these observations are consistent with the role of TGF-β1 as the key profibrotic cytokine in Fabry nephropathy and that it indirectly triggers apoptosis in renal tubular cells.

Proteinuria is the major predictor of GFR loss in patients with Fabry disease and has been linked to increased glomerular sclerosis[71,72]. This suggests a critical role of podocyte injury in Fabry nephropathy. The podocytes, being terminally differentiated and thus not able to replicate, have a greater cell burden of glycolipid deposits than other kidney cells and become significantly enlarged. As such, they may suffer earlier and greater injury than other renal cell types in Fabry disease. For this reason, Fabry disease can be thought of as a podocytopathy.

Najafian *et al.* have performed quantitative stereoscopic morphometric electron microscopic studies of kidney biopsies in Fabry disease patients that showed Gb3 accumulation in podocytes in males associated with progressive podocyte injury and loss, with increased foot process width, decreased podocyte density, and increased proteinuria[73,74]. They also showed that in females with Fabry disease, Gb3 accumulation in podocytes progresses with age in association with podocyte loss and proteinuria, and this process is similar to that in males[75].

There are secondary processes triggered by Fabry disease that are subsequently unresponsive to ERT and contribute to ongoing cell and tissue injury, including in the kidney[63]. These may also contribute to the suboptimal response of the kidneys to treatment with ERT or pharmacologic chaperone therapy. Recently, increased alpha-synuclein protein (SNCA) was identified in human podocytes in Fabry nephropathy. This accumulation appears to be independent of increased Gb3 levels and does not respond to ERT. Studies in a human podocyte cell line with *GLA* knockout confirmed the role of SNCA in podocyte injury with lysosomal impairment in Fabry disease. SNCA inhibition improved both lysosomal structure and function beyond that of ERT alone[76]. It is unknown whether SNCA accumulates in tissues other than the kidney in Fabry disease and whether this is a maladaptive or adaptive response[77]. Doubt has been raised as to the role of SNCA in Fabry nephropathy based on the lack of renal damage in Parkinson's disease, a condition with SNCA overexpression, and evidence of an SNCA protective effect in renal tubular disease[77]. Adjunctive treatments targeting these secondary processes in Fabry nephropathy need to be developed in the future to accompany ERT and chaperone therapy.

PATHOLOGY OF FABRY NEPHROPATHY

The pathology in Fabry disease has been described in detail by several authors. The changes are pan renal in nature, involving glomeruli, the tubulointerstitial compartment, and blood vessels of all sizes. These changes occur in early childhood in both sexes with Gb3 deposits identified as lamellar bodies in all renal cell types[24,69,78-80].

Macroscopic features

The glomeruli may appear pale and bulging due to lipid accumulation. In cores obtained on renal biopsy, the glomeruli may appear bloodless and pale instead of the normal pink due to excess lipids[81]. Parapelvic cysts are frequently described and the prevalence of these can be over 45% as patients age[22].

Light microscopic features

Glomerular podocyte cytoplasm may appear pale and vacuolated due to lipid deposits dissolving during processing [Figure 3]. The lipid deposits are seen as blue granules on electron microscopy thin sections stained with toluidine blue [Figure 4]. Glomeruli often show focal segmental sclerosis progressing to global sclerosis. Other concurrent glomerular diseases may be present. Distal tubular epithelial cells may also appear pale and vacuolated. Interstitial foam cells may be seen. The proximal tubular epithelial cells appear to be minimally involved. Vessels may show nodules of hyalinization. Endothelial cells may show large glycolipid deposits, which can enlarge the cells with encroachment on the lumen of capillaries; this has been hypothesized to contribute to downstream ischemia in the kidney, skeletal muscle, and myocardium. Whether renal ischemia occurs is unknown. Vascular sclerosis is common with thickening of the intima and media in small arterioles and large arteries. As the disease progresses, non-specific chronic changes may be present, such as global sclerosis, interstitial fibrosis, tubular atrophy, interstitial lymphocytic infiltration, and arteriosclerosis.

Immunofluorescence features

Non-specific positivity for IgM and C3 may be present in segmental scars, but there are no immune complex deposits. The lipid deposits exhibit a yellow fluorescence under UV light[82].

Electron microscopic features

Electron-dense laminated lipid deposits, known as zebra bodies, myeloid bodies, or lamellar bodies, are present most prominently in the podocytes [Figure 5], but may also be seen in almost any cell in the kidney, including mesangial cells, the parietal layer of Bowman's capsule, tubular epithelial cells, interstitial fibroblasts, endothelial cells, and vascular media. These lamellar bodies are enlarged lysosomes 1-3 μm in diameter with accumulated Gb3 and other glycolipids that are in concentric alternating layers of light and dark with a periodicity of 35-50 Å[24]. Such deposits can be identified before birth and increase in size and number with age[83].

While lamellar bodies are a distinctive feature of Fabry nephropathy, they are not diagnostic by themselves. They are reported in a number of other acquired or hereditary renal conditions including radiocontrast injury and other lysosomal diseases, e.g., Neimann-Pick disease, among others [Table 3].

In a single-center retrospective analysis of 4,400 renal biopsies over 11 years, there were 32 cases (0.73%) with lamellar bodies, only 6 of which were ultimately shown to be Fabry nephropathy while the majority was a mixture of possible DIP and other renal pathology[50]. While the pathology of DIP is very similar to that of Fabry nephropathy, there may be fewer podocytes involved with fewer lamellar bodies and no involvement of other renal cell types compared with Fabry nephropathy[50].

Table 3. Kidney diseases with lamellar bodies

Condition	Reference
Fabry disease	Gubler *et al.*, 1978[80]
Drug-induced renal phospholipidosis	Reasor *et al.*, 2006[84]
Neimann-Pick disease	Grafft *et al.*, 2009[85]
Silicosis	Banks *et al.*, 1983[86]
LMX1B-associated nephropathy (nail patella syndrome)	Lei *et al.*, 2020[87]
LCAT deficiency	Hirashio *et al.*, 2014[88]
CoQ2 nephropathy	Ni *et al.*, 2021[89]
Radiocontrast	Su *et al.*, 2018[90]
Renal cell carcinoma	Hull *et al.*, 1988[91]
Idiopathic	Kadosawa *et al.*, 2020[92]

LCAT: Lecithin cholesterol acyltransferase; CoQ2: co-enzyme Q2.

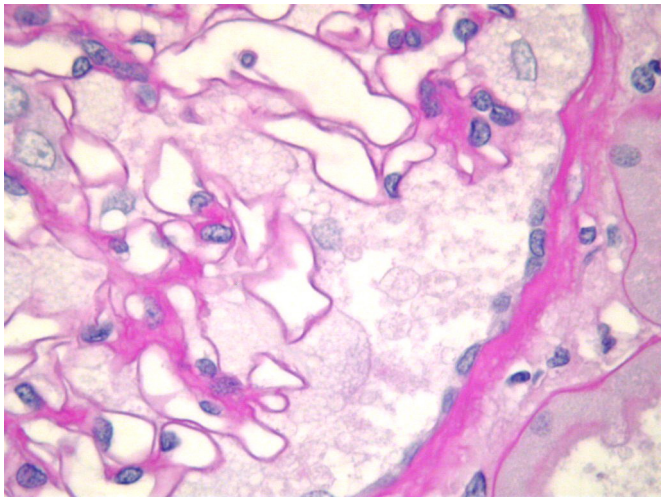

Figure 3. Light microscopy showing foamy podocyte cytoplasm, PAS, 400x.

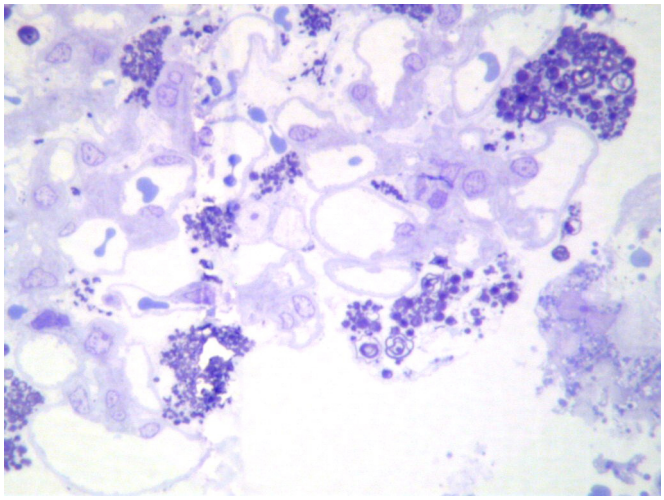

Figure 4. Light microscopy showing intracytoplasmic granules, electron microscopy thin section, toluidine blue, 400x.

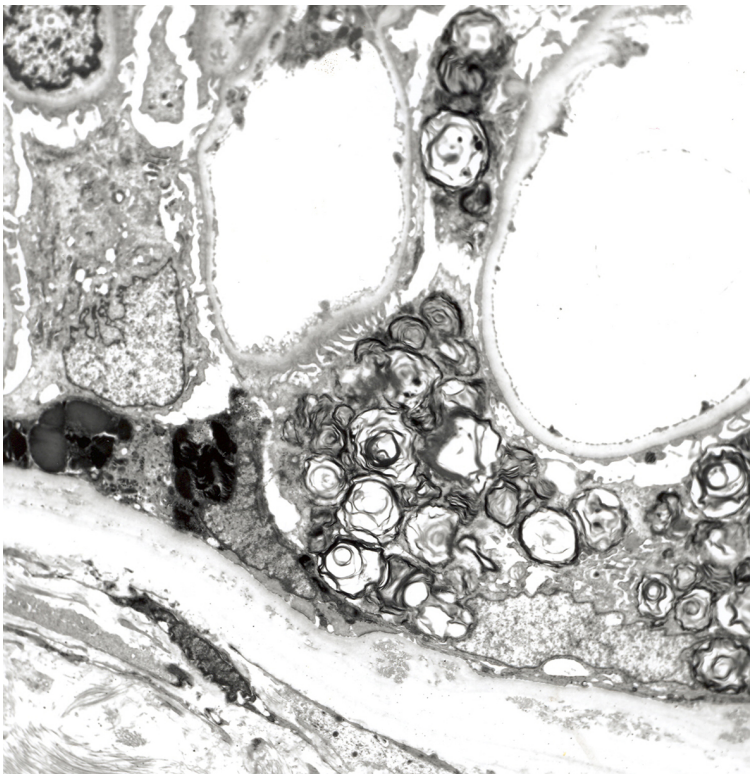

Figure 5. Electron microscopy showing lamellated lipid deposits in a podocyte, 4,000x.

Foot process effacement in Fabry nephropathy is also frequently present. Early in childhood, this process may be focal and associated with underlying lamellar bodies[93]. In addition, the presence of lamellar bodies and foot process effacement, as well as vascular and glomerular sclerosis, can antedate the development of microalbuminuria[94].

Immunohistochemistry

A monoclonal antibody to Gb3, or CD77, is available, which stains all renal cell types to varying degrees[95].

Other staining properties

In frozen sections, fresh or formalin-fixed, the lipid deposits are birefringent, autofluorescent, sudanophilic, and positive for oil red O, and PAS. The lipid deposits also stain with Luxol fast blue.

Fabry disease subtypes and classification

Two Fabry disease subtypes are identified, classic and late-onset. In the classic version, lipid deposits are in all renal cell types, whereas in the late-onset, they are mainly present in podocytes, distal tubular epithelial cells, and vessels, but not endothelial or mesothelial cells. Males, as hemizygotes, will have Fabry disease affecting all renal cells in contrast to female heterozygotes who are mosaics due to the Lyon hypothesis with inactivation of one of the X chromosomes in each cell. This mosaicism of the podocyte has been confirmed in Fabry nephropathy in females[96].

The International Study Group of Fabry Nephropathy developed a scoring system of light microscopic changes that can be used for assessment of prognosis as well as response to treatment[97].

Differential diagnosis

Chloroquine and many other amphiphilic drugs that cause DIP can lead to glycolipid deposits indistinguishable from Fabry disease[98].

FSGS may develop in Fabry disease and lead to a misdiagnosis when electron microscopy is not used to reveal the lamellar bodies. This has the risk of the use of inappropriate immuno-suppressive therapy that is ineffective with a possibility of adverse effects.

I-cell disease is a rare lipidosis caused by a lack of cellular mannose-6-phosphate needed to direct hydrolases into the lysosome; it is characterized by distended podocytes with numerous intracytoplasmic clear vacuoles containing lipids. On electron microscopy, there are no lamellar bodies; the lipid vacuoles stain positive for colloidal iron, which is not seen in Fabry disease[99].

MANAGEMENT

Non-specific therapy

The principles of CKD management have been recently reviewed and will not be repeated here[100].

A multidisciplinary team is useful with a cardiologist, neurologist, gastroenterologist, ophthalmologist, medical geneticist, pediatrician, social worker, and nurses, in addition to a nephrologist. Regular assessments are required, particularly as patients age. As this is a rare disease, management is best done in a medical center of excellence with experience in all aspects of Fabry disease.

In addition, a dietitian is helpful in counseling patients on a diet with low salt intake to minimize proteinuria, and control hypertension and heart failure. A diet low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols has been proposed to help reduce GI symptoms similar to irritable bowel syndrome[101].

Fabry patients have an excess of psychiatric disease, which may be part of this disease spectrum and not just the result of chronic pain[102]. They may benefit from regular access to a mental health team.

Fabry patients should all receive genetic counseling to help with understanding of the X-linked pattern of inheritance and reproductive choices. Additionally, a genetic counsellor can create a family pedigree which is useful in screening family members at risk for Fabry disease. If there is any question as to the pathogenicity of the *GLA* variant, then a medical geneticist should be consulted.

Limiting proteinuria

While ERT and pharmacologic chaperone therapy are valuable in stabilizing eGFR in Fabry nephropathy, they have little impact on proteinuria. This mean that antiproteinuric therapy must be used in Fabry nephropathy in conjunction with specific therapy. There have only been a few studies addressing CKD therapies in Fabry disease to date.

Tahir *et al.* (2007) reported on the use of ACEinh and ARB as antiproteinuric therapy plus ERT in 11 patients with Fabry nephropathy[103]. Sustained reductions in proteinuria with stabilization of kidney function were achieved in six patients with stage III/IV CKD; the eGFR slope was -0.23 mL/min/1.73 m^2/yr over 30 months of follow-up. Warnock *et al.* (2015) conducted a prospective study of the antiproteinuric effect of ACEinh and ARB on 24 adult classical genotype patients with Fabry nephropathy receiving recombinant agalsidase-beta therapy[104]. A urine protein to creatinine ratio (uPCR) < 0.5 g/g or a 50%

reduction in baseline uPCR was achieved in 75% of patients; eGFR slopes were significantly better than in those patients who did not achieve the uPCR goal, -3.6 *vs.* -7.0 mL/min/1.73 m^2/yr, respectively. Low blood pressure and hyperkalemia were limiting factors to the use of ACEinh and ARB in some patients.

There are no publications on the use of mineralocorticoid receptor antagonists (MRA) in Fabry nephropathy. As a result, nephrologists must rely on data about many of these drugs from studies carried out in other proteinuric diseases. Diabetic nephropathy may be a reasonable model for Fabry nephropathy given the commonalities of metabolic disease, renal glycosphingolipid abnormalities, glomerular hyperfiltration, hypertension, small vessel vasculopathy, and multisystem features with retinal, cardiac, gastrointestinal disease and sensory neuropathy. There are reports of significant antiproteinuric effects in diabetic nephropathy of MRAs such as spironolactone, eplerenone, and finerenone[105]. Thiazide diuretics may also have a modest effect[106]. There are two reports of amiloride-induced reduction of proteinuria, one in diabetic kidney disease[107] and one in Fabry disease[108].

Newer agents such as sodium-glucose cotransporter 2 inhibitors (SGLT2) are also of proven benefit in both diabetic nephropathy and nondiabetic proteinuric kidney disease in terms of stabilization of eGFR and reduction of proteinuria[109]. Their benefits also extend to both systolic and diastolic heart failure, which can complicate Fabry cardiomyopathy[110].

It should be noted that the benefits of MRAs and SGLT2 inhibitors are additive to those of ACEinh and ARBs. While there are no data yet on the use of SGLT2 inhibitors in Fabry disease, there is a clinical trial in the planning stage to study the effects of this drug on heart and kidney function in this condition. (NCT05710367 clinicaltrials.gov)

Vitamin D and its analogs may also be considered for the treatment of proteinuria in Fabry nephropathy. Vitamin D deficiency is common in Fabry disease; in one study, 46.8% of 77 patients had vitamin D deficiency, even though half of those were already taking cholecalciferol[111]. Vitamin D deficiency has been identified as a factor worsening both kidney and heart disease in Fabry disease with increasing proteinuria[31]. In a human podocyte model exposed to lyso-Gb3, vitamin D receptor activation was also shown to be of benefit through the blocking of an increase in secondary mediators of renal injury, such as TGF-β1, induced by lyso-Gb3[65]. The addition of the vitamin D analog paricalcitol to ACEinh or ARB in a randomized controlled trial in 15 adults with Fabry nephropathy further reduced proteinuria over 6 months from a mean of 1.4 g/day to 0.4 g/day[112]. Caution must be taken as secondary hypercalcemia with worsening cardiac and renal function can occur with high-dose vitamin D[113]. Vitamin D and its analogs have also been used successfully to lower proteinuria in small short-term studies of diabetic nephropathy and other proteinuric kidney diseases[114,115]. Overall, these data suggest that vitamin D deficiency should be identified and corrected wherever possible and that low-dose vitamin D therapy can be added to reduce proteinuria in Fabry nephropathy.

Adjunctive therapies

Other important therapies relate to minimizing risks for cardiovascular disease, such as dyslipidemia, smoking, hypertension, and obesity. Blood pressure control can be challenging in patients with Fabry disease due to significant variability, partially attributed to autonomic neuropathy present in some patients[116]. Ambulatory blood pressure monitoring can be useful to diagnose masked hypertension. Beta-blocker use is to be avoided as this may worsen the resting sinus bradycardia that many adults with Fabry disease exhibit[117].

Secondary stroke prophylaxis with ASA or clopidogrel may be required. Patients with Fabry disease have a greatly increased risk of stroke compared with the general population[118]. There is evidence of endothelial damage, abnormalities in cerebral blood flow velocity, a prothrombotic state, and increased production of reactive oxygen species that may contribute to the development of stroke in Fabry disease. However, there are no studies in this patient population of any stroke therapies, so clinicians are left to extrapolate from data from other larger patient groups. As atrial fibrillation is a common complication of cardiomyopathy in Fabry disease, it is important to provide appropriate anticoagulation to minimize the risk of subsequent stroke.

Control of neuropathic pain is very important and a variety of agents are available for use, including carbamazepine, gabapentin, pregabalin, duloxetine, and others. Narcotic analgesics are to be avoided due to their limited effectiveness in treating neuropathic pain and high potential for addiction[119].

Monitoring

Patients with Fabry nephropathy should be monitored for kidney function (eGFR) and proteinuria (urine albumin to creatinine ratio (ACR)/PCR/24-h urine), along with other biochemical parameters as per CKD care standards. Blood pressure control and the presence of edema should be noted at each clinic visit.

Extrarenal complications of Fabry disease should be monitored in adults. An annual highly sensitive troponin T or I, EKG, echocardiogram, and Holter monitor or loop recorder should be done to detect any signs of cardiac involvement. N-terminal pro-brain natriuretic peptide is useful for patients with heart failure. Cardiac MRI done every 2 to 3 years is important to identify fibrosis via late gadolinium enhancement, which can precede LVH in heterozygotes and males with late-onset cardiac variant disease. Brain MRI should be done every 2 to 3 years to identify silent infarctions, as well as dolichoectasia and white matter lesions. Audiograms and pulmonary function tests every 5 years will document the slow loss of hearing and progression of Fabry pulmonary changes.

Plasma lyso-Gb3 should be monitored in all patients undergoing therapy to ensure a therapeutic response and to verify drug compliance. As plasma lyso-Gb3 level is now recognized as a risk factor for Fabry disease complications including decreased eGFR and proteinuria, monitoring these levels at least annually may be of benefit to help guide therapeutic decisions[68,120].

Anti-drug antibodies, including neutralizing antibodies, should be done in all males receiving ERT; women do not need such testing unless they have infusion-associated reactions to ERT or are homozygotes.

Dialysis

Patients with Fabry disease can be managed with either hemodialysis or peritoneal dialysis. ERT can be given safely during hemodialysis treatments with no appreciable loss of enzyme through the dialyzer membrane or due to binding to the plastic tubing[121]. Gb3 is not removed by hemodialysis. It is unknown whether any Gb3 is removed on peritoneal dialysis, but this could occur due to the loss of peritoneal cells in the dialysate. Removal of lyso-Gb3 has not been studied during any type of dialysis. Peritoneal dialysis may have the advantage of better fluid and blood pressure control for Fabry disease patients who often have severe LVH with diastolic dysfunction due to Fabry cardiac disease. Mignani *et al.* (2008) noted that some Fabry patients on hemodialysis suffered a 20% increase in left ventricular mass (LVM) index despite ERT, while those with a kidney transplant had stable LVM index, suggesting that the increasing LVM index was a complication of ESRD in Fabry disease patients[122]. Anecdotally, some Fabry patients may show extremely rapid progression of LVH with hemodialysis probably in part due to contributions from anemia and

hypertension. Echocardiogram done annually is especially important during hemodialysis to identify these patients early, enabling timely implementation of additional therapies. While highly sensitive Troponin-I and -T values are useful for assessing myocardial inflammation in Fabry cardiac disease, the troponin-T level is also increased due to kidney failure, complicating their interpretation in this setting. Cardiac MRI can be performed to identify myocardial fibrosis with late gadolinium enhancement, but only with an approved ACR class II type of gadolinium-based contrast agent to minimize the risk of systemic nephrogenic fibrosis[123].

Kidney transplantation

Men with classical Fabry disease often receive a kidney transplant as they are usually among the younger adults starting dialysis at an average age of 42 years[2]. Kidney transplant graft survival and patient survival in Fabry disease are similar to those of patients transplanted for other nephropathies[124]. There is no evidence that Fabry disease recurs in the renal allograft in a significant manner. There are a few reports of benign endothelial glycosphingolipid deposits[124]. Fabry males can receive a living-related transplant with the caveat that neither their mother nor sister, as Fabry heterozygotes, should donate to them[125,126]. ERT is well tolerated by renal transplant recipients[127].

Sertraline-induced phospholipidosis has caused CKD with proteinuria in a renal transplant patient. Such cases are probably underestimated and underreported but could be misdiagnosed as Fabry nephropathy[128].

There are reports of undiagnosed Fabry patients as deceased donors wherein the transplant kidney showed changes of Fabry nephropathy on post-transplant biopsy[129]. In one case, the graft had stable function 12 years later with persistent lamellar bodies but a decrease in the overall Gb3 deposits on repeat biopsy[130]. This suggests that α-Gal from the transplant recipient may have some effects on the graft Gb3 burden.

Immunosuppressive therapy

Immunosuppressive therapy with high-dose prednisone has been used to induce remission in Fabry nephropathy patients who suddenly develop high-grade proteinuria. This does not represent a response of this metabolic disease to immunosuppression but rather a case of concurrent minimal change disease or FSGS usually in children or young adults[131].

Routine treatment of patients with Fabry nephropathy with immunosuppressive drugs is not recommended other than for post kidney transplant. It is of interest that immunosuppressive therapy in that situation has been reported to result in a lower prevalence of neutralizing anti-drug antibodies in adult males receiving ERT[132]. In addition, the *in vivo* neutralization of ERT was significantly reduced by this treatment but tended to increase as immunosuppression was reduced.

Specific therapy

Before therapy, physicians should always consider the whole clinical picture relevant to an individual. This includes key affected organ systems, i.e., cardiac, renal, and cerebrovascular, symptoms such as pain and gastrointestinal manifestations, concurrent medical conditions, and negative impacts on mental health and quality of life.

Fabry-specific therapies available in Canada include: (1) ERT with agalsidase-beta (Fabrazyme®, Sanofi-Aventis LLC) approved for patients with Fabry disease aged ≥ 8 years[133]; (2) ERT with agalsidase-alfa (Replagal®, Takeda Pharmaceuticals) approved for use in patients with Fabry disease, with dosage recommendations for ages 7-65 years[134]; and (3) Migalastat (Galafold®, Amicus Therapeutics), an oral

pharmacologic chaperone therapy approved for use in Fabry disease patients ≥ 12 years with an amenable *GLA* variant and eGFR > 30 mL/min/1.73 m^2[135]. Amenability is determined in an *in vitro* HEK assay, with a positive response defined as an absolute increase in α-Gal activity of at least 3% and a relative increase of at least 1.2-fold[136].

The cost of these long-term treatments is of importance for any health care system since treatment with ERT at a cost of CAN$280,000/patient/year per Fabry patient results in a significant burden. Chaperone therapy is priced only slightly less than ERT in Canada.

Enzyme replacement therapy

The introduction of ERT in 2001 was a landmark event, as prior to that, there was no specific therapy for Fabry nephropathy[137,138]. ERT was shown to reduce both urine and plasma Gb3[138,139], as well as Gb3 in kidney and skin[140].

In a randomized controlled study of agalsidase-beta in Fabry adults with chronic kidney disease, ERT was shown to delay the time to first clinical Fabry event including a 33% increase in serum creatinine and the development of ESRD with dialysis or transplantation[141]. Patients with baseline eGFR 55 mL/min/1.73 m^2 or less had a poorer response than those with eGFR over 55 mL/min/1.73 m^2, suggesting that early treatment was better. Mean proteinuria was unchanged with treatment.

Hyperfiltration confirmed by measured GFR can occur in some men with Fabry nephropathy. With ERT, GFR quickly fell into the normal range in all 9 men, but it is unknown whether this represented rapid disease progression despite ERT or normalization of eGFR, given the limited follow-up[26].

In a study of 58 adults (97% male) on agalsidase-beta, Germain (2007) noted that 10 patients with higher proteinuria > 1 g/day had a more rapid decline in eGFR, average -7.4 mL/min/1.73 m^2/yr compared with eGFR slope of -1.0 mL/min/1.73 m^2/yr in 42 patients with proteinuria ≤ 1.0 g/day[72]. Similarly, 8 patients with > 50% baseline glomerular sclerosis had eGFR slope of -8.9 mL/min/1.73 m^2/yr compared with 32 patients who had baseline glomerular sclerosis ≤ 50% and eGFR slope of -1.4 mL/min/1.73 m^2/yr. Baseline proteinuria and sclerosis were both shown to be important risk factors for the development of progressive Fabry nephropathy.

In a ten-year follow-up of these 52 patients, 20 had decreased eGFR and proteinuria[142]. It was recognized that early treatment and thus younger age, and a low renal involvement (LRI), defined as proteinuria < 0.5 g/24 h and the absence of fibrosis on renal biopsy, were associated with preservation of renal function in patients receiving agalsidase-beta. Mean eGFR slopes for LRI and high renal involvement (HRI proteinuria ≥ 0.5 g/day, and/or fibrosis on renal biopsy) were -1.89 mL/min/1.73 m^2/yr and -6.82 mL/min/1.73 m^2/yr, respectively.

These results have been confirmed in a larger study with 165 patients treated for up to 21 years with agalsidase-alfa[143]. The 51 patients with high baseline proteinuria > 0.5 g/24 h, compared with 114 patients with low baseline proteinuria < 0.5 g/24 h, had a lower baseline mean eGFR (89.1 *vs.* 106.6 mL/min/1.73 m^2) and faster mean eGFR decline (-3.62 *vs.* -1.61 mL/min/1.73 m^2/yr; $P < 0.0001$). The patients with high baseline proteinuria > 0.5 g/24 h. were projected to have a progressive decrease in eGFR, estimated at -36 mL/min/1.73 m^2 at 10 years and -72 mL/min/1.73 m^2 over 20 years.

Nephrogenic diabetes insipidus (NDI) resolved with ERT and use of an ACEinh in a 7-year-old boy with biopsy-proven Fabry nephropathy. It seems unlikely that the ACEinh would bring about a resolution of NDI, leaving one to conclude that ERT was responsible[23].

Early manifestations of Fabry nephropathy and early renal biopsy sampling

Males with severe classical mutations in *GLA*, defined as a very low or undetectable leukocyte α-Gal measurement and a disease-causing mutation, develop the classic phenotype with early and marked accumulation of Gb3 and its metabolite, lyso-Gb3, in the plasma and in a variety of cell types, such as vascular endothelial cells, smooth muscle cells, multiple kidney cell types, and cardiomyocytes. Najafian *et al.*, through quantitative renal electron-microscopic studies, have consistently shown that after ERT[144] or chaperone treatment[145], there is a proportional loss of Gb3 and podocyte shrinkage, resulting in unchanged Gb3 density.

With the advent of intravenous ERT, there is now the capability to remove Gb3 from cells and, indeed, endothelial cells are cleared of such deposits. Early ERT has also shown a tendency to clear some podocytes of deposits in young children, as well as reducing foot process effacement[146]. However, while the podocytes show rarefication and a decrease in Gb3 deposits with ERT in adults, complete clearance is hindered[72,140], partly because these cells bear a significantly greater burden of Gb3 due to aging and terminal differentiation, rendering them unable to replicate. This situation is similar to that in the cardiomyocyte.

In the FIELD study, 31 male Fabry patients, 30 with classic variants, aged 5-18 years, were randomized to receive agalsidase-beta at 0.5 mg/kg 2-weekly (n = 16) or 1.0 mg/kg 4-weekly (n = 15) for 5 years. Extensive podocyte Gb3 deposits were observed, despite normal eGFR and no proteinuria[147]. Fabry arteriopathy worsened after 5 years in 5 of 6 patients. Podocyte Gb3 content and foot process width showed variable responses. eGFR and proteinuria remained normal. Plasma and urine Gb3 levels normalized rapidly. Plasma lyso-Gb3 levels substantially decreased but fluctuated after year 2. Angiokeratomas worsened in 5 of 16. These findings suggested that boys should be treated with agalsidase-beta at a dose of 1.0 mg/kg every 2 weeks. Important and irreversible renal pathologic changes (arteriopathy) antedated any clinical signs of nephropathy in males with classic variant disease.

Treatment with pharmacologic chaperone

Hughes *et al.* reported that Fabry adults receiving migalastat show a similar eGFR over 18 months of treatment to those randomized to ERT. Migalastat was well tolerated[148].

In patients bearing amenable mutations, migalastat resulted in stable renal function for up to 8.6 years irrespective of treatment status (ERT-naïve and ERT-experienced), sex, or phenotype[149].

In a retrospective analysis of adult patients with Fabry disease amenable to migalastat treatment in Switzerland, in males, the achieved leukocyte α-Gal enzyme activity differed from that in HEK cells after incubation with migalastat, for example: 33% in leukocytes *vs.* 41% HEK cells for p.F113L variant; 43% in leukocytes *vs.* 36% in HEK cells for p.N215S variant: 24%-30% in peripheral leukocytes *vs.* 96% in HEK cells for S238N variant[150]. These differences reflect the variations in these parameters rather than any inaccuracies in the assays.

In a recent report, stable renal function was documented in adults on migalastat and it was suggested that female patients should not be treated differently than males[151]. In this study, 125 patients, comprising both males and females, demonstrated generally stable renal function while receiving migalastat for 3.9 years. The

median annualized rate of eGFR change was -1.4 mL/min/1.73 m^2/yr for males and -1.1 mL/min/1.73 m^2/yr for females.

Real-world evidence of migalastat confirmed its safety and tolerability in 59 adults, along with a significant reduction in LVM index in both genders[152]. However, eGFR declined in both females (-6.9 mL/min/1.73 m^2/yr) and males (-5.0 mL/min/1.73 m^2/yr). The cause of this decrease in renal function was unclear but was confounded by patients with lower blood pressure[152]. A recent preliminary report of migalastat use in 55 adults from Canada did not find any untoward eGFR change associated with chaperone use[153].

As patients with advanced Fabry kidney disease were excluded from the migalastat studies, there are limited data on the effectiveness of chaperone therapy in such patients. An anecdotal case report of eGFR stabilization and reduction in proteinuria in stage IIIb CKD needs to be confirmed with further studies[154].

Early treatment initiation

Some Fabry guidelines recommend initiating ERT in young asymptomatic males with classic *GLA* variants by age 15 or 16 years, or even younger; this recommendation stems from the argument that once fibrosis occurs, the response to ERT diminishes in both the kidneys and the heart[155]. This position, while initially based upon expert opinion, is supported by recent observations. Previous studies used age as the benchmark for starting treatment. In contrast, Hughes *et al.* considered time elapsed since symptom onset, as this may provide a more precise indicator for treatment initiation given the variable ages at the onset of disease progression in Fabry disease patients[156]. In a multivariate Cox regression analysis of data from 1,374 Fabry patients, prompt treatment initiation (defined as treatment initiation within < 24 months from symptom onset) significantly reduced the probability of cardiovascular events (HR = 0.83; P = 0.003) after adjusting for history of cardiovascular events, sex, and age at treatment initiation. Univariate analysis indicated a significantly lower likelihood of renal events in the prompt treatment group (P = 0.018); this finding was attenuated in the multivariate Cox regression analysis [Figure 6].

van der Veen *et al.* (2023) reported on 30 men with Fabry disease 13 to 27 years old; 7 received ERT via the FIELD study for about 10 years and the rest were untreated[157]. Baseline characteristics did not differ between the two groups. The treated patients had lower median ACR (P = 0.02) and lower LVM index by both echocardiography and cardiac MRI (P = 0.02) than those untreated. eGFR did not differ between the two groups. These results support the idea of early ERT initiation in Fabry disease. While a definitive randomized controlled study is unlikely to be done to answer the question regarding the optimal time to start ERT, there is an increasing body of evidence supporting early treatment.

ERT dose

There is ongoing debate as to the optimal dose of ERT for Fabry disease, given the five-fold difference in dose between agalsidase-alfa (0.2 mg/kg) and agalsidase-beta (1.0 mg/kg). The products also differ in the cell type used in culture to make the enzyme (human fibroblasts *vs.* Chinese hamster ovary cells) and mix of surface carbohydrate moieties. It is unknown if these manufacturing details contribute to outcome differences. The marked phenotypic heterogeneity of Fabry disease makes comparisons difficult. There has been one randomized controlled study comparing the two versions of ERT at the usual dose, namely the CFDI study in Canada[9]. This study was disrupted by the failure of randomization to ERT during the three-year global shortage of agalsidase-beta. Although 132 patients were enrolled over 10 years, the study ended due to lack of funding and inadequate enrollment. A number of studies have compared the two treatments, but most are flawed, retrospective, and short-duration, with differences in the mix of genotypes and phenotypes. While there are studies showing good renal outcomes after 10 and 20 years of ERT with both

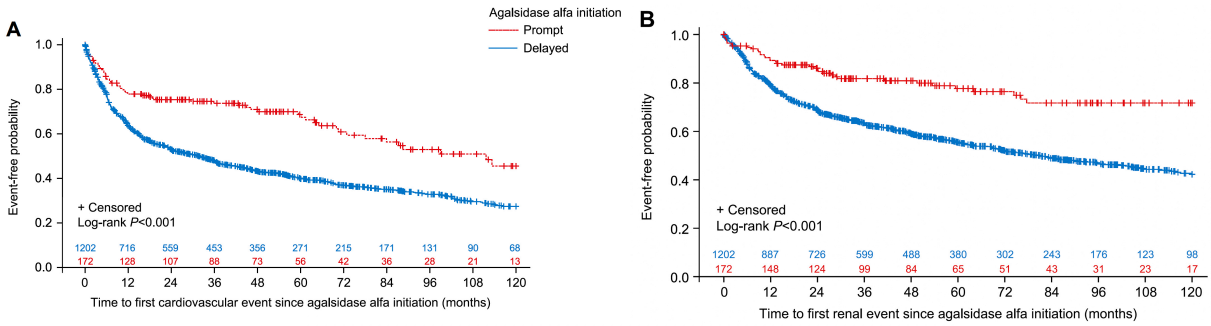

Figure 6. Kaplan-Meier curves with log-rank test showing (A) time to first cardiovascular event and (B) time to first renal event for prompt versus delayed agalsidase-alfa initiation cohorts, based on time elapsed from symptom onset (analysis A). From Hughes *et al.* (2021)[156] with permission.

doses of ERT in younger patients[142,143], older patients starting ERT late, with eGFR < 60 mL/min/1.73 m^2 and proteinuria > 0.5 g/day, often progress to ESRD despite receiving either ERT dose, due to pre-existing extensive glomerulosclerosis.

A renal biopsy study in children on ERT indicates that a higher cumulative dose gives better clearance of Gb3 from various kidney cells including podocytes and reduces urine ACR more than a lower cumulative dose[146]. There was no difference in eGFR between the two versions of ERT. ERT dose is thus suggested to be more important in younger male patients with classical disease. While girls were included in this study, their risk of ending up on dialysis is far lower than boys; it remains uncertain when to start treatment in young heterozygotes.

The higher dose of ERT is known for its greater efficacy in reducing plasma lyso-Gb3 levels compared to a lower dose. Given the mounting evidence that baseline plasma lyso-Gb3 level predicts the risk of future Fabry complications[68,120], this is an argument for using a higher dose of ERT. However, more studies are required to understand the relationship between plasma lyso-Gb3 and patient outcomes. Specifically, it is crucial to investigate whether lowering the plasma lyso-Gb3 through treatment directly mitigates the risk of organ dysfunction.

Plasma lyso-Gb3

While plasma Gb3 does not reflect Fabry disease activity[158], plasma lyso-Gb3 is considered more indicative, despite somewhat conflicting data. In the cross-sectional data analysis by Talbot *et al.* (2017)[159], patients with higher plasma lyso-Gb3 levels were more prone to clinical events. Several patients experiencing acute end-organ events did not show increases in lyso-Gb3 levels over the 18-month observation period. No specific "cutoff" level of lyso-Gb3 was determined to be predictive of organ damage.

In a retrospective study of 293 Fabry patients, neither the plasma lyso-Gb3 level at baseline or during treatment, the absolute decrease in lyso-Gb3, nor the relative decrease in lyso-Gb3 predicted the risk of events in Fabry patients[29].

In an analysis of 97 treatment-naïve and ERT-experienced patients with migalastat-amenable *GLA* variants from prior studies, no significant correlations were identified between changes in plasma lyso-Gb3 and changes in LVM index, eGFR, or pain[160]. Furthermore, neither baseline plasma lyso-Gb3 levels nor the rate of change in lyso-Gb3 levels during treatment predicted Fabry-associated clinical event occurrences.

However, changes in plasma lyso-Gb3 correlated with changes in kidney interstitial capillary Gb3 inclusions in treatment-naïve patients[161].

van der Veen *et al.* (2023) reported on the stability of baseline plasma lyso-Gb3 levels in 237 untreated patients with Fabry disease from a single center in the Netherlands[68]. The plasma lyso-Gb3 levels correlated with Fabry outcomes (cardiac, renal, and brain parameters) during ERT. Both more rapid eGFR slope and a greater rate of albuminuria rise were associated with a higher plasma lyso-Gb3. The large number of patients, long study duration, and multiple plasma lyso-Gb3 measurements for each make this the most important study of plasma lyso-Gb3 and outcomes to date.

Risk factors identified through Cox proportional hazards modeling for the development of renal disease progression in a study of 117 Chinese Fabry disease patients also included baseline plasma lyso-Gb3, in addition to the male gender, proteinuria > 1 g/day, and CKD stage III or greater[120].

The conflicting data on the plasma lyso-Gb3 and Fabry disease activity are probably due to a number of study factors, including small patient numbers, short follow-up, use of different outcome measures, and study populations that vary in terms of gender ratios, *GLA* variants, and phenotypes. Whether there is a different lyso-Gb3 response to chaperone therapy compared with ERT is uncertain. It has been speculated that as studies in mouse models suggest that lyso-Gb3 is either actively formed or preferentially stored in the liver and spleen, elevated plasma lyso-Gb3 could be a spillover from these organs and, therefore, may not reflect the substrate levels in clinically relevant organs such as the heart, kidney, or peripheral nerves[160]. In addition, as migalastat and lyso-Gb3 occupy distinct compartments (lysosomes versus plasma), not all lyso-Gb3 may be subject to catalysis by migalastat-stabilized α-Gal[160].

It is important to monitor plasma lyso-Gb3 in all Fabry patients on therapy and when switching therapies, in particular ERT to chaperone therapy, as there have been a few reports of inadequate clinical and biomarker responses despite *in vitro* amenability[162].

Table 4 summarizes the response of Fabry nephropathy to treatment.

Anti-drug antibodies

Both agalsidase-alfa and agalsidase-beta have been associated with anti-drug antibodies (ADA), with the prevalence being slightly greater for the beta version, at least partly due to the 5-fold higher dose[169]. Most ADA are IgG. IgE antibodies are quite rare and have only been reported in males receiving agalsidase-beta[170]. Desensitization protocols have been successful for agalsidase-beta[170,171]. Females have a lower prevalence of ADA than males, perhaps due to the fact that they have higher residual α-Gal levels than males[172]. ADA assays reported by various laboratories differ in terms of sensitivity and specificity, and thus, the results cannot be compared. Some males with Fabry disease will develop neutralizing ADA, which will bind the α-Gal enzyme in an *in vitro* assay[173]. To date, the only female reported to have developed neutralizing antibodies in the CFDI registry was a homozygote (unpublished data M. West). Some neutralizing ADA have been shown to bind the α-Gal enzyme *in vivo*[173]. Nonsense and frameshift mutations, higher baseline plasma lyso-Gb3 and agalsidase-beta as the first treatment were reported as risk factors for the development of neutralizing ADA[174]. Many of these patients will have undetectable or extremely low α-Gal activity. This can result in a blunted response to ERT with higher urine Gb3 and plasma lyso-Gb3 levels, as well as a worsening clinical state with lower eGFR and higher proteinuria[173]. Switching to a higher ERT dose of 1.0 mg/kg to give more antigen to overcome the ADA binding of enzyme has been of infrequent or transient benefit so far[175]. While induction of immune tolerance to ERT with

Table 4. Fabry nephropathy: response to treatment

Treatment	Outcome	Reference
Hypertension control	Not studied	
RAS inhibitors	Reduced proteinuria, improved eGFR slope	Warnock *et al.*, 2015[104]
ERT	Decreased plasma Gb3, urine Gb3	Schiffmann *et al.*, 2001[138] Eng *et al.*, 2001[137]
	Decreased plasma lyso-Gb3, urine lyso-Gb3	Aerts *et al.*, 2008[64] Rombach *et al.*, 2012[163]
	Decreased Gb3 in podocytes, PTC, endothelium, tubular cells	Tøndel *et al.*, 2013[146] Thurberg *et al.*, 2002[140]
	eGFR slope improved or stable	Germain *et al.*, 2015[142], Cybulla *et al.*, 2022[143]
	Decreased Fabry renal events	Banikazemi *et al.*, 2007[141]
	Reversed nephrogenic diabetes insipidus	Wornell *et al.*, 2006[23]
Migalastat	Increased α-Gal kidney	Germain *et al.*, 2012[164]
	Decreased plasma Gb3, urine Gb3	Germain *et al.*, 2012[164]
	Decreased plasma lyso-Gb3	Germain *et al.*, 2016[161]
	Decreased Gb3 in renal PTC, kidney	Germain *et al.*, 2012[164] Germain *et al.*, 2016[161]
	eGFR slope improved or stable	Bichet *et al.*, 2021[149]
	Decreased Fabry renal events	Hughes *et al.*, 2023[165]
Pegunigalsidase	Decreased plasma Gb3	Schiffmann *et al.*, 2019[166]
	Decreased plasma lyso-Gb3	Schiffmann *et al.*, 2019[166]
	Decreased Gb3 in renal peritubular capillaries	Schiffmann *et al.*, 2019[166]
	eGFR slope improved or stable	Linhart *et al.*, 2023[167], Wallace *et al.*, 2023[168]

RAS: Renin-angiotensin system; ERT: enzyme replacement therapy; Gb3: globotriaosylceramide; lyso-Gb3: globotriaosylsphingosine; PTC: peritubular capillaries; eGFR: estimated glomerular filtration rate; α-Gal: alpha-galactosidase A.

neutralizing ADA has been successful via immune modulatory therapy (IMT) in infant Pompe disease[176], no cases of IMT has been published in Fabry disease to date.

Infusion-associated reactions

Infusion-associated reactions (IARs) can occur with any form of ERT. Most IARs are immediate, but some can be delayed by up to 24 h. Most of these reactions are mild and brief in nature and are easily dealt with by slowing or interrupting the ERT infusion briefly with the administration of antihistamines and acetaminophen. Prophylaxis with these agents is often successful in preventing an IAR recurrence. Occasionally, low-dose prednisone will need to be added prior to ERT to prevent IARs. While most of these patients will have ADA, IARs can occasionally be T cell-mediated in patients who lack ADA. As ADAs will usually cross-react, patients with ADAs and IARs will usually experience IARs if switched to another form of ERT. Most patients with IARs can be reassured that their reactions will be mild, self-limited, and easily brought under control. It is extremely rare for IARs to be so severe and persistent that ERT must be withdrawn. In Canada, there are currently only three (< 1%) such patients who have had ERT stopped due to ongoing IARs out of the 306 that have received ERT since 2007 in the Canadian Fabry Disease registry (unpublished data M. West). These patients had very high titers of neutralizing ADA and were withdrawn from ERT also due to lack of effectiveness[177].

Investigational therapies

Modified enzyme replacement therapy

Modified ERT (pegunigalsidase, 1.0 mg/kg iv every 2 weeks, Elfabrio®, Chiesi Farmaceutici S.p.A) is now licensed in several jurisdictions (USA, UK, and EU) based on non-inferiority to agalsidase-beta[166,167,169,178,179]. This product is an α-Gal A dimer with a number of surface polyethylene glycol (PEG) molecules that result in a prolonged plasma half-life and increased Cmax. Whether these altered pharmacokinetics provide an

advantage over pre-existing forms of ERT is unknown. ADA have been described to this ERT with transient altered pharmacokinetics with lower Cmax and $T_{½}$ blood levels that returned to normal as the antibodies spontaneously disappeared[166]. In a study of 22 patients on agalsidase-alfa switched to pegunigalsidase, eGFR slope went from -5.90 to -1.19 mL/min/1.73 m^2/yr and mean plasma lyso-Gb3 fell by 31%[167]. This improvement was possibly due to the dose differences (0.2 *vs.* 1.0 mg/kg) between these ERTs, as another study of 77 patients comparing agalsidase-beta at 1.0 mg/kg dose with pegunigalsidase did not find any difference in eGFR slopes or plasma lyso-Gb3 between these ERTs[168]. A potential advantage of pegunigalsidase is that there may be less cross-reactivity with ADA to the other forms of ERT. Lenders *et al.* (2022) has reported that ADA to agalsidase versions of ERT have decreased affinity for the pegunigalsidase molecule; other data suggested possible masking of some epitopes by the PEG moieties[180].

Substrate reduction therapy

Substrate reduction therapy (SRT) involves the inhibition of the enzyme glucosylceramide synthase upstream in the glycosphingolipid metabolic pathway. This results in a reduction in Gb3 levels in plasma and urine independent of α-Gal A activity. Several of these oral iminosugars are in phase III trials (ClinicalTrials.gov NCT03425539 Lucerastat, Idorsia Pharmaceuticals Ltd; NCT06114329 AL01211, AceLink Therapeutics Inc; NCT05206773 Venglustat Sanofi-Aventis LLC)[181,182]. These drugs have the advantage that they are taken orally and should not be influenced by ADA. While SRTs might also work synergistically with ERT or chaperone to lower substrate and metabolite levels even more, current research focuses solely on their efficacy as single agents. Using two drugs to treat Fabry disease could well double the cost, which might outstrip any combined benefit.

Gene and cell therapies

The goal in gene therapy is to bring about continuous α-Gal production from a transduced cell population (hematopoietic stem cells, cardiomyocytes, hepatocytes) to enable α-Gal delivery to non-transduced cells in target organs such as the kidney and heart at levels sufficient to reverse the metabolic defect of Fabry disease. This is also dependent on the ability of the target cells to take up α-Gal from the circulation via various transmembrane transport systems. Pre-existing antibodies to ERT and to the adeno-associated virus (AAV) vector may complicate these studies. To date, however, none of the human studies have provided direct evidence of delivery of over-expressed or engineered *a*-gal into the kidney or heart tissues. Gene therapy by *ex vivo* lentivirus-mediated autologous hematopoietic stem cells or *in vivo* AAV delivery has been tried with some early positive results[183,184]. Duration of the effect from gene therapy is unknown, but the FACTS study using *ex vivo* lentivirus-mediated autologous hematopoietic stem cells has shown sustained effects at the 5-year follow-up (unpublished data. M. West). A number of pharmaceutical companies have quickly joined and then withdrawn from this field in the last 4 years, perhaps reflecting the challenges of gene therapy in both Fabry disease and other metabolic diseases, including significant complications such as atypical hemolytic uremic syndrome and death[185,186]. As gene therapies are also being developed for other lysosomal diseases, continued progress is expected in this field.

Recent T cell- and B cell-mediated techniques for delivery of enzyme therapy have been proposed, which may be simpler and less expensive than gene therapy but await human trials[187,188]. As these techniques do not involve immunosuppression or *in vivo* cell transduction, fewer adverse effects and hospital days would be required with reduced cost, but the duration of effect might be less than with gene therapy.

Nucleoside-modified messenger RNA was used successfully to restore α-Gal activity with Gb3 reduction and restoration of lysosomal protein levels in a model of cardiac Fabry disease, using induced pluripotent stem cells-derived human cardiomyocytes[189].

CRISPR/Cas techniques have been successfully applied to both create and treat animal models of Fabry diseases[190]. With this technique, DNA variants can be corrected *in vivo*. There remain concerns about the safety of these techniques in humans as well as the number of cells needed to be corrected to effect clinical benefits. Some investigations in animal models suggest that achieving 10% of normal α-Gal activity may be sufficient[191], but the optimal target α-Gal activity in human studies remains undefined.

QUESTIONS ABOUT FABRY NEPHROPATHY STILL TO BE ANSWERED

Important questions remain to be answered about Fabry kidney disease. These are some for future research.

1. Define the natural history of Fabry disease including nephropathy; why is the prevalence of kidney disease in heterozygotes less than that of cardiac disease or stroke?

2. Determine how to predict which heterozygotes will go on to develop progressive kidney disease so that treatment can be started early;

3. Determine which ERT is best for different patient groups - males, females, classic *vs*. late-onset disease; is dose important in adults?

4. Determine if combination therapy is superior to monotherapy, i.e., ERT + chaperone; are these treatments of additional benefits?

5. Determine the optimal age to start therapy, recognizing that this will vary among different patient groups;

6. Determine how to best deal with patients who have high levels of neutralizing anti-drug antibodies that are limiting treatment response;

7. Determine what the target α-Gal activity should be with gene and cell therapies;

8. Determine if plasma lyso-Gb3 or another biomarker can serve as a surrogate marker for clinical trials in Fabry nephropathy.

SUMMARY

Fabry nephropathy is a rare cause of CKD due to the deficiency of the lysosomal enzyme α-Gal. Its importance lies in the fact that it is a treatable cause of CKD. It is an X-linked disorder, with heterozygous females being less affected than males. It is difficult to recognize due to its rarity but also its shared clinical features with more common conditions. Clinical features are highly variable, with an early-onset classic severe phenotype and a later-onset phenotype. Family screening and high-risk screening of patients with CKD or on dialysis is best done with a combination of lyso-Gb3, α-Gal activity, and *GLA* analysis, as many females have normal α-Gal activity. Pathogenesis relates to the deposition of the enzyme substrate Gb3 and its metabolite lyso-Gb3, which together cause widespread abnormalities in cell signaling and homeostasis. In the kidney, the podocyte is the main target due to its inability to replicate and large accumulation of Gb3 over time. Podocyte damage from Gb3 results in podocyturia with focal glomerulosclerosis and increasing proteinuria. Control of proteinuria as the major factor promoting loss of GFR is one of the cornerstones of management. Treatment with recombinant ERT or pharmacologic chaperone is available. The best results come with early diagnosis and initiation of treatment, although the optimal age for start is still uncertain. Treatment will slow or stabilize the eGFR slope but does not lower proteinuria. ERT effect may be limited

by neutralizing anti-drug antibodies in some males. Patients generally fare well with all dialysis modalities and following a kidney transplant. Emerging treatments include oral substrate reduction inhibitors, modified ERT, plus gene and T and B cell therapies. Recognition that many aspects of Fabry disease do not respond to current therapy will drive more research to find adjunctive treatments.

DECLARATIONS

Authors' contributions

Design, writing, and review of this work: West ML, Geldenhuys L, Bichet DG

Availability of data and materials

Not applicable.

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Conflicts of interest

West ML has received research funding, speaker's fees and/or consultant fees from the following: Alexion, Amicus, Chiesi, Idorsia, Protalix, Sanofi, Sumitomo, and Takeda; he shares IP in Fabry gene therapy and Fabry cardiac biomarkers. Bichet DG has received research funding, speaker's fees and/or consultant fees from Amicus Therapeutics, Sanofi, and Takeda. Geldenhuys L declares no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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