Introduction
Fabry disease (FD) arises from an X-linked defect in lipid storage, whereby deficient or absent lysosomal α-galactosidase A (α-gal A) activity leads to systemic deposition of glycosphingolipids, mainly globotriaosylceramide (known as Gb3 or GL3). Deposition mainly affects the cardiovascular, renal, and neurologic systems, but can occur in all organs, and despite specific enzyme replacement therapy (ERT) with human recombinant α-galactosidase, kidney disease is progressive.1,2 Accordingly, FD is considered a genetic risk factor for kidney disease, cardiomyopathy, stroke, and early death.3 Kidney disease is a hallmark feature in male patients, with microalbuminuria and proteinuria as the initial presentation.4 The etiology of FD nephropathy is not completely understood, but vascular, glomerular, and tubular changes are probably all implicated early on in the disease; these changes have been noted in children, even in those who have only scant microalbuminuria or normoalbuminuria. For this reason, the role of podocyturia as both a biomarker and an early treatment target in preventing kidney damage is gaining prominence.4-6

FD occurs in all racial and ethnic groups, with none of the groups exhibiting a higher propensity for the disease than others.7 Though FD is considered rare, actual prevalence may be higher than originally thought due to delayed diagnosis. Because complaints are varied and often non-specific, clinicians should consider FD for a variety of differential diagnoses.8 This is especially important because ERT, the cornerstone of management, is currently available. Although mostly observational, evidence suggests that renal outcomes may be more favorable if ERT is initiated early on, and especially in those who have preserved kidney function, and who also receive antiproteinuric therapy.9-13

This clinical update on FD highlights renal manifestations, as well as epidemiology, diagnosis and screening, and the impact of treatment on renal outcomes.
Etiology and Pathophysiology

The inborn error of metabolism in FD is deficient lysosomal hydrolase enzyme, α-galactosidase A (α-galA; EC 3.2.1.22), which catalyzes the hydrolytic cleavage of the terminal galactose from Gb3. The enzyme is encoded by the GLA gene on Xq22, with more than 600 different mutations identified so far. Residual enzyme activity ranges from 0% to about 30% of mean normal value; newly identified GLA gene variants have higher residual enzyme activity, and may therefore be benign. Even in accepted FD mutations, however, it is not known how the disease will be expressed.

Without sufficient α-gal A activity, Gb3 accumulates in cells and tissues, which causes inflammation, ischemia, hypertrophy, and the development of fibrosis, thus promoting cellular damage and progressive organ dysfunction. Many cell types are affected, including vascular (endothelial and smooth muscle cells), cardiac and renal (tubular and glomerular cells, and podocytes), and nerve cells. Because of impaired autonomic function, vasculopathy, myopathy, and gastrointestinal symptoms are some of the most frequently reported symptoms.

Progressive endothelial accumulation of glycosphingolipids such as Gb3 accounts for the associated clinical abnormalities of skin, eye, kidney, heart, brain, and peripheral nervous system. This cellular process starts early in life, and, if untreated, eventually leads to organ failure and premature death. However, Gb3 deposition may only be partly responsible for the manifestations of FD, since signs and symptoms may occur without the presence of severe deposits. Another molecule, lyso-Gb3 is also found to be circulating in high concentrations in FD, and reproduces some of the effects of high glucose in podocytes.

Clinical manifestations of FD can include skin lesions (angiokeratomas, Figure 1), pain and burning in the hands and feet (acroparesthesia), fatigue, impaired sweating, and gastrointestinal problems such as diarrhea, constipation, nausea, and vomiting. Corneal opacities that progress to a characteristic “whorled” pattern are found in most hemizygotes, but generally do not impact vision. Many of these symptoms are observed during childhood and adolescence, and tend to be prominent in hemizygous males. Heterozygous females were believed to be asymptomatic carriers, however, it is now acknowledged that heterozygous females can also be affected and may develop either a partial or full spectrum of clinical manifestations associated with FD. In adulthood, progressive Gb3 accumulation in the microvasculature causes ischemic damages that can result in cardiovascular disease, stroke and kidney failure. Cardiac symptoms include left ventricular hypertrophy (LVH), arrhythmia, angina, and dyspnea.

Kidney disease is a major complication of FD related to glycosphingolipid accumulation throughout the nephron. Approximately 50% of men and 20% of women by the age of 35 years have proteinuria in the presence of FD. Regular assessments of kidney function in FD should include estimates of the glomerular filtration rate (eGFR), total protein and albumin excretion, and urinary sodium excretion. Biopsy studies have shown that glomerular and vascular changes are present before progression to proteinuria. Podocytoxin has been observed in patients before the appearance of albuminuria and proteinuria, and therefore researchers suggest that it be considered an even earlier biomarker of kidney damage, and as an indication to begin ERT at a younger age. Patients who start treatment later in life still have disease progression. Aside from these established effects on kidney pathology and function, parapelvic kidney cysts have also been found in some patients, but their cause is unknown. Identifying these cysts may help with earlier recognition of FD.

Epidemiology

The classic epidemiology of FD based on enzyme activity has estimated prevalence at 1:17,000 - 1:117,000 in Caucasian males, and 1:40,000 in males and females. However, evidence suggests that atypical and late-onset phenotypes are underdiagnosed, so the actual prevalence is most likely higher. The results from large genetic screening programs, which are based on DNA sequencing and do not rely upon the development of clinical symptoms, indicate that FD is more prevalent than previously thought. Mutations that cause classic manifestations of FD are found in approximately 1:22,000 to 1:40,000 males, and mutations that cause atypical presentations are found in approximately 1:1000 to 1:3000 males and 1:6000 to 1:40,000 females.

The incidence of FD is being reevaluated because variant forms of FD and newborn screening numbers challenge classic epidemiology. Incidence ranges are derived from the high prevalence of a presumed cardiac Fabry mutation (IVS4 + 919G), reported at 1:600 males in Taiwan to the 1:3,000 reported from newborn screening data in Illinois and 1:10,000 (males and females) in Washington state. Studies also suggest an increased incidence of FD in patients with cryptogenic strokes, hypertrophic cardiomyopathy, and dialysis patients, ranging from 1:20 to 1:1000. Newly identified GLA gene variants have higher residual enzyme activity, but it is questionable if these variants are clinically significant.

Diagnosis

Onset of the signs and symptoms associated with FD should warrant prompt diagnosis and intervention. However, observed symptoms are heterogeneous among many patients, making a timely diagnosis a challenge. In addition, some symptoms can resemble other more common diseases. Diagnostic delays have been estimated at ~15 years for both genders, because initial diagnosis is frequently inaccurate. In the 366 European patients from the Fabry Outcome Survey, the mean delay until correct diagnosis after symptom onset was estimated at 13.7 and 16.3 years for males and females, respectively. Appropriate biochemical and/or genetic confirmation could be considered for confirmation if physical and clinical examination raises a suspicion of FD.

When there is a clear family history and classic phenotype, FD can be confirmed in males if there is low α-gal A activity in leukocytes or plasma. Assays of plasma alpha-galactosidase activity may be less sensitive than in leukocytes. Mutation analysis of the α-Gal A gene is needed to diagnose female carriers (unless the woman is an obligate heterozygote, whereby the father...
Differential Diagnosis

Although FD is considered highly penetrant in males and females, its expression can vary greatly, thus contributing to its high rate of misdiagnosis. It is therefore necessary to begin with a comprehensive medical and family history, and to include FD in the differential diagnosis according to the hallmark clinical and familial features in Table 1.

Table 1: When to consider FD as a diagnosis

<table>
<thead>
<tr>
<th>Test ANY patient who has:</th>
<th>In the absence of above two factors, test patients with at least two of the features below:</th>
</tr>
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<tbody>
<tr>
<td>1. A family history of Fabry disease OR</td>
<td>1. Decreased sweating (anhidrosis or hypohidrosis)</td>
</tr>
<tr>
<td>2. Corneal verticillata (“whorls”) on slit lamp exam</td>
<td>2. Reddish-purple skin rash in the bathing trunk area (angiokeratomas)</td>
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<tr>
<td>3. Personal and/or family history of kidney failure</td>
<td>3. Personal and/or family history of kidney failure</td>
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<tr>
<td>4. Personal or family history of “burning” or “hot” pain in the hands and feet, particularly during fevers (acroparesthesias)</td>
<td>4. Personal or family history of exercise, heat, or cold intolerance</td>
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<tr>
<td>5. Patients with sporadic or non-autosomal dominant (no male-to-male) transmission of unexplained cardiac hypertrophy</td>
<td>6. Patients with sporadic or non-autosomal dominant (no male-to-male) transmission of unexplained cardiac hypertrophy</td>
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Additionally, FD must be considered in differential diagnoses when a young male presents with signs and symptoms of stroke, along with other characteristic lesions. Conditions that mimic the symptoms of FD include the following: acute stroke; basilar artery thrombosis; cardioembolic stroke; cavernous, dissection and lacunar syndromes; posterior cerebral artery stroke; transient global amnesia. Consider FD in adults with unexplained proteinuria or kidney failure, especially if LVH is present or there is a history of stroke. In children, other possible causes of pain such as rheumatoid arthritis, rheumatic fever, systemic lupus erythematosus, Raynaud’s disease, and “growing pains” (a frequent misdiagnosis in children with FD) must be ruled out. In adults, celiac disease and multiple sclerosis are the most common differential diagnoses, particularly in females. Likewise, when no mutation of the GLA gene has been found, the possibility of a phenocopy mimicking FD should be considered.

There are cases of hemizygous males with disease manifestations seen only in the kidney. These renal variants have been found among Japanese dialysis patients whose kidney disease had been misdiagnosed as chronic glomerulonephritis. These patients had absent or deficient α-galactosidase A activity, and were then found to have GLA gene mutations. These findings suggest that FD may be underdiagnosed among dialysis and kidney transplant patients. Early detection of these renal variants is important, since these patients may later develop vascular disease of the heart or brain.

Treatment

Supportive Care and Antiproteinuric Therapy

Supportive care usually consists of pain relief, blood pressure control/nephroprotection, antiarrhythmic agents, gastrointestinal agents, and lifestyle modifications. Renal replacement therapy (dialysis or kidney transplantation) is available for patients with kidney failure. Patients with neuropathic pain may benefit from avoiding any possible triggers of their acute pain attacks. Certain antidepressants and anticonvulsants can be considered.

Figure 2. Electron micrograph of a renal biopsy in Fabry Disease

Glycosphingolipid is deposited in cytoplasmic vacuoles in glomerular visceral epithelial cells. Insert: Cytoplasmic vacuoles contain electron-dense material in parallel arrays (zebra bodies) and in concentric whorls (myelin figures).

Analgiesics are also an option, but nonsteroidal anti-inflammatory drugs are generally not considered effective, and can negatively impact kidney function.55 Treatments for gastrointestinal symptoms can include changes in eating habits (e.g., smaller, more frequent meals), H-2 blockers, and metoclopramide.18 Hypertension is not common during earlier onset of FD, but more so as the disease progresses, particularly in the presence of kidney disease.38,51,52 Hypertension, along with LVH, is most strongly associated with cardiovascular events in the presence of proteinuria, given their antiproteinuric effect.50,53 One prospective observational study examined the safety and efficacy of controlling proteinuria with ACEi or ARB therapy in FD patients who were receiving ERT with agalsidase beta. Results showed that proteinuria in most classical FD patients was controlled, but that kidney function was not preserved in patients who did not achieve the urine protein to creatinine ratio (UPCR) treatment goal of 0.5 g/g. Kidney function was preserved in a minority of patients who started ERT at a younger age, and who maintained a UPCR ≤ 0.5 g/g with antiproteinuric therapy.10

**Enzyme Replacement Therapy**

To address the underlying metabolic error in FD, ERT was made available in 2001. Agalsidase beta is the only currently available ERT in the United States, whereas agalsidase alfa and agalsidase beta are both available in Europe. Agalsidase alfa has not been approved for use in the United States. No strong evidence demonstrates superiority of either one in the treatment of FD.54-56 A 10-year observational study of 52 patients with classic FD demonstrated the effectiveness of agalsidase beta therapy (1mg/kg/2 weeks) in regards to renal, cardiac, and overall outcomes. Mean left ventricular posterior wall thickness and interventricular septum thickness remained unchanged and normal in patients who had less kidney damage and had started ERT at a younger age (mean 25 years versus 38 years). Patients who began ERT at age ≥ 40 years exhibited significant increase in left ventricular posterior wall thickness and interventricular septum thickness. Most patients remained alive and event-free, and mean plasma Gb3 normalized within 6 months. Mean slopes for estimated glomerular filtration rate (eGFR) were -1.89 mL/min/1.73 m²/year and -6.82 mL/min/1.73 m²/year for starting ERT patients with less and greater kidney damage, respectively.12

Proteinuria is an early marker of kidney disease, but it may also reflect an already damaged and denuded filtration barrier due to a decreased number of podocytes; under this circumstance, proteinuria could be considered a late biomarker of glomerular damage.57 Research shows that the presence of increased podocytopenia in the absence of proteinuria and CKD may indicate that the detachment of damaged podocytes in FD precedes proteinuria.28,64,45 In the first morphologic study to show relationships between proteinuria and early glomerular lesions of FD nephropathy in young patients, podocyte Gb3 inclusion volume density increased progressively with age, but there were no significant relationships between age and endothelial or mesangial inclusion volume densities. Foot process width, greater in male patients, also progressively increased with age and correlated directly with proteinuria. This is important because once proteinuria presents, the response to ERT may be inadequate, and proteinuria usually does not abate.64

ERT produces a rapid and significant decrease in mesangial and endothelial cell Gb3 inclusions, whereas podocyte inclusions and proteinuria persist despite treatment.41 Some experts have therefore advocated for initiating ERT at an earlier age before podocytopenia and effacement have begun.58 In order to determine the effect of early ERT on renal morphology in FD, one study evaluated the effect of 5 years of treatment with agalsidase alfa or agalsidase beta in 12 consecutive patients, age 7-33 years (median age of 16.5 years). Results showed that long-term ERT in young patients with either drug can result in complete Gb3 clearance of mesangial and glomerular endothelial cells across all dosage regimens, and that effective clearance of podocyte inclusions is possible, and that it is dose-dependent.9

Only one placebo-controlled trial has studied the prevention of end organ complications with ERT, after a per-protocol analysis that adjusted for baseline proteinuria, a reduction in complications using agalsidase beta as compared with placebo was found in patients with advanced disease at baseline.59,57 Interpreting long-term studies is difficult because a control arm is usually absent, or because only historical controls are used for comparison with treatment groups.9 Aside from potential in reducing end organ complications, agalsidase beta has been associated with symptomatic benefits, including pain and health-related quality of life.60,61

Deriving benefit from ERT must be balanced by individual patient needs, along with the consideration that the burdens of treatment may be too great to either initiate or continue treatment. The European Fabry Working Group has therefore examined risks versus benefits of ERT in patient groups, and has created a consensus document on when to initiate, not initiate, or stop ERT.62

**Pharmacological Chaperones**

Pharmacological chaperones (PCs) bind to and stabilize some mutant forms of α-gal A in the endoplasmic reticulum, which facilitates proper protein folding, and allows for correct trafficking. Migalastat hydrochloride, currently in clinical trials, is an analog of the terminal galactose of Gb3 that binds and stabilizes wild type and mutant forms of α-gal A. The benefits of PCs include: being non-invasive because they are taken orally, exhibiting broad tissue distribution, and gaining access to the central nervous system. However, PCs are genotype specific, and it is estimated that only one-third to one-half of mutations may be amenable to currently available PCs. Aside from stabilizing misfolded proteins, PCs may improve the physical stability, and maybe the efficacy, of the recombinant enzymes in ERT. Pre-clinical studies have suggested that combination therapy of PCs and ERT maybe be helpful in FD, but such combination therapy has not yet been developed.54

**Substrate Reduction Therapy**

Substrate reduction therapy (SRT) aims at decreasing synthesis of the substrate Gb3 by targeting the enzymes involved in the production of cellular Gb3. An investigational drug that is in clinical trials is the glucosylceramide synthase (GCS) inhibitor, Genz-682452. In preclinical studies, GCS inhibition by Genz-682452 reduced the tissue level of Gb3 in mice. SRT most likely will not be used as a monotherapy, but maybe in combination with ERT.54,63

**Stem Cell Transplant and Gene Therapy**

Difficulties with finding a matching donor and treatment related side effects limit the use of stem cell transplants. Additionally, enzymes secreted by non-modified transplanted cells and their progeny do not attain therapeutic levels that could influence uncorrected cells in other organs.54 No gene therapies have yet been approved by the FDA, although many clinical trials are underway. Gene therapy can provide a non-immunogenic, sustained, and balanced supply of α-gal A.
Pre-clinical studies of gene therapy in FD have reported production of functional α-Gal A and reduction of Gb3 in Fabry mice and patient bone marrow mononuclear cells.54,64,65

Conclusion

Expanded newborn screening with advanced diagnostic techniques has led to identifying more FD patients. The advent of ERT has heralded a new era in treatment and improved outcomes for more patients. Morphological studies have pointed to the importance of kidney biopsy in confirming or ruling out FD, while also allowing for earlier ERT and hopefully preventing damage to podocytes. This constitutes a paradigm shift from finding patients much later on after a series of misdiagnoses and having missed a window of opportunity for optimizing ERT therapy. Study of other therapeutic targets such as lyso-Gb3 may lead to more individualized therapies, along with refinement of therapies already under investigation, such as PCs and SRT. The availability of combination therapies and earlier biomarkers of kidney damage such as podocyturia will likely enhance renal and overall outcomes, but large randomized controlled trials are required in order to determine the long-term benefits of ERT and other interventions. Newborn screening, along with earlier diagnosis and treatment will require greater involvement from neonatologists, pediatrics, and genetic counselors.

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