Assessment of Renal Pathology and Dysfunction in Children with Fabry Disease

Uma Ramaswami,* Behzad Najafian,† Arrigo Schieppati,‡ Michael Mauer,§ and Daniel G. Bichet

*Paediatric Metabolic Unit, Addenbrooke’s University Teaching Hospital, Cambridge, United Kingdom; Departments of Laboratory Medicine and Pathology and Pediatrics, University of Minnesota, Minneapolis, Minnesota; †Clinical Research Centre for Rare Disease, Aldo e Cele Daccò, Mario Negri Institute for Pharmacological Research, Bergamo, Italy; and ‡Departments of Medicine and Physiology and Groupe d’étude des protéines membranaires, Université de Montréal, Hôpital du Sacré-Coeur de Montréal, Montréal, Québec, Canada

Overt renal disease often first presents in male individuals with Fabry disease in early to middle adulthood, but proteinuria and reduced GFR may occur in adolescents and in young children. More recently, kidney biopsy data have shown early renal histologic changes in pediatric patients, and kidney dysfunction, primarily proteinuria, seems to be more common in girls. Renal investigations and their timing in children remain poorly defined. A consensus on renal investigations is necessary to understand the natural progression of the disease and to evaluate the efficacy of treatments such as enzyme replacement therapies. This article addresses three main categories: Use of GFRs, measuring albuminuria, and renal biopsies in children.

Progressive nephropathy is one of the main features of Fabry disease (1–3) and is marked by an insidious development. By adulthood, renal failure frequently becomes a major complication of Fabry disease, with more than half of male and more than 20% of female patients eventually developing advanced renal disease or ESRD (4). Although typically occurring by the third to fifth decade of life in men with Fabry disease (1), ESRD can occur as early as 16 years of age (5). Renal pathology can arise in pediatric patients with Fabry disease, but indicators are difficult to assess. Microalbuminuria is one of the first signs of impairment of renal function (6,7), and overt proteinuria may start as early as 10 years of age (5); however, chronic kidney lesions may already be present (8). In young patients, glomerular hyperfiltration (9–11) can mask the detection of early decline in GFR to the extent that a critical number of nephrons are damaged and cannot maintain adequate glomerular filtration. The decline in GFR typically commences once proteinuria is established (1) but may precede it (4).

In adults, there is compelling evidence that proteinuria is an indicator of renal dysfunction in Fabry disease (2,3) that requires immediate intervention with enzyme replacement therapy (ERT) and/or antiproteinuric medications (12); however, there are no clear guidelines for initiation of ERT in children with Fabry disease, and these decisions are usually based either on national guidelines in some countries such as the United Kingdom National Guidelines (13) on the basis of specific criteria for starting ERT or on clinical judgment also on the basis of symptoms and signs that significantly affect the quality of life of children with Fabry disease. There are currently no early biomarkers or predictors of disease progression in practice. The objective of this article is to summarize the diagnostic measures in the early stages of nephropathy in Fabry disease and to assess their value as outcome measures for therapeutic decisions.

What Do We Know?

The glomerular barrier is a complex biologic membrane with high filtration rates of water (despite the absence of water channels), nonrestricted passage of small- and middle-sized molecules, and almost total restriction of serum albumin and larger proteins (14). α-Galactosidase A (α-Gal A) deficiency results in accumulation of globotriaosylceramide (GL-3) in lysosomes and in other cellular compartments, perhaps as part of retrograde transport secondary to lysosomal accumulation, in all kidney cell types (15), a process that over time can cause irreversible functional damage to the glomerular barrier (16). Kidney cell GL-3 inclusions have been detected in fetuses that are affected with Fabry disease, especially in podocytes (17,18), and Gubler et al. (16) showed abundant GL-3 inclusions in all glomerular cells and in vessels in three children who were 8 to 12 years old and did not have proteinuria, albeit with a more...
heterogeneous distribution in females. Arteriopathy was present in some children with Fabry disease. More recently, renal lesions in biopsies from nine children with Fabry disease who were aged 7 to 18 years and had normal GFR have been described (8). There were large numbers of GL-3 inclusions in podocytes and distal tubules in all nine patients. Importantly, there was segmental foot process effacement, consistent with podocyte injury in all cases including those without proteinuria or microalbuminuria. Four of the nine children had arteriopathy, similar to the lesions described by Gubler et al. (16), and three of nine children who were aged between 16 and 18 years had FSGS. It is noteworthy that all nine patients in this study were symptomatic with acroparesthesia, and >80% (seven to eight of nine) of patients also had gastrointestinal symptoms, ophthalmologic findings, and autonomic dysfunction with hypohydrosis before the renal biopsies. In the seven children with Fabry disease reported in this study who did not have renal biopsies, only four had acroparesthesia and one had decreased sweating. These seven patients also did not have microalbuminuria or proteinuria. Conversely, although three of the nine patients who had renal biopsies did not have microalbuminuria, of these nine patients, six (85%) of seven boys and one (50%) of two girls had microalbuminuria. These nine patients would therefore fall into a group of children who had Fabry disease and presented with early onset of significant and severe symptoms. Podocyte inclusions, per se, were present in all nine patients and therefore cannot be discriminatory and used to assess treatment response on the basis of this study. These are important findings nevertheless, and longitudinal data on renal biopsies as part of a clinical trial will help address disease progression and the impact of early ERT to prevent the progression. With limited evidence-based literature available with respect to the progression of renal disease, microalbuminuria seems to be a helpful and noninvasive marker to evaluate renal disease in young children, which can be easily performed by every clinician treating Fabry disease worldwide. Wilcox et al. (4) showed that urinary protein levels in the microalbuminuria or proteinuria ranges are present in almost all adult Fabry registry patients for whom this was measured. Thus, in adults, microalbuminuria cannot be a precise predictor of serious Fabry nephropathy, because not all patients, especially female patients would be expected to progress to advanced kidney disease (4). Conversely, earlier onset of microalbuminuria in children may well prove to be of stronger predictive value, although larger numbers of patients are required to confirm this.

In adult patients with Fabry disease, the natural progression of renal disease has been delineated extensively (1–3). Recently, Schiffmann et al. (3) showed that the yearly decline in estimated GFR (eGFR) is −3.0 ml/min per 1.73 m² in males with baseline eGFR >60 ml/min per 1.73 m² and −6.8 ml/min per 1.73 m² in males with advanced CKD at baseline. Progression rates for females were 0.9 and −2.1 ml/min per 1.73 m², respectively. As previously demonstrated by Branton et al. (1), more rapid progression was found in patients who had higher baseline proteinuria and/or chronic renal insufficiency at baseline. No such large data sets on progression rates are available for children with Fabry disease. The contribution of children, both boys and girls, to the total number of patients with Fabry disease included in the registries (19,20) is only approximately 20%, and asymptomatic young patients may not be included at some centers; therefore, the present registry data might be biased toward more severely affected children. Among children and adolescents with Fabry disease included in four published analyses (19–22), kidney dysfunction, primarily proteinuria, seemed to be more common in girls (18% of 97) than in boys (8% of 127). In the renal biopsy study of nine symptomatic pediatric patients (seven boys, two girls; 7 to 8 years) who all had electron microscopically detected lesions, mean albumin-creatinine ratio was increased at 38 mg/mg (range 5.3 to 104.3 mg/mg; mean 53 mg/mg in six of nine patients who had microalbuminuria), whereas measured GFR (mGFR; iohexol-GFR) was normal in all patients (8).

It is generally recommended that the eGFR slope be monitored as an important outcome measure in Fabry nephropathy; however, recent reports demonstrated that eGFR, usually by the Modification of Diet in Renal Disease (MDRD) formula in adults (23) and the Schwartz formula (24–26) in children (10,20,27) may result in a substantial overestimation of the true GFR in adult male patients with Fabry disease (28,29). Most of these methods rely on serum creatinine, which is measured in different ways, including kinetic and Endpoint Jaffe method and enzymatic assays. Variations in serum creatinine measured by different assay methods can cause significant differences in eGFR values (30), and these discrepancies are even greater in children (31,32). The new Schwartz 09 formula (33) using creatinine measured by the enzymatic method reported that nearly 80% of eGFR values were within 30% of the mGFR, and similar results were also seen with the Counahan-Barratt method first described in 1976 (34). mGFR is only rarely performed (29,35), presumably because these investigations are time-consuming procedures that require intravenous catheters to be placed, with some using radioactive probes; however, Schwartz and Work (36) reported that there is no dependable substitute for an accurately mGFR, with iohexol plasma disappearance offering the best combination of safety, accuracy, and reproducible precision.” Iohexol is not available routinely in all centers that measure GFR. Given the difficulty in measuring GFR repeatedly in children, in a recent study (37), the correlation of eGFR and mGFR was analyzed in 82 measurements from 42 children from three European centers. In that study, it was shown that the old Schwartz formula overestimated GFR by 50.6 ml/min per 1.73 m², whereas the new Schwartz 09 formula correlated well with a mean overestimation of 5.3 ml/min per 1.73 m² and was similar to calculations made using the Counahan-Barratt method. On this basis, it may be reasonable to recommend a baseline GFR measurement before ERT initiation in children with correlation with the serum creatinine determined eGFR and repeated GFR measurement as clinically indicated using the Schwartz 09 or Counahan-Barratt method. Likewise, a better standardization of albumin measurements is to be considered, because urine albumin has been measured by nephelometry (8) or by routine RIA (22), which can give negative results.
ERT has been shown to reduce GL-3 rapidly in endothelial and mesangial cells (38) in adult patients, and, if started early, it may prevent or slow renal dysfunction (12,39,40); however, once proteinuria is established, responsiveness to ERT is incomplete and proteinuria generally does not normalize (41), although it has been demonstrated that enzyme substitution therapy will reach podocytes and renal endothelial cells (42). Studies of ERT in children (23 to 48 weeks) has shown that treatment was safe and well tolerated (9–11). ERT generally resulted in stable or reduced microalbuminuria and no worsening in eGFR in those with abnormal baseline values.

**What Do We not Know?**

The renal biopsy studies of pediatric patients strongly suggest that Fabry nephropathy, similar to many other slowly progressive chronic kidney diseases, such as diabetic nephropathy or chronic hypertension, may progress in clinical silence; however, the number of patients in the Fabry renal biopsy studies is very small (<10 patients) and represent a cohort of patients who presented early with a severe phenotype, and the patients in this study who did not have a renal biopsy also had fewer systemic symptoms as discussed. Two thirds of the patients in the study by Tøndel et al. (37) presented with microalbuminuria; in particular, six of seven boys with abnormal histologic findings had microalbuminuria, as discussed previously. We do not know how and at what pace the disease progresses in individual patients. For example, in males who have Fabry disease with kidney disease susceptibility, serious renal disease may manifest as early as the teenage years or as late as the seventh or eighth decade of life (4). Proteinuria may not be a sensitive marker of early injury, because advanced lesions have been detected in biopsies from patients with Fabry disease and normoalbuminuria (8,16). A fraction of patients with Fabry disease develop GFR loss before proteinuria (4). In addition, hyperfiltration may complicate detection of GFR loss because if GFR is normal, then this could represent a relative decline (43). Furthermore, we do not know when to initiate treatment and how much ERT is enough for each patient and whether reduction in small amounts of albumin in the urine is a surrogate for treatment efficacy. As yet, no studies allow us to conclude that, given current dosing guidelines, initiation of ERT in childhood will prevent or markedly delay the development of serious kidney disease in adulthood.

Renal biopsy is part of routine standard of care for many childhood and adult renal diseases to determine diagnosis or prognosis or to provide guidelines for management. Biopsies are regularly performed for their value as predictors of disease progression and to adjust treatment options in chronic kidney conditions in children, such as lupus nephritis, nephrotic syndrome, kidney transplant rejection, etc. (44,45) and in some centers in the United States and Europe to provide reassurance for patients with longstanding hematuria or low-grade proteinuria and normal GFR (46); however, although Fabry nephropathy carries a far greater risk for progression to renal failure than these other disorders, renal biopsy has not yet become the mainstream of routine clinical care. Renal biopsy is a low-risk procedure when performed in centers with experience in performing these tests regularly. There were no major adverse events for renal biopsies that were performed under anesthesia by interventional radiologists on 30 children aged 7.0 ± 2.7 years (47). In another study of 260 renal biopsies from children, adolescents, and young adults, there was no incidence of permanent injury; only three (1.2%) patients developed postbiopsy hemorrhage that required intervention (one embolization of a small artery and two bladder catheterization), 1.9% had mild postbiopsy discomfort, and 2.3% had postbiopsy pain (48).

Although the relative safety of renal biopsy has been established, many children, especially those who are younger than 10 years, will require general anesthesia for a renal biopsy to be performed, and the risks of general anesthesia and indeed the need for hospitalization have to be considered carefully given that there is no clear evidence that renal biopsies are predictive of therapeutic responses to ERT in children with Fabry disease. It is recommended that such studies be done as part of a clinical trial in selected centers where the safety of renal biopsies is established. We emphasize the importance of conducting long-term studies to address the value of renal biopsies to monitor treatment effects of ERT in children with Fabry disease before it can be recommended as a routine investigation for children with Fabry disease.

Careful measurement of urinary albumin excretion, estimation of GFR, and monitoring of BP must be included in the standard care of patients with Fabry disease, and, for reasons stated already, baseline formal measurement of GFR should be strongly considered. Some centers in the United States and in Norway include renal biopsy as part of their standard care for children with Fabry disease. These biopsies are used to determine the extent of kidney involvement by the disease at baseline and, assuming that early treatment in children may represent the best way to correct early kidney damage and prevent progressive disease, whether ERT should be initiated. Despite currently available ERT protocols, heterogeneity of disease manifestations and progression in Fabry nephropathy does not support the strategy of uniform ERT dosage or other treatment for all patients (one size fits all). This was reflected in the study by Schiffmann et al. (41), in which more frequent ERT resulted in slowing of the GFR decline. An early intervention and dosing study during a 5-year period in young treatment-naive male patients with Fabry disease is being undertaken to address this (49). Although the benefit of serial renal biopsies for children with respect to therapeutic benefits of Fabry disease has not been established, it is an important question that requires further evaluation before it is recommended as best practice in the management of Fabry disease.

**What Can We Do with What We Know?**

The recommendation is to evaluate systematically all children in registries and to measure albumin excretion rates regularly and precisely. Total urinary protein (mg/24 h) or microalbuminuria (μg/min) obtained in at least two of three consecutively timed urine collections are regularly measured in Fabry reference centers. As mentioned, mGFR methods require intravenous cannulation and sampling, and radioisotopic methods should be avoided in children. eGFR calculated by the
Counahan-Barratt and Schwartz 09 methods are acceptable methods to calculate eGFR in children and are useful for both monitoring disease and assessing treatment effects; however, it is important to consider mGFR at baseline and monitoring when treatment modalities such as ERT are initiated in children.

Renal biopsy could potentially give us insight about disease progression and responsiveness to treatment. Although when to do a follow-up biopsy is not yet clear, studies show that clearance of endothelial GL-3 inclusions were present in many patients within 5 to 54 months of ERT in adult patients (39,50). In the absence of adequate data on the timing of ERT and the benefits of renal biopsies, it is essential to perform meticulous and regular follow-up assessments, particularly noninvasive renal function tests such as three consecutive early morning urine samples for microalbuminuria, the most readily available marker of early renal disease.

Key Research Questions
The identification of biomarkers of disease vulnerability, progression, and treatment adequacy is a necessity. Discovery of noninvasive biomarkers will ease the need for repetitive renal biopsies in children. Histologic changes, assessed on renal biopsies, may correlate, precede, or predict albuminuria and GFR changes in chronic renal disease (51). Establishment of such structural-functional relationships usually requires careful measurement of these parameters. Early predictors of disease progression can be chosen as primary end points of clinical trials and as guides to monitor ERT efficiency. Nevertheless, long-term studies are required to answer how ERT may affect the natural history of nephropathy in children with Fabry disease.

Acknowledgments
We thank Ellen Buschman (Research Center, Hôpital du Sacré-Coeur) and Hans Ebels (Genzyme Corp.) for editorial assistance. All authors had full responsibility for the contents and views of the submitted manuscript.

Disclosures
None.

References


49. FierceBiotech: Genzyme begins enrollment in post-market-
