

# Nephropathy in Fabry disease: the importance of early diagnosis and testing in high-risk populations\*

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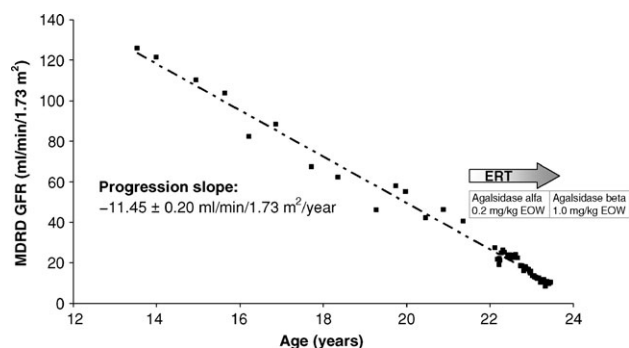
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## Introduction

Fabry disease is an X-linked lysosomal storage disorder caused by the deficiency of the enzyme alpha-galactosidase A (alpha-Gal A). Glycosphingolipids, predominantly globotriaosylceramide (GL-3), progressively accumulate in vascular endothelial cells, a variety of renal and cardiac cells and neural cells, and symptoms often first appear in childhood or adolescence [1–3]. Progressively worsening, life-threatening complications developing in adulthood may include chronic kidney disease (CKD), end-stage renal disease (ESRD), heart disease and stroke [1,4,5]. Nephropathy is a dominant feature of advanced Fabry disease. The average age for the development of clinical nephropathy is 27 years, with up to half of the patients with Fabry disease developing ESRD by the time they reach their 50s. Only a few survive past the age of 60 years [6]. Figure 1 provides an extreme example of progressive loss of kidney function in a young male with Fabry disease.

Due to the non-specific nature of early signs of Fabry disease, diagnosis is often delayed—commonly for more than 15 years after the onset of the first clinical symptoms [7,8]. Also in CKD patients, even in patients undergoing haemodialysis, the diagnosis of Fabry nephropathy may be missed [9–12]. This is worrisome, particularly because Fabry disease now represents one of the forms of proteinuric CKD that can specifically be treated with enzyme replacement therapy (ERT) [13–18], in combination with ancillary treatments, if required [19]. Guideline recommendations for the diagnosis and comprehensive management



**Fig. 1.** Extreme example of progressive loss of kidney function in a young male with Fabry disease. The urine albumin/creatinine index averaged 25 mg/mmol (221 mg/g) until age 18 and 188 mg/mmol (1663 mg/g) thereafter (normal 3.8 mg/mmol; 33.6 mg/g). The systemic blood pressure averaged 114/76 until age 18, and 139/92 thereafter despite multiple anti-hypertensive agents. Agalsidase alfa was instituted at age 21.6 at 0.2 mg/kg every other week when the eGFR was 41 ml/min/1.72 m<sup>2</sup>. The patient was switched to agalsidase beta at 1 mg/kg every week at age 23 when the eGFR was 15 ml/min/1.73 m<sup>2</sup>. Kidney function steadily declined, attributable to late institution of enzyme replacement therapy and inadequate control of the blood pressure and proteinuria. The rate of decline of kidney function was  $-11.45 \pm 0.2$  ml/min/1.73 m<sup>2</sup>/year between the ages of 13.5 and 23.4 years.

of Fabry nephropathy, as proposed by the participants of the 2007 Fabry Nephropathy Round Table, are summarized in Table 1 [20].

Prompt diagnosis of individuals affected by Fabry disease among patients with CKD is the key as it provides the opportunity to intervene, attempting to halt further renal deterioration and to prevent late Fabry disease-related cardiac and cardiovascular complications. Moreover, affected relatives may be identified at a relatively early stage of the disease, before late complications and organ damage have emerged, by means of active family screening [21,22].

**Table 1.** Recommendations for the management of Fabry nephropathy (adapted, with permission from Ortiz *et al.* [20])

Diagnosis and assessments
Confirm diagnosis of Fabry disease
Initial assessment of Fabry nephropathy
GFR < 90 ml/min/1.73 m <sup>2</sup> (CKD stages 2–5)
Albuminuria > 30 mg/day or 30 mg/g creatinine
Proteinuria > 300 mg/day or > 300 mg/g creatinine
Kidney biopsy
Presence of histological injury may precede clinical signs and provides a compelling indication for institution of ERT
Excludes other conditions, especially in patients with atypical Fabry phenotype. A biopsy is needed for rigorous exclusion of other renal diseases
Biopsy confirms Fabry diagnosis and disease stage and can be used to assess the response to therapy
Follow-up assessments
Measure serum creatinine and use modification of diet in renal disease study equation to estimate GFR on a regular basis
Use isotopic methods if estimated GFR > 60 ml/min/1.73 m <sup>2</sup> and more precise measures of GFR are required
Follow standard CKD assessment schedule
Quantify urine albumin, protein and creatinine values at each visit
Calculate estimated GFR slope throughout the follow-up; goal is to maintain at ≤ 1 ml/min/1.73 m <sup>2</sup> /year
Comprehensive treatment
Enzyme replacement therapy
Agalsidase alfa or beta (approved dose regimens only)
Initiate ERT as soon as a definite diagnosis is made in patients with no residual alpha-Gal A enzyme activity
Initiate ERT as soon as definite diagnosis is made in patients with residual alpha-Gal A activity if there is evidence of kidney involvement
ERT will not reduce proteinuria therefore additional measure required
Control of proteinuria
Use angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers in addition to ERT
Titrate doses to achieve urine protein < 500 mg/day, even if blood pressure is < 130/80 mmHg
Positive effects on progression are only likely to occur if dosing and timing of ERT are optimal
Other therapy
Initiate all applicable aspects of standard CKD care in the management of Fabry nephropathy (lipid reduction, smoking cessation, 2.4 g/day sodium diet, etc.)

alpha-Gal A, alpha-galactosidase A; CKD, chronic kidney disease; ERT, enzyme replacement therapy; GFR, glomerular filtration rate.

This review evaluates experiences of patients with Fabry nephropathy highlighting the necessity of early identification of individuals with Fabry disease. Issues in relation to early diagnosis and testing of high-risk populations are discussed. The review is based on the discussion of international expert researchers and clinicians during the 8th International Fabry Renal Expert Meeting held in Stockholm, Sweden, in May 2008, and on published clinical evidence identified in a PubMed literature search of the period 2000–08.

## Enzyme replacement therapy for Fabry nephropathy

Until recently, the management of nephropathy in patients with Fabry disease was restricted to supportive interventions (e.g. dialysis, antiproteinuric and antihypertensive

treatment) aimed at alleviating symptoms caused by organ damage [1]. However, in 2001 specific therapy that could correct the metabolic deficiency in Fabry disease became available, i.e. agalsidase beta (Fabrazyme<sup>®</sup>, Genzyme Corp., Cambridge, MA, USA) and agalsidase alfa (Replagal<sup>®</sup>, Shire Human Genetic Therapies, Boston, MA, USA) [16,23]. These recombinant enzyme preparations have been approved in Europe and many other countries (agalsidase alfa is not approved in the USA) although at a different intravenous dose: 1.0 mg/kg every other week (EOW) for agalsidase beta and 0.2 mg/kg EOW for agalsidase alfa. Both products have comparable amino acid compositions and *in vitro* pharmacodynamics [24]. Evidence of therapeutic efficacy of both forms of ERT has recently been thoroughly reviewed, including the issues of dosing of ERT, immunogenicity of ERT and generation of anti-agalsidase IgG antibodies and the use of adjunctive angiotensin-converting enzyme (ACE) inhibitors and/or angiotensin receptor blocker (ARB) therapy to control overt proteinuria to a target of 500 mg/day, thereby slowing the rate of renal function decline [25].

The importance of diagnosing patients with Fabry disease as early as possible is highlighted by the disease stage-dependent renal outcomes observed during the placebo-controlled studies with ERT and multiple years of open-label treatment. In patients with early Fabry nephropathy, ERT stabilized glomerular filtration rate [14,17].

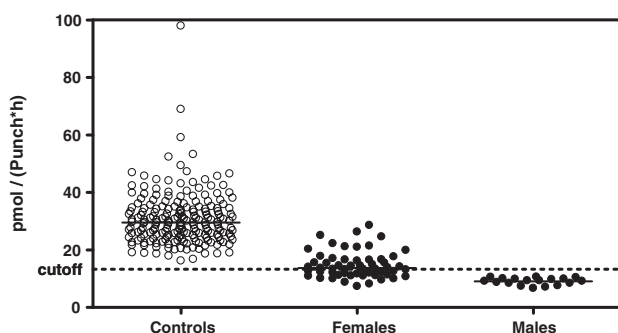
## Laboratory assays for diagnosing Fabry disease

Several laboratory assays have been developed to test for Fabry disease, including the alpha-Gal A enzyme activity in leukocytes, plasma and dried blood spots (DBS), molecular genetic analysis and GL-3 accumulation in urine and plasma.

### Diagnostic alpha-Gal A enzyme assays

Measuring the alpha-Gal A enzyme activity in leukocytes using the fluorogenic substrate 4-methylumbelliferyl- $\alpha$ -D-glucopyranoside (4-MUGal) has become the gold standard enzyme assay for diagnosing Fabry disease in males [5,26]. Most affected males can also be readily diagnosed by determining the alpha-Gal A activity in plasma [27]. However, the use of plasma as a sample in the alpha-Gal A enzyme activity assay has been shown to be associated with a higher rate of false-negative test results, which is particularly excessive in females [5,26]. The leukocyte enzyme assay can also be used to rule out an alpha-Gal A pseudo-deficiency in plasma, which may result from neutral pH-sensitive mutations. An example of the latter is the p.D313Y coding sequence variant [28,29], whose pathogenic role is still controversial [28–32].

Alternatively, males can be diagnosed using molecular genetic analysis to identify a mutation in the *GLA* gene. Many female heterozygotes have a normal alpha-Gal A enzyme activity in leukocytes and plasma (Figure 2) and the diagnosis requires molecular genetic analysis. Several groups have attempted to improve the detection of female patients and it has been suggested that the ratio of alpha-Gal



**Fig. 2.** The activity of alpha-galactosidase A in dried blood spot samples from 300 controls and 55 female and 18 male Fabry disease patients (Genzyme Corp., data on file).

A enzyme activity to beta-glucuronidase activity [33,34] could improve the differentiation of female heterozygotes from normal controls. Others have suggested measuring the secondary elevation of other lysosomal proteins, including glucuronidase and saposin C [34,35]. A prospective study is needed to determine the clinical utility of these methods.

#### *Molecular genetic analysis*

The identification of a pathologic GLA mutation confirms the diagnosis of Fabry disease [5,21,36] and facilitates the diagnosis of family members, irrespective of gender, age and symptoms (also refer to the section 'Pedigree analysis') [37]. Mutation analysis can also be used for prenatal diagnosis.

A mutation in the *GLA* gene is found in almost all patients with Fabry disease. About 75% of affected families have point mutations, including missense (>50%), nonsense and splicing mutations [37]. The remaining patients have partial gene deletions and insertions. A partial gene duplication containing exons 2 through 6 has also been reported [38]. Deletions and duplications may be missed on routine mutational diagnosis by genomic DNA sequencing, particularly in heterozygotes. Current technologies to detect deletion/duplication mutations include quantitative polymerase chain reaction (PCR), real-time PCR, multiplex amplifiable probe hybridization, multiplex ligation-dependent probe amplification and array comparative genome hybridization.

### **Laboratory methods for screening Fabry disease**

#### *Measuring the alpha-Gal A enzyme activity in DBS*

The use of leukocyte and plasma samples to measure alpha-Gal A enzyme activity has disadvantages for screening; it requires relatively large volumes of blood, subsequent laboratory processing and special storage, shipping and handling. Alpha-Gal A enzyme activity assays using DBS samples have been developed for high-throughput screening [39]. DBS samples are easy to obtain using a heel stick in infants and finger prick in children and adults. DBS can be prepared by spotting blood collected in an EDTA tube on filter paper. Samples can be sent to specialty labs for analysis by mail at room temperature. DBS stored at  $-20^{\circ}\text{C}$

for 8 months and stored at room temperature for 2 weeks showed no decrease in the alpha-Gal A enzyme activity or protein concentration [35]. Leukocyte alpha-Gal A enzyme activity assays can be used to confirm positive results obtained from DBS assays and to check negative results in patients with a clinical suspicion of Fabry disease [26]. The accuracy of alpha-Gal A enzyme activity assays using DBS was comparable with leukocyte assays [33], which suggests that DBS may be a useful diagnostic assay. The introduction of quality control and quality assurance programmes for alpha-Gal A enzyme activity assays in DBS will facilitate the assessment of the diagnostic utility of DBS samples [40]. Measuring the activity of a second lysosomal enzyme (for example, beta galactosidase) should be done in large screenings to control for sample quality [41].

#### *Multiplex enzyme activity assays*

A multiplex assay using tandem mass spectrometry is developed to measure the enzyme activities deficient in Pompe, Krabbe, Gaucher, Niemann-Pick type A/B and Fabry disease [42–44]. This assay appears to be particularly useful for newborn screening, as it allows simultaneous testing for multiple diseases from a single sample, with a high throughput and low cost. However, it has no advantage over alpha-Gal A fluorescence assays when testing for a single disease, such as Fabry disease. The Centers for Disease Control and Prevention, USA, are currently involved in setting up a quality control for this assay.

#### *GL-3 assays*

Methods to measure GL-3 accumulation using tandem mass spectrometry were developed for urine and plasma [45–47]. Plasma and urine levels of GL-3 in hemizygotes with 'classic' Fabry disease (characterized by little or no detectable alpha-Gal A enzyme activity) were clearly elevated over levels in normal controls. 'Cardiac variant' Fabry disease patients with the *N215S* mutation did not have elevated GL-3 in plasma or urine [46]. The mean GL-3 level in Fabry disease urine samples had a greater elevation over normal than that in Fabry disease plasma samples (66-fold and 2.6-fold over normal, respectively) [46]. However, there is a significant overlap of urine GL-3 levels in females with Fabry disease and normal controls. The method to measure GL-3 in urine has been adapted for use in urine dried on a filter paper [48]. Urine samples dried on filter paper are much easier to store and ship than liquid specimens and make the method attractive for Fabry disease screening initiatives.

#### *Combined measurement of urinary GL-3 and alpha-Gal A protein*

A recent study described improved sensitivity for detecting heterozygotes by measuring both urinary alpha-Gal A protein using ELISA and urinary GL-3 with tandem mass spectrometry (Table 2) [49]. Both methods could be adapted for high-throughput screening. The utility of this combined approach needs to be confirmed in a prospective study.

**Table 2.** Reliability of methods to identify Fabry disease hemizygotes and heterozygotes among patients at high risk for Fabry disease (data from Kitagawa *et al.* [49])

Measurements	Sensitivity (%)		Specificity (%)	
	Hemizygotes	Heterozygotes	Hemizygotes	Heterozygotes
Alpha-Gal A in urine	97.5	73.0	94.1	94.1
GL-3 in urine	87.5	96.7	96.4	96.4
Alpha-Gal A + GL-3 in urine	97.5	97.3	90.5	90.5

## Testing high-risk populations for Fabry disease

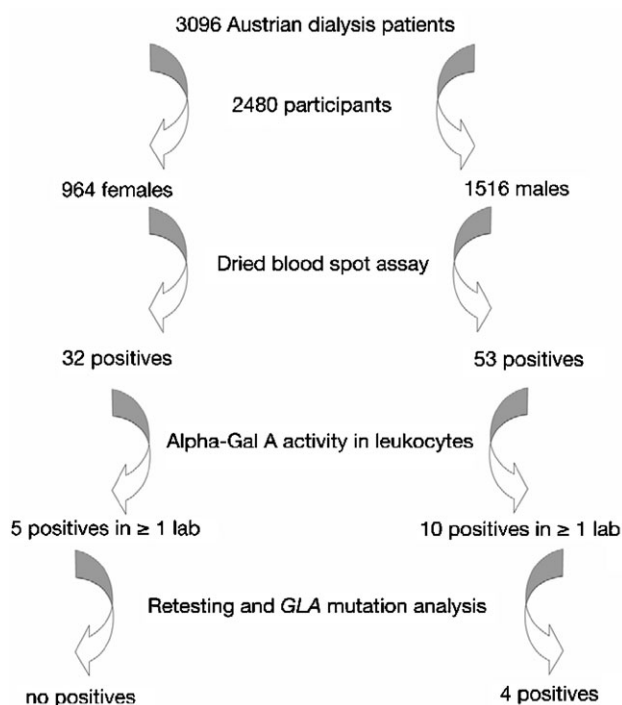
### Patients undergoing dialysis

Nationwide testing programmes for dialysis patients have been carried out in Austria [11] and the Czech Republic [12]. In Austria, 2480 patients (80% of the haemodialysis population) were tested using a sequential approach involving DBS alpha-Gal A activity assays, confirmation of alpha-Gal A enzyme activity levels in leukocytes and mutation analysis (Figure 3) [11]. In this study, one patient was diagnosed with Fabry disease; the three previously diagnosed Fabry disease patients included as controls were identified. The prevalence of 0.16% in the Austrian haemodialysis population is 10-fold higher than that previously reported [50,51]. A similar approach in the Czech Republic resulted in the diagnosis of five Fabry disease patients (prevalence of 0.15%) [12]. Mutation analysis was used to diagnose 10 family members in the study. Other smaller scale testing programmes among patients undergoing haemodialysis have reported prevalence ranges from 0 to 1.2% [9,10,26,52–55].

The mean alpha-Gal A enzyme activity measured in DBS and leukocytes is 40–50% lower in haemodialysis patients than in normal controls regardless of the presence of Fabry disease [41]. When the alpha-Gal A enzyme activity assay is used to detect Fabry disease among patients with ESRD, the rate of false positive results will most likely be increased unless a haemodialysis reference range is used. To minimize the number of haemodialysis patient samples needing confirmatory analysis, Fabry disease testing could focus on patients with ESRD of unknown cause [11]. Because a percentage of women with Fabry disease will have a normal activity, all reports should contain the phrase that ‘although deficient alpha-Gal A enzyme activity is diagnostic for Fabry disease in women, normal alpha-Gal A enzyme activity does not exclude that diagnosis of Fabry disease in women’. Women may be excluded from some testing programmes [11,52] and consequently the affected families will not be found.

### Other high-risk testing opportunities

A multidisciplinary approach should be adopted in the evaluation of Fabry disease high-risk populations, as the disease is multisystemic and there are a range of symptoms which can indicate the presence of the condition. Key symptoms include neuropathic pain, a distinct whorled ocular opacity detectable via slit lamp examination (‘cornea verticillata’), premature cerebrovascular disease, left ventricular hyper-

**Fig. 3.** Results of a nationwide programme for the testing of dialysis patients in Austria [11].

trophy, hypohidrosis and angiokeratomas of the skin. Testing programmes by ophthalmologists have been successful in identifying patients with Fabry disease [56,57]. Testing programmes for high-risk patients should also involve neurologists [58,59], cardiologists [32,60–62], rheumatologists [63] and other specialties.

### Testing and diagnosis of Fabry disease in patients with CKD

#### (a) Criteria for testing patients with CKD:

- Fabry disease nephropathy should be considered in the differential diagnosis of any patient presenting with CKD, especially in those with proteinuria. Peripelvic kidney cysts are also suggestive of Fabry disease [64].
- In such patients, other clinical manifestations of Fabry disease should be specifically sought as diagnostic clues.

- In patients undergoing kidney biopsy, the diagnosis of Fabry disease should be kept in mind to avoid overlooking the histopathological evidence.
- (b) Criteria for testing patients with ESRD or renal transplants:
- Specific diagnostic tests—alpha-Gal A assay for males, genotyping for both sexes—should be used for any patient with possible manifestations of Fabry disease, particularly angiokeratomas, acroparesthesias, hypohidrosis, left ventricular hypertrophy, arrhythmias, and evidence of premature cerebrovascular disease.
- (c) Criteria for the systematic testing of patients with ESRD or renal transplants:
- Men only (women cannot be reliably tested with current non-molecular diagnostic assays).
  - Testing should be focused on patients with ESRD of unknown cause or with a diagnosis of chronic glomerulonephritis.  
A clinical diagnosis of nephropathy associated with diabetes mellitus type 2 should not be used as exclusion criteria for testing.
  - Patients with left ventricular hypertrophy and relatively normal blood pressure should be tested.

### *Pedigree analysis*

As recommended by the National Society of Genetic Counselors in the USA and others, pedigree analysis in families of Fabry disease patients should be used to identify relatives that need Fabry disease diagnostic testing [12,22,65,66]. Laney *et al.* found that, on average, for every index case of Fabry disease diagnosed, five family members with Fabry disease were identified through family history [22].

Supportive tools, such as the 'Fabry Indicator<sup>®</sup>', software (distributed without charge by Genzyme Corp.) or the 'Fabry Pedigree Facilitator' (distributed without charge by TKT Europe—5S AB), have been developed for nephrologists and other healthcare professionals to aid in pedigree analysis that should involve the following steps [22].

Step 1: A detailed family history should be collected and a pedigree constructed focusing on the X-linked inheritance patterns of Fabry disease [66]; a minimum of three generations should be included.

Step 2: The patient initially identified with Fabry disease (the 'proband') should be encouraged to provide details of any possible Fabry-related symptoms expressed in their family in order to help to identify other at-risk members. The proband can also provide invaluable help in contacting family members, some of whom may otherwise be difficult to trace.

Step 3: Contacting family members at risk, as well as screening asymptomatic individuals, should be done in compliance with the regulations set out by the Health Insurance Portability Accountability Act (HIPAA) [22] and any applicable legal requirements.

Step 4: Once related individuals are evaluated and definitively diagnosed, a monitoring and treatment schedule can be implemented. Asymptomatic women with a Fabry disease mutation should be recalled for full clinical evaluations every 1–5 years depending on their age and clinical situation.

### *Family tree work-up*

- Support services from geneticists and genetic counsellors should be made available to the nephrologist in order to carry out a comprehensive pedigree analysis.

### *Newborn screening (NBS)*

Screening newborns within a few days of birth for a panel of treatable diseases is internationally recognized as an important public health programme. The development of DBS enzyme activity assays makes NBS for Fabry disease and other lysosomal storage disorders technically feasible.

NBS has the potential to identify Fabry disease patients early, before irreversible tissue and organ damage occur and therefore allowing timely therapeutic intervention [44]. This will improve the health outcome of the patient and avoid the burden of a lengthy and costly diagnostic process. NBS also permits families to make informed reproductive choices.

A Fabry disease NBS pilot programme in Italy [67] screened 37 104 consecutive male newborns infants for alpha-Gal A enzyme activity in DBS. Infants who were deficient in alpha-Gal A enzyme activity were retested and those with a 'double-positive' screening result had a diagnosis of Fabry disease confirmed using mutation analysis. Pedigree analysis of affected infants identified undiagnosed cases of Fabry disease. The incidence of positive screens in this study was 1 in 3100 and 1 in 4600 when only previously known disease-causing mutations were considered. Of the 12 mutations, only 1 was a previously described mutation associated with the 'classical' phenotype, and the other 11 were mutations with residual enzyme activity, which raises the possibility of clinical presentation later in life [68]. These results confirm the feasibility of NBS for Fabry disease and support the importance of pedigree analysis in conjunction with NBS programmes. NBS may result in the identification of a broader clinical spectrum of Fabry disease.

### **Kidney biopsy**

A renal biopsy is recommended in Fabry patients with *GLA* mutations associated with a high residual enzyme activity if they have reduced GFR, microalbuminuria or overt proteinuria. A kidney biopsy of a patient with Fabry disease will show typical deposits, if an appropriate fixative (e.g. glutaraldehyde) for light microscopy and electron microscopy is applied. A diagnosis of Fabry disease may be missed in incidental biopsies if only routine light microscopy is done. In clinical renal biopsy practice, it is observed that in all patients with Fabry disease, glomeruli will have a striking

white colour under illumination in a stereomicroscope as a result of lipid-laden podocytes [69–71] in contrast to the usual red colour of the glomeruli. This observation can be made as early as in childhood or adolescence; even in children with minimal albuminuria [70]. White glomeruli observed during bedside stereomicroscopic inspection of the biopsy material in patients with renal disease of unknown cause should immediately raise the suspicion of Fabry disease [71]. It needs to be borne in mind that patterns of deposits in renal biopsies similar to those of Fabry disease can be caused by silicosis [72] or can be drug induced; cases have been described of iatrogenic renal phospholipidosis caused by amiodarone, chloroquine and hydroxychloroquine [73].

## Conclusions

Nephropathy due to Fabry disease is now a treatable form of CKD with the availability of ERT and control of overt proteinuria with ACE inhibitors or ARB therapy. However, to be maximally effective, ERT must be initiated early in the disease course, before irreversible damage occurs. It is, therefore, important to develop accurate diagnostic assays and testing programmes for identifying individuals with Fabry disease. Biochemical and enzyme assays for Fabry disease should be standardized, and diagnostic tests that can reliably identify affected women should be developed. Testing high-risk patient populations can lead to the identification of previously undiagnosed patients with Fabry disease. Identification of other at-risk family members can be optimized through genetic analysis, particularly once the alpha-Gal A gene mutation is defined in the proband. Inclusion of Fabry disease screening as part of the genetic screening of newborns has been proposed as a method of identifying all families affected by the disease.

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