Fabry or not Fabry: From genetics to diagnosis
van der Tol, L.

Citation for published version (APA):
van der Tol, L. (2015). Fabry or not Fabry: From genetics to diagnosis

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
CHAPTER 4

Chronic kidney disease and an uncertain diagnosis of Fabry disease: approach to a correct diagnosis

Linda van der Tol ¹, Einar Svarstad ², Alberto Ortiz ³, Camilla Tøndel ⁴, João Paulo Oliveira ⁵, Liffert Vogt ⁶, Stephen Waldek ⁷, Derralynn A. Hughes ⁸, Robin H. Lachmann ⁹, Wim Terryn ¹⁰, Carla E. Hollak ¹, Sandrine Florquin ¹¹, Marius A. van den Bergh Weerman ¹¹, Christoph Wanner ¹², Michael L. West ¹³, Marieke Biegstraaten ¹, Gabor E. Linthorst ¹

¹. Department of Endocrinology and Metabolism, Academic Medical Center, Amsterdam Lysosome Center ‘Sphinx’, Amsterdam, Netherlands ². Department of Clinical Medicine, University of Bergen and Department of Medicine, Haukeland University Hospital, Bergen, Norway, ³. Unidad de Dialisis, IIS-Fundacion Jimenez Diaz/UAM, IRSIN, Madrid, Spain, ⁴. Department of Pediatrics, Haukeland University Hospital, Bergen, Norway, ⁵. Medical Genetics, Hospital São João, Faculty of Medicine of University of Porto, Porto, Portugal, ⁶. Department of Pediatrics, Haukeland University Hospital, Bergen, Norway, ⁷. Medical Genetics, Hospital São João, Faculty of Medicine of University of Porto, Porto, Portugal, ⁸. Department of Pathology, Academic Medical Center, Amsterdam, Netherlands, ⁹. Independent Medical Consultant, Manchester, UK, ¹⁰. Department of Haematology, Royal Free London NHS Foundation Trust, & University College London, UK, ¹¹. Charles Dent Metabolic Unit, National Hospital for Neurology and Neurosurgery, London, UK, ¹². Department of Internal Medicine, Division of Nephrology, Ghent University Hospital, Ghent, Belgium, ¹³. Department of Pathology, Academic Medical Center, Amsterdam, Netherlands, ¹². Department of Medicine, Division of Nephrology, University of Würzburg, Würzburg, Germany, ¹³. Department of Nephrology, Dalhousie University, Halifax, Nova Scotia, Canada

Molecular Genetics and Metabolism 2015; 114(2):242-247
ABSTRACT

Background and objectives
Screening for Fabry disease (FD), an X-linked lysosomal storage disorder, reveals a significant number of individuals with a genetic variant of unknown significance without classical FD manifestations; these variants in the α-galactosidase A gene often result in a high residual leukocyte α-galactosidase A and it is unclear whether these individuals suffer from FD. Therefore, a structured diagnostic approach is warranted. We present a diagnostic algorithm on how to approach adults with chronic kidney disease and an uncertain diagnosis of FD nephropathy.

Design, setting, participants, and measurements
A modified Delphi procedure was conducted to reach consensus among 11 FD experts. A systematic review was performed to identify possible criteria that could confirm or exclude FD nephropathy.

Results
The gold standard for FD nephropathy was defined as characteristic storage on electron microscopy (EM) in a kidney biopsy, in the absence of medication that may induce similar storage. The suggested criteria to confirm FD nephropathy: ‘renal cysts’, ‘Maltese cross sign’, ‘immunohistochemical staining of Gb3 in urine’ and ‘high urinary Gb3’; and to exclude FD nephropathy: ‘absence of renal cysts’, ‘small kidneys’ and ‘high protein excretion’, were rejected because of low or uncertain specificity. Urinary Gb3 may be increased in other kidney diseases and there was no agreement on this criterion, although a third of the panel indicated that it is sufficient to diagnose FD nephropathy. The ‘Maltese cross sign’ and ‘high urinary Gb3’ were selected as red flags to suggest the possibility of FD nephropathy, but are not sufficient for a definite diagnosis of FD nephropathy.

Conclusions
In adults with chronic kidney disease, an α-galactosidase A gene variant and an uncertain diagnosis of FD, a kidney biopsy with EM analysis should be performed to confirm or reject the diagnosis of FD nephropathy. Other criteria currently cannot substitute for a biopsy in these cases.
INTRODUCTION

Fabry disease (McKusick 301500; FD) is an X-linked, lysosomal storage disorder caused by deficient activity of α-galactosidase A (αGalA). The estimated birth prevalence has been reported to be between 1:40,000-170,000 for males. More than 670, mostly private, mutations/variants in the α-Galactosidase A (GLA) gene have been described. Classical FD is characterized by angiokeratoma, neuropathic pain, cornea verticillata, an- or hypohidrosis, and in males by absent or near absent αGalA activity and very high globotriaosylceramide (Gb3) and lysoGb3 in plasma and urine. The kidneys, heart, and central nervous system are often affected. Females can also be affected although, in general, organ involvement is less severe.

Since the availability of enzyme replacement therapy (ERT) with recombinant AGAL-A (agalsidase alpha, Shire HGT and agalsidase beta, Genzyme, a Sanofi company) an increasing number of studies have been performed in which high risk populations (i.e. with a nonspecific symptom such as chronic kidney disease or left ventricular hypertrophy) as well as newborns are screened for FD, for a review see. These screening studies as well as individual case finding revealed a higher than expected number of individuals with a GLA gene variant (defined as any alteration of the wild type GLA gene, irrespective of its suspected pathogenicity) and/or a deficiency of the αGalA enzyme. Approximately 80% of these identified individuals with a GLA gene variant are lacking characteristic FD features such as cornea verticillata. Males who are identified by screening most often have a higher residual αGalA activity and normal or slightly elevated (lyso)Gb3 levels than classically affected males. While the pathogenicity of some variants is well described, this is not the case for most of the GLA variants identified by screening. Thus, individuals often have an uncertain diagnosis of FD in the presence of a genetic variant of unknown significance (GVUS). For example, Terryn et al. showed that subjects identified by screening with the GLA variant p.A143T had no characteristic FD features and kidney biopsies of three individuals showed no Gb3 storage, while in classically affected males, kidney Gb3 deposits are already present during childhood. In Taiwan the splice site variant IVS4-919G>A has a high prevalence of 1 in 1300 newborns, but adults harbouring this variant did not demonstrate a classical FD phenotype. Yet, characteristic storage is reported in some tissue biopsies, but there is to date no systematic evaluation of structural changes in these individuals. Similar clinico-pathological variability has been reported for other GLA variants: e.g. N215S, R112H.

In the wake of our growing understanding of the spectrum of phenotypes associated with GLA variants, difficult dilemmas have emerged as these individuals may be misdiagnosed with FD. Misdiagnosis of FD can cause distress and inappropriate initiation of costly ERT. Often, individuals with a GLA variant of unknown significance are simply diagnosed as FD patients and treated as such without further consideration of other contributing (cardiovascular) risk factors and the pathogenicity of the GLA variant. Increased awareness for FD will inevitably lead to an increasing number of such cases. In contrast, early diagnosis of a true FD patient is of equal importance, since this will lead to adequate support and family counseling.

To address these diagnostic dilemmas, we initiated ‘The Hamlet study: Fabry or not Fabry’ to valorize clinical and laboratory assessments in order to improve the diagnosis of FD.
trial register [www.trialregister.nl] NTR3840 and NTR3841]. As part of this study, international consensus was reached among experts on criteria for a definite diagnosis of FD \(^{14}\). For individuals with an uncertain diagnosis of FD, organ-specific diagnostic algorithms were developed.

Here we report on a diagnostic algorithm based on the currently best available evidence regarding adults presenting with chronic kidney disease (CKD) and a variant in the GLA gene with an uncertain diagnosis of FD nephropathy. This algorithm was constructed by a panel of nephrologist and international experts on FD.

**MATERIALS AND METHODS**

**Panel and Delphi procedure**
Experts on FD were invited to participate in the study panel via email. A consensus document was presented to the experts with the rationale of the study and the adopted results from the previous consensus study on general diagnostic criteria for FD, chapter 3, table 2 \(^{14}\). The results of the literature study and the preselected items were described in detail.

The modified Delphi procedure consisted of online survey rounds, and a telephonic survey round (by investigator LT) to address outstanding arguments. The panelists were asked to criticize the proposed criteria on a five-point Likert scale, and were invited to add comments and suggestions for additional criteria. Additional analyses were performed in the Dutch FD cohort to gather additional data on features that the expert panel deemed possible entry or exit criteria.

Anonymized results were presented to the panel (absolute scores and comments) after every round and the online survey was adapted following these results. Clarification and additional data were provided. Rounds were repeated until consensus was reached or if consensus was deemed not feasible.

**Systematic review and pre-selection of voting items**
PubMed and Embase were searched for studies involving adults in peer reviewed journals that investigated kidney specific features in FD patients and/or healthy or diseased controls. Clinical trials as well as studies that investigated kidney specific features as the main subject were included. The prevalence of kidney specific features in FD patients and control groups was calculated and specified by gender. Sensitivity and specificity were calculated wherever possible. Unfortunately, data to calculate specificity were often absent, since most studies did not include a control group with non-FD kidney diseases. As a surrogate, specificity was calculated based on prevalence in healthy controls. Candidate criteria to exclude (exit criteria) or confirm (entry criteria) the diagnosis of FD nephropathy were selected by the study team (LT, MB, GEL). We aimed to select features that were compared to other kidney diseases with a high specificity of N90%. Because the number of criteria that fit this requirement was very limited, all studies were reported to the experts to assess the possible diagnostic criteria. The candidate items that were initially selected as possible entry criteria were subsequently selected as candidate criteria to serve as red flags. A red flag indicates that the presence of a feature makes a diagnosis of
FD nephropathy more likely, but it is not sufficient for a definite diagnosis of FD nephropathy by itself. Details on the literature search and criteria are presented in the online supplement.

**Statistical considerations**
As customary for a Delphi procedure, criteria were accepted if ≥75% (n ≥9/11 panelists) of the panelists agreed, and no one disagreed. Neutral votes were accepted if the other conditions were met. Data are presented as absolute numbers, or median and range. Cronbach's α was calculated to assess consensus among experts on a scale of 0-1, where 1 indicated full consensus.

### RESULTS

**Adopted results**
The panel re-emphasized the results of a previous consensus study on general diagnostic criteria for FD. These criteria include biochemical data (residual leukocyte enzyme activity of αGalA, plasma Gb3 and lysoGb3) as well as clinical data, see chapter 3, table 2.

**Systematic Literature search and pre-selection of voting items**
The literature search revealed 25 articles that studied kidney specific features in FD patients of which 4 also studied a control group with non-Fabry kidney diseases. Four features were selected as possible criteria to confirm or exclude FD nephropathy for survey round one (table 1). Details on the literature search are presented in the online supplement.

| Table 1. Possible entry and exit criteria that were rejected by the panel. |
|---|---|---|
| **Assessment** | **Entry criteria** | **Exit criteria** |
| Literature | Kidney Ultrasound/MRI/CT | Renal cysts |
| Urine light microscopy | Absence of renal cysts |
| Immunohistochemical staining of Gb3 in urine |
| Maltese cross sign in urine |
| Suggested by panel | Urine Mass spectroscopy | High urinary Gb3 (in range of classical males) |
| Kidney Ultrasound/MRI/CT | Small kidneys |
| High proteinuria * |

* Because the panel decided ‘High proteinuria’ is not a valid a criterion for diagnostic purpose, no cutoff value was defined.
Panel
Thirteen FD experts (8 nephrologists and 5 general FD experts) were invited to participate. Two experts supported the initiative, but were unable to participate due to time constraints. The panel consisted of three internists (CH, DH, RL) and eight nephrologists (AO, JO, ES, CT (pediatric nephrologist), WT, CW, MW, SW). All panelists participated in all rounds.

Delphi procedure and diagnostic criteria
Rounds 1 and 2 were online questionnaires. Because some issues required further clarification, the questionnaire of the second round was repeated in an individual telephonic round (round 3). Overall consensus, measured by Cronbach’s $\alpha$, increased from 0.85 in round 1 to 0.97 in round 3, indicating excellent consensus. The panel fully agreed that the current diagnostic algorithm is developed to aid in the diagnosis for individuals with CKD in whom a GLA variant was already found, who do not fit the criteria for a definite diagnosis of classical FD, see chapter 3, table 2. Thus, these individuals have an uncertain diagnosis of FD.

Initially, criteria for CKD were discussed according to the level of proteinuria and estimated GFR. Based upon feedback from the expert panel, the 2012 ‘Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease’ by the KDIGO working group was adopted to define CKD.

Full agreement was reached on the current gold standard, defined as concentric multi-lamellated myelin bodies with a zebra like pattern (zebra bodies) with a periodicity of approximately 5 nm on electron microscopy (EM), in a kidney biopsy, in the absence of medication use that may induce similar storage. The biopsy should be assessed by an expert team on FD pathology. It was emphasized that in case of an uncertain diagnosis, a kidney biopsy should always be considered as it is currently the only assessment that can confirm or exclude the diagnosis in individuals with an uncertain diagnosis of FD nephropathy and CKD.

Table 1 lists the preselected diagnostic criteria from the literature in round 1. The panel commented that renal cysts have a high prevalence, ranging from 5 to 41% in the general population. It was suggested that the presence of medullary cysts may imply the presence of congenital kidney disease, while Ries et al. reported a 50% prevalence of parapelvic cysts among 24 FD patients. Because of the non-specific nature and limited data, renal cysts were rejected by all experts as a diagnostic criterion.

The ‘Maltese cross sign’ and ‘immunohistochemical staining of Gb3 in urine’ may be helpful as diagnostic assessments, but results may be difficult to interpret. The differentiation between different Maltese cross types is challenging and the Maltese cross sign may also occur in patients with other causes for high urinary lipid content. The ‘Maltese Cross sign’ criterion was rejected by 10/11 experts, 1 voted neither agree nor disagree. Staining urine sediment immunohistochemically for Gb3 is hampered by the natural presence of Gb3 in several cell-types and limited data are available on urinary Gb3 in other, non FD, kidney diseases. Following discussion, 9/11 experts rejected ‘immunohistochemical staining of Gb3 in urine’ as a criterion, 1 agreed and 1 voted neither agree nor disagree.
Three additional criteria were suggested (table 1). First, ‘urinary Gb3 (in the range of classical males)’, measured in whole urine, was suggested to confirm FD nephropathy. Others suggested that urinary and/or plasma Gb3 levels may be significantly increased in other diseases than FD. Consequently, there was no agreement, although 4/11 of panelists were convinced that this criterion, in the context of chronic kidney disease and a GLA variant, is sufficient to confirm FD nephropathy. The remaining 7 panelists rejected this criterion.

Second, it was postulated that small kidneys could possibly exclude FD nephropathy. It was discussed that in all causes for CKD, including FD, small kidneys occur at end stage disease. The most frequent cause of small kidneys is hypertension, which may also coexist with FD, although in the experience of the experts classical FD patients often have normal or low blood pressure in early phases of the disease. All panelists rejected ‘small kidneys’ as an exit criterion in the absence of hypertension, while 3/11 experts agreed that in the presence of hypertension, small kidneys exclude FD nephropathy. As a result, the ‘small kidneys’ criterion was not included in the diagnostic algorithm. Finally, ‘high level of proteinuria’ was suggested to be rarely found in FD patients and to serve as a possible exit criterion. Because this feature was not studied in the literature, we assessed the maximum 24 hour protein excretion in 102 (39 males) Dutch FD patients with a definite diagnosis of classical FD, in accordance with previously defined criteria (chapter 3, table 2). Median total protein excretion was 0.31 g/24 h (range 0.07–4.8), 0.62 g/24 h in males (0.13–4.39) and 0.24 g/24 h (0.07–4.80) in females. Few patients had proteinuria in the nephrotic range (>3 g/24 h) (see figure 1 and table 2). An applicable cutoff value was debated and different opinions were shared by the experts. Most experts reported that some FD patients can have very high proteinuria. Proteinuria can also be influenced by concomitant diseases, e.g. hypertension. Because of limited data and absence of a cutoff value, this exit criterion was rejected by most experts (8/11). One voted neither agree nor disagree and 2 experts agreed that high proteinuria could serve as an exit criterion. The ‘Maltese cross sign’ and ‘high urinary Gb3’ were depicted as red flags, indicating that the presence of these features raises the suspicion of FD nephropathy, but further assessments are mandatory. It was deemed not necessary to perform these 2 assessments in all individuals with an uncertain diagnosis of FD nephropathy.

Ultimately, the diagnostic algorithm was constructed (figure 2).

Table 2. Specificity of proteinuria as an exit criterion in FD patients with a definite diagnosis of FD.

<table>
<thead>
<tr>
<th>Cut off value proteinuria g/24h</th>
<th>All, n=102</th>
<th>Male, n=39</th>
<th>Female, n=63</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specificity (n)</td>
<td>Specificity (n)</td>
<td>Specificity (n)</td>
</tr>
<tr>
<td>&gt; 3.0</td>
<td>94% (6)</td>
<td>90% (4)</td>
<td>97% (2)</td>
</tr>
<tr>
<td>&gt; 3.5</td>
<td>96% (4)</td>
<td>95% (2)</td>
<td>97% (2)</td>
</tr>
<tr>
<td>&gt; 4.0</td>
<td>98% (2)</td>
<td>97% (1)</td>
<td>98% (1)</td>
</tr>
</tbody>
</table>
DISCUSSION

An international panel of experts agreed on the diagnostic approach for individuals with CKD and an uncertain diagnosis of FD nephropathy. Group consensus was excellent, represented by a Cronbach’s \( \alpha \) of 0.97. The experts indicated that a biopsy of the kidney with EM assessment is currently the only available tool that can reliably confirm or exclude FD nephropathy and should be considered in all patients without a classical pattern of disease manifestations, a GLA variant and CKD who have an uncertain diagnosis of FD (predefined criteria, chapter 3, table 2 \(^1\)).

This group endorses the diagnostic value of a biopsy of an affected organ (i.e. heart, kidney) to assess a true diagnosis of FD, as proposed in a previous study on individuals with LVH and an uncertain diagnosis of FD \(^1\). However, use of medication that may induce similar storage at any time during the medical history should always be excluded. The importance of morphological evidence in cases where the diagnosis of FD remains uncertain was stressed by the panel. In clinical practice, a nephrologist may not always be involved in the care of FD patients and a kidney biopsy is not routinely performed. However, we strongly recommend that those individuals with
Consensus recommendation: chronic kidney disease

an uncertain diagnosis of FD nephropathy are assessed by a nephrologist and a kidney biopsy should be considered.

The storage pattern in FD, with characteristic lysosomal inclusions on EM, is specific for FD in the absence of medication (such as chloroquine and amiodarone) that may induce similar storage. It is crucial that the kidney pathology is assessed by an expert team including a pathologist and nephrologist. Although similar storage to that of FD has been reported in other lysosomal storage diseases, the clinical presentation is distinct from that of FD. Thus, the specificity is not disputed in the clinical context of symptoms that are compatible with FD. Furthermore, it is encouraged that the conclusion of the clinico-pathological examination should also state if the amount of storage is compatible with the renal involvement of that particular patient. A detailed scoring system of the amount and characteristics of Fabry-specific storage in various kidney cells has recently been developed. Because not all centers routinely perform EM on kidney biopsies, adequate routines and collaboration with specialized pathology departments are essential. The risks of a kidney biopsy are small when general contraindications are respected.

Figure 2. Diagnostic algorithm for individuals with an uncertain diagnosis of Fabry disease (FD). * In the absence of medication use that may induce similar storage.
and complications most often fully resolve. The indication and possible risks should be assessed carefully in each case. Clearly, the benefits of a correct diagnosis (either confirming or excluding FD nephropathy) are substantial. This study focused on diagnosis only, neither treatment indications nor the benefit of repeated renal biopsies to evaluate treatment for FD was discussed.

Our study was hampered by very limited and insufficient data on the prevalence of certain parameters in non-FD kidney diseases. Because of this, the specificity of most features could almost never be determined. Furthermore, expertise on some assessments is likely to be present only in few centers (e.g. staining of urinary cells for Gb3, interpretation of Maltese cross signs) and these assessments are therefore not widely applicable. The limited data emphasize the need to investigate the diagnostic accuracy of parameters in FD nephropathy and non-FD kidney disease in more detail, especially those tests that are minimally invasive.

The panel extensively discussed the value of increased levels of urinary Gb3, which is historically seen as a hallmark of FD. Data from previous studies indicate that Gb3 may be increased in plasma and/or urine in other diseases as well. As early as in 1978, reports on urinary glycosphingolipids in the urine of patients without sphingolipidoses were published. The specificity of the diagnostic criteria that were part of this study and that (indirectly) assess Gb3 in urine (‘immunohistochemical staining of Gb3 in urine’, ‘Maltese cross sign in urine’ and ‘high urinary Gb3 (in range of classical males)’) was therefore debated by the panel. It is quite likely that urinary Gb3 levels in the high range as usually seen in classical males can differentiate between classical FD and other diseases. The deacylated form of Gb3, lysoGb3, may be of even more importance as it can clearly differentiate classical patients from non-classical FD patients.

The ‘Maltese cross sign’ and ‘high urinary Gb3 (in the range of classical males)’ were selected as red flags. In individuals with an uncertain diagnosis of FD, who are not suitable for a kidney biopsy, these features may be helpful, but not conclusive, to address the diagnostic dilemma.

The current study does not serve to advocate screening for FD in cohorts or individuals with CKD. It is solely intended to provide the best possible knowledge on the diagnostic process in an individual with CKD and a GVUS in the GLA gene. Screening of individuals to identify FD patients is advocated by some to be able to initiate early treatment. It is important to realize that the majority of individuals who are identified through screening do not present with the characteristic pattern of FD and possibly do not suffer from FD. This causes unnecessary burden for the individual and family members. Moreover, for individuals with a non-classical FD phenotype with biopsy proven characteristic storage in the kidney, the natural history is yet unclear, and the effect of ERT has not been studied independently. While ERT may delay complications in patients with classical disease, a careful risk/benefit consideration should be made on an individual basis before initiating ERT in non-classical FD. Ideally, this group should be further studied within a well-defined study protocol.
In conclusion, a kidney biopsy with EM analysis should be considered for all individuals with CKD, a variant in the GLA gene and an uncertain diagnosis of FD, as it is currently the only diagnostic procedure to confirm or reject the diagnosis of FD nephropathy. With this approach, the unnecessary burden of inappropriate diagnosis of FD, counseling and treatment with costly ERT can be avoided. Equally important, individuals with true FD can be identified to initiate appropriate individualized counseling, treatment and family screening.

**FUNDING AND ACKNOWLEDGEMENTS**

This study was performed within the framework of the Dutch Top Institute Pharma (TIPharma, project number T6-504: ‘Fabry or not Fabry: valorization of clinical and laboratory tools for improved diagnosis of Fabry disease’. TIPharma is a non-profit organization that catalyzes research by founding partnerships between academia and industry. Partners: Genzyme, a Sanofi company; Academic Medical Center, University of Amsterdam; Subsidizing Party: Shire HGT. http://www.tipharma.com/pharmaceutical-research-projects/drug-discovery-development-and-utilisation/hamlet-study.html. The industry partners had no role in the content of this manuscript, or selection of panel members. RHL and DH are is supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. AO is supported by Intensificacion and REDINREN 012/0021 of the Fondo de investigaciones sanitarias-ISCIII

We want to thank Ben JHM Poorthuis, for his valuable advice on the biochemical aspects of Fabry disease and Bouwien E. Smid for her contribution during the preparation of the consensus document.
REFERENCES


23. Selvarajah M, Nicholls K, Hewitson TD, Becker GJ. Targeted urine microscopy in Anderson-Fabry disease: a cheap, sensitive and specific
Consensus recommendation: chronic kidney disease


SUPPLEMENT

Literature search and pre-selection of items

PubMed and Embase (1980-april 2013) were searched with the search terms Fabry disease, kidney, urine, ultrasound, MRI, pathology, biopsy, (electron/polarized) microscopy and Maltese crosses, using synonyms and mesh terms or headings were added. English, German and French studies were selected based on title and abstract, references were cross checked. Studies in adults in peer reviewed journals that investigated kidney specific features in Fabry disease (FD) patients and/or healthy or diseased controls were included. Reviews, case reports or case series, editorials and letters without original data were excluded. Reports on specific features of kidney biopsies were excluded because full consensus was already reached on electron microscopy assessments of biopsy material from an affected organ as the current gold standard for FD in another consensus meeting 1. Enzyme replacement therapy studies were included if biochemical assessments were reported in the abstract.

Baseline data were used (i.e. before initiation of enzyme replacement therapy) or it was indicated otherwise. Data on prevalence in FD patients and control groups of kidney specific features were calculated and specified for gender. Sensitivity and specificity were calculated wherever possible. Unfortunately data to calculate specificity (no control group with kidney disease) were often absent. As a surrogate, specificity was calculated based on prevalence in healthy controls. A qualitative description of the study results was used if data were not sufficient. If the authors reported details on the non-classical nature of FD patients in the cohort, this data was also captured, with specific attention for globotriaosylceramide and globotriaosylsphingosine ([lyso]Gb3), angiookeratoma, cornea verticillata (CV) and neuropathic pain. Corresponding authors were contacted if additional clarification was required.

The tables below summarize the results of the systematic literature search. The selected articles are categorized by the (type of) criterion that was studied. A study may appear more than once if two criteria were studied by the authors. See figure A.

In most studies specificity is not reliable for patients with kidney disease, since the feature was not studied in groups with non-Fabry kidney disease. Unfortunately, data on comparison with non-Fabry kidney disease is very limited (4 studies). In addition studies were hampered by low sensitivity, and were mostly performed in a combined population of FD patients with or without kidney disease. Where possible, details on the presence of kidney disease in the specific study were given.

Definitions

Entry criterion: the presence of this entry criterion confirms a diagnosis of Fabry disease
Exit criterion: the presence of this exit criterion excludes Fabry disease

Urine Gb3

Urine Gb3 (as well as urine Gb3 isoforms) is believed to be indicative of general disease burden and not specific for the kidney only. It is therefore uncertain to which extend the level of urine Gb3 is determined by kidney involvement. In the process to define criteria for a definite diagnosis, urine Gb3 was considered to be of use for these criteria. Initially Gb3 and/or plasma LysoGb3 were considered not to be kidney specific (or indicative of systemic disease) and therefore urine Gb3 was not selected as an entry criterion in round one. However, the panel suggested to add the feature as an entry criterion, and it was subsequently discussed in round two.
Figure A. Literature search.
Summary of selected possible criteria from the literature

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Entry criteria</th>
<th>Exit criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney Ultrasound/MRI/CT</td>
<td>Renal cysts</td>
<td>Absence of Renal cysts</td>
</tr>
<tr>
<td>Urine light microscopy</td>
<td>Immunohistochemical staining of Gb3 in urine</td>
<td>Maltese cross sign in urine</td>
</tr>
</tbody>
</table>

### Ultrasound and MRI of the kidney

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients (controls)</th>
<th>Method</th>
<th>Results</th>
<th>Comments/ limitations</th>
</tr>
</thead>
</table>
| Glass 2004 | FD patients: n=116, n=76 males, n=40 females  
N=6 cardiac variant patients were not included in calculation since definite diagnosis is possibly uncertain  
Controls: - | MRI 1.5T & Ultrasound:  
- decreased cortical thickness;  
- cortical cysts;  
- decreased corticomedulory differentiation;  
- increased echogenecity;  
- parapelvic cysts;  
- renal atrophy;  
- scarring; | Ultrasound  
Sensitivity 27%  
Males 53%  
Females 28%  
MRI  
Sensitivity 54%  
Males 57%  
Females 50% | Combined sensitivity for different features. Features develop with increasing age. |
| Ries 2004 | FD patients: n=24 males  
Low enzyme activity, 22 confirmed with GLA mutation  
FD protein excretion 84-3909 mg/24u. Inulin clearance 33-130 ml/min. No difference in uGb3 between FD with and without cysts.  
Controls: n= 19 healthy age matched | MRI 1.5T | Sensitivity 50%  
Specificity 95% | Not compared to other kidney disease. Small control group. |

Comments

- Renal cysts are described in FD patients and occur mainly in the parapelvic area. The sensitivity is, low and seems correlated with age.
- High prevalence of cysts are reported in healthy individuals and increases with age [21, 22]
- The sensitivity is low in FD patients.
## Microscopy of urine

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients (controls)</th>
<th>Method</th>
<th>Results</th>
<th>Comments/ limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selvalrajah 2010 1</td>
<td>FD patients: n=35 n=16 males, 19 females no kidney transplant, GLA mutation unknown. n=2 male and n=4 female have no kidney involvement ERT treated patients included. Controls: n=20 patients n=11 males, n=9 females across a spectrum of biopsy-proven renal disease: • lupus nephritis (Classes 3–5, n=5), • diabetic nephropathy (n=2), • nephrotic minimal-change disease (n=1), • focal glomerular sclerosis (n=2), • membranous nephropathy (n=2), • crescentic nephritis (n=2), • Antineutrophil cytoplasmic autoantibody (ANCA) vasculitis (n=2), • thrombotic thrombocytopenic purpura (TTP, n=1), • acute tubular necrosis (n=3), • IgA nephropathy (n=1) one normal subject (no renal biopsy, no Microalb., Normal serum creatinine)</td>
<td>Phase contrast microscopy using a polarized filter. Number of maltese crosses, three distinct Maltese cross particle morphologies: MC1, MC2, MC3</td>
<td>For MC2 particles: Sensitivity 100% Specificity 100% Combined ERT and untreated patients. Experimental method but widely available</td>
<td></td>
</tr>
<tr>
<td>Selvalrajah 2010 4</td>
<td>See above</td>
<td>Anti CD77 staining of urine</td>
<td>Sensitivity 97% Specificity 100%</td>
<td>See above</td>
</tr>
<tr>
<td>Utsumi 1999 5</td>
<td>FD patients: n=23 ERT unknown. N=15 males, n=8 females. Of which 5 males and 1 female with ‘variant disease’. Controls: healthy n= 49 n=29 males, n=20 females Proteinuria n=23 , n=10 males, n=13 females</td>
<td>Immunoblotting, enzyme-linked immunosorbent assaying (ELISA), and immunofluorescent microscopy. Vitronectin receptor</td>
<td>Sensitivity and specificity unknown Sensitivity could not be calculated from the available data, a cutoff value was not defined. there is a slight overlap with healthy and proteinuria controls</td>
<td></td>
</tr>
<tr>
<td>Utsumi 2005 6</td>
<td>FD patients: n=4 males, enzyme activity &lt;1%. N=2 GLA mutation, N=2 mutation unknown Controls: -</td>
<td>Polarized light microscopy Maltese crosses, not specified for type</td>
<td>Sensitivity 100% specificity is unknown</td>
<td></td>
</tr>
</tbody>
</table>

### Comments
- The applied light microscopy techniques are widely available methods.
- Limited data available for each method
### Urine Gb3 (uGb3)

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients (controls)</th>
<th>Method</th>
<th>Results</th>
<th>Comments/ limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baehner 2003</td>
<td>FD patients: n= 15 females with multi organ involvement</td>
<td>HPLC</td>
<td>Sensitivity and specificity unknown</td>
<td>Data on decline of uGb3 on ERT treatment only</td>
</tr>
<tr>
<td></td>
<td>Mean creatinine clearance values varied from 65 to 73 ml/min per 1.73 m2, proteinuria not given</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls: -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cable 1982</td>
<td>FD patients: n=24, n=10 males, n=12 females. Of the females 9 were obligate heterozygotes.</td>
<td>HPLC of uGb3 and Digalactosyl-ceramide</td>
<td>Sensitivity 100% Sensitivity males: 100% Sensitivity females: 100% Specificity 100%</td>
<td>Specificity based on reference value of healthy controls Unknown if the 3 non obligate carriers also had an increased glycolipid pattern.</td>
</tr>
<tr>
<td></td>
<td>Controls: healthy n=6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choi 2008</td>
<td>FD patients: n=11, n=8 males, n=3 females 1 male patient with renal disease (normal uGb3) Five patients had increased protein excretion of more than 150 mg/24u</td>
<td>MS/MS</td>
<td>Sensitivity 65% Sensitivity males: 75% Sensitivity females: 33% Specificity 100%</td>
<td>Specificity based on reference value of healthy controls</td>
</tr>
<tr>
<td></td>
<td>Controls: healthy n=unknown</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gupta 2005</td>
<td>FD patients: n= 57 females with GLA mutation and signs or symptoms of FD N=4 no GLA mutation n=23 proteinuria &gt;150mg/24u n=24 GFR&lt;90</td>
<td>HPLC</td>
<td>Sensitivity and specificity unknown</td>
<td>Patient Gb3 in range 25-1493 nmol/g Cr. No reference value available</td>
</tr>
<tr>
<td>Hughes 2008</td>
<td>FD patients: n= 15 males with GLA mutation</td>
<td>HPLC</td>
<td>Sensitivity and specificity unknown</td>
<td>No raw data on uGb3 and no reference value available Data on decline of uGb3 on ERT only</td>
</tr>
<tr>
<td></td>
<td>Controls: -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kitagawa 2005</td>
<td>FD patients n=19 males and females with FD, genotyping “if necessary” 2 cardiac variant patients not included Pediatric patients were included: Age range 3-65 years Low GFR &lt;30ml/min in 3 hemizygous patients and proteinuria in 4 patients.</td>
<td>MS/MS</td>
<td>Sensitivity 89% Sensitivity males: 100% Sensitivity females: 75% Specificity 100%</td>
<td>Specificity based on reference value of healthy controls</td>
</tr>
<tr>
<td></td>
<td>Controls: Healthy n= 1140</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schiffmann 2000</td>
<td>FD patients: n= 10 males, 1 patient with a high (12%) residual enzyme activity</td>
<td>HPLC</td>
<td>Sensitivity and specificity unknown</td>
<td>No reference value available, Decline of uGb3 on ERT only</td>
</tr>
<tr>
<td></td>
<td>Controls: -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Utsumi 2005</td>
<td>FD patients: n=4 male with an enzyme activity &lt;1%. N=0 R112A, N=2 mutation unknown</td>
<td>HPLC followed by MS</td>
<td>Sensitivity 100% Specificity 100%</td>
<td>Sensitivity and specificity are 100% based on value of healthy controls, however raw data is only available from 1 patient as an example. It was assumed that other patients also have a high uGb3.</td>
</tr>
<tr>
<td></td>
<td>Controls: Healthy n=5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vedder 2007</td>
<td>FD patients: n=51 males n=22, females n=29</td>
<td>HPTLC</td>
<td>Sensitivity 82% Sensitivity males: 86% Sensitivity females: 79% Specificity 100%</td>
<td>Calculation based on previously known normal reference value.</td>
</tr>
<tr>
<td></td>
<td>Controls: -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vedder 2007</td>
<td>FD patients: n=34 males n=18, females n=16 confirmed with low enzyme activity in males and GLA mutation in females.</td>
<td>HPLC</td>
<td>Sensitivity 92% Sensitivity males: 100% Sensitivity females: 88% Specificity 100%</td>
<td>Calculation based on previously known normal reference value.</td>
</tr>
<tr>
<td></td>
<td>Controls: -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vedder 2008</td>
<td>FD patients: n=39 confirmed with low enzyme activity in males and GLA mutation in females</td>
<td>HPLC</td>
<td>Sensitivity 95% Specificity 100%</td>
<td>Calculation based on previously known normal reference value.</td>
</tr>
</tbody>
</table>
### Study Patients (controls) Method Results Comments/ limitations

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients (controls)</th>
<th>Method</th>
<th>Results</th>
<th>Comments/ limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whitfield 2005 17</td>
<td>FD patients: n=8, males n=6, females n=2 Controls: -</td>
<td>MS/MS</td>
<td>Sensitivity and specificity unknown</td>
<td>Data on decline of uGb3 on ERT treatment only</td>
</tr>
<tr>
<td>Whybra 2009 18</td>
<td>FD patients: N=36 females with a GLA mutation mean (±SD) urinary protein excretion was 377 ± 546 mg/24 hours</td>
<td>HPLC</td>
<td>Sensitivity 56% Specificity 100%</td>
<td>Calculation based on previously known normal reference value.</td>
</tr>
<tr>
<td>Auray-Blais 2008 19</td>
<td>FD patients n=78, ERT n=33 Controls: healthy n=47 females n=24, males n=23</td>
<td>MS/MS</td>
<td>Sensitivity and specificity unknown</td>
<td>Children were tested, but excluded in the data capture. Upper limit of normal for adults 25µg/mmol/Cr. Only graph available showing an overlap of patients with the normal control range, sensitivity and specificity were not calculated.</td>
</tr>
</tbody>
</table>

### Comments
- Urine Gb3 is measured routinely in many clinical centers and is a widely available assessment.
- The data were collected in both patients with and without kidney failure and or proteinuria.
- Data were not compared to other kidney diseases. Thus specificity is non-informative for the use in case of kidney disease. (elevation of uGb3 was based on healthy individuals as the comparative group).

### Urine Gb3 isoforms

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients (controls)</th>
<th>Method</th>
<th>Results</th>
<th>Comments/ limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fauler 2005 18</td>
<td>FD patients: n=4, n=2 male, n=2 female Controls: Healthy n=63</td>
<td>MS/MS, Isoforms Gb3-24:0/24:1</td>
<td>Sensitivity 100% Specificity 100%</td>
<td>isoforms respectively were found to be &gt;2.5- and &gt;5.2-fold above the highest female and male controls. ERT unknown, not widely available, Experimental method.</td>
</tr>
<tr>
<td>Paschke 2011 21</td>
<td>FD patients: n=26 females, with a GLA mutation GFR 70-92 ml/min/1.73m2 Protein/dreatinine ration: 76.1-337 mg/g Cr, Controls: Healthy n=112 CKD n=170</td>
<td>MS/MS, Isoforms Gb3-24 Diagnostic ranking revealed Gb3-24 as best marker, sensitivity and specificity for cutoff 34.8 ng/mgCr</td>
<td>Sensitivity 100% Specificity 87%</td>
<td>Uncertain analytical performance samples were excluded. Experimental and not widely available</td>
</tr>
</tbody>
</table>

### Comments
- Urine Gb3 isoforms are not measured routinely.
- The measurement is accessible to centers that use MS/MS for quantification of urinary Gb3.
Urine Lyso-Gb3

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients (controls)</th>
<th>Method</th>
<th>Results</th>
<th>Comments/ limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auray-Blais</td>
<td>FD patients: n=83, n=41 males, n=42 females. Markedly decreased enzyme activity of mutation in GLA gene. ERT: n=27 males, n=18 females</td>
<td>Tandem MS/MS</td>
<td>Sensitivity 87% Specificity 100%</td>
<td>Difficult and experimental assay</td>
</tr>
<tr>
<td></td>
<td>Controls: Healthy, n=77, n=30 males, n=47 females</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comments
- Urine Lyso-Gb3 is not measured routinely.
- Limited data available.
- Data were not compared to other kidney diseases. Thus specificity is non-informative for the use in case of kidney disease.
- LysoGb3 is possibly reacylated to Gb3, resulting in a possible underestimation of lysoGb3 excretion.

Other

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients (controls)</th>
<th>Method</th>
<th>Results</th>
<th>Comments/ limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vylet' al 2008</td>
<td>FD patients: n=15 with low enzyme activity and GLA mutation</td>
<td>SDS-PAGE and western blot; MS; immunohistochemistry; Deglycosylation experiment.</td>
<td>Sensitivity and specificity unknown</td>
<td>Unknown Uromodulin has been described in many kidney disorders. There is no value available in the article to calculate sensitivity or specificity.</td>
</tr>
<tr>
<td></td>
<td>Controls: -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kistler 2011</td>
<td>FD patients: n=17 females, not specified if GLA mutation was present, no ERT</td>
<td>CE-MS proteomics/Capillary electrophoresis coupled to mass spectrometry</td>
<td>Sensitivity 88% Specificity 98%</td>
<td>Specificity for other diseases 89-100%. Highly experimental method, not widely available.</td>
</tr>
<tr>
<td></td>
<td>Controls: Healthy age(gender matched n=45 renal, metabolic and cardiovascular diseases. n=8-78)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fuller 2005</td>
<td>FD patients: n=28 males n=15, females N=13</td>
<td>MS lipid profiling (Gb3 and other lipids)</td>
<td>Sensitivity and specificity unknown</td>
<td>Only mean and standard deviations given. Sensitivity and specificity could not be calculated.</td>
</tr>
<tr>
<td></td>
<td>Controls: -</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comments
- The assessments are experimental and were not added as an entry or exit criterion.
REFERENCES


