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

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Uncertain diagnosis of Fabry disease: consensus recommendation on diagnosis in adults with left ventricular hypertrophy and genetic variants of unknown significance

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*International Journal of Cardiology 2014; 177(2):400-8*
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ABSTRACT

Background
Screening in subjects with left ventricular hypertrophy (LVH) reveals a high prevalence of Fabry disease (FD). Often, a diagnosis is uncertain because characteristic clinical features are absent and genetic variants of unknown significance (GVUS) in the α-galactosidase A (GLA) gene are identified. This carries a risk of misdiagnosis, inappropriate counselling and extremely expensive treatment. We developed a diagnostic algorithm for adults with LVH (maximal wall thickness (MWT) of > 12 mm), GLA GVUS and an uncertain diagnosis of FD.

Methods
A Delphi method was used to reach a consensus between FD experts. We performed a systematic review selecting criteria on electrocardiogram, MRI and echocardiography to confirm or exclude FD. Criteria for a definite or uncertain diagnosis and a gold standard were defined.

Results
A definite diagnosis of FD was defined as follows: a GLA mutation with ≤ 5% GLA activity (leucocytes, mean of reference value, males only) with ≥ 1 characteristic FD symptom or sign (neuropathic pain, cornea verticillata, angiokeratoma) or increased plasma (lyso)Gb3 (classical male range) or family members with definite FD. Subjects with LVH failing these criteria have a GVUS and an uncertain diagnosis. The gold standard was defined as characteristic storage in an endomyocardial biopsy on electron microscopy. Abnormally low voltages on ECG and severe LVH (MWT>15mm) < 20 years exclude FD. Other criteria were rejected due to insufficient evidence.

Conclusion
In adults with unexplained LVH and a GLA GVUS, severe LVH at young age and low voltages on ECG exclude FD. If absent, an endomyocardial biopsy with electron microscopy should be performed.
INTRODUCTION

Fabry disease (FD; OMIM 301500) is an X-linked lysosomal storage disorder caused by a deficiency of α-galactosidase A (AGAL-A). Estimated birth prevalence range between 1:40,000 and 110,000. Over 670 mutations in the α-galactosidase A (GLA) gene have been described, mostly appearing in single families. Since the availability of enzyme replacement therapy (ERT) screening in newborns, high risk populations, as well as individual case finding is increasing. These screening studies report a surprisingly high prevalence of FD in subjects with left ventricular hypertrophy (LVH) (range 0 - 12%). However, while the pathogenicity of some GLA mutations is well described, the subjects identified through screening often have a GLA genetic variant/ mutation of unknown significance (GVUS). Interestingly, most males with such GVUS demonstrate significant residual AGAL-A enzyme activity, in contrast to the absent or near absent enzyme activity in classically affected males. Moreover, most subjects identified through screening are lacking characteristic classical Fabry signs or symptoms such as neuropathic pain, angiokeratoma or cornea verticillata, but present with a single, non-specific Fabry sign such as cryptogenic stroke, proteinuria or LVH, all associated with other more common diseases. Because these subjects have symptoms restricted to single organs, they were coined as cardiac, renal or late onset variants of the disease. In addition, while classically affected males invariably have significant elevations in plasma globotriaosylsphingosine (lysoGb3), non-classical FD patients and subjects with a non-pathogenic GLA mutation, such as p.D313Y, have low or normal levels. While some still consider the p.D313Y mutation pathogenic, it has been shown that this mutation results in a pseudo-deficiency of AGAL-A in plasma, with only minimally reduced enzyme activity in cell expression models. Another example of advancing insight concerns the p.A143T mutation, which is frequently identified through screening studies. However, in subjects with this variant presenting with LVH or kidney failure, no characteristic Gb3 deposits were found in biopsies.

As part of the Hamlet study, designed to address the uncertainties related to diagnosing FD, we aimed to gain international consensus on a diagnostic algorithm for adult subjects presenting with LVH (maximal wall thickness in diastole (MWTd) of > 12 mm) with an uncertain diagnosis of FD, harbouring a GVUS in the GLA gene.

METHODS

Delphi participants

We used a modified Delphi procedure to gain a consensus. The voting panel consisted of internists with expertise in the diagnosis and general management of FD and cardiologists with expertise in FD cardiomyopathy.
Pre-selection of voting items

A proposal was made for definitions of a definite and uncertain diagnosis of FD, and the gold standard (by MB, CH, BS, and LT). A systematic review was performed to find criteria on electrocardiogram (ECG), cardiac magnetic resonance imaging (CMR) or echocardiography that could be used to either exclude FD (exit criteria) or confirm a diagnosis of FD (entry criteria). PubMed and EMBASE were searched from 1980 till October 2012 with the following search terms: Fabry disease, heart, cardiac, cardiomyopathy, cardiac hypertrophy, LVH, ECG, ultrasound and CMR, including synonyms and MeSH terms. Included were peer reviewed English written studies in adult human subjects. Titles and abstracts were screened and cross-referencing was performed. Corresponding authors were contacted if additional clarification was required. Criteria qualified when they were directly compared to other subtypes of hypertrophic cardiomyopathies (HCM) and when sensitivity and specificity could be calculated. We accepted an entry criterion for a diagnosis of FD only if there was a specificity of >90% (i.e. the presence of this criterion confirms a diagnosis of FD; there are no or only very few false positives) and an exit criterion only if the prevalence of this criterion was < 10% in FD (i.e. the presence of this criterion in FD is very unlikely, and is specific for other subtypes of HCM).

Validation of pre-selected criteria

Validation of the pre-selected criteria in patients similar to those identified through screening for LVH is of importance, since the selected criteria from the literature were primarily based upon patients with classical FD versus controls. Criteria that are specific or sensitive in a classically affected group may not necessarily have similar diagnostic accuracy in non-classical FD patients. To determine specificity and sensitivity, the pre-selected criteria were applied to Dutch patients presenting with LVH only (LVH defined as interventricular septal wall thickness of ≥ 12 mm and/or left ventricular mass of ≥ 48 g/height in m²⁻⁷ for females, and ≥ 51 g/height in m²⁻⁷ for males). These patients were divided into two groups. A ‘positive group’ consisted of patients presenting with LVH only and histological evidence of a specific storage pattern, or with a definite (classical) diagnosis based upon the following predefined criteria: a GLA mutation (defined as any abnormality found in the GLA gene) and ≤ 5% GLA activity (of the mean reference value in leucocytes, males only) with ≥ 1 characteristic FD sign or symptom (neuropathic pain, cornea verticillata, clustered angiookeratoma) or increased plasma (lyso)Gb3 (in the classical male range) or a family member with a definite diagnosis of FD carrying the same GLA mutation. A ‘negative group’ consisted of patients with unexplained LVH who did not fulfil the criteria of a definite diagnosis of FD and therefore have a GVUS in the GLA gene (defined as a variant / mutation in the GLA gene of unknown clinical significance) in whom a biopsy of an affected organ excluded FD, or expression studies showed AGAL-A pseudo deficiency (p.D313Y) in index patients. All data were gathered with (written) informed consent. Pre-treatment ECGs were retrospectively assessed by a single investigator (PP) using digitized ECGs and on-screen callipers with the ImageJ program (http://rsb.info.nih.gov/ij/). Data on the following parameters were retrieved from 3 consecutive sinus beats: heart rate, P wave duration, PQ-interval, QRS-duration and QT-interval, QTc, Sokolow-Lyon index to assess left ventricular hypertrophy and the sum of the QRS amplitudes in lead I + II + III < 1.5mV as well as a Sokolow-Lyon index of < 1.5mV to
Consensus recommendation: left ventricular hypertrophy

Assess low voltages. All available echocardiography and CMR reports (baseline and treatment) were retrospectively scored for the presence of pericardial effusion, left ventricular outflow tract obstruction (LVOTO) and late enhancement by gadolinium on CMR.

**Delphi voting rounds**

The procedure consisted of two voting rounds and a face-to-face meeting. During the first voting round panellists received the results of the systematic review and validation cohort. Through an online anonymous survey (Survey monkey) they could criticize the validity of the pre-selected criteria. Comments and new criteria could be added. Results of the first round were reviewed, items were adapted or added and the results were provided during the second round. During the face-to-face meeting each criterion was discussed and adapted when necessary. We admitted the possibility for supplementary analyses in panellist’s cohorts in case the panel would conclude that the level of evidence of the pre-selected criteria is insufficient.

**Statistical considerations: selection of final items in diagnostic algorithm**

In keeping with previous studies, we decided to accept criteria in the diagnostic algorithm only when at least 75% of the panel agreed, and none of the panellists disagreed (i.e. only two neutral votes were acceptable). To assess overall consensus, Cronbach’s α was calculated, with 0 indicating no consensus and 1 full consensus. The recommendations by Bland and Altman were applied: Cronbach’s α should be above 0.9, but preferably above 0.95 for clinical applications.

SPSS version 19 was used for statistical analyses.

**RESULTS**

**Delphi procedure and participants**

Nine FD experts were invited to participate; seven FD experts (FC, PE, DH, JT, GL, FW and MW) completed all three rounds. At the face-to-face meeting, five experts were present and two were involved by telephone.

**Pre-selection of voting items: systematic review**

To preselect voting items proposed to the panel, a systematic review was performed. Our search retrieved 140 articles of which 88 were excluded (supplementary figure 1). From the remaining 52 articles, 9 entry or exit criteria were pre-selected (table 1). A summary of all articles reviewed and the results of the Dutch validation cohort were presented to the panel (online supplementary data).

**Voting items**

Overall consensus on all voting items, measured by Cronbach’s α, increased from 0.87 in round 1 to 0.97 and 0.99 in rounds 2 and 3, respectively.

**Definitions of a definite and uncertain diagnosis of FD**

There was 100% agreement that a diagnosis of FD in patients presenting with LVH only (defined as a MWT > 12 mm), cannot always be made by biochemical (AGAL-A activity) and/or GLA
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mutation analysis alone. To determine to whom the cardiac diagnostic algorithm would apply (i.e. the patients with an uncertain FD diagnosis) definitions of a definite and uncertain FD diagnosis were made (see table 2). A definite diagnosis of FD (i.e. classical FD) was defined as follows: a GLA mutation with ≤5% AGAL-A activity (of the mean of reference value in leucocytes 48, in males only) with either ≥1 characteristic FD symptom or sign or increased plasma (lyso)Gb3 (in the classical male range) or a family member with a definite diagnosis of FD carrying the same GLA mutation. Patients presenting with LVH and a GLA mutation not fulfilling these criteria have an uncertain diagnosis of FD.

Diagnostic biochemical analyses
AGAL-A deficiency should preferably be measured in leucocytes 48. While this can reliably be established in other enzyme sources like dried blood spots and plasma, leukocytes are superior in estimating residual AGAL-A activity. Characteristic FD signs and symptoms should be assessed by a physician with extensive experience in FD. Fabry neuropathic pain was defined as pain in hands and/or feet with an onset of pain in childhood or adolescence (i.e. < 18 years of age), and/or a course characterized by exacerbations that are provoked by fever, exercise or heat, as well as a decreased cold sensation and an abnormal intra epidermal nerve fibre density 49. Cornea verticillata should be evaluated in the absence of amphiphilic drug use 50. Clustered angiokeratoma should be present in the bathing trunk, peri-umbilical and/or peri-oral regions (for examples see 51,52).

Plasma (lyso)Gb3 assays are not widely available but are very helpful when elevated to the level as found in classically affected males, e.g. with the assay used at the AMC either plasma lysoGb3

Table 1. Summary of pre-selected criteria, with sensitivity and specificity calculation.

<table>
<thead>
<tr>
<th>Fabry diagnosis</th>
<th>Entry criteria</th>
<th>Sen. %</th>
<th>Spec. %</th>
<th>Exit criteria</th>
<th>Prev. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECG</td>
<td>PQ interval minus P wave duration &lt; 40 ms 35</td>
<td>82</td>
<td>99</td>
<td>Low voltages: Sokolow-Lyon index of ≤1.5 mV total QRS amplitude in I, II, III &lt; 1.5mV 35,35</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Corrected PQ interval &lt; 144 ms 35</td>
<td>82</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PQ &lt; 120 ms 35</td>
<td>24</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac echocardiography</td>
<td>Increased papillary muscle LV wall ≥ 12mm when LV wall &gt; 13 mm 39</td>
<td>75</td>
<td>86</td>
<td>Severe LVH without right ventricle hypertrophy 40</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>ND</td>
<td>LVOTO 41-43</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pericardial effusion 53</td>
<td>0</td>
</tr>
<tr>
<td>CMR</td>
<td>Late enhancement in papillary muscles 43-47</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: CMR: cardiac magnetic resonance imaging, ECG: electrocardiogram, LV: left ventricle, LVOTO: left ventricular outflow tract obstruction (in rest), LVH: left ventricular hypertrophy, ND: no data, prev.: prevalence, sens.: sensitivity, spec.: specificity.
Consensus recommendation: left ventricular hypertrophy

values of > 50 nmol/L (normal reference range 0.3-0.5 nmol/L) or plasma Gb3 values of > 2.9 nmol/mL (normal reference range 0.45-2.46 nmol/mL)\(^53\). However, because different assays of plasma (lyso)Gb3 are available, no laboratory independent cut-off values could be generated.

**Gold standard for a diagnosis of FD in uncertain cases is EMB**

The panellists all agreed that the gold standard for a diagnosis of FD in subjects presenting with a non-specific FD sign (such as LVH, renal failure, proteinuria) and an uncertain diagnosis of FD (table 2) is the demonstration of characteristic storage in the affected organ (e.g. heart, kidney, aside from skin) by electron microscopy analysis, according to the judgement of an experienced pathology team. Storage should preferably be evaluated in the affected organ, as the expected diagnostic yield of skin biopsies in non-classical FD patients is low. While the majority of classical FD patients demonstrate a ubiquitous storage pattern including dermal storage\(^54,55\), studies in patients with non-classical FD (or cardiac variant) illustrate that storage is restricted to the endomyocardium\(^20,56-58\). In addition, other explanations for the cardiomyopathy might be found on endomyocardial biopsy (EMB). So in case a patient, presenting with a non-specific sign such as LVH, does not fulfil the diagnostic criteria for a definite diagnosis of FD, but has a confirmative biopsy, the diagnosis is considered definite and defined as non-classical, biopsy proven FD (figure 1). In other words, a GLA mutation can be considered disease causing if the patient fulfils the definite FD diagnostic criteria, or if in a symptomatic patient not fulfilling the definite diagnostic criteria, a characteristic storage pattern in the affected organ is demonstrated.

<table>
<thead>
<tr>
<th>Table 2. Definitions of a definite and uncertain Fabry diagnosis.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Definite diagnosis of FD</strong></td>
</tr>
<tr>
<td><strong>Males</strong></td>
</tr>
<tr>
<td>GLA mutation</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>AGAL A deficiency of ≤5% of mean reference value in leukocytes(^48)</td>
</tr>
<tr>
<td>A or B or C</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td><strong>Uncertain diagnosis of FD in subjects presenting with a non-specific FD sign</strong></td>
</tr>
<tr>
<td><strong>Males/Females</strong></td>
</tr>
<tr>
<td>All patients presenting with a non-specific FD sign (such as LVH, stroke at young age, proteinuria) who do not fulfil the criteria for a definite diagnosis of FD have a GLA GVUS</td>
</tr>
</tbody>
</table>

**Abbreviations:** AGAL A: lysosomal α-galactosidase A enzyme, GLA: α-galactosidase A gene, GLA mutation: defined as any abnormality found in GLA gene, LVH: left ventricular hypertrophy defines as MWT >12 mm). GVUS: genetic variant of unknown significance, is defined as a GLA mutation that has unknown clinical significance because it does not fulfil the criteria of a definite diagnosis of FD.
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Characteristic storage was defined as concentric multi lamellated myelin bodies with a zebra like pattern (zebra bodies) with a periodicity of approximately 5 nm, in the absence of drug use known to induce these inclusion bodies (such as chloroquine or amiodarone). Although these inclusion bodies can be found in other lysosomal storage disorders, there is no clinical overlap and therefore these inclusions are considered specific in the context of a clinical presentation compatible with FD.

Following the protocol of the Association of European Cardiovascular Pathology and Society for Cardiovascular Pathology, a minimum of 5 endomyocardial fragments should be obtained for electron microscopy (EM) and light microscopy (LM) analysis (for a detailed description of processing biopsy material see 63). As the storage pattern in FD is diffuse throughout the ventricles, EMB can be performed in both ventricles 13,64,65. Furthermore, it was concluded that in patients with an uncertain diagnosis of FD, genetic (over) expression studies are informative (especially in females without an affected male family member), but cannot be used as a gold standard for a diagnosis of FD.

![Diagnostic Algorithm Diagram](image)

**Figure 1.** Proposal for a diagnostic algorithm for subjects presenting with isolated LVH and an uncertain diagnosis of FD. *LVH: left ventricular hypertrophy defined as an MWTd > 12 mm, ** see table 2, *** low voltages on ECG defined as the total sum of the amplitude of the QRS complex in I, II, III < 1.5 mV 34, **** severe LVH was defined as a MWT > 15 mm. Abbreviations: EM: electron microscopy, FD: Fabry disease, GLA: α-galactosidase A gene.

**Exit and entry criteria on ECG, echocardiography, CMR to exclude or confirm FD**

Table 1 shows the nine criteria that were pre-selected based on the systematic review. The supplementary data shows all criteria that were analysed (n=20) and the reasons for inclusion or rejection. Most criteria were not selected because they were not specific enough e.g. specificity.
Consensus recommendation: left ventricular hypertrophy

< 90% (concentric LVH, binary sign, myocardial infero-postero-lateral late enhancement), they were insufficiently compared to other subtypes of HCM (global and circumferential strain pattern, thoracal aortic dilatation), there were no clear cut off values available to discern FD from other subtypes of HCM (increased T2 relaxation time on CMR, extracellular volume measurement on CMR), or they were not reproducible (short p-wave on ECG). Furthermore, the expert panel suggested ‘severe LVH at young age’ as an exit criterion for a diagnosis of FD.

The panel rejected seven of the pre-selected criteria. The main reason for rejection was unsatisfactory data on specificity; specificity was based on limited studies with small cohorts (PQ minus P wave < 40 ms, corrected PQ interval < 144 ms, hypertrophied papillary muscle), criteria were insufficiently compared to other subtypes of HCM (hypertrophied papillary muscle, severe LVH without RVH, late enhancement in papillary muscle), or the criterion was negated by the data of our validation cohort (PQ minus P wave < 40 ms). Other reasons were: imprecise and or impractical tool in daily practice (PQ minus P wave < 40 ms, corrected PQ interval < 144 ms, severe LVH without RVH), or the presence of concomitant disease possibly inducing this criterion (LVOTO, pericardial effusion).

Supplementary cohort analyses on exit and entry criteria
The panel concluded that three criteria possibly qualified for the diagnostic algorithm, but required additional analyses: “PQ interval < 120 ms” as an entry criterion, and “severe LVH at young age” and “presence of abnormally low voltages on ECG (defined as the total sum of the QRS amplitude in I, II, III < 1.5 mV)” as exit criteria. Therefore, additional baseline ECGs and echocardiographic data were gathered from Dutch, German and Italian FD cohorts. All available patients fulfilling the criteria for a definite (classical) diagnosis (see table 2) and those with a non-classical, biopsy proven diagnosis were included. At least all index patients of non-classical families (n=5) showed evidence of a characteristic storage pattern in an affected organ in kidney or heart (7 out of 15 patients had a confirmative biopsy), within families the pathogenicity of a mutation was extrapolated to family members carrying the same GLA mutation. Patients with an uncertain diagnosis of FD in whom no biopsy was available (or of any family member) were excluded. An exception was made for patients with a p.N215S GLA mutation who only had a confirmatory histology in 1 of 11 patients. The pathogenicity of this mutation is well-established: this is a prevalent mutation associated with a non-classical phenotype of which confirmative histology has been described in several papers. In addition, an Italian HCM cohort was investigated (defined as maximal wall thickness (MWT) > 15 mm) in which a diagnosis of FD was ruled out either by mutation analysis (females) or AGAL-A activity (males). All data were gathered with informed consent following the Declaration of Helsinki.

Severe LVH at young age excludes FD
Figure 2 shows the distribution of the baseline IVSd thickness in 69 FD patients (27 males, 90% classical phenotype, median age of 18 years, range of 5-25). The maximum IVSd thickness was 15 mm. The highest value was found in a 24 year old classically affected male. The panel decided that a cut-off IVSd thickness of >15 mm below 20 years could serve as an exit criterion for FD (figure 2).
Abnormally low voltages on ECG excludes FD

In 158 adult FD patients (see table 3 for baseline characteristics) the presence of abnormally low voltages on ECG was 0.6% (n=1). This single female patient with a classical FD phenotype also had a concomitant phospholamban mutation (p.Arg14del) causing a dilated cardiomyopathy, which probably caused her abnormally low voltages on ECG. In 5% (n=3/66) of an Italian HCM cohort abnormally low voltages on ECG were present. As abnormally low voltages were found in this HCM cohort, and also in other subtypes of HCM, but not in FD patients, the panel decided that the presence of low voltages on ECG < 1.5 mV could be used to exclude a diagnosis of FD, which was implemented in the diagnostic algorithm (figure 1).

Table 3. Baseline characteristics of FD and HCM patients for supplementary data analysis.

<table>
<thead>
<tr>
<th></th>
<th>FD patients</th>
<th>HCM patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=158</td>
<td>n=66</td>
</tr>
<tr>
<td>Male/ Female, % (n)</td>
<td>38 (59) / 62 (97)</td>
<td>49(35) / 51(57)</td>
</tr>
<tr>
<td>Age median [years]</td>
<td>43 (18-90)</td>
<td>52 (20-80)</td>
</tr>
<tr>
<td>Classical/ non-classical phenotype with a positive biopsy % (n)</td>
<td>84 (132) / 17 (26, n=11 p.N215S)</td>
<td></td>
</tr>
<tr>
<td>IVSd / MWTd [mm]</td>
<td>12 (6-28)</td>
<td>20 (15-44)</td>
</tr>
<tr>
<td>median (range)</td>
<td>n=4 missing data</td>
<td>n=4 missing data</td>
</tr>
<tr>
<td>IVSd / MWTd &gt; 12 mm, % (n)</td>
<td>42 (64/154)</td>
<td>100</td>
</tr>
<tr>
<td>IVSd / MWT ≥ 15 mm, % (n)</td>
<td>23 (36/154)</td>
<td>100</td>
</tr>
<tr>
<td>ECG low voltages of &lt; 1.5 mV, % (n)</td>
<td>0.6 (1/158)</td>
<td>5 (3/66)</td>
</tr>
<tr>
<td>ECG PQ &lt; 120 ms, % (n)</td>
<td>15 (13/84)</td>
<td>7 (4/60)</td>
</tr>
</tbody>
</table>

Consensus recommendation: left ventricular hypertrophy

**Red flag to suspect FD: PQ interval of < 120 ms in FD**

In a subset of 84 FD patients, the presence of a short PQ interval was investigated. Their baseline characteristics were comparable to the total FD cohort. Fifteen percent (n=13/84) of the adult FD patients (n=5 males, n=5 with LVH) had a PQ interval of < 120 ms, the majority having a classical phenotype (n=12/13). Thirteen percent of the FD patients with LVH and 18% without LVH had a PQ interval of < 120 ms. In the HCM cohort 7% (n=4/60) had a PQ interval of < 120 ms. In addition, shortening of PQ interval has been described in glycogen storage disorders such as Danon disease. Danon disease can present with a late onset cardiomyopathy. Therefore, the panel concluded that a PQ interval < 120 ms is not specific enough to be included in the diagnostic algorithm.

The panel considered the presence of a PQ interval < 120 ms, sinus bradycardia, hypertrophied papillary muscle, myocardial late enhancement in the infero-postero-lateral region (table 4) useful in daily practice as a red flag to suspect a diagnosis of FD, but the data on specificity were insufficient to include these criteria in the diagnostic algorithm.

<table>
<thead>
<tr>
<th>Red flags to suspect Fabry disease</th>
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<tbody>
<tr>
<td>ECG</td>
</tr>
<tr>
<td>PQ interval &lt; 120 ms</td>
</tr>
<tr>
<td>Sinus bradycardia</td>
</tr>
<tr>
<td>Echocardiography</td>
</tr>
<tr>
<td>Hypertrophied papillary muscle</td>
</tr>
<tr>
<td>CMR</td>
</tr>
<tr>
<td>Myocardial late enhancement infero-postero-lateral region</td>
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</table>

**DISCUSSION**

Among an international group of experts a consensus was reached on a diagnostic algorithm for patients presenting with isolated LVH and an uncertain diagnosis of FD, harbouring a GVUS in the GLA gene. First of all, a consensus on definitions of a definite and an uncertain diagnosis was reached, emphasizing that in cases with an uncertain, non-classical phenotype, enzymatic or genetic tests cannot always confirm a definite diagnosis of FD. In these uncertain cases, additional studies are warranted. Agreement was reached that in these cases histology of the heart should be considered as the gold standard for a diagnosis of FD; an endomyocardial biopsy (EMB) showing characteristic lamellated inclusion bodies on electron microscopy, with a periodicity of approximately 5 nm, in the absence of drug use known to induce a similar storage pattern.

In the literature, many characteristics of FD cardiomyopathy, with regard to ECG and cardiac imaging, have been claimed. This is the first time that a systematic analysis of the specificity of these criteria has been reviewed and studied for replication for their usefulness in a diagnosis of Fabry disease. None of the criteria were specific enough (> 90%) to be used as an entry criterion, i.e. a test that confirms a definite diagnosis of FD. After confirmation in a relatively large
international Fabry disease cohort, two items were identified that could be used as exit criteria i.e. a test that can exclude FD. These were the presence of abnormally low voltages on ECG and severe LVH at young age (MWTd > 15 mm below the age of 20 years). However, in most cases with LVH and an uncertain FD diagnosis, an EMB should be performed. This conclusion is not new and has already been proposed by both the Association of European Cardiovascular Pathology and the Society for Cardiovascular Pathology. The proposed diagnostic criteria complement these recommendations by giving a detailed prescription in which cases EMB is warranted. It more specifically emphasizes that in a clinical context compatible with FD, in the absence of medication use inducing FD-like storage, EM analysis of an affected organ is considered as the gold standard. Although the performance of EMBs is already endorsed by some institutions and (FD screening) studies, many did not or performed EMB without EM analysis.

Safety issues often restrain clinicians from performing EMB. The serious adverse event rate of EMB is reported to be 0.12-2%. In our opinion this does not outweigh the importance of a correct diagnosis. It should be stressed, however, that EMB should only be performed in experienced hands in symptomatic patients, after extensive diagnostic work-up has been performed.

Although genetic testing for FD has become part of routine diagnostic tests in some cases, in patients with unexplained LVH other more common diseases should be excluded first (for review see). The presence of low voltages on ECG and severe LVH in young patients should discourage physicians to screen for FD. While we were unable to determine non-invasive tests that could prove a diagnosis of FD, there are several criteria that can serve as a red flag and raise clinical suspicion of FD (table 4). Of note, some of these criteria can demonstrate a dynamic behaviour in the course of FD’s cardiomyopathy. For instance, the presence of left atrial dilatation confounds the presence of short PQ intervals, and cardiac fibrosis can be more prominent in later disease stages, suggesting that the sensitivity and specificity can be variable throughout the disease course. As part of the diagnostic work-up, a careful history of drug use, such as amiodarone, chloroquine or tamoxifen, needs to be recorded. These drugs are capable to induce a similar storage pattern as seen in FD. As amiodarone is widely used in cardiology practice, EMB will not be a panacea for all uncertain FD cases. Furthermore, should characteristic storage on EMB be found (confirming FD), it cannot predict the disease course. This especially holds for family members of a subject with a non-classical, but biopsy proven diagnosis of FD. They may have a milder disease course or even remain asymptomatic, depending on each individual genetic and environmental background. Also, when a characteristic storage pattern in an affected organ is found, this cannot automatically be extrapolated to other non-specific signs in other organs in a family member carrying the same GLA mutation.

This study has several limitations. First of all, the criteria for a definite diagnosis of FD applied here are quite strict. For instance, we agreed that the level of plasma (lyso) Gb3 needs to be in the range of classically affected males, instead of two standard deviations above the mean reference value, to fulfil the criteria of a definite diagnosis. We know that the diagnostic sensitivity
of plasma lysoGb3 in classical FD patients is high. In addition, several centres have published mass spectrometric methods capable to detect even small increases of lysoGb3 above normal. A classification of pathogenicity of GLA mutations based on plasma lysoGb3 levels has recently been proposed. In our experience slightly elevated levels of lysoGb3 are often found in non-classical patients with a biopsy proven diagnosis of FD. However, the use of plasma lysoGb3 to identify non-classical FD patients with certainty needs further validation. We need additional data to prove that these small increases are always accompanied by the characteristic storage pattern on EM in tissue biopsies. If so, tissue biopsies might become superfluous. Secondly, not all non-classical patients have had a confirmative biopsy. Of the included families, at least all index patients showed a characteristic storage pattern of an affected organ and we assume that this result can be extrapolated to family members with the same GLA mutation. This is supported by a study showing characteristic storage pattern throughout non-classical families. Even if this would have led to the inclusion of non-Fabry patients, this would not have altered our conclusions, because this would not have led to a higher number of false negatives: i.e. FD patients in whom one of the exit criteria is present.

Another limitation of this study was the small size of the expert panel. We specifically sought the expertise of FD cardiologists for the panel, but only few cardiologists have specific interest in this rare disease. Lastly, the diagnostic algorithm provides a snapshot of currently available data. After the consensus meeting interesting study results were published, showing that a low signal on CMR T1 mapping of the septum was considered highly specific for FD patients with LVH. Unfortunately, we have not yet been able to validate this in non-classical FD patients presenting with LVH. Many of the investigated criteria on ECG, CMR, or echocardiography were rejected because of low level of evidence. As soon as some of these criteria have been studied in larger cohorts, in non-classical patients with LVH and compared to other subtypes of HCM, the diagnostic algorithm should be updated.

Many screening studies suggest that the identification of FD patients is beneficial as (early) ERT treatment can be initiated. We would like to emphasize that this assumption is not sufficiently supported by evidence. The natural history and effects of ERT in non-classical, biopsy proven FD patients (i.e. late onset, cardiac and renal variants) are currently still unclear and should be the focus of future research. Up to then, non-classical FD patients should be fully informed over the lack of evidence of benefits of ERT at this stage, and its initiation should be considered on an individual basis and only with their consent. Furthermore, we should be reluctant to perform population screening (especially in new-borns), while individual screening in patients presenting with a HCM may be beneficial.

Conclusions
This study presents a diagnostic algorithm for patients presenting with unexplained LVH (MWT > 12 mm) with an uncertain diagnosis of FD. Via a Delphi procedure a consensus was reached on general diagnostic criteria for a definite diagnosis of FD. The gold standard was defined as characteristic storage in an endomyocardial biopsy on electron microscopy. The presence of abnormally low voltages on ECG and severe LVH (MWT > 15 mm) < 20 years can exclude FD.
and should discourage physicians to screen for FD. This algorithm aims to generate a structured approach for all subjects identified in screening studies with an uncertain diagnosis of FD.

ACKNOWLEDGEMENTS
We would like to acknowledge the following: B.J.H.M. Poorthuis, for his valuable advice on the biochemical analyses used in the disease criteria and for the revision of the manuscript, H.A.C.M. Bon and M. Groenink for echocardiography and CMR analyses in the Dutch FD patients.

FUNDING
This study is part of the Hamlet trial 2\textsuperscript{8} which is supported by TI Pharma, a non-profit organization that catalyses research by founding partnerships between academia and industry. Partners: Genzyme (a Sanofi company), Academic Medical Centre of the University of Amsterdam; Subsidizing Party: Shire. http://www.tipharma.com/pharmaceutical-research-projects/drug-discovery-development-and-utilisation/hamlet-study.html. The industry partners have no role in the development of the algorithm in this study.
REFERENCES


34. Roberts WC, Waller BF. Cardiac amyloidosis causing cardiac dysfunction: analysis of 54 necropsy patients. *Am J Cardiol* 1983;52(1):137-146.


43. Pieroni M, Chimenti C, De CF et al. Fabry’s disease cardiomyopathy: echocardiographic
Consensus recommendation: left ventricular hypertrophy


Consensus recommendation: left ventricular hypertrophy


Chapter 3


121. Bella JN, Wachtell K, Boman K et al. Relation of left ventricular geometry and function to aortic root dilatation in patients with systemic hypertension and left ventricular hypertrophy (the LIFE study). *Am J Cardiol* 2002;89(3):337-341.


SUPPLEMENT

Results of systematic review and Dutch validation cohort presented to panel

The pre-selected criteria from the literature (see figure S1 for systematic review results, and table 1 for selected criteria) were primarily based upon patients with classical Fabry disease versus controls. Since our aim is to make a definite diagnosis in patients with non-classical FD and an uncertain diagnosis of FD, the pre-selected criteria were applied to two groups of patients presenting with LVH only. The positive group comprised of n=22 FD patients with a definite (classical) diagnosis, including n= 16 females (either with characteristic FD signs or symptoms or a family member with a definite FD diagnosis) and n=6 patients with a biopsy proven diagnosis of the affected organ of the index patient (p.P389A (n=5), p.R112H (n=1), 3 females, 3 males). The negative group, with an initial uncertain FD diagnosis, comprised of n= 7 patients. (p.A143T variant (n=3), p.D313Y variant (n=2), p.P60L variant (n=2) 3 females, 4 males). All index patients with these mutations proved to have no FD by biopsy or showed a non-pathogenic GLA variant by expression analysis (p.D313Y). Results of the pre-selected criteria applied to the validation cohort are presented in the tables in the last column.

Supplementary Figure 1. Flow chart of identification, screening and inclusion of articles in the systematic review.
Chapter 3

1. Electrocardiogram: possible Electrocardiogram criteria

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Patients, (controls)</th>
<th>Methods</th>
<th>Results</th>
<th>Comments/ Limitations</th>
<th>Validation cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Namdar et al 35</td>
<td>FD, classical phenotype, pre-ERT; n=17; all LVH</td>
<td>Retrospective case-control study</td>
<td>PQ interval minus P wave duration &lt; 40 ms sensitivity 82% and specificity 99% for FD</td>
<td>Small sample sizes; Hypertensive and CAD FD patients excluded; PQ minus P wave &lt; 40ms and or QTc &lt; 440ms sensitivity 99% specificity 100% for FD</td>
<td>PQminusP &lt; 40ms Positive group: 57% (12/21, 1 missing data) Negative group: 86% (8/7)</td>
</tr>
<tr>
<td></td>
<td>Controls: HCM, cardiac amyloidosis, HDD, aortic stenosis</td>
<td></td>
<td>PQ minus P wave &lt; 40ms and or QTc &lt; 440ms sensitivity 99% specificity 100% for FD</td>
<td>Unclear if all consecutive FD patients are included, or selection is made; Parameters manually traced instead of digitally</td>
<td>PQminusP &lt; 40ms Positive group: 86% (8/7), Negative group: 86% (6/7)</td>
</tr>
<tr>
<td></td>
<td>Controls: age and LV mass matched</td>
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</tr>
<tr>
<td></td>
<td>Exclusion: all patients with CAD, WMA, atrial fibrillation, pacemaker, FD with hypertension, FD with cardiac variant</td>
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<tr>
<td>Namdar et al 89</td>
<td>FD without LVH n=30 Healthy controls Age and heart rate matched</td>
<td>Retrospective case-control study</td>
<td>No difference between FD without LVH and healthy controls on PQ interval minus P wave duration</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.1. PQ interval minus P wave duration as an entry criterion for FD diagnosis


- Comments
  - Studies:
    - Single study in rather small cohorts of different cardiomyopathies.
    - Exclusion of patients with CAD, pacemaker and hypertensive FD and only analysed in classical FD patients: representative sampling of study population is questionable.
    - PQ minus P interval < 40 ms is a small difference on ECG: possibly not a reliable parameter in clinical practice due to manual tracing.
    - Discrepancy between PQ minus P < 40 ms in FD patients with and without LVH (since shortening of PQ interval is found in FD patients with and without LVH). No pathophysiological explanation is given.
  - Validation cohort:
    - PQminusP < 40 ms: sensitivity (57%) and specificity (14%) in FD positive group, possibly due to milder cardiac phenotype in females than study Namdar et al 35.
1.2. Shortened PQ interval < 120 ms or corrected PQ < 144 ms as an entry criterion for FD diagnosis

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Patients, (controls)</th>
<th>Methods</th>
<th>Results</th>
<th>Comments/ Limitations</th>
<th>Validation cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Namdar et al 35</td>
<td>FD, classical phenotype, pre-ERT LVH n=17</td>
<td>Retrospective case-control study</td>
<td>PQ &lt; 120 ms Sensitivity 24% Specificity 100%</td>
<td>Small sample sizes</td>
<td>PQ &lt; 120 ms Positive group: 5% (1/21, 1 missing data) Negative group: 0%</td>
</tr>
<tr>
<td>Namdar et al 35</td>
<td>FD without LVH n=30 Healthy controls Age and heart rate matched</td>
<td>Retrospective case-control study</td>
<td>PQ &lt; 120 ms 13% (M+F)</td>
<td>Percentage of patients with LVH and PQ &lt; 120 ms unknown PQ interval similar in FD LVH+ and FD LVH-</td>
<td></td>
</tr>
<tr>
<td>Motwani et al 36</td>
<td>FD, pre-ERT LVH 46% n=207</td>
<td>Cross-sectional cohort study</td>
<td>PQ &lt; 120 ms 14% (M+F)</td>
<td>Percentage of patients with LVH and PQ &lt; 120 ms unknown</td>
<td></td>
</tr>
<tr>
<td>Niemann et al 37</td>
<td>FD, pre-ERT, LVH % unknown n=150 No controls</td>
<td>Cross-sectional cohort study</td>
<td>PQ &lt; 120 ms 16% males, 16% females</td>
<td>Percentage of patients with LVH and PQ &lt; 120 ms unknown</td>
<td></td>
</tr>
<tr>
<td>O’Mahony et al 38</td>
<td>FD, ERT 23% LVH 53% n=204 No controls</td>
<td>Retrospective longitudinal cohort study</td>
<td>PQ &lt; 120 ms 7% (M+F)</td>
<td>Percentage of patients with LVH and PQ &lt; 120 ms unknown</td>
<td></td>
</tr>
<tr>
<td>Sadik et al</td>
<td>FD, ERT unknown LVH 76% n=12 Controls n=42</td>
<td>Cross-sectional cohort study (controls for echocardiography only)</td>
<td>PQ &lt; 120 ms 17% (2 out of 12)</td>
<td>Percentage of patients with LVH and PQ &lt; 120 ms unknown</td>
<td></td>
</tr>
<tr>
<td>Senedchal et al 34</td>
<td>FD, classical males, ERT unknown LVH 50% n=20 No controls</td>
<td>Cross-sectional cohort study</td>
<td>PQ &lt; 120 ms 40% (M only)</td>
<td>Percentage of patients with LVH and PQ &lt; 120 ms unknown</td>
<td></td>
</tr>
<tr>
<td>Shah et al 34</td>
<td>FD, ERT 53%, LVH unknown % n=78 No controls</td>
<td>Longitudinal cohort study</td>
<td>PQ &lt; 120 ms 21% (M+F)</td>
<td>Percentage of patients with LVH and PQ &lt; 120 ms unknown</td>
<td></td>
</tr>
</tbody>
</table>

- Comments:
  - Studies:
    - Short PQ interval is not 100% specific for FD: shortening of PQ interval has been described in glycogen storage disorders 35 such as (late onset) Pompe 96,97 and Danon disease 71 sometimes presenting with a cardiomyopathy. These can easily be ruled out enzymatically or genetically.
  - Validation cohort:
    - PQ < 120 ms: sensitivity 5% (studies: sensitivity 7-40%), specificity 100%
    - Corrected PQ < 144 ms: sensitivity 28%, specificity 100%
  - General
    - Correction of PQ time for heart rate unusual
    - When short PQ interval or corrected PQ < 144 ms is present, FD diagnosis likely, when absent does not exclude FD.
    - No pathophysiological background?
1.3 Abnormally low voltages on ECG as an exit criterion for FD

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Patients, (controls)</th>
<th>Methods</th>
<th>Results</th>
<th>Comments/ Limitations</th>
<th>Validation cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Namdar et al 35</td>
<td>FD, classical phenotype, pre-ERT, all LVH, n=17</td>
<td>Retrospective case-control study</td>
<td>QTc &gt; 440ms + Sokolow-Lyon index ≤ 1.5 mV in FD: 0% in amyloidosis: 85%</td>
<td>Small sample sizes Sokolow-Lyon Index &lt; 1.5mV:  Positive group: 3/21 (2 with RBBB)</td>
<td>Negative group: 0/7</td>
</tr>
<tr>
<td></td>
<td>controls: HCM, cardiac amyloidosis, hypertensive heart disease, aortic stenosis age and LV mass matched</td>
<td>Abnormally low voltages defined as Sokolow-Lyon index ≤ 1.5 mV</td>
<td>Sokolow-Lyon index ≤ 1.5 mV in FD: 0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exclusion: all patients with CAD, WMA, atrial fibrillation, pacemaker, FD with hypertension and cardiac variant</td>
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</tr>
<tr>
<td>Hoigné et al 33</td>
<td>FD, classical phenotype 46% ERT all LVH n=13</td>
<td>Case-control study</td>
<td>Abnormally low voltages (total QRS amplitude in I, II, III &lt; 1.5mV) in FD: 0%</td>
<td>Small sample sizes Abnormally low voltages (total QRS amplitude in I, II, III &lt; 1.5mV)</td>
<td>Positive group: 0/21 Negative group: 0/7</td>
</tr>
<tr>
<td></td>
<td>controls: cardiac amyloidosis, non-obstructive HCM, hypertensive heart disease</td>
<td>Abnormally low voltages defined as total QRS amplitude in I, II, III &lt; 1.5mV</td>
<td>Absence of hypertension, pericardial effusion, abnormal papillary muscle or orthostatic hypotension: sensitivity 92%, specificity 87% for FD</td>
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<tr>
<td></td>
<td>not age gender or LV mass matched</td>
<td></td>
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<tr>
<td></td>
<td>Exclusion: transmural myocardial infarct, asymmetric septal hypertrophy, LVOTO</td>
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</tbody>
</table>

- Comments:
  - Studies
    - Sokolow-Lyon index: single study in rather small cohorts of different cardiomyopathies 35
    - Sokolow-Lyon index: a tool for LVH assessment, possibly unsuitable tool to assess abnormally low voltages.
    - Abnormally low voltages measured with total QRS amplitude in I, II, III < 1.5 mV 0% in FD. Single study on rather small cohorts.
  - Validation cohort
    - Sokolow-Lyon index used to assess low voltages presumably not reliable in patients with right bundle branch block, a frequent finding in FD.
2. Cardiac echocardiography: possibly echocardiography criteria

2.1. Pericardial effusion as an exit criterion for FD diagnosis

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Patients (controls)</th>
<th>Methods</th>
<th>Results</th>
<th>Comments/ Limitations</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoigné et al [33]</td>
<td>FD, classical phenotype 46% ERT all LVH n=13 controls: cardiac amyloidosis, non-obstructive HCM, hypertensive heart disease Exclusion: transmural myocardial infarct, asymmetric septal hypertrophy, LVOTO</td>
<td>Case-control study Pericardial effusion</td>
<td>0% in FD 38% amyloidosis 6% HCM 5% HDD</td>
<td>Small sample sizes HCM not genetically proven Controls not age gender or LV mass matched</td>
<td>Pericardial effusion: Positive Group: 0/22 Negative group: n=4/7 Limited amount of PE on echocardiography or CMR</td>
</tr>
</tbody>
</table>

- **Comments**
  - **Studies**
    - Single study with small sample sizes
  - **Validation cohort**
    - Small amount of pericardial effusion is present in 4/7 patients in negative group: when is amount of pericardial effusion clinically significant?
### 2.2. Left ventricular outflow tract obstruction (LVOTO) as an exit criterion for FD diagnosis

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Patients, (controls)</th>
<th>Methods</th>
<th>Results</th>
<th>Comments/ Limitations</th>
<th>Validation cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kounas et al 41</td>
<td>FD, ERT 100%; n=14; LVH 86%</td>
<td>Retrospective case-control study</td>
<td>LVOTO= LVOT gradient ≥30 mmHg</td>
<td>Small sample size Not all patients LVH</td>
<td>LVOTO Positive group: 1/22 possible LVOTO, coinciding with mild SAM</td>
</tr>
<tr>
<td></td>
<td>Controls: HCM</td>
<td></td>
<td>In FD: 0% In HCM: 29%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age gender maximal LV wall thickness matched</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mundigler et al 42</td>
<td>FD, ERT; n=14; LVH 100%</td>
<td>Retrospective case-control study</td>
<td>LVOTO not defined</td>
<td></td>
<td>Negative group: 0/7</td>
</tr>
<tr>
<td></td>
<td>Controls (n=295): valvular disease, CAD, HCM, hypertension, heart transplantation, arrhythmias, other</td>
<td></td>
<td>In FD: 0% In HCM:52%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not age gender or LV matched</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Exclusion: FD patients without LVH</td>
<td></td>
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</tr>
<tr>
<td>Pieroni et al 43</td>
<td>FD, ERT 100%; LVH: 83%; n=40</td>
<td>Case-control study</td>
<td>LVOT &gt; 30 mmHg at rest</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Controls: Hypertensive heart disease, HCM, age matched healthy controls</td>
<td></td>
<td>In FD: 0% In HCM: 25%</td>
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</tbody>
</table>

**Comments**

- **Other studies:**
  - Resting left ventricular outflow tract obstruction is a relatively frequent finding in HCM 41-43, and has rarely been described in FD. It might therefore be of interest as an exit criterion for FD. However, some FD cases with LVOTO have been described 93;96-101. In the majority of these cases this coincided with an asymmetrical septum instead of a concentric hypertrophy, which is less often found in FD 102. It is important to note that it is unclear whether HCM was genetically ruled out in these cases.
  - Validation cohort:
    - 1 patient with possible LVOTO. To be discussed: practical cut off value for LVOTO in practice
2.3 Hypertrophied papillary muscle as an entry criterion for FD diagnosis

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Patients, (controls)</th>
<th>Methods</th>
<th>Results</th>
<th>Comments/Limitations</th>
<th>Validation cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niemann et al 39</td>
<td>FD, ERT: unknown LVH 100% n=28 controls: Friedreich ataxia, HDD, amyloidosis and healthy controls. Exclusion: Valvular heart disease, DM, other endocrine and systemic disease</td>
<td>Prospective case-control study Prominent papillary muscle: (increase absolute papillary muscle area and relative to LV cavity)</td>
<td>LV mass was rather high in FD compared to some of the other CM Not investigated in HCM (asymmetric or concentric) Measurement of papillary muscle laborious. Eyeballing possibly sufficient, but sensitivity and specificity not assessed with eyeballing technique. Significant overlap with HDD No clear cut-off values for increased size</td>
<td>By eyeballing mentioned in two FD patients in positive group on CMR. Not structurally assessed in patients, papillary muscle size not routinely measured.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>papillary muscle measurement: absolute papillary muscle area, relative to LV cavity, also with eyeballing technique</td>
<td>FD: Sensitivity 75% specificity 86% In FD: when LV wall &gt; 13 mm: 100% of FD patients with increased papillary muscle, unknown in controls</td>
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<tr>
<td></td>
<td></td>
<td>By eyeballing: in FD 19/21 patients with increased papillary muscle were identified</td>
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</tbody>
</table>

**Comments**

- Study
- Not investigated in HCM (symmetrical and asymmetrical), which is in the differential diagnosis of FD.
- In comparison to some of the other investigated subtypes of hypertrophic cardiomyopathies, Fabry patients had rather large LV dimensions. Since LV mass correlated to papillary muscle size, it cannot be excluded that the specificity would have been lower when all patients would have been LV mass matched.
- No cut-off values could be generated since values of different cardiomyopathies were overlapping.
- Implication in practice: the authors conclude that eyeballing instead of exact measurement of papillary muscle is sufficient to recognize the hypertrophied muscle. But this was only done in FD patients. Sensitivity and specificity not assessed with eyeballing technique. Surprisingly, a study of Hoigné et al 33 found an increased papillary muscle by eyeballing technique in only 1/13 FD patients. In our validation cohort it is only described twice on CMR. This can possibly be explained by different severity of phenotype or that assessor of echocardiography/CMR should be focussed on papillary muscle.
3. Cardiac Magnetic Resonance Imaging (CMR): possible CMR criteria

3.1. Late enhancement (LE) in papillary muscle as an exit criterion for FD

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Patients, (controls)</th>
<th>Methods</th>
<th>Results</th>
<th>Comments/ Limitations</th>
<th>Validation cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pieroni et al</td>
<td>FD, ERT: 100%, LVH: 83%, n=40 (n=20 with MWT≥ 15 mm)</td>
<td>Case-control study</td>
<td>Late enhancement: In FD with LVH (MWT≥ 15 mm): 50%</td>
<td>LE papillary muscle: Positive group: 0/15</td>
<td>Negative group: 0/3</td>
</tr>
<tr>
<td>Controls: Hypertensive heart disease, HCM, age, gender matched healthy controls</td>
<td></td>
<td></td>
<td>All basal or mid-lateral or infero-lateral, some apical, none papillary</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>In HCM: 50% LE: mostly (no % given) interventricular and papillary, but 9/20 with concentric LVH also basal segment lateral inferior</td>
<td></td>
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</tr>
<tr>
<td>De Cobelli et al</td>
<td>FD, ERT: unknown LVH 100%, n=13</td>
<td>Retrospective case-control study</td>
<td>LE: FD: 77% (10/13)</td>
<td>HCM: 89% (8/9) LE mostly basal or mid inferior lateral wall</td>
<td>LE papillary muscle 1/8</td>
</tr>
<tr>
<td>Controls: Symmetrical HCM</td>
<td></td>
<td></td>
<td>LE: all mid and basal infero-lateral, in some with apical anterior and lateral extension, none papillary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age matched</td>
<td></td>
<td></td>
<td>HCM: asymmetric, LVOTO, septal alcohol ablation/myomectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excluded HCM: asymmetric, LVOTO, septal alcohol ablation/myomectomy</td>
<td></td>
<td></td>
<td>LE papillary muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beer et al</td>
<td>FD, pre-ERT LVH: unknown % n=35</td>
<td>Prospective longitudinal cohort study</td>
<td>FD LE 35% (11/35) location missing n=3</td>
<td>LE: 50%</td>
<td>None in papillary muscle</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>n=6 infero lateral n=2 septal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moon et al</td>
<td>FD, ERT: unknown LVH: 53% n=26</td>
<td>Prospective cohort study</td>
<td>LE: 50%</td>
<td></td>
<td>None in papillary muscle</td>
</tr>
<tr>
<td>Niemann et al</td>
<td>FD, pre-ERT LVH: ≤ 38% n=104</td>
<td>Cross-sectional cohort study</td>
<td>LE: 55% in FD with LVH</td>
<td></td>
<td>None in papillary muscle</td>
</tr>
</tbody>
</table>

Other studies:

- In FD in the majority of LE is found in LV basal en mid infero-lateral or postero-lateral segments \(^{43-47,72-77}\), it can also expand to the anterior and lateral apex \(^{45}\), mid and basal septum \(^{47}\) and is rarely found restricted to (antero) septal segments (n=2) \(^{73}\).

- Significant overlap LE pattern HCM and FD in study de Cobelli and Pieroni et al.

- Incidence of LE in papillary muscle in HCM differs greatly between these studies. When incidence of LE in papillary in HCM is low, LE in papillary muscle possibly unsuitable exit criterion.

- To be discussed: feasibility of assessment LE in papillary muscle; easily missed etc.
4. Onset of LVH in Fabry disease below the age of 25 as an exit criterion for Fabry disease

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Patients, (controls)</th>
<th>Methods</th>
<th>Results</th>
<th>Comments/ Limitations</th>
<th>Validation cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niemann et al 47</td>
<td>FD, ERT 0% 58 males (7-66 years), 46 females (10-71 years)</td>
<td>Definition LVH: LVWT &gt;12 mm on echocardiography</td>
<td>Youngest male 20 years</td>
<td>Precise data on severity of LVH not given</td>
<td>No longitudinal data available of positive group</td>
</tr>
<tr>
<td>Wu et al 162</td>
<td>FD patients &gt; 16 years, ERT 0% 92 males (13-75 years), 47 females (13-71 years)</td>
<td>Definition LVH: LVMi g/m2 &gt; 95 g/m2 for females and 115 g/m2 for males on echocardiography</td>
<td>LVH present in age group 10-29 years (male and female)</td>
<td>Number of patients with LVH, 25 years not given, precise data on severity of LVH not given</td>
<td>Negative group: 5 missing data, n=2 LVH below age of 25</td>
</tr>
<tr>
<td>Ramaswami et al 163</td>
<td>FD patients &lt; 7 year ERT 0% 7 males (2,5- 6 year), 1 females (4 year)</td>
<td>Definition of LVH: LVMi/m2.7 &gt; 95 % CI by echocardiography</td>
<td>LVH 1 male 4 year 52.3 g/m2.7</td>
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<tr>
<td>Kampmann et al 164</td>
<td>FD patients ERT 0% 55 females (6-70 years)</td>
<td>Definition LVH: LVMi &gt; 47 g/m2 body mass index (BMI) &lt; 26 kg/m2 Or &gt; 60 g/m2 in BMI &gt; 26 kg/m2 by echocardiography</td>
<td>Youngest female 14 years</td>
<td>No precise data on severity of LVH in this patient</td>
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</tr>
<tr>
<td>Kampmann et al 167</td>
<td>FD patients ERT 0% 100 females, 66 males (3.3-70.8 years)</td>
<td>Definition LVH: LVMi &gt; 48 g/m2 for females; &gt; 51 g/m2 for males by echocardiography</td>
<td>Youngest male 24 years, female 14 years</td>
<td>No precise data on severity of LVH in this patient</td>
<td></td>
</tr>
<tr>
<td>Kampmann et al 166</td>
<td>FD patients, &lt; 18 year ERT unknown 12 females (10-15 years), 8 males (6-16 years)</td>
<td>Definition LVH: LVMi &gt; 50 g/m2.7 by echocardiography</td>
<td>LVH below age 18: 2 males 5 females</td>
<td>No precise data on severity of LVH in these patients</td>
<td></td>
</tr>
<tr>
<td>Ries et al 165</td>
<td>FD patients ERT unknown 25 males (6-18 years)</td>
<td>Definition LVH: LVMi &gt; 61 g/m2 for adults and &gt; 95th percentile for children by echocardiography</td>
<td>7 patients &lt; 18 year with LVH (40.4-55.6 g/m2), youngest male 6 years with LVH: 55.6 g/m2.7</td>
<td>No precise data on severity of LVH in this patient</td>
<td></td>
</tr>
<tr>
<td>Hughes et al 167</td>
<td>FD patients &gt; 18 year ERT 0% 15 males (23.1-50.8 year)</td>
<td>Definition LVH: LVM &gt; 134 g/m2 by echocardiography</td>
<td>Youngest patients with LVH 23 year</td>
<td>No precise data on severity of LVH or gender in this patient</td>
<td></td>
</tr>
<tr>
<td>Weidemann et al 164</td>
<td>FD patients ERT 0% 14 males (24-55year), 2 females (5-58years)</td>
<td>Definition LVH: not defined</td>
<td>Youngest male patient with LVH 24 year</td>
<td>No precise data on severity of LVH in this patient</td>
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</tbody>
</table>

- **Comments**
  - **Studies:**
    - Only cross-sectional studies available: this may cause an underestimation of age of onset.
    - Different definitions of LVH are being used
    - Data on precise severity of LVH, contribution of other cardiac diseases often lacking.
Chapter 3

The age at which LVH occurs in Fabry disease has been reported in many studies. Although it is believed that LVH generally develops at adulthood, LVH has been described in a substantial number of patients < 25 years old, the youngest being a 4 year old male \(^{103}\) and a 14 year old female \(^{102}\). Unfortunately, most studies use different LVH definitions, this troubles comparing LVH severity between studies. Furthermore, many studies do not comment whether other cardiac diseases were ruled out in these patients. Lastly, there are many cross-sectional, but no longitudinal studies that report on the age of onset of LVH. Therefore an underestimation of LVH at young age is probably made. Whether severe LVH (i.e. MWTd > 15 mm) below the age of 25 is present at young age could not be answered by literature since articles use different units of expressing LVH and data on severity of LVH are often lacking.

Other criteria

5. Other ECG parameters

5.1. Sinus bradycardia

In some studies it has been found that the heart rate of Fabry patients in comparison to other cardiomyopathies is generally low \(^{33,35,39}\). However, sinus bradycardia is not a specific sign. The prevalence of sinus bradycardia in FD ranges from 16.4 -72% in males and females (defined as heart rate < 60 bpm in \(^{109}\), not defined in \(^{100,110}\)). It was not reported whether the prevalence is higher in FD patients with LVH. Whether absence of sinus bradycardia in an FD patient precludes a diagnosis of FD has not been investigated.

5.2 Short p-wave

In two studies the prevalence of a short p-wave (< 80 msec.) in FD with and one without LVH, ranged from 85-92% \(^{89}\). However, in the previously mentioned study of Namdar et al \(^{35}\) no differences in mean p-wave duration could be found between FD and other cardiomyopathies. This difference can possibly be explained by the larger left atrial size in the latter study, which is known to influence p-wave length. Since p-wave is influenced by atrial size, Namdar et al concluded that this marker is less reliable than PQ interval minus P wave duration to detect FD.

6. Other echocardiography parameters

6.1. Absence of RVH with presence of LVH

Right ventricular hypertrophy (RVH) was present in 65-94% of FD patients with LVH \(^{40,75,111}\). The degree of RVH in FD was never compared to other cardiomyopathies with echocardiography. One study investigates RVH with ECG \(^{35}\), wherein FD RVH was less pronounced than in other cardiomyopathies. Since all FD patients with severe LVH (> 75 g/m²) also had RVH (right ventricle anterior wall thickness > 6mm) the absence of RVH when severe LVH is present, could therefore serve as an exit criterion for FD diagnosis \(^{40}\). However, whether this is specific for FD is unknown since this was never compared to other cardiomyopathies. Furthermore, severe LVH defined as > 75 g/m² is an impractical clinical tool and assessment if RVH by echocardiography is not very reliable.

6.2. Speckle tracking

One study investigated longitudinal and rotational mechanics with speckle tracking in FD, versus HCM \(^{112}\). In contrast to FD patients, HCM patients had an increase of systolic global circumferential strain (GCS), whereas FD patients, with or without LVH, had a decrease of systolic global longitudinal and circumferential strain (GCS and GSL). However, other studies reported an increase as well as a decrease of GCS in HCM \(^{112}\). Although the findings of this study are very interesting, we did not include GSL or GSL as robust feature to discern FD from HCM because of the following reasons; Gruner et al could not (yet) generate cut-off values to discern FD
Consensus recommendation: left ventricular hypertrophy

from HCM, FD was only compared to HCM and not to other cardiomyopathies and values appeared variable in different HCM cohorts.

6.3. Tissue Doppler imaging (TDI)
TDI can detect early changes of systolic and diastolic dysfunction in FD patients even without LVH \(^{102,113-115}\). Zamorano et al showed that early TDI decreases later progresses to LVH. Similar findings have been found in other cardiomyopathies \(^{115}\) and are therefore not specific for FD. In a report by Sadick et al\(^{93}\), especially the E’ velocity was decreased with a corresponding decrease in E’/A’ ratio, compared to controls. However, decreased E’ velocity is also described in other cardiomyopathies.

6.4. Concentric versus asymmetrical septal or eccentric hypertrophy
The presence of an asymmetrical septal or eccentric hypertrophy does not rule out the presence of FD, although the majority of FD patients suffer from a concentric hypertrophy. A rather large study of untreated FD patients (n=166) showed that roughly 5% of FD patients had an asymmetrical septal hypertrophy and 33% an eccentric hypertrophy \(^{102}\).

6.5. Binary sign
Multiple studies have investigated a binary appearance of the left endomyocardial border as a pathognomonic sign of Fabry disease \(^{41,42,116-118}\). A study of Pieroni et al showed a high sensitivity of 94% and a specificity of 100% for FD patients with maximal wall thickness of ≥ 15 mm, compared to a cohort of healthy controls, hypertensive LVH and HCM. Other studies found much lower sensitivities in FD, ranging from 12.5- 44%. In contrast to the study of Pieroni et al, the binary sign was present in 20% in a study with a rather large control group with LVH of another cause than FD \(^{42}\). Possible explanations for the divergent results are the poor reproducibility, variability caused by echocardiography settings and different software, phenotypic variation of FD, and size of the control groups \(^{42}\). Because the data of Pieroni et al were poorly reproducible and the binary sign was found in a significant number of other cardiomyopathies we did not consider the binary sign a reliable parameter for FD diagnosis.

6.6. Other
The following identified factors were (sometimes) present in FD but are not specific for FD: diminished ejection fraction or (severity of) diastolic dysfunction \(^{33,39,116}\) or mitral valve thickening \(^{33}\).

7. Other CMR parameters

7.1. Aortic dilatation
Thoracic aortic dilatation in FD patients has been reported in different studies with echocardiography and CMR, albeit with different definitions of dilatation \(^{91,94,98,119,120}\). The prevalence of aortic dilatation in FD, irrespective of LVH, ranged from 10-56% in classically affected males and 0-21.1% in females. The largest study (n=106) reported a prevalence of 5.6% and 21.1% of dilatation of the aorta respectively at the sinus of Valsalva or the ascending aorta in females, and 32.7 % and 29.6% in males \(^{120}\). Since LV mass correlates with aortic dilatation \(^{120}\), we expect that the percentage of an aortic dilatation to be even higher in FD patients with LVH. The percentage of aortic dilatation in FD patients with LVH could only be assessed from a rather small study in which 63% (n=5 out of 8) male and 33% (n=1 out of 3) female FD patients with LVH presented with aortic dilatation. FD Aortic dilatation was never actively compared to other cardiomyopathies; therefore no specificity could be calculated. Importantly, aortic dilatation is frequently described in hypertensive patients with LVH (±10%) \(^{121}\), but also in those with mitochondrial cardiomyopathy \(^{122}\) and Noonan’s syndrome\(^{123}\).
7.2. 

**T₂ relaxation time**

Imbroaco et al. found an increased T₂ relaxation time in a relatively small FD cohort as compared to HCM and healthy controls. Interestingly, increased T₂ relaxation times could also be found in three women without LVH. However, albeit less pronounced, T₂ relaxation time was also increased in HCM and overlapped with some FD patients. Since no clear cut-off values could be generated to distinguish FD from HCM, data would preferably have to be confirmed in larger cohorts and compared to other cardiomyopathies.

7.3. Extracellular volume (ECV)

Sado et al. hypothesized that the extracellular volume (ECV) could be increased in different cardiomyopathies in equilibrium contrast CMR. They compared ECV between healthy controls, Fabry disease (80% with LVH), HCM, dilated and hypertrophied cardiomyopathy, severe aortic stenosis, cardiac amyloidosis and myocardial infarction. While septal ECV was markedly elevated in patients with cardiac amyloidosis and myocardial infarcts, FD did not differ from healthy controls. Septal ECV might therefore be an interesting tool to discern cardiac amyloidosis from Fabry disease. Limitations of this study are that all FD patients were on ERT and that ECV was measured only in the septum. The authors note that it cannot be excluded that ECV when measured in the total myocardium could be increased in FD. Also, there was a large overlap between FD, HCM DCM and AS. Although the findings of this study are very interesting, we did not adopt in the algorithm because the technique is not yet implemented in daily care and earlier mentioned limitations.

**Unspecific CMR parameters for FD**

7.4. Late enhancement: location and distribution throughout the wall (excluding LE in papillary muscles)

The presence of late enhancement in FD has been investigated in many studies and has been found in FD males and females with LVH, as well as in FD females without LVH. Selecting only patients with LVH (which was defined differently within studies) LE presence ranged from 48-77% (males and females combined). Although it has been suggested that the localization and distribution of LE has a specific pattern for FD distinguishable from symmetrical HCM, this has been refuted by larger studies. Two studies showed that the LE pattern in HCM often significantly overlaps with FD. In contrast to HCM, LE was never detected in papillary muscles in FD, which might serve as an exit criterion.

It has been suggested that the distribution of LE within the wall in FD is always mesocardial, whereas in HCM both subendocardial and mesocardial. This contradicts to other (larger) cohort studies where LE was also found subendocardial, epicardial and even transmural.

7.5. CMR-tagging

CMR tagging is a powerful method to assess subtle changes in cardiac motion. Long axis shortening, circumferential contraction as well as apical rotation and torsional deformation appear abnormal in FD cardiomyopathy. But since this has also been found in other cardiomyopathies e.g. as the consequence of aortic stenosis or hypertension, it is not a specific finding for FD.