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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 ☆

B.E. Smid [a], L. van der Tol [a], F. Cecchi [e], P.M. Elliott [f], D.A. Hughes [g], G.E. Linthorst [a], J. Timmermans [h], F. Weidemann [i], M.L. West [j], M. Biegstraaten [a], R.H. Lekanne Deprez [b], S. Florquin [c], P.G. Postema [d], B. Tomberli [e], A.C. van der Wal [c], M.A. van den Bergh Weerman [c], C.E. Hollak [a,*]

[a] Department of Endocrinology and Metabolism, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands
[b] Clinical Genetics, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands
[c] Department of Pathology, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands
[d] Department of Cardiology, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands
[e] Department of Clinical and Experimental Medicine, University of Florence, Italy
[f] Department of Cardiology, Heart Hospital, London, UK
[g] Department of Haematology, Royal Free & University College Medical School, London, UK
[h] Department of Cardiology, RadboudUMC, Nijmegen, The Netherlands
[i] Department of Cardiology, Comprehensive Heart Failure Centre, University Hospital Würzburg, Germany
[j] Department of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada

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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 > 15 mm) <20 years exclude FD. Other criteria were rejected due to insufficient evidence.
Conclusions: 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.

1. Introduction

Fabry disease (FD; OMIM 301500 [2]) 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 [3–5]. Over 670 mutations in the α-galactosidase A (GLA) gene have been described [6], 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 [7–16]. 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) [17–19].

Interestingly, most males with such GVUS demonstrate significant residual AGAL-A enzyme activity, in contrast to the absent or near

Abbreviations: FD, Fabry disease; LVH, left ventricular hypertrophy; HCM, hypertrophic cardiomyopathy; AGAL-A, alpha-galactosidase A; GLA, alpha-galactosidase A gene; MWT, maximal wall thickness; GVUS, genetic variant of unknown significance; ERT, enzyme replacement therapy; lysoGb3, globotriaosylsphingosine.

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

* Corresponding author. Tel.: +31 20 5666071; fax: +31 20 6917682.
E-mail address: c.e.hollak@amc.uva.nl (C.E. Hollak).

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absent enzyme activity in classically affected males [5]. 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 [20]. Because these subjects have symptoms restricted to single organs, they were coined as cardiac, renal or late onset variants of the disease [21,22]. 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 [23–25]. While some still consider the p.D313Y mutation pathogenic [26,27], 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 [28]. 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 [17]. These examples show that the lack of unequivocal definitions for a definite FD diagnosis leads to difficult clinical dilemmas with a risk of misdiagnosis. Early diagnosis of a true FD patient is of great importance to offer adequate support, but prompt identification of those without FD is of equal importance to avoid distress in families and inappropriate initiation of ERT, an invasive and extremely expensive treatment.

As part of the Hamlet study [1], 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.

2. Methods

2.1. Delphi participants

We used a modified Delphi procedure [29] 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.

2.2. 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) [30] 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).

2.3. 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 $m^{2.7}$ for females, and ≥51 g/height in $m^{2.7}$ for males [31]). 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 angiokeratoma) 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 [32], Sokolow–Lyon index to assess left ventricular hypertrophy and the sum of the QRS amplitudes in lead I + II + III < 1.5 mV [33,34] as well as a Sokolow–Lyon index of <1.5 mV [35] to 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.

2.4. 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 [36]. 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.

2.5 Statistical considerations: selection of final items in diagnostic algorithm

In keeping with previous studies [37] 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 [38], with 0 indicating no consensus and 1 full consensus. The following recommendations by Bland and Altman were applied: Cronbach's α should be above 0.9, but preferably above 0.95 for clinical applications [38]. SPSS version 19 was used for statistical analyses.

3. Results

3.1. 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.

3.2. 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 Fig. 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 [39-47,72-77,89-126]).

3.3. 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.

3.3.1. 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 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.

3.3.2 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 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.

3.3.3 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 [21,56–58]. 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 (Fig. 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.

Characteristic storage was defined as concentric multi-lamellated myelin bodies with a zebra-like pattern (zebra bodies) with a periodicity of approximately 5 nm [59], in the absence of drug use known to induce these inclusion bodies (such as chloroquine or amiodarone [50,60]). Although these inclusion bodies can be found in other lysosomal storage disorders [61–63], 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 [14,56,64,65]. 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.

3.3.4 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.

Table 2
Definitions of a definite and uncertain Fabry diagnosis.

Definite diagnosis of FD	
Males	**Females**
GLA mutation	GLA mutation
+	+
AGAL–Adeficiency ≤5% of mean reference value in leukocytes (39)	normal or deficient AGAL–A in leukocytes (39)
+	+
A or B or C	
A	
≥1 characteristic FD sign/symptom (Fabry neuropathic pain, cornea verticillata or clustered angiokeratoma)	
B	
(when available) an increase of plasma (lyso) Gb3 (within range of males with definite FD diagnosis)	
C	
A family member with a definite FD diagnosis carrying the same GLA mutation	
Uncertain diagnosis of FD in subjects presenting with a non–specific FD sign	
Males/Females	
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	

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.

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

Fabry diagnosis	Entry criteria	Sen. %	Spec. %	Exit criteria	Prev. %
ECG	PQ interval minus P wave duration < 40 ms (35)	82	99	Low voltages: Sokolow–Lyon index of ≤1.5 mV total QRS amplitude in I, II, III < 1.5 mV (33, 35)	0
	Corrected PQ interval < 144 ms (35)	82	90		
	PQ < 120 ms (35)	24	100		
Cardiac echocardiography	Increased papillary muscle			Severe LVH without right ventricle hypertrophy (40)	ND
	-LV wall ≥ 12 mm	75	86		
	-When LV wall is >13 mm (39)	100	ND		
				LVOTO ((41-43)	0
				Pericardial effusion (33)	0
CMR				Late enhancement in papillary muscles (43-47)	0

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.

Most criteria were not selected because they were not specific enough e.g. specificity < 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).

3.4 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) [34]" 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 [14,65–68]. 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.

3.4.1 Severe LVH at young age excludes FD

Fig. 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 (Fig. 2).

3.4.2 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 [69]. 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 [33], 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 (Fig. 1).

3.4.3 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

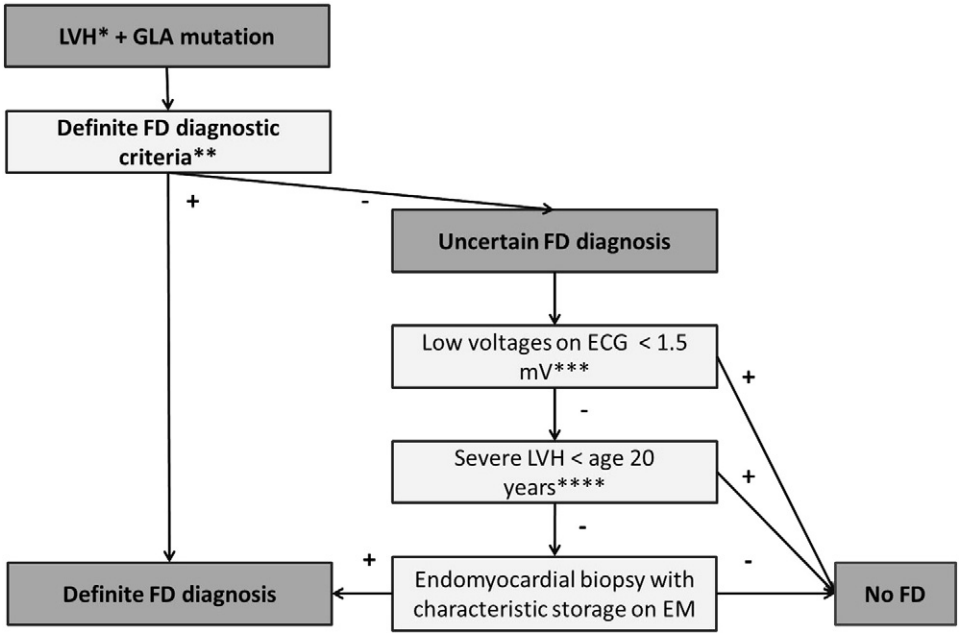

Fig. 1. Proposal for a diagnostic algorithm for subjects presenting with isolated LVH and an uncertain diagnosis of FD. *LVH: left ventricular hypertrophy defined as an MWTd > 12 mm, **see Table 2, ***low voltages on ECG defined as the total sum of the amplitude of the QRS complex in I, II, III < 1.5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

males, n = 5 with LVH) had a PQ interval < 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 [70] such as Danon disease [71]. 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.

4. 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 [59], 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 [63]. 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 [14,17,18,22,65,78,79], many did not [7,8,15,80] or performed EMB without EM analysis [9,17].

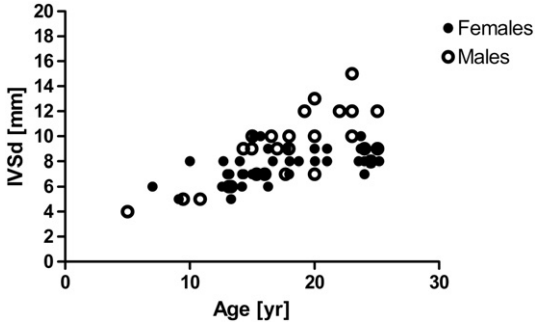

Fig. 2. Distribution of interventricular septum thickness in diastole (IVSd) in FD patients' age of ≤25 years (n = 67).

Table 3
Red flags suggestive of a diagnosis of FD. These criteria make a diagnosis of FD likely, but are not fully specific for FD.

Red flags to suspect Fabry disease	
ECG	PQ interval < 120 ms (35) Sinus bradycardia (33, 35, 39)
Echocardiography	Hypertrophied papillary muscle (39)
CMR	Myocardial late enhancement infero–postero–lateral region (43-47, 72-77)

Safety issues often restrain clinicians from performing EMB. The serious adverse event rate of EMB is reported to be 0.12–2% [63,65,81]. 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 [20]). 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 [50,60]. 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 [66,68,82], 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 [23]. In addition, several centres have published mass spectrometric methods capable to detect even small increases of lysoGb3 above normal [83–86]. A classification of pathogenicity of GLA mutations based on plasma lysoGb3 levels has recently been proposed [25]. In our experience slightly elevated levels of lysoGb3 are often found in non-classical patients with a biopsy proven diagnosis of FD [87]. 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

Table 4
Baseline characteristics of FD and HCM patients for supplementary data analysis.

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

Abbreviations: AF: atrial fibrillation, FD: Fabry disease, HCM: hypertrophic cardiomyopathy, IVSd: interventricular septum thickness in diastole, MWTd: maximal wall thickness in diastole.

families [87]. 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 [88]. 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 [7–9,12–16]. 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.

5. 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.

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Contribution

BS: study design, systematic review, data acquisition and interpretation, preparation and analyses of Delphi voting rounds, drafting of the manuscript.

LT: study design, data acquisition and interpretation, preparation and analyses of Delphi voting rounds, revision of the manuscript.

MB: study design, preparation and analyses of Delphi voting rounds, revision of the manuscript.

CH: study design, data interpretation, preparation and analyses of Delphi voting rounds, group leader face-to-face meeting, helped in drafting of the manuscript.

GL, PE, DH, MW, JT, FW, FC: formed the expert panel, revision of the manuscript.

FC, BT, PP, FW: analysis of ECG and ultrasounds, revision of the manuscript.

SF, MBW, FW: advised on the interpretation of pathological analyses, revision of the manuscript.

LD: advised on the interpretation of genetic tests, revision of the manuscript.

Conflict of interests

No honorarium was provided for participation in this consensus recommendation.

BS and LT received travel support and reimbursement of expenses from Genzyme, a Sanofi company, Shire HGT and Actelion.

MB, GL and CH have received honoraria for consultancies and speakers fees from Actelion, Genzyme, Shire HGT, Protalix or Amicus. All fees are donated to the Gaucher Stichting or the AMC Medical Research BV for research support.

FC has received reimbursement of expenses and honoraria for lectures from Genzyme, a Sanofi company.

PE has received honoraria for speaking or consultancy fees from Genzyme, a Sanofi company and Shire HGT.

DH has received research and travel grants, honoraria for speaking or consultancy fees from Genzyme, a Sanofi company, Shire HGT, Amicus/GSK and Protalix.

FW received a reimbursement of expenses and honoraria for lectures on the management of lysosomal storage diseases from Genzyme a Sanofi company and Shire HGT.

MW received research funds, honoraria and consultant fees from Actelion Pharmaceuticals, Amicus Therapeutics, Genzyme a Sanofi company, GlaxoSmithKline, Shire Human Genetic Therapies and Sumitomo Pharma.

BT received travel support and reimbursement of expenses from Genzyme and Shire HGT.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2014.09.001.

References

[1] Hamlet study: NTR3840 and NTR3841. www.trialregister.nl. [31-5-2013. 31-5-2013. Ref Type: Internet Communication].
[2] Online Mendelian Inheritance of Man (OMIM). http://www.omim.org. [24-6-2013. Ref Type: Online Source].
[3] Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. JAMA Jan. 20, 1999;281(3):249–54.
[4] Poorthuis BJ, Wevers RA, Kleijer WJ, et al. The frequency of lysosomal storage diseases in The Netherlands. Hum Genet 1999 Jul;105(1-2):151–6.
[5] Desnick RJ, Ioannou YA, Eng ME. Alpha-galactosidase A deficiency: Fabry disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular bases of inherited disease. 8th ed. New York: McGraw-Hill; 2001. p. 3733–74.
[6] The Human Gene Mutation Database. http://www.hgmd.cf.ac.uk. [1-6-2013. Ref Type: Internet Communication].
[7] Sachdev B, Takenaka T, Teraguchi H, et al. Prevalence of Anderson–Fabry disease in male patients with late onset hypertrophic cardiomyopathy. Circulation Mar. 26, 2002;105(12):1407–11.
[8] Elliott P, Baker R, Pasquale F, et al. Prevalence of Anderson–Fabry disease in patients with hypertrophic cardiomyopathy: the European Anderson–Fabry Disease Survey. Heart Dec. 1, 2011;97(23):1957–60.
[9] Havndrup O, Christiansen M, Stoevring B, et al. Fabry disease mimicking hypertrophic cardiomyopathy: genetic screening needed for establishing the diagnosis in women. Eur J Heart Fail Jun. 1, 2010;12(6):535–40.
[10] Monserrat L, Gimeno-Blanes JR, Marin F, et al. Prevalence of Fabry disease in a cohort of 508 unrelated patients with hypertrophic cardiomyopathy. J Am Coll Cardiol Dec. 18, 2007;50(25):2399–403.
[11] Mechtler TP, Stary S, Metz TF, et al. Neonatal screening for lysosomal storage disorders: feasibility and incidence from a nationwide study in Austria. Lancet Jan. 28, 2012;379(9813):335–41.
[12] Hwu WL, Chien YH, Lee NC, et al. Newborn screening for Fabry disease in Taiwan reveals a high incidence of the later-onset GLA mutation c.936 + 919G > A (IVS4 + 919G > A). Hum Mutat Oct. 2009;30(10):1397–405.
[13] Spada M, Pagliardini S, Yasuda M, et al. High incidence of later-onset Fabry disease revealed by newborn screening. Am J Hum Genet Jul. 2006;79(1):31–40.
[14] Chimenti C, Pieroni M, Morgante E, et al. Prevalence of Fabry disease in female patients with late-onset hypertrophic cardiomyopathy. Circulation Aug. 31, 2004;110(9):1047–53.
[15] Hagege AA, Caudron E, Damy T, et al. Screening patients with hypertrophic cardiomyopathy for Fabry disease using a filter-paper test: the FOCUS study. Heart Jan. 2011;97(2):131–6.
[16] Palecek T, Honzikova J, Poupetova H, et al. Prevalence of Fabry disease in male patients with unexplained left ventricular hypertrophy in primary cardiology practice: prospective Fabry cardiomyopathy screening study (FACSS). J Inherit Metab Dis Oct. 31, 2013;37(3):455–60.
[17] Terryn W, Vanholder R, Hemelsoet D, et al. Questioning the pathogenic role of the GLA p.Ala143Thr 'mutation' in Fabry Disease: implications for screening studies and ERT. JIMD Rep 2013;8:101–8.
[18] Kobayashi M, Ohashi T, Fukuda T, et al. No accumulation of globotriaosylceramide in the heart of a patient with the E66Q mutation in the alpha-galactosidase A gene. Mol Genet Metab Dec. 2012;107(4):711–5.
[19] Tol van der L, Smid BE, Poorthuis BJ, et al. A systematic review on screening for Fabry disease: prevalence of individuals with genetic variants of unknown significance. J Med Genet Jan. 2014;51(1):1–9.
[20] Yousef Z, Elliott PM, Cecchi F, et al. Left ventricular hypertrophy in Fabry disease: a practical approach to diagnosis. Eur Heart J Jul. 24, 2012;11(34):802–8.
[21] von Scheidt W, Eng CM, Fitzmaurice TF, et al. An atypical variant of Fabry's disease with manifestations confined to the myocardium. N Engl J Med Feb. 7, 1991;324(6):395–9.
[22] Nakao S, Takenaka T, Maeda M, et al. An atypical variant of Fabry's disease in men with left ventricular hypertrophy. N Engl J Med Aug. 3, 1995;333(5):288–93.
[23] Aerts JM, Groener JE, Kuiper S, et al. Elevated globotriaosylsphingosine is a hallmark of Fabry disease. Proc Natl Acad Sci U S A Feb. 26, 2008;105(8):2812–7.
[24] Niemann M, Rolfs A, Giese A, et al. Lyso-Gb3 indicates that the alpha-galactosidase A mutation D313Y is not clinically relevant for Fabry Disease. JIMD Rep 2013;7:99–102.
[25] Niemann M, Rolfs A, Stork S, et al. Gene mutations versus clinically relevant phenotypes: Lyso-Gb3 defines Fabry disease. Circ Cardiovasc Genet 2014 Feb;7(1):8–16.
[26] Baptista MV, Ferreira S, Pinho-E-Melo, et al. Mutations of the GLA gene in young patients with stroke: the PORTYSTROKE study: screening genetic conditions in Portuguese young stroke patients. Stroke Mar. 2010;41(3):431–6.
[27] Rolfs A, Fazekas F, Grittner U, et al. Acute cerebrovascular disease in the young: the Stroke in Young Fabry Patients study. Stroke Feb. 2013;44(2):340–9.
[28] Froissart R, Guffon N, Vanier MT, Desnick RJ, Maire I. Fabry disease: D313Y is an alpha-galactosidase A sequence variant that causes pseudodeficient activity in plasma. Mol Genet Metab Nov. 2003;80(3):307–14.
[29] Dalkey N, Holmes O. An experimental application of the Delphi method to the use of experts. Santa Monica: Rand Corporation; 1962 [http://www.rand.org/content/dam/rand/pubs/research_memoranda/2009/RM727.1.pdf. 7-1962, Ref Type: Online Source].
[30] Elliott P, Andersson B, Arbustini E, et al. Classification of the cardiomyopathies: a position statement from the European Society of Cardiology working group on myocardial and pericardial diseases. Eur Heart J Jan. 2008;29(2):270–6.
[31] de Simone G, Devereux RB, Roman MJ, Alderman MH, Laragh JH. Relation of obesity and gender to left ventricular hypertrophy in normotensive and hypertensive adults. Hypertension May 1994;23(5):600–6.
[32] Postema PG, De Jong JS, Van der Bilt IA, Wilde AA. Accurate electrocardiographic assessment of the QT interval: teach the tangent. Heart Rhythm Jul. 2008;5(7):1015–8.
[33] Hoigne P, Attenhofer Jost CH, Duru F, et al. Simple criteria for differentiation of Fabry disease from amyloid heart disease and other causes of left ventricular hypertrophy. Int J Cardiol Aug. 28, 2006;111(3):413–22.
[34] Roberts WC, Waller BF. Cardiac amyloidosis causing cardiac dysfunction: analysis of 54 necropsy patients. Am J Cardiol Jul. 1983;52(1):137–46.
[35] Namdar M, Steffel J, Jetzer S, et al. Value of electrocardiogram in the differentiation of hypertensive heart disease, hypertrophic cardiomyopathy, aortic stenosis, amyloidosis, and Fabry disease. Am J Cardiol Feb. 15, 2012;109(4):587–93.
[36] Likert R. A Technique for the Measurement of Attitudes. Arch Psychol 1932;140:1–55.
[37] Glassel A, Kirchberger I, Kollerits B, Amann E, Cieza A. Content validity of the extended ICF Core Set for stroke: an international Delphi survey of physical therapists. Phys Ther Aug. 2011;91(8):1211–22.
[38] Bland JM, Altman DG. Cronbach's alpha. BMJ Feb. 22, 1997;314(7080):572.
[39] Niemann M, Liu D, Hu K, et al. Prominent papillary muscles in Fabry disease: a diagnostic marker? Ultrasound Med Biol Jan. 2011;37(1):37–43.
[40] Kampmann C, Baehner FA, Whybra C, et al. The right ventricle in Fabry disease. Acta Paediatr Suppl Mar. 2005;94(447):15–8.
[41] Kounas S, Demetrescu C, Pantazis AA, et al. The binary endocardial appearance is a poor discriminator of Anderson–Fabry disease from familial hypertrophic cardiomyopathy. J Am Coll Cardiol May 27, 2008;51(21):2058–61.
[42] Mundigler G, Gaggl M, Heinze G, et al. The endocardial binary appearance ('binary sign') is an unreliable marker for echocardiographic detection of Fabry disease in patients with left ventricular hypertrophy. Eur J Echocardiogr Oct. 2011;12(10):744–9.
[43] Pieroni M, Chimenti C, De CF, et al. Fabry's disease cardiomyopathy: echocardiographic detection of endomyocardial glycosphingolipid compartmentalization. J Am Coll Cardiol Apr. 18, 2006;47(8):1663–71.
[44] Moon JC, Sachdev B, Elkington AG, et al. Gadolinium enhanced cardiovascular magnetic resonance in Anderson–Fabry disease. Evidence for a disease specific abnormality of the myocardial interstitium. Eur Heart J Dec. 2003;24(23):2151–5.
[45] De Cobelli F, Esposito A, Belloni E, et al. Delayed-enhanced cardiac MRI for differentiation of Fabry's disease from symmetric hypertrophic cardiomyopathy. AJR Am J Roentgenol Mar. 2009;192(3):W97–W102.
[46] Beer M, Weidemann F, Breunig F, et al. Impact of enzyme replacement therapy on cardiac morphology and function and late enhancement in Fabry's cardiomyopathy. Am J Cardiol May 15, 2006;97(10):1515–8.
[47] Niemann M, Herrmann S, Hu K, et al. Differences in Fabry cardiomyopathy between female and male patients: consequences for diagnostic assessment. JACC Cardiovasc Imaging Jun. 2011;4(6):592–601.
[48] Mayes JS, Scheerer JB, Sifers RN, Donaldson ML. Differential assay for lysosomal alpha-galactosidases in human tissues and its application to Fabry's disease. Clin Chim Acta May 5, 1981;112(2):247–51.
[49] Biegstraaten M, Hollak CEM, Bakkers M, Faber CG, Aerts JMFG, van Schaik IN. Small fiber neuropathy in Fabry disease. Mol Genet Metab Jun. 2012;106(2):135–41.
[50] Hollander DA, Aldave AJ. Drug-induced corneal complications. Curr Opin Ophthalmol Dec. 2004;15(6):541–8.
[51] Fervenza FC, Torra R, Lager DJ. Fabry disease: an underrecognized cause of proteinuria. Kidney Int May 2008;73(10):1193–9.
[52] Charrow J. A 14-year-old boy with pain in hands and feet. Pediatr Ann Apr. 2009;38(4):190 [192].
[53] Groener JE, Poorthuis BJ, Kuiper S, Helmond MT, Hollak CE, Aerts JM. HPLC for simultaneous quantification of total ceramide, glucosylceramide, and ceramide trihexoside concentrations in plasma. Clin Chem Apr. 2007;53(4):742–7.
[54] Thurberg BL, Randolph BH, Granter SR, Phelps RG, Gordon RE, O'Callaghan M. Monitoring the 3-year efficacy of enzyme replacement therapy in Fabry disease by repeated skin biopsies. J Invest Dermatol Apr. 2004;122(4):900–8.
[55] Elleder M. Sequelae of storage in Fabry disease—pathology and comparison with other lysosomal storage diseases. Acta Paediatr Suppl Dec. 2003;92(443):46–53.
[56] Takenaka T, Teraguchi H, Yoshida A, et al. Terminal stage cardiac findings in patients with cardiac Fabry disease: an electrocardiographic, echocardiographic, and autopsy study. J Cardiol Feb. 2008;51(1):50–9.
[57] Elleder M, Bradova V, Smid F, et al. Cardiocyte storage and hypertrophy as a sole manifestation of Fabry's disease. Report on a case simulating hypertrophic non-

obstructive cardiomyopathy. Virchows Arch A Pathol Anat Histopathol 1990; 417(5):449–55.
[58] Ogawa K, Sugamata K, Funamoto N, et al. Restricted accumulation of globotriaosylceramide in the hearts of atypical cases of Fabry's disease. Hum Pathol Oct. 1990;21(10):1067–73.
[59] Takahashi K, Naito M, Suzuki Y. Lipid storage disease: Part III. Ultrastructural evaluation of cultured fibroblasts in sphingolipidoses. Acta Pathol Jpn Feb. 1987;37(2): 261–72.
[60] Roos JM, Aubry MC, Edwards WD. Chloroquine cardiotoxicity: clinicopathologic features in three patients and comparison with three patients with Fabry disease. Cardiovasc Pathol Sep. 2002;11(5):277–83.
[61] Grafft CA, Fervenza FC, Semret MH, Orloff S, sethi S. Renal involvement in Neimann–Pick disease. Nephrol Dial Transplant Plus Aug. 10, 2009;2:448–51.
[62] Ryazantsev S, Yu WH, Zhao HZ, Neufeld EF, Ohmi K. Lysosomal accumulation of SCMAS (subunit c of mitochondrial ATP synthase) in neurons of the mouse model of mucopolysaccharidosis III B. Mol Genet Metab Apr. 2007;90(4): 393–401.
[63] Leone O, Veinot JP, Angelini A, et al. 2011 Consensus statement on endomyocardial biopsy from the Association for European Cardiovascular Pathology and the Society for Cardiovascular Pathology. Cardiovasc Pathol Jul. 2012;21(4):245–74.
[64] Thurberg BL, Fallon JT, Mitchell R, Aretz T, Gordon RE, O'Callaghan MW. Cardiac microvascular pathology in Fabry disease: evaluation of endomyocardial biopsies before and after enzyme replacement therapy. Circulation May 19, 2009;119(19):2561–7.
[65] Frustaci A, Russo MA, Chimenti C. Diagnostic contribution of left ventricular endomyocardial biopsy in patients with clinical phenotype of hypertrophic cardiomyopathy. Hum Pathol Jan. 2013;44(1):133–41.
[66] Eng CM, Resnick-Silverman LA, Niehaus DJ, Astrin KH, Desnick RJ. Nature and frequency of mutations in the alpha-galactosidase A gene that cause Fabry disease. Am J Hum Genet Dec. 1993;53(6):1186–97.
[67] Meehan SM, Junsanto T, Rydel JJ, Desnick RJ. Fabry disease: renal involvement limited to podocyte pathology and proteinuria in a septuagenarian cardiac variant. Pathologic and therapeutic implications. Am J Kidney Dis 2004 Jan;43(1):164–71.
[68] Bekri S, Enica A, Ghafari T, et al. Fabry disease in patients with end-stage renal failure: the potential benefits of screening. Nephron Clin Pract 2005;101(1):c33–8.
[69] van der Zwaag PA, van Rijsingen IA, Asimaki A, et al. Phospholamban R14del mutation in patients diagnosed with dilated cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy: evidence supporting the concept of arrhythmogenic cardiomyopathy. Eur J Heart Fail Nov. 2012;14(11):1199–207.
[70] Wolf CM, Arad M, Ahmad F, et al. Reversibility of PRKAG2 glycogen-storage cardiomyopathy and electrophysiological manifestations. Circulation Jan. 15, 2008;117(2):144–54.
[71] Boucek D, Jirikowic J, Taylor M. Natural history of Danon disease. Genet Med Jun. 2011;13(6):563–8.
[72] Koeppe S, Neubauer H, Breunig F, et al. MR-based analysis of regional cardiac function in relation to cellular integrity in Fabry disease. Int J Cardiol Sep. 20, 2012;160(1):53–8.
[73] Weidemann F, Breunig F, Beer M, et al. The variation of morphological and functional cardiac manifestation in Fabry disease: potential implications for the time course of the disease. Eur Heart J Jun. 2005;26(12):1221–7.
[74] Niemann M, Breunig F, Beer M, et al. Tei index in Fabry disease. J Am Soc Echocardiogr Sep. 2011;24(9):1026–32.
[75] Niemann M, Breunig F, Beer M, et al. The right ventricle in Fabry disease: natural history and impact of enzyme replacement therapy. Heart Dec. 2010;96(23): 1915–9.
[76] Niemann M, Hartmann T, Namdar M, et al. Cross-sectional baseline analysis of electrocardiography in a large cohort of patients with untreated Fabry disease. J Inherit Metab Dis 2013 Sep;36(5):873–9.
[77] Weidemann F, Niemann M, Breunig F, et al. Long-term effects of enzyme replacement therapy on Fabry cardiomyopathy: evidence for a better outcome with early treatment. Circulation Feb. 3, 2009;119(4):524–9.
[78] Mitobe S, Togawa T, Tsukimura T, et al. Mutant alpha-galactosidase A with M296I does not cause elevation of the plasma globotriaosylsphingosine level. Mol Genet Metab 2012 Nov;107(3):623–6.
[79] Ommen SR, Nishimura RA, Edwards WD. Fabry disease: a mimic for obstructive hypertrophic cardiomyopathy? Heart Aug. 2003;89(8):929–30.
[80] Lin HY, Chong KW, Hsu JH, et al. High incidence of the cardiac variant of Fabry disease revealed by newborn screening in the Taiwan Chinese population. Circ Cardiovasc Genet Oct. 2009;2(5):450–6.
[81] Holzmann M, Nicko A, Kuhl U, et al. Complication rate of right ventricular endomyocardial biopsy via the femoral approach: a retrospective and prospective study analyzing 3048 diagnostic procedures over an 11-year period. Circulation Oct. 21, 2008;118(17):1722–8.
[82] Eng CM, Desnick RJ. Molecular basis of Fabry disease: mutations and polymorphisms in the human alpha-galactosidase A gene. Hum Mutat 1994;3(2): 103–11.
[83] Gold H, Mirzaian M, Dekker N, et al. Quantification of globotriaosylsphingosine in plasma and urine of Fabry patients by stable isotope ultraperformance liquid chromatography-tandem mass spectrometry. Clin Chem Mar. 1, 2013;59(3): 547–56.
[84] Boutin M, Gagnon R, Lavoie P, Auray-Blais C. LC-MS/MS analysis of plasma lyso-Gb3 in Fabry disease. Clin Chim Acta Dec. 24, 2012;414:273–80.
[85] Kruger R, Tholey A, Jakoby T, et al. Quantification of the Fabry marker lysoGb3 in human plasma by tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci Feb. 1, 2012;883–884:128–35.
[86] Lukas J, Giese AK, Markoff A, et al. Functional characterisation of alpha-galactosidase A mutations as a basis for a new classification system in Fabry disease. PLoS Genet 2013;9(8).
[87] Smid BE, Hollak CE, Poorthuis BJ, et al. Diagnostic dilemmas in Fabry disease: a case series study on GLA mutations of unknown clinical significance. Clin Genet Jul. 7, 2014 http://dx.doi.org/10.1111/cge.12449.
[88] Sado DM, White SK, Piechnik SK, et al. The identification and assessment of Anderson–Fabry disease by cardiovascular magnetic resonance non-contrast myocardial T1 mapping. Circ Cardiovasc Imaging Apr. 5, 2013;6(3):392–8.
[89] Namdar M, Steffel J, Vidovic M, et al. Electrocardiographic changes in early recognition of Fabry disease. Heart Mar. 2011;97(6):485–90.
[90] Namdar M, Kampmann C, Steffel J, et al. PQ interval in patients with Fabry disease. Am J Cardiol Mar. 1, 2010;105(5):753–6.
[91] Motwani M, Banypersad S, Woolfson P, Waldek S. Enzyme replacement therapy improves cardiac features and severity of Fabry disease. Mol Genet Metab Sep. 2012;107(1–2):197–202.
[92] O'Mahony C, Coats C, Cardona M, et al. Incidence and predictors of anti-bradycardia pacing in patients with Anderson–Fabry disease. Europace Dec. 2011;13(12): 1781–8.
[93] Sadick N, Thomas L. Cardiovascular manifestations in Fabry disease: a clinical and echocardiographic study. Heart Lung Circ Jun. 2007;16(3):200–6.
[94] Senechal M, Germain DP. Fabry disease: a functional and anatomical study of cardiac manifestations in 20 hemizygous male patients. Clin Genet Jan. 2003;63(1):46–52.
[95] Shah JS, Hughes DA, Sachdev B, et al. Prevalence and clinical significance of cardiac arrhythmia in Anderson–Fabry disease. Am J Cardiol Sep. 15, 2005;96(6): 842–6.
[96] Gillette PC, Nihill MR, Singer DB. Electrophysiological mechanism of the short PR interval in Pompe disease. Am J Dis Child Nov. 1974;128(5):622–6.
[97] Forsha D, Li JS, Smith PB, van der Ploeg AT, Kishnani P, Pasquali SK. Cardiovascular abnormalities in late-onset Pompe disease and response to enzyme replacement therapy. Genet Med Jul. 2011;13(7):625–31.
[98] Linhart A, Palecek T, Bultas J, et al. New insights in cardiac structural changes in patients with Fabry's disease. Am Heart J Jun. 2000;139(6):1101–8.
[99] Sachdev B, Elliott PM. Isolated cardiac manifestations in Fabry disease: the UK experience. Acta Paediatr Suppl 2002;91(439):28–30.
[100] Wu JC, Ho CY, Skali H, et al. Cardiovascular manifestations of Fabry disease: relationships between left ventricular hypertrophy, disease severity, and alpha-galactosidase A activity. Eur Heart J May 2010;31(9):1088–97.
[101] Magage S, Linhart A, Bultas J, et al. Fabry disease: percutaneous transluminal septal myocardial ablation markedly improved symptomatic left ventricular hypertrophy and outflow tract obstruction in a classically affected male. Echocardiography Apr. 2005;22(4):333–9.
[102] Kampmann C, Linhart A, Baehner F, et al. Onset and progression of the Anderson–Fabry disease related cardiomyopathy. Int J Cardiol Nov. 28, 2008;130(3):367–73.
[103] Ramaswami U, Parini R, Kampmann C, Beck M. Safety of agalsidase alfa in patients with Fabry disease under 7 years. Acta Paediatr Apr. 2011;100(4):605–11.
[104] Kampmann C, Baehner F, Whybra C, et al. Cardiac manifestations of Anderson–Fabry disease in heterozygous females. J Am Coll Cardiol Nov. 6, 2002;40(9): 1668–74.
[105] Kampmann C, Wiethoff CM, Whybra C, Baehner FA, Mengel E, Beck M. Cardiac manifestations of Anderson–Fabry disease in children and adolescents. Acta Paediatr Apr. 2008;97(4):463–9.
[106] Ries M, Gupta S, Moore DF, et al. Pediatric Fabry disease. Pediatrics Mar. 2005; 115(3):e344–55.
[107] Hughes DA, Elliott PM, Shah J, et al. Effects of enzyme replacement therapy on the cardiomyopathy of Anderson–Fabry disease: a randomised, double-blind, placebo-controlled clinical trial of agalsidase alfa. Heart Feb. 2008;94(2):153–8.
[108] Weidemann F, Breunig F, Beer M, et al. Improvement of cardiac function during enzyme replacement therapy in patients with Fabry disease: a prospective strain rate imaging study. Circulation Sep. 16, 2003;108(11):1299–301.
[109] Gupta S, Ries M, Kotsopoulos S, Schiffmann R. The relationship of vascular glycolipid storage to clinical manifestations of Fabry disease: a cross-sectional study of a large cohort of clinically affected heterozygous women. Medicine (Baltimore) Sep. 2005; 84(5):261–8.
[110] Lobo T, Morgan J, Bjorksten A, et al. Cardiovascular testing in Fabry disease: exercise capacity reduction, chronotropic incompetence and improved anaerobic threshold after enzyme replacement. Intern Med J Jun. 2008;38(6):407–14.
[111] Palecek T, Dostalova G, Kuchynka P, et al. Right ventricular involvement in Fabry disease. J Am Soc Echocardiogr Nov. 2008;21(11):1265–8.
[112] Gruner C, Verocai F, Carasso S, et al. Systolic myocardial mechanics in patients with Anderson–Fabry disease with and without left ventricular hypertrophy and in comparison to nonobstructive hypertrophic cardiomyopathy. Echocardiography Aug. 2012;29(7):810–7.
[113] Pieroni M, Chimenti C, Ricci R, Sale P, Russo MA, Frustaci A. Early detection of Fabry cardiomyopathy by tissue Doppler imaging. Circulation Apr. 22, 2003;107(15): 1978–84.
[114] Toro R, Perez-Isla L, Doxastaquis G, et al. Clinical usefulness of tissue Doppler imaging in predicting preclinical Fabry cardiomyopathy. Int J Cardiol Feb. 6, 2009;132(1):38–44.
[115] Toro R, Perez-Isla L, Doxastaquis G, et al. Clinical usefulness of tissue Doppler imaging in predicting preclinical Fabry cardiomyopathy. Int J Cardiol Feb. 6, 2009;132(1):38–44.
[116] Pieroni M, Crea F. Binary sign in Anderson–Fabry cardiomyopathy. J Am Coll Cardiol 2009;53:813–4.

[117] Koskenvuo JW, Engblom E, Kantola IM, et al. Echocardiography in Fabry disease: diagnostic value of endocardial border binary appearance. Clin Physiol Funct Imaging May 2009;29(3):177–80.
[118] Kovacevic-Preradovic T, Zuber M, Attenhofer Jost CH, et al. Anderson–Fabry disease: long-term echocardiographic follow-up under enzyme replacement therapy. Eur J Echocardiogr Nov. 2008;9(6):729–35.
[119] Bass JL, Shrivastava S, Grabowski GA, Desnick RJ, Moller JH. The M-mode echocardiogram in Fabry's disease. Am Heart J Dec. 1980;100(6 Pt 1):807–12.
[120] Barbey F, Qanadli SD, Juli C, et al. Aortic remodelling in Fabry disease. Eur Heart J Feb. 2010;31(3):347–53.
[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 Feb. 1, 2002;89(3):337–41.
[122] Brunetti-Pierri N, Pignatelli R, Fouladi N, et al. Dilation of the aortic root in mitochondrial disease patients. Mol Genet Metab Jun. 2011;103(2):167–70.
[123] Jefferies JL, Belmont JW, Pignatelli R, Towbin JA, Craigen WJ. PTPN11 mutation associated with aortic dilation and hypertrophic cardiomyopathy in a pediatric patient with Noonan syndrome. Pediatr Cardiol Jan. 2010;31(1):114–6.
[124] Imbriaco M, Spinelli L, Cuocolo A, et al. MRI characterization of myocardial tissue in patients with Fabry's disease. AJR Am J Roentgenol Mar. 2007;188(3):850–3.
[125] Sado DM, Flett AS, Banypersad SM, et al. Cardiovascular magnetic resonance measurement of myocardial extracellular volume in health and disease. Heart 2012;98:1436–41.
[126] Rutz AK, Juli CF, Ryf S, et al. Altered myocardial motion pattern in Fabry patients assessed with CMR-tagging. J Cardiovasc Magn Reson 2007;9(6):891–8.