In Patients with an α -Galactosidase A Variant, Small Nerve Fibre Assessment Cannot Confirm a Diagnosis of Fabry Disease

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Abstract *Background:* Fabry disease (FD) is an X-linked lysosomal storage disorder caused by an α -galactosidase A enzyme deficiency due to pathogenic variants in the α -galactosidase A gene (*GLA*). An increasing number of individuals with a *GLA* variant, but without characteristic FD features, are identified. A definite diagnosis of FD has important consequences for treatment and counselling.

Objectives: We assessed the diagnostic value of quantitative sensory testing (QST) and intraepidermal nerve fibre density (IENFD) for patients with an uncertain FD diagnosis.

Methods: All patients with a *GLA* variant who initially presented at the Academic Medical Center with an uncertain FD diagnosis were included. A biopsy of an affected organ in a patient or family member showing FD characteristic storage is used as a reference standard for a diagnosis of FD. All patients underwent a comprehensive QST protocol and IENFD assessment which was compared to age and gender-matched healthy controls. Sensitivity and specificity were calculated for a combination of ≥ 1 abnormal QST modality and an abnormal IENFD.

Results: Twenty-six patients participated (nonclassical FD $n = 18$, 9 males; no FD $n = 5$, 3 males; uncertain $n = 3$, 1 male). Of the patients classified as nonclassical FD, 28% had ≥ 1 abnormal QST modalities, and 83% had an abnormal IENFD. From the patients without FD, 20% had ≥ 1 abnormal QST modality, and IENFD was abnormal in 25% (1 not available). Sensitivity was 28% and specificity 80%.

Conclusions: In our study cohort, QST and IENFD could not reliably distinguish patients with FD from those without FD.

Introduction

Fabry disease (FD) is a lysosomal storage disorder with an X-linked pattern of inheritance caused by impaired function of the lysosomal hydrolase α -galactosidase A (α GalA) due to pathogenic variants in the α -galactosidase A (*GLA*) gene. Subsequent accumulation of globotriaosylceramide (Gb3) may lead to cornea verticillata, angiokeratoma and pain in the upper and lower limbs related to a small fibre neuropathy (Fabry neuropathic pain) in childhood. Later in life, complications of the heart, the kidney and the central nervous system may occur. This disease phenotype is reported as classical FD (Desnick et al. 2007). Females may also be affected although generally less severely. In the past few years, it has become clear that the phenotypical spectrum is broad. Patients with a proven disease-causing *GLA* gene variant may present at middle age with a nonspecific feature such as stroke, left ventricular hypertrophy or chronic kidney disease. This disease phenotype is currently known as nonclassical or late onset FD. A third group of individuals has been described with nonpathogenic

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variants in the *GLA* gene, who do not suffer from FD. A diagnosis can be erroneously made in these individuals, since nonclassical disease symptoms show overlap with more common diseases, such as cerebrovascular disease. This has become critical with the increase in screening initiatives in FD resulting in an increasing number of individuals with a *GLA* variant in whom it is unclear if they have either nonclassical FD or a nonpathogenic *GLA* variant (Terry et al. 2013; Smid et al. 2014; van der Tol et al. 2014). Uncertainty of the diagnosis may lead to considerable distress for the patients and their families. Additionally, this poses difficult dilemmas with regard to counselling, family screening and expensive treatment options (Terry et al. 2013; Smid et al. 2014). Reliable tools are needed to confirm or exclude FD in these cases in order to decide on the best clinical approach.

In previous studies, experts agreed that electron microscopy (EM) examination of biopsy specimen of the affected organ (i.e. kidney or heart) showing storage characteristic of FD, according to strict histological criteria, can confirm the diagnosis in most cases (see Table A1) (Smid et al. 2014; van der Tol et al. 2015). However, a biopsy is invasive and cannot be performed in every patient.

Previous studies have shown that all male and most female patients with (classical) FD have an abnormal quantitative sensory testing (QST) profile and a decreased intraepidermal nerve fibre density (IENFD) in a skin biopsy at the distal leg (Dutsch et al. 2002; Laaksonen et al. 2008; Biegstraaten et al. 2011, 2012; Üçeyler et al. 2011, 2013). We hypothesized that QST and IENFD could aid in the diagnosis of FD in patients who initially present with an uncertain diagnosis. Advantages are that QST and a skin biopsy are far less invasive than an organ biopsy and that both investigations can be done in patients in whom a biopsy of an affected organ is not an option.

Materials and Methods

Patients

The Academic Medical Center (AMC) is the single referral centre for patients with (suspected) FD in the Netherlands. Patients with a *GLA* variant who originally presented with an uncertain FD diagnosis were eligible for participation in the study.

Patients were included prospectively if they presented at the AMC between January 2012 and May 2013. In addition, the Dutch FD database (initiated in 1999), which contains clinical, biochemical and radiological data on all individuals with any *GLA* variant who ever visited the outpatient FD clinic at the AMC, was searched for additional eligible patients. Patients with the classical

phenotype as defined by absent or very low (<5%) residual enzyme activity in leukocytes (males) and the presence of FD-specific signs or symptoms (neuropathic pain, cornea verticillata and clustered angiokeratoma) or a very high plasma globotriaosylsphingosine (lysoGb3) (see Table A1 for details) were excluded. Patients who initially presented with an uncertain diagnosis were invited to participate. Patients with a confirmed small fibre neuropathy or polyneuropathy in the medical history were excluded because nerve fibre assessment is under investigation in this study.

Reference Standard

A biopsy of the affected organ in a patient or a family member showing storage characteristic of FD was considered confirmative for an FD diagnosis (Takahashi et al. 1987; Meehan et al. 2004; Hsu et al. 2014; Smid et al. 2014, 2015). Based upon the results of an organ biopsy using strict histological criteria (see Table A1 for details), patients were categorized as follows:

1. Nonclassical FD: FD is confirmed by storage characteristic of FD on electron microscopy in a biopsy of the affected organ (i.e. heart or kidney) of the patient or a family member.
2. No FD: FD is excluded based on a negative biopsy from the subject or a family member, or the individual carries the well-known neutral variant p.D313Y (Froisart et al. 2003; Yasuda et al. 2003).
3. Uncertain: A biopsy is not available from the patient or a family member.

Assessments

QST and IENFD data were extracted from the database if these tests were already performed in the context of a previous study (Biegstraaten et al. 2011, 2012). In all other study patients a QST protocol and skin biopsy for IENFD assessment were performed. The QST protocol consisted of the following measurements on the left hand and right foot: cold detection threshold (CDT), warm detection threshold (WDT) and thermal sensory limen (TSL). Patient data were compared to previously established reference data matched for site, age and gender from the German Research Network on Neuropathic Pain (Rolke et al. 2006a, b).

To assess intraepidermal nerve fibre density (IENFD), 3 mm circular skin biopsies were taken 10 cm above the lateral malleolus of the right foot. The biopsies were processed and nerve counts were performed by an experienced investigator (AK) as previously described (Bakkers et al. 2009; Lauria et al. 2010). In short, the biopsy specimen was fixed in cold fixative and kept in a

cryoprotective solution for 3 nights. The specimens were cut in 50 µm sections and were then stained with a polyclonal panaxonal marker, PGP 9.5. Three sections from each biopsy were examined using bright-field immunohistochemistry. Intraepidermal nerve fibres (IENFs) were counted under the light microscope at high magnification (40×). Only single IENFs crossing the dermal-epidermal junction were counted. The length of the section was measured with computerized software and the IENFD was subsequently calculated. Patient data were compared to reference values matched for age and gender (Lauria et al. 2010).

Information on medication history, neuropathic pain and medical history was retrieved from the medical charts. Laboratory data on plasma creatinine, glucose and lipid profile were collected within 1 year from the study assessment date. Glomerular filtration rate was estimated with the CKD-EPI formula (Levey et al. 2009). αGalA enzyme activity was measured in leukocytes at baseline and is represented as percentage of the mean of the normal reference value. If the mean was not available, the middle of the range was used. Globotriaosylsphingosine was assessed at baseline (before initiation of enzyme replacement therapy) as previously described (Gold et al. 2013).

Approval and Consent

The local institutional review board approved this study and all patients signed informed consent. Six patients participated in a previous study that included similar investigations and gave their consent to reuse their results in this study (Biegstraaten et al. 2011).

Statistical Analyses

Data were specified for gender and phenotype as described above.

To assess QST data independently from the specific reference values for site of assessment, age and gender, data were Z-transformed for each parameter. Absolute values were used to calculate the Z-score with the formula: $Z = (\text{value}_{\text{patient}} - \text{mean}_{\text{controls}}) / \text{SD}$. As recommended by Rolke et al. (2006a,b), logarithmic transformation was used for CDT, WDT and TSL values to calculate the Z-score. Z-scores below -1.96 indicate loss of function (abnormal). Thus, if higher temperatures compared to controls are needed to feel warmth, and lower temperatures are needed to feel cold, this will result in negative Z-scores; the patient is less sensitive for the applied stimulus.

Absolute data were given for IENFD, and IENFD was scored as normal or abnormal. Abnormal values are defined as an IENFD below the fifth percentile for age- and gender-matched reference values (Lauria et al. 2010).

Sensitivity and specificity for the combination of ≥ 1 abnormal QST modality (CDT, WDT or TSL; hand or foot) and an abnormal IENFD were calculated for the prediction of a positive outcome of the reference standard (nonclassical FD).

Results

Patients

Forty-three patients fulfilled the criteria of nonclassical FD, no FD or an uncertain FD diagnosis. Four patients were excluded because they presented with a small fibre neuropathy (p.D313Y, male, no FD and p.R118C, female, uncertain diagnosis) or a polyneuropathy (p.R112H, male, nonclassical and p.D313Y, male, no FD). Thirteen patients (nonclassical FD $n = 3$ females; no FD $n = 2$, 1 male; uncertain $n = 8$, 4 males) refused to participate. Time constraints were the most frequently mentioned reason. Twenty-six patients of nine families participated (nonclassical FD $n = 18$, 9 males; no FD $n = 5$, 3 males; uncertain $n = 3$, 1 male). Ultimately, one participant (patient 6) only consented to QST assessment. A few exceptions to the classification criteria were made for two patients (family 4) because they harboured the same *GLA* variant as family 3, in which two members had a heart biopsy with characteristic storage on electron microscopy. Based on this, these patients were grouped as nonclassical FD. Four patients (2.3, 2.4, 2.6 and 3.1) had atypical non-diffuse corneal abnormalities, very different from the diffuse cornea verticillata observed in patients with a classical phenotype. Because other FD characteristic signs or symptoms such as neuropathic pain and angiokeratoma were absent, these patients were grouped as nonclassical. Baseline patient characteristics are presented in Table 1.

Pain, QST and IENFD

None of the patients used pain medication for pain in the upper and/or lower limbs. Patient 2.9 had burning pains in her feet that had commenced in her 30s, not exacerbating with heat, fever or exercise. Patient 9 has had episodic pain in the limbs since early childhood, not clearly related to heat, fever or exercise. The remaining patients did not report pain in hands or feet.

In two patients, the QST assessments were performed on the right hand and left foot, because of a stroke that affected the sensory function of the left hand (patient 2.2) and due to discomfort of the right leg for patient 3.4.

Individual patient data on QST and IENFD are presented in Table 2 and Fig. A1.

Table 1 Baseline characteristics

Patient	Gender	Age	GLA	α GalA (%)	Characteristic storage ^a	Lyso Gb3 (nmol/L) ^b	≥ 1 Clinical event	eGFR (mL/min/1.73 m ²)	Lipid profile	Statin	DM
<i>Nonclassical</i>											
1	M	69	I319T	2.2	+	19.7	+	86	— ^c	—	— ^c
2.1	M	60	P389A	6.7	+	5	+	59	+ ^c	+	— ^c
2.2	M	70	P389A	6.7	nd	7.6	+	85	+ ^c	+	— ^c
2.3	M	66	P389A	3.3	nd	7.5	+	56	— ^c	—	— ^c
2.4	M	34	P389A	6.7	nd	7.4	—	103 ^d	— ^c	—	— ^c
2.5	F	70	P389A	73.3	nd	1.1	—	79 ^d	— ^c	+	— ^c
2.6	F	46	P389A	46.7	nd	1.3	—	91	— ^c	—	— ^c
2.7	F	64	P389A	76.7	nd	1.5	—	68	+ ^c	+	+ ^c
2.8	F	34	P389A	36.4	nd	1	—	117 ^d	— ^c	—	— ^c
2.9	F	38	P389A	38.0	nd	0.6	—	89	— ^c	—	— ^c
2.10	F	37	P389A	80.0	nd	1.2	—	105	— ^c	—	— ^c
3.1	M	66	R112H	2.7	+	2	+	9	+	+	—
3.2	M	49	R112H	4.5	+	1.8	—	82	—	—	—
3.3	M	46	R112H	2.7	nd	1.3	—	98	—	—	—
3.4	F	77	R112H	12.7	nd	0.7	—	29	— ^c	—	— ^c
3.5	F	37	R112H	55.6	nd	0.6	—	96	—	—	—
4.1	M	62	R112H	2.4	—	1.6	—	87	—	+	+
4.2	F	24	R112H	34.7	nd	0.5	—	120	—	—	—
<i>No FD</i>											
5.1	M	27	A143T	29.8	nd	0.4	—	118 ^d	— ^c	—	— ^c
5.2	M	22	A143T	35.8	nd	0.6	—	121	— ^c	—	— ^c
6	M	69	D313Y	108.4	nd	0.3	+	72	—	—	—
7.1	F	50	D313Y	142.2	nd	0.3	+	98	—	—	—
7.2	F	44	D313Y	156.0	nd	0.3	—	102	—	—	—
<i>Uncertain</i>											
8.1	M	69	L106F	5.6	nd	1.6	+	93	—	+	—
8.2	F	48	L106F	60.7	nd	0.4	—	101	—	—	—
9	F	61	P60L	38.9	nd	0.5	+	15	+	+	—

Clinical events were defined as stroke, transient ischaemic attack, symptomatic cardiac arrhythmias, pacemaker, implantable cardiac defibrillator, myocardial infarction, percutaneous transluminal coronary angioplasty or coronary artery bypass graft, admission to hospital due to heart failure, dialysis or kidney transplant

nd not done, M male, F female, GLA α -galactosidase A gene, α GalA α -galactosidase A activity in leukocytes, eGFR estimated glomerular filtration rate, lysoGb3 plasma globotriaosylsphingosine, DM diabetes mellitus, + present, — absent

^aCharacteristic storage observed in a heart or kidney biopsy

^bNormal value plasma lysoGb3 ≤ 0.6 nmol/L

^cLipid profile and glucose were assessed >1 year prior to study participation, range 13–41 months

^dGFR was assessed >1 year prior to study participation, range 16–25 months

Five patients (28%) with the nonclassical phenotype had a loss of function of one or more QST modalities, with 1–4 affected modalities per patient. The CDT, previously reported as the most severely affected QST value in FD patients with a classical phenotype (Biegstraaten et al. 2011), was abnormal in 1 (6%) and 2 (11%) patients at the foot and hand, respectively. In patient 2.9, who had complaints of burning pain, none of the QST modalities were abnormal.

One patient in the “no-FD group” had an abnormal CDT (20%). In the uncertain group, one female (33%) had an abnormal CDT and WDT at the foot in the absence of pain. The male patient had a normal QST profile.

IENFD was abnormal in most patients with a nonclassical phenotype (83%, $n = 15$, 7 males), in three patients in the “no-FD group” (75%, $n = 3$, 1 male) and in two patients in the “uncertain group” (66%, $n = 2$, 1 male).

Table 2 Quantitative sensory testing (QST) and intraepidermal nerve fibre density

Patient	Gender	Age (years)	Foot			Hand			IENFD	IENFD fibres/mm
			CDT	WDT	TSL	CDT	WDT	TSL		
Nonclassical										
1	M	69	−0.94	0.64	0.24	−0.58	0.26	0.43	Abnormal	0.3
2.1	M	60	−0.49	0.73	−0.22	−0.66	−1.23	−1.07	Abnormal	0
2.2	M	70	−0.52	−0.76	−0.37	−1.17	0.16	−1.67	Normal	4.8
2.3	M	66	−0.16	−1.10	−0.77	0.19	0.72	1.09	Normal	4.8
2.4	M	34	0.24	−0.86	−0.1	−1.95	−1.44	−1.03	Abnormal	0.2
2.5	F	70	0.51	−1.54	−0.83	−0.41	−0.37	0.92	Normal	2.9
2.6	F	46	0.79	1.17	0.59	1.08	1.25	2.22	Abnormal	2.2
2.7	F	64	−1.80	−1.07	−2.01	−1.16	−1.21	−0.77	Abnormal	0.6
2.8	F	34	0.55	−1.16	−0.52	0.24	−0.64	0.13	Abnormal	2.3
2.9	F	38	2.02	0.35	0.76	0.67	−0.11	0.66	Abnormal	0.8
2.10	F	37	−1.94	−0.48	−0.86	−1.67	−1.87	−1.88	Abnormal	1.9
3.1	M	66	−2.99	−1.32	−2.16	−2.9	−1.86	−2.69	Abnormal	0.4
3.2	M	49	−0.12	0.87	0.06	−1.78	−1.23	−1.65	Abnormal	2.0
3.3	M	46	0.89	0.97	0.55	−1.55	0.07	−0.26	Abnormal	2.7
3.4	F	77	1.90	−2.19	nd	−0.31	0.35	−3.95	Abnormal	0.7
3.5	F	37	0.02	−0.90	−0.40	0.06	−1.41	−0.89	Abnormal	1.4
4.1	M	62	1.25	−0.48	0.75	−0.90	−0.26	−3.80	Abnormal	0
4.2	F	24	−1.55	−1.91	−1.78	−2.85	−1.53	−1.56	Abnormal	0.5
No FD										
5.1	M	27	−0.98	−1.55	−0.77	0.60	0.66	0.66	Abnormal	5.6
5.2	M	22	0.71	−0.62	−0.10	0.38	0.83	1.21	Normal	9.1
6	M	69	−1.14	−1.55	−0.70	0.50	1.16	0.99	nd	nd
7.1	F	50	−1.23	−0.63	−0.68	−2.67	−1.80	−1.31	Abnormal	1.0
7.2	F	44	−0.38	−0.63	0.22	−1.30	−0.53	−0.20	Abnormal	1.1
Uncertain										
8.1	M	69	−0.58	−0.6	−1.62	−0.66	−0.54	−0.49	Abnormal	0.3
8.2	F	48	−2.72	−2.36	−1.67	−0.41	−1.84	−0.97	Abnormal	0.2
9	F	61	−1.63	0.3	−0.64	−0.7	−0.53	−0.42	Normal	8.0

For QST data, values represent Z-scores, abnormal values (loss of function) are presented in bold font

M male, *F* female, *nd* no data, *CDT* cold detection threshold, *WDT* warm detection threshold, *TSL* thermal sensory limen

All patients with a normal IENFD had a normal QST profile. Patients who had one or more abnormal QST modalities also had an abnormal IENFD. Ten patients in the nonclassical group (55%, 5 males), had a normal QST profile and an abnormal IENFD. Two patients (one male) in the no-FD group and one male in the uncertain group had a normal QST profile with an abnormal IENFD.

Diagnostic Accuracy

For ≥ 1 abnormal QST modality and an abnormal IENFD, sensitivity was 28% and specificity was 80%; see Table 3.

Discussion and Conclusion

We assessed the diagnostic value of QST and IENFD for patients with a *GLA* variant, who have an uncertain diagnosis of FD. These individuals may have a nonclassical FD phenotype or no FD at all. In this study on 18 patients with nonclassical FD and 5 patients without FD, as defined by strict biochemical and clinical criteria, an optimal separation between the groups could not be achieved with small nerve fibre assessment using QST and IENFD.

The interpretation of the results is hampered by the small number of individuals included in this study, especially in the no-FD group. However, with the finding that

Table 3 Two-by-two table for sensitivity and specificity calculations

QST and IENFD	Positive	Negative
Nonclassical	5	13
No FD	1	4

QST quantitative sensory testing, IENFD intraepidermal nerve fibre density

abnormalities were also found in the patients without FD (specificity 80%), it is safe to draw the conclusion that small nerve fibre assessment is most likely an unsuitable tool for establishing the diagnosis of FD in patients who present with an uncertain diagnosis. In previous studies, it has been shown that all male and most female patients with (mostly classical) FD have small nerve fibre pathology, as assessed with QST alone or in combination with an IENFD assessment (Dutsch et al. 2002; Laaksonen et al. 2008; Biegstraaten et al. 2011, 2012; Üçeyler et al. 2011, 2013). With this study, we confirmed our hypothesis that in patients with the nonclassical phenotype QST and/or IENFD may be abnormal, even in the absence of pain. However, the abnormalities are not as extensive as in patients with classical disease (Biegstraaten et al. 2011, 2012) and predominantly concern small nerve fibre structure as represented by the IENFD. Similarly, in patients with diabetes mellitus (DM), an abnormal IENFD in the distal leg and impaired cold sensation have been found, even without neuropathic symptoms (Umapathi et al. 2007; Loseth et al. 2008; Ragé et al. 2011). For both DM and nonclassical FD, it may be hypothesized that a reduced IENFD and abnormal QST modalities reflect a presymptomatic state. However, longitudinal studies to confirm the development of clinical apparent neuropathy in these patients are lacking. In our experience, however, patients with nonclassical FD do not have the characteristic neuropathic pain as seen in classical FD, probably due to the slow progressive nature of FD.

The reason for the abnormal results of the patients without FD remains unclear. It may be speculated that the presence of the *GLA* variant in the patients without FD may be a risk factor for the loss of small nerve fibres. Another possibility could be the presence of undetected comorbidities in these patients. We did not perform a complete workup of other causes of small fibre neuropathy that may possibly explain the abnormalities in the patients without FD. It is important to realize that the group of patients without FD is small, impeding the interpretation of the specificity.

Although small nerve fibre assessment may not aid in establishing the diagnosis of FD in patients who present with an uncertain diagnosis, it can still be helpful in the context of

neuropathic pain characteristic of FD. In a patient with a *GLA* variant who presents with chronic, burning pain in hands and feet starting at young age or increasing with heat, fever or exercise and who has a small fibre neuropathy as confirmed by abnormal QST and IENFD results, there is no doubt about the diagnosis of FD. This patient will generally have a classical FD phenotype.

In conclusion, there is a need for accurate diagnostic tools for patients with a *GLA* variant and an uncertain diagnosis of FD. In our cohort, small nerve fibre assessment using QST and IENFD could not reliably distinguish patients with nonclassical FD from those without FD. In those patients with an uncertain diagnosis of FD evaluation, follow-up at an expert centre is recommended and other diagnostic approaches should be applied (Van der Tol et al. 2014; Smid et al. 2014).

Synopsis

In a cohort of 26 individuals with a *GLA* variant, small fibre neuropathy assessments cannot reliably distinguish patients with FD from those without FD in the presence of a nonpathogenic *GLA* variant.

Compliance with Ethics Guidelines

Conflicts of Interest

LT has received travel support and reimbursement of expenses from Actelion, Shire HGT or Genzyme.

MB and CH have received travel support, honoraria for consultancies and educational grants from Actelion, Genzyme, Shire HGT, Protalix or Amicus. All financial arrangements are made with the AMC Medical research BV, in accordance with the AMC Research Code.

IS received departmental honoraria for serving on scientific advisory boards and a steering committee for CSL Behring, and in the past (>5 years) he received honoraria for lecturing and consultancy and research support from Actelion Pharmaceuticals Ltd. All consulting fees for IS were donated to the Stichting Klinische Neurologie, a local foundation that supports research in the field of neurological disorders.

CV and AK report no disclosures.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki

Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

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Contributions

LT study design, data acquisition, data analyses, data interpretation, and first draft of manuscript.

AK data acquisition, data interpretation, and revision of manuscript.

CV study design, data interpretation, and revision of manuscript.

IS data interpretation and revision of manuscript.

MB and CH study design, data interpretation, and revision of manuscript.

Appendix

Table A1 Diagnostic criteria for a definite diagnosis of FD

Definite diagnosis of FD	
Males	Females
<i>GLA</i> variant	<i>GLA</i> variant
+	+
α GalA deficiency of $\leq 5\%$ of mean reference value in leukocytes	Normal or deficient α GalA in leukocytes
+	+
A or B or C	
A:	
≥ 1 Characteristic FD sign/symptom (Fabry neuropathic pain, cornea verticillata or clustered angiokeratoma) ^a	
B:	
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 <i>GLA</i> variant	

Uncertain diagnosis of FD

Males/females:

All patients presenting with a nonspecific 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. Further evaluations are needed, following diagnostic algorithms^b

Gold standard for uncertain FD diagnoses

In subjects with an uncertain FD diagnosis, a GVUS and a nonspecific FD sign, 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 expert pathologist, in the absence of medication that can lead to storage, confirms FD

Adopted from Smid et al. with permission (Smid et al. 2014)
GLA α -galactosidase A gene, α GalA α -galactosidase A enzyme,
GVUS genetic variant of unknown significance

^a Fabry neuropathic pain meets the “characteristic clinical criteria” if there is neuropathic pain in hands and/or feet, starting before age 18 years, or increasing with heat, fever. Quantitative sensory testing (QST) reveals an increased cold detection threshold and the intraepidermal nerve fibre density is decreased. There is no other cause for neuropathic pain. Angiokeratomas meet the “characteristic clinical criteria” if they are clustered and present in characteristic areas: bathing trunk area, lips and umbilicus. There is no other cause for angiokeratoma. Cornea verticillata meets the “characteristic clinical criteria” if there is a whorl-like pattern of corneal opacities. There is no other cause (medication induced, among others: amiodarone, chloroquine)

^b For organ-specific algorithms, see Smid et al. (2014) for heart and Van der Tol et al. for kidney (van der Tol et al. 2015) and Van der Tol et al. for brain (van der Tol et al. 2014a)

Fig. A1 Z-scores for CDT (cold detection threshold), WDT (warm detection threshold), and TSL (thermal sensory limen) for hand and foot. Black dots represent males, and open dots represent females.

Dotted lines represent a Z-score of -1.96 . Horizontal lines represent the mean for males and females combined

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