Characterization of classical and non-classical Fabry disease: a large multicenter cohort study

Maarten Arends, Christoph Wanner, Derralynn Hughes, Atul Mehta, Daniel Oder, Oliver T. Watkinson, Perry M. Elliott, Gabor E. Linthorst, Frits A. Wijburg, Marieke Biegstraaten, Carla E. Hollak

Running title: Characterization of classical and non-classical Fabry disease

Abstract

Fabry disease leads to renal, cardiac and cerebrovascular manifestations. Phenotypic differences between classically and non-classically affected patients are evident but there are few data on the natural course of men and women with classical and non-classical disease. To describe the natural course of Fabry disease stratified for sex and phenotype we retrospectively assessed the event free survival from birth to the first visit (before enzyme replacement therapy) in 499 adult patients (mean age 43 years old, 41% men, 57% with the classical phenotype) from three international centers of excellence. We classified patients by phenotype on the basis of characteristic symptoms and enzyme activity. Men and women with classical Fabry disease had higher event rate than did those with non-classical disease (hazard ratio for men, 5.63, 95% confidence interval, 3.17 to 10.00; $p<0.001$; hazard ratio for women: 2.88, $p<0.001$, respectively). Furthermore, men with classical Fabry disease had lower eGFR, higher left ventricular mass and higher plasma globotriaosylsphingosine than men with non-classical Fabry disease or women with either phenotype ($p<0.001$). In conclusion, before treatment with enzyme replacement therapy men with classical Fabry disease had a history of more events than men with non-classical disease or women with either phenotype; women with classical Fabry disease were more likely to develop complications than women with non-classical disease. These data may support the development of new guidelines for the monitoring and treatment of Fabry disease and studies on the effects of intervention in subgroups of patients.

Introduction

Fabry disease (FD) (OMIM 301500) is a lysosomal storage disorder caused by deficiency of the enzyme alpha galactosidase A (aGAL) (enzyme commission number: 3.2.1.22) due to mutations in the GLA gene located on the X chromosome (Xq22.1). Deficiency of alpha galactosidase A leads to accumulation of glycosphingolipids, particularly globotriaosylceramide, in various cell types throughout the body.[1, 2] This accumulation of globotriaosylceramide can result in multi-system disease, mainly affecting the kidneys, heart and nervous system.

The disease can be divided into a severe, classical phenotype, most often seen in men without residual enzyme activity, and a generally milder non-classical phenotype. Patients with classical FD usually present with characteristic FD symptoms such as neuropathic pain, cornea verticillata and angiokeratoma. Long term disease manifestations include hypertrophic cardiomyopathy, cardiac rhythm disturbances, progressive renal failure and stroke.[3] Non-classical FD, also referred to as late onset or atypical FD, is characterized by a more variable disease course, in which patients are generally less severely affected and disease manifestations may be limited to a single organ. Men with non-classical disease typically have residual enzyme activity and lower levels of the deacetylated substrate (globotriaosylsphingosine [lysoGb3]).[4] Patients with FD identified in screening studies of individuals with stroke, renal failure or cardiomyopathy often have this more restricted phenotype.[5] Despite the X-linked inheritance pattern, women often have signs and symptoms of FD although they are in general less severely affected compared with men.[6] It is hypothesized that skewed X inactivation underlies the variability of the phenotype in women.[7-9]

Treatment of FD consists of enzyme replacement therapy (ERT) and adjunctive treatment including ACE-inhibitors or angiotensin receptor blockers, anti-platelet drugs and analgesics. Studies have shown that ERT can delay, but not always prevent, some of the clinical complications of the disease. [10, 11]

The interpretation of long term studies of ERT is hampered by insufficient information on the phenotypes of enrolled patients and the absence of a control group or reliable data on the natural course of the disease without ERT. Some studies conducted before the use of ERT showed that men with FD have a reduced life expectancy and an increased risk of developing complications such as end stage renal disease (ESRD).[12, 13] These cohorts most likely consisted mainly of patients with a classical FD phenotype since the wide spectrum of the disease was not fully recognized at that time. It is inappropriate, however, to study treatment effect in a combined classical and non-classical FD cohort and subsequently, compare the results to natural course data in classical FD patients. The limited availability of data on the disease course of untreated FD patients prompted us to study the natural history of untreated FD in men and women with classical and non-classical disease which can be used for interpretation of studies on effectiveness of ERT, but also for counseling of patients and their family members. Here we present the results of a retrospective study in a large cohort of FD patients recruited from three international centers of excellence in Germany, the United Kingdom and The Netherlands.

Concise methods

Study design

Data from three FD centers of excellence (Academic Medical Center, The Netherlands; Royal Free London NHS Foundation Trust, United Kingdom; and the University Hospital Wuerzburg, Germany) were merged into one database. For this study we used retrospectively gathered clinical data from before the first visit which were retrieved from medical records and clinical letters, as well as data collected at first visit.

To study the natural course of FD, we included data before start of ERT. No event data for untreated patients subsequent to the first visit were included since this would lead to bias by indication, i.e. only patients with less severe disease would be selected for untreated follow-up. Data at first visit were used to study the relation between phenotype, sex, age and clinical and laboratory measurements.

Data from consecutive patients with a confirmed FD diagnosis by combination of phenotypic features, enzymatic assay (men) and DNA analysis (men and women; see below) were used. Subjects who were classified as no FD and patients of whom insufficient data was available for phenotypic classification or to assess the occurrence of events were excluded from the analysis. Subjects with one of the following genetic variants, with exceptions outlined below, were classified as no FD, based on previous reports in which pathology studies confirmed that there is no characteristic storage in relevant organs: A143T [14, 15], P60L [14], D313Y [16-18], R118C [19]. Three additional mutations were considered neutral variants based on the high frequency in the general population [20, 21], >50% residual enzyme activity and/or lysoGb3 concentrations of <0,7 nmol/l: T385A, IVS0-10 C>T, the complex haplotype: IVS0-10 C>T/IVS4-16A>G/IVS6-22C>T.

Phenotype

Patients were classified as classical or non-classical FD on the basis of their enzyme activity (men only) and the presence or absence of characteristic symptoms **(table 3)**. [22] Men were considered to have a classical phenotype when they met the following criteria: (1) a GLA mutation, (2) enzyme activity ≤5% of the mean reference range, and (3) one or more characteristic FD symptoms (i.e. Fabry neuropathic pain, angiokeratoma and/or cornea verticillata, definitions are in Van der Tol et al.[23]). Men not fulfilling these criteria were categorized as non-classical FD.

Women with a GLA mutation and one or more characteristic FD symptoms (i.e. Fabry neuropathic pain, angiokeratoma and/or cornea verticillata[23]) were classified as having a classical phenotype. Women without these characteristic FD symptoms were classified as non-classical FD.

Classification on the basis of phenotypic features and residual enzyme activity was challenging in two groups of patients. It was decided that, in these patients, a final judgement was made by the treating physician. These groups were as follows:

(1) Patients with the N215S mutation: this group is especially prevalent in the United Kingdom. According to literature and physician experience, patients exhibit a non-classical (mostly cardiac) phenotype, but exceptions may occur. In this group of 90 patients, 12 had a characteristic symptom, but without confirmatory deficiency of GLA activity in leucocytes in men (*n=5)*. Furthermore, one of the N215S patients presented with renal disease at young age (with no other cause). Renal disease was observed in his family (not included in our cohort). According to the judgement of the treating physician this patient was classified as classical FD while the other N215S patients were all classified as non-classical FD. Similarly, three patients with characteristic symptoms and the P389A mutation (1 man, 1 woman) or R112H (1 woman) mutation were discussed with the treating physician.

These patients all had a late onset presentation, only minimal cornea verticillata (no other characteristic FD symptoms) and a family history of non-classical FD. Consequently they were classified as non-classical FD.

(2) Men with slightly higher than 5% enzyme activity in the presence of 1 or more characteristic symptoms (*n=13*). Residual enzyme activity ranged from 6% to 10% in leucocytes (*n = 10)*, and from 6% to 20% in plasma (*n = 3*). All had at least one characteristic FD symptom and the majority had a relative with classical FD and consequently were considered having classical FD. In four men, the enzyme activity and/or the data on characteristic FD symptoms were missing. These patients were classified as classical FD according to the opinion of the treating physician, which was mainly based on their family history.

We also included three patients (one man an two women, all from the same family) with the A143T mutation. They were classified as having classical FD based on the combination of characteristic deposits on renal biopsy or post mortem biopsy, the presence of one or more characteristic FD symptoms, low enzyme activity (3,9%, 21% and 38% respectively) and high plasma lysoGb3 concentrations (man: 35-50 nmol/l while receiving ERT; women 1: 16 nmol/l while receiving ERT; women 2: 8 nmol/l while not receiving ERT). In these cases, a combination of the A143T mutation and an unknown mutation and/or other (genetic) disease modifiers may have caused the classical FD presentation.

Outcomes

We assessed the clinical event rates from birth until first visit. It is important to note that this is not a mortality study, becuase patients were included at first visit. Furthermore, clinical and laboratory measurements at first visit were analyzed.

Clinical events

Renal events were defined as Chronic Kidney Disease (CKD) stage G5 (eGFR <15ml/min/1.73m^2), renal transplantation or renal replacement therapy. Cardiac events included atrial fibrillation, admission for any rhythm disturbance, admission for congestive heart failure, implantation of an implantable cardiac defibrillator (ICD) or pacemaker (PM), myocardial infarction, coronary artery bypass graft surgery or a percutaneous transluminal angioplasty. Cerebral events were defined as stroke or transient ischemic attack (TIA) diagnosed by a neurologist.

Clinical and laboratory measurements

Renal function was evaluated by the eGFR and the amount of protein excretion in urine. The eGFR was calculated using the CKD-EPI in adults [24] and the Schwartz formula in children up to 16 years of age [25]. The eGFR of patients who had received a renal transplant or were undergoing RRT was set at 10 ml/min/1.73m^2. Albuminuria/proteinuria excretion was categorized following CKD guidelines: A1 normal to mildly increased (albumin excretion rate (AER): <30 mg/24h; protein excretion rate PER: <150 mg/24h; albumin to creatinine ratio (ACR): <3 mg/mmol and/or protein to creatinine ratio (PCR): <15 mg/mmol); A2 moderately increased (AER: 30-300; PER: 150-500; ACR: 3-30 and/or PCR 15-50) and A3 severely increased (AER >300, PER >500, ACR >30 and/or PCR >50).[24]

Cardiac involvement was assessed by echocardiography and cardiac MRI. Left ventricular mass (LVM) on echocardiography was calculated using the Devereux formula and was corrected for height (m$^{2.7}$). [26] Left ventricular hypertrophy was defined as LVM ≥49 and ≥45 gram/m$^{2.7}$ in men and women, respectively.[26] The upper reference limit of the relative wall thickness (RWT) was defined as >0.42.[26] The LVM (not including papillary muscles) measured by cardiac MRI was corrected for body surface area (BSA) using the Dubois formula. The upper reference limit [27], adjusted for not including papillary muscles (9% on average) [28, 29], for men and women was estimated at 78 and 74 gram/m^2, respectively. In addition, the presence of late gadolinium enhancement (LGE) on cardiac MRI was assessed as marker of fibrosis.

The presence of white matter lesions (WMLs)/ischemic lesions was investigated by cerebral MRI.

Enzyme activity was expressed as percentage of the mean of the lower and upper reference value. Plasma lysoGb3 levels were measured with an (adjusted) method based on tandem mass spectrometry with glycine labeled (all samples from the Royal Free Hospital and the University Hospital Wuerzburg, as well as all samples after August 2015 from the Academic Medical Center) or isotope labeled (samples from before August 2015 from the Academic Medical Center) lysoGb3 as an internal standard.[30] Results from both internal standards correlated very well (**supplemental material A)**.

Statistical analysis

R (version 3.1.5) was used for statistical analysis. The retrospectively collected data on events were used to assess the event free survival by using survival curves and Cox proportional hazard models. Models were fitted for first renal, first cardiac and first cerebral events separately as well as for any first event. The proportional hazard assumption was visually tested by using Schoenfeld residuals and by including a time dependent variable as covariate. In addition, cross-sectional analyses were performed on data collected at or close to first visit. A linear regression model was used to analyze the relation between age, LVM and RWT. Data on LVM were first log transformed. Not meeting the assumption of normality, a robust linear regression model (package robustbase) using MM-type estimation [31] was used for the analysis of the relation between age and eGFR. Logistic regression was used to analyze the presence of CKD stage A3, LGE and WMLs. Sex and phenotype were included as covariates in all models. The Kruskal-Wallis test with Dunn's test for post-hoc comparison was used to compare lysoGb3 concentrations at first visit between subgroups. Logistic and linear regression models were used to assess the relation between lysoGb3 and disease severity. P-values <0.05 were considered statistically significant. Where appropriate, 95% confidence intervals (95% CI) are given.

Results

Patients

The merged database contained data on 596 patients, of whom 13 were excluded because of insufficient data. Another 42 patients were excluded since they harbored a neutral GLA variant (i.e. did not have FD). The remaining 541 patients were included in the analyses. **Table 1** shows the characteristics of the adult patients at first visit (n = 499). Pediatric patients (n = 42) are discussed separately.

Clinical events

Table 1 shows the differences per group for the occurrence of cardiac, renal and cerebral events. One hundred thirteen adult patients (22.5%) had experienced one or more events before their first visit to the referral center. Men with classical FD had the highest event rate, with a median event free survival of 49.5 years (**figure 1** and **supplemental figures A1-A3)**. The event rate of classically affected women and men with non-classical FD showed overlap. Hazard ratios (HRs) per sex and phenotype are depicted in **table 2**.

Renal function

Data on eGFR were available for 485 (97%) of the adult patients. There was an association between the eGFR at first visit and age in men with classical FD (β = -1.90, 95% confidence interval [95% CI]: -2.2 – -1.6, p<0.001). In the other subgroups this relationship was also present, although less strong ($\beta_{men,\ non\text{-}classical}$ = -1.14, $\beta_{women,\ classical}$ = -1.07, $\beta_{women,\ non\text{-}classical}$ = -1.03, all p<0.001), see **figure 2**.

Of 433 adult patients (85%) data were available on urinary excretion of protein and/or albumin. CKD stage A3 was present in 14-33% of the FD patients **(table 1)**. The odds ratio (OR) of having CKD stage A3 increased with age (OR = 1.035, 95% CI: 1.01 – 1.06, p<0.001). Men with classical FD had a higher risk of having CKD stage A3 than men with non-classical FD (age-adjusted OR = 3.38, 95% CI: 1.6 – 8.2, p<0.01), whereas no differences were found between men with non-classical FD and women with either classical or non-classical FD. Inclusion of CKD stage A3 as covariate in the previously mentioned linear regression model with eGFR as

dependent variable showed that CKD stage A3 was an important predictor of eGFR. This was most prominent in men with the classical phenotype: the eGFR in patients with CKD stage A3 was 27.1 ml/min/1.73m^2 lower (95% CI: -37.5 – -16.7, p<0.001) at age 50 compared with that in patients without CKD stage A3. Details per sex and phenotype are in **supplemental figures B1-B2**.

Cardiac involvement

Left ventricular mass (LVM) measured by echocardiography and cardiac MRI was available in 414 (83%) and 225 (45%) adult patients, respectively. The log transformed LVM was associated with age and phenotype, see **figure 3** (echocardiography) and **figure 4** (cardiac MRI)**.** The LVM measured by echocardiography in patients with classical FD was higher compared with the LVM measured by echocardiography in those with non-classical FD (men: 32%, p<0.001; women: 22% p<0.001). Men with classical FD had a 27% higher LVM compared with women with classical FD (p<0.001). Men with non-classical FD and women with classical FD had comparable LVMs. The relative wall thickness (RWT) increased with age and was dependent of sex and phenotype, see **supplemental figures C1-C2**. The RWT was elevated in the majority (83%) of patients with an increased LVM (i.e. concentric hypertrophy). Concentric remodeling (RWT >0.42 but normal LVM) was predominantly observed in men with classical FD and older patients **(supplemental figures C1-C2)**.

Information on LGE was available for 208 (92%) of the patients who had MRI performed. LGE was present in 30.3% of the cardiac MRIs. The older the patient, the higher the risk of LGE (OR = 1.16, 95% CI: 1.11 – 1.21, p<0.001). Also, men with classical FD had an increased risk of having LGE compared with men with the non-classical phenotype (age-adjusted OR = 7.11, 95% CI: 1.32 – 42.77, p<0.05) and women with classical FD (age-adjusted OR = 2.91, 95% CI: 1.22 – 7.29, p<0.05). The odds of having LGE did not differ between men with non-classical FD and women with either classical or non-classical FD.

In contrast to men, in whom left ventricular hypertrophy on cardiac MRI was a strong predictor for the presence of LGE (age-adjusted OR = 8.63, 95% CI: 2.43 – 36.01, p<0.01), the risk of having LGE was not

associated with left ventricular hypertrophy in women (age-adjusted OR = 1.26, 95% CI: 0.44 – 3.53, p=0.66) (**supplemental figures D1 and D2)**.

Patients with the non-classical phenotype were under-represented in the cardiac MRI data since no cardiac MRI data was available for the majority of patients with the N215S mutation, which most often results in non-classical FD.

Cerebrovascular manifestations

Information on the presence or absence of white matter lesions (WMLs) was available for 283 adult patients (57%). WMLs were present at first visit in 45% of the patients and the odds of having WMLs on cerebral MRI at first visit increased with age (OR = 1.12, 95% CI: 1.09 – 1.15, p<0.001). Men with classical FD were more likely to have WMLs than men with non-classical FD (age-adjusted OR = 8.72, 95% CI: 2.55 – 27.32, p<0.001) and there was a trend towards a higher risk compared with women with classical FD (OR = 2.19, 95% CI: 0.96 – 5.12, p=0.06). Women with classical FD had a higher risk of WMLs compared with women with non-classical FD (OR = 2.11, CI: 1.07 – 4.23, p<0.05). There was no difference between men and women with non-classical FD.

LysoGb3

Samples for analysis of plasma lysoGb3 concentration were available for 351 adult patients (70%). LysoGb3 concentrations differed between all groups (p<0.001), except for the comparison between men with non-classical FD and women with classical FD, see **figure 5**. Taking all patients together, higher lysoGb3 concentrations at first visit were associated with a higher event rate in the past (HR = 1.01, 95% CI 1.01 – 1.02, p<0.001). In the analyses of subgroups an association was found in men with non-classical FD (HR = 1.05, 95% CI: 1.01 – 1.10, p<0.05) and women with non-classical FD (HR = 1.13, 95% CI: 1.03 – 1.25), whereas no relation was found in men and women with classical FD. When data from men with non-classical FD and all women were combined, a similar relation was found (HR = 1.05, 95% CI: 1.03 – 1.08, p<0.001). In this

combined group of men with non-classical FD and all women a 10 point increase in lysoGb3 resulted in a more rapid decrease in eGFR (additional decline of -0.34 ml/min/1.73m^2 per year, 95% CI: -0.55 – -0.12, p<0.01).

In addition a 10 point increase in lysoGb3 was associated with a 20.7% higher LVM (95% CI: 14.6 – 27.1, p<0.001) on echocardiography. LysoGb3 was not associated with eGFR or LVM in men with classical FD phenotype. Of note, the lysoGb3 concentration was not available for the N215S patient with classical FD.

Pediatric cohort

Data on 42 pediatric patients (age <18 years) were available (boys with classical FD: *n* = 16, boys with non-classical FD: *n* = 4, girls with classical FD: *n* = 15, girls with non-classical FD: *n* = 7). Median age was 16.1 (range: 5 – 18). No events had occurred prior to the first visit in these children. Median eGFR was 123.8 ml/min/1.73m^2 (range: 92 – 165), median LVM on echocardiography was 28.1 gram/m$^{2.7}$ (range: 11 – 48, all within age and sex specific reference intervals [32]). The eGFR and LVM did not differ between children with the classical or non-classical phenotype. WMLs were found in four children with classical FD (3 boys, 1 girl) at age 11, 15, 17 and 15 old, respectively. The 15-year-old girl has been described before.[33]

Discussion

In this study, we compared the natural course of men and women with classical and non-classical FD. Our results confirm that the disease course of patients with the classical phenotype clearly differs from that of non-classical FD patients. In particular, men with classical FD have a much higher risk of developing events than both men with non-classical FD and women. In addition, renal function is worse and LVM is higher in this group of patients. Of note, natural history in classically affected women resembles that of non-classical disease in men, whereas non-classically affected women have the mildest disease course. Interestingly, there was a strong relationship between the presence of cardiac hypertrophy and LGE, indicating fibrosis, in men but not in women. Women often developed fibrosis in the absence of cardiac hypertrophy, which is in

line with earlier findings in FD.[34] The significance of an increased relative wall thickness but normal LVM in a subset of patients needs further, preferably longitudinal studies.

Although these outcomes are not unexpected, this study shows, for the first time, the slopes of deterioration for different variables in the different phenotypic groups. Taking these findings together, it should be stressed that it is very important to stratify results of therapeutic studies by sex and phenotype. Our findings may also be used to predict disease course in patients with either classical or non-classical FD, and as such may be helpful in counseling patients and their family members. However, the considerable variation in disease course, even within genotypes, should be taken into account.[35]

Most studies on the natural course of FD are from the pre-ERT era.[12, 13] Although not explicitly stated, the subjects in these studies were most likely to have a classical phenotype, because awareness and screening studies led to a marked increase in the identification of patients with non-classical FD.[5] These natural history studies showed that complications occurred at a mean age of approximately 40 years old[12] and that men with FD had a reduced life span with a median survival of 50 to 55 years.[12, 13] In a more recent study the median event free survival was 41 years.[36] In our cohort, men with classical FD had their first event at a median age of 50 years. This difference can be partly ascribed to the fact that a wider definition of clinical events was used in the previous studies, including bradyarrhythmia and an increase in creatinine of more than 50%.[36] Another explanation could be that the retrospective design of our study has resulted in an underestimation of the event rate. We collected data from before the first visit to estimate the number of clinical events. Consequently, we may have missed or misclassified clinical events if they were not properly documented.

Also, the phenomenon of immortal (or survivor) bias which is inherent to the study design we used, may have led to an underestimation of the event rate[37]: we calculated the event rate by using retrospective data from before the first visit. Consequently, deceased patients were not included, and these patients wer more likely to have severe disease and thus, a history of clinical events.

The problem of immortal bias probably plays a less prominent role in women with FD and men with non-classical FD, because a near normal life expectancy may be assumed in these subgroups. Interestingly, men with non-classical FD and women with classical FD showed substantial heterogeneity: some had only very few symptoms while others had severe cardiac or renal disease at first visit, which is in line with previous findings.[38-40] In these patients, it is important to rule out co-morbidities that could explain severe disease manifestations. However, if additional investigations have shown that organ involvement is, at least partly, due to FD, treatment with ERT may be considered.[41] Women with non-classical FD were in general very mildly affected with only a minority having clinical signs and symptoms.

Although it is clear that the disease course differs between patients with classical and non-classical FD, the definition of these phenotypes is still subject of debate. We used residual enzyme activity (men only) and the presence of characteristic FD symptoms to classify patients. It was considered inappropriate to base the classification on the type of mutation since it is known that phenotypes may differ between patients with the same mutation, and even between patients within the same family. This is assumed to be caused by disease modifiers or in case of women, skewed X inactivation.[7-9] As an alternative, plasma lysoGb3 concentrations are a valuable tool in the classification of FD.[4, 42] In line with earlier findings,[4] lysoGb3 concentrations can, indeed, be used to differentiate between phenotypes in men with FD. Concentrations of >45 nmol/l are strongly indicative for a classical FD phenotype in men with FD. Four men who were classified as classical FD patients had a plasma lysoGb3 concentration of below 45 nmol/l. Most likely, (part of) these four patients are misclassified due to misinterpretation of the retrospectively collected clinical information. Furthermore, one man with non-classical FD had a plasma lysoGb3 concentration of 47 nmol/l which is just

above the cut off value of 45 nmol/l. His brother who was also classified as non-classical had a lysoGb3 concentration of 40 nmol/l which suggests that their genotype (G325S) results in relatively high levels of plasma lysoGb3. More generally, similar lysoGb3 concentrations were found in men with non-classical FD and women with classical FD which is consistent with the observation that the disease course in women with classical FD resembles that of non-classical disease in men. In addition, we showed that plasma lysoGb3 concentrations were associated with the disease severity in men and women with non-classical FD. The absence of such an association in classical FD patients may be due to a ceiling effect; above a certain threshold concentration, higher lysoGb3 levels are not predictive for more severe disease. These results support previous findings that the concentration of lysoGb3 is a reliable predictor of the disease course.[43]

For the pediatric patients in our cohort no clinical events were reported. Also, we did not observe any abnormal value for the eGFR or LVM at first visit. However, four children with classical FD presented with one or more WMLs. The presence of early cerebrovascular disease in children with FD has been described in previous reports.[44, 45] Other early findings may include subtle electrocardiography abnormalities, mild left ventricular hypertrophy and mild albuminuria.[46-48]

In conclusion, sex, phenotype and plasma lysoGb3 concentrations are strongly associated with the rate of clinical events as well as cardiac, renal and cerebral involvement. Men with classical FD have an increased risk of developing complications, more severe cardiac and renal disease and higher lysoGb3 values compared with non-classical FD patients and women. Women with classical FD also have a higher risk to develop complications compared with women with non-classical FD. Of interest, women may develop cardiac fibrosis in the absence of left ventricular hypertrophy according to reference values. These data are of high importance to support the development of guidelines for follow-up and treatment and to study effects of intervention per subgroup of patients.

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Authors' contributions

MA: study design, data acquisition, data analyses, data interpretation, first draft of manuscript. MB and CH: study design, data interpretation, revision of manuscript. CW, DH, AM, DO, OW, PE, GL and FW: data acquisition, data interpretation, revision of manuscript.

Competing interests

MA and OW have no competing interests do declare. DO received travel assistance from Sanofi Genzyme and Shire HGT. MB, GL and CH have received travel support, honoraria for consultancies, and educational grants from Sanofi Genzyme, Shire HGT, Protalix, Actelion and Amicus. All financial arrangements are made with the AMC Medical Research BV, in accordance with the AMC Research Code. CW has received honoraria for lecturing from Sanofi Genzyme and grant to the institution from Sanofi Genzyme and Shire HGT. DH as received honoraria for speaking and advisory boards and support for research from Shire HGT, Sanofi Genzyme, Amicus and Protalix, consultancy arrangement through UCL consultants to support in part laboratory research. AM has received honoraria for consultancy and educational activities as well as research grant support from Shire HGT, Sanofi Genzyme, Protalix/Pfizer and Amicus. PE has received speaker fees from Shire HGT and consultancy and speaker fees from Sanofi Genzyme, Pfizer and Gilead Sciences. FW has received honoraria for presentations and board meetings, travel expenses to meetings and honoraria for consultancy work from Sanofi Genzyme and Shire HGT, and have received unrestricted educational grants and research grants from Sanofi Genzyme.

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Figure legends

Figure 1. Event free survival (any event) stratified for sex and phenotype. Shaded areas represent the 95% CI. Crosses indicate censoring (i.e. first visit).

Figure 2. Robust linear regression of eGFR, shaded areas represent the 95% CI for the fitted curves. Black dots represent classical FD patients, grey triangles represent non-classical FD patients.

Figure 3. Log-linear regression curve of the LVM measured by echocardiography corrected for height (m2.7), shaded areas represent the 95% CI for the fitted curves. The dashed horizontal lines represent the upper reference limits (men: 48 gram/m2.7, women: 44 gram/m2.7) [26]. Black dots represent classical FD patients, grey triangles represent non-classical FD patients.

Figure 4. Log-linear regression curve of the LVM measured by cardiac MRI corrected for BSA, shaded areas represent the 95% CI for the fitted curves. The solid black line represents the adjusted (for not including the papillary muscles) upper reference limit for men (83 gram/ m2), the dashed black line represents the upper reference limit for women (71 gram/ m2) [49]. Black dots represent classical FD patients, grey triangles represent non-classical FD patients.

Figure 5. Boxplot of lysoGb3 stratified for sex and phenotype. *** p<0.001

Tables

Table 1
Characteristics at first visit of patients ≥18 years old

	Men		Women	
	Classical	Non-classical	Classical	Non-classical
Patients	138 (27.7%)	66 (13.2%)	147 (29.5%)	148 (29.7%)
Age at first visit	38.4 (10.8, 19-65)	55.4 (13.1, 19-76)	41.5 (13.5, 18-75)	43.7 (15.0, 18-79)
Any event before first visit	42 (30.4%)	27 (40.9%)	28 (19.0%)	16 (10.8%)
Cardiac event before first visit	16 (11.6%)	19 (28.8%)	11 (7.5%)	9 (6.1%)
Arrhythmia related event	11 (8.0%)	18 (27.3%)	9 (6.1%)	5 (3.4%)
Ischemia related event	5 (3.6%)	1 (1.5%)	2 (1.4%)	4 (2.7%)
Renal event before first visit	12 (8.7%)	2 (3%)	2 (1.4%)	1 (0.7%)
Renal transplant	0	0	1 (0.7%)	1 (0.7%)
Renal replacement therapy	9 (6.5%)	1 (1.5%)	0	0
eGFR < 15 ml/min/1.73m^2	3 (2.2%)	1 (1.5%)	1 (0.7%)	0
Cerebral events before first visit	15 (10.9%)	6 (7%)	16 (10.9%)	6 (4.1%)
TIA	10 (7.2%)	3 (3.5%)	9 (6.1%)	5 (3.4%)
Stroke (symptoms >24h)	5 (3.6%)	3 (3.5%)	7 (4.8%)	1 (0.7%)
eGFR (ml/min/1.73m^2)	101 (7-139)	83 (10-136)	105 (10-145)	95 (10-131)
eGFR <60 ml/min/1.73m^2	38/131 (29.0%)	18/65 (27.7%)	8/142 (5.6%)	14/147 (9.5%)
CKD stage A3	39/131 (33.3%)	13/65 (23.6%)	20/142 (14.9%)	147/116 (13.8%)
LVM (gr/m$^{2.7}$)	42 (21-140)	56 (16-99)	42 (21-140)	36 (16-108)
Relative wall thickness	0.41 (0.24-1.45)	0.47 (0.24-0.95)	0.48 (0.21-0.93)	0.42 (0.24-1.45)
Left ventricular hypertrophy	66/105 (62.9%)	33/49 (67.3%)	59/132 (44.7%)	40/128 (31.2%)
Concentric hypertrophy	57/105 (54.3%)	28/49 (57.1%)	47/132 (35.6)	32/128 (25.0%)
Concentric remodeling	17/105 (16.2%)	6/49 (12.2%)	18/132 (13.6%)	17/128 (13.3%)
LysoGb3*	111 (32-175)	7.8 (1.2-47)	9.3 (0.7-42)	2.5 (0.4-20)

*Mean (SD, range) or median (range) of continuous variables, number of patients (%) for discrete variables. Events represent the number of patients with one or more events before first visit. Arrhythmia related event included: atrial fibrillation, implantation of an ICD or PM and admission for any rhythm disturbance. Ischemia related events included: myocardial infarction, coronary artery bypass graft surgery and a percutaneous transluminal angioplasty intervention. TIA = transient ischemic attack, eGFR = estimated glomerular filtration rate, LVM = left ventricular mass index on echocardiography. Left ventricular hypertrophy was defined as follows, a LVM: ♂ ≥49 / ♀ ≥ 45. Concentric remodeling was defined as left ventricular hypertrophy and a RWT >0.42.Upper reference limit lysoGb3 = 0.6. *LysoGb3 was not available for the N215S patient classified as classical FD.*

Table 2
Hazard ratios of events

	Any event	Cardiac events	Cerebral events	Renal events
Men, classical vs non-classical phenotype	5.63 *** (3.17 -10.00)	5.16 *** (2.22 – 12.04)	4.71 ** (1.74 – 12.76)	9.24 ** (1.73 -49.45)
Women, classical vs non-classical phenotype	2.88 *** (1.54 – 5.40)	2.34 (0.95 – 5.76)	3.51 ** (1.36 – 9.06)	2.27 (0.21 – 25.13)
Classical phenotype, men vs women	3.87 *** (2.32 – 6.55)	4.98 *** (2.13 - 11.62)	1.65 (0.81 – 3.39)	9.07 * (1.98 – 42.56)
Non-classical phenotype, men vs women	1.98 * (1.07 – 3.69)	2.26 * (1.02 – 5.02)	1.23 (0.40 – 3.82)	1.24 (0.20 – 25.72)

Hazard ratios (95% confidence interval)
** p<0.05, **p<0.01, ***p<0.001*

Table 3
Criteria for phenotypic classification

Classical FD

Men	Women
▪ A mutation in the GLA gene* ▪ ≥ 1 of the following characteristic Fabry disease symptoms: Fabry neuropathic pain, angiokeratoma and/or cornea verticillata ▪ Severely decreased or absent leukocyte AGAL activity (<5% of the normal mean)	▪ A mutation in the GLA gene ▪ ≥ 1 of the following characteristic Fabry disease symptoms: Fabry neuropathic pain, angiokeratoma and/or cornea verticillata

Non-classical FD

▪ A mutation in the GLA gene, and not fulfilling the criteria for classical FD

**The following genetic variants were considered no FD (neutral variants): A143T, P60L, D313Y, R118C, T385A, IVS0-10 C>T, the complex haplotype: IVS0-10 C>T/IVS4-16A>G/IVS6-22C>T. In patients in whom classification on the basis of these criteria was not feasible, the final judgement was made by the treating physician.*