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Fabry disease phenotyping in women from the complete Romanian cohort – time for early diagnostic awareness

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Abstract

Fabry disease (FD) is an X-linked rare disorder caused by mutations in the GLA gene. Women with FD have been less enrolled in studies and less treated compared with men. The aim of the present study is to describe the complete phenotype of the women cohort with FD diagnosed and evaluated in Romania and compare it to the male population.

This study included all consecutive patients diagnosed with FD referred to the Expert Center for Rare Genetic Cardiovascular Diseases between 2014–2023 which included 73 consecutive Romanian FD patients: 41 women (56.2%) and 32 men (43.8%) from 33 unrelated families.

Women with FD were diagnosed later and had a later symptom onset. Comparing with men, women were less often symptomatic, but with similar symptom severity. They had similar ophthalmologic and ENT involvement, but less angiokeratomas. Both women and men had similar heart failure symptoms, which were usually mild to moderate, with no difference between the age of developing of the heart failure symptoms. There were also similar rates of acroparesthesia and stroke between sexes, but women presented less renal involvement, with less requirement for renal transplant.

This study demonstrates that women with Fabry disease are not just carriers of the disease, they can present symptoms as severe as men, and they have less or later access to pathogenic therapy. Further studies with more female participations are needed to better understand the burden of Fabry disease in women.

Keywords: Fabry disease, women, heart failure, enzyme replacement therapy, gender.

INTRODUCTION

Fabry disease is an X-linked rare lysosomal storage disease caused by mutations in the GLA gene which lead to a decrease in alpha-galactosidase A (α-GalA) enzyme activity and finally to the accumulation of lysosomal globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3) in tissues. Gb3 and lyso-Gb3 accumulate mainly in kidneys, heart, blood vessels, and nervous system. The incidence of FD was reported to range from 1 in 37000 to 1 in 117000 [1] [2], but may be underestimated [3].

More than 1000 GLA variants have been identified [4] with some variants leading to a more severe phenotype. Depending on the variant, patients can present with a classical severe form of FD, or with later onset, more benign form of FD. Variants that lead to absent or low a-Gal A enzyme activity are usually associated with classic early-onset FD, while variants associated with residual a-Gal A activity cause later-onset FD, sometimes limited to one organ. Classic FD is rare and severe. It is usually present in hemizygous males, but females can also present with a classic form. The non classic FD is a later onset disease.

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Being an X linked disease, females are heterozygous, with one mutant and one non-mutant X chromosome. This has led to the belief that FD women are less affected than males. In fact, women can vary from asymptomatic or mildly symptomatic, to severely symptomatic but with a later onset of symptoms [5] [6]. The phenotypic heterogeneity in women is linked to skewed X chromosome inactivation (XCI), which is a process in which one of the X chromosomes is randomly inactivated during embryonic development [7]. Therefore, females can have both variant Fabry cells and normal cells and in varying proportion [6]. Women with FD have been less enrolled in studies and less treated compared to men [8]. The purpose of this study is to describe the complete phenotype of the FD women cohort resulted from the complete FD population diagnosed and evaluated in Romania, as well as compare it to the male population.

MATERIAL AND METHODS

STUDY POPULATION

The study included all consecutive patients diagnosed with FD referred to the Expert Center for Rare Genetic Cardiovascular Diseases from Bucharest Romania, between 2014–2023.

The study received the approval of the ethics committee of the "C. C. Iliescu" Emergency Institute for Cardiovascular Diseases and was performed in accordance with the principles of the Declaration of Helsinki. All patients signed an informed consent.

DIAGNOSTIC WORKUP

All patients went through a complete clinical and biological workup. They were evaluated by a multidisciplinary team, in order to obtain complete data on disease manifestations.

Patients went through a thorough clinical assessment, including a complete family history and pedigree. The enrollment method by proband status or family screening was documented for each patient. In case of a proband patient we also documented the department who first evaluated and diagnosed the patient (cardiology, nephrology, neurology, or dermatology), and reported it as a diagnostic pathway. Age at diagnosis and age of symptoms onset were noted. The main phenotype of disease was recorded in every patient.

All patients underwent genetic testing, and the gene variant was recorded in every patient. Genetic testing was performed using a dry blood spot test. Alpha galactosidase (αgalA) and lysoGb3 (globotriaosylsphingosine) levels were documented.

Cardiovascular assessment included screening for classical cardiovascular risk factors (hypertension, diabetes, smoking and dyslipidemia), for cardiovascular symptoms (angina, dyspnea, syncope, palpitation) and we also documented the age of onset of cardiovascular symptoms.

All patients had a standard 12-lead electrocardiogram (EKG) and a comprehensive transthoracic echocardiogram was performed according to the current international recommendations [9] using commercially available scanners (Vivid e95, GE Vingmed Ultrasound AS Horten, Norway). All parameters recorded are shown in the supplementary material.

Cardiac magnetic resonance (CMR) was performed in patients who did not have a contraindication (e.g. claustrophobia, presence of metallic bodies) using a 1.5T CMR scanner (Siemens Magnetom Sempra, Siemens Healthineers, Erlangen, Germania). All parameters recorded are shown in the supplementary material.

Neurological assessment included documenting the presence of acroparesthesia, transient ischemic attacks (TIA) or stroke. Also, the age at first cerebrovascular event was noted. Whenever deemed necessary, patients underwent cerebral magnetic resonance imaging (MRI).

Patients underwent a comprehensive renal evaluation, documenting also whether they had a kidney biopsy or a renal transplant. Renal function was assessed by measuring creatinine, presence of microalbuminuria (30–300 mg/24 h), or proteinuria (>300 mg/24 h). Estimated glomerular filtration rate (EGFR) was calculated using CKD-EPI formula in adults and Schwarts formula in children and the stage of the chronic kidney disease was established.

Dermatologic evaluation included recording the presence of angiokeratomas or hypohydrosis. We noted whether patients had tinnitus, deafness, or vertigo. Ophthalmologic evaluation including slit lamp examination for cornea verticillate was performed.

Biological workup included conventional blood and urine tests. Whenever available, natriuretic peptides (BNP or NTproBNP) and troponin levels were documented.

The complete prescribed treatment was recorded, noting whether patients were on pathogenic therapy. If pathogenic therapy was present, we documented the type, duration and starting age of therapy: enzyme replacement therapy (ERT) or migalastat.

STATISTICAL ANALYSIS

Statistical analysis was performed using a commercial software (IBM SPSS version 28, ICM Corporation, Armonk, NY). Continuous variables were expressed as mean±SD or median and interquartile range depending on the normality of their distribution. Categorical data were expressed as number and percentage of total patients in each group. T test or Wilcoxon rank-sum test was used for differences in continuous variables between 2 groups when normally or non-normally distributed, respectively. Normality was confirmed using the Kolmogorov-Smirnov test. Categorical variables were compared by using χ^2 tests. Survival at the last time point of follow-up was recorded and compared among genders using Kaplan Meyer analysis. A $p<0.05$ was considered of statistical significance.

RESULTS

STUDY POPULATION

During the inclusion interval, we collected data from all 73 consecutive Romanian FD patients evaluated in our center: 41 women (56.2%) and 32 men (43.8%) from 33 unrelated families. The 33 unrelated families included between 1 and 7 known affected family members. While the majority of families had different variants, 4 of them shared the same variant c.[644A>G] which led to p.[N215S] which was the most common variant in our group. There were also 2 families that shared the variant c.[334C>T] which led to p.[R112C], and 2 families that shared the debatable c.937G>T p.[D313Y] variant. (Table S1 in Supplementary Materials). We found 27 different variants from which 59.3% were missense, 22.2% nonsense, 18.5% deletions. The variants associated with less than 3% residual enzyme activity led to a classic phenotype (83.6% of patients), while the ones that associated with residual enzyme activity between 3–15% led to a late onset FD (13.7% of patients). The variant p. D313Y, controversial in terms of pathogenicity, was present in 2.7% of cases (2 women with neurological onset – stroke at young age). The classic variant was present in 33 women (80.5%), while the late variant was present in 6 cases (14.6%).

In our population most females were diagnosed through family screening (31 patients, 75.6%), while most men were diagnosed after evaluation for chronic kidney disease (14 patients, 43.7%) ($p<0.001$) (Table 1). Cardiology specialists had an important contribution in diagnosing women with FD, being the second entry pathway for women (7 patients – 17.1%), followed by neurology with 2 patients (4.8%).

The number of healthy carriers (G+/P-) was different between genders: in our group 11 women (26.8%) were G+/P- and only 1 man (3.1%) (p 0.007).

The main characteristics of patients with organ involvement are listed in the table 3 showing the main difference between women and men regarding age and systemic involvement.

In our cohort, women were older (p 0.005), diagnosed later ($p<0.001$) and had a symptom onset later compared with the men ($p<0.001$). Women with FD were less symptomatic comparing to men (p 0.007), but when symptoms were present, they could be as severe as in men.

CARDIAC INVOLVEMENT

There was no difference in conventional CV risk factors or symptoms between women and men (Table 2). Both women and men with FD developed cardiac symptoms later in life starting from the fifth decade (table 2). There was no difference in terms of symptoms of heart failure between women and men. Most of the women and men with FD had no symptoms of HF, while most of the symptomatic ones were in NYHA class I and II, and no patients were in NYHA class IV.

Women had more presyncope and more palpitations than men. Pacemaker implantation was necessary in 9 patients (12.3%), among whom 7 were women (17%) and 2 men (6.2%) (p 0.283).

Only one woman with FD received an implantable cardioverter defibrillator (ICD), which was implanted before the FD diagnosis was established, based on the high hypertrophic cardiomyopathy sudden cardiac death score which was estimated because she was first diagnosed with hypertrophic cardiomyopathy. None of the male patients required ICD implantation.

NEUROLOGIC INVOLVEMENT

Women in our cohort were equally affected as men from acroparesthesia and stroke. Stroke was diagnosed in a considerable proportion of patients of both genders, and age of first cerebrovascular event did not differ in women compared to men (Table 3).

Table 1

Diagnostic pathways in FD patients

		Gender		
		F (n=41)	M (n=32)	Total (n=73)
Diagnostic pathway	Cardiology n, %	7 (17.1%)	4 (12.5%)	11 (15%)
	Nephrology n, %	1 (2.4%)	14 (43.8%)	15 (20.5%)
	Neurology n, %	2 (4.9%)	6 (18.7%)	8 (11%)
	Dermatology n, %	0 (0 %)	2 (6.3%)	2 (2.8%)
	Family screening n, %	31 (75.6%)	6 (18.7%)	37 (50.7%)

Table 2

Clinical characteristics of patients (p for women vs men)

	All patients (n=73)	**Women (n=41)**	**Men (n=32)**	**p value**
Age at enrollment (years ±SD, min, max)	46.5±16.41 (7-79)	50.8±16.2 (12-79)	41±14.96 (7-71)	0.005
Age at Fabry diagnosis (years ±SD, min, max)	41.2±16.9 (4-77)	47.1±16.7 (6-77)	33.5±14.1 (4-64)	<0.001
Age at symptoms onset (years ±SD, min, max)	27.3± 17.1 (5-63)	35.5±15 (10-57)	20.1±15.8 (5-63)	<0.001
Deceased patients (n, %)	12 (16.4%)	3 (7.3%)	9 (28.1%)	0.017
Age of death (years ±SD, min, max)	52.8± 12.8 (33-71)	62.6± 2.1 (61-65)	49.6± 12.8 (33-71)	0.131
Symptomatic patients (n, %)	61 (83.5%)	30 (73.1%)	31 (96.8%)	0.007
Cardiovascular risk factors (n, % from available data)				
Smoking	14 (20.3%)	9 (23.7%)	5 (16.1%)	0.438
Hypertension	27 (39.1%)	17 (44.7%)	10 (32.2%)	0.291
Dyslipidemia	25 (36.2%)	17 (44.7%)	8 (25.8%)	0.104
Diabetes	4 (5.8%)	3 (7.9%)	1 (3.2%)	0.409
Cardiovascular involvement (n, % from available data)				
Angina	8 (10.9%)	4 (9.8%)	4 (12.5%)	0.711
Palpitations	20 (27.4%)	15 (36.6%)	5 (15.6%)	0.046
Syncope	5 (6.8%)	4 (9.8%)	1 (3.2%)	0.377
Lipothymia	5 (6.8%)	5 (12.2%)	0 (0%)	0.014
Dyspnea	22 (30.1%)	14 (34.1%)	8 (25%)	0.398
NYHA Class				
No HF symptoms	41 (56.2%)	23 (56.1%)	18 (56.2%)	0.611
NYHA I class	10 (13.7%)	4 (9.8%)	6 (18.8%)	
NYHA II class	18 (24.6%)	11 (26.8%)	8 (21.9%)	
NYHA III class	4 (5.5%)	3 (7.3%)	1 (3.1%)	
NYHA IV class	0 (0%)	0 (0%)	0 (0%)	
Age at cardiovascular symptoms onset (mean, SD, min, max)	53.5±10.2 (33-73)	53.3±11.7 (33-73)	53.8±8.2 (43-63)	0.462
Neurological involvement (n, % from available data)				
Acroparesthesia	42 (59.1%)	22 (55%)	20 (64.5%)	0.418
TIA	2 (2.7%)	0 (0%)	2 (6.2%)	0.189
Stroke	18 (24.6%)	11 (26.8%)	7 (21.9%)	0.626
Age at first cerebrovascular event (mean, SD, min, max)	48.8±11.2 (26-65)	51±10.4 (26-64)	45.3±12.2 (32-65)	0.148

Table 2 (continued)

	All patients (n=73)	Women (n=41)	Men (n=32)	p value
Renal involvement (n, % from available data)				
CKD stage n (%)				
no CKD	6 (8.8%)	2 (5.6%)	4 (12.5%)	**0.043**
Stage I+II	39 (57.4%)	24 (66.7%)	15 (46.9%)	
Stage IIIa+IIIb	10 (14.7%)	7 (19.4%)	3 (9.3%)	
Stage IV+V	13 (19.1%)	3 (8.3%)	10 (31.3%)	
Proteinuria level n (%)				**0.016**
No proteinuria	21 (42.9%)	14 (41.2%)	12 (46.2%)	
Microalbuminuria (30-300 mg/24 h)	11 (22.4%)	11 (32.4%)	1 (3.5%)	
Proteinuria (>300 mg/24 h)	17 (34.7%)	6 (26.5%)	13 (50%)	
Dialysis n (%)	10 (13.7%)	2 (4.9%)	8 (25%)	0.018
Kidney biopsy performed n (%)	25 (37.9%)	13 (36.1%)	12 (40%)	0.746
Kidney transplant performed n (%)	7 (9.6%)	1 (2.4%)	6 (18.8%)	**0.039**
Kidney transplant before diagnosis (n, % from all transplants)	3 (42.9%)	0 (0%)	3 (50%)	
Kidney transplant after diagnosis (n, % from all transplants)	4 (57.1%)	1 (100%)	3 (50%)	
Angiokeratomas (n, % from available data)	29 (43.3%)	10 (25%)	19 (63.3%)	**0.003**
Cornea verticillata (n, % from available data)	22 (48.9%)	12 (52.2%)	10 (45.5%)	0.652
Hypohydrosis (n, % from available data)	26 (40.6%)	11 (30.5%)	15 (53.6%)	0.063
Acroparesthesia (n, % from available data)	42 (59.1%)	22 (55%)	20 (64.5%)	0.418
ENT involvement (n, % from available data)				
Tinnitus	17 (25%)	10 (26.3%)	7 (23.3%)	0.778
Deafness	11 (16.4%)	6 (16.2%)	5 (16.6%)	0.961
Vertigo	12 (17.6%)	8 (21%)	3 (13.3%)	0.407

Abbreviations: HF heart failure, TIA transient ischemic attack, CKD chronic kidney disease

Table 3

αGalA and Lyso Gb3 levels (p for women vs men)

	All patients	Women	Men	p value
α Gal A levels μmol/L/h (mean ±SD, min, max)	1.03±0.92 (0-3.7)	1.35±0.89 (0.3-3.7)	0.61±0.83 (0-2.8)	**<0.001**
α Gal A levels < 2.8 μmol/L/h (n, % from available data)	47 (90.3%)	26 (92.8%)	21 (87.5%)	0.652
lyso GB3 ng/ml (mean ±SD, min, max)	22.6±31.3 (1.3-102.4)	6.3±2.9 (1.3-13.7)	54±37.3 (1.5-102.4)	**<0.001**
lyso GB3 >3.5 ng/ml (n, % from available data)	36 (87.8%)	23 (85.1%)	13 (92.8%)	0.645

RENAL INVOLVEMENT

When comparing milder kidney disease stages with advanced kidney disease stages, women had milder kidney involvement (stages 1 and 2), while men had more often severe kidney involvement (stages 4 and 5) (p 0.025). The mean eGFR in women was 79.2±29.0 ml/min/1.73 m², while in men was 65.3±41.9 ml/min/m² (p 0.045).

Women required less often renal transplant (one woman vs 6 men, p 0.039).

SERUM BIOMARKERS

The levels of αGalA and Lyso Gb3 are shown in Table 3. α Gal A levels at diagnosis were available in 70% of patients, and lysoGB3 levels were available in 56% of patients. Women had higher residual activity of αGal A and much lower levels of Lyso GB3 compared to men in our group.

We did not find a correlation between maximal wall thickness and lysoGb3 levels in our group (r 0.353, p=0.071).

The levels of BNP, troponin, and the number of patients with increased levels are listed in Table 4. There was no difference between the values of BNP and troponin between women and men.

We found a correlation between BNP and MWT in our group both in women and men (Spearman r =0.792, p<0.001 for women and Spearman r=0.753, p<0.001 for men). BNP also correlated with GLS both sexes (Spearman r =0.686, p=0.007 for women and Spearman r=0.815, p<0.001 for men). There were no differences between BNP levels in patients with or without LGE irrespective of their sex (median BNP 70.3 pg/ml in women without LGE and 142.5 pg/ml in women with LGE p 0.190 for women, and median BNP 12 pg/ml in men without LGE and 77 pg/ml in men with LGE p 0.381 for men).

In our cohort troponin also correlated with MWT both in women and in men (Spearman r= 0.740, p<0.001 for women and Spearman r=0.867, p<0.001), as well as with GLS in both genders (Spearman r =0.847, p<0.001 for women and Spearman r=0.654, p=0.008 for men). Men with LGE had higher levels of troponin compared with men without LGE (mean troponin in men with LGE present 47.28 ng/ml vs mean troponin in men without LGE 0.006 ng/ml, p 0.03). However, this was not true in women as both women with LGE present or absent had similar increase in troponin (mean troponin in women with LGE 0.004 ng/ml vs mean troponin in women without LGE 0.019 ng/ml, p 0.109).

ECG CHANGES

Sinus rhythm was the dominant heart rhythm in our cohort. Supraventricular or ventricular arrhythmias like atrial fibrillation or nonsustained ventricular tachycardia (NSVT) were not frequently seen, with no inter-gender differences (Table S2 in Supplementary materials).

The PR interval duration was similar in both genders, as well as the frequency of a short PR. Less women than men had LVH on ECG (33.3% of women vs 73.3% of men) (p <0.001). Right or left bundle branch block (BBB) is not a characteristic finding (14.4% overall prevalence), without gender differences. Negative T waves appear to be present often on FD patients EKGs, mostly in the lateral leads, being as frequently seen in women (29.3%) as in men (40.7%) (p NS).

ECHOCARDIOGRAPHY

Complete echocardiography data are presented in Table 5. LV hypertrophy (defined as wall thickness ≥ 12 mm) was present in a smaller number of women than men (19 women – 47.5% vs 22 men – 75.8%, p 0.018), but without significant difference for MWT between genders (p 0.079) (Figure 1 and 2). MWT correlated with age both in women and in men. (Spearman r =0.530, 0 <0.001 in women, Spearman r=0.713, p<0.001 for men).

Patients with FD with LVH had mainly nonobstructive hypertrophic cardiomyopathy (HCM) independent of their sex: 17 women (42.5%) and in 18 men (62%). Obstructive HCM in our group was present in a small number of patients 2 women (5%) and men 4 (13.7%) (p 0.203) and was due to systolic anterior movement of the mitral valve. Left atrial enlargement was seen in 18 women (47.3%) and 11 men (40.7%) (p 0.981) with similar LA indexed volumes. Most of the patients had a preserved EF (36 women, 97.3% and 28 men, 96.6%), with only one woman (2.7%) having moderate reduced EF 31–39%, and one man (3.4%) having mild reduced EF 40–49%. No patient had severely reduced EF less than 30%.

LV GLS was decreased (below 18% in absolute value) in 16 women (50%) and in 17 men (68%) but there was no intergender difference (p 0.190). Furthermore, an abnormal GLS was found in 22.2% of the women without LVH and in 42.8% of the men without LVH, but with no differences between genders (p 0.836) (Figure 3).

RV hypertrophy (defined as RV free wall thickness >5mm) was present in 16 women (48.4%) and 17 men (65.3%) (p 0.242), with similar average RV wall thickness. Furthermore, RVH increases with age, both women and men presenting a significant correlation between age and RVH (r =0.652, p <0.001 in women and r=0.457, p=0.019 in men). RV function appeared preserved in both sexes.

No significant valvular disease was present in women or men with FD from our cohort (28% in women and 44.8% in men had mild mitral regurgitation, p 0.457).

CMR

CMR was performed in 26 women (63.4%) and 19 men (59.3%). Based on CMR, LVH was more frequently seen in men than in women, being present in our group in 12 women (46.1%), and in 15 men (78.9%) (p 0.02). However, MWT was similar between sexes (p 0.167). As for echocardiography, MWT correlated with age and for both women and men (r= 0.698, p< 0.001 in women and r =0.942, p <0.001 for men).

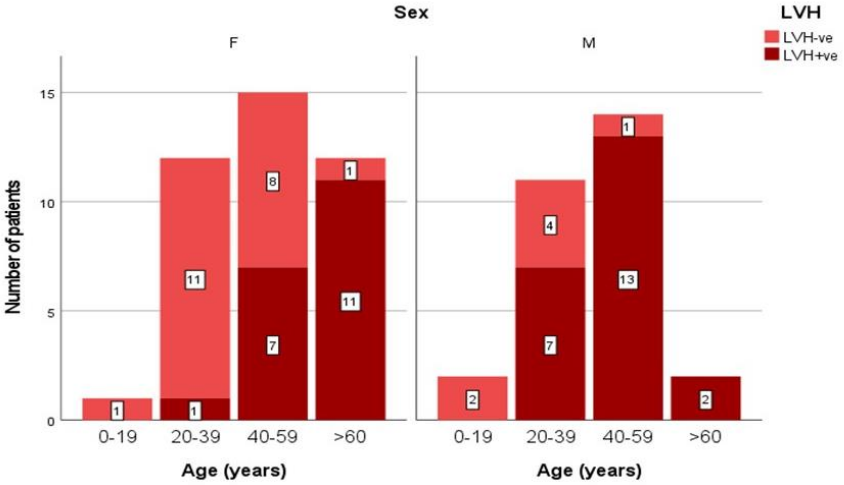

Figure 1. Left ventricular hypertrophy prevalence according to age and sex. Abbreviations: LVH, left ventricular hypertrophy.

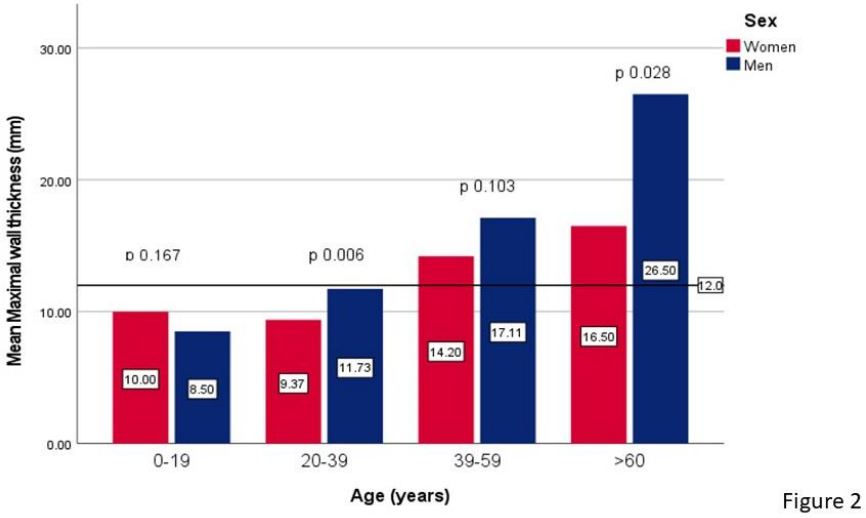

Figure 2. Maximal wall thickness according to age and sex.

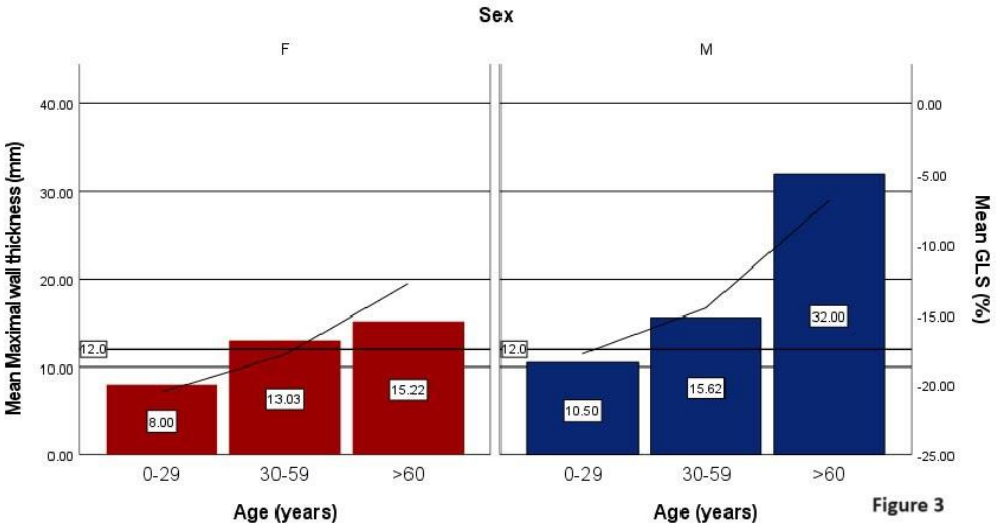

Figure 3. Maximal wall thickness and myocardial deformation indices relation.
Abbreviations: GLS, global longitudinal strain of the left ventricle; F, females; M, males

CMR with T1 mapping was available in 12 women (46.1%) and in 12 men (63.1%). Average T1 values and also decreased T1 values were similar between sexes. Furthermore 57.1% of females and 50% of males without LVH had reduced T1 values. Reduced T1 values were more frequent in patients with LVH, being found in 80% of women and in 77.7% of men with LVH.

LGE was not frequently seen in our FD patients independent of their sex (p 0.355). In our group LGE was not present in women without LVH. Patients with LGE had higher LVH compared with patients without LGE no matter of their sex (p<0.001 for both sexes) (for women with LGE+, MWT was 18.2±3.6 mm vs 11.3±4.2 mm for LGE-, while for men with LGE+ MWT was 20.9±6.1 vs 12±2.5 mm in men with LGE-.

TREATMENT

Women were less treated with pathogenic therapy as compared to men (19 women, 63.3% vs 29 men, 90% based on the population with an indication to treat, p 0.01). Among the 19 treated women, 16 (84.2%) were on ERT and the other 3 (15.8%) on migalastat. Meanwhile, 27 men (93.1%) received ERT and the other 2 (6.9%) migalastat. Of note, migalastat was only more recently available on national level.

Moreover, treatment initiation age was higher in women than men: mean age of starting pathogenic therapy 53.9 ± 10.6 (30–69) in women vs 36±12 (10–61 years) in men (p< 0.001).

From the 19 women that received pathogenic therapy: 5 (26.3%) had mixed cardiac, renal and neurologic involvement, 3 (15.8%) mixed cardiac and renal involvement, 1 (5.2%) mixed cardiac and neurologic involvement, 1 (5.2%) mixed renal and neurological involvement, 6 (31.5%) isolated neurologic involvement, 2 (10.5%) isolated cardiac involvement, 1 (5.2%) was asymptomatic with positive kidney biopsy.

Twenty-two women (53.6%) did not receive pathogenic therapy. These women were all thoroughly screened for organ involvement. Untreated women were either carriers (11 women, 50%), or with no major organ involvement (9 women, 40.9%) (e.g. only had mild neuropathic pain or electrocardiographic changes without cardiac hypertrophy), while 2 women (9%) refused the treatment despite medical recommendations and counselling.

Otherwise, there was no difference between sexes regarding administration of other cardiovascular drugs (e.g. angiotensin converting enzyme inhibitors, sartans or betablockers) (p 0.830, 0.884 and 0.306 respectively).

SURVIVAL

Survival of FD patients was different among genders. The median follow-up period for patients since complete evaluation in our center was 6 years. Less women compared with men died as seen in Table 2, with a trend towards higher age at death (62.6± 2.1 years in women vs 49.6± 12.8 in men, p=0.131). The causes of death in women were: 1 patient (33.3%) with sepsis, 1 patient (33.3%) with stroke due to stopping of oral anticoagulants, and 1 patient (33.3%) due to heart failure, while the causes of death in men were: 2 patients (22.2%) with stroke, 2 patients (22.2%) sudden cardiac death, 2 patients (22.2%) severe complications of chronic kidney disease (CKD), 2 patients (22.2%) sepsis and 1 patient (11.2%) COVID 19. Women had a significantly longer survival at last follow-up (mean age 75.1±1.9 years) than men (58.1±3.4 years) (p<0.001) (Figure 4).

Of note, 29 FD women (70%) in our cohort had pregnancies, uncomplicated in all cases, but without the possibility of a complete documentation of pregnancy evolution, as they all occurred before FD diagnosis.

Tabel 4

Levels of BNP and troponin (p for women vs men)

	All patients	**Women**	**Men**	**p value**
BNP pg/ml (median ±SD, min, max)	79.7±375.8 (10-1756.1)	142.5±227.3 (10-716)	25± 494.8 (10-1756.1)	0.067
Patients with high BNP >100 pg/ml (n, % from available data)	15 (42.8%)	11 (61.1%)	4 (23.5%)	**0.025**
Troponin ng/ml (median ±SD, min, max)	0.014±29.61 (0-160)	0.0175±0.065 (0.001-0.253)	0.014±41.93 (0-160)	0.935
Patients with high troponin >0.04 ng/ml (n, % from available data)	10 (28.5%)	6 (33.3%)	4 (23.5%)	0.711

Abbreviations: BNP brain natriuretic peptide

Table 5

Echocardiographic parameters (p for women vs men)

	All patients (n=73)	**Women (n=41)**	**Men (n=32)**	**P value**
IVS mm (mean ±SD, min, max)	13.6±5.9 (7-34)	12.4±5.3 (7-30)	14±5.5 (8-32)	0.059
PW mm (mean ±SD, min, max)	12±4.3 (6-28)	10.5±2.6 (6-16)	13.5±5.2 (8-28)	**0.016**
MWT mm (mean ±SD, min, max)	14.1±5.9 (7-34)	12.4±5.2 (7-30)	14.7±5.9 (8-32)	0.079
LVEDD mm (mean ±SD, min, max)	44.6±5.7 (33-60)	44.6±6.5 (35-60)	44.3±5 (33-55)	0.807
LVESD mm (mean ±SD, min, max)	28.9±4.9 (17-44)	28.2±5.1 (20-39)	29.9±5.3 (17-44)	0.189
Indexed LV mass g/m^2 (mean ±SD, min, max)	130.9±84.1 (41.5-442.6)	124.7±84.5 (41.5-442.5)	139.5±84.3 (63.8-442.6)	0.158
LVEF% (mean ±SD, min, max)	62.4±6.5 (36-77)	62.5±6.5 (36-77)	62.3±6.4 (40-74)	0.952
GLS % (mean ±SD, min, max)	-16.1±4.4 (-23,-4.9)	-16.7±4.4 (-23,-4.9)	-15.2±3.8 (-20.2,-6.9)	0.105
Indexed LA volume ml/m^2 (mean ±SD, min, max)	37.4±17.6 (14.5-105.7)	38±19.3 (14.5-91.2)	36.5±15.2 (15.2-69.8)	0.915
E cm/s (mean ±SD, min, max)	80.4±16 (40-110)	81.6±16.9 (53-110)	79.2±15.5 (40-101)	0.869
A cm/s (mean ±SD, min, max)	69.2±22.6 (20-135)	64± 20.4 (20-107)	71.4±20.1 (37-117)	0.246
E/A (mean ±SD, min, max)	1.3±0.7 (0.6-5.4)	1.4±0.8 (0.6-5.4)	1.1± 0.3 (0.6-2)	0.263
E' septal cm/s (mean ±SD, min, max)	8.2±3.5 (2-17)	8.2±3.7 (3-17)	8.3±3.4 (2-15)	0.944
E/E septal' (mean ±SD, min, max)	12.1±6.5 (5-32.5)	12.1±5.7 (5.5-26.6)	12.1±7.7 (5-32.5)	0.677
Mitral S cm/s (mean ±SD, min, max)	7.5±2 (3-15)	7.2±1.9 (3-10)	7.6±1.7 (4-11)	0.492
RVEDD mm (mean ±SD, min, max)	31.1±4.2 (17-40)	30±4.3 (17-38)	33.2±4.3 (26-40)	**0.004**
RV free wall thickness mm (mean ±SD, min, max)	6±1.6 (3-12)	5.7±1.3 (3-9)	6.4±1.9 (4-12)	0.135
TAPSE mm (mean ±SD, min, max)	22.7±3.4 (15-30)	22.2±2.9 (15-29)	23±4.1(15-30)	0.597
RV S cm/s (mean ±SD, min, max)	13±2 (7-16)	12.7±3.6 (7-19)	13.6±2.1 (9.4-18)	**0.029**
RV free wall strain % (mean ±SD, min, max)	-21.2±4.2%	-21.5±4.1%	-20.9±4.5%	0.710
sPAP mm Hg (mean ±SD, min, max)	28.9±6 (18-45)	29.3±6.1 (18-45)	28.5±7.3 (20-43)	0.654

Abbreviations: IVS interventricular septum, PW posterior wall, MWT maximal wall thickness, LVEDD end diastolic left ventricle diameter, LVESD end systolic left ventricle diameter, GLS global longitudinal strain, LV left ventricle, LA left atrium, RVEDD LVEDD end diastolic right ventricle diameter, RV right ventricle, TAPSE tricuspid annular plane systolic excursion, sPAP systolic pulmonary arterial pressure.

Table 6

CMR results

	All patients	**Women**	**Men**	**P value**
MWT mm (mean ±SD, min, max)	14.4±6.2 (6-34.3)	13.8±6.4 (6-34.3)	15.4±5.9 (8-33.6)	0.167
Indexed LVmass g/m^2 (mean ±SD, min, max)	79.9±44.8 (20.35-230.4)	72.8±34.6 (31.8-161)	88.8±54.8 (20.3-230.4)	0.367
LVEDVi ml/m^2 (mean ±SD, min, max)	82±20.2 (27-126)	74.3±18.1 (27-103)	91.9±18.7 (54-126)	**0.002**
LVESVi ml/m^2 (mean ±SD, min, max)	31.8±10.1 (9-56)	28.5±8.7 (9-42)	30.8±10.4 (14-56)	**0.008**
LVEF % (mean ±SD, min, max)	61.5±8.6 (34-77)	62.6±9.68 (34-77)	60.9±18.4 (55.7-69)	0.496
RVEDVi ml/m^2 (mean ±SD, min, max)	73.2±21.8 (23-134)	66.5±16.5 (23-92)	69.7±25.1 (39-134)	**0.01**
RVESVi ml/m^2 (mean ±SD, min, max)	30.3±11.9 (10-64)	27.1±10 (10-44)	34.6±13.2 (17-64)	**0.022**
RVEF % (mean±SD, min, max)	60.5±10.5 (32-90)	62.3±11.9 (32-90)	58.2±8 (46.6-75)	0.1

Table 6 (continued)

	All patients	Women	Men	P value
LGE present (n, %)	13 (29.5%)	6 (24%)	7 (36.8%)	0.355
T1 (mean ±SD, min, max)	912.9±47.8 (843-1032)	908.4±43.5 (843-974)	907.4± 53.3 (857-1032)	0.327
T1<930 ms prevalence (n, %)	16 (66%)	8 (66%)	8 (66%)	0.437
T2 (mean±SD, min, max)	45.7±2.9 (40-53)	46.6±3.1 (42-53)	43.2±7.1 (40-49)	0.087

Abbreviations: MWT maximal wall thickness, LV left ventricle, LVEDVi Indexed end diastolic left ventricle volume, LVESVi Indexed end systolic left ventricle volume, LVEF left ventricle ejection fraction, RVEDVi Indexed end diastolic right ventricle volume, RVESVi Indexed end systolic right ventricle volume, RVEF right ventricle ejection fraction, LGE late gadolinium enhancement

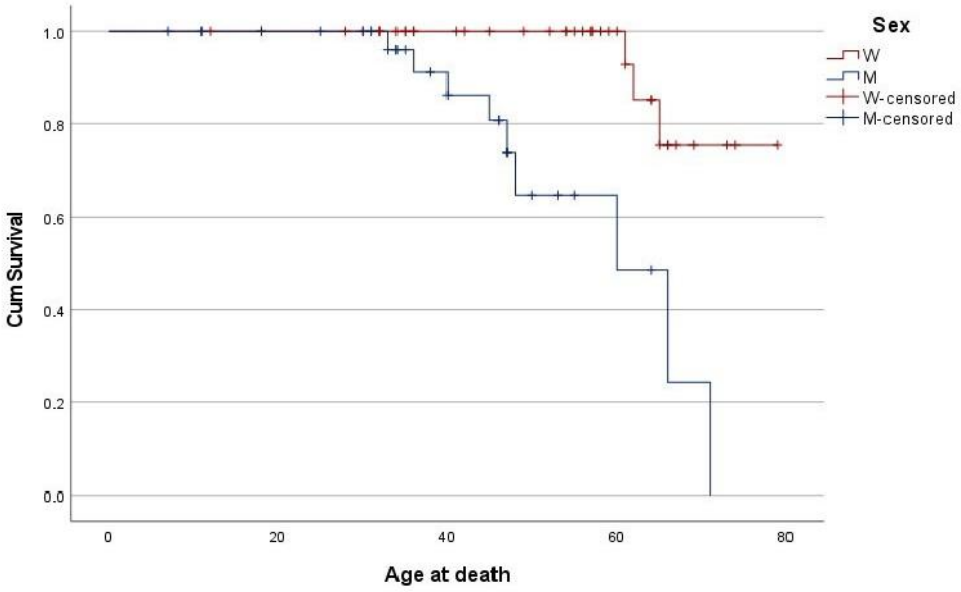

Figure 4. Survival curves for men and women with Fabry disease. Abbreviations: W, women; M, men.

DISCUSSION

Our study is the first to report the complete available data of the contemporary Romanian FD cohort focusing on the particularities of the affected women. We described that while women were more often diagnosed through family screening and at an older age, their cardiac and neurologic involvement appeared to be as important as in men, who only had more renal involvement. However, while less women were treated, and pathogenic therapy was introduced later, survival was still longer in women than men.

The mean age at diagnosis in women in the Romanian population is 41.1±16.7 years old, older than previously reported in the literature, where the mean age of diagnosis of FD female patients was 31 years old [10]. Similar to previously published data, Romanian FD women tend to be diagnosed later than men [11]. However, the time delay between onset of symptoms and diagnosis appeared to be shorter in women from our cohort then what was described in the literature (5.6 vs 16.3 years in literature) [12]. Furthermore, the time delay between symptoms onset and diagnosis was shorter for the women than for the men which is different from literature. This might be because most of the women in our group were diagnosed through family screening which led to a quicker diagnosis than in an index case. Another more probable cause could be represented by lack of self-recognition of onset symptoms (like acroparesthesia, often interpreted as rheumatic pain before the diagnosis, and considered trivial), only reporting the more prominent symptoms related to clinical events.

Women from our cohort reported less symptoms than men. When compared with the results from the natural history of the women with FD from the Fabry Outcome Survey (FOS), there were however some differences: our cohort of women presented more palpitations (36.6% vs 20% in FOS), but less angina (9.8% vs 15% in FOS) and less

angiokeratoma (25% vs 40% in FOS) [13]. The proportion of women and men with stroke was similar, with a similar age in both genders at first stroke occurrence. This is unlike some studies reporting stroke to be more common in women and women with stroke to be older than corresponding men [11]. In line with other published data [11], women in our cohort have less renal involvement than men, and they required less renal transplants. Regarding cardiovascular symptoms: dyspnea, angina, palpitations, and syncope were more frequent in our group than in literature for both women and men [14], with palpitations and fainting reported more often by women.

Of note, acroparesthesia and ENT symptoms were equally present in both genders, which is an important driver for lower quality of life independent of sex [15]. Less frequent angiokeratoma in women could contribute to the later recognition of the disease.

In our cohort, among the 41 women, 29 (70%) have had one or more pregnancies, all before FD diagnosis was established, and therefore none under specific FD therapy. While these issues were not addressed by our study, recent data in the literature suggest that there could be an increase of pain burden during pregnancies and a possibly increased risk for preeclampsia, prematurity, and neonates small for gestational age, leading to a high-risk pregnancy profile of FD mothers [16].

Unsurprisingly, women in our cohort had higher levels of αgalactosidase A and smaller levels of Gb3 than men [17], supporting the need for genetic testing when the clinical suspicion exists. One of the mechanisms linked to pathology in Fabry disease is GB3 and LysoGb3 deposition in various tissues. A clear link between Gb3 and lysoGb3 deposition and renal involvement was reported [18] and there are studies that correlated lysoGb3 with an increased risk of developing LVH in women with FD [19].

Cardiac biomarkers like BNP and high sensitive troponin should be measured in patients with FD as they are a marker of FD cardiomyopathy. BNP levels in our group correlated with LVH as in literature for both women and men. [20] Furthermore BNP levels and troponin correlated with GLS for both sexes this emphasizing the role of speckle tracking and biomarkers for early diagnosis even in women. While there are studies that showed Hs-troponin as a marker of fibrosis in FD patients, this was true only for male patients in our group, as women with LGE present had similar values of Hs-troponin as women without LGE in our group [21]. Both female and male patients with increased levels of troponin repeatedly presented increased values, emphasizing the fact that hs-troponin levels are related to a constant low grade injury related with cardiomyopathy and not to coronary events.

The ECG anomalies were similar between genders with only one difference. As in literature women had less LVH on ECG compared with men [14]. Rhythm disturbances like atrial fibrillation and NSVT were not frequent in our group in neither of the sexes and were less seen than in literature [22].

The need for pacemaker in our full cohort of FD population was similar to the one reported in the literature (12.3% in our study vs 12.5% in literature) [23]. However, there was a trend in our group for women to require more pacemakers, which is unlike other reports [24], which could be explained by the older age of FD women.

LVH is one of FD main features in echocardiography. Previous reports noted LVH to be more frequent in men [25], being present in approximately 50% men and in 30% women [26]. In our group LVH was diagnosed more often with a male predominance (75.8% men and 47.5% women), which could be explained by later diagnosis and CMR use with higher sensitivity for thickness measurements. However, the severity of LVH in our group is similar between sexes, which is different from literature [27]. Like in literature, patients with FD with LVH had mainly nonobstructive hypertrophic cardiomyopathy (HCM) (in our group 62% of men and 42.5% of women), while obstructive HCM was present in a small number of patients (13.7% of men and 5% of women). None of them had midventricular obstruction that has been noted in some FD patients [28]. This highlights the importance of cardiac evaluation of all FD patients since disease modifying therapies can reverse LVH in early phases and since FD is one HCM phenocopies that should be thought as a differential diagnosis. GLS could also represent a useful tool for identifying subclinical cardiac involvement in FD patients. In our group it was more impaired in patients with more advanced hypertrophy, without differences between genders. Similar to published data [29], RVH in FD was noted not to differ between sexes, neither in frequency, nor in severity, being present in more than half of the patients.

Cardiac MRI has an important contribution in early diagnosis and characterization of FD cardiomyopathy [30]. While administrating gadolinium-based contrast agents should better be avoided in patients advanced renal involvement, native T1 mapping can aid the diagnosis in these patients, with the advantage of early changes. Several studies showed that low native T1 values

were noted in 40% of patients with FD without LVH and in more than 90% of patients with FD with LVH [31, 32], suggesting T1 mapping is a valuable test for detection of cardiac involvement, even in the absence of LVH. In our group we found low native T1 values in 57.1% of females and 50% of males without LVH and in 80% of women and in 77.7% of men with LVH.

Treatment guidelines in women only recommend therapy initiation when organ involvement is present [33] therefore every FD patient should be carefully evaluated by a multidisciplinary team in order to fully diagnose the burden and organ involvement. It is known that organ involvement burden is important for both genders [34]. This is of high value as women both in literature as in our group, were less treated than men, or treated at an older age when compared to men [35],[36]. Furthermore, most of the treated women in our group showed multiple organ involvement, which highlights a more advanced disease, benefiting less from treatment initiation and leading to a higher burden of disease in women.

Mortality in women was lower than in men in our study, with a longer mean survival of higher age at death (62.6± 2.1 years in women vs 49.6± 12.8 in men). This is better than previously reported in the FOS cohort where the mean age at death in 53 female relatives was 49.8±12.3 years; this is probably explained by the fact that FOS data in women were published in 2006, in an era where awareness and treatment availability were lower than in recent years. Our results are similar to ones from the Fabry Registry, where mean age of death of women was 60.9 ±12.76 years [37].

STUDY LIMITATIONS

The patient population was small, but this is in accordance with the fact that FD is a rare disease and represented the full Romanian cohort of diagnosed FD up to date.

Also, as in any real-life cohort, based on accessibility or contraindications, some parameters could be lacking. Only part of the patients underwent CMR and even less T1 mapping, due either to the presence of CKD which prevented CMR with contrast, or to other contraindications (claustrophobia, metallic bodies). T1 mapping was recently available, and it was reported in all patients screened since its introduction.

This study did not include complete data about pregnancies in women with FD, and further data are needed to understand this evolution.

Even so, such a study is an important contribution to the yet sparse data on FD female patients.

CONCLUSIONS

In conclusion women with Fabry disease in the Romanian cohort were often phenotype positive with cardiac, renal and neurologic involvement. Awareness should be increased on the fact that women are not just carriers of the disease, as this might improve their access to timely diagnosis and pathogenetic therapy initiation. Further studies with more female subjects are needed to better understand the organ involvement particularities in women.

Boala Fabry (BF) este o afecțiune rară X-linkată cauzată de mutații în gena GLA. Femeile cu BF au fost mai puțin înrolate în studii și mai puțin tratate comparativ cu bărbații. Scopul acestui studiu este de a descrie fenotipul complet al femeilor cu BF diagnosticate și evaluate în România și de a-l compara cu cel al bărbaților.

Acest studiu a inclus toți pacienții diagnosticați consecutiv cu BF care au fost evaluați în Centrul de Expertiză pentru Boli Genetice Cardiovasculare Rare în perioda 2014–2023 și a inclus 73 de pacienți: 41 femei (56.2%) și 32 bărbați (43.8%) din 33 de familii neînrudite.

Femeile cu BF au fost diagnosticate mai târziu și au avut un debut al simptomelor mai tardiv comparativ cu bărbații. Femeile au fost mai rar simptomatice, dar cu o severitate a simptomelor asemănătoare cu a bărbaților. Ele au avut o afectare oftalmologică si ORL asemănătoare cu cea a bărbaților, dar mai puține angiokeratoame. Ambele sexe au fost afectate asemănător în ceea ce privește insuficiența cardiacă, cu simptome care au fost ușoare spre moderate, fără a exista vreo diferență între vârstele de apariție a fenomenelor de insuficiență cardiacă. Femeile din cohorta noastră au fost afectate la fel de mult ca bărbații de acroparestezii și AVC, dar au prezentat o afectare renală mai redusă, necesitând mai puține transplanturi renale.

Acest studiu demonstrează că femeile cu BF nu sunt doar purtătoare ale bolii, ele putând prezenta simptome la fel de severe ca ale bărbaților, dar au acces mai puțin sau mai târziu la tratament patogenic. Mai multe studii care să includă o participare mai numeroasă a femeilor sunt necesare pentru a înțelege povara bolii Fabry la femei.

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SUPPLEMENTARY FILES

Echocardiographic parameters:
Dimensions of the interventricular septum (IVS), posterior wall (PW), maximal wall thickness (MWT), left ventricle end diastolic diameter (LVEDD), left ventricle end systolic diameter (LVESD), indexed LV mass (LVMi), left ventricle ejection fraction (LVEF), left ventricle global longitudinal strain (GLS), left atrial (LA) volume were documented. Presence of obstruction or systolic anterior motion of the mitral valve (SAM), abnormalities of the inferolateral wall, presence, and type of valvulopathies were also noted. Doppler parameters (E, A, E/A) and tissue parameters (septal S, E', E/E'), right ventricle (RV) diameter, thickness and function (tricuspid annular plane systolic excursion – TAPSE, and myocardial velocities – S'), RV free wall strain, estimated pulmonary arterial systolic pressure (sPAP) were also noted. Image analysis was performed offline, using a vendor-dependent software (Echopac PC, GE Medical Systems, Milwaukee, WI, United States).

CMR parameters:
CMR allowed precise measurements of wall thickness (MWT was noted), LV end diastolic and systolic indexed volumes, LVEF, LV stroke volume, LV mass, RV end diastolic and end systolic indexed volumes, right ventricle ejection fraction (RVEF). Myocardial tissue characterization included T1 and T2 mapping, as well as presence and distribution of LGE.

Table S1

Study population: family, proband status and GLA gene mutations in the FD cohort

Patient no	Family	Sex (M/F)	Age at diagnostic	GLA variant (c)	GLA variant (p)	Type of variant	Phenotype	Status proband/ relative
1	1	F	62	c.[1031T>C]	p.[L344P]	missense	classic	**proband**
2	1	F	40	c.[1031T>C]	p.[L344P]	missense	classic	relative
3	2	M	19	c.[1224del66]		deletion	classic	**proband**
4	2	F	56	c.[1224del66]		deletion	classic	relative
5	3	M	36	c.[141G>A]	p.[W47*]	nonsense	classic	**proband**
6	3	F	59	c.[141G>A]	p.[W47*]	nonsense	classic	relative
7	4	F	67	c.[154T>A]	p.[C52S]	missense	classic	**proband**
8	4	M	43	c.[154T>A]	p.[C52S]	missense	classic	relative
9	5	M	15	c.[295C>T]	p.[Q99*]	nonsense	classic	**proband**
10	5	F	31	c.[295C>T]	p.[Q99*]	nonsense	classic	relative
11	5	M	4	c.[295C>T]	p.[Q99*]	nonsense	classic	relative
12	6	M	29	c.[334C>T]	p.[R112C]	missense	classic	**proband**
13	6	F	50	c.[334C>T]	p.[R112C]	missense	classic	relative
14	7	M	64	c.[416A>G]	p.[N139S]	missense	later-onset	**proband**
15	8	M	33	c.[485G>A]	p.[W162*]	nonsense	classic	**proband**
16	8	F	32	c.[485G>A]	p.[W162*]	nonsense	classic	relative
17	8	F	6	c.[485G>A]	p.[W162*]	nonsense	classic	relative
18	8	F	52	c.[485G>A]	p.[W162*]	nonsense	classic	relative
19	8	M	42	c.[485G>A]	p.[W162*]	nonsense	classic	relative
20	8	F	30	c.[485G>A]	p.[W162*]	nonsense	classic	relative
21	8	F	28	c.[485G>A]	p.[W162*]	nonsense	classic	relative
22	9	M	30	c.[548delG]		deletion	classic	**proband**
23	9	F	66	c.[548delG]		deletion	classic	relative

Table S1 (continued)

Patient no	Family	Sex (M/F)	Age at diagnostic	GLA variant (c)	GLA variant (p)	Type of variant	Phenotype	Status proband/ relative
24	9	F	64	c.[548delG]		deletion	classic	relative
25	9	F	46	c.[548delG]		deletion	classic	relative
26	10	F	58	c.[644A>G]	p.[N215S]	missense	later-onset	**proband**
27	11	M	40	c.[671A>G]	p.[N224S]	missense	classic	**proband**
28	11	F	26	c.[671A>G]	p.[N224S]	missense	classic	relative
29	11	F	30	c.[671A>G]	p.[N224S]	missense	classic	relative
30	11	F	28	c.[671A>G]	p.[N224S]	missense	classic	relative
31	11	M	60	c.[671A>G]	p.[N224S]	missense	classic	relative
32	12	M	15	c.[779G>A]	p.[G260E]	missense	classic	**proband**
33	12	F	47	c.[779G>A]	p.[G260E]	missense	classic	relative
34	13	F	49	c.[797A>C]	p.[D266A]	missense	classic	**proband**
35	13	F	46	c.[797A>C]	p.[D266A]	missense	classic	relative
36	13	F	17	c.[797A>C]	p.[D266A]	missense	classic	relative
37	13	M	9	c.[797A>C]	p.[D266A]	missense	classic	relative
38	13	M	29	c.[797A>C]	p.[D266A]	missense	classic	relative
39	13	F	29	c.[797A>C]	p.[D266A]	missense	classic	relative
40	13	M	30	c.[797A>C]	p.[D266A]	missense	classic	relative
41	14	M	48	c.[831G>A]	p.[W277*]	nonsense	classic	**proband**
42	15	M	24	c.[836A>G]	p.[Q279R]	missense	classic	**proband**
43	15	M	40	c.[836A>G]	p.[Q279R]	missense	classic	relative
44	15	F	50	c.[836A>G]	p.[Q279R]	missense	classic	relative
45	16	M	38	c.[863C>A]	p.[A288D]	missense	classic	**proband**
46	16	F	74	c.[863C>A]	p.[A288D]	missense	classic	relative
47	17	F	55	c.[937G>T]	p.[D313Y]	missense	pseudo deficiency	**proband**
48	18	M	16	c.[1121_1123delAAG]		deletion	classic	**proband**
49	18	F	46	c.[1121_1123delAAG]		deletion	classic	relative
50	18	F	24	c.[1121_1123delAAG]		deletion	classic	relative
51	19	M	51	c.[644A>G]	p.[N215S]	missense	later-onset	**proband**
52	19	F	77	c.[644A>G]	p.[N215S]	missense	later-onset	relative
53	21	M	42	c.[334C>T]	p.[R112C]	missense	classic	**proband**
54	21	F	63	c.[334C>T]	p.[R112C]	missense	classic	relative
55	22	M	43	c.[644A>G]	p.[N215S]	missense	later-onset	**proband**
56	22	F	58	c.[644A>G]	p.[N215S]	missense	later-onset	relative
57	22	F	65	c.[644A>G]	p.[N215S]	missense	later-onset	relative
58	23	M	16	c.[658C>T]	p.[R220T]	nonsense	classic	**proband**
59	23	F	53	c.[658C>T]	p.[R220T]	nonsense	classic	relative
60	24	M	39	c.[115C>T]	p.[Q260*]	nonsense	classic	**proband**
61	25	F	62	c.[1228A>G]	p.[T410A]	missense	later-onset	**proband**
62	25	F	36	c.[1228A>G]	p.[T410A]	missense	later-onset	relative
63	26	M	30	c.[955A>T]	p.[I319F]	missense	classic	**proband**
64	27	F	53	c.[937G>T]	p.[D313Y]	missense	pseudo deficiency	**proband**
65	28	M	28	c.[981A>C]	p.[Q327H]	missense	classic	**proband**
66	29	M	37	c.[671delA]	p.[N224Ifs*16]	deletion	classic	**proband**
67	30	M	31	c.[668G>A]	p.[C223Y]	missense	classic	**proband**
68	30	F	69	c.[668G>A]	p.[C223Y]	missense	classic	relative
69	30	F	34	c.[668G>A]	p.[C223Y]	missense	classic	relative
70	31	M	53	c.[644A>G]	p.[N215S]	missense	later-onset	**proband**
71	32	F	61	c.[44C>A]	p.[A15E]	missence	classic	**proband**
72	32	F	35	c.[44C>A]	p.[A15E]	missence	classic	relative
73	33	M	38	c.1156C>T	p.[Q386*]	deletion	classic	**proband**

Table S2

Electrocardiographic characteristics (p for women vs men)

	All patients	**Women**	**Men**	**P value**
PR (mean±SD, min-max)	139±34.8 (80-320)	137.3±38.4 (90-320)	141.22±30 (80-220)	0.273
Short PR<120 ms (n, % from available data)	14 (21.5%)	9 (24.3%)	5 (17.8%)	0.530
QRS (mean±SD, min-max)	97.9±16.7 (70-142)	92.6±15.3(70-130)	104.6±16.3 (80-142)	**<0.001**
LVH ECG criteria (n, % from available data)	35 (50.7%)	13 (33.3%)	22 (73.3%)	**<0.001**
Conduction defects (n, % from available data)				
no BBB (n, %)	59 (85.5%)	33 (86.8%)	26 (83.9%)	0.739
RBBB (n, %)	7 (10.1%)	4 (10.5%)	3 (9.7%)	
LBBB (n, %)	3 (4.3%)	1 (2.6%)	2 (6.5%)	
Depolarization abnormalities (n, % from available data)				
no negative T waves	35 (58.3%)	29 (70.7%)	19 (59.4%)	0.768
anterior negative T waves (n, %)	5 (8.3%)	2 (4.8%)	2 (6.2%)	
lateral negative T waves (n, %)	17 (28.3%)	9 (21.9%)	10 (31.2%)	
Inferior negative T waves (n, %)	5 (8.3%)	3 (4.2%)	1 (3.1%)	
Arrhythmias (n, % from available data)				
Atrial fibrillation (n, %)	5 (10.6%)	3 (11.5%)	2 (9.5%)	0.823
NSVT (n, %)	4 (8.7%)	3 (11.5%)	1 (5%)	0.423

Abbreviations: LVH left ventricular hypertrophy, RBBB right bundle branch block, LBBB left bundle branch block, NSVT nonsustained ventricular tachycardias.

REFERENCES

1. SPADA M., PAGLIARDINI S., YASUDA M., TUKEL T., THIAGARAJAN G., SAKURABA H., *et al., High incidence of later-onset fabry disease revealed by newborn screening.* Am J Hum Genet, 2006. **79**(1): p. 31–40.
2. MEIKLE P. J., HOPWOOD J. J., CLAGUE A. E. and CAREY W. F., *Prevalence of lysosomal storage disorders.* Jama, 1999. **281**(3): p. 249-54.
3. BURLINA A. B., POLO G., SALVIATI L., DURO G., ZIZZO C., DARDIS A., *et al., Newborn screening for lysosomal storage disorders by tandem mass spectrometry in North East Italy.* J Inherit Metab Dis, 2018. **41**(2): p. 209–219.
4. ORTIZ A., GERMAIN D. P., DESNICK R. J., POLITEI J., MAUER M., BURLINA A., *et al., Fabry disease revisited: Management and treatment recommendations for adult patients.* Mol Genet Metab, 2018. **123**(4): p. 416–427.
5. MAIER E. M., OSTERRIEDER S., WHYBRA C., RIES M., GAL A., BECK M., *et al., Disease manifestations and X inactivation in heterozygous females with Fabry disease.* Acta Paediatr Suppl, 2006. **95**(451): p. 30–8.
6. ECHEVARRIA L., BENISTAN K., TOUSSAINT A., DUBOURG O., HAGEGE A. A., ELADARI D., *et al., X-chromosome inactivation in female patients with Fabry disease.* Clin Genet, 2016. **89**(1): p. 44–54.
7. WANG R. Y., LELIS A., MIROCHA J. and WILCOX W. R., *Heterozygous Fabry women are not just carriers, but have a significant burden of disease and impaired quality of life.* Genet Med, 2007. **9**(1): p. 34–45.
8. BECK M., RAMASWAMI U., HERNBERG-STÅHL E., HUGHES D. A., KAMPMANN C., MEHTA A. B., *et al., Twenty years of the Fabry Outcome Survey (FOS): insights, achievements, and lessons learned from a global patient registry.* Orphanet J Rare Dis, 2022. **17**(1): p. 238.
9. EVANGELISTA Arturo, FLACHSKAMPF Frank, LANCELLOTTI Patrizio, BADANO Luigi, AGUILAR Rio, MONAGHAN Mark, *et al., European Association of Echocardiography recommendations for standardization of performance, digital storage and reporting of echocardiographic studies.* European Journal of Echocardiography, 2008. **9**(4): p. 438–448.
10. MEHTA A., CLARKE J. T., GIUGLIANI R., ELLIOTT P., LINHART A., BECK M., *et al., Natural course of Fabry disease: changing pattern of causes of death in FOS - Fabry Outcome Survey.* J Med Genet, 2009. **46**(8): p. 548–52.
11. MEHTA A., RICCI R., WIDMER U., DEHOUT F., GARCIA DE LORENZO A., KAMPMANN C., *et al., Fabry disease defined: baseline clinical manifestations of 366 patients in the Fabry Outcome Survey.* Eur J Clin Invest, 2004. **34**(3): p. 236–42.
12. GIUGLIANI Roberto, NIU Dau-Ming, RAMASWAMI Uma, WEST Michael, HUGHES Derralynn, KAMPMANN Christoph, *et al., A 15-Year Perspective of the Fabry Outcome Survey.* Journal of Inborn Errors of Metabolism and Screening, 2016. **4**.
13. DEEGAN P. B., BAEHNER A. F., BARBA ROMERO M. A., HUGHES D. A., KAMPMANN C. and BECK M., *Natural history of Fabry disease in females in the Fabry Outcome Survey.* J Med Genet, 2006. **43**(4): p. 347-52.
14. LINHART A., KAMPMANN C., ZAMORANO J. L., SUNDER-PLASSMANN G., BECK M., MEHTA A., *et al., Cardiac manifestations of Anderson-Fabry disease: results from the international Fabry outcome survey.* Eur Heart J, 2007. **28**(10): p. 1228-35.

15. ARENDS M., KÖRVER S., HUGHES D. A., MEHTA A., HOLLAK C. E. M. and BIEGSTRAATEN M., *Phenotype, disease severity and pain are major determinants of quality of life in Fabry disease: results from a large multicenter cohort study.* (1573-2665 (Electronic)).
16. HANINGER-VACARIU Natalja, ANASTOPOULOS Kyra, AIGNER Christof, SUNDER-PLASSMANN Raute, GATTERER Constantin, PONLEITNER Markus, *et al.*, *Pregnancy outcomes of Fabry disease in Austria (PROFABIA)-a retrospective cohort-study.* Orphanet Journal of Rare Diseases, 2024. **19**(1): p. 165.
17. LUKAS J., GIESE A. K., MARKOFF A., GRITTNER U., KOLODNY E., MASCHER H., *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): p. e1003632.
18. NAJAFIAN B., TØNDEL C., SVARSTAD E., GUBLER M. C., OLIVEIRA J. P. and MAUER M., *Accumulation of Globotriaosylceramide in Podocytes in Fabry Nephropathy Is Associated with Progressive Podocyte Loss.* J Am Soc Nephrol, 2020. **31**(4): p. 865–875.
19. ROMBACH S. M., DEKKER N., BOUWMAN M. G., LINTHORST G. E., ZWINDERMAN A. H., WIJBURG F. A., *et al.*, *Plasma globotriaosylsphingosine: diagnostic value and relation to clinical manifestations of Fabry disease.* Biochim Biophys Acta, 2010. **1802**(9): p. 741–8.
20. COATS C. J., PARISI V., RAMOS M., JANAGARAJAN K., O'MAHONY C., DAWNAY A., *et al.*, *Role of serum N-terminal pro-brain natriuretic peptide measurement in diagnosis of cardiac involvement in patients with anderson-fabry disease.* Am J Cardiol, 2013. **111**(1): p. 111–7.
21. SEYDELMANN N., LIU D., KRÄMER J., DRECHSLER C., HU K., NORDBECK P., *et al.*, *High-Sensitivity Troponin: A Clinical Blood Biomarker for Staging Cardiomyopathy in Fabry Disease.* J Am Heart Assoc, 2016. **5**(6).
22. YOUSEF Z., ELLIOTT PM FAU - CECCHI Franco, CECCHI F FAU - ESCOUBET Brigitte, ESCOUBET B FAU - LINHART Ales, LINHART A FAU - MONSERRAT Lorenzo, MONSERRAT L FAU - NAMDAR Mehdi, *et al.*, *Left ventricular hypertrophy in Fabry disease: a practical approach to diagnosis.* (1522–9645 (Electronic)).
23. SENÉ T., LIDOVE O FAU - SEBBAH Joel, SEBBAH J FAU - DARONDEL Jean-Marc, DARONDEL JM FAU - PICARD Hervé, PICARD H FAU - AARON Laurent, AARON L FAU - FAIN Olivier, *et al.*, *Cardiac device implantation in Fabry disease: A retrospective monocentric study.* (1536–5964 (Electronic)).
24. VIJAPURAPU Ravi, GEBERHIWOT Tarekegn, JOVANOVIC Ana, BAIG Shanat, NORDIN Sabrina, KOZOR Rebecca, *et al.*, *Study of indications for cardiac device implantation and utilisation in Fabry cardiomyopathy.* Heart, 2019. **105**(23): p. 1825–1831.
25. KAMPMANN C., WIETHOFF C. M., WHYBRA C., BAEHNER F. A., MENGEL E. and BECK M., *Cardiac manifestations of Anderson-Fabry disease in children and adolescents.* Acta Paediatr, 2008. **97**(4): p. 463–9.
26. PIERONI M., MOON J. C., ARBUSTINI E., BARRIALES-VILLA R., CAMPOREALE A., VUJKOVAC A. C., *et al.*, *Cardiac Involvement in Fabry Disease: JACC Review Topic of the Week.* (1558-3597 (Electronic)).
27. NIEMANN M., HERRMANN S., HU K., BREUNIG F., STROTMANN J., BEER M., *et al.*, *Differences in Fabry cardiomyopathy between female and male patients: consequences for diagnostic assessment.* JACC Cardiovasc Imaging, 2011. **4**(6): p. 592–601.
28. GRAZIANI Francesca, LILLO Rosa, PANAIOLI Elena, SPAGNOLETTI Gionata, PIERONI Maurizio, FERRAZZI Paolo, *et al.*, *Evidence of evolution towards left midventricular obstruction in severe Anderson–Fabry cardiomyopathy.* ESC Heart Failure, 2021. **8**(1): p. 725–728.
29. GRAZIANI F., LAURITO M., PIERONI M., PENNESTRI F., LANZA G. A., COLUCCIA V., *et al.*, *Right Ventricular Hypertrophy, Systolic Function, and Disease Severity in Anderson-Fabry Disease: An Echocardiographic Study.* J Am Soc Echocardiogr, 2017. **30**(3): p. 282–291.
30. MILITARU S., GINGHINA C., POPESCU B. A., SAFTOIU A., LINHART A. and JURCUT R., *Multimodality imaging in Fabry cardiomyopathy: from early diagnosis to therapeutic targets.* (2047–2412 (Electronic)).
31. DEVA Dp Auid-Orcid X., HANNEMAN K., LI Q., NG M. Y., WASIM S., MOREL C., *et al.*, *Cardiovascular magnetic resonance demonstration of the spectrum of morphological phenotypes and patterns of myocardial scarring in Anderson-Fabry disease.* (1532–429X (Electronic)).
32. THOMPSON R. B., CHOW K FAU - KHAN Aneal, KHAN A FAU - CHAN Alicia, CHAN A FAU - SHANKS Miriam, SHANKS M FAU - PATERSON Ian, PATERSON I FAU - OUDIT Gavin Y., *et al.*, *T_1 mapping with cardiovascular MRI is highly sensitive for Fabry disease independent of hypertrophy and sex.* (1942–0080 (Electronic)).
33. ORTIZ A., GERMAIN D. P., DESNICK R. J., POLITEI J., MAUER M., BURLINA A., *et al.*, *Fabry disease revisited: Management and treatment recommendations for adult patients.* (1096–7206 (Electronic)).
34. WILCOX W. R., OLIVEIRA JP FAU - HOPKIN Robert J., HOPKIN RJ FAU - ORTIZ Alberto, ORTIZ A FAU - BANIKAZEMI Maryam, BANIKAZEMI M FAU - FELDT-RASMUSSEN Ulla, FELDT-RASMUSSEN U FAU - SIMS Katherine, *et al.*, *Females with Fabry disease frequently have major organ involvement: lessons from the Fabry Registry.* (1096–7206 (Electronic)).
35. BARBA-ROMERO MÁ and PINTOS-MORELL G., *Gender Differences in the Application of Spanish Criteria for Initiation of Enzyme Replacement Therapy for Fabry Disease in the Fabry Outcome Survey.* Int J Mol Sci, 2016. **17**(12).
36. LENDERS M., HENNERMANN J. B., KURSCHAT C., ROLFS A., CANAAN-KÜHL S., SOMMER C., *et al.*, *Multicenter Female Fabry Study (MFFS) – clinical survey on current treatment of females with Fabry disease.* Orphanet J Rare Dis, 2016. **11**(1): p. 88.
37. WALDEK Stephen, PATEL Manesh R, BANIKAZEMI Maryam, LEMAY Roberta and LEE Philip, *Life expectancy and cause of death in males and females with Fabry disease: findings from the Fabry Registry.* Genetics in Medicine, 2009. **11**(11): p. 790–796.

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