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Experience with cascade screening: A comprehensive family pedigree analysis of two index patients with Fabry disease

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Abstract

The wide range of clinical symptoms observed in patients with Fabry disease (FD) often leads to delays in diagnosis and initiation of treatment. Delayed initiation of therapy may result in end-organ damage, such as chronic renal failure, hypertrophic cardiomyopathy, and stroke. Although some tools are available to identify undiagnosed patients, new comprehensive screening methods are needed. In this study, the outcomes of the cascade screening applied to three index cases with FD from 2 familes were investigated. In the pedigree analysis, 280 individuals were included; out of them, 131 individuals underwent genetic testing and cascade screening for FD. During the screening program, a total of 45 individuals were diagnosed, with a diagnostic ratio of 1:15. The average age at diagnosis for all individuals was 30.9 ± 17.7 years, and %25 were pediatric cases (mean age 9.5 ± 5.9 years). Thirty affected relatives were diagnosed from the two index cases in Family 1 and 15 individuals were diagnosed from one index case in Family 2. There were 13 consanguineous marriages observed among 2 pedigres, in two both spouses were affected, leading to two homozygous affected daughters in one couple. In regions where there is a high prevalence of consanguineous marriages, implementing the cascade screening approach to identify all individuals at risk can be beneficial for patients with FD, specifically women and children.

KEYWORDS
cascade screening, Fabry disease, homozygous females, pedigree analysis

1 | INTRODUCTION

Fabry disease (FD, OMIM 301500) is an X-linked, multisystemic, progressive lysosomal storage disease, caused by pathogenic variants in the *GLA* gene encoding the α-galactosidase A enzyme (α-Gal A, EC 3.2.1.22). The deficiency of the activity of α-Gal A enzyme, which is involved in the breakdown of glycosphingolipid, causes the accumulation of glycosphingolipids, globotriaosylceramide (Gb3), and its derivative globotriaosylsphingosine (lyso-Gb3) in various cell types including endothelial and smooth muscle cells, renal and cardiac parenchymal cells, and the central nervous system. This accumulation results in progressive and irreversible damage to vital organs (Germain, 2010; Yuasa et al., 2017).

In patients with classic FD, which is characterized by significantly reduced or no residual α-Gal A activity, the disease manifests present in childhood with non-specific findings such as acroparesthesia, hypohidrosis, abdominal pain, and fatigue (Schiffmann & Ries, 2016). Consequently, it is often difficult to consider the diagnosis of FD during this period. (Ezgu et al., 2022; Ries et al., 2005). Complications associated with FD, such as chronic kidney disease (CKD), left ventricular

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hypertrophy (LVH) and cerebrovascular events, are commonly observed during the third and fourth decades of life (del Pino et al., 2018; O'Mahony & Elliott, 2010; Sims et al., 2009). Asymptomatic or late-onset phenotype, which only affects one or a few organs, can be observed in female patients. In some cases, female patients may also exhibit the classic FD phenotype associated with severe findings (Juchniewicz et al., 2018). In the presence of classic signs and symptoms identified as red flags, such as neuropathic pain, anhidrosis/hypohidrosis, angiokeratomas, cornea verticillate, hearing loss, gastrointestinal problems (nausea, vomiting, abdominal pain, diarrhea or constipation), LVH of unknown origin (concentric and asymmetric), albuminuria, proteinuria, CKD, juvenile and/or cryptogenic transient ischemic attack or stroke. When these red flags are present, it is important to consult a health care professional, such as a geneticist or metabolic disorders specialist, for proper evaluation and diagnosis of FD (Citro et al., 2022; Pieroni et al., 2021).

The diagnosis can be established by α-Gal A enzyme activity assay and/or by molecular analysis in affected males. Men inherit the mutant *GLA* gene only from their mother, as it is located on the X chromosome. Consequently, they are always hemizygous in the GLA gene. In female patients, mainly consisting of heterozygotes in *GLA* gene, α-Gal A enzyme activity is often at similar levels to healthy controls (Yuasa et al., 2017). Therefore, the *GLA* gene sequence analysis is crucial for accurate diagnosis. *GLA* gene analysis is also used to test family members at risk (Ortiz et al., 2018).

Molecular studies have identified more than 1000 different GLA variants until now. Due to the phenotypic heterogeneity of this genetic disorder and the large number of pathogenic variants in the GLA gene, the treatment approach varies depending on factors such as genotype, sex, family history, phenotype, and specific clinical symptoms (Ezgu et al., 2022; Germain et al., 2020). Enzyme replacement therapy (ERT) has demonstrated efficacy in stabilizing and improving various manifestations associated with FD. Initiating treatment in the early stages of the disease may effectively prevent the progression toward irreversible organ damage and the subsequent development of associated life-threatening complications (Germain et al., 2019; Thomas & Hughes, 2014). While the prevalence of classical FD in males was formerly known as 1: 117,000, when the late-onset phenotype is included, it is known that the frequency varies between 1/1500–1/7800 and the disease is seen worldwide (Hsu & Niu, 2018). Considering the prevalence of FD, it is believed that there many undetected cases are still awaiting diagnosis.

Cascade genetic screening is a method used to identify individuals at high risk for hereditary diseases by genetic testing in relatives of people known to have specific genetic variants. Detection of the disease early, particularly in asymptomatic individuals, leads to improved clinical findings and outcomes. It is generally considered to be more cost-effective than population screening (Cernat et al., 2021). Many factors, such as the inheritance pattern of the disease, the depth of screening, and the degree of penetrance can influence the outcomes of cascade screening (George et al., 2015).

The present study evaluates the pedigree analysis of two patients diagnosed with end-stage renal disease, as well as the results of the cascade screening method applied to these patients. This method detected, 30 new FD patients from the first family and 15 new FD patients from the second family. Among them, there were two couples both diagnosed with FD and two female patients with homozygotes in the GLA gene.

2 | PATIENTS AND METHODS

2.1 | Subjects

We performed a retrospective study of two consecutive patients with FD, consulting at the Department of Inherited Metabolic Disorder. This cross-sectional descriptive study was conducted in accordance by the Declaration of Helsinki and was approved by the Dokuz Eylül University Ethics Committee (Approval No: 2020/26-51; Date: 26.10.2020). All participants gave written informed consent for the using their personal data. Considering the X-linked inheritance pattern of the disease, index cases were interviewed regarding their family members, and at least a three-generation family pedigree was drawn. Index cases were requested to contact family members who probably have FD and refer them to the diagnosis center. Individuals who could not be reached due to communication problems, such as poor communication or being adopted, were asked to be referred to the center by contacting their family physician and explaining the risk of having FD. Detailed information about the disease, including its hereditary trait and index case, was given to each new family member who applied for cascading screening.

The pedigree analysis was reconstructed each time a new proband was identified. For male patients, the mothers, daughters, and siblings were screened. For female patients, the parents, children, and siblings were screened. If a consanguineous relationship was detected between the couples, their spouses sample were also collected. In cases where it was not possible to obtain a sample from a first-degree relative of a newly diagnosed FD case (due to death, test refusal, transportation constraints, etc.), cascade screening was not terminated at that point. Instead, the risk of having FD was assessed by attempting to obtain samples from other first-degree relatives, including children, siblings, mother, and father. This process was repeated each time a new proband was detected. The total number of individuals screened, the number of individuals at risk of inheriting pathogenic variants, and the number of patients diagnosed with FD were determined. Age, sex, α-Gal A enzyme levels, plasma lyso-Gb3 levels (if available), and *GLA* gene analysis reports of family members examined within the scope of comprehensive family screening were examined (Table S1). When a family member who had the identical variant as the index case (index patient-1 and index patient-2; c.902G>A, index patient-3; c.1226C>T) was detected, they were considered to have FD following with the European Fabry Working Group Consensus report (Biegstraaten et al., 2015). The pedigree analysis was expanded to include these newly detected cases. By examining the family member relations among all individuals with FD, these pedigrees merged with the index case's pedigree.

Because of the wide clinical spectrum of FD, all probands were evaluated by a multidisciplinary team of experts experienced in FD, including specialists of inherited metabolic disorders, medical geneticists, neurologists, cardiologists, nephrologists, ophthalmologist. Patients' age at diagnosis, age at the symptom onset, sex, and FD-related findings (such as acroparesthesia, angiokeratoma, hypohidrosis) were questioned. The treatments administered before the diagnosis was questioned. Family members exhibiting at least one FD-specific sign, such as proteinuria, arrhythmia, sudden loss of hearing, and so on were classified as symptomatic. On the other hand, those without any signs were classified as asymptomatic. Deceased family members, who had a high probability of having FD due to X-linked inheritance, were not included in the study even if they had a history of FD-related conditions such as renal failure or transient ischemic attack (TIA), since there were no available samples for testing.

2.2 | GLA gene analysis

2.2.1 | Sample collection and DNA extraction

Minimum 2 mL of peripheral venous blood samples of the subjects were collected to the EDTA tubes. Total genomic DNA were extracted via magnetic bead purification method. MagPurix Blood DNA Extraction Kit (Zinexts, Taiwan) were used with MagPurix Automated Extraction System (Zinexts, Taiwan) according to the manufacturer's protocol. DNA quality and concentration measurements are performed by NanoDrop ND1000® Spectrophotometer (Thermo Fisher Scientific, USA). After the proper quality (50–100 ng/μL concentration and A260/A280:1.8-2.0 purity) of DNA is ensured, DNA is stored in −20°C prior to study.

2.2.2 | Sanger sequencing

The laboratory workflow included initial sequencing of all coding exons and exon-intron boundaries (±10 base pairs) of the *GLA* gene in index patients. After confirmation of pathogenic variants in index patients, cascade screening was performed in each family by sequencing of only the exon in which the mutation was found (exon 6 or exon 7).

Direct amplification and sequencing of *GLA* gene were performed using primer sequences designed according to the GRCh38 human genome *GLA* reference sequence (Ensembl transcript ID: ENST00000218516). Exons and exon-intron boundaries were amplified using a standard PCR procedure that used AmpliTaq Gold™ 360 DNA Polymerase (Applied Biosystems—Thermo Fisher Scientific, USA). Amplifications were performed using Eppendorf 5332 Mastercycler (Eppendorf AG, Germany). Sequencing reactions were performed with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems—Thermo Fisher Scientific, USA), and electrophoresed on an ABI 3130 Capillary Electrophoresis System (Applied Biosystems—Thermo Fisher Scientific, USA). Sequence alignment and evaluation were performed using CLC Genomics Workbench v3.6.5 (Qiagen, Germany). Detected variants were classified according to the current guidelines (Richards, et al., 2015). During variant annotation; rsID numbers were used for variants registered in the Single Nucleotide Polymorphism Database (dbSNP) (https://www.ncbi.nlm.nih.gov/snp/). Population frequency data were obtained from the gnomAD database (https://gnomad.broadinstitute.org/) and relevant pathogenicity submissions were obtained and evaluated from the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) (Table 1).

2.3 | Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics for Windows version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistical methods were used to analyze the results. The normality of the distribution of the numerical variables was evaluated using the Kolmogorov–Smirnov test. Values for the numerical variables were expressed as mean ± standard deviation or median (minimum-maximum), depending on the normality of distribution. Categorical variables were reported as numbers and percentages (%).

3 | RESULTS

3.1 | Patient 1 and patient 2

Index patient-1 (IV.67) was a 53-year-old male referred to the nephrology department due to high serum creatinine levels, proteinuria, and hyperkalemia. He was diagnosed with renal failure of unknown origin. Medical history revealed a consanguineous marriage between his parents, and it was learned that several relatives also had CKD. He had complaints of numbness and tingling in his hands and feet since childhood as well as heat intolerance, constipation, and recurrent abdominal pain. On physical examination, there were multiple angiokeratomas on the trunk of the patient. Ophthalmologically, corneal opacity was observed by slit-lamp examination while an otolaryngology examination identified sensorineural deafness. Echocardiography revealed LVH with markedly prominent papillary muscles. The interventricular septum measured 18 mm, and the papillary muscles were measured at 17 mm in diastole. FD was suspected, and enzyme levels were examined based on the patient's medical history and clinical findings. α-Gal A enzyme activity was below 0.1 μmol/L/h (normal >2.8 μmol/L/h), and lyso-Gb3 was 14.7 ng/mL (normal >1.30 ng/mL). Molecular analysis of *GLA* revealed that he was hemizygous for the previously described c.902G>A (p. Arg301Gln) pathogenic variant associated with the classic FD phenotype (Table 1). ERT was initiated due to the presence of arrhythmia, LVH, cornea verticillata, angiokeratoma, and CKD. As there were many individuals with CKD in his family history, pedigree analysis was performed (Figure 1a). While conducting the pedigree analysis, it was noticed that Patient 1 had a

TABLE 1 The details and pathogenicity of variants identified in this study.

Nucleotide sequence variant	Protein sequence variant	Location	Mutation type	dbSNP ID*	gnomAD (v.2.1.1)**	ClinVar***	ACMG pathogenicity ****
c.902G>A	p.Arg301Gln	Exon 6	Missense	rs104894828	-	Pathogenic	P (PM1, PM2, PM5, PP1, PP2, PP3, PP5 > Strong)
c.937G>T	p.Asp313Try	Exon 6	Missense	rs28935490	0.003040	Conflicting interpretations of pathogenicity	B (BS1, BS2)
c.1226C>T	p.Pro409Leu	Exon 7	Missense	-	-	-	LP (PM2, PM5, PP2, PP3)

*https://www.ncbi.nlm.nih.gov/snp/.
**(Karczewski et al., 2020).
***https://www.ncbi.nlm.nih.gov/clinvar/.
****(Richards et al., 2015).

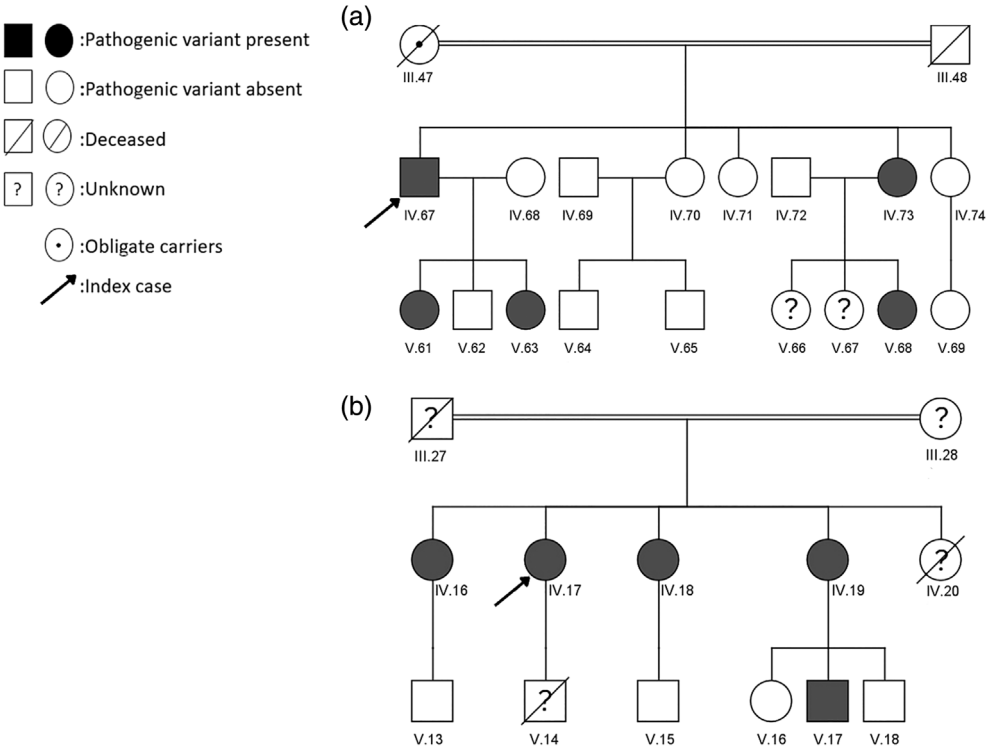


FIGURE 1 The family trees of Patient 1 and Patient 2.

consanguineous relationship with Patient 2 who had also sought medical attention at the Department of Inborn Errors of Metabolism. Patient 2 (IV.17), a 42-year-old female, was receiving peritoneal dialysis for CKD. In addition to renal failure, she presented with symptoms of heat intolerance and sporadic episodes of gastrointestinal distress. Echocardiography had revealed normal IVS thickness (10 mm) and normal left ventricular posterior wall thickness (9 mm). FD was investigated for the etiology of CKD, and c.902G>A variant was detected in the *GLA* gene. Family screening was performed by a pedigree analysis (Figure 1b). The diagnosis was established approximately 3 weeks prior to the identification of the index case, during which it was observed that she exhibited the same variant as the index case. Notably, these two patients resided in different cities and were unaware of their familial relationship. The pedigree was expanded using the cascade method employed for index patient 1 to explore the consanguineous connections between the patients. Consequently, it was determined that comprehensive screening of all family members was essential, and the two pedigrees were merged for this objective.

3.1.1 | Cascade screening—Family 1

The pedigree analysis of 214 individuals, spanning six generations, is illustrated in Figure 2. The samples could not be collected from 30 (14%) family members who had passed away (individuals from the first and second generations were no longer alive). Considering X-linked inheritance, it was determined that 57 (27%) individuals were

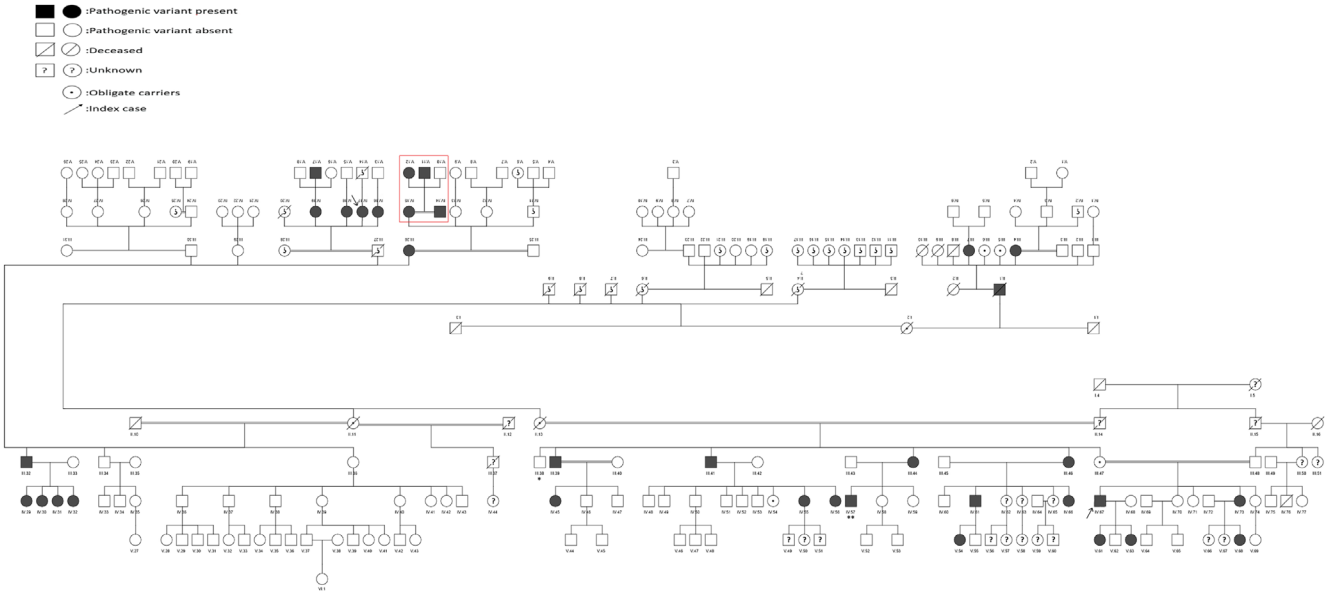
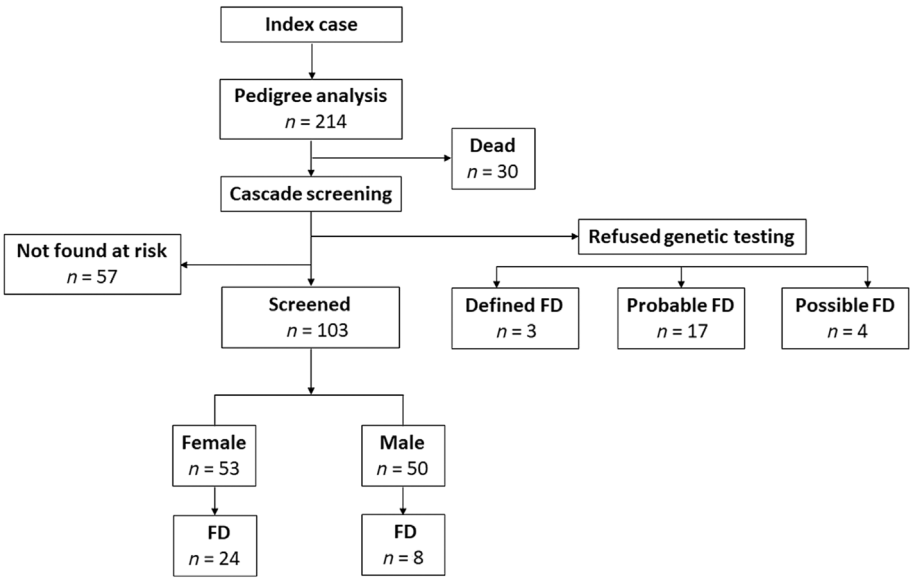


FIGURE 2 The pedigree analysis of Patient 1 and Patient 2. Red box indicates the family where both spouses are affected. *: This individual is also III.3. **: This individual is also IV.14.

FIGURE 3 Cascade screening of Family 1.



not at risk. Twenty-four (11%) individuals could not undergo screening due to various reasons, such as financial constraints, communication difficulties within the family, adoption, refusal to participate in the test, and settlement in remote areas (different countries or cities). Three (1.4%) female family members, who unable to be sampled, were identified as obligate carriers. 103 (48%) individuals were tested. c.902G>A variant was detected in 32 (15%) individuals including the index case (Figure 3). In addition, a c.937G>T (p. Asp313Tyr) variant was detected in a family member, and there was no FD-specific symptom in this 5-year-old girl. This was interpreted as a benign variant and no further action were taken for this variant. A total of 10 couples had consanguineous marriages. In one of these consanguinity marriages, the mother and daughter carried a heterozygous variant in a family, while the father and son carried a hemizygous variant in the *GLA* gene (Figure 2).

In Family 1, there were a total of 30 newly diagnosed patients. Among these patients, seven (22%) were under 18 years old. Out of the pediatric patients, five (71%) were girls and two (28%) were boys. None of the children had advanced organ damage seen in FD such as CKD or LVH. A nine-year-old male patient (V.11) had cornea verticillata. Three patients of pediatric patients had complaints of abdominal pain. One of these patients (V.17) was being investigated by the Pediatric Rheumatology Department with the suspicion of Familial Mediterranean Fever (FMF) due to recurrent abdominal pain. Six out of the 25 adult patients (24%) were male. Additionally, six patients declined systemic evaluation. Eight patients (25%), including the index case,

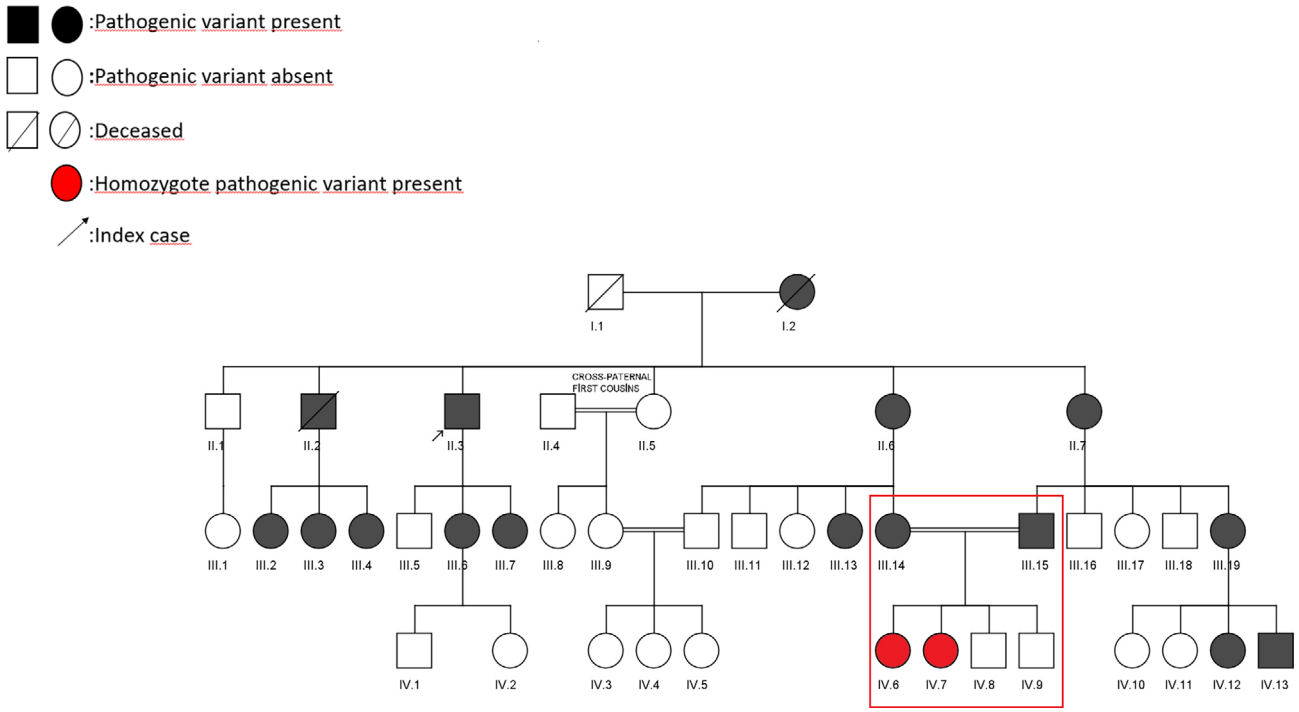


FIGURE 4 The pedigree analysis of Patient 3. The family with both parents with FD is framed in a red box.

had advanced organ involvement due to FD (CKD in five patients, LVH in seven patients, and TIA in one patient). Three patients were in dialysis treatment due to CKD, and one patient had undergone renal transplantation. ERT treatment was initiated in 14 patients, and three patients refused treatment. Fifteen patients remained asymptomatic and were regularly monitored. A family member who was learned to be adopted was diagnosed with FMF due to recurrent abdominal pain and was receiving colchicine treatment. After receiving ERT treatment, his symptoms significantly decreased.

3.2 | Patient 3

A 44-year-old male patient was referred to the hospital with evidence of CKD and LVH. His family history revealed that his mother, father, and one of his brothers died with the diagnosis of CKD and cerebrovascular events of unknown origin. Echocardiography showed LVH as his interventricular septum measured 21 mm and the posterior wall measured 20 mm in diastole. The α-Gal A enzyme activity was below 0.2 µmol/L/h (normal >2.8 µmol/L/h), Lyso GL3:42.5 ng/mL (normal >1.30 ng/mL). Molecular analysis of the *GLA* gene revealed the novel c.1226C>T variant (Table 1).

3.2.1 | Cascade screening—Family 2

The pedigree analysis of Family 2 is presented in Figure 4. Considering X-linked inheritance, 35 out of 66 family members were found to be at risk. Two of the individuals at risk had passed away. Samples could not be taken from seven individuals due to various reasons such as transportation difficulties, financial reasons, and unwillingness to get a diagnosis. Of the 28 screened family members, FD was detected in 15 relatives by screening. Cascade screening was limited to pedigree analysis involving four generations due to insufficient information about the family tree of patients. When the index case was evaluated together, 13 (81%) of the patients were female, and four (25%) of them were under 18 years of age. There was consanguineous marriage in three families.

When the cascade screening results were examined, considering the two pedigree analyses together, 131 out of 280 individuals in the pedigree analysis were tested, and 45 patients with FD were reached (34%). (Family 1; 2:30, Family 2; 1:15). There were 13 consanguineous marriages. In both families, both the mother and the father had FD. When the demographic and clinical findings of all members with *GLA* variant were examined together with the index cases, 11 (23%) were male, 37 (77%) were female, the average age was 32 ± 17.6 (median, 31 years, minimum-maximum, 1–68 years) (Table S1). The mean age at diagnosis of the patients (not including index cases) diagnosed by cascade screening was 30.9 ± 17.7 years (median, 29 years, minimum-maximum, 1–68 years). Fourteen patients refused systemic evaluation. Of the patients, 12 (25%) were children, 36 (75%) were adults. The mean age of the children was 9.5 ± 5.9 (median, 10 years, minimum–maximum, 1–18 years) (normally distributed). The distribution of clinical findings of patients grouped according to X linked inheritance were compared in Table 2. ERT was started in 20 patients (42%), five patients refused treatment, and 23 patients (48%) were recommended to have regular follow-up.

TABLE 2 Baseline characteristics of patients with FD in Family-1 and Family-2.

	Homozygote (IV-6/IV-7)	Heterozygote	Hemizygote
N (%)	2 (4)	35 (73)	11 (23)
Age			
Mean + SD	4/6	32.6 ± 15.9	34.9 ± 21.2
Med [min–max]		32 [3–66]	31 [1–68]
Alfa-Gal A (>1.2 µmol/L/h)	0.1/ 0.2	1.8 ± 0.8 2.1 [0.2–3.1]	0.4 ± 0.3 0.2 [0.1–0.9]
Lyso-Gl3 (<3.5 ng/mL)	49.3/50.3	4.3 ± 1.5 4.7 [0.5–7.9]	36.7 ± 16.4 38.8 [14.7–58]
Clinical findings[a]			
Asymptomatic	1/2	22/35	1/11
Angiokeratomas	-	10/33	6/10
Pain			
Dysesthesia/episodic crisis of burning in hands or feet	1/2	8/33	7/11
Reduced sweating			
Hypohidrosis or anhidrosis (not sweating enough)	1/2	0/33	6/11
Corneal signs			
Corneal whorls/cornea verticillata	-	8/27	8/9
Heat/cold/exercise intolerance	-	2/30	2/7
Hearing problems Hearing loss, tinnitus and vertigo	-	13/34	3/10
Gastrointestinal symptoms			
Nausea, vomiting, non specific abdominal pain, constipation, diarrhea	-	9/32	4/9
System Involvement[a]			
Kidney			
Proteinuria	1/2	10/33	9/11
Decreased glomerular filtration rate[b]	-	5/32	6/33
Progressive renal impairment	-	3/33	3/11
Cardiac			
Abnormal ECG	-	4/28	2/8
Shortened PR interval, non-sustained ventricular tachycardia			
Left ventricular hypertrophy	-	4/33	7/11
Stroke/Transient ischaemic attack	-	1/11	0/25
Number of patients on ERT		14/33	6/11

[a]Some of the patients could not be tested or very few tests could be performed, the number of patients with findings/total number of patients who evaluated were shown.
[b]Decreased glomerular filtration rate was defined as GFR <90 mL/min/1.73 m^2.

3.2.2 | The patients with homozygote variant in *GLA* gene

The index case's nephews had married each other, and the mother was heterozygous, and the father was hemizygous in terms of c.1126 C>T in *GLA* variant. The couple had four children, two homozygous affected daughters and two unaffected sons. The daughters had significantly low enzyme levels and highly elevated lysoGL3 levels, similar to classic Fabry disease. (Family member IV.6: α-Gal A; 0.1 µmol/L/h LysoGL3: 49.3 ng/mL (0–3.5); Family member IV.7: α-Gal A; 0.2 µmol/L/h LysoGL3: 50.3 ng/mL). At the time of diagnosis, systemic evaluation including urine sampling, abdominal USG, and hearing evaluation were normal. It was learned that the older sibling, previously examined in the general pediatric evaluation, had positive (+2) protein in her urine analysis with a simple dipstick test. Five years later, they applied to the outpatient clinic of inherited metabolic disorder. The 10-year-old younger sister had no complaint and was considered asymptomatic. Physical examination was normal, weight: 31.6 kg

(centile: 48, SD score: −0.05) height: 136 cm (centile: 45.22, SD score: −0.12; BMI: 17 kg/m^2). The older sister had tingling in the hands and feet, abdominal pain, and hypohidrosis. The family stated that she could not go to school several times a month due to abdominal pain. There were no remarkable findings on physical examination, weight: 42 kg (centile: 60, SD score: 0.27); height: 146 cm (centile: 34, SD score: −0.41; BMI: 20 kg/m^2). The routine laboratory panels revealed normal complete blood count, electrolyte levels, and liver and kidney function in both children. In the younger sibling, 24-h urine protein demonstrated a rate of 5.09 mg/m^2/h, while the Glomerular Filtration Rate (GFR) was measured at 117 mL/min/1.73 m^2. In the older sibling, 24-h urine protein demonstrated a rate of 6.4 mg/m^2/h, while the GFR was measured at 165 mL/min/1.73 m^2.

4 | DISCUSSION

The main goal of FD treatment is to prevent disease progression and irreversible organ damage. Early recognition of clinical signs and prompt initiation of ERT is crucial in preventing further progression and complications and improving long-term outcomes (Hsu & Niu, 2018). Cohort studies conducted on patients with FD have demonstrated that patients were followed for a long time with misdiagnoses and the average time between symptom onset and the accurate diagnosis was 14 years in men and 19 years in women (Eng et al., 2007). Although there has been a slight decrease in the duration of these periods due to increased awareness, there are still patients whose diagnosis period exceeds 30 years (Reisin et al., 2017). The prevalence of diagnosed patients, especially in the pediatric population, is low (Ries et al., 2005). Further research is required to explore screening modalities aimed at early detection of undiagnosed patients, thereby reducing the time for diagnosis. In screening high risk patients, specific populations, that is, patient groups with diseases carrying high risk for FD, such as LVH, end-stage renal disease, hemodialysis, and cerebrovascular event are screened. In studies conducted with this method in the Anatolian region, the prevalence of FD was found to be 1.05% in patients with idiopathic left ventricular hypertrophy (Barman, 2019), 0.31% in patients with a history of renal transplant surgery (Yalln et al., 2019), 1.8% in CKD (Turkmen et al., 2016), and 0.3% in hemodialysis patients (Sayilar et al., 2016). Although the patients can be detected with this method, it was seen that end organ damage has already developed in all of the patients. In addition, pediatric patients cannot be detected with this screening method, as it is applied to the population with advanced age. However, in FD, which was formerly called adult disease, it is now known that GB3 accumulation begins before the age of five, and the findings can be seen in childhood (Laney et al., 2015; Liern et al., 2019).

With newborn screening and family screening, FD can be diagnosed earlier (Schiffmann & Ries, 2016). Numerous later-onset forms and unclassified and uncertain genetic variants (A143T, D313Y) are detected by newborn screening method. However, by increasing diagnostic laboratory testing and follow-up visits, they lead to increased scanning costs (Gragnaniello et al., 2021; Viall et al., 2022). A large number of patients are reached with the cascade screening method (Moiseev et al., 2022). In particular, approximately 80% of pediatric patients are found using this method (Hopkin et al., 2008). When it is used together with neonatal screening or screening of at-risk populations, (CKD, LVH), even more new FD patients are reached (Germain et al., 2021).

In a literature analysis examining the benefits of family screening in FD, it was shown that an average of 4.8 additional affected family members were diagnosed from each proband, and two-thirds of these patients were women (Germain et al., 2021). In a study conducted in USA, a total of 27 patients were reached with two index FD, and the ratio was reported as 1:13 (Maron et al., 2018). Fifteen patients with FD were detected in a family residing in Argentina, with a positive case detection rate of 33% (Rozenfeld et al., 2019). This rate is 35% in the study of Herrera et al (Herrera & Miranda, 2014). In the present study, a total of 45 patients with FD from two families (Family 1; 2:30, Family 2; 1:15) were reached by comprehensive family screening. Similar to other studies, the disease detection rate was 34%, 25% of the patients were under the age of 18 years and 50% of them were asymptomatic. The family members were diagnosed approximately 20 years earlier compared to index cases. In a study that screened relatives of index patients with cascade screening, 20% of the cases were under 18 years of age and 40% were asymptomatic (Germain et al., 2021; Moiseev et al., 2022). In a study conducted in Denmark, the age of diagnosis of index cases was 42 years, while the age of diagnosis of family members was 26 years. With cascade screening, the age of diagnosis is decreases and patients can be diagnosed in the asymptomatic period (Effraimidis et al., 2022).

The diagnosis and treatment strategies have generally focused on men who are affected, while female patients have long been characterized as carriers (MacDermot et al., 2001). Although it is now known that the disease may also have a severe effect on women, the diagnosis time for symptomatic female patients is much longer than that for men (Ortiz et al., 2018). Similiar to pediatric patients, most female FD patients can be diagnosed through family screening (Gutiérrez-Amavizca et al., 2014; Juchniewicz et al., 2018). In this study, 37 women were diagnosed FD and by the time they were diagnosed with FD, severe organ damage had already developed in two heterozygous female patients (progressive renal failure). The present study made a valuable contribution to the literature by describing the novel c.1226C>T variant and its clinical manifestations and also by identifying the first homozygous female FD patients in Family 2. Both of their parents had findings of FD and they were initiated on ERT. Unlike heterozygous female FD patients, the enzyme level was found to be significantly low in these patients, and they had findings such as hypohidrosis, proteinuria, and acroparesthesia in the first decade of life.

The study's first limitation is the inability to reach all family members in the pedigree. Several reasons contribute to this, including low social ties with those in the upper generation, the unwillingness of certain family members to participate in screening (resulting from fear of receiving a diagnosis, challenges in accessing the hospital, financial difficulties, or living in a different country), and death

of family members. A study examining pedigree analysis in which all at-risk individuals are screened will offer more significant insights into the outcomes of the cascade screening method. Another limitation is that organ involvement could not be evaluated as some family members refuse extensive diagnostic procedures and treatment.

Experienced physicians in FD play a crucial role in increasing clinicians' awareness about the disease, offering support during the diagnostic process, conducting systematic patient reviews post-diagnosis, performing family screenings for the index case, and determining the initiation of treatment (Thomas & Mehta, 2013).

5 | CONCLUSION

Implementing the cascade screening approach to screen all individuals at risk can prove beneficial in identifying patients with FD, specifically women and children.

AUTHOR CONTRIBUTIONS

Conception and design: Pelin Teke Kisa, Mehmet Kocabey, Burcu Ozturk Hismi, Nur Arslan. *Method development*: Pelin Teke Kisa, Mehmet Kocabey, Burcu Ozturk Hismi. *Clinical data collection and interpretation*: Zumrut Arslan Gulten, Bulent Huddam, Selim Ekinci, Evrim Bozkaya, Harun Akar, Ozge K. Karalar Pekuz, Ayca Aydogan. *Analysis and interpretation*: Pelin Teke Kisa, Mehmet Kocabey, Burcu Ozturk Hismi. *Writing and revising draft*: Pelin Teke Kisa, Mehmet Kocabey, Burcu Ozturk Hismi. *Reviewing draft*: All authors.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PATIENT CONSENT STATEMENT

All participants gave their written informed consents for the use of their personal data.

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SUPPORTING INFORMATION

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