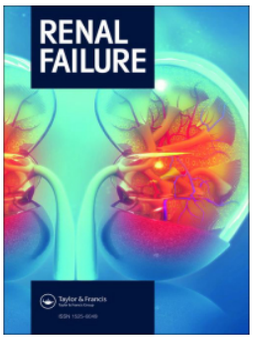

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Expanded screening for Fabry disease in patients with chronic kidney disease not on dialysis: a multicenter Italian experience

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ABSTRACT

Fabry disease (FD) is a progressive, multisystemic X-linked disorder caused by mutations in the GLA gene, often leading to renal failure. Although several screening programs have been conducted, the prevalence of FD in patients with chronic kidney patients who are not dependent on dialysis (NDD-CKD) is likely underestimated due to restrictive inclusion criteria and methodological shortcomings. This study aims to assess the prevalence of FD in NDD-CKD patients using an expanded screening approach. Ongoing outpatients attending Italian nephrology clinics were screened by assay of plasma α-galactosidase A (α-Gal A) activity. Genetic testing was also performed in all females and males with low α-Gal A activity. Inclusion criteria were: (1) females ≥18 years old; (2) males aged between 18 and 70 years; (3) NDD-CKD stages 1–5. Patients with histological diagnosis of glomerulonephritis or diagnosis of autosomal dominant polycystic kidney disease (ADPKD) were excluded. Demographic data and laboratory results were also collected. Among 385 NDD-CKD outpatients, 173 underwent screening. One patient with three family members carrying a novel mutation (c.320 A>G, p.Q107R); one patient with three family members carrying a silent mutation (c.48 T>G, p.L16L) and two patients with a missense mutation (c.376A>G, p.S126G), were identified. Overall, the prevalence of FD was 2.3%, increasing to 5.4% (10 in 183) with family screening. FD may be more common than previously believed, particularly within NDD-CKD populations. FD screening should be expanded to include NDD-CKD patients with known causes of CKD, such as hypertension and diabetes mellitus, and genetic testing should be routinely used for female patients.

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1. Introduction

Fabry disease (FD) is a rare X-linked hereditary lysosomal disease caused by pathogenic variants in the GLA gene, leading to the accumulation of glycosphingolipids in several organs and tissues [1]. This multisystemic disease presents with two phenotypes [2]: the classic form, which is more severe and typically manifests earlier in life (<16 years), and the late-onset form, characterized by a later onset (50–60 years) and involving typically one organ (e.g., renal or cardiac) [3]. The severity of FD phenotypes depends on gene mutations, enzyme activity levels, and gender.

The primary clinical manifestations of FD affect multiple organs, causing chronic renal disease, hypertrophy cardiomyopathy, and cerebrovascular stroke [4–6]. Despite these life-threatening complications, FD is often underdiagnosed. Patients often consult multiple medical specialists before receiving a correct diagnosis, leading to delayed treatment initiation with enzyme-replacement therapy (ERT) or chaperon therapy.

According to the latest data from the Fabry Outcome Survey (FOS), the mean diagnostic delay in FD was 14.0 years (95% CI 9.0–20.0) during 2001–2006, with a slight reduction to 10.5 years (95% CI 8.0–13.0) during 2007–2013 (p = NS).

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Conversely, the delay in treatment onset showed a statistically significant improvement, decreasing from 2.1 years (95% CI 1.3–3.2) during 2001–2006 to 0.9 years (95% CI 0.8–1.1) during 2007–2013 ($p < .001$) [7].

These diagnostic and treatment delays frequently result in increased morbidity and premature mortality due to renal, cardiovascular, or cerebrovascular complications, often around the fifth decade of life for men and seventh for women [8, 9].

A recent population-based study by Kermond-Marino et al. identified a prevalence of one in 3225 undiagnosed men and women with FD based on predicted pathogenic GLA variants in the general population [10].

The classically reported birth prevalence of FD in the general population (one in 476,000 to one in 117,000) significantly underestimates the true frequency of the disease [1]. More recent global prevalence estimates based on clinical ascertainment suggest a substantially higher frequency (one in 40,000 to one in 170,000) [11]. Knowing the true population frequency of FD provides clinicians with essential insights into how often it is likely to affect their patients.

Screening for FD is a valuable tool for identifying affected individuals [12]. This approach offers key benefits: (a) early diagnosis, which also permits FD detection in other family members [13]; (b) timely treatment, which can slow disease progression and improve survival [14–16]. A long-term study on ERT efficacy in adults demonstrated a 10-year survival rate of 94%, with no severe clinical events reported in 81% of patients. Moreover, the greatest benefits from ERT were observed in younger patients, who had a slower decline in renal function [17].

The 2023 European Renal Best Practice (ERBP) guidelines [18] and the 2017 KDIGO Controversies Conference [19] recommend screening for FD in patients with chronic kidney disease (CKD) of unknown origin. However, emerging evidence suggests that FD may also occur in patients affected by CKD caused by hypertension or type 2 diabetes [20]. To date, nine studies have focused on not dialysis-dependent chronic kidney disease (NDD-CKD) patients, as identified by a systematic review [21]. The pooled prevalence of FD in this population was reported at 0.22% (95% CI 0.06–0.44), with a notable reduction in studies including females (0.12% [95% CI 0.01–0.32]) compared to excluding females (0.41% [95% CI 0.12–0.84]) ($p = .046$) [21].

These studies, however, are limited by either methodological shortcomings, including the use of α-galactosidase A (α-Gal A) activity as a screening test, despite it typically being normal in females, or selection bias, such as the inclusion of only males, or patients with unknown CKD causes. As a result, the prevalence of FD in NDD-CKD patients remains uncertain and is likely underestimated.

By addressing these limitations, a more inclusive and rigorous methodology should be adopted. Consequently, the primary aim of this multicenter, cross-sectional Italian study was to evaluate, for the first time, the prevalence of FD in a cohort of NDD-CKD patients using an expanded screening approach that included an enzymatic activity testing for both genders and a genetic test for females. As a secondary aim, the prevalence of FD was investigated through familiar screening of index cases.

2. Methods

2.1. Population

Ongoing outpatients attending Italian nephrology clinics were recruited between 1 January 2023 and 1 January 2024. This work was conducted according to the Helsinki Declaration of 1975, revised in 2013, and approved by the Research Ethics Committee of the University of Palermo. Inclusion criteria were: (1) females aged 18 years or older; (2) males aged 18–70 years; and (3) a diagnosis of CKD in stages 1–5 according to the Kidney Disease: Improving Global Outcomes (KDIGO 2024) guidelines [22]. Patients (1) on dialysis; (2) with an electron beam histological diagnosis of glomerulonephritis; (3) with a diagnosis of autosomal dominant polycystic kidney disease (ADPKD); or (4) kidney transplant recipients were excluded.

Expert nephrologists determined the etiology of CKD based on the patient's medical and family history, clinical and biochemical data, and, when indicated, renal biopsy.

Demographic data were recorded, including age, sex, cause of CKD, family history (e.g., renal disease, hypertrophic cardiomyopathy, sudden death, or stroke disease), comorbidities (hypertension and juvenile stroke), electrocardiographic rhythm abnormalities, hypertrophic cardiomyopathy, cysts on renal ultrasound, hypoacusis, tinnitus, adnominal pain, angiokeratomas, acroparesthesia, hypohidrosis, and corneal opacities. Routine laboratory tests, such as serum creatinine (sCr), estimated glomerular filtration rate (eGFR), and 24-h proteinuria, were also collected. eGFR was calculated using the CKD-EPI 2021 equation [23]. According to eGFR value, CKD was classified as stage 1, eGFR >90 mL/min/1.73 m^2; stage 2, eGFR of 60–89 mL/min/1.73 m^2; stage 3a, eGFR of 45–59 mL/min/1.73 m^2; stage 3b, eGFR of 30–44 mL/min/1.73 m^2; stage 4 eGFR of 15–29 mL/min/1.73 m^2; and stage 5, eGFR <15 mL/min/1.73 m^2 [22].

2.2. Alfa-galactosidase A activity assay

Plasma α-Gal A activity was performed in all patients. Blood samples were collected via capillary puncture and dropped onto filter paper. α-Gal A activity assays were performed using the dried blood filter paper (DBFP) test described by Chamoles et al. [24], with some modifications (unpublished data). Normal α-Gal A activity was defined as >3.0 nmol/ml/h with an upper limit of 15 nmol/ml/h.

2.3. Molecular analysis of the GLA gene

Genetic testing was also performed on all females and males with α-Gal A activity below the reference values (3 nmol/ml/h). Genomic DNA was extracted from dried blood spots (DBS) using silica-coated magnetic particles in a robotic

workstation for automated nucleic acid purification (Qiagen, Hilden, Germany). DNA concentrations were measured using a biophotometer (Eppendorf, Hamburg, Germany). Mutations in the GLA gene were identified via Sanger sequencing. Eight pairs of primers were designed to target eight regions containing the seven exons of the GLA gene, including flanking regulatory sequences and the cryptic exon. PCR products were purified and sequenced using an automated DNA sequencer.

2.4. Lyso-Gb3 dosage

In cases where GLA gene mutations were detected, additional clinical exams and complementary tests, such as serum globotriaosylsphingosine (LysoGb3) measurement, were performed. The standard reference value for LysoGb3 was <2.3 nmol/l. LysoGb3 levels in blood were determined via tandem mass spectrometry (MS/MS), following the methodology previously described by Polo et al. [25].

2.5. Statistical analysis

Statistical analysis was carried out using the SPSS-30 software package (Chicago, IL). Dichotomous and categorical variables were expressed as absolute values, percentages, and proportions. Continuous variables were reported as mean ± standard deviation (SD). Normality was assessed using the Shapiro–Wilk test. No variable fitted a non-normal distribution. Student's *t*-test and Chi-square test were used to compare continuous and categorical variables between groups. Additionally, α-Gal A activity was categorized using an upper limit of 15.0 nmoL/ml/h. A *p* value less than .05 was considered statistically significant.

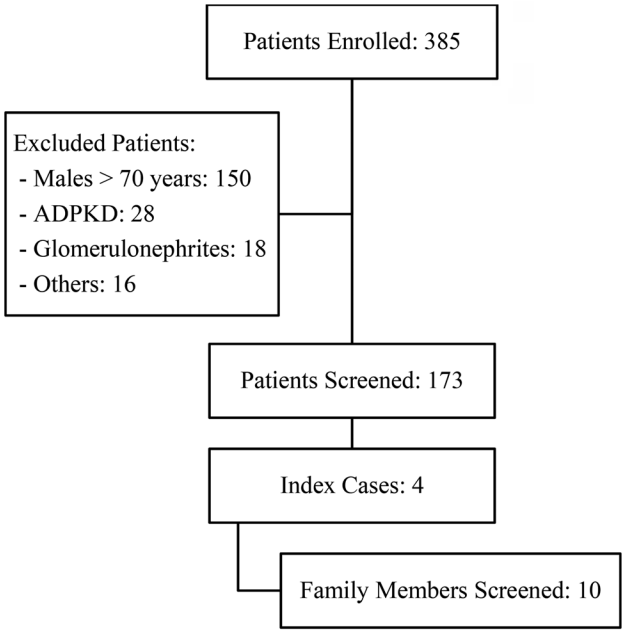

Figure 1. Flowchart of Fabry disease screening in NDD-CKD patients. ADPKD: autosomal dominant polycystic kidney disease; NDD-CKD: non-dialysis-dependent chronic kidney disease.

3. Results

Among 385 NDD-CKD outpatients, a high percentage (38.9%) were excluded, being males older than 70 years. 173 patients underwent FD screening, as illustrated in the flow diagram (Figure 1). Additionally, 10 family members from two index patients were screened for FD. The demographic characteristics of the entire cohort are reported in Table 1. Briefly, 48.6% of the patients were male, with a mean age of 57.15 (SD 11.02) years, which was lower than the mean age (63.54, SD 17.92) years of the females ($p < .03$). According to the KDIGO 2024 classification [22], 4.6% of patients were in stage 1, 19.7% in stage 2, 23.1% in stage 3a, 35.3% in stage 3b, 11.6% in stage 4, and 5.8% in stage 5. The mean eGFR was 47.1 (SD 21.0) ml/min with a mean sCr level of 1.7 (SD 1.0) mg/dl. More than half of the patients (55%) had a proteinuria between 0 and 0.5 g/24 h. The main cause of CKD was hypertension (37.6%), followed by unknown (36.4%) and diabetic nephropathy (18.5%). Only a low percentage (34.7%) of patients had a positive familial history of renal disease. A no-pathognomonic renal FD sign, pelvic cysts [26], were detected in 45 (26%) patients by renal ultrasound. Electrocardiographic and echocardiographic abnormalities suggestive of FD were more frequent in males compared with females. However, only 16.9% and 9.1% of females and 31% and 36.9% of males had previously undergone an electrocardiogram and an echocardiogram, respectively, at the screening visit.

Table 1. Clinical characteristics of screened NDD-CKD patients divided based on sex.

Variables	Males (*n* = 84)	Females (*n* = 89)	Statistics (*p* value)
Age, years	57.15 (11.02)	63.54 (17.92)	.03[c]
Serum creatinine, mg/dL	1.85 (1.08)	1.63 (0.98)	.17
eGFR, mL/min/1.73 m^2	48.66 (20.54)	45.57 (21.53)	.33
Proteinuria			
0–0.5 g/24h, %	45.7	62.1	.10
0.5–1 g/24h, %	32.9	27.3	
>1 g/24h, %	21.4	10.6	
CKD cause			
Hypertension, %	41.7	33.7	
Diabetes, %	19.0	18.0	.52
Unknown, %	31.0	41.6	
Others, %	8.3	6.7	
Family history for CKD, %	33.3	32.6	.91
Family history of stroke, %	26.2	21.3	.45
Family history for CV, %	26.2	16.9	.13
Family history for SD, %	16.7	18.0	.82
Hypertension, %	65.5	58.4	.34
ECG abnormalities[a], %	14.3	2.2	.01[c]
Left ventricular hypertrophy[b], %	21.4	4.5	.03[c]
Type 2 diabetes, %	17.9	20.2	.69
Parapelvic cysts[b], %	32.1	20.2	.07
Other FD findings			
Hearing loss/tinnitus, %	1.2	2.2	.59
Abdominal pain, %	3.6	11.2	.05[c]
Juvenile cerebri stroke, %	0	2.2	.16
Juvenile acroparesthesia, %	6.0	3.4	.41

NDD-CKD: non-dialysis-dependent chronic kidney disease; CV: cardiovascular disease; SD: sudden death.
[a]Presence of bradycardia and PQ-interval shortening.
[b]Detecting by ultrasound.
[c]Statistically significance.

Table 2. Demographic, clinical, and laboratory findings of four Fabry disease (FD) cases.

	Index patient 1	Index patient 2	Index patient 3	Index patient 4
Age, years	62	70	56	56
Sex	Female	Female	Female	Female
CKD cause	Diabetic Nephropathy	Diabetic Nephropathy	Unknown	Hypertension
Serum creatinine, mg/dL	1.5	1	2.5	2.4
eGFR, mL/min/1.73 m^2	37	60	21	22
Proteinuria, g/24 h	0–0.5	0–0.5	0.5–1	0.5–1
Parapelvic cysts	No	No	Yes	Yes
Family CKD history	No	Microhematuria	No	No
Comorbidities	Type 1 diabetes Arterial hypertension Hypertrophic cardiomyopathy	Type 2 diabetes Microhematuria	None	Arterial hypertension Sinus bradycardia
α-Gal A activity, nmol/ml/h	14.8	>15	6.3	7.0
LysoGB3, nM/L	1.65	1.40	1.40	1.02
GLA variant	c.320 A > G, p.Q107R	c.48 T > G, p.L16L	c.376A > G, p.S126G	c.376A > G, p.S126G
FD phenotype	GVUS	Benign	GVUS	GVUS

CKD: chronic kidney disease; eGFR: estimated glomerular filtration rate; GVUS: genetic variant of unknown significance.

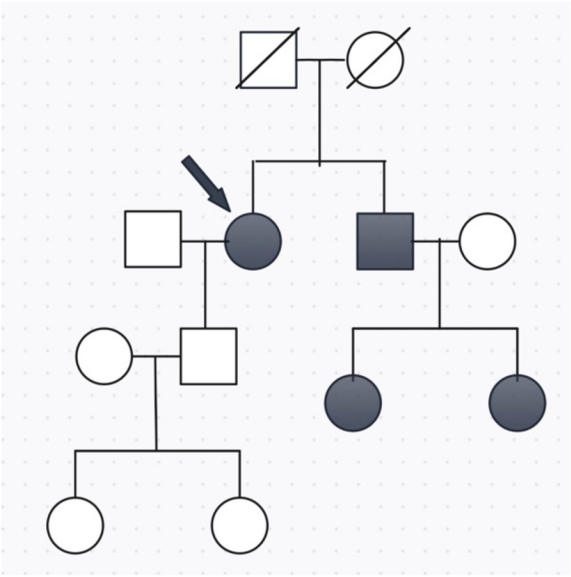

Figure 2. Pedigree family chart of index patient 1.

87.3% of the sample had α-Gal A activity greater than 15 nmol/ml/h, including 83/89 females compared with 68/84 males (df = 1, p = .015). Higher serum α-Gal A activity (>15 nmol/mL/h) was associated with older age (df = 171, p = .003) and lower proteinuria (df = 2, p = .037).

Among the comorbidities, hypertension was detected in 61.8% of the entire cohort, although only 12.7% had an imaging-documented hypertrophic cardiomyopathy. A significant percentage (17.3%) of patients reported a family history of sudden death. Regarding type 2 diabetes, it was found in 19.1% of patients, but none underwent renal biopsy to confirm the presence of diabetic nephropathy.

Other specific findings of FD, such as hearing loss and/or tinnitus (1.8%), adnominal pain (7.5%), juvenile acroparesthesia (4.6%), and juvenile cerebri stroke (1.2%), were rare. No cases of hypohidrosis, corneal opacities, and angiokeratomas were observed.

Four patients presented with an FD mutation; their clinical features are listed in Table 2.

In a 62-year-old woman (index patient 1) with CKD stage 3b due to non-biopsy diabetic nephropathy also affected by hypertension, a novel mutation (c.320 A > G, p.Q107R) was identified. This mutation has not been previously reported in the HGMD [27]. α-Gal A activity (14.8 nmol/mL/h) and LysoGb3 plasma concentration (1.65 nM/L) resulted within the normal levels. Other typical FD findings, such as angiokeratoma, acroparesthesia, hypohidrosis, and corneal opacities, were absent in this patient. This picture was compatible with late-onset or GVUS (genetic variant of unknown significance) variants. Three patients were identified through family screening: her brother with CKD and her two nephews (family tree in Figure 2), all of whom did not exhibit any FD signs and symptoms.

In a 70-year-old woman (index patient 2) with CKD stage 2 of unknown origin, a silent mutation (c.48 T > G, p.L16L) associated with a benign phenotype [28], was detected. Normal range of α-Gal A activity (>15 nmol/ml/h) and LysoGb3 plasma concentration (1.40 nM/l) was found. The same FD mutation was found in her brother and two sons (family tree in Figure 3).

In a 56-year-old woman (index patient 3) with CKD stage 4 of unknown origin and a 56-year-old woman (index patient 4) with CKD stage 4 due to hypertension, an FD mutation (c.376A > G, p.S126G), described as a GVUS variant [29], was identified. Both patients' α-Gal A activity and LysoGb3 plasma concentration were within normal range. Index patients 3 and 4 did not present with any typical clinical manifestation of FD, except for parapelvic cysts. Unfortunately, both families declined FD screening.

Overall, the prevalence of FD was 5.4% (10/183), with four case indexes on 173 patients (2.2%) identified in the primary screening and 6/10 family members (60%) in two out of four family screening. Index patient 1 had six family members (two males and four females), while index patient 2 only four (three males and one female).

4. Discussion

This is the first Italian study that found a high prevalence of FD in NDD-CKD patients using a broader screening

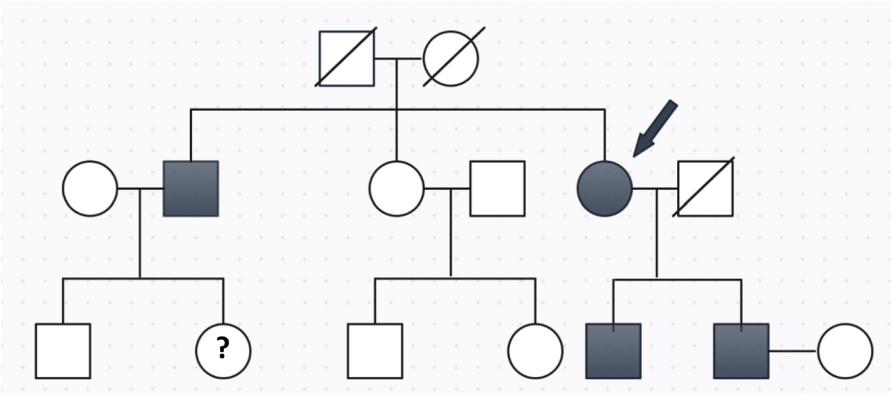

Figure 3. Pedigree family chart of index patient 2.

strategy with expanded selection criteria and appropriate enzymatic/genetic testing across both genders. Excluding six family members of index cases, our prevalence among the screened population was 2.2% (4/173), all of whom were female. Our findings suggest that this approach may be necessary to identify more FD cases and the true FD prevalence in the NDD-CKD population. Additionally, this strategy is crucial to avoid misdiagnosis, especially in patients with CKD due to hypertension and diabetes, where the cause is often based solely on clinical judgment. While the long-term benefits for patient outcomes are evident, cost-effectiveness studies are needed to evaluate the burden of expanded screening on healthcare systems, given the limited resources available.

Our results contrast with previous screening studies conducted in patients with NDD-CKD. A recent systematic review by Linares et al. [21], involving 7423 NDD-CKD patients, reported an FD pooled prevalence of 0.22% (0.06–0.44). In female subgroups, the FD prevalence decreased significantly to 0.009% (0.00–0.229).

The first potential explanation is due to our expanded selection criteria, which included females of any age, males younger than 70, and patients with CKD stages 1–5. Only patients with ADPKD or glomerulonephritis confirmed by electronic bean microscopy were excluded. In contrast, previous studies restricted the screening to CKD stages 3–5 [30, 31], male patients [20, 32, 33], and CKD of unknown causes [34]. Among these studies, the highest FD prevalence (0.95%; 3/313) was reported by Turkmen et al. [34], who recruited stage 1–5 CKD patients of both genders, aged 18–70 years, but limited to patients with CKD of unknown etiology.

The index cases in the present study included four women aged 56, 62, and 70 years with CKD stages 3–4. The CKD etiology was attributed to diabetic nephropathy or hypertension in three out of four patients based on clinical judgment. None of the four patients exhibited other characteristic signs of FD except for mild abdominal discomfort and parapelvic cysts in two of them. Without this screening, diagnosis of FD would likely have been challenging in these four cases, restricting the screening only in patients with CKD of unknown origin risks missing FD cases. Only patients with a confirmed CKD diagnosis via electronic beam microscopy would be excluded from an efficient screening protocol for CKD patients.

Second, the difference in FD prevalence might be related to the screening test. We quantified α-Gal A enzyme activity in all patients and conducted a molecular analysis of the GLA gene in females, regardless of their enzyme levels. It is acknowledged that a significant proportion of females who are heterozygous for the pathogenic GLA mutation exhibit normal values of α-Gal A activity [33]. Therefore, the prevalent FD studies which had performed genetic analysis only in females with reduced α-Gal A activity [32, 33, 35], may have underdiagnosed FD. Indeed, our study showed a higher FD prevalence in women, with 4.5% (4/89) rising to 7.44% (7/94) when including family members, compared with previous findings [21].

Similarly, other screening tests, such as Gb3 and Lsyso-Gb3 urinary assays, still need to be validated and may underestimate the FD prevalence. For instance, in a study of 397 CKD patients, Auray-Blais et al. failed to identify any FD cases using Gb3 analysis in dried urine spots.

It is worth noting that FD underdiagnosis has also been highlighted in neonatal screenings. In 2006, Spada et al. reported the highest incidence of FD, one in 3100 male births, in northwestern Italy, using a fluorometric enzyme assay [36]. More recently, Gragnaniello et al. confirmed a higher overall incidence of FD, one in 7879 rising to one in 4068 males [37, 38], through neonatal screening in northeastern Italy, which measured α-Gal A activity in DBS. Compared with our screening in CKD patients, neonatal screening is universal and symptom-independent, reducing the risk of missed diagnosis, particularly in patients with late-onset forms and atypical FD phenotypes. However, it should be noted that the neonatal screening data still exclude heterozygous females with normal enzyme function.

Finally, genetic mutations may account for differences in FD prevalence. In our screening, three mutations were classified as GVUS variants (Q107R, pS126G), while the fourth was a silent mutation. GVUS variants can be reclassified as benign or pathognomonic over time, potentially changing the prevalence of FD. To date, more than 1000 types of GLA variants, including classic, benign, and GVUS, have been reported [4, 39]. While benign variants do not require treatment, GVUS

variants must be interpreted and evaluated case-by-case due to their unknown pathogenicity [39, 40]. In order to define the pathogenicity of GVUS variants, cardiac or kidney biopsies, although invasive, are valuable diagnostic tools that can provide insights by revealing lysosomal storage damage [40]. Unfortunately, no additional data from renal biopsies were available in our cases, as patients refused to undergo these procedures.

Nevertheless, a comprehensive screening program should facilitate the early identification of patients with GVUS variants, who should be closely monitored. This approach can improve the classification of GVUS variants, reduce the risk of underestimating FD prevalence, and optimize management and treatment strategies [41, 42]. Indeed, when a GVUS variant is reclassified as a pathogenic mutation, a prompt start of specific treatment (ERT or chaperon therapy) should be considered alongside adjuvant treatments [43] to delay the progression of kidney disease. This approach should be applied not only for males but also for females, given the presence of kidney damage [44]. Furthermore, a more rigorous patient follow-up plan should be implemented, including procedures to timely identify damage to other organs.

The strength of this study lies in its finding, for the first time, a higher FD prevalence in the NDD-CKD population by using a screening approach with expanded inclusion criteria (i.e., patients with known CKD cause due to hypertension and diabetes) and employing appropriate enzymatic and genetic testing for both genders. However, implementing such expanded screening programs in other healthcare settings could have logistical and financial challenges. Therefore, innovative approaches, such as artificial intelligence tools [45] leveraging structured medical history data, could help overcome these barriers. Further large-scale studies should focus on optimizing screening strategies to improve the early detection of FD in the NDD-CKD population.

This study has several limitations. First, we excluded patients with a diagnosis of ADPKD, despite Esposito et al. [46] having described a case of co-occurrence between ADPKD and FD. However, only three such cases have been reported in the literature. Second, we excluded male patients over 70 for practical reasons, including costs and sampling logistics. Notably, male patients with the FD classic typically become symptomatic at a younger age, and those with the late-onset form generally present symptoms by age 70 [21]. Third, the small sample size of our study population does not allow us to generalize our results, even though we conducted a systematic screening of outpatients attending the participating nephrology clinics. Additionally, we could not study the families of two index patients due to their refusal to participate. Finally, we did not perform renal biopsies in patients with GVUS variants.

5. Conclusions

A high prevalence (2.2%) of FD was detected among NDD-CKD patients through expanded screening, including patients with a known CKD cause. This expanded screening used enzymatic activity testing for both genders and a genetic test for females. Additionally, six FD cases were found by family screenings, raising the overall FD prevalence to 5.4%. This approach is practical for the early identification of FD patients, which may help reduce disease complications and allow timely initiation of treatment.

Author contributions

Conceptualization, Y.B.; investigation, M.S, C.S., S.R., G.C., M.E., G.S., L.S., and G.T.; formal analysis, Y.B.; writing and editing – original draft, C.S., P.C., and F.B.; writing – review and editing, Y.B.; supervision, G.D. and M.A.; validation, R.M. and F.P. All authors have read and agreed to the published version of the manuscript.

Ethical approval

The Hospital Ethics Committee for Human Research granted the research protocol and ethical approval. All study procedures are following the Declaration of Helsinki (Finland).

Consent form

Informed consent was obtained from all subjects involved in the study.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data underlying this article will be shared upon reasonable request to the corresponding author.

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