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Fabry Disease Screening in Patients with Proteinuria or Chronic Kidney Disease and Defining a Novel Mutation: A Single-Center Experience

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ABSTRACT

Background: Fabry disease is an X-linked inheritance lysosomal storage disorder caused by mutations in the GLA gene and a deficiency of the α-galactosidase A enzyme. Globotriaosylsphingosine deposition causes tissue fibrosis, and eventual organ failure. Guidelines recommend screening for Fabry disease in high-risk populations, such as individuals with familial early-diagnosed kidney disease and kidney failure, with replacement therapy. This approach enables the identification of affected family members at earlier stages, before the development of chronic organ damage. This study aimed to investigate the prevalence of Fabry disease in patients with proteinuria and chronic kidney disease, and to report a novel mutation found in a patient diagnosed with Fabry disease, adding to the existing literature.

Methods: We screened 494 patients with chronic kidney disease (proteinuria or decreased kidney function) and 23 patients with a family history of Fabry disease mutation. Patients with mutations underwent electrocardiography, echocardiography, cardiac magnetic resonance imaging, and electromyography.

Results: A total of 3 patients (0.6%) were diagnosed with Fabry disease, among whom 1 patient exhibited a novel Fabry mutation (c.645T>A(p.N215K)). Fabry disease mutation was detected in 1 (0.64%) of 155 patients with proteinuria. Eight patients with Fabry mutation were identified in family screening.

Conclusion: The screening for Fabry disease holds significant importance in promptly diagnosing and treating individuals with proteinuria or chronic kidney disease. Our evidence-based findings provide evidence supporting the pathogenic nature of the newly identified N215K mutation.

Keywords: Fabry disease, chronic kidney disease, novel mutation, proteinuria

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INTRODUCTION

Fabry disease (FD) is an X-linked lysosomal storage disease caused by mutations in the GLA gene, resulting in a deficiency of the α-galactosidase A enzyme and subsequent intracellular accumulation of terminal α-D-galactosyl residual glycosphingolipids, especially globotriaosylsphingosine (lyso Gb3) and globotriaosylceramide (GL3). The deposition of GL3 leads to tissue fibrosis, ultimately resulting in kidney, cardiac, cerebrovascular diseases, and organ failure.[1]

The prevalence of FD in males is estimated to range from 1:22 000 to 1:40 000. Atypical or acquired mutations occur at a rate of 1:1000 to 1:3000 in males and 1:6000 to 1:40 000 in females.[2] Fabry disease is more frequently observed in males than females, with males experiencing a more severe clinical presentation. The severity of symptoms in females is influenced by X chromosome inactivation, which can vary widely from asymptomatic to symptomatic phenotypes. It is important to note that not all GLA mutations are pathogenic, and most

mutations are specific to individual families. Furthermore, there may be no clear correlation between genotype and phenotype, and the disease course can vary among individuals.[3,4]

The classical form of FD can manifest in childhood, displaying characteristic symptoms such as acroparesthesia, angiokeratoma, cornea verticillata, hearing loss, hypo/hyperhidrosis, abdominal neuropathic pain, and Fabry painful crises. In adulthood, patients may develop kidney failure, cardiac issues, and neurological complications. The nonclassical form typically presents at a later age, and patients may experience kidney or cardiac complications alone or in combination with other organ involvement. Cardiovascular manifestations of FD are left ventricular hypertrophy, heart valve regurgitation, conduction defects, hypertrophic or restrictive cardiomyopathy, coronary artery disease, and hypertension. Cardiac causes are the most common cause of death in FD for both genders.[5]

Kidney manifestations of FD exhibit greater prominence in hemizygous males compared to heterozygous females. However, due to X chromosome inactivation, females also experience a significant disease burden, including the risk of kidney disease. The kidney can enlarge due to the accumulation of lysoGb3, a specific substance. Radiographic imaging has revealed the presence of cortical or parapelvic cysts in some cases. Light microscopy shows characteristic changes in the kidney. Glomeruli display foamy vacuoles, mesangial enlargement, and hypertrophic visceral epithelial cells (podocytes). Moreover, lipid material accumulation is observed mostly in podocytes within the glomeruli. Subsequently, progressive glomerulosclerosis, characterized by ischemic changes in the kidney microcirculation, results in proteinuria, thickening of capillary walls, tubular atrophy, and interstitial fibrosis. Podocytes filled with osmophilic granular lamellar membrane structures can be seen by electron microscopy.[6] In classically affected patients, proteinuria usually appears in the second decade of life and is a major contributor to the progression of FD nephropathy; in fact, it is one of the most important indicators of kidney disease progression. Proteinuria is therefore generally accepted as a biomarker for Fabry nephropathy. Although rare cases of kidney failure have been reported in the second decade, kidney failure and requirement of kidney replacement therapies (KRT) typically develop between the third and fifth decades of life.[7]

Enzyme replacement therapies (ERT) reduce the progression of cardiac hypertrophy and prevent heart failure in the absence of fibrosis. Additionally, ERT has proven beneficial in slowing the progression of kidney failure.[8] Guidelines recommend screening for FD in high-risk populations, such as individuals with familial kidney disease diagnosed at an early age and those on KRT.[9] Implementing this screening strategy allows for the identification of affected family members at earlier disease stages. In this study, we aimed to investigate the prevalence of FD in patients with proteinuria over 300 mg/day or chronic kidney disease (Stage 2-5).

MAIN POINTS

- Fabry disease screening is recommended in patients with unknown etiology, early onset, or familial chronic kidney disease.
- In the differential diagnosis of patients with proteinuria who have vacuolated/foamy cells, mesangial enlargement, or segmental sclerosis findings in kidney biopsy, Fabry disease should also be considered.
- Detection of the mutation by screening and enzyme replacement treatment can prevent organ failure in both the patient and family members with the mutation.
- Cardiac magnetic resonance imaging can be used to investigate the cardiac involvement of Fabry Disease.

MATERIAL AND METHODS

Between 2018 and 2022, 517 patients under the care of our hospital's nephrology clinic underwent screening. Male patients were screened using an enzyme test measuring α-Gal A activity. GLA gene mutation analysis was performed in all female patients and male patients with α-Gal A activity below 2.5 nmol/mL/h. Patients with positive genetic analysis underwent measurement of plasma globotriaosylsphingosine (lyso Gb3) levels. Fabry disease-related signs and symptoms were evaluated in patients with identified mutations. The analyses were conducted at the Düzen Laboratory in Ankara. Consultations with cardiology, neurology, and ophthalmology specialists were done, and additional diagnostic tests such as electrocardiography, echocardiography, cardiac magnetic resonance imaging, and electromyography were performed on patients with confirmed mutations. Patients were given detailed written information about the study, and doctors obtained written informed consent.

Ethical standards were followed in all procedures performed in the study (Haydarpaşa Numune Education and Research Hospital Ethics Committee (HNEAH-KEAK/KK/2020/13), which approved the protocols in this study, and the 1964 Declaration of Helsinki).

Inclusion Criteria

Between 2018 and 2022, all patients who were diagnosed with chronic kidney disease (CKD) and/or proteinuria in our clinic and provided consent were included in the screening, regardless of the possible etiology. Screening tests were performed before the histopathological diagnosis (before kidney biopsy). Patients with known causes of kidney failure were also included. All patients with chronic kidney disease were divided into two groups in order to be able to study the frequency of FD separately in the groups.

Groups:

- Patients with decreased kidney function (CKD Stage 2-5):

estimated glomerular filtration rate (eGFR) less than 90 mL/min/1.73 m^2, lasting 3 months or longer (based on the CKD-EPI 2021 calculation)

- Patients with proteinuria (CKD Stage 1):

Normal kidney function but proteinuria exceeding 300 mg/day.

- Individuals with a known family history of Fabry mutations.

Exclusion Criteria

- Cases without any chronic kidney damage or family history of FD
- Patients who do not provide consent for Fabry screening.

α-Gal Activity Testing

The analysis was performed using the fluorimetric method. 4-Methylumbelliferyl α-D-Galactopyranoside (TRC; M334475) was used as the substrate, and *N*-acetyl-D-galactosamine (Sigma-Aldrich; A2795) was used as the inhibitor. Incubation was carried out at 37°C for 17 hours with a 3 mm DBS punch, inhibitor, and substrate. Fluorescent molecules were labeled with Ex: 366 nm and Em: 442 nm in fluorimetry (BioTek Synergy). The calibration curve was established with 4-methylumbelliferone (Sigma M1381). A cutoff value of 2.5 nmol/mL per hour, corresponding to 25% of the enzyme level distribution, was used as the trigger value for confirmatory genetic analysis.

Lyso-Gb3 Method Measurement

The tests were performed using liquid chromatography–mass spectrometry (LC-MS) or mass spectroscopy (MS) technique.

Standard: Lyso-ceramide trihexoside (Matreya, Cat. No. 1520)

Internal standard: *N*-Glycinated lyso-ceramide trihexoside (Matreya, Cat. No. 1530)

GLA Sequence Analysis

Deoxyribonucleic acid extracted from the samples was sequenced using the Next Generation Sequencing (MiSeq next-generation sequencing platform). Mutations identified through this analysis are further validated using the Sanger method. All coding exons of the gene and their flanking splice site junctions were amplified using PCR primers designed with PRIMER © – Primer Designer version 2.0 software (Scientific and Educational Software, Denver, CO, USA). The library was prepared using the Nextera XT kit (Illumina Inc.), following the instructions of the manufacturer.

Test (reference values)

- Alpha-galactosidase, Card (>2.5 nmol/mL/h)
- Alpha-galactosidase, leukocyte (>23.1 nmol/mg/h)
- Lyso-Gb3, Plasma (<1.0 ng/mL)
- Lyso-Gb3, Card (<1.3 ng/mL)

Statistical Package for the Social Sciences Statistics software, version 23.0 (IBM SPSS Corp.; Armonk, NY, USA) was used for all statistical analyses. For descriptive statistics, mean ± standard deviation was used for continuous variables regardless of normality status, while numbers with percentages were used for categorical variables.

RESULTS

A total of 517 people were screened for FD. The mean age of the patients was 51 ± 15 years. Among them, 231 (44.7%) were female. The order of screening indications for FD in these patients was as follows: 339 (65%) were screened due to decreased kidney function (CKD Stage 2-5), 155 (31%) due to proteinuria, and 23 (4%) due to a familial Fabry mutation (Figure 1). The prevalence of hypertension was 352 (68.1%) and diabetes mellitus was 138 (26.7%). Among those with CKD Stage 2-5, 215 (63%) were undergoing KRT. Specifically, 146 (68%) were on hemodialysis, 58 (27%) had undergone kidney transplantation, and 11 (5%) were receiving peritoneal dialysis.

In the analysis of the patients who were screened for decreased kidney function, 139 (41%) of the patients were female. The mean age of these patients was 53.7 ± 14.9 years, and the mean duration of diagnosis was 59.1 ± 60 months. The mean creatinine level was 4 ± 2.8 mg/dL, and eGFR was 26.3 ± 16.3 mL/min. In patients with decreased kidney function who did not receive KRT, the creatinine level was 1.9 ± 0.83 mg/dL, and eGFR was 40.6 ± 13.2 mL/min. When the patients were classified according to the possible etiology, the etiology was unclear in 96 (28.3%), diabetes mellitus in 61 (18%), hypertension in 32 (9.4%), glomerulonephritis in 49 (14.4%), focal segmental glomerulosclerosis (FSGS) in 26, IgA nephritis in 10, membranous nephropathy in 7, other glomerulonephritis in 6, atrophic kidney in 30 (8.8%), postrenal etiology in 22 (6.5%), solitary kidney in 14 (4.1%), and amyloidosis in 12 (3.5%) patients. Etiological causes such as cystic kidney disease and drug-related nephropathy were found in 23 of the remaining patients.

Seventy-eight (50.3%) of the patients screened for proteinuria were female. The mean age of the patients was 46 ± 13.6 years. Of the 78 patients with proteinuria and kidney biopsy, 26 were diagnosed as FSGS, 12 as IgA nephritis, 8 as diabetic nephropathy, 7 as membranous nephropathy, 4 as minimal change disease, 7 as normal findings, and 3 as lupus nephritis. The remaining 11 patients had multiple myeloma, amyloidosis, tubulointerstitial nephritis, chronic nonspecific changes, and

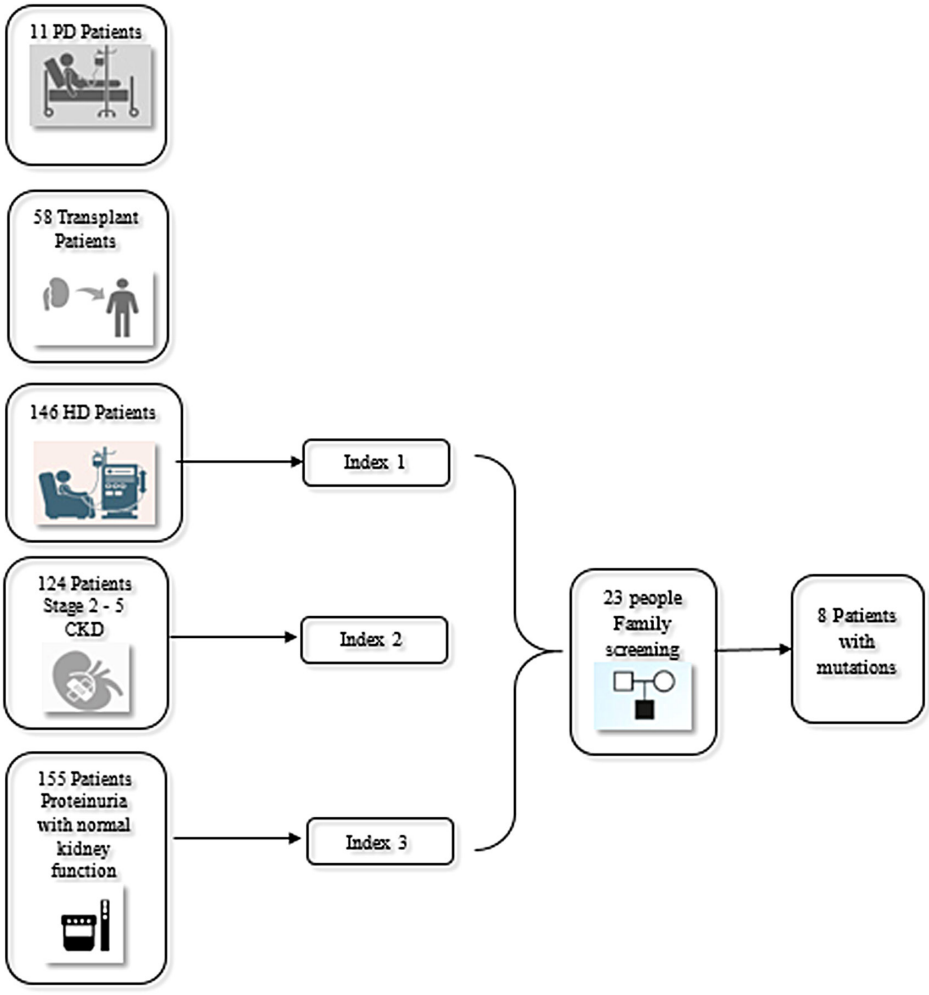

Figure 1. Screened patients and their clinical characteristics. PD, peritoneal dialysis; HD, hemodialysis; CKD, chronic kidney disease.

others. Moreover, among the 155 patients with proteinuria, the mean proteinuria was 3.8 ± 3.7 g/day.

Fabry disease mutation was detected in 3 (0.6%) of 494 patients with decreased kidney function or proteinuria (referred to as index cases), and an additional 8 patients with Fabry mutations were detected within 3 families that have been screened thus far. Our center conducted family screening for 2 index cases, while the screening for the third index case (referred to as index 3) was performed at the hospital where her daughters were being monitored. The pedigree of the index families for which Fabry screening was performed by our center, along with the clinical characteristics of the patients with mutations, are given below.

A 43-year-old man with no history of chronic disease was our first index case. He presented to the emergency department with symptoms consistent with uremia. However, a kidney biopsy could not be performed due to the small size of his kidney. Given his early-onset CKD and hypertrophic cardiomyopathy with heart failure, he underwent screening for FD. The α-galactosidase card test showed a result of 1.1 nmol/mL/h (normal: >2.5). Moreover, mutation analysis revealed the c.1079G>A (p.G360D) mutation, which is known to be associated with FD. Additionally, the Lyso-GB3 card test yielded a result of 10.9 ng/mL (normal: <1.30). Since cardiac findings were considered to be related to FD, ERT was initiated to prevent further organ involvement. The patient received hemodialysis and exhibited improved echocardiography findings and the ejection fraction of the patient within the first year of treatment. The family tree of our first index case (Figure 2) and the clinical findings of patients with FD mutations within their families are shown in Table 1.

Our second index case was a 50-year-old female patient with CKD, hypertension, and proteinuria of 2 g/day. A kidney biopsy revealed findings consistent with accelerated hypertension. Moreover, echocardiography indicated hypertrophic cardiomyopathy. We suspected the FD and conducted the lyso-GB3 card test, which yielded a result of 0.75 ng/mL (normal: <1.30). Furthermore, mutational analysis detected a c.645T>A (p.N215K) mutation. Therefore, we reexamined the previously

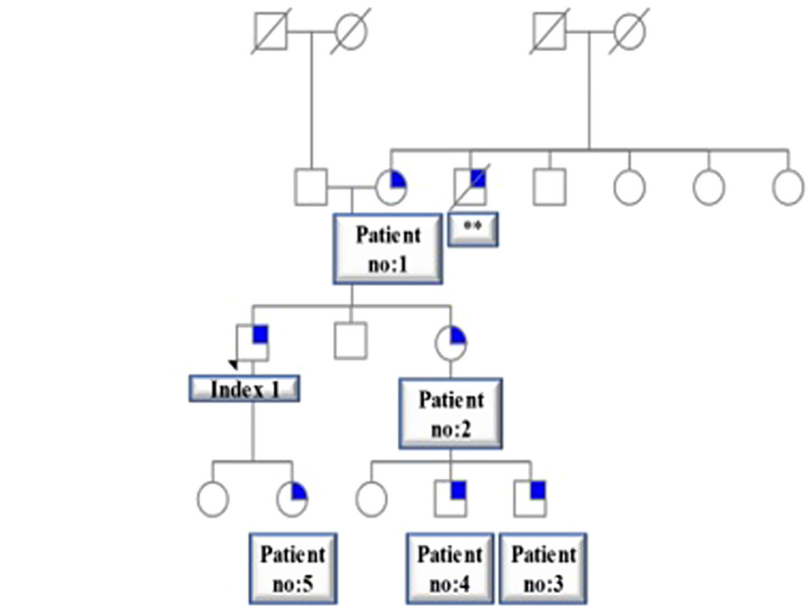

Figure 2. Pedigree of the first index case. **The uncle of our index case died at an early age due to cardiac disease. He was considered a probably affected case.

performed kidney biopsy materials for signs of FD involvement and observed the eosinophilic material indicative of FD involvement (Figure 3). The identified mutation was deemed significant for the diagnosis of FD and was reported as a novel mutation in the FD literature. ERT was initiated in this patient because of cardiac and kidney involvement. As kidney failure progressed to the end stage, the patient opted for peritoneal dialysis as her KRT. The patient's father experienced early cardiac death, implying a possible genetic transmission of FD. Her mother died at the age of 72 due to a hematological malignancy. None of the patient's uncles and aunts exhibited symptoms related to FD. The mutation was also detected in the patient's daughter, although clinical, laboratory, and imaging studies did not reveal any signs of FD involvement. The pedigree of our second index case is presented in Figure 4, and the findings of patients with FD mutation in the family are shown in Table 2. Despite efforts to contact the entire family, many members declined FD screening.

The third index case was a 58-year-old woman with proteinuria of 1.7 g/day and normal kidney functions. One of her daughter had CKD-KRT of unknown etiology, and the other had FSGS. A kidney biopsy showed no significant findings. The c.1207T>C (rs869312239) (p.L403=) mutation was consistent with FD. Moreover, cardiac MR taken for cardiac evaluation revealed FD involvement. Enzyme replacement therapy was initiated due to the current involvement. The FD mutation was positive in both of the patient's daughters with CKD. No additional mutations were observed in the first-degree relatives. The family screening was performed at the hospital where the daughters were being monitored. Since patients other than the 3rd index case were scanned outside our hospital, their data were not included in our study. The findings of our third index case are shown in Table 3.

DISCUSSION

Since FD has various and nonspecific clinical manifestations, its diagnosis is often challenging and delayed, resulting in organ damage and potential organ failure. The literature demonstrates that screening patients with organ damage, starting with the index case, can identify other affected family members before the onset of organ failure. Therefore, ERT can be initiated to prevent irreversible organ damage.

Table 1. Fabry Disease Test Results and Clinical Findings of the First Index Case and Mutation-Identified Family Members

Patient	Age	Alpha-Galactosidase (N: >2.5 nmol/mL/h)	LysoGb3 (N:<1.30 ng/mL)	Mutation	Kidney Findings	Cardiac Findings	Eye Findings	Neurological/ Joint/ Skin Findings	Follow-Up
Index 1	43	1.1	10.9	c.1079G>A (p.G360D)	Yes, ESRD/ HD	EF %40 ECHO: HCMP	None	None	ERT started, cardiac findings regressed
Patient 1	64		3	c.1079G>A (p.G360D)	None	CMR: Septal hypertrophy, myocardial pathological contrast enhancement	None	Neuropathy (EMG) and muscle/joint pain	ERT started, pain regressed
Patient 2	47		0.7	c.1079G>A (p.G360D)	MA	None	Cornea verticillata	None	ERT started
Patient 3	21	0.1	10.3	c.1079G>A (p.G360D)	MA	None	Cornea verticillata	None	ERT started
Patient 4	24	0,2	8.3	c.1079G>A (p.G360D)	MA	CMR: myocardial pathological contrast enhancement	Cornea verticillata	None	ERT started
Patient 5	The 12-year-old daughter of the index case. The enzyme level was 0.9, and mutation was detected. Referred to pediatric metabolic diseases department								

CMR, cardiac magnetic resonance; ECHO, echocardiography; EF, ejection fraction; EMG, electromyography; ERT, enzyme replacement therapy; ESRD, end stage kidney disease; FD, Fabry disease; HCMP, hypertrophic cardiomyopathy; HD, hemodialysis; MA, microalbuminuria; N, Normal range.

In a 2013 study, although there is no recommended evidence level, FD screening was suggested for men under 50 years of age with unexplained CKD and women with CKD of unclear etiology and potential systemic involvement associated with FD.[9] Turkmen et al[10] conducted a 10-center study called TURKFAB and reported a 0.95% (3/313) prevalence of FD in patients with stage 1-4 CKD of unknown etiology and stage 5 CKD without KRT. Furthermore, through family screening of the index case, an additional 8 patients with both FD and CKD were identified. In a study involving 1453 CKD without KRT in Türkiye, mutations were identified in 3 male patients, resulting in an FD prevalence of 0.2%.[11] Screening studies conducted globally within the same population have reported FD prevalence ranging from 0% to 0.95%.[12-15] In our study, 1 out of 124 patients with CKD not undergoing KRT tested positive for an FD mutation, yielding a prevalence of 0.8%, consistent with existing literature.

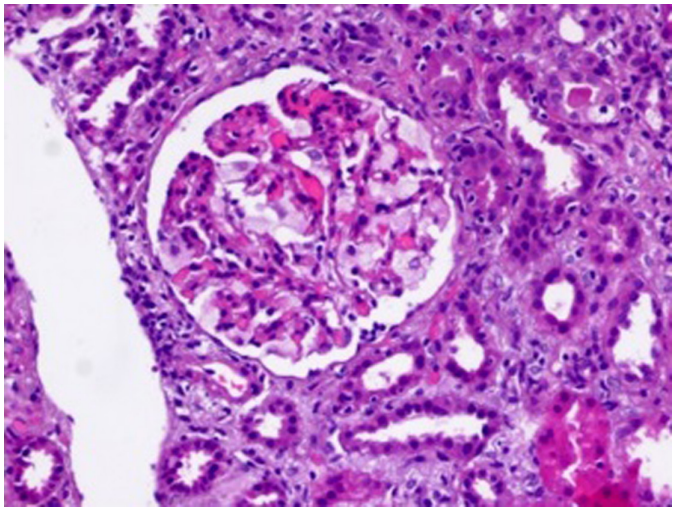

Figure 3. Eosinophilic material and proliferative pattern in glomeruli in kidney biopsy material of index 2 (hematoxylin and eosin; original magnification ×400/light microscopy)

In our country, Kalkan et al[16] reported a prevalence of 0.24% in screening 808 male patients undergoing hemodialysis. Moreover, Yalın et al[17] identified FD mutations in 17 out of 5657 patients receiving KRT, resulting in a prevalence of 0.3%.[17] Prevalence studies conducted in other countries with similar populations showed rates of 0.36% in Brazil, 0.23% in China, 0.3%-0.55% in Spain, and 0.02% in Japan.[18-20] Our study found a prevalence of 0.68% in the hemodialysis group and 0.46% in the group of patients with CKD-KRT.

Proteinuria is a risk factor for the progression of CKD, including Fabry nephropathy. Several studies have demonstrated a correlation between higher baseline proteinuria levels and a faster decline in kidney function.[21] In a large multicenter study involving Fabry patients from 5 countries, proteinuria was observed in 90% of patients who progressed to CKD-KRT. In addition, proteinuria was more common in patients with an eGFR <60 mL/min/1.73 m^2 than in those with an eGFR >60 mL/min/1.73 m^2.[22] Proteinuria prevalence rates of 91% in men and 72% in women with eGFR <60 mL/min/1.73 m^2 were reported by Ortiz et al.[23]

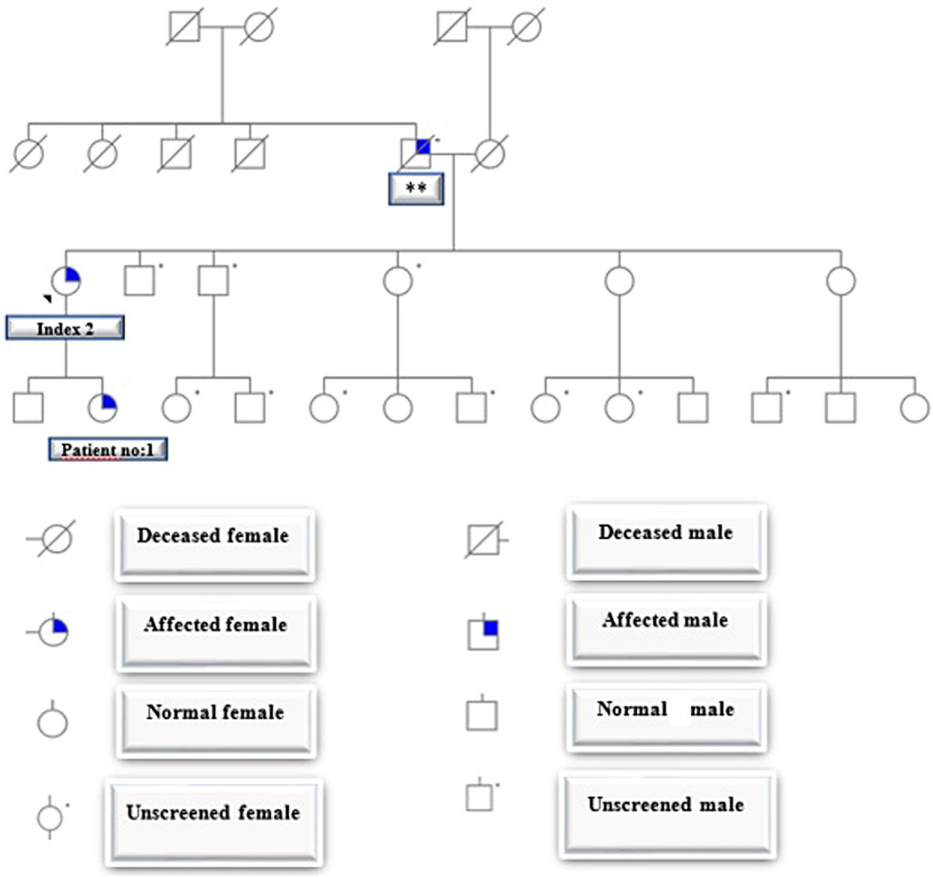

Figure 4. Pedigree of the second index case. **The father of the index case died of cardiac causes at an early age. He was considered a probable affected case.

Proteinuria is one of the early kidney manifestations of FD. Therefore, in patients with proteinuria, Fabry disease is also included in the differential diagnosis. FD may be considered in the initial diagnosis, especially in patients with systemic disease findings in their families and in patients with an unclear etiology of proteinuria (e.g., patients with a diagnosis of FSGS but no etiological factor). Previous reports have described cases where FD mutations were detected in patients with proteinuria who underwent a kidney biopsy.[24] This study identified an FD mutation in 1 (0.64%) out of 155 patients with proteinuria but no kidney dysfunction. Mutations were also detected in her 2 daughters, who had previously been diagnosed with FSGS and

Table 2. Fabry Disease Test Results and Clinical Findings of the Second Index Case and Mutation-Identified Family Members

Patient	Age	Alpha-Galactosidase (N: >2.5 nmol/mL/h)	LysoGb3 (N:<1.30 ng/mL)	Mutation	Kidney Findings	Cardiac Findings	Eye Findings	Neurological/ Joint/ Skin Findings	Follow-up
Index 2	50		0.75	c.645T>A (p.N215K)	Hypertension, proteinuria/ accelerated hypertension findings (reevaluation of the kidney biopsy, vacuolization and intraglomerular accumulation indicative of FD) → ESRD /peritoneal dialysis	EF %60 ECHO: HCMP (apical/ septal)	None	None	ERT started
Patient 1	21			c.645T>A (p.N215K)	None	No findings on CMR	None	None	Stable

CMR, cardiac magnetic resonance; ECHO, echocardiography; EF, ejection fraction; ERT, enzyme replacement therapy; ESRD, end stage kidney disease; FD, Fabry disease; HCMP, hypertrophic cardiomyopathy; N, normal range.

Table 3. Test Results and Clinical Findings of the Third Index Case

Patient	Age	Alpha-Galactosidase (N: >2.5 nmol/mL/h)	LysoGb3 (N:<1.30 ng/mL)	Mutation	Kidney Findings	Cardiac Findings	Eye Findings	Neurological/ Joint/ Skin Findings	Follow-Up
Index 3	58		0.8	c.1207T>C (rs869312239) (p.L403=)	Proteinuria	CMR: myocardial pathological contrast enhancement	None	Muscle/joint pains	ERT started

CMR, cardiac magnetic resonance; ERT, enzyme replacement therapy; FD: Fabry disease; MR, magnetic resonance; N: normal range.

were undergoing hemodialysis treatment. Furthermore, a retrospective examination of the pathology samples from index 2, who had chronic kidney failure and proteinuria, revealed signs of FD kidney involvement.

Early detection of cardiac involvement is crucial, as it significantly contributes to mortality in FD. Left ventricular (LV) hypertrophy and regional fibrosis are common in Fabry cardiomyopathy. Cardiac magnetic resonance is an imaging technique that has become very popular for the early detection of cardiac involvement in patients with FD. The technique of late gadolinium enhancement (LGE) provides additional valuable information that can be correlated histologically with the focal fibrosis in FD. In up to 50% of FD patients, LGE is typically observed in a distinct midwall pattern in the basal inferolateral LV wall.[25] In our study, cardiac MRI revealed midmyocardial pathological contrast enhancement in 3 patients. Two of these patients were diagnosed with FD after the family screening, and no overt kidney disease was detected in either patient. Although publications recommend the use of CMR in the management and follow-up of FD, the potential of CMR as a tool for early diagnosis of cardiac involvement and optimization of the initiation of ERT as the leading therapy in FD remains questionable and needs further evaluation.[26]

c.645T>A (p.N215K) Mutation—Novel Mutation

Our second index case initially received a kidney biopsy report of hypertensive nephrosclerosis. However, mutation analysis revealed the presence of the c.645T>A (p.N215K) mutation upon screening. Genetic consultation and subsequent new-generation sequencing confirmed the pathogenic nature of the mutation. The patient was diagnosed with FD and initiated on ERT. Upon reevaluation of the kidney biopsy, vacuolization and intraglomerular accumulation indicative of FD involvement were observed. The patient experienced improved quality of life, increased exercise capacity, and positive physical examination findings following the initiation of ERT. Uremic symptoms developed in the 11th month of ERT treatment, leading to the commencement of peritoneal dialysis as kidney replacement therapy.

In our patient, a novel heterozygous mutation, p.N215K, was identified, which exhibited cardiac and kidney involvement, with symptoms appearing in the fourth decade and progressing to ESKD in the fifth decade. This novel mutation showed normal lyso-GB3 levels, suggesting X chromosome inactivation during embryogenesis. Based on our findings, the c.645T>A (p.N215K) mutation may be classified as nonclassical FD, characterized by kidney and cardiac involvement with no clear genotype-phenotype correlation. However, further research is needed to understand the manifestation of this new mutation, and the field will continue to expand as more cases are reported. Currently, more than 900 FD-associated mutations in the human GLA gene have been documented in the literature, with novel mutations still being discovered.

Our study has limitations. First, regardless of etiology, we screened patients who presented to our clinic within a certain time period and whose consent was obtained. The fact that mutation analysis was not performed only in the high-risk population for whom FD screening is recommended in the literature may have led to an underestimation of the true frequency of the disease. However, we preferred to screen all patients because of the potential for overlap and the lack of clear criteria for which patients to screen. Secondly, family screening of index cases could not be completed because family members did not give consent or requested that it be done at the hospital where they were followed up. As a result, the FD phenotype of the patient with a novel mutation could not be definitively investigated. Thirdly, a single-center study with a relatively small number of patients may have reduced the statistical power.

Increased awareness of FD among clinicians and the implementation of screening programs has facilitated early diagnosis before the onset of progressive organ damage. Apart from the classical and nonclassical forms, patients present diverse clinical presentations and display variations in genotypic and phenotypic features, which are influenced by genetic mutations. While we have compelling evidence that our newly identified N215K mutation is pathogenic, the lack of family screening has left unanswered questions regarding the potential variability of clinical presentations associated with this mutation. Our understanding will expand as new mutations are identified in the literature, whether they are similar or distinct. Numerous studies and updated recommendations emphasize the significance of early treatment

initiation in both sexes. Early initiation of ERT plays a crucial role in achieving early diagnosis and preventing organ failure. Therefore, we believe that screening for FD is important for early diagnosis and treatment in patients with proteinuria and CKD, especially in those of uncertain etiology or with kidney biopsy findings such as vacuolated cells, mesangial enlargement, FSGS, interstitial fibrosis, and unexplained tubular atrophy.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Haydarpaşa Numune Education and Research Hospital (Approval no:HNEAH-KAEK/KK/ 2020/13, Date: 2020).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

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