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Original Paper

The Prevalence of Fabry Disease in Patients with Chronic Kidney Disease in Turkey: The TURKFAB Study

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Key Words
Chronic kidney disease • Fabry disease • Agalsidase therapy • Screening • ERBP

Abstract
Background/Aims: Fabry disease is a treatable cause of chronic kidney disease (CKD) characterized by a genetic deficiency of α-galactosidase A. European Renal Best Practice (ERBP) recommends screening for Fabry disease in CKD patients. However, this is based on expert opinion and there are no reports of the prevalence of Fabry disease in stage 1-5 CKD. Hence, we investigated the prevalence of Fabry disease in CKD patients not receiving renal replacement therapy. ***Methods:*** This prospective study assessed α-galactosidase activity in dried blood spots in 313 stage 1-5 CKD patients, 167 males, between ages of 18-70 years whose etiology of CKD was unknown and were not receiving renal replacement therapy. The

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diagnosis was confirmed by GLA gene mutation analysis. ***Results:*** Three (all males) of 313 CKD patients (0.95%) were diagnosed of Fabry disease, for a prevalence in males of 1.80%. Family screening identified 8 aditional Fabry patients with CKD. Of a total of 11 Fabry patients, 7 were male and started enzyme replacement therapy and 4 were female. The most frequent manifestations in male patients were fatigue (100%), tinnitus, vertigo, acroparesthesia, hypohidrosis, cornea verticillata and angiokeratoma (all 85%), heat intolerance (71%), and abdominal pain (57%). The most frequent manifestations in female patients were fatigue and cornea verticillata (50%), and tinnitus, vertigo and angiokeratoma (25%). Three patients had severe episodic abdominal pain attacks and proteinuria, and were misdiagnosed as familial Mediterranean fever. ***Conclusions:*** The prevalence of Fabry disease in selected CKD patients is in the range found among renal replacement therapy patients, but the disease is diagnosed at an earlier, treatable stage. These data support the ERBP recommendation to screen for Fabry disease in patients with CKD of unknown origin.

Introduction

Fabry disease (OMIM # 301500) is a systemic X-linked disease characterized by the accumulation of globotriaosylceramide (GL-3) secondary to decreased activity of the lysosomal enzyme α-galactosidase A (α-GAL A, EC 3.2.1.22) [1]. The classical form of Fabry disease is observed in males with very low α-GAL A activity. Early manifestations of Fabry disease include episodic-intermittent pain crisis (acroparesthesia), vascular lesions (angiokeratomas), vascular tortuosity and corneal brown subepithelial linear deposits (cornea verticillata), hypohidrosis, and proteinuria [2]. In the following years, end-stage renal disease (ESRD), left ventricular hypertrophy (LVH) and occlusive cerebro-vascular events may occur [3, 4], leading to early mortality when compared with age-and gender-matched healthy subjects [5]. Renal involvement can be seen in 48-55% of patients with Fabry disease and progresses to ESRD in most male patients [6]. However, the mean age at ESRD is 40 years both in males and in the females who do progress to this stage [2, 4].

Disease presentation in female heterozygotes may be as severe as in males although women may also remain asymptomatic. The recent introduction of enzyme replacement therapy (ERT) to address the underlying pathophysiology of Fabry disease has focused attention on the early diagnosis of Fabry disease. Guidelines have been proposed for the recognition, evaluation, and surveillance of disease-associated morbidities, as well as therapeutic strategies, including ERT and other adjunctive therapies, to optimize patient outcomes [7].

The prevalence of Fabry disease varies between 1/3100-1/117.000 in Europe [8]. However, there are no data on the prevalence of Fabry disease in stage 1-5 chronic kidney disease (CKD), despite an expert-based ERBP recommendation to screen for Fabry disease in such population [5]. Hence, we investigated the prevalence of Fabry disease in a CKD population in Turkey. We also defined the characteristics of CKD patients with Fabry disease and compared these with the results obtained in CKD patients without Fabry disease.

Material and Methods

The study protocol was approved by the Medical Ethics Committee of GATA (Ankara, Turkey). Written informed consent was obtained from all subjects included in the study.

This prospective study recruited 313 stage 1-5 CKD patients not receiving renal replacement therapy (hemodialysis, peritoneal dialysis or transplantation) between December 2014 and March 2016 from 6 different region and 10 centers of Turkey. A review of medical records (including information on age; sex;

weight; medications; primary disease of CKD) was undertaken. Inclusion criteria were: (1) stage 1-5 CKD patients not receiving renal replacement therapy ; (2) unknown etiology for CKD: patients did not have diabetic kidney disease or other kidney disease including glomerulonephritis, lupus nephritis, systemic vasculitis proven by kidney biopsy; (3) age 18-70 years; (4) patients gave written inform consent.

Four hundred and three patients were evaluated and 82 patients were excluded from the study: 47 refused to participate in the study, 24 had biopsy-proven glomerulopathies (including systemic lupus erythematosus and microscopic polyangiitis); and in 11 Fabry disease was considered very unlikely based on clinical manifestations. The remaining 313 CKD patients were enrolled in the study (Table 1). Additionally, 73 family members from 3 index patients were also screened for Fabry Disease.

Table 1. Demographic and Clinical data of CKD patients that were screened and of index patients found in the screening

Parameters	CKD screening population (n: 313)	Fabry index patients (n: 3)
Age (years)	43±14	48±21
Gender (M/F)	167/146	3/0
CKD stages, n (M/F)		
1	64 (33/31)	2 (2/0)
2	56 (32/24)	0 (0/0)
3	101 (60/41)	0 (0/0)
4	50 (26/24)	1 (1/0)
5	42 (17/25)	0 (0/0)

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the upright sitting position after ≥5 minutes of rest using an Erka sphygmomanometer (PMS Instruments Limited, Berkshire, UK) with an appropriate cuff size. Two readings were recorded for each individual and the mean value was defined as the blood pressure. CKD patients with SBP and DBP >140 and >90 mmHg, respectively, or who were already on antihypertensive treatment were assumed to be hypertensive.

Echocardiography

M-mode and two-dimensional echocardiographic measurements were performed using a Vingmed System Five, Norway echocardiographic system equipped with 2.5-MHz transducers (Vingmed Sound, Horten, Norway) in accordance with methods recommended by the American Society of Echocardiography [9]. Cardiac mass was calculated by means of the Devereux and Reichek formula [10]. Left ventricular hypertrophy (LVH) was defined as left ventricular mass index (LVMI) ≥125 g/m^2 for men and ≥110 g/m^2 for women.

Screening and Diagnosis of Fabry Disease

Screening for Fabry was performed by assessing α-Gal A activity in dried blood spots (DBS). Blood was spotted directly onto the filter paper after lancet finger stick or venipuncture syringe draw. Blood spots were dried for at least 4 h at room temperature and were stored in sealed plastic bags at 4 °C for up to 1 week in a cabinet and at −20 °C with desiccant and a humidity indicator for longer periods [11].

The cut-off to trigger confirmatory genetic analysis was 1.2 micromol/L/h in the CKD screening Fabry disease was confirmed by GLA gene mutation analysis. This cut-off point was determined from Receiver Operating Characteristic (ROC) testing by the Archimed Life Science GmbH laboratory. The same cut-off value was used for females and males. For the study of family members, genetic analysis both enzymatic analysis and genetic analysis were performed, given the higher probability of Fabry disease and the fact that enzymatic activity may be normal in Fabry females. Plasma lyso-Gl-3 levels were measured via tandem mass spectrometry method from DBS before ERT in 4 CKD patients with Fabry disease.

GLA Gene Sequencing

GLA gene was sequenced using the MiSeq next generation sequencing (NGS) platform, a FDA approved diagnostic system (Illumina, San Diego, CA, USA). Genomic DNA was extracted according to the manufacturer's standard procedure using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). All coding exons and their flanking splice site junctions were amplified using PCR primers, designed with PRIMER© – Primer Designer v.2.0 (Scientific & Educational Software programme) software. PCRs were validated by agarose gel electrophoresis. After PCR amplification, the libraries were prepared with the NexteraXT kit (Illumina Inc.), according to the manufacturer's instructions. Sequences were aligned to the hg19 genome within MiSeq Reporter software (Illumina Inc.). Visualization of the data was performed with IGV 2.3 (Broad Institute) software.

Biochemical analyses

Venous blood samples for biochemical analyses were drawn after an overnight fast in all CKD patients. Serum glucose, creatinine, urea, LDL cholesterol, and triglyceride concentrations were assessed by an oxidase-based technique at Roche/ Hitachi Modular System (Mannheim, Germany) in the biochemistry laboratories.

Statistical analysis

The Statistical Package for Social Sciences for Windows version 15.0 (SPSS, Chicago, IL, USA) was used for statistical analyses. Data are expressed as mean ± standard deviation. Dichotomous variables were compared using the chi-squared test. Statistical differences between parametric data of two groups were analyzed using the Student's t-test. The Mann-Whitney U test was used to determine differences between nonparametric data. Linear associations between continuous variables were assessed using the Spearman correlation test. A p value <0.05 was considered significant for all tests.

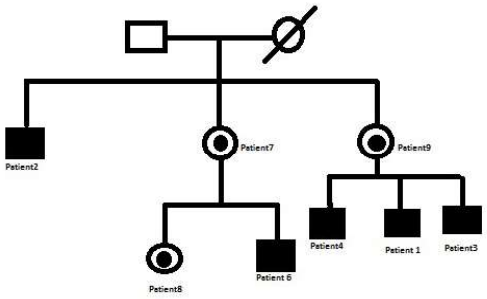

Fig. 1. Family tree of index patient 1.

Results

Demographic Characteristics

A total of 3 of 313 (0.95 %) Caucasian CKD patients were diagnosed of Fabry disease in primary screening (patients 1, 5, 11). All the three were males and thus, the prevalence of Fabry disease in CKD males was 1.80% (Table 1). Family screening of 73 family members from the 3 index patients disclosed that 8/73 (10.9%) had Fabry disease. Index patient 1 had 7 family members (4 male, 3 female) with both CKD and Fabry disease (Figure 1) and index patient 11 had one daughter (patient 10) with both CKD and Fabry disease. Baseline demographic and biochemical data for CKD patients are shown in Tables 2 and 3. 7 out of 11 Fabry patients were male and 4 were female. As expected Fabry patients had more frequently symptoms associated with Fabry disease than CKD patients without Fabry disease.

Table 2. Demographic and clinical data of CKD patients with and without Fabry Disease and family members with Fabry Disease

Parameters	Fabry disease (n:11)	No fabry disease (n: 310)	p value
Age (years)	41.3±14.5	43.2±14.9	0.64
Gender (M/F)	7/4	164/146	0.483
Hypertension, n (%)	1 (9%)	132 (42%)	0.005
FMF gene mutation n (%)	0 (0%)	11 (3.5%)	0.524
Microscopic Hematuria, n (%)	2 (18%)	69 (22%)	0.631
CAD n (%)	0 (0%)	16 (5%)	0.439
LVH n (%)	2 (18%)	9 (3%)	0.449
Hypo-anhidrosis n (%)	7 (63%)	1(0.3%)	<0.0001
Heat intolerance n (%)	5 (45%)	40 (13%)	0.002
Cold intolerance n (%)	4 (36%)	36 (12%)	0.016
Acroparesthesia n (%)	7 (63%)	0 (0%)	<0.0001
Angiokeratoma n (%)	6 (54%)	0(0%)	<0.0001
Cornea verticillata n (%)	6 (54%)	0 (0%)	<0.0001
Tinnitus n (%)	7 (63%)	30(10%)	<0.0001
Vertigo n (%)	7 (63%)	31(10%)	<0.0001
Exercise intolerance n (%)	1 (9%)	65 (21%)	0.334

CAD: coronary artery disease. LVH: left ventricular hypertrophy assessed with echocardiography.

Fabry Patient Symptoms

Median age at onset of first symptoms of Fabry disease was lower in males than in females (26 years vs 38 years, re-

spectively). The median age at diagnosis of Fabry disease was 31 years (range 25-71) for males and 49 years (range 30-55) for females. Table 4 provides a summary of the characteristics of patients with Fabry disease. The most frequent presenting symptoms in male Fabry disease patients were fatigue (100%), tinnitus (85%), acroparesthesia (85%), vertigo (85%), heat intolerance (71%), abdominal pain (57%), cold intolerance (57%) Six male patients with Fabry disease (85%) had cornea verticillata, cataract, hypohidrosis, and angiokeratomas at dorsal and lumbar region. The most frequent presenting symptoms in female Fabry disease patients were fatigue (50%), tinnitus (25%) and vertigo (25%). Male patients with Fabry disease (85%) had cornea verticillata (50%), cataract (25%), and angiokeratoma (25%).

Table 3. Biochemical Parameters of CKD Patients with and without Fabry Disease

Parameters	Fabry disease (n:11)	No Fabry disease (n: 310)	P value
Glucose (mg/dL)	95 (80-124)	89 (56-256)	0.059
Urea (mg/dL)	26 (16-95)	50 (10-370)	0.001
Creatinine (mg/dL)	0.82 (0.57-2.70)	1.49 (0.93-4.32)	0.004
eGFR (ml/min/1.73 m^2)	87 (23-126)	44 (12-121)	0.003
LDL cholesterol (mg/dL)	90 (57-185)	118 (80-263)	0.193
Triglycerides (mg/dL)	108 (54-283)	153 (101-345)	0.006
Albumin (g/dL)	4.1 (4.0-4.2)	4.2 (2.0-4.3)	0.724
Hemoglobin (g/dL)	13.3 (12.4-16.7)	13.5 (8.6-18.2)	0.991
Spot urine total protein/ creatinine ratio (g/g)	0.26 (0.07-5.6)	0.68 (0.08-14)	0.351
Alfa-galactosidase activity (micromol/L/hour)	1.2 (0.7-2.5)	3.1 (1.9-7.8)	<0.001

Data expressed as median (min-max).

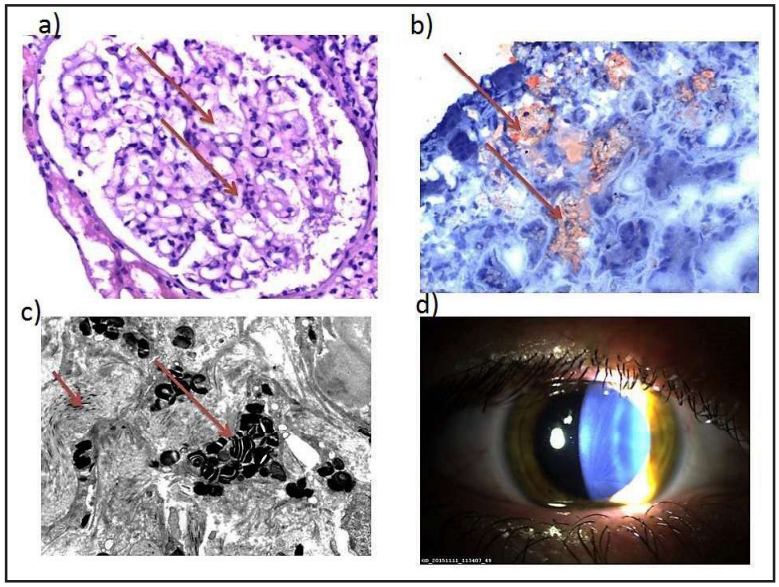

Fig. 2. Light and Electronic Microscopic Findings of Patient 1 with Fabry Disease. a) Hematoxylin and eosin (HE) staining of kidney biopsy specimen showed empty vacuoles in glomerular cells (arrows). Original magnification 4x0. b) Oil-red 10 staining disclosed glomerular lipid vacuoles (arrows). Original magnification 100x. c. Electron microscopy revealed zebra bodies and myelin figures in podocytes (large arrow) and interstitial fibrosis (small arrow). Original magnification 5680x. d). Subepithelial brown lines (cornea verticillata) are seen on slit-lamp photographs of cornea.

Index patient 1 had two brothers with Fabry disease (patients 3 and 4) and the three of them had severe episodic abdominal pain attacks and proteinuria, hence they were misdiagnosed as familial Mediterranean fever (FMF) and secondary amyloidosis even though they did not have FMF gene mutations. Kidney biopsy in the three siblings disclosed glomerular lipid vacuoles by that appeared empty with hematoxylin eosin and stained with oil-red 10 (Figure 2a,b). Electron microscopy revealed zebra bodies and myelin figures in podocytes (Figure 2c), but no evidence of amyloidosis.

Table 4. Characteristics of Patients with Fabry Disease. * Normal values <3.5 ng/ml

Patient No	Age/ Gender	Main Symptoms	Physical Examination	Serum Creatinine (mg/dL)	Proteinuria prior to treatment (g/day)	eGFR (ml/min/ 1.73 m²)/CKD stage	α-GAL A activity (micromol/ L/h)	Gene Mutation	Lyso-Gl-3 levels before ERT (ng/mL)*	ERT type and cumulative dose (mg)	Proteinuria after 6 months of ERT (g/day)
1	29/M	Fatigue, hypohidrosis abdominal pain, heat intolerance, acroparesthesia, tinnitus, vertigo	Cornea verticillata, cataract, angiokeratoma	0.89	5.6	101/1	0.9	p.N34H	41.3	Agalsidase-α 144	1.6
2	35/M	Fatigue, Hypohidrosis, heat/cold intolerance acroparesthesia, tinnitus, vertigo	Cornea verticillata, cataract, angiokeratoma	1.08	0.08	77.8/2	1.1	p.N34H	55.1	Agalsidase-α 110	0.1
3	31/M	Fatigue, Hypo-anhydrosis, abdominal pain, heat/cold intolerance acroparesthesia, tinnitus, vertigo	Cornea verticillata, cataract, angiokeratoma	1.17	2.2	72/2	1.4	p.N34H	N/A	Agalsidase-α 156	1.46
4	25/M	Fatigue, Hypohidrosis, abdominal pain, heat/cold intolerance acroparesthesia, tinnitus, vertigo	Cornea verticillata, cataract, angiokeratoma	1.04	1.1	87/2	1.3	p.N34H	53.9	Agalsidase-α 148	1.1
5	49/M	Vertigo, Fatigue, mild deafness, acroparesthesia, hypohidrosis, tinnitus	Cornea verticillata, cataract, SVA, LVH	0.78	0.2	105/1	1.1	p.358delE	N/A	Agalsidase-α 130	0.2
6	28/M	Fatigue, Exercise intolerance, hypohidrosis abdominal pain, heat/cold intolerance acroparesthesia, tinnitus, vertigo	Hypertension, Cornea verticillata, cataract, Angiokeratoma, hematuria	0.74	0.2	125/1	1.2	p.N34H	42.2	Agalsidase-α 120	0.1
7	55/F	Fatigue	Cornea verticillata	0.81	0.19	78/2	2.5	p.N34H	N/A	No ERT	0.07
8	30/F	None	None	0.57	0.26	124/1	2.4	p.N34H	N/A	No ERT	N/A
9	53/F	Fatigue, vertigo, tinnitus	Cornea verticillata, cataract, angiokeratoma	0.68	0.31	90/1	2.2	p.N34H	N/A	No ERT	N/A
10	45/F	None	None	0.82	0.13	75/2	0.8	p.F229V	N/A	No ERT	N/A
11	70/M	Fatigue	Pretibial edema	2.7	2.76	23/4	0.7	p.F229V	N/A	Agalsidase-β 980	1.5

Our second index patient (Patient 5) had premature stroke in his forties and developed CKD. He had severe acroparesthesias and angiokeratomas that suggested a diagnosis of Fabry disease.

GLA gene sequencing

GLA gene sequencing results are presented in tables 4 and 5. Patients 1-4 and 6-9 had NM_000169.2 (GLA): c.100A>C (p.N34H), Patient 5 had NM_000169.2 (GLA): c.1072_1074del (p.358delE) and patients 10 and 11 had NM_000169.2 (GLA): c.685T>G (p.F229V) gene mutations.

Treatment and follow-up

Of 11 patients with Fabry disease, 6 males (patients 1-6) started agalsidase-α 0.2 mg/kg/2 weeks and 1 male (patient 11) started agalsidase-β 1.0 mg/kg/2 weeks as ERT along with previously prescribed renin-angiotensin-aldosterone blockers (RAAS blockers) (Table 4). Patient 1 also started non-dihydropyridine calcium channel blocker

Table 5. GLA Gene Mutation Characteristics of 3 index FD Patients

Index Patient Number Mutation	Mutation Taster	Polyphen2 HDivPred	Polyphen2 HVarPred	SIFT	SIFT Provean	HGMD-Public	HGMD mutation in same codon	fabry-database.org
Patient 1 NM_000169.2(GLA): c.100A>C (p.N34H)	Disease Causing (0.9999)	Probably damaging (1.0)	Probably damaging (0.999)	Damaging (0.000)	Deleterious (-4.21)		PHE-LEU ASN-SER	Not listed
Patient 5 NM_000169.2(GLA): c.1072_1074del (p.358delE)	Disease Causing (0.9999)					CD962019	CM066856 GLU-ALA and CM024297 GLU-GLY	Listed, no phenotype assigned
Patient 11 NM_000169.2(GLA): c.685T>G (p.F229V)	Disease Causing (0.9910)	Probably damaging (0.969)	Possibly damaging (0.547)	Damaging (0.006)	Deleterious (-5.31)		CM024297 PHE-LEU	Not listed

(diltiazem 90 mg/day) to decrease proteinuria. Female patients did not accept ERT and they are being monitored. No patient developed anti-agalsidase antibody after 6 months of ERT. No drug-related adverse events were observed in 6 patients, however patient 5 developed a mild infusion reaction including fever, malaise and skin rash that responded to slowing infusion, methylprednisolone, and anti-histaminic medication.

Discussion

The main findings of the present study were as follows: 1) the prevalence of Fabry disease was found to 0.95% (3/313) in a selected Turkish CKD cohort, 2) FMF was a frequent misdiagnosis that delayed the final diagnosis by about 5 years.

The diagnosis of Fabry disease is frequently delayed, as illustrated by the present report and Registry data [12]. In some cases this is due to misdiagnosis. In the present study, patients 1, 3 and 4 had severe episodic abdominal pain attacks and proteinuria, hence they were misdiagnosed as familial Mediterranean fever (FMF) and secondary amyloidosis despite later shown that neither a FMF gene mutation nor pathologic evidence of amyloidosis were present. Both FD and FMF patients might exhibit similar symptoms and clinical manifestations. In Turkey, patients with FMF have recurrent episodes of fever, abdominal pain, joint pain, and gastrointestinal disorders as well as kidney damage. Recently, 23 of 177 (13%) patients with symptoms of FMF were found to have α-Gal A deficiency in the Central Anatolia region of Turkey [13]. These results suggest that Fabry disease may be misdiagnosed as FMF in endemic regions and should be suspected especially in patients with symptoms of FMF without FMF gene mutation.

Most of our male patients with Fabry disease had severe and debilitating neuropathic pain. Their pain crises frequently coincided with warm weather which might be associated with increased body temperature, as previously described in Fabry disease [6, 14]. The beneficial effect of ERT on neuropathic Fabry pain within 6 months was reported by several authors [15, 16]. In the present study, after 6 months of ERT, all of our male patients with Fabry disease reported improvement in neuropathic pain.

Proteinuria, especially if >0.5 g/24h, is a risk factor for CKD progression in Fabry disease, even in patients receiving ERT [17, 18]. In this regard, 4/7 (57%) males already proteinuria above 0.5 g/24h when Fabry disease was diagnosed. Interestingly, RAAS blockers were recently found to be associated with stabilization of GFR when used in conjunction with ERT if proteinuria decreased below 0.5 g/day [19].The GLA gene mutation of patient 5 has not been previously defined in the literature. This variant was not found in 500 clinical exome sequencing data of our patients. It was reported as a likely pathogenic variant by Mutation Taster software. In this regard, the presence of cornea verticillata, hypohidrosis and neuropathic pain are consistent with classical Fabry disease. However, the α-Gal A activity was just below the cut-off point and lyso-Gb levels were not available.

The main limitation of the study was the small sample size. Another limitation is that the screening threshold for α-Gal A activity may have been too low. Two male siblings of an

index case had enzymatic activity just above the threshold, despite Fabry symptoms, and genetic and histological evidence of Fabry disease. Thus, we may have missed some male Fabry patients, in addition to the well known fact that females may be missed in enzymatic screening efforts. Indeed, no female was diagnosed in the original CKD screening. Only the combination of genetic and enzymatic studies allowed the diagnosis of female family members of the index male patients found in the original CKD screening.

Conclusion

Our data demonstrated that Fabry disease was not uncommon among selected patients with CKD and support the ERBP recommendation to screen for Fabry disease in CKD patients in whom the etiology is unclear. Fabry disease should be considered in CKD patients with unknown origin and appropriate physical examination including eye, cardiovascular and central nervous system examination should be performed. Another population that merits special attention is that with nephropathy and abdominal pain without FMF gene mutation.

Disclosure Statement

AO is a consultant for Genzyme and has received speaker fees from Shire HGT. KT has received speaker fees for lectures on Fabry disease from Shire HGT. Other authors declare no competing interest.

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