Screening of Fabry disease in patients with chronic kidney disease in Japan

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ABSTRACT

Background. Fabry disease (FD), an X-linked lysosomal storage disorder caused by a deficiency in alpha-galactosidase A (α-Gal A) activity due to mutations in the GLA gene, has a prevalence of 0–1.69% in patients undergoing haemodialysis; however, its prevalence in patients with chronic kidney disease (CKD) Stages 1–5 is unknown.

Methods. Serum α-Gal A activity analysis and direct sequencing of GLA were used to screen for FD in 2122 male patients with CKD, including 1703 patients with CKD Stage 5D and 419 with CKD Stages 1–5. The correlation between serum α-Gal A activity and confounding factors in patients with CKD Stages 1–5 was evaluated.

Results. FD prevalence rates in patients with CKD Stage 5D and CKD Stages 1–5 were 0.06% (1/1703) and 0.48% (2/419), respectively. A patient with CKD Stage 5D exhibited a novel GLA mutation, p.Met208Arg, whereas two patients with CKD Stages 1–5 had c.370delG and p.Met296Ile. p. Met208Arg caused moderate structural changes in the molecular surface region near the substituted amino acid residue but did not affect the catalytic residues Asp170 and Asp231 in α-Gal A. Serum α-Gal A activity in patients with CKD Stages 1–5 was inversely correlated with age (P < 0.0001) but directly correlated with estimated glomerular filtration rate (P < 0.0001).

Conclusions. FD prevalence was much higher in male patients with CKD Stages 1–5 than in those with CKD Stage 5D. FD screening in patients with CKD Stages 1–5 may improve patient survival, decreasing the number of patients with CKD Stage 5D.

Keywords: Fabry disease, screening, chronic kidney disease, haemodialysis, nephropathy

INTRODUCTION

Fabry disease (FD) is an X-linked lysosomal storage disorder that leads to the accumulation of globotriaosylceramide (Gb3) in cells with decreased alpha-galactosidase A (α-Gal A) activity [1]. Poor clearance of Gb3 stored in the kidney and heart contributes to tissue damage, leading to conditions such as end-stage kidney disease (ESKD) and heart failure [2, 3]. Based on the presence or absence of characteristic symptoms, age of onset, sex and gene mutations, FD phenotypes are classified into three main categories: classic FD males, late-onset FD males and FD females.

Screenings for FD revealed that the FD prevalence in male patients undergoing haemodialysis (HD) is 0–1.69% [4, 5]. To date, although several studies on FD screenings have included female patients, α-Gal A activity in heterozygous FD is sometimes within the normal range. Notably, Sakuraba et al. [6] reported that the average values of serum and leukocyte α-Gal A activity in female patients with FD are approximately half of the control mean. Therefore female patients with FD cannot be clearly identified through screening based on the measurement of α-Gal A activity. Thus only male patients were included in this study of FD screening to avoid obtaining high false-negative results. Furthermore, although some screenings for FD in patients with chronic kidney disease (CKD) Stages 1–5 have been conducted [7, 8], FD prevalence in Japan is not yet known.

In this study, male patients with CKD Stage 5D and those with CKD Stages 1–5 were screened for FD by measuring serum α-Gal A activity followed by GLA gene analysis. Leukocyte α-Gal A assay and measurement of plasma globotriaosylsphingosine (lyso-Gb3) were performed to determine the diagnosis. We also compared the FD prevalence in these patients and...
evaluated the correlation between serum α-Gal A activity and confounding factors in patients with CKD Stages 1–5.

MATERIALS AND METHODS

Patients and data collection

The workflow of this study is summarized in Figure 1. From June 2014 to August 2018, a total of 2122 male patients with CKD Stages 1–5 were recruited, including 1703 patients undergoing HD or peritoneal dialysis in 43 dialysis hospitals and clinics and 419 patients with CKD Stages 1–5 in Kurume University Hospital (Figure 1). Patients with CKD Stage 5D were dialysed for 4–5 h with high-flux dialysers three times a week. Fasting blood was drawn from the antecubital vein of patients with CKD Stages 1–5 to determine blood urea nitrogen (BUN) and serum creatinine (SCr) levels. Spot urine test was performed to measure the level of proteinuria (g/g Cr). Additionally, estimated glomerular filtration rate (eGFR) was calculated using the following formula: 

\[
eGFR = 0.741 \times 175 \times SCr^{-1.154} \times \text{age}^{-0.203} \times (0.742 \text{ if female}) \quad [9, 10].
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To avoid a high rate of false-negative results, female patients were excluded from this screening. CKD was defined according to the criteria stated in the Kidney Disease: Improving Global Outcomes (2012) Clinical Practice Guidelines. Written informed consent was obtained from all patients and the study protocol was approved by the Institutional Ethics Committee of Kurume University School of Medicine, Japan (no. 13296).
This work was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013, and was registered with the University Hospital Medical Information Network clinical trials database (UMIN000040203).

Measurement of α-Gal A activity in serum and leukocytes

α-Gal A activity in serum and leukocytes was measured as described previously [6, 11]. In brief, serum samples obtained at the start of dialysis were centrifuged at 3000 rpm for 10 min, then kept at 4°C for 24 h. The serum samples were stored at −30°C until α-Gal A activity measurement. α-Gal A activity was measured using a substrate solution containing 5 mmol/L 4-methylumbelliferyl α-D-galactopyranoside (Calbiochem, La Jolla, CA, USA) as a substrate and 117 mmol/L N-acetyl-D-galactosamine (Sigma-Aldrich, St. Louis, MO, USA) as a specific inhibitor of α-N-acetylgalactosaminidase in 0.1 mol/L citrate–phosphate buffer, pH 4.6 [12]. In the first screening, 20 μL of serum was mixed with 40 μL of the substrate solution in a 96-well plate. The mixture was then incubated at 37°C for 4 h, after which the reaction was stopped by adding 200 μL of 0.2 mol/L glycine buffer, pH 10.7; the released 4-methylumbelliferone was measured using a Wallac 1420 ARVO MX Multilabel Counter (PerkinElmer, Waltham, MA, USA) at excitation and emission wavelengths of 355 and 460 nm, respectively. When the serum α-Gal A activity was <1.5 nmol/h/mL, the leukocyte α-Gal A activity was measured. In the second examination, 10 μL of leukocyte homogenate (10 μg protein) was mixed with 40 μL of the substrate solution in a 1.5-mL microtube and the mixture was incubated at 37°C for 30 min. Then the reaction was stopped by adding 950 μL of 0.2 mol/L glycine buffer, pH 10.7, and the released 4-methylumbelliferone was measured using a spectrofluorometer (F2700; Hitachi, Tokyo, Japan) at excitation and emission wavelengths of 365 and 450 nm, respectively.

GLA gene analysis

GLA gene analysis was performed for subjects that exhibited serum α-Gal A activity <1.5 nmol/h/mL (mean 1.75 SD) [6]. In brief, genomic DNA was purified from leukocytes using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer’s instructions and all seven exons, intron/exon boundaries and specific intronic regions containing IVS4 + 919 of the GLA gene were amplified by polymerase chain reaction (PCR) using appropriate primers [6, 13]. Then the PCR fragments were directly sequenced. The Fabry database (http://fabry-database.org/) and the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/) were assessed for variant filtering and classification.

Measurement of lyso-Gb3 concentration in plasma

Lyso-Gb3 concentration in plasma was measured using liquid chromatography–tandem mass spectrometry as described previously [14]. Briefly, an LCMS-8040 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization interface was used in the positive ion mode and the multiple reaction monitoring (MRM) conditions were optimized with an automatic MRM optimization function. In the MRM mode, the following transitions were monitored using a stable isotope labelled lyso-Gb3 as an internal standard: m/z 786.8→282.3 for lyso-Gb3 and m/z 790.8→286.2 for the labelled lyso-Gb3. Serum and leukocyte α-Gal A assays, plasma lyso-Gb3 measurement and GLA gene analysis were performed in the research laboratory at the Department of Clinical Genetics, Meiji Pharmaceutical University, Tokyo, Japan.

Statistical analysis

To compare the α-Gal A activity between patients with CKD Stage 5D and CKD Stages 1–5, a Wilcoxon rank test was performed, as the α-Gal A activity was not normally distributed. To determine the correlation between α-Gal A activity and age, BUN, Cr, eGFR or proteinuria, regression analysis was conducted. All statistical analyses were performed using JMP Pro version 14 software (SAS Institute, Cary, NC, USA). Data are presented as mean ± SD. P-values <0.05 were considered statistically significant.

RESULTS

Serum α-Gal A activity

Male patients with CKD Stage 5D and CKD Stages 1–5 were distributed according to their serum α-Gal A activity (Figure 2). Most patients with CKD Stage 5D and CKD Stages 1–5 had serum α-Gal A activities of 4–<4.5 (Figure 2A) and 3.5–<4.0 (Figure 2B) nmol/h/mL, respectively. However, 10 patients with CKD Stages 5D and 4 with CKD Stages 1–5 showed serum α-Gal A activity <1.5 nmol/h/mL (Figure 3).

Screening of FD in HD patients

We evaluated serum α-Gal A activity in 1703 male patients with CKD Stage 5D. The mean serum α-Gal A activity was 4.5 ± 1.3 nmol/h/mL and 10 patients undergoing dialysis showed low serum α-Gal A activity (Table 1). These patients had ESKD due to chronic glomerulonephritis (n = 3), diabetic nephropathy (n = 4) or unknown aetiologies (n = 3). Among them, two patients refused further examination and eight patients were tested for leukocyte α-Gal A activity, plasma lyso-Gb3 and gene mutations. Patient 1 showed very low serum and leukocyte α-Gal A activities (0.2 nmol/h/mL and 0.2 nmol/h/mg protein, respectively; normal range 1.6–14.6 nmol/h/mL and 20–62 nmol/h/mg protein, respectively) and elevated plasma lyso-Gb3 level (54 nmol/L; normal range 0.14–0.75 nmol/L) (Table 1).

Furthermore, seven patients had the p.Glu66Gln allele, which is known as a functional GLA variant [15, 16] (Table 1). The FD prevalence in patients with CKD Stage 5D was 0.06%, which corroborated the previous report [11]. In a 49-year-old patient undergoing HD (Patient 1), a novel GLA missense mutation, p. Met208Arg (ATG→AGG), was detected; when this patient was 26 years old, proteinuria was detected; at 39 years of age the kidney function of the patient decreased (BUN 55 mg/dL, SCr 6.4 mg/dL); and at 44 years of age the patient started undergoing dialysis. However, typical FD findings, such as angiokeratoma, acroparesthesia, hypohidrosis and corneal opacities, were absent in this patient. These findings were...
compatible with the late-onset type of FD. Furthermore, the mother of the patient had been admitted to a psychiatric hospital due to bipolar disorder and was diagnosed with FD based on genetic analysis. Enzyme replacement therapy (ERT) (agalsidase-β 1 mg/kg, 50 mg every 2 weeks) was initiated intravenously during HD sessions. Moreover, the older brother of the patient was diagnosed with proteinuria and left ventricular hypertrophy (LVH). His SCr level was within the normal range (0.97 mg/dL), proteinuria level was slightly high (1.47 g/gCr) and mulberry bodies and cells were noted. Additionally, leukocyte α-Gal A activity was <0.1 nmol/h/mg protein and plasma lyso-Gb3 was 32 nmol/L. Kidney biopsy demonstrated vacuolization and foamy changes in podocytes, while electron microscopy revealed abundant lamellar bodies in the podocyte cytoplasm.

Predicted p.Met208Arg-induced structural change in α-Gal A

To predict p.Met208Arg-induced structural changes in α-Gal A, we performed in silico structural analysis, as described previously [17]. Briefly, a structural model of the mutant α-Gal A protein was generated by homology modelling using the molecular modelling software TINKER (http://dasher.wustl.edu/tinker/). The crystal human α-Gal A structure (PDB: 1R46) [18] was used as a template and energy minimization was performed. The root mean square gradient was set at 0.05 kcal/mol Å. To determine the effect of the mutation, the mutant α-Gal A model was superimposed on the wild-type α-Gal A structure based on the Ca atoms using the least square mean fitting algorithm. An atom was considered as affected by the mutation when the position of the atom in a mutant differed from that in the wild-type structure by >0.15 Å, based on the total root mean square distance.

Figure 4 shows the affected atoms in the 3D α-Gal A structure. The mutation is located on the surface of the (β/α)8-barrel domain in human α-Gal A. Moreover, p. Met208Arg caused moderate structural change on the molecular surface region near the substituted amino acid residue but did not affect the catalytic residues Asp170 and Asp231 in α-Gal A.

Screening of FD in patients with CKD Stages 1–5

We screened for FD in 419 male patients with CKD Stages 1–5. The characteristics of the patients were as follows: mean age 60.9 ± 16.1 years, mean BUN 40.3 ± 32.9 mg/dL, SCr 3.5 ± 3.0 mg/dL and mean eGFR 35.2 ± 30.2 mL/min/1.73 m². Additionally, the mean proteinuria level of the patients was 4.2 ± 4.3 g/g Cr and the serum α-Gal A activity was 4.3 ± 1.6 nmol/h/mL. In total, four patients showed serum
a-Gal A activity <1.5 nmol/h/mL; gene analysis of these patients revealed that Patient 2 had a c.370delG mutation (a classic FD variant), Patient 3 had a p.Glu66Gln mutation (a functional variant) and Patient 4 had a p.Met296Ile mutation (a late-onset variant) (Table 2). Patient 1 was excluded from gene analysis because he did not present with any typical clinical manifestation of FD, including high leukocyte a-Gal A activity.

Patient 2 exhibited typical symptoms of FD and was administered agalsidase-a at a dose of 0.2 mg/kg. However, his kidney function gradually decreased. Patient 3 did not show any typical symptoms of FD. Patient 4 exhibited nephrotic syndrome, with completely diminished proteinuria due to prednisolone administration, as recently reported [19]. FD prevalence in male patients with CKD Stages 1–5 [0.48% (2/419)] was much higher than in those with CKD Stage 5D (4.3 ± 1.5 and 4.5 ± 1.2 nmol/h/mL, respectively; P = 0.004). Furthermore, the serum a-Gal A activity in the patients with CKD Stages 1–5, except for the four aforementioned patients, was inversely correlated with age ($R^2 = 0.082$, $P < 0.0001$), BUN ($R^2 = 0.128$, $P < 0.0001$) and SCR ($R^2 = 0.129$, $P < 0.0001$), but directly correlated with eGFR ($R^2 = 0.210$, $P < 0.0001$) (Figure 5A–D). Conversely, serum a-Gal A activity and proteinuria levels were not significantly correlated (Figure 5E).

**DISCUSSION**

This is the first report comparing FD prevalence between male patients with CKD Stage 5D and those with CKD Stages 1–5. FD prevalence was much higher in male patients with CKD Stages 1–5 [0.48% (2/419)] than in those with CKD Stage 5D [0.06% (1/1703)]. In addition, we identified a novel mutation of

### Table 1. Serum and leukocyte a-Gal A activity, plasma lyso-Gb3 concentration and GLA gene variants in 10 patients with CKD Stage 5D

| Patient no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|-------------|---|---|---|---|---|---|---|---|---|----|
| Serum a-Gal A (nmol/h/mL) | 0.2 | 1.0 | 1.1 | 0.8 | 1.4 | 0.9 | 1.4 | 1.3 | 1.1 | 1.2 |
| Leukocyte a-Gal A (nmol/h/mg protein) | 0.2 | 25 | NA | 18 | NA | 19 | 20 | 21 | 12 | 12 |
| Plasma lyso-Gb3 (nmol/L) | 54 | 0.27 | NA | 0.13 | NA | 0.46 | 0.34 | 0.14 | 0.54 | 0.34 |
| GLA variant | p.Met208Arg | p.Glu66Gln | p.Glu66Gln | NA | p.Glu66Gln | p.Glu66Gln | p.Glu66Gln | p.Glu66Gln | p.Glu66Gln | p.Glu66Gln |
| ACMG classification | PS3, PM2, PM5, PP2, PP4 | BS2, BS3 | BS2, BS3 | NA | BS2, BS3 | BS2, BS3 | BS2, BS3 | BS2, BS3 | BS2, BS3 |

NA, not applicable.

### Table 2. Serum and leukocyte a-Gal A activity, plasma lyso-Gb3 concentration and GLA gene variants in four patients with CKD Stages 1–5

| Patient no. | 1 | 2 | 3 | 4 |
|-------------|---|---|---|---|
| Age (years) | 61 | 40 | 63 | 67 |
| Serum a-Gal A (nmol/h/mL) | 0.9 | <0.1 | 1.1 | 0.2 |
| Leukocyte a-Gal A (nmol/h/mg protein) | 54 | <0.1 | 7 | 1.0 |
| Plasma lyso-Gb3 (nmol/L) | NA | 262 | NA | 7.4 |
| GLA variant | NA | c.370delG | p.Glu66Gln | p.Met296Ile |
| ACMG classification | – | PV51, PS3, PM2 | BS2, BS3 | PS1, PS3, PM2, PP1 |

NA, not applicable.
The clinical findings of the patient are consistent with those of late-onset variants. Although several screenings for FD have been conducted among male patients undergoing HD, there is a notable difference in the FD prevalence (Table 3). The FD prevalence observed in this study was consistent with that reported by Maruyama et al. [35]. However, functional GLA variants are sometimes included in the analysis of FD prevalence rate [25, 29]. Here we detected p.Glu66Gln in seven patients with CKD Stage 5D whose α-Gal A activity was <1.5 nmol/h/mL. According to the American College of Medical Genetics (ACMG) classification, p.Met208Arg and p.Glu66Glu are considered to be pathogenic and benign variants, respectively. FD is known as a congenital metabolic disease, and the diagnosis of this disease should involve both analysis of genetic variants and detection of biochemical and symptomatic abnormalities. Since a patient with the p.Met208Arg variant showed an extreme decrease in α-Gal A activity, with massive accumulation of Gb3 and lyso-Gb3 in the kidney and plasma, this variant is pathogenic. Lee et al. [15] reported that considerable residual α-Gal A activity was shown both in the leukocytes of p.Glu66Gln patients (19.0–30.3% of normal activity) and in transiently overexpressed COS-7 cells (43.8 ± 3.03% of normal activity). Furthermore, as the allele frequency of p.Glu66Gln determined in 833 unrelated Korean individuals was remarkably high, at 1.046%, they concluded that p.Glu66Gln is a functional polymorphism rather than a pathogenic mutation. Later, Sueoka et al. [43] confirmed that the plasma lyso-Gb3 level, a biomarker of FD, in patients with p.Glu66Gln was apparently lower than in FD patients and that there was no difference between the p.Glu66Gln patients and the healthy subjects. In this study, none of the patients with the p.Glu66Gln allele showed any typical clinical manifestations of FD. Doi et al. [11] eliminated 8 of 10 patients with low α-Gal A activity and the p.Glu66Gln gene variant from their prevalence analysis. Therefore the handling of functional or partially functional alleles such as p.Glu66Gln requires attention and if the α-Gal A activity is low, gene mutation analysis is strongly recommended to identify the cause of pathological FD.
| No. | Authors | Country | Date of publication | No. of total male patients | Screening method | Red: Classic (No. of patients) | Blue: Late-onset (No. of patients) | Green: Unknown (No. of patients) | Black: Functional variants (No. of patients) | Prevalence of FD without functional variants (%) |
|-----|----------|---------|---------------------|---------------------------|-----------------|-------------------------------|---------------------------------|---------------------------------|-----------------------------------------------|---------------------------------------------|
| 1   | Utsumi K et al. [20] | Japan | 2000 | 440 | Plasma | p.Gln357Xaa (1) | N/A (1) | N/A (1) | N/A (1) | 2/440 (0.45) |
| 2   | Nakao S et al. [21] | Japan | 2003 | 514 | Plasma | p.Gly373Asp (1) | p.Met296Ile (3) | p.Ala97Val (1) | p.Glu66Gln (1) | 5/514 (0.97) |
| 3   | Linthorst GE et al. [22] | Netherlands | 2003 | 508 | Whole blood | p.Ala121Pro (1) | p.Trp162Arg (1) | p.Ile239Thr (1) | p.Arg112His (1) | 1/508 (0.20) |
| 4   | Kotanko P et al. [23] | Austria | 2004 | 1,516 | DBS test | p.Ala121Pro (1) | p.Trp162Arg (1) | p.Ile239Thr (1) | p.Arg112His (1) | 4/1,516 (0.26) |
| 5   | Ichinose M et al. [24] | Japan | 2005 | 450 | Plasma | c.761_763delTTG (1) | N/A (1) | N/A (1) | N/A (1) | 1/450 (0.22) |
| 6   | Tanaka M et al. [25] | Japan | 2005 | 401 | Plasma | p.Tyr365Xaa (2) | 288AAT-AT(DelA) (1) * | p.Ala97Val (1) | p.Glu66Gln (1) | 4/401 (1.00) |
| 7   | Bekri S et al. [5] | France | 2005 | 59 | Blood leukocytes | p.Asn215Ser (1) | N/A (1) | N/A (1) | N/A (1) | 1/59 (1.69) |
| 8   | Merta M et al. [26] | Czech Republic | 2007 | 1,521 | DBS test | p.Gly360Ser (1) | p.Ile317Thr (1) | p.Arg112His (1) | p.Ala143Thr (1) | 3/1,521 (0.20) |
| 9   | Porsch DB et al. [27] | Brazil | 2008 | 558 | DBS test | p.Ala143Thr (1) | N/A (1) | N/A (1) | N/A (1) | 2/558 (0.36) |
| 10  | Terryn W et al. [28] | Belgium | 2008 | 180 | DBS test | p.Try356Xaa (2) | 288AAT-AT(DelA) (1) * | N/A (1) | N/A (1) | 1/180 (0.56) |
| 11  | Andrade J et al. [4] | Canada | 2008 | 499 | Plasma | p.Leu275Phe (1) | N/A (1) | N/A (1) | N/A (1) | 0/499 (0) |
| 12  | Fujii H et al. [29] | Japan | 2009 | 635 | DBS test | p.Glu66Gln (1) | c.1037delG (1) | p.Asp313Thr (1) | p.Ala143Thr (1) | 3/635 (0) |
| 13  | Gaspar P et al. [30] | Spain | 2010 | 543 | DBS test | p.Glu66Gln (1) | c.1037delG (1) | p.Asp313Thr (1) | p.Arg112His (1) | 1/543 (0.19) |
| 14  | Wallin EF et al. [31] | UK | 2011 | 155 | DBS test | N/A (0) | N/A (0) | N/A (0) | N/A (0) | 0/155 (0) |
| 15  | Nishino T et al. [32] | Japan | 2012 | 557 | DBS test | p.Glu66Gln (1) | c.335+1(1)* | p.Ala143Tyr (1) | p.Try236Arg (1) | 0/557 (0) |
| 16  | Kalkan Uc et al. [33] | Turkey | 2012 | 808 | Plasma | p.Glu66Gln (1) | c.335+1(1)* | p.Ala143Tyr (1) | p.Try236Arg (1) | 1/808 (0.12) |
| 17  | Okur I et al. [34] | Turkey | 2013 | 615 | DBS test | p.Glu66Gln (1) | c.1037delG (1) | p.Asp313Thr (1) | p.Arg112His (1) | 2/615 (0.33) |
| 18  | Doi K et al. [11] | Japan | 2013 | 1,080 | Plasma | p.Glu66Gln (1) | c.1037delG (1) | p.Asp313Thr (1) | p.Arg112His (1) | 2/1,080 (0.19) |
| 19  | Maruyama H et al. [35] | Japan | 2013 | 1,453 | Plasma | p.Glu66Gln (1) | c.1037delG (1) | p.Asp313Thr (1) | p.Arg112His (1) | 1/1,453 (0.07) |
| 20  | Herrera J et al. [36] | Spain | 2014 | 2,239 | DBS test | p.Glu66Gln (1) | c.1037delG (1) | p.Asp313Thr (1) | p.Arg112His (1) | 2/2,239 (0.09) |
| 21  | Sayilar EI et al. [37] | Turkey | 2016 | 847 | DBS test | p.Glu66Gln (1) | c.1037delG (1) | p.Asp313Thr (1) | p.Arg112His (1) | 4/847 (0.47) |
| 22  | Saito O et al. [38] | Japan | 2016 | 5,408 | Plasma | p.Glu66Gln (1) | c.1037delG (1) | p.Asp313Thr (1) | p.Arg112His (1) | 2/5,408 (0.04) |
| 23  | Silva CA et al. [39] | Salvador | 2016 | 2,583 | DBS test | p.Glu66Gln (1) | c.1037delG (1) | p.Arg112His (1) | p.Arg112His (1) | 3/2,583 (0.12) |
| 24  | Veloso VSP et al. [40] | Brazil | 2018 | 108 | DBS test | p.Glu66Gln (1) | c.1037delG (1) | p.Arg112His (1) | p.Arg112His (1) | 1/108 (0.93) |
| 25  | Moiseev S et al. [41] | Russian Federation | 2019 | 3,551 | DBS test | p.Glu66Gln (1) | c.1037delG (1) | p.Arg112His (1) | p.Arg112His (1) | 19/3,551 (0.54) |

Continued
variant [44, 45]. Notably, the patient harbouring the GLA gene mutation had kidney dysfunction as well as LVH, but showed no subjective symptoms of typical FD, suggesting that it might be a late-onset variant. Interestingly, his older brother was also diagnosed with FD, although his kidney function was within the normal range despite harbouring the same mutation. Related adult males with FD can have different kidney phenotypes despite the same classic genotype [46], owing to different epigenetic and environmental factors and/or the coexistence of other kidney disease. Thus further genetic and/or epigenetic research is warranted. Moreover, two patients could not be examined for gene mutation; however, they were speculated to possess the p.Glu66Gln variant because they have not-so-low α-Gal A activity levels and low plasma lyso-Gb3 levels.

Table 3. Continued

| No. | Authors               | Country | Date of publication | No. of total male patients | Screening method of α-Gal A activity | Gene mutations (No. of patients) | Prevalence of FD without functional variants (%) |
|-----|-----------------------|---------|---------------------|---------------------------|--------------------------------------|---------------------------------|-----------------------------------------------|
| 26  | Nagata A et al. [42]  | Japan   | 2020               | 1,703                     | Serum                                | p.Arg227Xaa (1) p.Cys56Ser (1) p.Gly183Ser (1) p.Gly328Arg (1) p.Tyr134Asp (1) p.Tryp304Cys (1) p.Asp170Tyr (1) p.Glu7Xaa (1) p.Glu273Ser (1) p.Leu54Pro (3) p.Leu668Pro (1) p.Asn215Ser (1) p.Ala37Ser (1) p.Met208Arg (1) | N/A (2) p.Glu66Gln (7) | 1/1,703 (0.06) |
| Total |                      |         |                     |                           |                                      |                                 | 63/28,931 (0.22) |

*These descriptions were in line with the originally published data. However, in these GLA gene variants: 1) the 288AAT-AT (DelA) in No. 6 does not match the GLA cDNA sequence, and 2) the c.335>T in No. 16 might be wrongly described.

DBS, dried blood spot; NA, not applicable.

Table 4. Summary of the recent screening for FD in male patients with CKD Stages 1-5

| No. | Authors               | Country | Date of publication | No. of total male patients | Screening method of α-Gal A activity | Gene mutations (No. of patients) | Prevalence of FD without functional variants (%) |
|-----|-----------------------|---------|---------------------|---------------------------|--------------------------------------|---------------------------------|-----------------------------------------------|
| 1   | Turkmen K et al. [7]  | Turkey  | 2016               | 167                       | Plasma                               | p.Asn34His (1) c.1072_1074delGAG (1) | 3/167 (1.80) |
| 2   | Lin CJ et al. [8]     | Taiwan  | 2018               | 1,012                     | DBS test                             | p.Thr410Ala (1) p.Gly389Glu (1) c.639>G>A (3) p.Pro210Ser (1) | 6/1,012 (0.59) |
| 3   | Nagata A et al. [42]  | Japan   | 2020               | 419                       | Serum                                | c.370delG (1) p.Met296Ile (1) p.Glu66Gln (1) N/A (1) | 2/419 (0.48) |
| Total |                      |         |                     | 1,601                      |                                      | No. of patients as FD: total 13 Classic type (%): 5 (38.5) Late-onset type (%): 6 (46.2) Functional variants (%): 2 (15.3) | 11/1,598 (0.06) |

NA, not applicable.
In patients with CKD Stages 1–5, the FD prevalence was 0.48% (2/419), including one classic and one late-onset phenotype, corroborating a previous report from Taiwan [0.59% (6/1012)] that included two classic and four late-onset phenotypes from the high-risk CKD population [8]. However, that study excluded patients with diabetic kidney disease (DKD) and autosomal dominant polycystic kidney disease (ADPKD). FD may coexist with other kidney diseases such as ADPKD [47], DKD [38], amyloidosis [48] and minimal change nephrotic syndrome [19], hence we included all CKD patients regardless of the kidney disease.

To date, the FD prevalence rates in patients with CKD Stage 5D and CKD Stages 1–5 have not been compared. In this study, the FD prevalence in patients with CKD Stage 5D was much lower than in patients with CKD Stages 1–5. The risk of mortality in FD patients with CKD Stage 5D may be much higher than in those with CKD Stages 1–5. Indeed, the European Renal Association–European Dialysis and Transplant Association registry demonstrated that the survival rate of FD patients undergoing dialysis was 41% at 5 years [49], whereas there was a 10% risk of mortality by 50 years of age in non-dialysis FD patients [50]. Therefore the number of FD patients with CKD Stage 5D could be reduced due to death, which is one of the explanations. Another explanation is that the progression of kidney dysfunction in FD may be much slower than in other kidney diseases. In fact, Schiffmann et al. [50] reported that the mean rate of eGFR decline in male FD patients is 2.93 ml/min/1.73 m²/year [50]; however, it is relatively faster in patients with other types of CKD, including DKD [51], leading to increased prevalence of FD in patients with CKD Stages 1–5. ERT has been shown to improve patients’ quality of life and possibly ameliorate the progression of typical Fabry cardiomyopathy, even in patients undergoing HD [52]. Therefore, although there is lack of clear evidence regarding the effectiveness of ERT in ameliorating kidney disease progression, early screening for FD is recommended in patients with CKD Stages 1–5 to improve patient survival, thereby decreasing the number of FD patients with CKD Stage 5D.

Serum or plasma α-Gal A activity in patients undergoing HD can be higher [21], unchanged [25, 30] or lower [33] than in healthy subjects. In this study, the serum α-Gal A activity in patients with CKD Stages 1–5 was slightly lower than in those with CKD Stage 5D. Moreover, serum α-Gal A activity may fluctuate because of differences in confounding factors such as age, sex and kidney function. In patients with CKD Stages 1–5, serum α-Gal A activity might be influenced by ageing and progression of kidney dysfunction. Since the measurement of leukocyte α-Gal A activity may be more accurate than that of serum α-Gal A activity, future studies should measure leukocyte α-Gal A activity to clarify the precise correlation.

Up to date, 25 screenings for FD in patients with CKD Stages 5D and 2 screenings for FD in patients with CKD Stages 1–5 have been reported (Tables 3 and 4). The phenotypes of the FD patients were determined according to their manifestations and family history as follows: classic, late onset, functional variants and unknown. In Japan, the prevalence of p.Glu66Gln is higher than in other countries. Here we present for the first time the FD prevalence in males, excluding possible functional polymorphisms. While the global FD prevalence in male patients with CKD Stage 5D may be 0.22% (63/28 931) (Table 3), it may be only 0.14% (18/12 641) in Japan. In Turkey, Taiwan and Japan, the total FD prevalence rate in patients with CKD Stages 1–5 could be 0.69% (11/1601) (Table 4) [7, 8]. In Japan, total FD prevalence in both types of CKD patients was lower than in other countries, which may be ascribed to the different racial and genetic backgrounds.

Nevertheless, there are several limitations to this study. First, the patient sample size was small and the target population for the CKD Stages 1–5 study was much smaller than the CKD Stage 5D population, consequently the FD prevalence may not be significant. Second, we recruited patients only from the south coast of Japan. Third, we did not examine female patients with CKD. Fourth, we could not evaluate the differences in confounding factors between male patients with CKD Stages 1–5 and CKD Stage 5D because the data could not be obtained from patients with CKD Stage 5D. Therefore a large and nationwide clinical screening for FD in both male and female CKD patients is required to confirm the accurate FD prevalence.

In conclusion, the FD prevalence in male patients with CKD Stages 1–5 was much higher than in those with CKD Stage 5D. Additionally, we identified a novel missense mutation, p. Met208Arg, in GLA that corresponds to the late-onset FD phenotype. Although symptoms and extra-kidney complications are important for early diagnosis of FD, it is sometimes misdiagnosed. Indeed, three FD patients were not diagnosed with FD before the screening in this study. Therefore early screening for FD is recommended in patients with CKD Stages 1–5 to improve patient survival, which may decrease the number of FD patients at CKD Stage 5D.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the following investigators: Yoshifumi Wada, Wada Naika Cardiovascular Clinic, Ryotaro Ando, Sugi Cardiovascular Hospital; Michiaki Usui, Usui Clinic; Michio Chiba, Chiba Naika Junkankika; Atsuo Moriyama, Moriyama Naika; Hiroshi Miyazaki, Miyazakinaika Medical Clinic; Motoko Tanaka, Akebono Clinic; Shuji Iida, Iida Clinic; Shunichi Imadachi, Imadachi Naika Clinic; Keisuke Kono, Imamura Hospital; Keiichiro Uemura, Uemura Urological Clinic; Kazuhiro Sonoda, Saiseikai Hita Hospital; Makio Nagano, Omuta City Hospital; Syunmon Kasuga, Kasuga Clinic, Masako Onzuka, Kano Clinic; Hideharu Tanaka, Kurume Ekimae Clinic; Atsuko Ohara, Yame General Hospital; Yoshiimi Takamiya, Yokokura Hospital; Shoji Sakai, Shimonoseki City Hospital; Tetsuya Tajiri, Jinseikai Clinic Otsu; Tetsuya Tajiri, Jinseikai Clinic Kurokami; Tetsuya Tajiri, Jinseikai Clinic Nagamine; Tetsuya Tajiri, Jinseikai Clinic Hikarimonori; Harumichi Higashi, St Mary’s Hospital; Yoshiyuki Tomiyoshi, Takagi Hospital; Sakuya Ito, Tanushimaru Central Hospital; Yosuyuki Toyama, Toyama Naika; Yoshiharu Hori, Chikugo Clinic; Akiko Nagata, Nagata Hospital; Yosuhiko Nakano, Nakano Naika Junkankika; Hirosaburo Naganuma, Naganuma Clinic; Yoshifumi
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Received: 26.6.2020; Editorial decision: 24.10.2020