Screening of Male Dialysis Patients for Fabry Disease by Plasma Globotriaosylsphingosine

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Summary

Background and objectives Previous reports of Fabry disease screening in dialysis patients indicate that α-galactosidase A activity alone cannot specifically and reliably identify appropriate candidates for genetic testing; a marker for secondary screening is required. Elevated plasma globotriaosylsphingosine is reported to be a hallmark of classic Fabry disease. The purpose of this study was to examine the usefulness of globotriaosylsphingosine as a secondary screening target for Fabry disease.

Design, setting, participants, & measurements This study screened 1453 patients, comprising 50% of the male dialysis patients in Niigata Prefecture between July 1, 2010 and July 31, 2011. Screening for Fabry disease was performed by measuring the plasma α-galactosidase A enzyme activity and the globotriaosylsphingosine concentration, by high-performance liquid chromatography. Genetic testing and genetic counseling were provided.

Results A low level of plasma α-galactosidase A activity (≤ 4.0 nmol/h per milliliter) was observed in 47 patients (3.2%). Of these, 3 (0.2%) had detectable globotriaosylsphingosine levels. These patients all had *α-galactosidase A* gene mutations: one was p.Y173X and two were the nonpathogenic p.E66Q. The patient with p.Y173X started enzyme replacement therapy. Subsequent screening of his family identified the same mutation in his elder sister and her children. Genetic testing for 33 of the other 44 patients detected 7 patients with p.E66Q. Thus, the plasma lyso-Gb3 screen identified Fabry disease with high sensitivity (100%) and specificity (94.3%).

Conclusions Plasma globotriaosylsphingosine is a promising secondary screening target that was effective for selecting candidates for genetic counseling and testing and for uncovering unrecognized Fabry disease cases.

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Introduction

Fabry disease (FD) is an X-linked lysosomal storage disorder resulting from a deficiency in the activity of α-galactosidase A (α-Gal A) (1), a lysosomal enzyme. This enzyme deficiency causes the systemic lysosomal accumulation of glycolipids, primarily globotriaosylceramide (Gb3), in the vascular endothelium and other tissues. Morbidity and mortality from FD, caused by renal failure, cardiac disease, and early onset stroke, increase with age, and screens of male patients on dialysis show an FD prevalence of 0.2%–1.7% (2–11). The advent of an effective treatment, enzyme replacement therapy (ERT), has increased the importance of identifying people with FD.

α-Gal A deficiency screens have used plasma (2,3,6,7) and dried blood spot (5,9–11) samples; low leukocyte α-Gal A activity is also used to identify candidates for further testing (3,5–7,9). Patients positive for the α-Gal A activity screen (*i.e.,* with low activity) are subsequently tested for mutations in the *α-Gal A* gene (*GLA*) (2–11). The genetic tests revealed that α-Gal A activity screening lacks specificity, in that some patients without *GLA* mutations test positive (5,8,10,11). In addition, many females who are heterozygous for pathogenic *GLA* mutations have normal plasma α-Gal A activity, so screening studies for FD have predominantly focused on male dialysis patients (3,4,6). A secondary screening method to identify FD patients is needed. Ideally, this method would avoid re-sampling or unnecessary genetic testing for FD, which can increase patients' psychologic distress.

Although Gb3, found in the plasma of control participants (12–15), is not a suitable target for secondary screening, globotriaosylsphingosine (lyso-Gb3) is dramatically increased in the plasma of male patients with classic FD (12–14), and might therefore be a good secondary screening target (12).

In this study, we screened the male dialysis population of Niigata Prefecture for FD by measuring the plasma α-Gal A activity and lyso-Gb3 concentration and verified the sensitivity and specificity of the lyso-Gb3 screen. The study design included methodologic strategies for reducing patients' distress.

Materials and Methods

Study Design

Lyso-Gb3 Screen. Male dialysis patients in Niigata Prefecture, Japan, of unknown FD status, were screened for FD between July 1, 2010 and July 31, 2011. All 51 dialysis centers in Niigata Prefecture were invited to participate. Dialysis patients were informed about the study specifics, and those who agreed to participate provided informed consent. The study design involved four steps: (1) Patients were screened for FD by measuring their plasma α-Gal A activity (activity $\leq$4 nmol/h per milliliter was considered a positive result); (2) patients with positive results for the α-Gal A activity screen were re-tested by measuring their plasma lyso-Gb3; (3) patients with elevated lyso-Gb3 were informed in the context of genetic counseling that they might have FD; and (4) with patient consent, the diagnosis of FD was confirmed by genetic testing of *GLA*, and familial diagnosis was performed.

Screen Verification. To determine whether the lyso-Gb3 screen missed patients who had FD, we performed genetic testing on the patients who tested positive in the α-Gal A activity screen, but negative in the lyso-Gb3 screen.

Plasma Samples

To minimize patient distress, we used plasma obtained from a single blood sample collection for both the primary and secondary screening. This procedure reduced the number of times that patients had to endure sampling, permitted the secondary screen to be performed without alarming the patients unnecessarily, and reduced the burden of labor on the institutional staff.

Determination of Plasma α-Gal A Activity

The α-Gal A activity was assayed with an artificial substrate, 4-methylumbelliferyl-α-D-galactoside, as described elsewhere (16). The low cutoff value for a positive plasma α-Gal A activity was set as the third percentile value (*i.e.*, 4 nmol/h per milliliter).

Measurement of Plasma Lyso-Gb3

Lyso-Gb3 was extracted from plasma as described (14). The *O*-phthaldialdehyde–derivatized lyso-Gb3 was separated by high-performance liquid chromatography (Jasco Co., Hachioji, Tokyo, Japan) with a Unison UK-C18 reversed-phase column (150×4.6 mm, 3 μm; Imtakt Co., Kyoto, Kyoto, Japan), and quantified by fluorescence detection at λ_{ex} 340 nm and λ_{em} 435 nm, at a 1.0 ml/min flow rate. The mobile phase was methanol/H_2O [88:12 (v/v)]. For quantification, authentic lyso-Gb3 (Sigma-Aldrich Co., St. Louis, MO) was added to normal plasma.

Genetic Testing

The human *GLA* consists of seven exons. Exons 1 and 7 were amplified from genomic DNA by PCR, and a fragment containing full-length exons 2–6 and all of the exon-intron boundaries was obtained by RT-PCR using total RNA. The DNA and RNA were extracted from white blood cells. Genetic testing was performed as described elsewhere (17).

Informed Consent and Ethics

The study protocol was approved by the Ethics Committee of the Niigata University School of Medicine, Oita University, and the collaborating dialysis centers, and informed consent was obtained from the patients. Separate approval and informed consent were obtained for the verification protocol. The study protocols were in accordance with the Declaration of Helsinki.

Genetic Counseling before and after Genetic Testing

Patients with detectable plasma lyso-Gb3 were considered candidates for genetic testing. The genetic counseling team consisted of two certified specialists in medical genetics, a nephrologist, two certified genetic counselors, two clinical psychologists, and a nurse, all from Niigata University. The principal issues were explained to the examinee, whose consent and comprehension were confirmed by the team before and after genetic testing. The genetic testing performed in this study met the recommendations of the Japanese Association of Medical Sciences (18).

Statistical Analyses

Values are shown as the median and interquartile range (25th to 75th percentiles) or the mean $\pm$ SD. A two-sided Fisher's exact test and chi-squared test were used to evaluate the categorical data. *P* values < 0.05 were considered significant.

## Results

Study Population

Of the 51 Niigata Prefecture dialysis centers, 31 (61%) participated in this study. Of the total population of male dialysis patients in Niigata Prefecture (*n*=2905), 1453 (50%), including 1428 on hemodialysis and 25 on peritoneal dialysis, were screened. Participation rate among eligible patients in the dialysis centers was 86.7% (1453 of 1676). Their underlying kidney diseases were chronic GN in 685 patients (47.1%), diabetic nephropathy in 488 patients (33.6%), nephrosclerosis in 142 patients (9.8%), polycystic kidney disease in 62 patients (4.3%), other disease in 63 patients (4.3%), and unknown in 13 patients (0.9%). Their mean age at the time of blood sampling was 66.1±12.0 years (range, 25.3–95.0 years), and was 57.7±15.5 years (range, 12.8–91.4 years) at the start of dialysis. The duration of dialysis was 8.5±8.4 years (range, 0–42.5 years).

### Plasma α-Gal A Activity in the Primary Screening

The median plasma α-Gal A activity of all patients was 7.0 nmol/h per milliliter (5.9–8.4 nmol/h per milliliter). In 47 patients, the activity was $\leq$4.0 nmol/h per milliliter, with a median of 3.4 nmol/h per milliliter (2.9–3.9 nmol/h per milliliter). The median plasma α-Gal A activity in the remaining 1406 patients was 7.1 nmol/h per milliliter (6.0–8.5 nmol/h per milliliter) (Figure 1).

Plasma Lyso-Gb3 Levels in the Secondary Screening

Plasma lyso-Gb3 was detected in 3 of the 47 patients with low α-Gal A activity (6.4%). Figure 2 shows representative high-performance liquid chromatography profiles of lyso-Gb3 in plasma samples from the patients in cases 1, 2, and 3, the elder sister of the patient in case 1, and a control participant. The median plasma lyso-Gb3 level in the three patients was 3.1 nM (1.9–128 nM). Their plasma α-Gal A activity was lower than that of the patients without
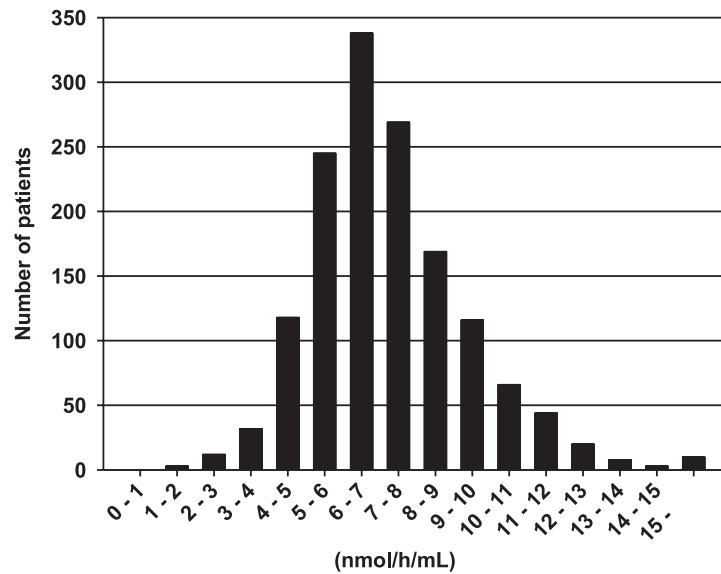
Figure 1. | **Distribution of plasma α-Gal A activities for all patients tested (n=1453) in increments of 1 nmol/h per milliliter, plotted against the number of patients with activity in each interval (observed range of activity 1.3–88.3 nmol/h per milliliter; median activity 7.0 nmol/h per milliliter [5.9–8.4 nmol/h per milliliter]).** Each interval in the x axis scale refers to the range beginning with the minimum value, but not including the minimum value and extending up to the maximum value. For example, 1–2 means the range beginning at 1, but not including 1 and containing the values up to 2. In 47 patients, the activity was ≤4.0 nmol/h per milliliter, the low cutoff for the test; the median activity in these 47 patients was 3.4 nmol/h per milliliter (2.9–3.9 nmol/h per milliliter). α-Gal A, α-galactosidase A.
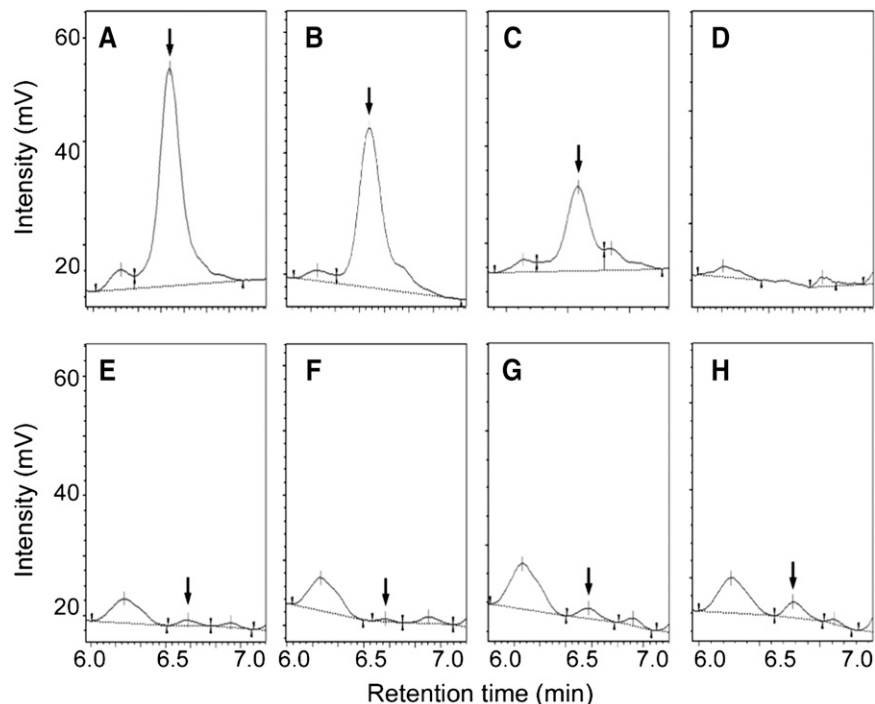
Figure 2. | **High-performance liquid chromatography profiles of OPA-derivatized lyso-Gb3 extracted from human plasma.** The peak identified as OPA-derivatized lyso-Gb3 is indicated by the arrow. (A) Case 1 (p.Y173X hemizygote, 128 nM lyso-Gb3); (B) 100 nM authentic lyso-Gb3 in normal plasma; (C) 50 nM authentic lyso-Gb3 in normal plasma; (D) normal plasma; (E) case 2 (p.E66Q hemizygote, 3.1 nM lyso-Gb3); (F) case 3 (p.E66Q hemizygote, 1.9 nM lyso-Gb3); (G) case 1's elder sister (p.Y173X heterozygote, 6.9 nM lyso-Gb3); and (H) 10 nM authentic lyso-Gb3 in normal plasma. OPA, O-phthaldialdehyde; lyso-Gb3, globotriaosylsphingosine.

detectable lyso-Gb3 (*n*=44), 2.2±0.8 nmol/h per milliliter (range, 1.3–2.9 nmol/h per milliliter) versus 3.4±0.6 nmol/h per milliliter (range, 1.9–4.0 nmol/h per milliliter), respectively ($P<0.02$).

Case Presentation

A 28-year-old man had suffered from hypohidrosis from early childhood. Acroparesthesia was not detected (Table 1). At age 27 years, he suffered from loss of appetite, head-ache, leg edema, and vertigo. One month later, severe kidney dysfunction, hypertension, anemia, and atrophy of the kidneys were detected at the first medical examination. The diagnosis was stage 5 CKD due to an unknown cause; he was unfamiliar with FD. He started dialysis immediately. One month later, he developed restless legs syndrome, which can be associated with FD (19,20) and insomnia. His plasma α-Gal A activity was 1.3 nmol/h per milliliter, and lyso-Gb3 was 128 nM. In his first genetic counseling session, he stated that he hoped the cause of his sickness could be determined.

The physical examination disclosed abdominal angio-keratoma and bilateral lower limb lympho-edema. Genetic testing showed a nonsense mutation in *GLA* (c.519C>A, p.Y173X) (21). The Mainz Severity Score Index (MSSI) score (22) was 32. Thus, his CKD was caused by FD. He started ERT within 5 months after the primary screening, and his plasma lyso-Gb3 level was 20 nM after 2 months of treatment. The patient's parents were divorced, and he had lived with his father. He was not in contact with his mother, who was suspected to be a heterozygote of the *GLA* mutation.

The patient's elder sister, who did not present any clinical signs of FD, requested genetic counseling and testing. Her plasma α-Gal A activity and lyso-Gb3 were 4.5 nmol/h per milliliter and 6.9 nM, respectively. She had the same *GLA* mutation as the patient in case 1, as a heterozygote. All of the sister's four children had the same *GLA* mutation, and the three youngest showed only mild heat intolerance as a sign of FD. The children's characteristics were as follows: 10-year-old female, 5.2 nmol/h per milliliter plasma α-Gal A activity, and no detectable lyso-Gb3; 8-year-old female, 1.3 nmol/h per milliliter plasma α-Gal A activity, and 8 nM lyso-Gb3; 4-year-old male, 0.1 nmol/h per milliliter plasma α-Gal A activity, and 83 nM lyso-Gb3; 2-year-old male, 0.0 nmol/h per milliliter plasma α-Gal A activity, and 140 nM lyso-Gb3.

Verification of Lyso-Gb3 Accuracy

Although this study aimed to avoid excess genetic testing, to determine whether the lyso-Gb3 screen captured all cases of FD in the study population, it was necessary to perform genetic testing on patients who had tested positive in the α-Gal A activity screen, but negative in the lyso-Gb3 screen. Forty-four of our original patients met this criterion.

The genetic testing was performed between February 1, 2012 and July 31, 2012. Of the original 44 patients, 9 had died, 1 could not provide informed consent due to dementia, and 1 did not consent to participate. Thus, we performed genetic testing for 33 of the original 44 patients. In 26 patients, there was no mutation in *GLA*. Seven had a missense mutation in *GLA* (c.196G>C, p.E66Q) (Table 2) that occurs at an unexpectedly high frequency in Japanese and Korean patients screened for FD. It is not a pathogenic mutation, but a functional polymorphism (23,24).

Including the 3 patients who were positive for lyso-Gb3, we performed genetic testing for a total of 36 male dialysis patients with low α-Gal A activity. Among them, we found one patient whose FD had not been diagnosed previously (case 1; see Table 3 for summary). Thus, the plasma lyso-Gb3 screen identified FD with high sensitivity (100%) and specificity (94.3%).

Discussion

This is the first report on the screening of dialysis patients for FD by measuring plasma lyso-Gb3. Our findings showed that of 1453 male dialysis patients, this method identified 1 new case of FD, and 2 cases with functional *GLA* mutation; thus, the prevalence of FD in male dialysis patients was 0.07%. The patient with FD had a nonsense mutation (p.Y173X), and the other two had a nonpathogenic missense mutation (p.E66Q). All patients with detectable plasma lyso-Gb3 had a *GLA* mutation. Finally, 33 patients with positive screening results for α-Gal A activity but negative results for lyso-Gb3 had a normal *GLA* gene or carried

Table 1. Characteristics of three patients with a positive result in the lyso-Gb3 screen

	Case 1	Case 2	Case 3
Age (yr)	28	49	74
Cause of CKD	Fabry disease	Focal glomerulosclerosis	Chronic GN
Age dialysis started (yr)	27	30	58
Plasma α-Gal A (nmol/h per milliliter)	1.3	2.9	2.3
Plasma lyso-Gb3 (nM)	128	3.1	1.9
GLA mutation			
Nucleotide change	c.519C>A	c.196G>C	c.196G>C
Amino acid change	p.Y173X	p.E66Q	p.E66Q
MSSI	32	30	30
ERT started	Yes	No	No

The cause of CKD in case 1 was considered unknown before study participation. *GLA*, α-galactosidase A gene; MSSI, Mainz Severity Score Index; ERT, enzyme replacement therapy.

Table 2.   Characteristics of the 7 patients with *GLA* mutation among 33 patients with positive results in the α-Gal A activity but negative findings in the lyso-Gb3 screen

Patient	1	2	3	4	5	6	7
Age (yr)	76	80	75	76	58	62	83
Cause of CKD	IgA GN	Chronic GN	Chronic GN	Bilateral ureteral cancer	DM	Chronic GN	NS
Age dialysis started (yr)	52	56	73	71	53	62	79
Plasma α-Gal A (nmol/h per milliliter)	2.9	3.4	3.8	2.4	3.8	1.9	2.0
GLA mutation							
Nucleotide change	c.196G>C	c.196G>C	c.196G>C	c.196G >C	c.196G>C	c.196G>C	c.196G>C
Amino acid change	p.E66Q	p.E66Q	p.E66Q	p.E66Q	p.E66Q	p.E66Q	p.E66Q
MSSI	18	36	24	24	22	32	26
ERT started	No	No	No	No	No	No	No

GLA, α-galactosidase A gene; α-Gal A, α-galactosidase A; lyso-Gb3, globotriaosylsphingosine; IgA GN, IgA glomerulonephropathy; DM, diabetes mellitus; NS, nephrosclerosis; MSSI, Mainz Severity Score Index; ERT, enzyme replacement therapy.

Table 3.   Sensitivity and specificity of plasma lyso-Gb3 screening for FD

	FD (+)	FD (−)	Total
Lyso-Gb3 (+)	1	2	3
Lyso-Gb3 (−)	0	33	33
Total	1	35	36
Sensitivity (%)	100		
Specificity (%)		94.3	

In total, we performed *α-galactosidase A* gene analysis for 36 male dialysis patients with low α-galactosidase A activity. We found one FD (+) patient with class 1 mutation (p.Yl73X) and 35 FD (−) patients consisting of 9 FD (−) patients with class 2 mutation (p.E66Q) and 26 FD (−) patients without a *GLA* mutation. The sensitivity was calculated by dividing the number of lyso-Gb3 (+) patients by the total number of total FD (+) patients. The specificity was calculated by dividing the number of lyso-Gb3 (−) patients by the number of total FD (−) patients. Lyso-Gb3, globotriaosylsphingosine; FD, Fabry disease; *GLA*, *α-galactosidase A* gene.

the functional polymorphism p.E66Q. FD was identified exclusively in a patient with detectable plasma lyso-Gb3.

Published reports on male dialysis patients screened for FD (summarized in Table 4) show that when 0.5% or fewer of the patients were given genetic testing, 100% were positive for FD (2,4,6,7,9). In those in which ≥4% of the patients were tested, 33% or fewer had diagnosable FD (8,10,11). These results show that α-Gal A activity alone does not permit the selective identification of patients who are at high risk for FD. Furthermore, because additional sampling and tests can be upsetting to the patient, we strongly feel that reducing the number of false positives is important for patients' peace of mind. In published

studies, all but one (3) had a very high rate of re-sampling in cases that were identified as false positives: 66.7% (6), 90.9% (7), 92.5% (5), and 93.1% (9). If the genetic test from which the *GLA* mutation was not detected was defined as the unnecessary genetic tests, similarly, unnecessary genetic tests were carried out in a high percentage of patients in some studies: 60.0% (5), 66.7% (8), 86.7% (11), and 96.0% (10). Unnecessary genetic tests of some studies were 0% (2–4,6,7,9). To avoid false negative results, the screening cutoff α-Gal A level, the percentage of the control mean or cohort mean was recently set at approximately ≥50% (7–11). Secondary screening was needed to realize 0 unnecessary genetic tests (7,9). Finally, to educate patients and help them manage stress, we strongly feel that genetic counseling should be given before genetic testing, but only to patients who are strongly suspected of having or carrying a mutation for a genetic disease.

In this study, a *GLA* mutation was found in all of the patients with detectable plasma lyso-Gb3 levels. Although our case sample was small, our findings indicate that plasma lyso-Gb3 is an effective secondary screening target for refining and verifying positive results in an α-Gal A activity screen. In support of the need for a secondary screen, Andrade *et al.* (25) reported false negative results in an α-Gal A activity screen. Secondary screening for plasma lyso-Gb3 levels may solve this problem.

Two classes of *GLA* mutations are recognized according to their pathogenicity (26). Class 1 mutations have a high probability of causing disease. Class 2 mutations are nonpathogenic polymorphisms and include five missense changes (p.E66Q, p.S102L, p.S126G, p.D313Y, p.F396Y) (23,27). Therefore, the two patients in this study with detectable lyso-Gb3 levels and the p.E66Q mutation (cases 2 and 3 in Table 1) have not begun ERT.

p.E66Q is a class 2 mutation that retains a high residual enzyme activity (17). Therefore, plasma lyso-Gb3 could be difficult to detect in patients with this mutation. However,

Table 4. Reports on FD screening of male dialysis patients

Authors, Year	First Sampling		Re-Sampling		Positive before Genetic Testing	Positive by Genetic Testing	New Discovery Frequency	Unnecessary Re-Sampling (%)	Unnecessary Genetic Testing (%)
	Screening Cutoff α-GalA Level[a]	α-Gal A	α-Gal A	Plasma Gb3, Lyso-Gb3					
Utsumi et al., 2000 (2)	5.0	Plasma, 2/440 (0.5)			2/440 (0.5)	2/2 (100.0)	2/440 (0.5)		0/2 (0.0)
Nakao et al., 2003 (3)	29.8	Plasma, 6/514 (1.2)	WBC, 6/6 (100.0)	Gb3, 6/6 (100.0)	6/514 (1.2)	6/6 (100.0)	6/514 (1.2)		0/6 (0.0)
Linthorst et al., 2003 (4)	9.6	Whole blood, 1/508 (0.2)			1/508 (0.2)	1/1 (100.0)	1/508 (0.2)		0/1 (0.0)
Kotanko et al., 2004 (5)	35.0	DBS, 53/1516 (3.6)	WBC, 10/53 (18.9)		10/1516 (0.7)	4/10 (40.0)	4/1516 (0.3)	49/53 (92.5)	6/10 (60.0)
Ichinose et al., 2005 (6)	15.4	Plasma, 3/450 (0.7)	WBC, 1/3 (33.3)		1/450 (0.2)	1/1 (100.0)	1/450 (0.2)	2/3 (66.7)	0/1 (0.0)
Tanaka et al., 2005 (7)	58.2	Plasma, 11/398 (2.8)	WBC, 1/11 (9.1)		1/398 (0.3)	1/1 (100.0)	1/389 (0.3)	10/11 (90.9)	0/1 (0.0)
Bekri et al., 2005 (8)	55.0	WBC, 3/59 (5.1)			3/59 (5.1)	1/3 (33.3)	1/59 (1.7)		2/3 (66.7)
Merta et al., 2007 (9)	62.6	DBS, 58/1521 (3.8)	Plasma, WBC, 4/58 (6.9)		4/1521 (0.3)	4/4 (100.0)	4/1521 (0.3)	54/58 (93.1)	0/4 (0.0)
Fujii et al., 2009 (10)	66.0	DBS, 25/625 (4.0)			25/625 (4.0)	1/25 (4.0)	1/625 (0.2)		24/25 (96.0)
Gaspar et al., 2010 (11)	48.0	DBS, 30/543 (5.5)			30/543 (5.5)	4/30 (13.3)	4/543 (0.7)		26/30 (86.7)
This study	54.1	Plasma, 47/1453 (3.2)		Lyso-Gb3, 3/47 (6.4)	3/1453 (0.2)	3/3 (100.0)	3/1453 (0.2)		0/3 (0.0)

Data are presented as sample (% positive), unless otherwise indicated. FD, Fabry disease; α-Gal A, α-galactosidase A; Gb3, globotriaosylceramide; lyso-Gb3, globotriaosylsphingosine; WBC, white blood cell; DBS, dried blood spot.
[a]Data are presented as the % of control mean or cohort mean.

we detected plasma lyso-Gb3 in two different families with the p.E66Q mutation, suggesting that detectable plasma lyso-Gb3 may directly reflect the existence of a *GLA* mutation, a point that is very important to verify.

Among our patients testing positive in the α-Gal A activity screen but negative for lyso-Gb3, the only *GLA* mutation detected was p.E66Q. The fact that the lyso-Gb3 screen missed these individuals may indicate that some patients with class 2 mutations produce low levels or no lyso-Gb3. Although there is little downside to lyso-Gb3 being undetectable in some patients with class 2 mutations (ERT is not currently indicated for these patients), it is important not to overlook class 1 mutations. Further investigations are necessary to clarify whether all class 1 mutations result in the production of lyso-Gb3.

Of seven reports on the FD screening of Japanese male dialysis patients, including the two most recent (2,3,6,7,10,28,29), the p.E66Q mutation was found in four studies (3,10,28,29). Thus, the p.E66Q mutation in this population is frequently detected. If we exclude the FD patients with p.E66Q in the previous reports, the prevalence of FD is 0% (10,28,29), 0.2% (6), 0.3% (7), 0.5% (2), and 1.0% (3). The low prevalence of FD in this study was concerned with excluding a patient with FD from study population or a study limitation that not all patients were genotyped, leaving open the possibility that there were some cases of FD missed.

In the pedigree analysis for the family of the patient in case 1, the children who were hemizygotes for the class 1 FD mutation and the adult heterozygote all had detectable lyso-Gb3 concentrations. Plasma lyso-Gb3 may also be a promising screening target for adult heterozygotes. By contrast, lyso-Gb3 was not detected in the 10-year-old asymptomatic heterozygote, probably because lyso-Gb3 accumulates more slowly in heterozygotes than in hemizygotes (12). Rombach *et al.* (14) reported that some very young asymptomatic children who were heterozygous for a class 1 FD mutation showed normal lyso-Gb3 levels. Thus, plasma lyso-Gb3 levels vary with the mutation and among individuals. Therefore, the detection of lyso-Gb3 may become a useful marker for even nonpathologic *GLA* mutations, if the detection sensitivity of the test can be increased.

Plasma lyso-Gb3 is a promising screening tool that was effective for identifying candidates for genetic counseling and genetic testing, and for uncovering unrecognized FD among male dialysis patients.

Disclosures
None.

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