ORIGINAL ARTICLE

Metabolic Disorder

Plasma Globotriaosylsphingosine Level as a Primary Screening Target for Fabry Disease in Patients With Left Ventricular Hypertrophy

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Background: Although previous studies have suggested a certain prevalence of Fabry disease (FD) in left ventricular hypertrophy (LVH) patients, the screening of FD is difficult because of its wide-ranging clinical phenotypes. We aimed to clarify the utility of combined measurement of plasma globotriaosylsphingosine (lyso-Gb3) concentration and α-galactosidase A activity (α-GAL) as a primary screening of FD in unexplained LVH patients.

Methods and Results: Between 2014 and 2016, both lyso-Gb3 and α-GAL were measured in 277 consecutive patients (male 215, female 62, age 25–79 years) with left ventricular wall thickness >12 mm on echocardiogram: 5 patients (1.8%) screened positive (2 (0.7%) showed high lyso-Gb3 and 4 (1.4%) had low α-GAL levels). Finally, 2 patients (0.7%) were diagnosed with clinically significant FD. In 1 case, a female heterozygote with normal α-GAL levels had genetic variants of unknown significance and was diagnosed as FD by endomyocardial biopsy. The other case was a male chronic renal failure patient requiring hemodialysis, and he had a p.R112H mutation. In both cases there were high lyso-Gb3 levels.

Conclusions: The serum lyso-Gb3 level can be relevant for clinically significant FD, and combined measurement of lyso-Gb3 and α-GAL can provide better screening of FD in unexplained LVH patients.

Key Words: α-galactosidase A; Fabry disease; Left ventricular hypertrophy; Lyso-Gb3; Primary screening

Fabry disease (FD) is a rare X-linked lysosomal storage disease caused by abnormalities in the α-galactosidase A gene (*GLA*) leading to a partial or absolute deficiency of lysosomal enzyme, α-galactosidase A, activity (α-GAL). This deficiency results in an accumulation of globotriaosylceramide (Gb3), which in turn disturbs the functioning of various organs, predominantly the kidneys, central nerve system, and the heart.[1] Because a previous report suggested beneficial effects of enzyme replacement therapy (ERT) if patients receive it before advanced disease stage,[2] early detection of FD by screening is important.

The estimated prevalence of FD in the general population is 0.02–0.09/10,000 individuals,[3] and it is much higher in high-risk populations (with renal failure, stroke and left ventricular hypertrophy [LVH]). Numbers of screening studies, which have been conducted to assess the prevalence of FD in populations of patients with LVH,[4–7] have reported a prevalence varying markedly from 0% to 12%,

reflecting referral bias as well as different criteria for patient inclusion.

Because FD shows a wide range of clinical phenotypes, it is well known that screening for FD is difficult.[1,8–10] Although the precise causes for the remarkably heterogeneous manifestation of FD remains unklnown,[11] relatively high residual α-GAL is often noted in these atypical cases.[1] The measurement of α-GAL activity is the most well-known screening test for FD, but it sometimes gives false-positive results in normal subjects and often shows borderline or normal results in atypical male phenotypes and female heterozygotes.[1,8,9,12]

Emerging evidence suggests that deacylated form of Gb3, globotriaosylsphingosine (lyso-Gb3) is a feasible marker for the diagnosis of FD. In female heterozygotes, a clearly increased plasma lyso-Gb3 concentration with a concomitantly normal Gb3 concentration has been detected.[13,14] In addition, Niemann et al revealed that patients with atypical mutations of *GLA*, who showed lower lyso-Gb3 levels than patients with classical mutations, had no classic organ involvement.[15] Thus, the measurement of the lyso-Gb3 level may be beneficial for the diagnosis of clinically significant forms of FD in unexplained LVH patients.

Recently, we assessed the value of lyso-Gb3 as an FD biomarker for screening of Japanese male and female patients with suspected FD based on clinical symptoms and we showed that the lyso-Gb3 level is a promising primary screening target for identifying both classic and late-onset FD probands, even in female patients not identified through serum α-GAL screening.[16] Prior studies that have evaluated patients with FD have not studied the usefulness of the lyso-Gb3 level for the screening of probands.

The present study is a subanalysis of the study performed by Maruyama et al[16] with respect to the value of measuring the lyso-Gb3 level in FD, focusing on a clinically non-selected cohort of patients with LVH, by combined α-GAL and lyso-Gb3 level measurements, and genetic analysis.

Methods

Patients

This was a prospective multicenter study conducted between April 2014 and September 2016 in 9 institutes belonging to the Hamamatsu Circulation Forum. All patients of both sexes aged 15–79 years referred for transthoracic echocardiography (TTE) were considered eligible for the FD screening program. Patients were included if there was an evidence of LVH, defined as interventricular septal thickness (IVST) and/or LV posterior wall thickness (PWT) >12 mm, or focal LV wall hypertrophy >12 mm including the apex. The exclusion criteria were the presence of severe aortic valve disease and uncontrollable hypertension. The study protocol was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board. All study participants provided informed consent before inclusion.

Clinical Assessments

After the inclusion criteria were defined, a brief history focused on the investigation of symptoms suggestive of FD and multi-organ involvement, especially cardiac, was recorded. The questionnaire included history of pain in the extremities, gastrointestinal transit and sweating abnormalities, stroke, kidney and heart diseases, hypertension, diabetes, lipid disorders, and the presence of shortness of breath/orthopnea and chest pain. Clinical assessment also included family history for stroke, and kidney and heart diseases.

The cardiac investigation was complemented by 12-lead ECG, which allowed assessment of rhythm, the voltage criteria for LVH (Sokolow-Lyon criteria), and ST-T abnormalities. Some patients without renal dysfunction underwent cardiac magnetic resonance imaging (cMRI) with late gadolinium enhancement (LGE). The details of cMRI have been described elsewhere.[17] For the investigation of renal involvement, serum creatinine levels were determined with further estimation of the glomerular filtration rate (eGFR). Random urine specimens were also collected to rule out proteinuria.

Measurement of α-GAL and Lyso-Gb3 Levels

For the primary screening, blood specimens were collected in Venoject II collection tubes (Terumo, Tokyo, Japan) containing ethylenediaminetetraacetic acid (EDTA). The α-GAL level was measured at GlycoPharma Co. (Oita, Japan), using assays with the artificial substrate 4-methylumbelliferyl-α-D-galactoside, as described elsewhere,[16] and the serum cut off value in this screening was set as 4.0 nmol/h/mL for both sexes. The lyso-Gb3 level was measured by ultraperformance liquid chromatography/tandem mass spectrometry at GlycoPharma Co., as described elsewhere,[16] and the coefficient of variation of lyso-Gb3 values from those of normal subjects (0.4 ng/mL), female heterozygotes (20 ng/mL), and classical male Fabry patients (150 ng/mL) was 9.0%, 5.8%, and 4.5%, respectively. The serum cutoff value in this screening was set as 2.0 ng/mL for both sexes, as in the previous study.[16] Elevated levels of α-GAL and/or reduced lyso-Gb3 was defined as screening positive, and all results were explained to the participants or family members, as appropriate.

Genetic Analysis

Whenever the α-GAL level was reduced and/or the lyso-Gb3 level was elevated, genetic screening was recommended and performed after obtaining the patient's approval. To obtain DNA and RNA samples, blood specimens were collected in Venoject II collection tubes containing EDTA and in PAXgene Blood RNA tubes (PreAnalytiX, Hombrechtikon, Switzerland).

Genetic analysis was performed at the Department of Clinical Nephroscience, Niigata University Graduate School of Medical and Dental Sciences (Niigata, Japan). DNA and RNA were extracted from white blood cells using a QIAamp DNA Blood Mini Kit (Qiagen, Tokyo, Japan) and PAX gene Blood RNA Kit, respectively. Details of the genetic analysis are described elsewhere.[16]

Statistical Analysis

Continuous data are expressed as mean±standard deviation (SD) or as a median with range, as appropriate. Categorical data are expressed as numbers and percentages. Continuous variables were compared using the two-tailed unpaired t-test, and P-values <0.05 were considered statistically significant. All statistical analyses were performed using a statistical software package (SPSS ver. 18.0; SPSS Inc., Chicago, IL, USA).

Table 1. LVH Patients' Characteristics (Clinical Status)	
n	277
Sex (M/F)	215/62
Age (years)	61.0±12.9
Initial heart disease	
HCM/HHD/IHD/DCM/others	166/78/19/9/5 (59.9/28.2/6.9/3.3/1.8)
Symptoms	
NYHA ≥II	75 (27.1)
Chest pain	12 (4.3)
Pain in extremities	17 (6.1)
Abnormalities in gastrointestinal transit	21 (7.6)
Sweating abnormalities	26 (9.4)
Complications	
Kidney disease	133 (48.0)
Stroke	40 (14.5)
Family history	
Heart disease	53 (19.1)
Kidney disease	33 (11.9)
Stroke	37 (13.4)

Data are presented as number (%) or mean±standard deviation. DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; HHD, hypertensive heart disease; IHD, ischemic heart disease; LVH, left ventricular hypertrophy; NYHA, New York Heart Association.

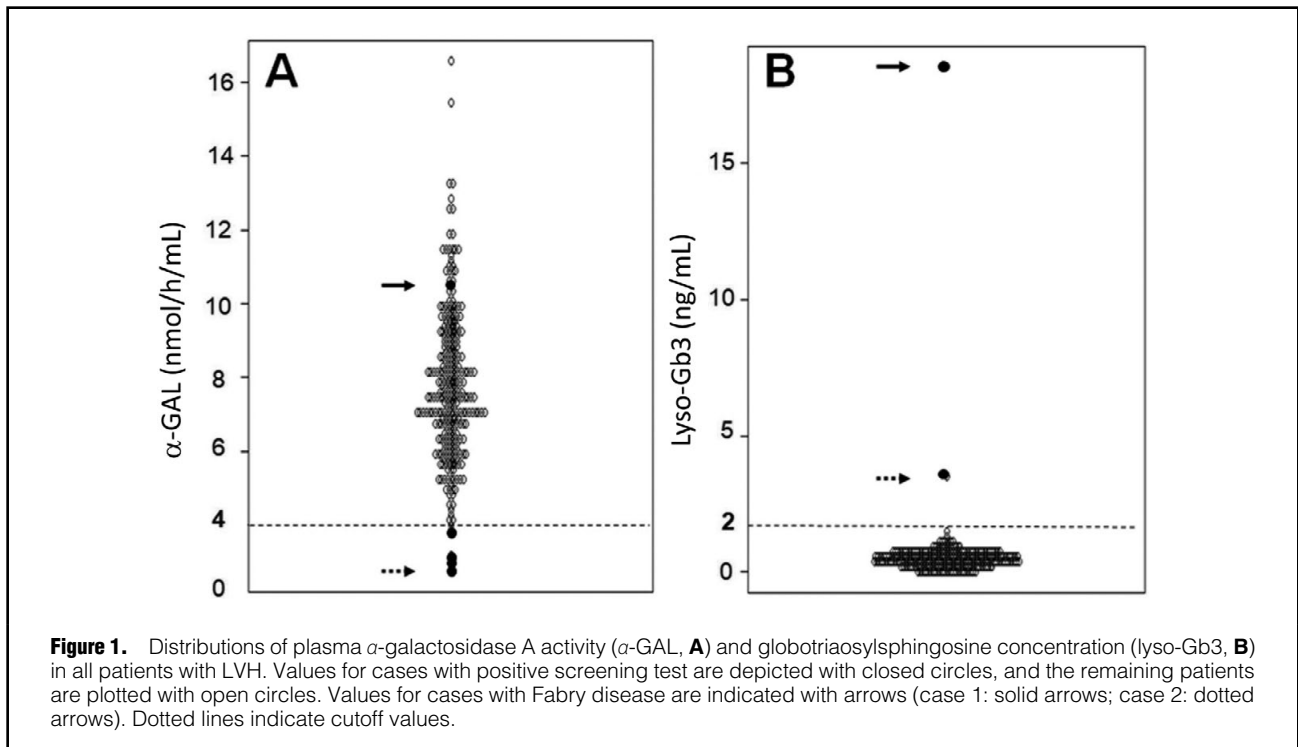| Table 2. LVH Patients' Characteristics (Examinations)	
Laboratory data	
pCr (mg/dL)	1.77±2.38
eGFR (mL/min/1.73 m²)	57.9±27.0
Proteinuria	89 (32.3)
NT pro-BNP (median, range)	657.5 (19–65,104)
TTE	
LVDd (mm)	45.6±7.5
LVDs (mm)	29.2±8.3
IVST (mm)	14.4±3.0
PWT (mm)	12.7±2.2
LVEF (%)	65.4±14.2
E/A ratio	1.00±0.58
E/e' ratio	14.9±6.7
12-lead ECG	
AF	45 (16.3)
Voltage criteria for LVH	122 (44.0)
ST-T abnormalities	175 (63.2)
Cardiac MRI	
Number	82 (29.6)
LGE (sep-ant / inf-post)	50/18

Data are presented as number (%), mean±standard deviation, or median with range. AF, atrial fibrillation; IVST, interventricular septum; LGE, late gadolinium enhancement; LVDd/Ds, left ventricular diastolic/systolic dimensions; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; NT pro-BNP, N-terminal pro-B-type natriuretic protein; pCr, plasma creatinine concentration; PWT, posterior wall thickness; sep-ant / inf-post, septum-anterior/inferior-posterior left ventricular wall.

Results

Patients' Characteristics

During the study period of 2.5 years, 280 patients fulfilled the inclusion criteria and 3 of them were excluded because of severe aortic stenosis or regurgitation. Finally, 277 patients underwent FD screening (**Table 1**): 62 patients (22.4%) were female, and the age ranged from 25 to 79 years old. The initial diagnoses of LVH were mainly hypertrophic cardiomyopathy (HCM), hypertensive heart disease, ischemic heart disease, and dilated cardiomyopathy. The prevalence of symptoms suggesting the classic type of FD was relatively low in this cohort. Some patients were symptomatic (New York Heart Association class ≥II or chest pain) and had a history of kidney disease and/or stroke; 33 patients (11.9%) were on a regular dialysis program. A few patients had a family history of heart disease, kidney disease, or stroke.

Table 2 summarizes the examination data of the patients. TTE revealed normal mean LV dimensions and LV ejection fraction in all patients, despite LV wall thickening and relatively high E/e' ratios. ECG assessment showed normal sinus rhythm in most patients, with the remaining showing atrial fibrillation (AF: 16.3%) or pacemaker rhythm (0.7%). Approximately half of the patients presented with voltage criteria for LVH and ST-T abnormalities. cMRI was performed in 82 patients (29.6%), and LGE in the inferior-lateral LV wall, which is a typical finding in FD, was observed in 18 patients (22.5%).

Combined Measurement α-GAL With Lyso-Gb3 Level and Gene Mutation Analysis

Primary screening for FD using α-GAL measurement (**Figure 1A**; 7.8±2.0 nmol/h/mL) revealed that 4 patients (1.4%) had lower α-GAL levels than the cutoff (4 nmol/h/mL) value, and the lyso-Gb3 (**Figure 1B**; 0.56±1.26 ng/mL) level was higher in 2 patients (0.7%) than the cutoff value (2 ng/mL). Thus, 5 patients (1.8%) were screen-positive for FD (**Table 3**).

All 5 screen-positive patients were referred to genetic study and in 3 of them (60%) α-galactosidase gene mutations in exons were detected. In case 1, any putative mutations by ordinary genetic analyses were not found, but an intronic analysis revealed genetic variants of unknown significance (GVUS); in case 2 the patient had an R112H mutation, which has been previously defined as relevant for FD; in cases 3 and 4 the patients showed an E66Q mutation, which is known as a benign polymorphism; in case 5 there was no variation (a non-pathogenic gene promoter variant).

Case Presentation and Diagnosis of FD

Case 1 Patient complained of shortness of breath and fatigue, but had no history of symptoms suggesting FD and was initially diagnosed with HCM and AF. TTE showed asymmetrical LVH without obstruction and normal systolic function. The screening test revealed a high lyso-Gb3 level without a decrease in the α-GAL level (**Table 3**). cMRI demonstrated transmural LGE in the inferior-lateral wall of the LV and endomyocardial biopsy revealed vacuolar degeneration and lamellar inclusion bodies in cardiomyocytes on both light and electron microscopy (**Figure 2**). Thus, the patient was diagnosed with advanced stage of a cardiac variant of FD, and ERT with α-galactosidase β (1 mg/kg, every other week) was started in June 2016. Her lyso-Gb3 level decreased from

Figure 1. Distributions of plasma α-galactosidase A activity (α-GAL, **A**) and globotriaosylsphingosine concentration (lyso-Gb3, **B**) in all patients with LVH. Values for cases with positive screening test are depicted with closed circles, and the remaining patients are plotted with open circles. Values for cases with Fabry disease are indicated with arrows (case 1: solid arrows; case 2: dotted arrows). Dotted lines indicate cutoff values.

Table 3. Summary of Positive Cases Detected by Screening

	Case no.				
	1	2	3	4	5
Sex (M/F)	F	M	M	M	M
Age (years)	67	61	26	79	76
Initial heart disease	HCM, AF	IHD, As	HCM	HHD	HCM
Renal disease	–	CRF/HD	–	–	CKD
Stroke	–	–	–	–	–
NYHA class	II	II	I	I	II
Family history	None	NA	AVB (PM)	None	None
LVDd/Ds (mm)	50.0/32.0	39.5/23.3	38.8/19.2	42.7/27.8	41.3/21.0
IVST/PWT (mm)	17.0/12.0	15.1/12.3	13.8/10.8	12.8/11.3	13.8/12.8
LVEF (%)	69.0	72.4	82.4	64.5	72.0
LAD (mm)	51.0	35.2	30.3	35.8	50.1
LGE-MRI	inf-lat LV	NA	No LGE	NA	NA
α-GAL (nmol/h/mL)	10.9	2.7	3.2	3	3.9
Lyso-Gb3 (ng/mL)	18.5	3.6	0	0.5	0.9
GLA mutation	GVUS	c.335 A>G (p.R112H)	c.196 C>G (p.E66Q)	c.196 C>G (p.E66Q)	No mutation

α-GAL, α-galactosidase A activity; As, mild aortic stenosis; AVB (PM), atrioventricular block with pacemaker implantation; CKD, chronic kidney disease; CRF, chronic renal failure; GVUS, genetic variants of unknown significance; HD, hemodialysis; inf-lat LV, inferior to lateral left ventricular wall; LAD, left atrial diameter; lyso-Gb3, globotriaosylsphingosine; MRI, magnetic resonance imaging; NA, not available. Other abbreviations as in Tables 1,2.

18.5 ng/mL to 5.2 and 3.4 ng/mL after 3 and 12 months, respectively, of therapy. The secondary family analysis suggested that she was a de novo case of FD.

Case 2 Patient had chronic renal failure and was on hemodialysis for 22 years. TTE showed a concentric LVH, normal systolic function, and mild aortic valve stenosis. Coronary angiography revealed significant stenotic lesions in the right coronary artery and the left anterior descending artery. The systolic pressure gradient between the LV and ascending aorta was 12 mmHg. Although the patient refused further examination or ERT, he was diagnosed with clinically significant FD, because he exhibited both increased lyso-Gb3 and decreased α-GAL levels and showed a clinical phenotype of chronic renal failure requiring hemodialysis.

Case 3 Patient with no family history of LVH was referred to hospital because of ECG abnormalities
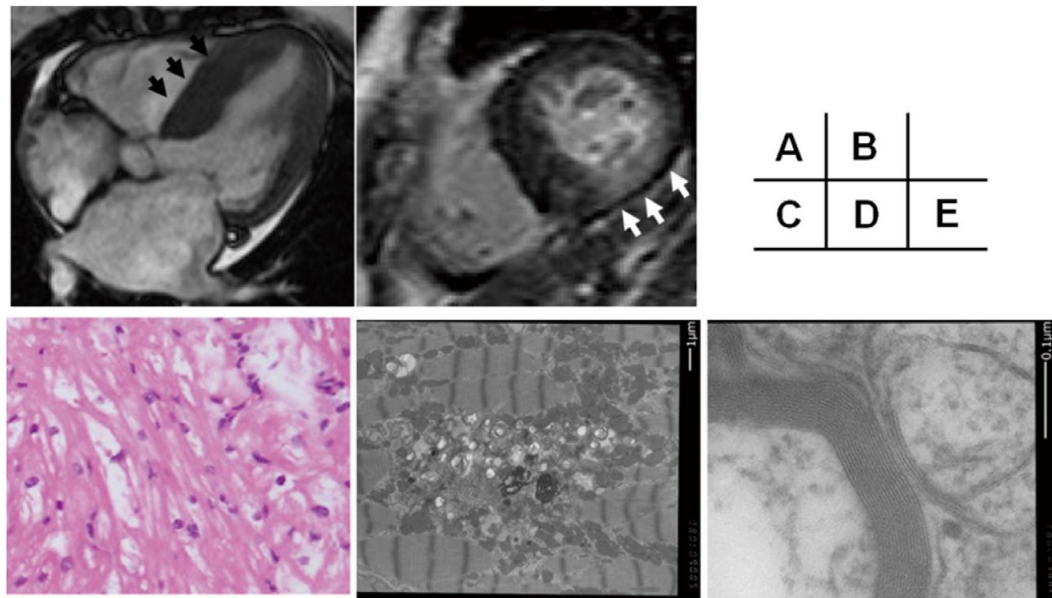
Figure 2. Cardiac magnetic resonance imaging (cMRI) and histological findings in case 1. The horizontal axis (4-chamber) view of cine-MRI (**A**) and mid-ventricular short-axis view of late gadolinium enhancement (LGE)-cMRI (**B**). Arrows indicate asymmetrical septal wall hypertrophy (black) and transmural LGE in the inferior-lateral wall of LV (white). Images for endomyocardial biopsy from the right ventricle on (**C**) light microscopy (H&E staining, ×100) and (**D**,**E**) electron microscopy.

(undetermined QRS axis). Although TTE demonstrated asymmetrical septal hypertrophy with outflow obstruction, cMRI showed no LGE in the myocardium. Cardiac biopsy did not exhibit any typical changes of FD.

Case 4 Patient was treated for hypertension and TTE revealed mild concentric LVH. Despite screening positive for FD, he refused further examination and family analysis.

Case 5 Patient was treated for chronic kidney disease (serum creatinine level 2.2 mg/dL). Although he complained of pretibial pitting edema, he had no history of symptoms suggesting FD. He was initially diagnosed with HCM, because TTE revealed concentric LVH with normal systolic function and enlarged left atrium. Genetic analysis did not show any mutation in *GLA*, and he was later diagnosed with systemic amyloidosis.

Although there was not strong evidence, because of the lack of supportive cardiac histological changes of FD, we considered that in 3 cases (nos 3, 4, and 5) we can at least exclude clinically significant FD, because they showed neither genetically relevant polymorphism for FD nor typical clinical phenotypes of FD. Finally, the prevalence of FD was 0.7% (2 patients: cases 1 and 2) in our cohort of 277 LVH patients, and of note, both cases screened positive by serum lyso-Gb3. Our results suggested that combined measurement of α-GAL and lyso-Gb3 is more beneficial for FD screening than the usual single α-GAL measurement.

Discussion

This study investigated the utility of combined measurement of lyso-Gb3 and α-GAL as a primary screening for FD in a cohort of Japanese patients with unexplained LVH. Our finding suggested that (1) plasma lyso-Gb3 is a promising marker in female heterozygotes who are usually not identified by α-GAL screening, and (2) may also be beneficial in male LVH patients for screening clinically significant FD.

The prevalence of FD in LVH patients varies according to the different study regions, inclusion criteria, and methods of screening.[4–7] Although previous screening studies used α-GAL in leucocytes, genetic analysis, and/or urinary Gb3 level, those tests (enzymatic and/or genetic tests) cannot always identify FD in atypical male phenotypes and/or in female heterozygotes who lack the characteristic clinical features.[1,8,9,12,15] Our study cohort included patients with a relative wide range of clinical manifestations, such as mild to severe LVH, kidney diseases (even hemodialysis and renal transplantation), hypertension, and valve diseases; however, we found a similar prevalence of FD to that reported by previous studies. Given the particularly high incidence of LVH in older female FD patients,[16] our study might have found more positive cases if the analysis had focused on female LVH patients.

Although serum α-GAL is a common tool for primary screening for FD, it sometimes gives false-positive results in normal subjects and often shows borderline or normal results in atypical male phenotypes and female heterozygotes.[1,8,9,12] Even evaluation of leucocyte α-GAL sometimes gives false-negative results as well, because α-galactosidase activity differs with cell type because of "random X-chromosomal inactivation".[8–10] This existing unsatisfactory screening test carries the risk of misdiagnosis and inappropriate counselling for FD.

Emerging evidence suggests that the lyso-Gb3 level is relevant for FD.[13–15,18] Although the likelihood of using the lyso-Gb3 level for primary screening has remained elusive, our study results suggested that it could provide better screening of clinically significant FD in patients with unex-

plained LVH. Notably, the measurement of the lyso-Gb3 level for FD screening was beneficial for female heterozygotes. In fact, in case 1, in which the patient showed a normal range for both α-GAL and Gb3, an increased lyso-Gb3 level was screen-positive and the patient was finally diagnosed by endocardial biopsy as having an advanced stage of a cardiac variant of FD. To define the cutoff value of lyso-Gb3, we referred to a previous investigation in which normal subjects without *GLA* mutation were screened by lyso-Gb3 value,[19] and we set their highest lyso-Gb3 value of those normal subjects (2.0 ng/mL) as our cutoff. Unfortunately, we could not provide the predictive values of lyso-Gb3 in the present study, because not all patients underwent *GLA* mutation analysis or endocardial biopsy. Lukas et al reported that, when they defined the pathological cutoff value as 0.9 ng/mL, which was the 95th percentile of normal subjects, the positive predictive value of lyso-Gb3 to screen FD was 95%.[19] Because we set a higher cutoff value up to 2.0 ng/mL in our study, our positive predictive value would seem to be better than 95%.

In addition, because the patient in case 1 had GVUS, even a genetic analysis was less useful in the diagnosis of FD. The lyso-Gb3 level should be beneficial for male FD screening, as well. Although in 3 cases (cases 3, 4, and 5) the patients were screened positive by low α-GAL levels, they did not show increased lyso-Gb3 and so were excluded from having clinically significant FD because they exhibited a p.E66Q mutation, which is a known benign polymorphism or non-pathogenic gene promoter variant in genetic analysis. Because both the p.E66Q mutation and non-pathogenic gene promoter variant can retain enzyme activities, the lyso-Gb3 levels did not increase in these patients.[16,20] Although the gene mutation in case 2 (p. R112H) remains controversial regarding its clinical relevance for FD,[15,21] the patient should be diagnosed with clinically significant FD because there was both increased lyso-Gb3 and decreased α-GAL levels, as well as chronic renal failure requiring hemodialysis. Thus, our results suggested that combined measurement of lyso-Gb3 and α-GAL levels is beneficial for FD screening.

Lyso-Gb3 is a useful biomarker of the severity of FD and the effectiveness of ERT, because it reflects the overall total body substrate accumulation.[22] Even in female patients with FD, a recent study suggested that an elevated lyso-Gb3 level in ERT-naive females was associated with significantly higher incidences of organ manifestations even if the patients were ~8 years younger than females with normal lyso-Gb3 levels.[23] However, it is difficult to define the FD burden and progression by lyso-Gb3 level measurement among atypical male phenotypes and heterozygote females.[18,24] As for case 1, a reduction in the lyso-Gb3 level was achieved with ERT, but unfortunately no improvement in either LVH or cardiac fibrosis on cMRI after 3 years treatment of ERT (**Supplementary Figure**).

Study Limitations
(1) We did not examine FD-related genes other than *GLA*, (2) we did not perform histological assessment of the endomyocardial biopsy sample in many cases, and (3) the family history was not obtained in some cases because of the patient's refusal. Further investigations should explore the most effective tools for FD screening, using a combination of the α-GAL level, lyso-Gb3 level, FD-related genetic tests, and other new techniques.

Conclusions
The combined evaluation of lyso-Gb3 and α-GAL could more beneficial for primary screening of FD in patients with LVH.

Disclosure
The authors declare no conflicts of interests.

References
1. Desnick R, Ioannou Y, Eng C. α-Galactosidase A deficiency: Fabry disease, 8th edn. New York: McGraw-Hill, 2001.
2. Weidemann F, Niemann M, Breunig F, Herrmann S, Beer M, Stork S, et al. Long-term effects of enzyme replacement therapy on Fabry cardiomyopathy: Evidence for a better outcome with early treatment. *Circulation* 2009; **119**: 524–529.
3. Terryn W, Deschoenmakere G, De Keyser J, Meersseman W, Van Biesen W, Wuyts B, et al. Prevalence of Fabry disease in a predominantly hypertensive population with left ventricular hypertrophy. *Int J Cardiol* 2013; **167**: 2555–2560.
4. Nakao S, Takenaka T, Maeda M, Kodama C, Tanaka A, Tahara M, et al. An atypical variant of Fabry's disease in men with left ventricular hypertrophy. *N Engl J Med* 1995; **333**: 288–293.
5. Sachdev B, Takenaka T, Teraguchi H, Tei C, Lee P, McKenna WJ, et al. Prevalence of Anderson-Fabry disease in male patients with late onset hypertrophic cardiomyopathy. *Circulation* 2002; **105**: 1407–1411.
6. Chimenti C, Pieroni M, Morgante E, Antuzzi D, Russo A, Russo MA, et al. Prevalence of Fabry disease in female patients with late-onset hypertrophic cardiomyopathy. *Circulation* 2004; **110**: 1047–1053.
7. Havndrup O, Christiansen M, Stoevring B, Jensen M, Hoffman-Bang J, Andersen PS, et al. Fabry disease mimicking hypertrophic cardiomyopathy: Genetic screening needed for establishing the diagnosis in women. *Eur J Heart Fail* 2010; **12**: 535–540.
8. MacDermot KD, Holmes A, Miners AH. Anderson-Fabry disease: Clinical manifestations and impact of disease in a cohort of 60 obligate carrier females. *J Med Genet* 2001; **38**: 769–775.
9. MacDermot KD, Holmes A, Miners AH. Anderson-Fabry disease: Clinical manifestations and impact of disease in a cohort of 98 hemizygous males. *J Med Genet* 2001; **38**: 750–760.
10. Vedder AC, Linthorst GE, van Breemen MJ, Groener JE, Bemelman FJ, Strijland A, et al. The Dutch Fabry cohort: Diversity of clinical manifestations and Gb3 levels. *J Inherit Metab Dis* 2007; **30**: 68–78.
11. Eng CM, Fletcher J, Wilcox WR, Waldek S, Scott CR, Sillence DO, et al. Fabry disease: Baseline medical characteristics of a cohort of 1765 males and females in the Fabry Registry. *J Inherit Metab Dis* 2007; **30**: 184–192.
12. Linthorst GE, Vedder AC, Aerts JM, Hollak CE. Screening for Fabry disease using whole blood spots fails to identify one-third of female carriers. *Clin Chim Acta* 2005; **353**: 201–203.
13. van Breemen MJ, Rombach SM, Dekker N, Poorthuis BJ, Linthorst GE, Zwinderman AH, et al. Reduction of elevated plasma globotriaosylsphingosine in patients with classic Fabry disease following enzyme replacement therapy. *BBA-Mol Basis Dis* 2011; **1812**: 70–76.
14. Aerts JM, Groener JE, Kuiper S, Donker-Koopman WE, Strijland A, Ottenhoff R, et al. Elevated globotriaosylsphingosine is a hallmark of Fabry disease. *Proc Natl Acad Sci USA* 2008; **105**: 2812–2817.
15. Niemann M, Rolfs A, Stork S, Bijnens B, Breunig F, Beer M, et al. Gene mutations versus clinically relevant phenotypes: Lyso-Gb3 defines Fabry disease. *Circ Cardiovasc Genet* 2014; **7**: 8–16.
16. Maruyama H, Miyata K, Mikame M, Taguchi A, Guili C, Shimura M, et al. Effectiveness of plasma lyso-Gb3 as a biomarker for selecting high-risk patients with Fabry disease from multispe-

cialty clinics for genetic analysis. *Genet Med* 2019; **21:** 44–52.

17. Matoh F, Satoh H, Shiraki K, Saitoh T, Urushida T, Katoh H, et al. Usefulness of delayed enhancement magnetic resonance imaging to differentiate dilated phase of hypertrophic cardiomyopathy and dilated cardiomyopathy. *J Card Fail* 2007; **13:** 372–379.

18. Smid BE, van der Tol L, Biegstraaten M, Linthorst GE, Hollak CE, Poorthuis BJ. Plasma globotriaosylsphingosine in relation to phenotypes of Fabry disease. *J Med Genet* 2015; **52:** 262–268.

19. Lukas J, Giese AK, Markoff A, Grittner U, Kolodny E, Mascher H, et al. Functional characterisation of alpha-galactosidase a mutations as a basis for a new classification system in fabry disease. *PLoS Genet* 2013; **9:** e1003632.

20. Maruyama H, Takata T, Tsubata Y, Tazawa R, Goto K, Tohyama J, et al. Screening of male dialysis patients for Fabry disease by plasma globotriaosylsphingosine. *Clin J Am Soc Nephrol* 2013; **8:** 629–636.

21. Tsukimura T, Nakano S, Togawa T, Tanaka T, Saito S, Ohno K, et al. Plasma mutant alpha-galactosidase A protein and globotriaosylsphingosine level in Fabry disease. *Mol Genet Metab Rep* 2014; **1:** 288–298.

22. Nowak A, Mechtler TP, Desnick RJ, Kasper DC. Plasma lysogb3: A useful biomarker for the diagnosis and treatment of Fabry disease heterozygotes. *Mol Genet Metab* 2017; **120:** 57–61.

23. Lenders M, Hennermann JB, Kurschat C, Rolfs A, Canaan-Kuhl S, Sommer C, et al. Multicenter Female Fabry Study (MFFS): Clinical survey on current treatment of females with Fabry disease. *Orphanet J Rare Dis* 2016; **11:** 88.

24. Germain DP. Fabry disease. *Orphanet J Rare Dis* 2010; **5:** 30.

Supplementary Files

Please find supplementary file(s);
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