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ORIGINAL ARTICLE

Fabry disease in patients with hypertrophic cardiomyopathy: a practical approach to diagnosis

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This study aimed to develop a new set of screening criteria that is easily applicable and highly sensitive for the detection of patients at high risk of Fabry disease (FD) among hypertrophic cardiomyopathy (HCM) patients. We prospectively studied 273 consecutive unrelated patients who were referred to HCM clinic for unknown left ventricular hypertrophy. Among the 273 patients, we selected 65 high-risk patients who fulfilled at least one of our newly proposed screening criteria. All 273 patients were assayed for plasma α-galactosidase A (α-GAL A) activity. The new screening criteria were: (1) atypical HCM, (2) history or presence of documented arrhythmia, (3) short PR interval defined as <120 ms on electrocardiogram, and (4) symptoms of autonomic dysfunction. From this screening study, three unrelated patients (4.6%; 2 females and 1 male) were newly diagnosed with FD using α-GAL A activity and mutation analysis of the *GLA* gene. Using the screening method based on the newly proposed criteria, the prevalence of FD in our HCM population was 4.6% if at least one criterion was met and 18.8% if $\geqslant 3$ criteria were met. Therefore, our proposed criteria are easily applicable and highly sensitive for classifying patients at high risk of FD from HCM patients.
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INTRODUCTION

Fabry disease (FD) is an X-linked lysosomal storage disorder resulting from α-galactosidase A (α-GAL A) enzyme deficiency. This results in accumulation of glycosphingolipids in various tissues and consequent multi-organ manifestations, including stroke, renal insufficiency or left ventricular hypertrophy (LVH). Cardiac FD is a variant form of FD that primarily manifests as LVH,[1,2] and the prevalence of FD in LVH patients is reported to be approximately 1–3%.[3–6] However, studies have raised the possibility that FD prevalence is underestimated.[7,8] Recent studies have revealed that multi-organ effects caused by FD, including LVH, are reversible to a significant extent through enzyme replacement therapy, particularly during the early stages.[9–11] Therefore, it is important to effectively screen and accurately diagnose FD patients. FD screening can be performed using either enzyme assay or gene mutation studies. A study conducted by Matawari *et al.*[4] screened α-GAL A enzyme activity in all LVH patients, and Elliot *et al.*[6] investigated the prevalence of FD in hypertrophic cardiomyopathy (HCM) patients by screening all HCM patients for mutations in *GLA*, the gene that encodes α-GAL A. However, considering the low incidence of FD, FD screening of all HCM patients is neither efficient nor cost-effective.

HCM patients with FD present with various cardiac manifestations, and concentric or diffuse hypertrophy is one of the most common patterns observed.[12,13] Other frequently reported cardiac characteristics in this population include significant arrhythmias, PR interval shortening and autonomic dysfunction symptoms.[14,15] Although several studies have reported these cardiac features in FD,[12–15] there are currently no specific screening criteria that include cardiac features in the HCM population. Therefore, the aim of this study was to develop new screening criteria that are easily applicable and highly sensitive for the detection of patients at high risk of FD among HCM patients.

MATERIALS AND METHODS

Study population

From March 2012 to August 2014, 273 consecutive unrelated patients (age, 58 ± 16 years) who were referred to HCM clinic at Severance Hospital (Yonsei University College of Medicine, Seoul, Republic of Korea) with a diagnosis of HCM or LVH of unknown origin were prospectively enrolled in this study. All patients had unexplained LVH with a maximum LV wall thickness >13 mm in the absence of abnormal loading conditions. We divided the 273 patients into two groups, low- and high-risk group, respectively, according to our newly proposed cardiac FD screening criteria. Low-risk group consisted of patients who did not fulfill any of our FD screening criteria, while

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high-risk group consisted of patients who fulfilled at least one of our newly proposed screening criteria. We assayed plasma α-GAL A activity in all 273 patients.

The four criteria for FD screening among HCM patients were as follows: (1) atypical HCM, which was distinguished from typical HCM by an absence of asymmetric septal hypertrophy, and was specifically defined as diffuse, symmetric mid-ventricular, biventricular or predominant LV free wall hypertrophy thickness >13 mm; (2) the presence or a documented history of arrhythmia, including atrial tachycardia (AT), atrial fibrillation (AF), ventricular tachycardia (VT), symptomatic premature ventricular complex or high-degree atrioventricular (AV) block; (3) short PR interval (<120 ms) on resting electrocardiogram (ECG); and (4) symptoms related with autonomic dysfunction, including unexplained syncope, orthostatic hypotension (OH), recurrent dizziness or chronotropic incompetence (CI). Clinical information, including referral reason, symptoms and laboratory data, was acquired by obtaining careful medical histories and reviewing medical records. All patients provided written informed consent for the clinical, molecular biology and genetic studies. The institutional ethics committee of Severance Hospital approved the protocol.

Echocardiography

Comprehensive transthoracic echocardiography was performed using commercially available equipment. Standard two-dimensional measurements were obtained as recommended by the American Society of Echocardiography.[16] The LV ejection fraction was calculated using the modified Simpson method from the apical four- and two-chamber views.[16] Wall thickness was measured at the level of the mitral valve, papillary muscle and apex using the parasternal short axis view. The maximum LV wall thickness was defined as the greatest thickness in any single segment. LV hypertrophy was defined as a mean LV mass index (LVMI) $>131\ g\,m^{-2}$ in men and $>113\ g\,m^{-2}$ in women.[16,17] Diagnosis criteria for HCM included demonstration of asymmetric LV hypertrophy with a wall thickness of >13 mm and a maximal wall-to-normal wall thickness ratio >1.5 at end-diastole assessed using standard two-dimensional transthoracic echocardiography. Mitral inflow velocities were traced to obtain the peak velocity of early (E) and late (A) filling and the deceleration time of the E velocity. Mitral annular velocities were measured by Doppler tissue imaging using the pulsed-wave mode. The LV outflow tract area was determined as $(D/2)$, where D is the LV outflow tract diameter measured from a zoomed systolic freeze frame in the parasternal long-axis view. The LV outflow tract (LVOT) gradient was measured using continuous-wave Doppler in the apical three-chamber view. HCM type was classified by four variants in echocardiography: (1) asymmetric septal hypertrophy, (2) symmetric mid-ventricular hypertrophy, (3) diffuse concentric LVH, and (4) predominantly free wall hypertrophy.

ECG and exercise test

In all study subjects, ECGs were performed on the same day as transthoracic echocardiography (TTE). ECGs (12-lead) were digitally downloaded from the GE Marquette MUSE system (GE Medical Systems, Milwaukee, WI, USA). The heart rate (HR) and PR intervals were automatically measured, and short PR interval was defined as <120 ms from at least one ECG lead. Two trained cardiologists who were unaware of clinical characteristics of the patients and their disease status independently reviewed the ECGs. Twenty-four-hour Holter monitoring was performed in all patients to detect significant arrhythmias, including symptomatic premature ventricular complexes, AT, AF, non-sustained VT (NSVT) or high-degree AV block. Symptom-limited exercise tests using a treadmill were conducted as recommended according to the Bruce protocol.[18] During each exercise and recovery stage, we recorded symptoms, blood pressure (BP), HR, cardiac rhythm, ST-segment displacement and metabolic equivalents. Testing was terminated if the patient experienced fatigue, dyspnea, leg discomfort, chest pain, achieved the maximal predicted HR, an exaggerated hypertensive response (systolic BP >250 mm Hg or diastolic BP >115 mm Hg) or drop in systolic BP of >10 mm Hg, severe arrhythmias (including second- or third-degree AV block) or marked ST-segment displacement. After peak exercise, the test was almost immediately terminated, and measurements were obtained while the patient was in a standing position. Treadmill exercise tests were performed in all patients to demonstrate exercise-related arrhythmias and CI.

Tests for autonomic dysfunction

When patients had suspicious symptoms that correlated with autonomic dysfunction, such as recurrent dizziness, syncope or OH, we performed specific tests to confirm the diagnosis. Head-up tilt test (HUT) was performed in patients with unexplained syncope following a standard protocol. After a 4-h fast, subjects were placed in a supine position on a tilt table under continuous ECG and BP monitoring. At baseline, patients were in the supine position for 10 min, and their hemodynamic parameters, including BP and HR, were monitored every minute. Subsequently, subjects were tilted to an 80° head-up position for 30 min with their feet resting on the footboard for support. BP, HR and subjective symptoms were monitored and recorded every 2 min. If the results of the baseline tilting were negative, patients were returned to the supine position. After 5 min of rest, intravenous isoproterenol was administered ($1\ \mu g\ min^{-1}$). The dosage was increased $1\ \mu g\ min^{-1}$ every 3 min until a HR of $120\ min^{-1}$ or a maximum dosage of $5\ \mu g\ min^{-1}$ was reached. Next the head-up tilt to 80° was repeated for 10 min. A positive result (with or without concomitant isoproterenol infusion) was defined as follows: (1) syncope or (2) the development of presyncope in association with an abrupt fall in systolic BP to <70 mm Hg, bradycardia ($HR<40\ min^{-1}$) and reproduction of the patient's relevant clinical symptoms.[19] For the detection of OH, upright BP tests were performed. Upright BP tests for OH measure the patient's BP after being in a supine position for 5 min, then 1 min after standing and 3 min after standing. OH is defined as a decrease in systolic BP of at least 20 mm Hg and/or in diastolic BP of at least 10 mm Hg between the supine reading and the upright reading. All screening patients underwent exercise testing with a treadmill to detect CI. CI was defined as a diminished HR response to exercise based on the following two criteria,[20,21] (1) failure to achieve 85% of the maximum age-predicted HR (% Max PHR), where % Max PHR was calculated as 220 − age (years); and (2) percentage of HR reserve of $\leqslant 80\%$, calculated using the equation (HR at peak exercise − resting HR)/((220 − age) − resting HR)).[20–22]

Enzyme assay and gene analyses

Samples were collected from all subjects who fulfilled at least one out of the four screening criteria for dried blood spot and plasma analyses for α-GAL A enzyme levels. On the basis of previously published data, an α-GAL A activity $<2.5\ \mu mol\ l^{-1}h^{-1}$ (0.5 percentile) was considered a positive dried blood spot result. Plasma α-GAL A activity was measured using 4-methylumbelliferyl-α-D-galactopyranoside as the substrate and *N*-acetyl-D-galactosamine as the inhibitor. An α-GAL A activity $<75.1\ mmol\ h^{-1}\ mg^{-1}$ protein was considered a positive result. The same cutoff value was used for both males and females. Those who had enzyme levels lower than the cutoff value had their peripheral blood sample sent for genetic study of the GLA gene. Mutation analysis was based on direct sequencing of exons 1–7 of PCR products of the GLA gene using the 3730xl sequencer (Illumina Inc, San Diego, CA, USA). Primer specification and reaction conditions are available upon request.

Statistical analyses

Continuous variables are presented as the mean ± s.d., and categorical variables are given as a percentage of the group total. For comparison of continuous variables among groups according to screening, we used Student's *t*-tests. Comparisons of nonparametric data were performed using χ^2 tests. The results are expressed as the mean ± s.d. $P<0.05$ was considered statistically significant.

RESULTS

The baseline characteristics of the study population are summarized in Table 1. Among 273 consecutive patients, low-risk group consisted of 101 patients (36.9%) with apical HCM and 107 patients (39.2%) who did not fulfill any of our FD screening criteria, while high-risk group was composed of 65 (23.8%) patients who fulfilled at least one of our newly proposed screening criteria. The mean age of the 273 subjects was 58 ± 16 years (169 males), and of the 208 low-risk subjects and 65

Table 1 Baseline characteristics of the study population

	All patients (n=273)	*Low risk* (n=208)	*High risk* (n=65)
Demographics			
Age, years	58 ± 16	63 ± 13	44 ± 15[a]
Male gender, n (%)	169 (61)	134 (64)	35 (54)
BMI, kg m^{-2}	24.2 ± 3.4	24.6 ± 3.1	23 ± 3.8
Hypertension, n (%)	145 (53)	113 (54)	32 (49)
Diabetes mellitus, n (%)	46 (17)	37 (18)	9 (14)
Chronic kidney disease, n (%)	34 (12)	20 (10)	14 (22)
ESRD, n (%)	6 (2.2)	4 (1.9)	2 (3.1)
Laboratory tests			
Serum creatinine, mg dl^{-1}	0.79 ± 0.28	0.79 ± 0.21	0.8 ± 0.44
GFR, ml min^{-1}/1.73 m^{-2}	83 ± 19.4	85 ± 15.8	79 ± 28.2[a]
Total cholesterol, mg dl^{-1}	147 ± 33	148 ± 34	145 ± 33
Echocardiographic parameters			
LVEDD, mm	47.0 ± 4.7	47.2 ± 4.4	46.2 ± 5.5
LV mass index, g m^{-2}	132 ± 37	125 ± 31	153 ± 47[a]
IVSd, mm	14.9 ± 4.5	14.2 ± 4.1	16.9 ± 5.2[a]
PWd, mm	10.7 ± 2.5	10.5 ± 1.7	11.4 ± 4.1[a]
Maximal LV wall thickness, mm	18.5 ± 3.7	18.2 ± 3.3	19.5 ± 4.7[a]
LVEF, %	68.6 ± 8.8	69.3 ± 7.3	66.1 ± 12.3
E/e′	14.6 ± 5.7	14.2 ± 5.4	16.1 ± 12.3[a]
Ischemic findings, n (%)	126 (46)	102 (49)	24 (37)
Medications			
Beta blocker, n (%)	187 (68)	138 (66)	49 (75)
Non-dihydropyridineCCB, n (%)	99 (36)	70 (34)	29 (44)
ACEi/ARB, n (%)	143 (52)	104 (50)	39 (60)
Statin, n (%)	122 (45)	97 (47)	25 (38)

Abbreviations: ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; CCB, calcium channel blocker; ESRD, end stage renal disease; GFR, glomerular filtration rate; Ischemic findings, ischemic findings in treadmill exercise tests or 24 h Holter monitoring; IVSd, interventricular septal width at diastole; LV, left ventricle; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; PWd, posterior wall width at diastole. Data are expressed as the number (%) or mean ± s.d.
[a]$P<0.05$ vs the unscreened group. A full color version of this table is available at the *Journal of Human Genetics* journal online.

high-risk subjects it was 63 ± 13 years (134 males) and 46 ± 13.3 years (36 males), respectively. Their mean body mass index (BMI) value was 24.2 ± 3.4. The number of patients who had either current hypertension or a history of hypertension, diabetes, chronic kidney disease or end-stage renal disease were 145 (53%), 46 (17%), 34 (12%) and 6 (2.2%), respectively. The mean estimated glomerular filtration rate was 83 ± 19.4 ml min^{-1} 1.73 m^{-2} and the level of serum creatinine was 0.79 ± 0.28 mg dl^{-1}. The high-risk group was significantly younger (44 ± 15 vs 63 ± 13 years, $P<0.001$) and had lower glomerular filtration rate values (79 ± 28.2 vs 85 ± 15.8, $P=0.049$) than the low-risk group. However, the mean level of serum creatinine was not significantly different between the two groups (0.79 ± 0.21 vs 0.8 ± 0.44 mg dl^{-1}, $P=0.82$). The mean maximum LV wall thickness and the mean LV ejection fraction of the study population were 18.0 ± 3.9 mm and 68.6 ± 8.8%, respectively. The mean LV end diastolic dimension and LVMI were 47.0 ± 4.7 mm and 132 ± 37 g m^{-2}, respectively. The mean *E*/*e*′ value was 14.6 ± 5.7, and 45 (16%) patients had LVOT obstruction. Maximum LV wall thickness (19.5 ± 4.7 vs 18.2 ± 3.3 mm, $P=0.012$), inter-ventricular septal thickness (16.9 ± 5.2 vs 14.2 ± 4.1 mm, $P<0.001$) and posterior wall thickness (11.4 ± 4.1 vs 10.5 ± 1.7 mm, $P=0.009$) were significantly higher in the high-risk group compared with the low-risk group. LVMI (125 ± 31 vs 153 ± 47 g m^{-2}, $P=0.002$) and *E*/*e*′ (16.1 ± 12.3 vs 14.2 ± 5.4, $P=0.031$) were also significantly higher in the high-risk group. Among 273 patients, 126 patients had positive findings in their treadmill exercise tests. The results of treadmill exercise test did not show significant difference between the high- and low-risk groups. There was no difference in the usage of medications such as beta blockers, calcium channel blockers or angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers between the two groups.

Table 2 shows the screening result characteristics of the high-risk group. In the high-risk population, HCM was of asymmetric septal hypertrophy type in 12 patients (18%) and atypical in 53 patients (82%). Sixteen patients (25%) had documented arrhythmia, 10 patients (15%) had AF, 5 patients (8%) had NSVT, 2 patients (3%) had complete AV block and 1 patient (1%) had AT. The mean PR interval on ECGs was 166 ± 34 ms, and the mean HR was 63 ± 8 b.p.m. Significant PR shortening (<120 ms) was noted in ECGs from 5 patients (8%). Thirty-eight patients (58%) had symptoms related to autonomic dysfunction: 35 patients (54%) had recurrent dizziness of unknown origin, 12 patients (18%) had OH confirmed by upright BP tests, 5 patients (8%) had vasovagal syncope that was confirmed by HUT, and 2 patients (3%) had CI in treadmill exercise tests. Based on our proposed screening criteria, the number of patients who fulfilled one, two, three and four of our criteria were 31 (48%), 18 (28%), 15 (23%) and 1 (1%), respectively.

Eight of the 65 high-risk patients (12.3%) and 10 of the 208 low-risk patients (4.8%) had lower than normal α-GAL A enzyme values and underwent gene mutation studies for the final diagnosis of FD. After the gene study, 3 patients (4.6%) from the high-risk group were confirmed with FD, whereas none were found to have FD in the low-risk group. The detection rates of FD in the high-risk group using the proposed screening criteria were 4.6% (3/65) for one matched criterion, 8.8% (3/34) for ⩾2 matched criteria, 18.8% (3/16) for ⩾3 matched criteria and 100% (1/1) for all four matched criteria. The clinical characteristics of three HCM patients with FD are shown in Table 3. Two of these patients were female and one was male. Their ages were 49, 44 and 45 years (46 ± 2.6 years). The maximum LV wall thickness ranged from 16 to 23 mm (18.3 ± 4.0 mm). LVOT obstruction was not observed in any of the patients. Two patients had diffuse HCM pattern, whereas the other had ASH (Figure 1). A past documented history of arrhythmia or current arrhythmia was noted in two patients. All patients had short PR (108, 104 and 110 ms). Moreover, all patients complained of symptoms related to autonomic dysfunction.

Clinical characteristics and results of the genetic analysis are presented in Table 3. Patients 1 and 2 did not show any systemic complications of FD other than cardiac involvement. Patient 1 had a previously described nonsense *GLA* mutation c.678G>A (p.Trp226*),[23] whereas patient 2 had a previously described missense mutation c.671A>G (p.Asn224Ser).[24] In patient 3 (male), however, a novel variant was detected. A splice site mutation in intron 3, c.547 +3A>G, was noted. The pathogenicity of this variant was supported by the patient's medical history, clinical symptoms and the result of *in silico* analyses using MutationTaster.[25] This patient had extremely low α-GAL A enzyme activity (1.7 nmol per mg protein per h; normal range: 17.6–57.6 nmol per mg protein per h) and he presented with typical symptoms of classic FD, including angiokeratoma, anhidrosis, end-stage renal disease, hearing loss, stroke and polyneuropathy. He also had a family history of sudden cardiac death in his mother and maternal aunt. In addition, based on the MutationTaster analyses, the site of alteration is a working splice site. Thus the variation is likely to disturb normal splicing and is predicted to be a disease-causing mutation.

DISCUSSION

We proposed four screening criteria for the detection of patients at high risk of FD among those with diagnosed or suspected HCM. We found 3 patients (1.1%) with FD out of 273 consecutive HCM

Table 2 Characteristics of screening criteria in high-risk population

	High-risk population (n = 65)
Type of HCM	
Typical (ASH), *n* (%)	12 (18)
Atypical, *n* (%)	53 (82)
Mid-ventricular hypertrophy	3 (5)
Diffuse concentric LV hypertrophy	48 (74)
Predominantly free wall hypertrophy	2 (3)
Arrhythmia, *n* (%)	16 (25)
AF, *n* (%)	10 (15)
NSVT, *n* (%)	5 (8)
Complete AV block, *n* (%)	2 (3)
AT, *n* (%)	1 (1)
PR shortening (<120 ms), *n* (%)	5 (8)
PR interval, ms	165.5±34.2
Heart rate, b.p.m.	63.4±7.9
Symptoms of autonomic dysfunction, *n* (%)	38 (58)
Recurrent dizziness, *n* (%)	35 (54)
Orthostatic hypotension, *n* (%)	12 (18)
Syncope, *n* (%)	5 (8)
Chronotropic incompetence, *n* (%)	2 (3)

Abbreviations: AF, atrial fibrillation; ASH, asymmetric septal hypertrophy; AV, atrioventricular; AT, atrial tachycardia; HCM, hypertrophic cardiomyopathy; LV, left ventricle; NSVT, non-sustained ventricular tachycardia. Data are expressed as the number (%) or mean±s.d.

Table 3 Clinical characteristics of patients with Fabry disease

	Patient 1	*Patient 2*	*Patient 3*
Age, years	49	44	45
Sex	Female	Female	Male
Max. LV thickness, mm	16	16	23
Type of Fabry disease	Cardiac variant	Cardiac variant	Classic
α-Gal A enzyme activity[a] (nmol per mg protein per h)	0.32	<8.5	1.7
GLA gene mutation	c.678G>A	c.671A>G	c.547+3A>G
HCM type	Diffuse	Diffuse	ASH
Arrhythmia	Paroxysmal AF	None	NSVT
Short PR interval (ms)	Yes (108)	Yes (104)	Yes (110)
Autonomic symptoms	Recurrent dizziness	Syncope	Orthostatic hypotension
Renal function	Normal	Normal	ESRD
Clinical events	AF ablation	None	KT, ICD
Angiokeratoma	None	None	Yes
Stroke	None	None	Yes
ERT	+	+	+
Total number of criteria	4	3	3

Abbreviations: AF, atrial fibrillation; ASH, asymmetric septal hypertrophy; ERT, enzyme replacement therapy; ESRD, end-stage renal disease; HCM, hypertrophic cardiomyopathy; I CD, implantable cardioverter defibrillator; KT, kidney transplantation; LV, left ventricle; NSVT, non-sustained ventricular tachycardia; α-GAL A, α-galactosidase A.
[a]Normal range of α-Gal A enzyme activity in leukocytes, 17.6–57.6 nmol per mg protein per h.

patients. This is similar to the prevalence of FD reported in previous studies in which a large number of unselected HCM patients were screened for FD.[5,6] Using our new screening criteria, we found three FD patients (4.6%) among 65 selected high-risk patients. In the highly selected groups who fulfilled ⩾3 criteria, we achieved a high positive predictive value (3/16 patients; 18.8%) for FD.

Based on previous studies, we included patients with HCM who did not have typical asymmetric septal hypertrophy and classified these patients with an atypical HCM pattern. Analyses of echocardiographic data from studies that investigated FD among unexplained LVH or late-onset HCM patients show that FD occurs more frequently in concentric LVH patients compared with asymmetric LVH patients.[12,13,26] Nakao *et al.*[2] screened for FD in male patients with LVH and found 7 FD patients out of 230 study subjects. However, no FD patient was found among 27 ASH patients. In our study, two cardiac variant FD patients (patients 1 and 2) showed diffuse HCM pattern and one classic FD patient (patient 3) showed ASH (Figure 1). However, this classic FD patient had an early-onset type of FD, with a history of long-term hemodialysis and severe myocardial fibrosis and calcification in the posterior wall detected on cardiac MRI. This may be a compatible finding with the burnout stage of the posterior wall owing to myocardial fibrosis, with a relatively thin wall compared with other walls.

Accumulation of globotriaosylceramide (GL3) affects cardiomyocytes, vascular endothelial cells and smooth muscle cells, as well as cells of the conduction system, eventually causing irreversible cardiac damage even in the early stages.[27,28] Consequently, various arrhythmias, such as AF and NSVT, occur more frequently in cardiac FD patients compared with normal subjects.[29,30] In this study, among the 65 high-risk patients, 16 patients (25%) showed significant arrhythmias either in the resting ECG or during 24-h Holter monitoring. Two FD patients, patients 1 and 3, showed AF and NSVT, respectively.

PR interval shortening is considered an early detectable marker of myocardial storage diseases.[31,32] Moreover, many observational studies and registry data show that PR interval shortening might be a diagnostic symptom of FD.[6,13] In this study, PR interval shortening (<120 ms) was observed in 5 patients (7.7%). However, all FD patients had short PR intervals on their resting ECGs (Figure 1). These results conflict with results from a previous study. Namdar *et al.*[33] identified 29 patients with short PR intervals among 207 (14%) newly diagnosed FD patients. Because all 65 high-risk patients underwent at least five 12-lead ECGs before FD screening, we observed more frequent PR shortening compared with previous studies.

Because several studies have shown small fiber damage and accumulation of lipids in the autonomic ganglia in Fabry patients, symptoms compatible with autonomic dysfunction have generally been attributed to autonomic neuropathy.[34,35] Autonomic nervous system dysfunction accounts for unexplained syncope, dizziness, OH, CI, gastroparesis, diarrhea, constipation and bladder disorders. In this study, 38 patients (58.5%) had symptoms related to autonomic dysfunction. The three FD patients had autonomic dysfunction symptoms, including unexplained dizziness, syncope and OH. However, symptoms of autonomic dysfunction are vague and frequently cannot be clearly distinguished. Therefore, we took careful health histories and performed thorough examinations, including treadmill exercise tests, HUT and upright BP tests. Unexplained syncope (patient 2) and OH (patient 3) were confirmed by positive HUT and upright BP tests, respectively. Although thorough cardiological, neurological and ear examinations were performed, we could not find any cause of recurrent dizziness in patient 1. Therefore, we concluded that her recurrent dizziness is related to autonomic dysfunction.

The prognosis of patients with cardiac FD is significantly improved through enzyme replacement therapy, particularly if initiated during the early stages. Hence, it is of paramount importance to effectively screen for and detect FD in patients with HCM. However, in previous large cohort screening studies, the prevalence of FD in patients with HCM ranged from 0.5% to 1%.[5,6] Because of this low frequency, it is

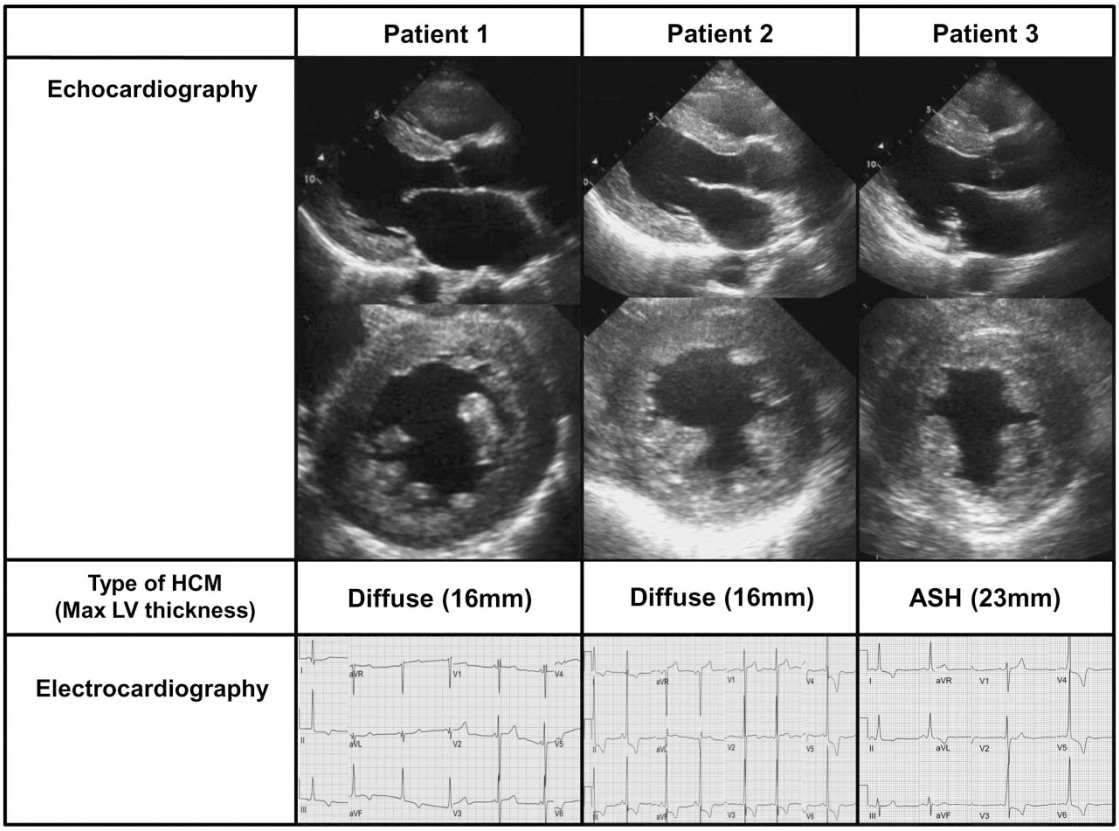

Figure 1 Echocardiography and ECG findings of cardiac Fabry disease patients. A parasternal long-axis (upper panel) and short-axis (middle panel) view of trans-thoracic echocardiogram and 12-lead ECG (lower panel) before diagnosis of the FD. Patients 1 and 2 had diffuse concentric LV hypertrophy and patient 3 had asymmetric septal hypertrophy on echocardiogram. All three patients had short PR intervals on ECG. ASH = asymmetric septal hypertrophy; HCM = hypertrophic cardiomyopathy; LV = left ventricle. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

debatable whether biochemical testing of α-GAL A activity and *GLA* gene mutation analyses should be standard in the clinical evaluation of all HCM patients. Therefore, we need more sensitive screening criteria to select patients within the HCM population who are at the highest risk of FD. Using our proposed screening criteria, we selected 65 high-risk patients from 273 HCM patients. After screening all 273 patients for α-GAL A activity, we found 18 patients with low enzyme activity. Of these, three patients from the high-risk group were confirmed by gene test to have FD. Our detection rate of 4.6% (3/65) is significantly higher than those reported in previous screening studies. Moreover, if this screening is confined to 16 very-high-risk patients who fulfilled $\geqslant 3$ of our criteria, the positive predictive value for FD is extremely high (18.8%, 3/16). Therefore, our new screening criteria might serve as a tool to select patients from the HCM population as having a high risk of FD.

There are limitations that should be considered when interpreting our findings. We did not include other systemic factors for FD, including gender, onset age, family history or renal impairment, in our criteria. Although inclusion of such systemic factors would have further increased the detection rate, we wanted to specifically distinguish high-risk patients and effectively diagnose FD in clinical practice based solely on cardiac factors and symptoms. Also, we included symptoms of autonomic dysfunction as one of our screening criteria but did not quantify the degree of autonomic dysfunction. Autonomic nervous system dysfunction is very common in FD. However, it is very difficult to delineate symptoms caused by autonomic dysfunction vs other various non-specific symptoms. HUT, upright BP tests and treadmill exercise ECG tests were performed to objectify autonomic dysfunction in the study participants. Furthermore, neurological, psychological and ear examinations were used to rule out other causes of autonomic dysfunction symptoms.

The aim of the newly proposed criteria was to detect patients at high risk of FD from unexplained LVH population simply by observing cardiac factors alone. Unselective screening for FD in all HCM patients would have clinical benefits for detecting FD in the entire HCM population.[5] However, the detection rate of FD in unselected HCM patients is very low (0.5–1%).[5,6] Therefore, such screening is neither cost-effective nor efficient. When patients to be screened are selected based upon consideration of various clinical factors, the cost of screening decreases. This concept helped us to develop a novel set of screening criteria to select patients at the highest risk for FD in this HCM population. We diagnosed 3 FD patients out of the total 273 HCM patients (1.1%), which is comparable with results from previous large cohort studies.[5,6] However, the new criteria for FD effectively increased the detection rate (4.6–18.8%).

In conclusion, using our newly proposed criteria, HCM patients were classified as having either high or low risk of FD. The prevalence of FD in the high-risk group was 4.6% in patients with at least one criterion and 18.8% for patients with $\geqslant 3$ criteria. Therefore, our proposed criteria are easily applicable and highly sensitive for the selection of patients at high risk for FD in patients with HCM.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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