Fabry Disease in Families With Hypertrophic Cardiomyopathy
Clinical Manifestations in the Classic and Later-Onset Phenotypes

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Background—The screening of Icelandic patients clinically diagnosed with hypertrophic cardiomyopathy resulted in identification of 8 individuals from 2 families with X-linked Fabry disease (FD) caused by GLA (α-galactosidase A gene) mutations encoding p.D322E (family A) or p.I232T (family B).

Methods and Results—Familial screening of at-risk relatives identified mutations in 16 family A members (8 men and 8 heterozygotes) and 25 family B members (10 men and 15 heterozygotes). Clinical assessments, α-galactosidase A (α-GalA) activities, glycosphingolipid substrate levels, and in vitro mutation expression were used to categorize p.D322E as a classic FD mutation and p.I232T as a later-onset FD mutation. In vitro expression revealed that p.D322E and p.I232T had α-GalA activities of 1.4% and 14.9% of the mean wild-type activity, respectively. Family A men had markedly decreased α-GalA activity and childhood-onset classic manifestations, except for angiotokeratoma and cornea verticillata. Family B men had residual α-GalA activity and developed FD manifestations in adulthood. Despite these differences, all family A and family B men >30 years of age had left ventricular hypertrophy, which was mainly asymmetrical, and had similar late gadolinium enhancement patterns. Ischemic stroke and severe white matter lesions were more frequent among family A men, but neither family A nor family B men had overt renal disease. Family A and family B heterozygotes had less severe or no clinical manifestations.

Conclusions—Men with classic or later-onset FD caused by GLA missense mutations developed prominent and similar cardiovascular disease at similar ages, despite markedly different α-GalA activities. (Circ Cardiovasc Genet. 2017;10:e001639. DOI: 10.1161/CIRCGENETICS.116.001639.)

Key Words: cardiomyopathy, hypertrophic ▫ Fabry disease ▫ gadolinium ▫ magnetic resonance ▫ mutation ▫ phenotype ▫ stroke

Fabry disease (FD) is an X-linked lysosomal storage disease caused by pathogenic GLA (α-galactosidase A gene) mutations that result in the absent or markedly decreased activity of the encoded lysosomal hydrolase, α-galactosidase A (α-GalA). This enzyme deficiency results in progressive accumulation of glycosphingolipids with terminal α-galactosyl moieties, primarily globotriaosylceramide and its deacetylated derivative, lysoglobotriaosylceramide, in fluids and cellular lysosomes throughout the body. There are 2 major clinical subtypes of FD: the early-onset classic and later-onset phenotypes. Cardiac involvement is common in affected men and to a lesser extent in female heterozygotes with both phenotypes. Globotriaosylceramide accumulation occurs in all cardiac cells, particularly in cardiomyocytes, leading to left ventricular hypertrophy (LVH), which can morphologically and clinically mimic autosomal dominant hypertrophic cardiomyopathy (HCM). Studies of large cohorts of HCM patients have identified FD as the cause of LVH in 0.5% to 1% of cases.

See Editorial by Elliott
See Clinical Perspective
Men with the classic phenotype have little or no functional \(\alpha\)-GalA enzymatic activity and marked microvascular endothelial globotriaosylceramide accumulation. In childhood or adolescence, these men typically develop early-onset symptoms, including acroparesthesia, angiokeratoma, hypohidrosis, gastrointestinal symptoms, and the characteristic corneal dystrophy. With age, they develop LVH, renal failure, or cerebrovascular disease. Before the advent of renal replacement therapy, the average age of death of classically affected men was 41 years.

By contrast, men with the later-onset phenotype have residual \(\alpha\)-GalA activity, less substrate accumulation, little or no microvascular endothelial globotriaosylceramide accumulation, and lack the early manifestations observed in men with the classic phenotype. They typically develop cardiac or kidney disease in the third to seventh decades of life and often remain unrecognized throughout life. Later-onset patients are typically identified by screening performed in cardiac, hemodialysis, kidney transplant, and stroke clinics and more recently by newborn screening.17,8,10-13

To date, >850 mutations in the \(\alpha\)-GalA gene have been reported (Human Gene Mutation Database; http://www.hgmd.org).14 Nonsense, frame-shift, consensus splice site and some missense mutations cause the classic phenotype, whereas some missense mutations and alternative splicing mutations that result in residual \(\alpha\)-GalA activity cause the later-onset phenotype. Although many \(\alpha\)-GalA missense mutations have been reported for various lesions, the description of their associated clinical phenotypes are limited or absent.15,16 In particular, few previous studies have reported the manifestations of the later-onset phenotype, with the notable exception of the common Taiwanese later-onset \(\alpha\)-GalA alternative splicing mutation c.936+919G>A (IVS4+919G>A).13,17 Moreover, within a given family, phenotypic variation may be present,18 which may be because of other presumptive modifying/protective genes, as well as lifestyle or environmental factors.

A study of the genetic causes of HCM in Iceland identified 8 FD patients from 2 unrelated families.19 Family A had the \(\alpha\)-GalA mutation, c.966G>A, encoding p.D322E (designated D322E), and family B had the \(\alpha\)-GalA mutation, c.695T>C, encoding p.I232T (designated I232T). Recently, D322E was reported in a German patient.22 No clinical information was reported for either of these cases, and no evaluations of at-risk family members were performed. Here, we describe the enzymatic activities, substrate concentrations, and clinical manifestations of 8 index patients, initially diagnosed as HCM, and 33 affected family members. Our data illustrate the spectrum and variability of manifestations among affected men with classic and later-onset FD.

**Methods**

This study was approved by the National Bioethics Committee of Iceland (NBC 13-086) and the Icelandic Data Protection Authority. Informed consent was obtained from the participants.

**Subjects and FD Diagnosis**

Eight index patients with FD were identified in a nationwide study of the genetic causes of HCM in Iceland (all without mutations in the genes encoding 8 sarcomere proteins, protein kinase AMP-activated noncatalytic subunit \(\gamma_2\) [PRKAG2] and lysosomal-associated membrane protein-2 [LAMP2]).23 They were members of 2 large families whose pedigrees were obtained. All at-risk relatives were invited to undergo targeted mutation analyses for the respective D322E and I232T mutations (PreventionGenetics, Marshfield, WI) and subsequent analyses, including routine clinical laboratory examinations, \(\alpha\)-GalA enzyme analyses, and measurements of urinary globotriaosylceramide or plasma lyso-globotriaosylceramide concentrations. Selected patients underwent kidney biopsy to establish their diagnosis based on pathological evidence of lysosomal globotriaosylceramide storage. The diagnoses of classic and later-onset (non-classic) FD were in accordance with published FD diagnostic criteria.21

**\(\alpha\)-GalA Activity and Globotriaosylceramide Analysis**

Leukocyte \(\alpha\)-GalA activities and urinary globotriaosylceramide concentrations were assayed at the Laboratory for Clinical Chemistry, Sahlgrenska University Hospital, Gothenburg, Sweden. \(\alpha\)-GalA activity was expressed as a percentage of the mean value in normal control subjects. Lyso-globotriaosylceramide was measured in 19 subjects by Centogene (Rostock, Germany).

**In Vitro \(\alpha\)-GalA Expression Analysis**

The full-length wild-type \(\alpha\)-GalA cDNA was cloned into a pASc8 vector.24 Constructs carrying the mutation encoding D322E or I232T were generated by site-directed mutagenesis using polymerase chain reaction (Stratagene, La Jolla, CA). The sequences of both constructs were confirmed by resequencing, and plasmids were prepared using a Qiagen Plasmid Midi Kit (Valencia, CA). The wild-type control and mutant constructs were individually transfected into COS-7 cells, and analysis of intracellular \(\alpha\)-GalA activity was performed as previously described.25

**Clinical Assessment**

Comprehensive evaluations of FD manifestations included recording of medical history, physical examination, assessment of kidney function, standard clinical biochemical testing, 12-lead electrocardiography, and imaging studies (Table I in the Data Supplement). The serum creatinine levels were measured, and the estimated glomerular filtration rate (GFR; n=30) was determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. In addition, the GFR was directly measured in a subset of 8 patients residing in Denmark. Assessment of proteinuria was performed by determining the albumin-to-creatinine ratio (ACR; n=36) in spot morning urine specimens or by measuring the total protein excretion in 24-hour urine samples (n=8). Microalbuminuria was defined as an ACR of >2.5 (heterozygotes) or >3.5 mg/mmol (men) or a urinary albumin excretion rate of >30 mg per 24 hours. Overt albuminuria was defined as an ACR of >30 mg/mmol. Chronic kidney disease was classified according to the KDIGO (Kidney Disease: Improving Global Outcomes) classification system.26

**Imaging Studies**

Cardiac and brain magnetic resonance imaging (MRI) examinations were performed using a clinical 1.5T MRI scanner (Siemens Magnetom Avanto B17 and Siemens Magnetom Avanto fit; Siemens, Erlangen, Germany). All MRI analyses were performed by the investigators (Drs. Appelbaum, Maron, Gardarsdottir, and Neisius), who were blinded to patient identities.

Maximal left ventricular wall thickness (mLVWT) was defined as the greatest dimension at any site within the left ventricular myocardium. LVH was defined as an mLVWT ≥12 mm or an increased left ventricular mass index according to published data on the normal limits for age and sex.27 The mLVWT was based on standard echocardiographic measurements in a subset of patients (n=14) for whom cardiac MRI was unavailable (Methods section in the Data Supplement). Longitudinal left ventricular wall thickness (LVWT) measurements were available for the 8 index patients initially diagnosed with HCM. Septal-to-lateral wall ratios (SLRs) were calculated by determining the average segment wall thicknesses and dividing the thickest septal segment by the thickest lateral segment. Asymmetrical hypertrophy was defined as an SLR of ≥1.3, and concentric wall thickening was defined as an SLR of <1.3.
Cerebral white matter signal intensity changes on fluid attenuated inversion recovery (FLAIR) images were rated using the modified Fazekas scale\textsuperscript{38,39} and categorized as mild, moderate, or severe white matter hyperintensities (WMHs). The MRI protocols are described in detail in Methods section and Figure 1 in the Data Supplement.

Statistical Analysis
Descriptive data are presented as mean±SD or mean (range) for continuous variables and as proportions for categorical variables. Spearman correlation coefficients were calculated to assess correlations between continuous variables. To account for relatedness between individuals, we calculated the kinship of all relative pairs and used the method proposed by Parisseaux and Bernardo\textsuperscript{30} as implemented in the R package linear mixed effects models for genome-wide association studies (https://CRAN.R-project.org/package=lmem.gwaser). \textit{P} values <0.05 were considered significant.

Results
Subjects
Evaluation of the pedigrees of the 8 index patients initially diagnosed with HCM resulted in the identification of an additional 33 mutation-confirmed individuals. Of the 41 total patients, 16 family A members (8 affected men and 8 female heterozygotes) had the \textit{GLA} mutation encoding D322E and fulfilled the criteria for classic FD, and 25 family B members (10 affected men and 15 female heterozygotes) had the \textit{GLA} mutation encoding I232T that causes later-onset FD (Figure 1). The major clinical and biochemical characteristics of the patients in each family are summarized in Table 1. The detailed findings for all patients with \(\alpha\)-GalA activity <40\% of the mean reference value in leukocytes are shown in Table IIA and IIB in the Data Supplement.

The mean delay in correctly diagnosing the 8 index patients was 14 years (range, 5–26 years) from the initial HCM diagnosis to the FD diagnosis. The 3 family A index men were older (50, 54, and 61 years of age; mean, 55 years) and had more advanced disease than the 5 men identified by pedigree analyses (20, 22, 30, 48, and 51 years of age; mean, 32 years; \(P=0.03\)). Similarly, the 2 family B index men were older (59 and 61 years of age; mean, 60 years) and had more advanced disease than the 8 men identified by pedigree analyses (9, 10, 12, 39, 42, 46, 46, and 50 years of age; mean, 34 years; \(P=0.02\)).

\(\alpha\)-GalA Activity and Globotriaosylceramide Analysis
The affected men in family A (D322E mutation) had lower leukocyte \(\alpha\)-GalA activity (mean, 1.5\% of mean normal activity; range, 0.7–2.5\%) than the family B men (mean, 7.4\%; range, 3.6\% to 11\%; \(P<0.0001\); Figure 2) and higher plasma lysoglobotriaosylceramide (mean, 106; range, 91–125 versus mean, 12; range, 1.7–16 ng/mL; \(P<0.0001\)) and urinary globotriaosylceramide levels (mean, 369; range, 108–981 versus mean, 13; range, 1.0–27 \(\mu\)mol/mol creatinine; \(P=0.02\); Table 1). Family A heterozygotes had similar \(\alpha\)-GalA activities (mean, 56\%; range, 29\% to 72\%) to family B heterozygotes (mean, 45\%; range, 12\% to 72\%; \(P=0.44\)). Only 1 heterozygote from family A (A-IV:2) had markedly increased urinary globotriaosylceramide (216 \(\mu\)mol/mol), whereas all other heterozygotes had normal or slightly elevated urinary globotriaosylceramide levels.

\textbf{In Vitro \(\alpha\)-GalA Expression Analysis}
The intracellular \(\alpha\)-GalA activities expressed in COS-7 cells by the mutant constructs encoding the D322E and I232T mutations...
### Table 1. Baseline Characteristics of Affected Men and Female Heterozygotes in Family A (D322E) and Family B (I232T)

<table>
<thead>
<tr>
<th>GLA Mutation/Phenotype</th>
<th>Family A</th>
<th>Family B</th>
<th>P Value, D322E vs I232T, Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men (n=8)</td>
<td>42 (20–61)</td>
<td>37 (13–69)</td>
<td></td>
</tr>
<tr>
<td>Heterozygotes (n=8)</td>
<td>38 (9–61)</td>
<td>39 (8–72)</td>
<td></td>
</tr>
<tr>
<td>Leukocyte α-GalA activity,* μkat/kg protein</td>
<td>0.4 (0.2–0.7)</td>
<td>16 (8.4–21)</td>
<td></td>
</tr>
<tr>
<td>α-GalA, % of mean normal activity</td>
<td>1.5 (0.7–2.5)</td>
<td>56 (30–72)</td>
<td></td>
</tr>
<tr>
<td>Urinary globotriaosylceramide,† μmol/mol creatinine</td>
<td>369 (108–981)</td>
<td>55 (11–216)</td>
<td>0.02</td>
</tr>
<tr>
<td>Lyso-globotriaosylceramide,‡ ng/mL</td>
<td>106 (91–125)</td>
<td>13§</td>
<td>12 (1.7–16)</td>
</tr>
</tbody>
</table>

#### Classical signs/symptoms

- ≥1 Classical sign/symptoms, n (%) 7 (88) 2 (25) 1 (10) 1 (6.7) 0.0002
- Angiokeratoma, n (%) 0 0 0 0 1.00
- Hypohidrosis, n (%) 6 (75) 0 1 (10) 0 0.002
- Acroparesthesia, n (%) 7 (88) 1 (13) 0 0 0.0001
- Corneal dystrophy, n (%) 1 (13) 0 0 0 0.24
- Abdominal discomfort, n (%) 6 (75) 1 (13) 0 0 0.0002
- Hypoacusis, n (%) 2 (25) 1 (13) 0 1 (6.7) 1.00

#### Cerebrovascular manifestations

- mLVWT ≥12 mm, n (%) 5 (63) 2 (25) 7 (70) 3 (20) 0.74
- PM/ICD, n (%) 2 (25) 0 1 (10) 0 0.39
- Atrial fibrillation, n (%) 4 (50) 1 (13) 2 (20) 1 (6.7) 0.64
- Coronary artery disease,§ n (%) 2 (25) 0 2 (20) 1 (6.7) 1.00
- Hypertension, n (%) 0 1 (13) 0 1 (6.7) 1.00

#### Cerebral manifestations

- Cerebral infarct, n (%) 4 (50) 1 (13) 1 (10) 3 (20) 0.05
- Mild WMH, n (%) 2 (25) 1 (13) 3 (30) 3 (20) 0.81
- Moderate WMH, n (%) 0 1 (13) 1 (10) 2 (13) 1.00
- Severe WMH, n (%) 3 (38) 1 (13) 1 (10) 0 0.47
- Dolichoectasia, n (%) 2 (25) 1 (13) 0 0 0.10
- Basilar artery diameter, mm 43 (32–64) 33 (24–44) 0 0 0.11
- Pulvinar hyperintensity, n (%) 0 0 0 0 1.00

#### Renal manifestations

- GFR, mL/min per 173 m² 103 (68–122) 108 (66–133) 107 (81–134) 101 (62–136) 0.59
- Serum creatinine, μmol/L 74 (60–82) 62 (48–79) 68 (71–89) 57 (37–82) 0.43
- Microalbuminuria, n (%) 2 (25) 1 (13) 3 (30) 1 (6.7) 0.81
- Overt albuminuria, n (%) 1 (13) 1 (13) 1 (10) 0 0.87
- Enzyme replacement therapy,¶ n (%) 8 (100) 0 3 (30) 0 0.0004

Continuous variables are expressed as the mean (range). α-GalA indicates α-galactosidase A; GFR, glomerular filtration rate; ICD, implantable cardioverter defibrillator; mLVWT, maximal left ventricular wall thickness; PM, pacemaker; and WMH, white matter hyperintensities.

*Reference range for α-GalA activity in leukocytes: 22–36 (mean, 28.5) μkat/kg protein.
†Reference range for urinary globotriaosylceramide: <10 μmol/mol creatinine.
‡Reference range for lyso-globotriaosylceramide: <1 ng/mL.
§Defined as ≥50% narrowing in ≥1 epicardial vessel.
¶Decision concerning enzyme replacement therapy had not been made for several of the patients at the time of the study.
missense mutations were 1.5±0.4% and 14.9±2.1% of the mean expressed wild-type activity, respectively. These levels of expression are consistent with mutations causing the classic and later-onset phenotypes, respectively.25

**Signs and Symptoms**

In family A, the classical FD signs and symptoms, including hypohidrosis, acroparesthesia, abdominal discomfort, or hypoacusis, were present in 7 of 8 affected men and in 2 heterozygotes. Surprisingly, only 1 man had cornea verticillata, and none had angiokeratoma.

By contrast, in family B, no patient had classical FD signs or symptoms, although 1 man reported hypohidrosis.

**Cardiac Manifestations**

**Classic Family A (D322E)**

As shown in Figure 3A, all 5 men aged ≥34 years had LVH, with mLVWTs ranging from 13 to 24 mm. The mean age at LVH diagnosis for the men was 46 years (range, 34–51 years). Among the 8 heterozygotes, only the 2 oldest had LVH (aged 49 and 56 years at LVH diagnosis), with mLVWTs of 17 and 20 mm, respectively. Table 2 shows the cardiac MRI findings for 8 men and 5 heterozygotes who underwent the study. Late gadolinium enhancement (LGE) was observed in 5 men and 1 heterozygote, all of whom had LVH, occupying between 0.5% and 21% (mean, 8.5%) of the total myocardial mass. The LGE burden increased with age (Figure 3B) and mLVWT (Figure 3C). Based on the American Heart Association 16-segment model for the distributions of LVH and LGE, all subjects had LGE but normal LVWT in the basal inferolateral wall (Figure 4A and 4B). The pattern of LVH was asymmetrical in 5 (83%) of the subjects (4 men and the heterozygote), whereas only 1 man had concentric hypertrophy, with an SLR of <1.3. LGE was confined to the basal inferolateral wall in only 1 man and 1 heterozygote, whereas 3 men and extensive widespread LGE, the third man had mild widespread LGE, and 1 heterozygote had LGE predominantly in the apex.

Three individuals with LVH had a history of intermittent or persistent atrial fibrillation, including 2 men and 1 heterozygote. One of the men had an implanted pacemaker because of a third-degree atrioventricular block.

**Later-Onset Family B (I232T)**

All 7 men aged ≥38 years had LVH, with mLVWTs ranging from 12 to 26 mm (Figure 3A). The mean age at LVH diagnosis for the men was 46 years (range, 38–54 years). Among the 15 heterozygotes, 3 had LVH (aged 59, 67, and 71 years at LVH diagnosis), with mLVWTs of 15, 19, and 20 mm, respectively. The cardiac MRI findings for 6 men and 5 heterozygotes are summarized in Table 2. Three men and 1 heterozygote with LVH and 1 heterozygote without LVH displayed midmyocardial LGE, occupying between 0.3% and 21% (mean, 4.9%) of the total myocardial mass. Similar to family A, the burden of LGE increased with age (Figure 3B) and mLVWT (Figure 3C). The distributions of LVH and LGE are shown in Figure 4C and 4D. The pattern of LVH was asymmetrical in 4 (57%) subjects (3 men and the heterozygote), and 3 men had concentric hypertrophy, with an SLR of <1.3. LGE was confined to the basal inferolateral wall in only 1 man and 1 heterozygote, whereas the second man had extensive widespread LGE, the third man had mild widespread LGE, and 1 heterozygote had LGE predominantly in the apex.

Of the subjects with LVH, 4 men and 1 heterozygote had a history of intermittent or persistent atrial fibrillation. Two of the men had an implantable cardioverter defibrillator because of ventricular tachycardia, of whom 1 also had a third-degree atrioventricular block.

**Cerebrovascular Manifestations**

**Classic Family A (D322E)**

Among the 8 men, 4 had a history of ischemic stroke, and 3 (aged 48, 50, and 61 years) had underlying atrial fibrillation and LVH. The fourth and youngest man (A-V:8) had a silent cerebral infarct diagnosed by MRI at 20 years of age. One heterozygote (A-IV:2) had a fatal ischemic stroke at the age of 55 years because of an underlying large fusiform aneurysm (16.7 mm
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Vertebrobasilar dolichoectasia was present in 2 men and 1 heterozygote, and a borderline increased basilar artery diameter (4.4–4.5 mm) was observed in one 51-year-old man and one 16-year-old heterozygote. At least mild WMHs were observed in all men and heterozygotes ≥47 years of age (Table 1). Three men (aged 51, 54, and 61 years) and a 69-year-old heterozygote had severe, confluent WMHs. The 61-year-old man (A-IV:1) had multiple small infarcts and an arteriovenous malformation in the cerebellum that was discovered after he had experienced subarachnoid hemorrhage.

**Later-Onset Family B (I232T)**

Among the 10 men, 1 had ischemic stroke at the age of 59 years and had underlying atrial fibrillation and LVH. Three heterozygotes (aged 43, 71, and 72 years) had suffered ischemic stroke, and the 2 older men had underlying LVH or atrial fibrillation. None of these individuals had dolichoectasia. Mild, moderate, or severe WMHs were observed in all patients aged ≥50 years (Table 1). In addition, a 59-year-old man (B-III:4) had severe WMHs and a history of unexplained severe progressive dementia that was detected 7 years before the FD diagnosis.

**Renal Manifestations**

**Classic Family A (D322E)**

Only 3 family A men, aged 30, 50, and 51 years, had mild albuminuria, with ACRs of 18, 40, and 29 mg/mmol, respectively. The 51-year-old man had a mildly reduced GFR of 68 mL/min per 1.73 m². All other individuals had normal GFR. Of the heterozygotes, 2 had mild albuminuria;
the 56-year-old had an ACR of 41.6 mg/mmol and a normal GFR of 105 mL/min per 1.73 m², whereas the 69-year-old had a slightly increased ACR of 3.9 mg/mmol and a mildly decreased GFR of 66 mL/min per 1.73 m². Hence, only 1 man and 1 heterozygote had stage 2 chronic kidney disease, whereas all other individuals had normal kidney function (Table 1; Table IIB in the Data Supplement).

**Later-Onset Family B (I232T)**

One man, aged 39 years, had overt albuminuria of 1.9 g per 24 hours and a normal GFR of 105 mL/min per 1.73 m². Three men, aged 42, 46, and 61 years, had a normal GFR and mildly increased urinary albumin excretion, with ACRs of 28, 32, and 20 mg/mmol, respectively. These 4 men underwent kidney biopsy, which revealed varying degrees of globotriaosylceramide accumulation in podocytes and modest interstitial fibrosis but no endothelial deposition. Three of the 15 heterozygotes, aged 27, 67, and 71 years, had mild albuminuria, with ACRs of 17, 31, and 23 mg/mmol and GFRs of 93, 81, and 92 mL/min per 1.73 m², respectively. The 67-year-old heterozygote underwent kidney biopsy, which revealed globotriaosylceramide accumulation in podocytes, with modest interstitial and no endothelial globotriaosylceramide deposition. All other family members had normal kidney function (Table 1; Table IIB in the Data Supplement).

**Discussion**

We describe 2 large Icelandic families with the classic (D322E) and later-onset (I232T) phenotypes, for which the probands were identified by screening of Icelandic HCM patients for causative genes, including GLA.19 Although there are many reports describing patients with FD, few have characterized and compared the manifestations in large families with the classic and later-onset phenotypes. Importantly, the findings reported in this study for the 2 families illustrate the shared and variable clinical manifestations that can be present among family members with a specific mutation and phenotype. Specifically, the men in the classic family did not have the typical early manifestations of angiokeratoma and cornea verticillata and did not have significant renal involvement. However, the men from both the classic and later-onset families developed significant cardiac manifestations at similar ages, even though their causative mutations resulted in significantly different α-GalA activities (1.5% versus 7.4% of the normal mean) and lyso-globotriaosylceramide substrate levels (91–125 versus 1.7–16). To our knowledge, this is the first report in the FD literature that has documented both LVH and LGE patterns and their distributions by cardiac MRI in classic and later-onset families. Previous publications have been mainly based on heterogeneous cohorts with various mutations and often inconclusive phenotypic information.

We found that men with both classic and later-onset FD who were >30 years of age had all developed LVH and that LVWT increased with age. Moreover, our data showed that age, mLVWT, and LGE were strongly correlated, but the development of LGE occurred later than that of LVH. Notably, similar LVH and LGE patterns were observed in the subjects with classic and later-onset FD. There was no significant difference in the mean mLVWT, left ventricular mass index, or total LGE burden.

We observed a higher proportion of patients with asymmetrical hypertrophy than previous studies;11 specifically, 83% and 57% of the classic and later-onset patients. Indeed, 8 of the 10 patients with asymmetrical septal hypertrophy were index cases from the HCM cohort. This finding likely accounted for the misdiagnosis of HCM and emphasizes the need to consider FD in sarcomere mutation-negative HCM.

Strikingly, the distribution of LVWT and LGE showed opposite patterns (Figure 4). All of the classic and the majority of the later-onset FD patients with LVH had increased LVWT in the basal anteroseptal wall but normal LVWT in the basal inferolateral wall, whereas LGE was prominent in the basal inferolateral wall but generally absent in the basal anteroseptal...
Previous cardiac MRI literature on FD has documented the basal inferolateral wall as a typical location of LGE. 

This LGE distribution differs from what is usually observed in HCM, in which enhancement is often localized along the thickened myocardium, typically within the anteroseptal wall. Despite these distinctions, our results indicate that the distribution of LGE in FD can overlap with that in HCM, which is in agreement with a recent report by Deva et al. In our cohort, 50% and 25% of the LGE-positive classic and later-onset FD patients, respectively, had prominent LGE in 1 or more left ventricular segments in addition to the basal inferolateral wall, and 17% and 25% had atypical LGE with septal or apical predominance. Likewise, in HCM the LGE distribution can vary, and LGE can be observed in the inferolateral wall only or in the inferolateral wall and other myocardial segments. We suggest that the differences in the LGE distribution between FD and HCM reflect distinct pathophysiological mechanisms. Myocyte hypertrophy and disarray are the typical histological findings in HCM, with overt fibrosis that can be detected by LGE emerging later. By contrast, globotriaosylceramide accumulation in FD cardiomyocytes has been estimated to account for 1% to 2% of the cardiac mass, suggesting that LVH in FD may result from activation of complex signaling pathways that lead to hypertrophy. Moreover, clinical and histological studies have suggested that replacement fibrosis contributes to thinning of the inferolateral wall. LGE may also be detected before the development of LVH as the first sign of cardiac involvement in FD. We observed minor LGE in 1 female patient without LVH; however, that finding does not necessarily reflect a scar and could be related to extracellular matrix expansion alone. The dichotomous findings of increased fibrosis in hypertrophy and focal wall thinning in HCM and FD highlight the need for better understanding of the molecular pathogenesis of myocardial fibrosis and its impact on cardiac physiology.

Prominent cerebrovascular manifestations were observed in this study, particularly in men with classic FD. The family A men had a tendency toward a higher ischemic stroke incidence compared with the family B men (50% versus 10%; P=0.05), but this increase was probably largely secondary to cardiac disease because most of the stroke patients had underlying LVH and documented atrial fibrillation. At least mild WMHs were observed in all patients aged ≥50 years. WMHs have been frequently described in FD in association with the risk of stroke. Whereas WMHs are not specific to FD and are commonly observed in normal aging, severe, confluent
WMHs are associated with a poor prognosis and are rarely observed before the age of 70 years, particularly in the absence of hypertension and other cardiovascular risk factors.\(^4\) Severe WMHs were detected in 38% and 10% of the family A and family B men (aged 51–61 years), respectively, including 1 family A man with mild cognitive dysfunction and 1 family B man with severe progressive dementia presenting at the age of 52 years. Limited data are available on the mechanisms of cognitive impairment in FD patients; findings ranging from mild cognitive deficits to vascular dementia have been correlated with the load of WMHs.\(^4\)

Although men with classic FD frequently develop progressive chronic kidney disease by the second to fifth decade of life,\(^4\) we did not observe this in family A men. Despite other classic FD symptoms, the kidney function in family A men was preserved, although several had mild proteinuria. Longitudinal studies will determine whether they subsequently develop progressive chronic kidney disease.

We recognize several limitations of this study, including the relatively small sample size and incomplete phenotypic data for several of the patients. Notably, the cardiac MRI studies were not performed in 17 subjects for various reasons, as outlined in the Methods section in the Data Supplement. We cannot exclude coronary artery disease as a contributing factor to LGE in the 2 classic and 3 later-onset patients with LVH and coronary artery disease; however, none had a history of myocardial infarct or typical infarction scars on cardiac MRI. Furthermore, FD patients without apparent coronary artery disease can exhibit ischemia from the presence of microvascular lesions and increased myocardial oxygen requirements associated with cardiac FD.\(^4\)

Conclusions
In summary, we evaluated the clinical and biochemical features of affected men and heterozygotes from 2 large Icelandic families with the classic and later-onset phenotypes of FD, discovered by the genetic screening of HCM patients. Importantly, affected men of both the classic and later-onset families developed prominent and similar cardiovascular manifestations, whereas the kidneys were only mildly affected. Clearly, FD remains an important differential diagnosis in patients with unexplained LVH, even in those with asymmetrical hypertrophy, and it is easily diagnosed by GLA mutation analysis.

Acknowledgments
We thank the Danish Fabry Team for providing the data for the family B Danish patients and Daniel F. Gudbjartsson (deCODE genetics, Inc., Reykjavik, Iceland) for statistical assistance.

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Disclosures
Dr Madsen has received an unrestricted research grant from Genzyme. Dr Desnick is a consultant for Genzyme/SanoFil, Amicus Therapeutics, and Sangamo Bioncs and has founder’s shares of Amicus Therapeutics and stock options of Sangamo BioSciences. Dr Feldt-Rasmussen has received speaker honoraria, unrestricted research grants, and served at advisory boards of Genzyme/SanoFil, Shire, and Amicus.

References
Adalsteinsdottir et al. Fabry Disease in HCM Families


**CLINICAL PERSPECTIVE**

This study describes the cardiac and systemic manifestations of classic and later-onset FD in 2 large families who were detected through a nationwide study of the genetic causes of hypertrophic cardiomyopathy in Iceland. Our findings illustrate the shared and variable clinical manifestations that can be present among family members with a specific mutation and phenotype. The men in the class family did not have the typical early manifestations of angiokeratoma and cornea verticillata nor evidence for significant renal involvement. However, men from both the classic and later-onset families developed similar cardiovascular manifestations at similar ages, despite markedly different α-galactosidase A activities. We observed a higher proportion of patients with asymmetrical left ventricular hypertrophy than in previous studies; specifically, 83% and 57% of the patients with classic and later-onset FD, respectively. By contrast, late gadolinium enhancement was most prominent in the basal inferolateral wall, whereas 17% and 25% of the late gadolinium enhancement-positive patients had atypical late gadolinium enhancement with septal or apical predominance, respectively. These results indicate that both the left ventricular hypertrophy pattern and the distribution of late gadolinium enhancement in FD can overlap with that of hypertrophic cardiomyopathy. This article reinforces the importance of accurate evaluation of cardiovascular involvement in FD to better understand the possible genotype-phenotype correlations and possible pathogenic pathways shared with sarcomeric hypertrophic cardiomyopathy. Clearly, FD remains an important differential diagnosis in patients with unexplained left ventricular hypertrophy, even in those with asymmetrical hypertrophy, and it is easily diagnosed by GLA mutation analysis.
Fabry Disease in Families With Hypertrophic Cardiomyopathy: Clinical Manifestations in the Classic and Later-Onset Phenotypes

Berglind Adalsteinsdottir, Runolfur Palsson, Robert J. Desnick, Marianna Gardarsdottir, Polakit Teekakirikul, Martin Maron, Evan Appelbaum, Ulf Neisius, Barry J. Maron, Michael A. Burke, Brenden Chen, Silvere Pagant, Christoffer V. Madsen, Ragnar Danielsen, Reynir Arngrimsson, Ulla Feldt-Rasmussen, Jonathan G. Seidman, Christine E. Seidman and Gunnar Th. Gunnarsson

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Supplemental Methods

Magnetic Resonance Imaging Studies

The cardiac protocol included true fast imaging with steady-state free precession (TrueFISP), dark blood (DB) half-Fourier acquisition single-shot turbo spin-echo (HASTE) transverse and coronal imaging, TrueFISP cine imaging in the 2-chamber, 4-chamber, short-axis (SA) and 3-chamber views, and DB T1 imaging in the 2-chamber, 4-chamber and SA views. In addition, after administration of gadolinium contrast, the protocol included an inversion time (TI) series and late gadolinium enhancement (LGE) series in the 4-chamber, 2-chamber and 3-chamber views, as well as a TurboFLASH phase-sensitive inversion recovery single-shot series in the SA view. The images were transferred to a core laboratory (PERFUSE, Boston, Massachusetts) for centralized and blinded analyses. Cardiac MRI analyses were performed by experienced readers (EA, UN and MM). The left ventricular (LV) volume, ejection fraction, and wall and papillary muscle mass were measured using standard volumetric techniques and were analyzed using commercially available software (QMASS, v7.4; Medis Inc., Leiden, The Netherlands). To quantify LV trabeculations a two-step approach was chosen. First, the area of blood in the LV cavity was visually defined by manual adjustment of a grayscale threshold (Supplemental Figure 1). Secondly, the intra-cavity volume was added to the papillary muscle and LV wall volumes, and the result was subtracted from the volume inside the epicardial border. The LV chamber was assessed according to the American Heart Association 17-segment model. The LV volume and mass data were indexed to body surface area. Quantification of LGE was carried out by manually
adjusting a grayscale threshold to define areas of visually detectable LGE. These areas were then summed to determine the total LGE volume and were expressed as a proportion of the total LV myocardial mass (%LGE). Cardiac MRI studies were not performed in 7 patients residing in Denmark due to lack of availability of the study, 6 children aged 8-15 years without any signs or symptoms of FD, 3 heterozygotes without any signs or symptoms of FD declined to have the study performed and 1 heterozygote who died shortly after the FD diagnosis was established.

The brain protocol included acquisition of sagittal and transverse T1-weighted spin-echo, transverse T2-weighted turbo spin-echo, T2 turbo inversion recovery magnitude and T2 TSE coronal images and a time-of-flight three-dimensional series. Images were assessed with respect to the presence of lacunar and cortical infarcts, old hemorrhages, pulvinar hyperintesity or verebrobasilar dolichoectasia (defined as an increase in the length of the basilar artery and a diameter greater than 4.5 mm on time-of-flight three-dimensional images).
### Supplemental Table I. Examinations Performed at Baseline

<table>
<thead>
<tr>
<th>Investigation/Parameter:</th>
<th>Family A/D322E</th>
<th>Family B/I232T</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n=8)</td>
<td>Female (n=8)</td>
<td>Male (n=10)</td>
</tr>
<tr>
<td>GLA mutation analysis</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Medical history and physical examination</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>α-GAL activity in leukocytes and urinary Gb3</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Lyso-Gb3</td>
<td>63%</td>
<td>13%</td>
<td>70%</td>
</tr>
<tr>
<td>Echocardiography</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Cardiac MRI</td>
<td>100%</td>
<td>75%</td>
<td>60%</td>
</tr>
<tr>
<td>Brain MRI</td>
<td>100%</td>
<td>88%</td>
<td>80%</td>
</tr>
<tr>
<td>Serum creatinine/GFR</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Urinary protein</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Kidney biopsy</td>
<td>0%</td>
<td>0%</td>
<td>40%</td>
</tr>
</tbody>
</table>

The percentage of patients for whom the investigation was performed is shown. α-GAL, α-galactosidase A; Gb3, globotriaosylceramide; GLA, α-galactosidase A gene; GFR, glomerular filtration rate; MRI, Magnetic resonance imaging.
**Supplemental Table IIA.** Baseline Characteristics of Subjects with α-GAL Activity <40% of the Mean Reference Value in Leukocytes

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Case (from pedigree)</th>
<th>Age</th>
<th>Gender</th>
<th>GLA variant</th>
<th>α-GAL activity in leukocytes (ukat/kg protein)</th>
<th>α-GAL activity (%)</th>
<th>Urinary Gb3 (umol/mol creatinine)</th>
<th>Plasma lyso-Gb3 (ng/ml)</th>
<th>Angiokeratoma</th>
<th>Hypohidrosis</th>
<th>Acroparesthesia</th>
<th>Corneal opacity</th>
<th>Abdominal discomfort</th>
<th>Hypoacusis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A-IV:1</td>
<td>61</td>
<td>M</td>
<td>p.D322E</td>
<td>0.7</td>
<td>2.5%</td>
<td>271</td>
<td>91</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>A-V:4</td>
<td>22</td>
<td>M</td>
<td>p.D322E</td>
<td>0.3</td>
<td>1.1%</td>
<td>108</td>
<td>114</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>A-IV:3</td>
<td>54</td>
<td>M</td>
<td>p.D322E</td>
<td>0.5</td>
<td>1.8%</td>
<td>316</td>
<td>104</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>A-IV:4</td>
<td>51</td>
<td>M</td>
<td>p.D322E</td>
<td>0.5</td>
<td>1.8%</td>
<td>981</td>
<td>N/A</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>A-IV:5</td>
<td>50</td>
<td>M</td>
<td>p.D322E</td>
<td>0.3</td>
<td>1.1%</td>
<td>698</td>
<td>125</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>A-IV:6</td>
<td>48</td>
<td>M</td>
<td>p.D322E</td>
<td>0.3</td>
<td>1.1%</td>
<td>178</td>
<td>N/A</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>A-V:3</td>
<td>30</td>
<td>M</td>
<td>p.D322E</td>
<td>0.2</td>
<td>0.7%</td>
<td>291</td>
<td>96</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>8</td>
<td>A-V:8</td>
<td>20</td>
<td>M</td>
<td>p.D322E</td>
<td>0.6</td>
<td>2.1%</td>
<td>112</td>
<td>N/A</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>9</td>
<td>A-IV:2</td>
<td>56</td>
<td>F</td>
<td>p.D322E</td>
<td>8.4</td>
<td>29%</td>
<td>216</td>
<td>N/A</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>B-III:2</td>
<td>61</td>
<td>M</td>
<td>p.I232T</td>
<td>3.1</td>
<td>11%</td>
<td>21</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>11</td>
<td>B-III:4</td>
<td>59</td>
<td>M</td>
<td>p.I232T</td>
<td>1.8</td>
<td>6.3%</td>
<td>23</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>12</td>
<td>B-IV:5</td>
<td>50</td>
<td>M</td>
<td>p.I232T</td>
<td>1.9</td>
<td>6.7%</td>
<td>7</td>
<td>16</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>13</td>
<td>B-IV:6</td>
<td>46</td>
<td>M</td>
<td>p.I232T</td>
<td>2.2</td>
<td>7.7%</td>
<td>4.5</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>Family</td>
<td>Age</td>
<td>Sex</td>
<td>p.I232T</td>
<td>Specific Activity</td>
<td>% Activity</td>
<td>Gb3</td>
<td>GLA</td>
<td>Gender</td>
<td>Specific Activity</td>
<td>% Activity</td>
<td>Gb3</td>
<td>GLA</td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>--------</td>
<td>-----</td>
<td>-----</td>
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<tr>
<td>14</td>
<td>B-IV:9</td>
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<td>M</td>
<td>p.I232T</td>
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<td>8.8%</td>
<td>N/A</td>
<td>N/A</td>
<td>-</td>
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<tr>
<td>15</td>
<td>B-IV:10</td>
<td>42</td>
<td>M</td>
<td>p.I232T</td>
<td>1.6</td>
<td>5.6%</td>
<td>N/A</td>
<td>N/A</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>16</td>
<td>B-IV:11</td>
<td>39</td>
<td>M</td>
<td>p.I232T</td>
<td>3.1</td>
<td>11%</td>
<td>27</td>
<td>N/A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>B-V:2</td>
<td>12</td>
<td>M</td>
<td>p.I232T</td>
<td>1.0</td>
<td>4%</td>
<td>2.6</td>
<td>8.6</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td>18</td>
<td>B-V:4</td>
<td>10</td>
<td>M</td>
<td>p.I232T</td>
<td>1.6</td>
<td>5.6%</td>
<td>14</td>
<td>16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>B-V:3</td>
<td>9</td>
<td>F</td>
<td>p.I232T</td>
<td>2.7</td>
<td>9.6%</td>
<td>1</td>
<td>1.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>20</td>
<td>B-IV:7</td>
<td>43</td>
<td>F</td>
<td>p.I232T</td>
<td>11</td>
<td>39%</td>
<td>N/A</td>
<td>N/A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>B-IV:2</td>
<td>39</td>
<td>F</td>
<td>p.I232T</td>
<td>6.6</td>
<td>23%</td>
<td>10</td>
<td>3.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>B-V:7</td>
<td>27</td>
<td>F</td>
<td>p.I232T</td>
<td>10</td>
<td>35%</td>
<td>N/A</td>
<td>N/A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>B-III:6</td>
<td>67</td>
<td>F</td>
<td>p.I232T</td>
<td>3.4</td>
<td>12%</td>
<td>2.1</td>
<td>N/A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

α-GAL, α-galactosidase A; F, females; Gb3, globotriaosylceramide; GLA, α-galactosidase A gene; M, males; N/A, not applicable. - indicates negative finding; + indicates positive finding.

*The specific activity was expressed as percentage of the mean values of normal control subjects (α-GAL activity in leukocytes).
**Supplemental Table IIB.** Cardiac, Cerebrovascular and Renal Manifestations of Subjects with α-GAL Activity <40% of the Mean Reference Value in Leukocytes

<table>
<thead>
<tr>
<th>Case number</th>
<th>mLVWT (mm)</th>
<th>LGE (% of LV mass)</th>
<th>AF</th>
<th>PM/ICD</th>
<th>CAD</th>
<th>Cerebral infarct</th>
<th>White matter hyperintensities</th>
<th>Dolicho ectasia</th>
<th>GFR* (umol/L)</th>
<th>Serum creatinine (umol/L)</th>
<th>Albuminuria</th>
<th>Urinary albumin excretion (mg/24h)†</th>
<th>Kidney biopsy</th>
<th>ERT</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-IV:1</td>
<td>23</td>
<td>21</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>severe</td>
<td>-</td>
<td>64</td>
<td>&gt;60</td>
<td>72</td>
<td>-</td>
<td>3.1</td>
<td>16</td>
</tr>
<tr>
<td>A-V:4</td>
<td>normal</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>42</td>
<td>&gt;60</td>
<td>75</td>
<td>-</td>
<td>&lt;3,0</td>
<td>19</td>
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<tr>
<td>A-IV:3</td>
<td>18</td>
<td>7.9</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>severe</td>
<td>-</td>
<td>54</td>
<td>&gt;60</td>
<td>77</td>
<td>-</td>
<td>&lt;3,0</td>
<td>&lt;8,0</td>
</tr>
<tr>
<td>A-IV:4</td>
<td>16</td>
<td>2.1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>severe</td>
<td>-</td>
<td>44</td>
<td>68*</td>
<td>79</td>
<td>+</td>
<td>29</td>
<td>43</td>
</tr>
<tr>
<td>A-IV:5</td>
<td>25</td>
<td>18</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>mild</td>
<td>-</td>
<td>39</td>
<td>&gt;60</td>
<td>74</td>
<td>+</td>
<td>40</td>
<td>684</td>
</tr>
<tr>
<td>A-IV:6</td>
<td>16</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>mild</td>
<td>-</td>
<td>35</td>
<td>&gt;60</td>
<td>82</td>
<td>-</td>
<td>&lt;3,0</td>
<td>&lt;8,0</td>
</tr>
<tr>
<td>A-V:3</td>
<td>normal</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
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ACR, albumin-to-creatinine ratio; AF, atrial fibrillation; CAD, coronary artery disease; ERT, enzyme replacement therapy; GFR, glomerular filtration rate; ICD, implantable cardioverter-defibrillator; LGE, late gadolinium enhancement; LV, left ventricular; mLVWT, maximal left ventricular wall thickness; N/A, not applicable; PM, pacemaker. - indicates negative finding; + indicates positive finding.

*Measured GFR values (n=7). Estimated GFR values (n=16) are without a label.

†Total protein excretion in a 24-hour urine collection (n=6). Values from spot morning samples (n=17) are without label.

Of the patients not included in the Table (α-GAL activity >40%), 5 females aged 47-72 years had one or more of the following positive findings: LVH, LGE, cerebral infarct, WMHs, and microalbuminuria.
**Supplemental Figure 1.** Severe left ventricular hypertrophy of a Family B male. Depicted are end-diastolic short axis cardiac MRI images in consecutive order (base to apex), covering the complete left ventricle. The epicardial border (green line) and intra-cavity blood area (red area) are defined by manual tracing and grey scale threshold adjustment, respectively.
References
