Single-Gene Mutations and Increased Left Ventricular Wall Thickness in the Community

The Framingham Heart Study

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Background—Mutations in sarcomere protein, *PRKAG2*, *LAMP2*, α-galactosidase A (*GLA*), and several mitochondrial genes can cause rare familial cardiomyopathies, but their contribution to increased left ventricular wall thickness (LVWT) in the community is unknown.

Methods and Results—We studied 1862 unrelated participants (52% women; age, 59±9 years) from the community-based Framingham Heart Study who had echocardiograms and provided DNA samples but did not have severe hypertension, aortic prosthesis, or significant aortic stenosis. Eight sarcomere protein genes, 3 storage cardiomyopathy–causing genes, and 27 mitochondrial genes were sequenced in unrelated individuals with increased LVWT (maximum LVWT >13 mm). Fifty eligible participants (9 women) had unexplained increased LVWT. We detected 8 mutations in 9 individuals (2 women); 7 mutations in 5 sarcomere protein genes (*MYH7, MYBPC3, TNNT2, TNNI3, MYL3*), and 1 *GLA* mutation. In individuals with increased LVWT, participants with sarcomere protein and storage mutations were clinically indistinguishable from those without mutations.

Conclusions—In a community-based cohort, about 3% of eligible participants had increased LVWT, of whom 18% had sarcomere protein or lipid storage gene mutations. Increased LVWT in the community is a very heterogeneous condition, which sometimes may arise from single-gene variants in one of a number of genes. **(*Circulation*. 2006;113: 2697-2705.)**

Key Words: epidemiology ■ genetics ■ hypertrophy ■ myosin

Left ventricular (LV) hypertrophy (LVH) is associated with the development of stroke, coronary heart disease, heart failure, and cardiovascular and all-cause mortality (for reviews, see Vakili et al[1] and Gosse[2]). The poor prognosis has created intense interest in understanding the determinants of LVH. The risk factors for LVH include increased blood pressure, elevated body mass index, insulin resistance, myocardial infarction, and valvular heart disease.[3] However, between 50% and 75% of the variability in LV mass remains unexplained.[4] LVH results from increases in LV wall thickness (LVWT), LV mass, and/or LV volume. Given the modest heritability of LV mass and LVWT, researchers have looked to genetic factors to elucidate the unexplained causes of LVH (for a review, see Arnett et al[5]). On the basis of the hypothesis that LVH is a common complex disorder, many genetic polymorphisms have been examined; however, no heritable sequence variants responsible for LVH have yet been reported in community-based studies.

Clinical Perspective p 2705

Hypertrophic cardiomyopathy (HCM) is a life-threatening autosomal dominant disorder caused by sarcomere protein gene mutations that produce otherwise unexplained LVH due to increased LVWT in the absence of increased LV volume (reviewed in Seidman and Seidman[6] and Ahmad et al[7]). Histological examination of cardiac tissues demonstrates myocyte hypertrophy, disarray, and fibrosis. Pathological findings can vary widely from mild or focal hypertrophy to marked septal hypertrophy and LV outflow tract obstruction. Similarly, clinical symptoms in HCM can range from asymp-

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tomatic status to heart failure, atrial fibrillation, angina, syncope, and sudden death.[8]

Over the past decade, molecular genetic approaches have identified >300 disease-causing defects in genes encoding 8 sarcomere proteins: β-cardiac myosin heavy chain (*MYH7*), cardiac myosin binding protein-C (*MYBPC3*), cardiac troponin T (*TNNT2*), cardiac troponin I (*TNNI3*), α-tropomyosin (*TPM1*), and, more rarely, the essential or regulatory myosin light chains (*MYL3, MYL2*), and cardiac actin (*ACTC*) (data summarized on the Web[9]). The contribution of genetic heterogeneity to clinical variability among patients with HCM is uncertain.[6,7] All of the HCM-causing sarcomere protein gene mutations are predicted to alter the structure of the encoded sarcomere protein.

Storage cardiomyopathy may mimic HCM and may be responsible for disease in patients previously diagnosed as having HCM (reviewed in Ahmad et al[7] and Arad et al[10]). Mutations in genes encoding the γ2-subunit of AMP-activated protein kinase (*PRKAG2*), α-galactosidase A (*GLA*), and lysosome-associated membrane protein-2 (*LAMP2*) can cause profound myocardial hypertrophy as the result of accumulated glycogen. Recent studies have reported that 4% to 12% of probands diagnosed as having HCM have glycogen storage diseases caused by either *PRKAG2* or *LAMP2* mutations. Furthermore, mutations in mitochondrial-encoded tRNAs, ATP synthase, and cytochrome b cause a variety of syndromes, such as Kearns-Sayre syndrome accompanied by LVH.

The prevalence of clinical HCM in young adults is estimated at 1 in 500.[11] However, the contribution of sarcomere protein gene mutations, storage cardiomyopathy–causing genes, and mitochondrial mutations to LVH in the community is unknown. We tested the hypothesis that single-gene mutations are responsible for some cases of increased LVWT in the community. Our objective was to identify the prevalence and clinical features of single-gene mutations in adults in the community with increased LVWT in the absence of obvious secondary forms of hypertrophy. We screened individuals in the Framingham Heart Study with increased LVWT for mutations in 8 sarcomere protein genes, 3 storage cardiomyopathy–causing genes, and 27 mitochondrial genes.

Methods

Clinical and Echocardiographic Evaluation

Procedures for collecting Framingham Heart Study participant clinical information are outlined in detail in the online-only Data Supplement Methods section. The Boston Medical Center Institutional Review Board approved the Framingham Heart Study protocol, and all participants gave written informed consent.

Genetic Studies

Sarcomere protein genes, mitochondrial genes, and storage cardiomyopathy genes were characterized by DNA sequence analyses, as described in the Data Supplement Methods.

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

The mean age of the 1862 eligible Framingham Heart Study participants was 59±9 years (range, 33 to 85), and 52% were women (Data Supplement Methods and Table). In this sample, 50 unrelated individuals (18% women) had increased LVWT, defined for the present study as maximum LVWT >13 mm. Of those with increased LVWT, the mean interventricular and LV posterior wall thicknesses were 14.2±1.4 mm and 12.6±1.6 mm, respectively. The individuals with increased LVWT were more likely to be older, male, and taking antihypertensive medication; to have higher mean body mass index; and to have higher mean left atrial diameter (Data Supplement Table). The participants with increased LVWT did not have ventricular preexcitation or atrioventricular conduction disturbance. Of the individuals excluded because of severe hypertension, aortic stenosis, aortic prosthesis, or unavailability of DNA, 5% (n=10/196), 27% (n=3/11), 29% (n=2/7), and 2% (n=2/118), respectively, had maximum LVWT >13 mm.

Sarcomere Protein Gene Mutations in Participants With Increased LVWT

DNA sequences encoding 8 sarcomere proteins, *MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, *TPM1*, *MYL3*, *MYL2*, and *ACTC*, were determined from all 50 participants with increased LVWT (see Data Supplement Methods). Three types of sequence variants were identified. First, 62 synonymous sequence variants observed (n=26 exons, n=36 introns) were excluded from further evaluation because such synonymous variants both are very unlikely to alter cardiac morphology and have never been shown to correlate with increased LVWT in HCM families[9] (additional data not shown here). Second, among the 50 individuals with increased LVWT, there were 3 sequence variants (Val158Met [n=13], Ser236Gly [n=5] in *MYBPC3,* and Lys253Arg [n=1] in *TNNT2*) predicted to alter the structure of the encoded sarcomere protein but that were excluded from further investigation because they had been described previously as polymorphisms that do not cause HCM.[12] Third, there were 7 sequence variants predicted to alter the structure of the encoded polypeptides, which we hypothesized might cause increased LVWT in these individuals (Table 1 and Figure 1).

The 7 potential LVH-causing sequence variants were identified in 5 sarcomere protein genes, *MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, and *MYL3* (Table 1, Figure 1), in 8 individuals or 16% of those with unexplained increased LVWT (8/50=16%; exact 95% CI, 7.2% to 29.1%). No sequence variants predicted to alter the structure of the encoded *TPM1*, *MYL2*, and *ACTC* were detected in any participants (data not shown). Three (*MYBPC3* mutations Gly5Arg, Gly490Arg, and Val753fs) of these 7 sequence variants previously were reported as HCM-causing mutations.[9,13] One sequence variant, Val753fs in exon24 of *MYBPC3*, was found in 2 individuals. All 7 sequence variants were confirmed independently by restriction enzyme digestion of PCR-amplified sequences from participant DNA.

Sarcomere proteins are highly conserved during evolution. We compared the sequence variants with protein sequences from a variety of species (Figure 2). All observed amino acid residue–altering sequence variants,

TABLE 1. Eight Sequence Variants Identified Among 50 Individuals With Maximum LVWT >13 mm

Designation	Gene	Sequence Change	Probable Consequences	Frequency
Met982Thr	*MYH7*	T→C	Met→Thr at codon 982	2/4078
Gly5Arg	*MYBPC3*	G→C	Gly→Arg at codon 5	0/4050
Val753fs	*MYBPC3*	T insertion at nt 2259	Encodes an 831 aa polypeptide (deletes 443 aa)	0/4032
Gly490Arg	*MYBPC3*	G→A	Gly→Arg at codon 490	0/2186
Ala28Val	*TNNT2*	C→T	Ala→Val at codon 28	0/4032
Gln130Arg	*TNNI3*	A→G	Gln→Arg at codon 130	0/4020
Val156Met	*MYL3*	G→A	Val→Met at codon 156	2/4068
Asp313Tyr	*GLA*	G→T	Asp→Tyr at codon 313	0/463

Frequencies of sarcomere protein gene variants were determined among a cohort of Framingham Heart Study participants and 300 other individuals of European descent. The X chromosome *GLA* variant Asp313Tyr was not found in any of 300 non-Framingham participants (163 women). aa indicates amino acid.

except Ala28Val of *TNNT2*, affect residues that have been conserved at least since mice or rats and humans diverged, and some since frogs or chickens and humans diverged. The sequence variants in *MYH7*, *TNNI3*, and *MYL3* affect residues that have been conserved since skeletal muscle isoforms separated from cardiac isoforms.

The frequencies of the sequence variants were assessed further in 2 additional samples. First, we searched for and did not find the 7 identified genetic variants in 300 asymptomatic individuals not in the Framingham Heart Study (600 chromosomes). Second, the frequency of all 7 sequence variants was further determined in randomly selected unrelated Framingham Heart Study Offspring participants (Table 1).[9] Two subjects, ages 51 and 54 years, had *MYL3* Val156Met variant. Although neither of these subjects had LVWT >13 mm, one had left atrial

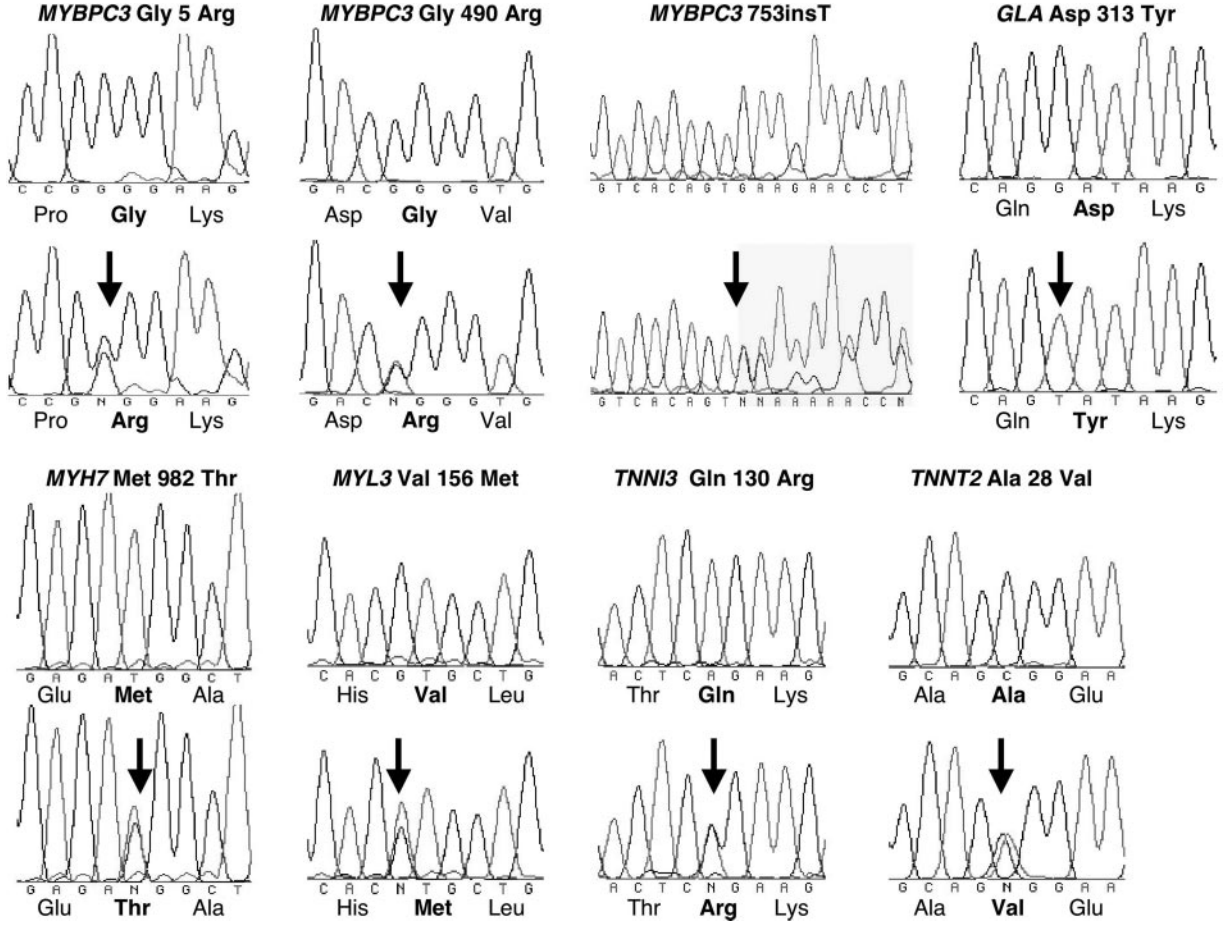

Figure 1. Sequence analyses of genomic DNA from 8 variants; cardiac myosin binding protein-C (*MYBPC3*) Gly5Arg, Gly490Arg, and Val753fs and α-galactosidase A (*GLA*) Asp313Tyr (upper row); β-cardiac myosin heavy chain (*MYH7*) Met982Thr; cardiac troponin T (*TNNT2*) Ala28Val; cardiac troponin I (*TNNI3*) Gln130Arg; and essential myosin light chain (*MYL3*) Val156Met (lower row). Arrows indicate the substituted or inserted nucleotides. The altered residues are displayed in bold.

Figure 2. Conservation of amino acid residues altered by 6 sarcomere protein gene missense mutations, cardiac myosin binding protein-C (*MYBPC3*) Gly5Arg, and Gly490Arg; essential myosin light chain (*MYL3*) Val156Met; β-cardiac myosin heavy chain (*MYH7*) Met982Thr; cardiac troponin I (*TNNI3*) Gln130Arg; and cardiac troponin T (*TNNT2*) Ala28Val. The altered residue and 8 flanking amino acid residues are displayed in single-letter code.

enlargement and enhanced fractional shortening (52%), features consistent with the hypothesis that this younger individual might develop increased LVWT in the future. Two subjects had the *MYH7* Met982Thr variant: One had an echocardiographic pattern consistent with "burnt out" cardiac hypertrophy with a dilated left ventricle (LV diastolic diameter >56 mm) and enlarged atrium, whereas the other had a mildly enlarged left atrium and ECG voltages suggestive of HCM. These 4 subjects were normotensive and had no history of treatment for hypertension. None of the other 5 variants was found among eligible Framingham Heart Study subjects (Table 1).

Additionally, to verify that our exclusion criteria were not too stringent, we determined the nucleotide sequence of the 8 sarcomere protein genes in 34 individuals who either did not have increased LVWT (ie, LVWT ≤13 mm, n=29) or had increased LVWT caused by aortic stenosis (n=3) or aortic prosthesis (n=2). None of the 34 participants had a sarcomere protein gene mutation predicted to cause increased LVWT.

Because a first-degree relative of an individual carrying a sarcomere protein gene mutation has a 50% chance of carrying the same mutation, we searched for the sequence variant in other family members who were Framingham Study participants. Only the probands with the *MYBPC3* Gly5Arg and the *MYBPC3* Val753fs variants had first-degree relatives with available DNA specimens (n=9); only 1 of the 9 carried the same sarcomere protein gene mutation as the proband. The proband with the *MYBPC3* Gly5Arg mutation had an elderly sibling with this sequence variant, but this sibling did not have LVH (maximum LVWT <11 mm). The subjects with the *MYBPC3* Gly5Arg and *MYH7* Met982Thr each had a family member who died suddenly before collection of DNA.

With the exception of borderline-significantly smaller LV end-diastolic diameters ($P=0.05$), the distribution of LVWT, age, sex, cardiac symptoms, and ECG findings in participants with sarcomere protein gene mutations did not differ significantly from those individuals without mutations (Table 2 and Figure 3). Other echocardiographic features associated with HCM, such as premature aortic valve closure, LV outflow tract obstruction, systolic anterior mitral motion, anterior papillary muscle displacement, and LV outflow tract obstruction, did not vary significantly by sarcomere protein mutation status. The participants with increased LVWT and sarcomere protein gene mutations were less likely to be treated for hypertension than were those without mutations (25% versus 71%, $P=0.04$). Remarkably, 6 of 18 subjects with increased LVWT but without a history of hypertension treatment had a sarcomere protein gene mutation.

Storage Cardiomyopathy–Causing Gene Mutations in Participants With Increased LVWT

The *PRKAG2*, *LAMP2*, and *GLA* genes of all 50 individuals with increased LVWT were characterized by nucleotide sequence analysis. Synonymous sequence variants (eg, Arg531Arg in *PRKAG2*) and intronic polymorphisms were identified but excluded from further consideration. No sequence variants predicted to alter the structure of the encoded *PRKAG2* and *LAMP2* were detected in any participants (data not shown).

An α-galactosidase A gene (*GLA*) nonsynonymous sequence variant (Asp313Tyr) was identified in one male subject with maximum LVWT of 13.7 mm, for a frequency of 2.0% (1/50=2.0%; exact 95% CI, 0.1% to 10.6%; Figures 1 and 3 and Table 1). The charge of the encoded amino acid was altered (acidic to uncharged). The ECG of this participant showed normal sinus rhythm without either conduction disturbance or LVH. The Asp313Tyr sequence variant was not found in 300 unrelated individuals.

Because *GLA* is encoded on the X chromosome, the proband's mother and 50% of his brothers should carry the same *GLA* variant (unless this mutation arose de novo). DNA samples were unavailable from family members, but 2 relatives of the individual with the Asp313Tyr missense mutation had increased LVWT. His mother's maximum LVWT was 12 mm in her 60s and increased to 14 mm in her 80s. One brother had a maximum LVWT of 13 mm in his 40s.

Mitochondrial Gene Mutations in Framingham Heart Study Participants With Increased LVWT

We identified 75 sequence variants in the 50 participants with increased LVWT after determining the DNA sequences of all

TABLE 2. Characteristics of Participants With Increased LVWT by Presence or Absence of Sarcomere Protein Mutations

	No Mutation (n=42)	Mutation (n=8)	P*
Clinical			
Age, y	63±9	59±8	0.17
Women, n (%)	7 (17)	2 (25)	0.35
Body mass index, kg/m^2	31.9±4.9	29.5±5.0	0.09
Physical exam ≥3/6 systolic murmur, n (%)	4 (10)	0 (0)	0.38
Systolic blood pressure, mm Hg	132±13	131±11	0.86
Diastolic blood pressure, mm Hg	76±8	83±6	0.11
Blood pressure treatment, n (%)	30 (71)	2 (25)	0.04
Statin treatment, n (%)	6 (14)	1 (13)	0.98
Heavy physical activity, n (%)	4 (10)	0 (0)	0.39
Prior myocardial infarction or coronary insufficiency, n (%)	4 (10)	0 (0)	0.47
Angina, n (%)	2 (5)	0 (0)	0.60
Syncope, n (%)	5 (12)	3 (38)	0.15
Prior stroke, n (%)	0 (0)	0 (0)	...
Heart failure, n (%)	0 (0)	0 (0)	...
New York Heart Association class I or higher, n (%)	5 (12)	0 (0)	0.38
Atrial fibrillation, n (%)	2 (5)	0 (0)	0.56
LVH on ECG			
With repolarization changes, n (%)	3 (7)	0 (0)	0.55
Without repolarization changes, n (%)	6 (14)	1 (13)	0.96
ECG PR interval <120 ms, n (%)	0 (0)	0 (0)	...
Estimated glomerular filtration rate, mL/min per 1.73 m^2	80±25	98±35	0.18
Chronic kidney disease, n (%)	6 (14)	0 (0)	0.43
Echocardiography			
Left atrial diameter, mm	46.9±6.4	42.1±5.9	0.11
LV diastolic diameter, mm	48.7±5.5	43.9±3.8	0.05
LV fractional shortening, %	38.1±5.7	36.3±7.4	0.26
Interventricular septal thickness, mm	14.1±1.2	14.9±2.2	0.25
LV posterior wall thickness, mm	12.5±1.7	12.8±1.0	0.67
Maximal LV wall thickness, mm	14.1±1.2	14.9±2.2	0.27
IVS/PW ratio	1.14±0.15	1.19±0.12	0.62
Doppler LV outflow tract obstruction, n (%)	1 (2)	0	0.31
Premature atrioventricular closure, n (%)	0	0	...
Chordal systolic anterior motion, n (%)	5 (12)	3 (38)	0.60
Mitral leaflet systolic anterior motion, n (%)	1 (2)	0	0.64
Presence of anterior papillary muscle displacement (>0.40), n (%)	7 (17)	2 (25)	0.99
Anterior papillary muscle displacement, mean	0.360±0.051	0.394±0.064	0.20

Continuous variables are given as mean±SD. Ellipses (. . .) indicate no statistical test done (no participants having a particular characteristic).

Anterior papillary muscle displacement was unmeasurable in 2 subjects without mutations. Systolic anterior papillary muscle displacement=[(LV dimension)−(septal to APM dimension)/LV diameter], with LV diameter measured at papillary muscle level.

*P values are age and sex adjusted but not corrected for multiple statistical testing.

22 transfer RNA genes, 2 ribosomal RNA genes (12S and 16S), ATP synthase 6 gene, ATP synthase 8 gene and cytochrome b gene. In the protein-encoding sequences, 24 synonymous variants were excluded from further consideration. Eleven of the mitochondrial variants—4 in transfer RNA genes, 1 in ribosomal RNA genes, 5 in ATP synthase 6 gene, 1 in cytochrome b gene—had not been reported among 713 mitochondrial genomes from European subjects.[14] Because none of the 10 Framingham Heart Study participants with 11 mitochondrial gene variants had either variants in sarcomere protein genes or storage cardiomyopathy–causing genes, we considered the hypothesis that these variants might contribute to their increased maximum LVWT. To test this hypothesis, we performed 2 experiments. First, we asked if

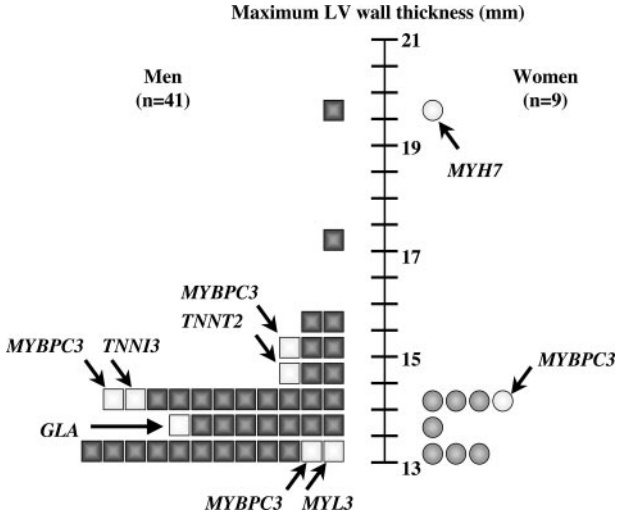

Figure 3. Distribution of individuals with maximum LVWT >13 mm and sarcomere protein gene mutations among Framingham Heart Study participants. The interventricular septum was usually, but not universally, the thicker LV wall. ■Men and ●women.

such novel sequence variants were found in randomly selected individuals. We randomly selected 60 mitochondrial DNA sequences from the mitochondrial database of 2064 published mitochondrial sequences[14] and searched these 60 mitochondrial sequences for novel sequence variants comparable to those found in the mitochondrial sequences assessed in the Framingham Heart Study participants with increased LVWT. Twelve sequence variants were found in the mitochondrial genomes of the 60 randomly selected individuals, analogous to the 11 sequence variants found in the 50 Framingham Heart Study participants with increased LVWT were detected. Because 12 novel sequence variants among 60 individuals are not significantly different from 11 sequence variants in 50 Framingham Heart Study participants with increased LVWT ($P=0.67$), we strongly suspect that the novel sequence variants found in the Framingham Heart Study participants with increased LVWT are unlikely to be responsible for increased LVWT. Second, among 16 mothers and siblings of Framingham Heart Study participants with increased LVWT who had the 11 mitochondrial variants, all of whom should have inherited the same mitochondrial sequence variants, only 1 had increased LVWT (unpublished results). Hence, the inheritance patterns of increased LVWT and mitochondrial sequence variants are quite different.

Discussion

We demonstrated that sarcomere protein gene mutations or *GLA* mutations were present in an appreciable fraction of individuals with unexplained increased LVWT in the community. About 3% (n=50) of the 1862 eligible Framingham Heart Study participants had maximum LVWT >13 mm. Eight of 50 participants with increased LVWT (16%; exact 95% CI, 7.2% to 29.1%) carried 7 sarcomere protein gene mutations. Furthermore, one of these 50 individuals had a mutation in *GLA* (2%; exact 95% CI, 0.1% to 10.6%), which was predicted to cause a lipid storage cardiomyopathy. We estimate that about 1 of 200 persons, or about 0.5% of middle-aged to elderly adults in the community, have a sarcomere protein or lipid storage gene mutation (9/1862=0.5%; exact 95% CI, 0.2% to 0.9%). A more conservative estimate of the community prevalence of single-gene mutations associated with LVH arises if we include the probands' excluded siblings and assume that no sibling had a genotyped mutation; the estimated prevalence would be about 1 in 300 (9/2579=0.3%; exact 95% CI, 0.2% to 0.7%).

One question that needs to be addressed is whether the sarcomere protein and *GLA* mutations detected among subjects with increased LVWT were responsible for their altered cardiac morphology. Because mutations in the sarcomere protein genes cause unexplained increased LVWT, the primary diagnostic criteria for HCM, we began with the strong conviction that any sarcomere protein gene mutation that alters the structure of the encoded protein discovered in participants with increased LVWT would be responsible for their increased LVWT. Because previous studies in many laboratories have involved the sequencing of these genes in at least 1000 HCM probands, most non–HCM-causing sequence polymorphisms should have been identified previously.[9,13,15,16] The assertion that the sequence variants identified in this study were responsible for increased LVWT is further strengthened by several observations. First, the *MYBPC3* mutations Gly5Arg, Gly490Arg, and Val753fs were previously shown to be responsible for HCM, as evidenced by coinheritance with the disease.[9,13] All except one (*TNNT2* Ala28Val) of the mutations identified here as potentially causing increased LVWT alter residues highly conserved during the course of evolution (Figure 2). Not only do these mutations affect conserved residues, but in 3 instances the altered amino acids change the charge of the encoded amino acid (gly→arg [n=2], gln→arg [n=1]). Furthermore, we demonstrated that 5 of the 7 variants were found only among individuals who have increased LVWT. We strongly suspect but cannot prove that the 2 additional variants, *MYH7* Met982Thr and *MYL3* Val156Met, are mutations, as they were observed among 4 individuals from >1734 (Table 2) randomly selected unrelated Framingham Heart Study Offspring participants without increased LVWT. However, 3 of these individuals have some clinical features associated with HCM. Finally, no rare nonsynonymous variants were identified in 34 Framingham Heart Study individuals without unexplained increased LVWT. The finding of 8 individuals bearing variants among 50 subjects with increased LVWT was significantly different ($P<0.02$) from finding of no variant-holders among 34 Framingham participants with aortic stenosis or prosthesis (n=5) or without diagnostic increased LVWT (n=29).

The distribution of variants in sarcomere protein genes among patients with HCM has been described in several independent studies of referral-based cohorts.[16,17] In several studies, about 60% of patients with earlier-onset HCM have mutations in one of the 8 sarcomere protein genes studied here (Table 3). However, among individuals with elderly-onset HCM, only ≈20% have a mutation in one of these sarcomere protein genes.[17] In the middle-aged to

TABLE 3. Sarcomere Protein Gene Mutation Distribution in 8 Framingham Heart Study Participants With Increased LVWT Versus Other HCM Cohorts

GENE	HCM*	Elderly-Onset HCM[17]	Present FHS Study
MYH7	79 (22.3)	0	1 (2.0)
MYBPC3	81 (22.9)	5 (16.1)	4 (8.0)
TNNT2	15 (4.2)	0	1 (2.0)
TNNI3	19 (5.4)	2 (6.5)	1 (2.0)
TPM1	2 (0.6)	0	0
MYL3	2 (0.6)	NE	1 (2.0)
MYL2	5 (1.4)	NE	0
ACTC	0	NE	0
Total with mutations	203	7	8
Total	354	31	50

*Results of 192 patients taken from Richard et al[16] and 162 HCM patients[9] (unpublished results). NE denotes not evaluated.

The presence of mutations was higher in HCM patients than in Framingham Heart Study (FHS) participants with increased LVWT (57% vs 16%, exact $P<0.0001$), but among those with mutations, the distribution by type did not differ between these groups (2-sided exact $P=0.18$). Comparing elderly-onset HCM vs FHS participants with increased LVWT, neither the mutation frequencies (23% vs 16%, $P=0.56$) nor the distribution by type (2-sided exact $P=1.0$) differed significantly between groups.

elderly Framingham Heart Study community-based study, 16% of individuals with increased LVWT have a sarcomere protein gene mutation (Tables 2 and 3). We acknowledge that elderly hypertrophy series and the Framingham series estimates will need to be replicated in larger studies, but the lower prevalence of sarcomere protein mutations compared with HCM series (≈60%) is consistent. One possible explanation for the reduced percentage (compare 60% to 16%) of individuals with sarcomere protein gene mutations is that in older community-based cohorts a higher proportion of individuals may have hypertension-induced LVH, even though their hypertension has been treated and at least partially controlled at the time of echocardiography. Of the 50 subjects with increased LVWT, 32 were taking antihypertensive medication (Data Supplement Table). Perhaps LVH developed in some of these individuals in response to their hypertension despite treatment or before the initiation of treatment. Alternatively, there may be some other age-related cause of increased LVWT. Remarkably, among 18 subjects with increased LVWT but without a history of hypertension, 6 subjects had a sarcomere protein gene mutation, suggesting that sarcomere protein gene mutation may play an important role in LVH in the normotensive individuals.

We identified one individual with the *GLA* variant Asp313Tyr, which alters the charge of the encoded amino acid and is not observed in normal chromosomes. The Asp313Tyr missense mutation was previously reported to be responsible for classic Fabry disease in affected members of a German family.[18] Furthermore, the Asp313Tyr mutation was identified in a subject with cardiac Fabry disease in an HCM-referral cohort, who also had low enzymatic activity of α-galactosidase A.[19] Thus, we suggest that the Asp313Tyr variant in *GLA* was most likely the cause of increased LVWT in our subject. We speculate that the Asp313Tyr missense mutation can produce different Fabry phenotypes—systemic classic Fabry manifestations, isolated cardiomyopathy, and mild LVH. Previous studies have demonstrated that patients with Fabry within the same family, who share the identical mutation, can exhibit a significant degree of variability in the phenotypic expression of the disease.[20] Two other first-degree relatives of our subject with the Asp313Tyr missense mutation (DNA unavailable for analysis) had increased LVWT. Among 50 subjects with increased LVWT from the Framingham Heart Study, only 1 male subject had a variant in a storage cardiomyopathy disease gene. A frequency of 2.4% in male subjects (1/41) with maximum LVWT >13 mm (exact 95% CI, 0.1% to 12.9%) is similar to that reported among Japanese men with LVH.[21] Although based on a small data set, our findings are consistent with a frequency of *GLA* mutations of 1 in 2000. This is potentially an important observation because enzyme replacement therapy is proven to decrease LVH in individuals with *GLA* mutations.[22]

We also investigated the contribution of mitochondrial sequence variants to increased maximum LVWT in Framingham Heart Study subjects. Whereas 11 novel sequence variants were detected in among 50 Framingham Heart Study participants, a similar number of novel sequence variants were found among a cohort of 60 normal individuals (see Results). Furthermore, 16 siblings of Framingham Heart Study subjects with maximum LVWT >13 mm were studied, and only one of these had a maximum LVWT >13 mm; this distribution of increased LVWT is not consistent with a mitochondrial mode of inheritance. We conclude that mitochondrial gene variants do not cause a large fraction of increased LVWT in the community.

The present study had several strengths, including the routine ascertainment of echocardiography with the use of a rigorous quality control protocol, the dense standardized phenotypic characterization of coexistent risk factors and symptoms, and the community basis of the cohort. Nevertheless, limitations must be acknowledged. Because the Framingham Heart Study cohort is almost exclusively white, we do not know if the estimated prevalence of single-gene mutations will be similar in other ethnic/racial groups. We do not expect to find sex differences in the prevalence of sarcomere protein gene mutations, but we lacked power to detect such differences. Additionally, the cohort had a mean age of 59 years, raising the possibility of survival bias; the percentage and distribution of sarcomere protein gene mutations in a community-based cohort of young adults may differ. With 50 participants with increased LVWT sequenced and only 8 individuals with sarcomere protein gene mutations, we had very limited power to detect differences in clinical and echocardiographic characteristics and gene–environment interactions. However, the overlap in clinical characteristics

between the 2 groups underscores the concept that, in the community, it may be difficult to distinguish individuals with sarcomere protein gene mutations on the basis of clinical features. Similarly, the subject with *GLA* mutation had no specific clinical findings. Because of the community-based ascertainment of probands, we lacked the detailed family segregation typical of genetic studies reported from referral-based clinics. Our informed consent precluded us from contacting family members who were not enrolled in the Framingham Study, leading to this study limitation. Because of our reliance on short-axis quantification of LVWT, we cannot report the prevalence of mutations with isolated apical or lateral LVH. Finally, we may have underestimated the prevalence of single-gene mutations in individuals with increased LVWT because we did not sequence titin (*TTN*), α-myosin heavy chain (*MYH6*), or cardiac troponin C (*TNNC1*) in this study.

Most of our knowledge of sarcomere protein gene mutations is derived from referral-based samples, which appropriately have drawn attention to potential complications of HCM. The clinical outcome of community-based individuals with increased LVWT and sarcomere protein gene mutations is unknown and requires further follow-up. However, the mean age of 59 years and apparent lack of major differences in symptoms might imply that the prognosis of the individuals with sarcomere protein gene mutations detected in the community may be more benign than those detected by screening referred HCM cases and their families. A similar pattern has been observed with HCM diagnosed clinically; Spirito and colleagues[23] reported that individuals identified by outpatient echocardiographic screening (but not genotyped) have a more benign prognosis than would be anticipated from referral-based series.

Some sarcomere protein gene mutations produce more severe disease associated with a significant reduction in survival, whereas other mutations produce a much milder disease.[6,9,24] Perhaps some of the Framingham Heart Study participants identified in this study have mutations that produce milder disease. Alternatively, we know that some individuals with "malignant" mutations have milder disease than other individuals with the same mutation (referred to as partial penetrance). Presumably, the wide range of responses to the same mutation may be due to differences in genetic modifier genes or differences in environmental factors that exacerbate the hypertrophic process.[25] As discussed above, the identical *GLA* mutation could cause different Fabry phenotypes—systemic classic Fabry manifestations, isolated cardiomyopathy, and mild increases in LVWT. Further study in humans and in animals should help us determine the consequences of the mutations identified in community-based individuals. Additional investigation of the prevalence, clinical features, and prognosis of single-gene mutations in other community-based cohorts will improve our understanding of the broad spectrum of genetic causes of increased LVWT.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Left ventricular hypertrophy is an independent risk factor for cardiovascular events as well as all-cause mortality. Unexplained increased left ventricular wall thickness (LVWT) (>1.3 cm) contributes to left ventricular hypertrophy and occurs in about 3% of the general population over age 45. Because mutations in sarcomere protein genes *PRKAG2* and *LAMP2* can present as unexplained increased LVWT, we hypothesized that mutations in these genes might be found in Framingham Heart Study participants with LVWT >1.3 cm. Among 1862 Framingham Heart Study participants, 50 had unexplained increased LVWT. Eight subjects (16%) had a sarcomere protein gene mutation, and 1 (2%) had a *GLA* mutation. Hence, ≈0.5% of the general population over age 45 years has increased unexplained LVWT and a single-gene mutation that probably accounts for their hypertrophy.