The Burden of Early Phenotypes and the Influence of Wall Thickness in Hypertrophic Cardiomyopathy Mutation Carriers: Findings From the HCMNet Study

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**IMPORTANCE** Sarcomere mutations and left ventricular (LV) hypertrophy (LVH) are cardinal features of hypertrophic cardiomyopathy (HCM). However, little is known about the full spectrum of phenotypic manifestations or how LVH influences disease expression.

**OBJECTIVES** (1) To characterize and assess phenotypic burden in sarcomere mutation carriers (genotype positive [G+]) and (2) to investigate the correlation between LV wall thickness (LVWT) and other disease features in mutation carriers.

**DESIGN, SETTING, AND PARTICIPANTS** This investigation was a cross-sectional, multicenter observational study in the setting of the HCMNet network of HCM clinical centers. Mutation carriers with LVH (G+/LVH+), mutation carriers without LVH (G+/LVH−), and healthy related control individuals (G−/LVH−) were enrolled through HCMNet sites. A total of 193 participants were enrolled and underwent study procedures. Participants were enrolled between April 9, 2010, and January 30, 2012. Study analysis was performed between June 2015 and May 2016.

**EXPOSURES** The primary stratifying variables were the presence of a sarcomere mutation and measures of LVWT.

**MAIN OUTCOMES AND MEASURES** Variables from standardized exercise testing, echocardiography, cardiac magnetic resonance imaging, serum biomarker measurement, and electrocardiography were compared across study cohorts.

**RESULTS** Analyses were performed in 178 participants, including 81 G+/LVH+ (mean [SD] age at baseline, 27 [14] years), 55 G+/LVH− (20 [10] years), and 42 G−/LVH− (18 [8] years). All mutation carriers had smaller LV cavity, higher ratio of LVWT to diastolic diameter, and higher echocardiographic LV ejection fraction than controls. A phenotypic burden score was evaluated as the cumulative number of 7 traits (changes on electrocardiography; decreased LV systolic, diastolic diameter, or septal E' velocity; higher ratio of LVWT to diastolic diameter; serum troponin level; and natriuretic peptide level) in each individual. The mean (SE) phenotypic burden was 4.9 (0.2) phenotypes per individual in G+/LVH+, 2.4 (0.2) in G+/LVH−, and 1.3 (0.2) in controls (P < .001). Classification and regression tree analysis identified an LV end-diastolic dimension z score less than −1.85 or the combination of an LV end-diastolic dimension z score of −1.85 or higher and a septal E’ velocity z score less than −0.52 as having 74% accuracy in discriminating G+/LVH+ participants from controls. In mutation carriers, clinical variables demonstrated a continuous correlation with LVWT, generally without a clear cutoff signifying pathologic transition.

**CONCLUSIONS AND RELEVANCE** G+/LVH− individuals demonstrated altered cardiac dimensions and function and a higher burden of early phenotypes than healthy G− controls. Two methods discriminated phenotypic subgroups, namely, a sum across 7 traits and a regression tree-based rule that identifies constellations of distinguishing factors. Greater LVWT is associated with more prominent cardiac abnormalities in a continuous, although not always linear, manner. A single value of LVWT could not dichotomize the presence or absence of disease.

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Hypertrophic cardiomyopathy (HCM) is caused by mutations in sarcomere genes. Clinically, left ventricular (LV) hypertrophy (LVH) has been the focal point for diagnosing and describing the disease. In addition to being the most obvious phenotype, LVH carries prognostic importance. Symptoms and adverse clinical sequela are rare in sarcomere mutation carriers with normal LV wall thickness (LVWT). However, LVH is not specific for HCM and does not fully encompass the entire range of disease. Wall thickness is usually normal in early childhood and may remain so for decades despite the presence of a pathogenic sarcomere mutation.\(^1\)\(^2\) Moreover, other conditions, such as infiltrative and metabolic cardiomyopathies, hypertension, and athletic remodeling, can mimic the crude phenotype of HCM. These distinct clinical entities have fundamentally different biological features and thus divergent management strategies, prognosis, and implications to family members.

Sarcomere mutation carriers (genotype positive [G+]) without LVH (LVH−) are referred to as having “preclinical” or “subclinical” HCM. Studies in G+/LVH− cohorts have demonstrated that myocardial structure, function, and biochemistry are perturbed in the absence of LVH. Impaired LV relaxation,\(^3\)\(^4\) increased LV ejection fraction (LVEF),\(^3\)\(^4\) altered myocardial energetics,\(^6\) electrocardiographic abnormalities,\(^7\) increased mitral valve leaflet length,\(^8\) myocardial crypts on cardiac magnetic resonance (CMR) imaging,\(^9\)\(^12\) and evidence of a profibrotic state\(^13\)\(^14\) may be present in mutation carriers when LVWT is normal. However, little is known about the full spectrum of phenotypic manifestations in mutation carriers.

A collaborative network of HCM specialty centers (HCMNet) was established to advance understanding of disease pathogenesis and foster development of novel disease-modifying therapies. To better characterize the phenotypic spectrum of sarcomere mutations beyond LVH, rigorous and standardized evaluations were performed in a diverse genotyped population. Rather than focusing on individual traits, cumulative phenotypic burden was also assessed as a potentially more accurate reflection of disease expression. Similarly, recognizing the limitations of diagnosing HCM based on a single arbitrary threshold for pathologic LVH, the influence of greater LVWT in sarcomere mutation carriers was analyzed by treating LVWT as a continuous variable rather than a binary variable.

Methods

Study Design and Participants

The first participant enrollment date was April 9, 2010, and the last participant final visit was January 30, 2012. The analysis for this study was performed between June 2015 and May 2016. A cross-sectional, multicenter observational study was performed from April 2010 to January 2012. The analysis for this study was performed between June 2015 and May 2016. The goal of this study was to evaluate sarcomere mutation carriers with clinically overt HCM (G+/LVH+), mutation carriers with normal maximal LVWT (G+/LVH−), and healthy relatives who do not carry the family’s mutation (G−/LVH− control individuals). Left ventricular hypertrophy was based on echocardiographic core laboratory measurements and defined as a maximal LVWT of at least 12 mm in adults or a z score of at least 3 in participants younger than 18 years. These criteria were chosen rather than those standardly used to diagnose HCM clinically to avoid including mutation carriers with borderline LVH and potentially emerging or mild HCM in the G+/LVH− group. Participating sites were 11 HCM specialty centers in the United States (eTable 1 in the Supplement). The institutional review boards at all participating sites approved the study protocol. All adult participants provided written informed consent, and parental consent or youth assent was obtained for younger participants.

Inclusion and Exclusion Criteria

Criteria for inclusion in the study were (1) a likely pathogenic or pathogenic sarcomere mutation or a healthy genotype-negative relative and (2) age older than 5 years. Exclusion criteria were (1) hypertension (systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg or receiving treatment), (2) coronary artery disease, (3) greater than mild intrinsic valvular heart disease, (4) congenital heart disease, (5) prior invasive septal reduction, (6) medical conditions associated with increased collagen turnover, (7) pregnant or lactating status, and (8) age older than 40 years for G+/LVH− and controls.

Study participants had full sequencing of at least the 8 sarcomere genes definitively associated with HCM (MYH7 [OMIM 160760], MYBPC3 [OMIM 600958], TNNT2 [OMIM 191045], TNNT3 [OMIM 191044], MYL2 [OMIM 160781], MYL3 [OMIM 160790], TPM1 [OMIM 191010], and ACTC [OMIM 102540]). Variants were classified using standard criteria accounting for segregation, conservation, literature review, review of publicly available databases (including ClinVar\(^15\)), and very low frequency in appropriate control populations\(^16\)\(^17\) (Exome Aggregation Consortium [ExAC]; http://exac.broadinstitute.org). Mutations were required to be classified as likely pathogenic
or pathogenic at enrollment. Before data analysis, genotypes were reviewed, and only participants with variants fulfilling current criteria as pathogenic or likely pathogenic were included.

**Study Procedures**
Participants underwent a history and physical examination, electrocardiography, standardized echocardiography, and exercise testing using a symptom-limited, standard Bruce treadmill exercise protocol. Standardized CMR imaging was performed when possible based on the presence of intracardiac devices, local institutional review board regulations, and participant or parental consent or assent. Blood was drawn for serum biomarker assessment.

**Echocardiographic Analysis**
Standard 2-dimensional images, spectral and color Doppler, and tissue Doppler interrogation were obtained following a standardized protocol. Measurements were obtained from the mean of 3 cardiac cycles in accord with guidelines of the American Society of Echocardiography. Further details are available in the eMethods in the Supplement. All echocardiographic studies were analyzed off-line by staff at the echocardiographic core laboratory (Johns Hopkins Echocardiography Research Laboratory) who were blinded to genotype status.

**CMR Imaging Analysis**
Images were acquired using electrocardiogram gating and breath holding following a standardized protocol consisting of cine steady-state free precession imaging for LV function and mass. Late gadolinium enhancement (LGE) imaging was used to detect focal myocardial fibrosis, quantified using a semi-automated gray-scale threshold technique with manual correction. Further details are available in the eMethods in the Supplement. Staff at the CMR imaging core laboratory (Radiology and Imaging Sciences at the Clinical Center of the National Institutes of Health) who were blinded to genotype performed off-line analyses.

**Electrocardiography and Exercise Testing**
Standard 12-lead electrocardiograms were obtained at rest in the supine position. Symptom-limited treadmill exercise tolerance testing was performed using a standard Bruce protocol. Q waves were considered abnormal if present in at least 2 contiguous leads exceeding one-third the height of the R wave and with duration greater than 30 milliseconds. ST changes consisted of ST segment elevation or ST segment depression and with duration greater than 30 milliseconds. ST changes indicating that the variable discriminated G+/LVH− from controls (decreased septal E′ velocity or the presence of Q waves or ST changes on electrocardiography). For septal E′ velocity, LVESD, and LVEDD, a z score less than −1.5 was chosen as a clinically relevant cutoff. For ratio of LVWT to diastolic diameter and serum biomarkers, the optimal threshold was identified by testing candidate cutoffs and choosing the one with the smallest P value in a χ² test comparing the 3 groups in a covariate-adjusted logistic regression model (ratio of LVWT to diastolic diameter >0.23, NTproBNP level >100 pg/mL, and cardiac troponin I level >3.0 pg/mL). Classification and regression tree (CART) analysis was performed to identify subgroups that were most likely to be G+/LVH− vs controls to assist in the discrimination of these cohorts. The input candidate variables to the CART were continuous (where applicable) versions of the 7 traits used in the phenotypic burden score. Further details are available in the eMethods in the Supplement.

In mutation carriers, the correlations between maximal LVWT and clinical variables were examined using generalized estimating equation models with these variables as the outcome (y) and maximal LVWT z score as the predictor (x), adjusted for age, sex, and within-family correlation. Linear spline analysis was performed to identify physiologically relevant thresholds of maximal LVWT. Knots for the splines were
identified by testing candidates at small increments and choosing the knot that produced the lowest Akaike information criterion. All analyses were performed using statistical software (SAS, version 9.3; SAS Institute Inc).

Results

Study Population

A total of 193 participants were enrolled and underwent study procedures. At the time of data analysis, the sarcomere variant present in 13 G+ participants was reclassified from likely pathogenic to uncertain significance. Accordingly, these participants and 2 participating G−/LVH− relatives were excluded. Therefore, analyses were performed in 178 genotyped participants, with a mean (SD) age of 23 (12) years (age range, 5-60 years). Basic demographic and genetic characteristics are summarized in Table 1. Study participants were generally asymptomatic (95.1% [77 of 81] of G+/LVH+ were New York Heart Association class I-II, and all G+/LVH− and controls were New York Heart Association class I). As is typical, more than 86% of mutations were in MYH7 and

<table>
<thead>
<tr>
<th>Variable</th>
<th>G+/LVH+ (n = 81)</th>
<th>G+/LVH− (n = 55)</th>
<th>Control (n = 42)</th>
<th>P Value</th>
<th>G+/LVH+ vs G+/LVH−</th>
<th>G+/LVH+ vs Control</th>
<th>G+/LVH− vs Control</th>
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<tr>
<td>Age at baseline, mean (SD), y</td>
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<td>20 (10)</td>
<td>18 (8)</td>
<td>&lt;.001</td>
<td>.003</td>
<td>&lt;.001</td>
<td>.12</td>
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<td>.02</td>
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<td>39 (92.9)</td>
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<td>.11</td>
<td>.42</td>
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<td>1 (2.4)</td>
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<td>.02</td>
<td>.005</td>
<td>.33</td>
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<td>.03</td>
<td>.01</td>
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<td>.01</td>
<td>.005</td>
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<td>Systolic</td>
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<td>113 (12)</td>
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<td>.50</td>
<td>.54</td>
<td>.99</td>
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<td>68 (9)</td>
<td>66 (10)</td>
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<td>.80</td>
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<td>.28</td>
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<tr>
<td>I</td>
<td>67 (82.7)</td>
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<td>NA</td>
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<td>29 (52.7)c</td>
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<td>NA</td>
<td>NA</td>
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<td>NA</td>
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<td>NA</td>
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<td>MYL2</td>
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</table>

Abbreviations: G+, genotype positive; LVH, left ventricular hypertrophy; NA, not applicable; NYHA, New York Heart Association.

* P < .017 was considered statistically significant, applying post hoc Bonferroni correction.

† One patient also had a TNNT2 mutation, and another patient had a secondary MYBPC3 mutation.

‡ One patient also had a secondary MYBPC3 mutation.

§ Both patients also had a secondary MYBPC3 mutation.
### Table 2. Cardiac Imaging Findings

| Variable | G+/LVH+ | G+/LVH− | Control | P Value
|-----------|---------|---------|---------|---------|
| No. of participants | 81 | 55 | 42 | Style
| Interventricular septum thickness z score, mean (SE) | 6.2 (0.6) | 0.7 (0.2) | 0.4 (0.2) | NA | NA | .15
| Posterior wall thickness z score, mean (SE) | 1.3 (0.3) | −0.5 (0.2) | −0.5 (0.3) | NA | NA | .80
| Maximal LVWT z score, mean (SE) | 9.6 (0.8) | 1.5 (0.2) | 1.3 (0.3) | NA | NA | .18
| LVESD z score, mean (SE) | −2.7 (0.2) | −1.8 (0.1) | −1.1 (0.2) | .001 | <.001 | .007
| LVESD z score, mean (SE) | −2.5 (0.2) | −1.6 (0.2) | −0.6 (0.2) | .004 | <.001 | .001
| Ratio of LVWT to diastolic diameter, mean (SE) | 0.41 (0.02) | 0.21 (0.01) | 0.20 (0.01) | <.001 | <.001 | .001
| Left atrial diameter z score, mean (SE) | −0.8 (0.2) | −1.4 (0.1) | −1.6 (0.1) | <.001 | <.001 | .06
| LVEF, mean (SE), % | 66.7 (1.1) | 66.1 (1.1) | 62.1 (1.1) | .97 | .02 | .02
| Peak LV outflow tract gradient, mean (SE), mm Hg | 10.3 (0.8) | 7.8 (0.4) | 8.0 (0.4) | .002 | <.001 | .75
| Patients with peak gradient >30 mm Hg, No./total No. (%) | 4/78 (5.1) | 0/55 | 0/42 | NA | NA | NA
| Septal E′ velocity z score, mean (SE) | −1.5 (0.2) | −0.3 (0.1) | 0.0 (0.2) | <.001 | <.001 | .21
| Lateral E′ velocity z score, mean (SE) | −1.2 (0.2) | −0.4 (0.1) | −0.5 (0.2) | <.001 | .01 | .58
| Septal S′ velocity z score, mean (SE) | 0.2 (0.2) | 0.7 (0.2) | 0.4 (0.2) | .15 | .91 | .15
| Lateral S′ velocity z score, mean (SE) | −0.3 (0.2) | 0.1 (0.1) | −0.1 (0.2) | .07 | .76 | .16
| Septal E/E′ velocity, mean (SE) | 9.7 (0.3) | 7.8 (0.3) | 7.4 (0.3) | <.001 | <.001 | .45
| Lateral E/E′ velocity, mean (SE) | 6.8 (0.3) | 5.8 (0.2) | 6.0 (0.3) | .001 | .01 | .51

**CMR Imaging Measures**

| No. of participants | 42 | 34 | 23 | Style
| Age, mean (SD), y | 30 (13) | 21 (8) | 22 (7) | NA | NA | NA
| Female, No./total No. (%) | 13/42 (31.0) | 16/34 (47.1) | 15/23 (65.2) | NA | NA | NA
| LVEF, mean (SE), % | 67.8 (1.1) | 60.5 (2.2) | 58.3 (1.2) | .006 | <.001 | .38
| Maximal LVWT, mean (SE), mm | 15.4 (0.8) | 10.1 (0.4) | 9.9 (0.3) | NA | NA | .64
| LV mass, mean (SE), g | 158 (6) | 115 (4) | 121 (4) | <.001 | <.001 | .24
| LVEDV, mean (SE), mL | 138 (5) | 130 (4) | 146 (4) | .17 | .25 | <.001
| LVESV, mean (SE), mL | 45 (2) | 49 (2) | 61 (2) | .23 | <.001 | <.001
| LGE present, No./total No. (%) | 18/41 (43.9) | 0/31 | 0/23 | <.001 | .01 | .99
| LGE mass, mean (SE), g | 12 (14) | NA | NA | NA | NA | NA
| LGE, mean (SE), % LV mass | 6 (8) | NA | NA | NA | NA | NA

**Abbreviations:** CMR, cardiac magnetic resonance; G+, genotype positive; LGE, late gadolinium enhancement; LV, left ventricular; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; LVEF, left ventricular ejection fraction; LVWT, left ventricular wall thickness; NA, not applicable.

**Footnotes:**

* Results were adjusted for age, sex, and within-family correlation.
* Echocardiographic variables were also adjusted for the manufacturer of the equipment used. P < .02 was considered statistically significant, applying post hoc Bonferroni correction.
* The CMR imaging dimensions also adjusted for age squared and body surface area.
* Adjusted by exact logistic regression for age and sex but not within-family correlation.

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**MYBPC3.** eTable 2 in the Supplement contains the full list of genetic variants.

**Findings of Cardiac Imaging Studies**

Cardiac imaging findings are summarized in Table 2. Mutation carriers with and without LVH had smaller LV cavity (echocardiographic LVEDD and CMR imaging LV volumes), higher ratio of LVWT to diastolic diameter, and higher echocardiographic LVEF compared with controls. In addition, G+/LVH+ participants had lower mean tissue Doppler E′ velocity z scores and higher E/E′ ratios compared with G+/LVH− and control participants.

Ninety-nine participants (55.6% of the cohort) underwent CMR imaging (42 G+/LVH+, 34 G+/LVH−, and 23 controls). Based on pediatric institutional review board guidelines, CMR imaging studies were not performed in G−/LVH− and most G+/LVH− children. Late gadolinium enhancement was present in 43.9% (18 of 41) of G+/LVH+ participants but was absent from all G+/LVH− and control participants. Older age was significantly related to the presence but not the extent of LGE, with a mean age of 35 years (age range, 13-56 years) for the 18 patients with overt HCM with LGE vs 26 years (age range, 10-54 years) for the 23 patients without LGE (P = .02).
Findings From Exercise Testing and Electrocardiography

Results from exercise testing and the electrocardiographic analysis are summarized in Table 3 and Table 4 in the Supplement. Effort tolerance was preserved, with a mean exercise duration exceeding 11 minutes using a standard Bruce protocol. In total, 66.4% (37 of 54) of G+/LVH− and 64.6% (29 of 42) of control participants had normal electrocardiogram tracings compared with 38.6% (31 of 81) of G+/LVH+ participants (unadjusted percentages). No exercise or electrocardiographic metrics discriminated G+/LVH− from controls.

Burden of Early Phenotypes of Sarcomere Mutations

A composite measure including 7 variables (ratio of LVWT to diastolic diameter >0.23, LVEDD z score less than −1.5, LVESD z score less than −1.5, septal E′ velocity z score less than −1.5, serum high-sensitivity cardiac troponin I level exceeding 3.0 pg/mL, NTproBNP level >100 pg/mL, and Q waves or ST changes on electrocardiogram) was created to score the number of early manifestations present in each participant. Each variable was more prevalent among sarcomere mutation carriers compared with mutation-negative controls (Table 3). Phenotypic burden was scored as the total number of early phenotypes in each participant, ranging from 0 to 7. There was a significant stepwise increase in burden comparing the 3 cohorts (Figure 1). Burden was highest in G+/LVH+ participants, with a mean of 4.9 phenotypes per individual. No G+/LVH+ participants had 0 abnormalities, and 76.3%

### Table 3. Prevalence of Early Phenotypic Abnormalities Among Mutation Carriers and Controls*

<table>
<thead>
<tr>
<th>Variable</th>
<th>G+/LVH+ (n = 81)</th>
<th>G+/LVH− (n = 55)</th>
<th>Control (n = 42)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio of LVWT to diastolic diameter &gt;0.23</td>
<td>80 of 81 (98.9)</td>
<td>20 of 55 (34.7)</td>
<td>6 of 42 (12.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LVEDD z score less than −1.5</td>
<td>66 of 81 (81.6)</td>
<td>37 of 55 (67.6)</td>
<td>18 of 42 (36.9)</td>
<td>.002</td>
</tr>
<tr>
<td>Serum high-sensitivity cardiac troponin I level &gt;3.0 pg/mL</td>
<td>60 of 76 (73.1)</td>
<td>21 of 50 (40.1)</td>
<td>7 of 41 (20.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LVESD z score less than −1.5</td>
<td>57 of 81 (72.0)</td>
<td>31 of 54 (57.2)</td>
<td>13 of 42 (30.2)</td>
<td>.09</td>
</tr>
<tr>
<td>NTproBNP level &gt;100 pg/mL</td>
<td>46 of 76 (58.2)</td>
<td>7 of 50 (14.1)</td>
<td>3 of 41 (8.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Q waves or ST changes on electrocardiogram</td>
<td>41 of 81 (45.8)</td>
<td>7 of 54 (14.4)</td>
<td>1 of 42 (3.5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Septal E′ velocity z score less than −1.5</td>
<td>36 of 81 (42.5)</td>
<td>7 of 55 (13.3)</td>
<td>2 of 42 (5.0)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: G+, genotype positive; LVH, left ventricular hypertrophy; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; LVWT, left ventricular wall thickness; NTproBNP, N-terminal brain-type natriuretic peptide.

SI conversion factors: To convert NTproBNP level to nanograms per liter, multiply by 1.0; troponin I level to micrograms per liter, multiply by 1.0.

* P values and percentages were adjusted for patient age, sex, and within-family correlation. P < .017 was considered statistically significant, applying post hoc Bonferroni correction.
(58 of 76) had 4 or more abnormalities, including 21.1% (16 of 76) with all 7 traits. The G+/LVH− participants had an intermediate phenotypic burden score, with a mean of 2.4 phenotypes per individual, and 12.2% (6 of 49) had 0 abnormalities. Control participants had the lowest phenotypic burden score, with a mean of 1.3 phenotypes per individual, and 34.2% (14 of 41) had 0 abnormalities. A phenotypic burden score of 3 or higher had 47% sensitivity and 88% specificity in distinguishing G+/LVH− from controls. No significant differences in phenotypic burden scores were identified when comparing MYH7 and MYBPC3 mutation carriers (mean, 3.8 vs 3.9), including separate analyses restricted to G+/LVH+ and G+/LVH− participants (mean, 2.5 vs 2.3).

**Discriminating G+/LVH− and Control Participants**

The 7 traits comprising the phenotypic burden score were also evaluated with CART analysis, with the goal of discriminating G+/LVH− and control participants without a requirement of prespecified threshold values as input (eMethods in the Supplement). The model that maximized sensitivity is shown in the eMethods in the Supplement and indicates that using the combination of an LVEDD score and a septal E′ velocity z score to identify subgroups has a sensitivity of 76% and a specificity of 71% in identifying mutation carriers and true noncarriers. Specifically, an LVEDD z score less than −1.85 or the combination of an LVEDD z score of −1.85 or higher and a septal E′ velocity z score less than −0.52 had 74% accuracy in discriminating G+/LVH− participants from controls (eTable 5 in the eMethods in the Supplement).

**Increasing Maximal LVWT and Disease Manifestations in Mutation Carriers**

Rather than dichotomizing mutation carriers as LVH+ or LVH−, linear regression and spline analysis were used to characterize how phenotypic manifestations and burden change with increasing LVWT, analyzed as a continuous variable. Analyses were restricted to mutation carriers. Linear regression is shown in eTable 6 in the Supplement. With the exception of the extent of LGE and exercise duration and hemodynamic response, almost all metrics of LV structure, function, and electrocardiographic manifestations become significantly more abnormal, and the overall burden of early phenotypic traits increased with greater maximal LVWT z score.

Spline analysis was performed to identify physiologically relevant inflection points of maximal LVWT z scores. Most measures had significant linear associations across the continuum of maximal LVWT, without obvious knots. However, inflection points were identified for septal E′ velocity, exercise capacity (peak metabolic equivalent tasks), and phenotypic burden (Figure 2). Each of these metrics initially became more abnormal as LVWT increased, but when maximal LVWT z scores exceeded 10, 5.5, and 8 (corresponding to an absolute LVWT in the average adult of approximately 18, 13.5, and 16 mm, respectively), the association leveled off. These metrics no longer worsened with increasing maximal LVWT z score.

**Discussion**

Determining that sarcomere mutations cause HCM was a seminal discovery in the study of genetic cardiomyopathies. However, much remains unknown regarding the full phenotypic spectrum of sarcomere mutations, the natural history of apparently healthy, at-risk mutation carriers identified from cascade family testing, and how LVH relates to disease expression. Key findings from this study include (1) mutation carriers without LVH have a higher burden of early phenotypes compared with healthy controls and (2) cardiac abnormalities become more prominent with greater LVWT. However, the presence or absence of disease is not accurately reflected by a single measurement.

**Early Phenotypes of Sarcomere Mutations, Including Individual Traits and Collective Burden**

Left ventricular hypertrophy is not the initial manifestation of HCM. Other facets of LV function and geometry differ significantly between controls and mutation carriers with normal LVWT. For example, echocardiographic LV diameter was almost 1 SD smaller in G+/LVH− individuals compared with their healthy genotype-negative relatives. Smaller CMR imaging LV volumes and increased ratios of LVWT to diastolic diameter of G+/LVH− individuals are further evidence of altered LV geometry in mutation carriers with normal LVWT. These findings expand on earlier, largely single-center studies. Other metrics that differentiated G+/LVH− and G− controls in smaller, more homogeneous studies, including E′ velocity and electrocardiographic abnormalities, were not discriminating in this cohort. These results underscore that the G+/LVH− cohort, like the G+/LVH+ cohort, is heterogeneous, with variability in phenotypic expressivity and penetrance throughout all stages of disease development.

We also emphasize that the presence of a sarcomere mutation is not equivalent to the clinical diagnosis of HCM. Although phenotypic manifestations can be identified in G+/LVH− individuals, these findings are of greater value as markers of the underlying biological features of disease and future clinical course rather than providing information about current clinical consequences. Careful longitudinal study is needed to more fully characterize disease evolution and to accurately identify factors that influence prognosis and disease expression.

Recognizing that individual early phenotypic “abnormalities” can also be found in healthy controls, we tested the hypothesis that mutation carriers manifest a greater composite burden of phenotypes. We constructed a phenotypic burden score that discriminated the 3 participant groups: G+/LVH+ participants had the highest burden, and G− controls had the lowest burden. Similarly, CART analysis demonstrated that preclinical mutation carriers could be differentiated from controls by virtue of smaller LV cavity and lower E′ velocity. In a smaller single-center study, Gandjbakhch et al described the value of assessing the collective influence of 4 echocardiographic measures (ratio of interventricular septum thickness to posterior wall thickness,
relative wall thickness, and septal E/E' velocity). They found that an echocardiographic tissue Doppler imaging score had higher sensitivity and specificity in delineating G+/LVH− individuals from controls compared with looking individually at E' velocity. Although composite burden approaches will need to be refined and prospectively validated in other genotyped populations, we propose that assessing phenotypic burden will be a more accurate and biologically relevant means of following disease development or progression rather than focusing on any individual trait.

The Influence of LVWT on Phenotype in Sarcomere Mutation Carriers

Hypertrophic cardiomyopathy has traditionally been defined by arbitrary and binary thresholds of LVWT based on population norms. Once the threshold is exceeded, pathologic LVH is deemed to be present, and HCM is diagnosed in the appropriate context. This method is an imperfect approach because disease likely develops along a continuum, and simply measuring LVWT cannot fully capture its presence or absence. When analyzing LVWT as a continuous variable, the correlation with maximal LVWT z score and other clinical metrics appeared generally continuous and linear in sarcomere mutation carriers. A single threshold of LVWT did not cleanly delineate mutation carriers as “normal” or “abnormal.” While practical necessity often drives the use of dichotomous cut points and definitions in clinical practice, our data underscore that HCM does not develop in a binary manner.

Limitations

This study has several important limitations. Although our cohort was larger, more comprehensively investigated, and more diverse than prior studies, it was small owing to the rarity of genotyped individuals. We did not specifically select for participants with mild disease; however, the G+/LVH− cohort was young (mean [SD] age, 27 [14] years) and generally healthy and thus may not be fully representative of the adult overt HCM population. Because of the small size of specific genetic subgroups, all mutations were pooled together for analysis. Individual phenotypes could have stronger associations with certain genes or mutations than for others, but these signals can be diluted by pooled analysis. Finally, this investigation was a cross-sectional study. We compared individuals anticipated to be in the early (G+/LVH−) or established (G+/LVH+) stages of disease and used LVWT as a crude reflection of disease severity and progression. Performing true longitudinal studies of larger genotyped populations will be crucial to accurately characterize the natural history and the transition to disease.

Conclusions

Abnormalities of LV geometry and a greater burden of phenotypic manifestations can be detected in sarcomere mutation carriers without LVH. Two methods that integrated composite features were able to discriminate phenotypic subgroups, namely, a sum across 7 traits and a regression

Figure 2. Spline Analysis for Correlation Between Maximal Left Ventricular Wall Thickness (LVWT) z Score and Septal E’ Velocity, Exercise Capacity, and Early Phenotypic Burden in Sarcomere Mutation Carriers

A. Septal E’ velocity tended to worsen with increasing LVWT but the association diminished beyond a maximal LVWT z score of 10 (corresponding to an absolute LVWT of ~18 mm in the average adult). B, Peak metabolic equivalent tasks (METs) on exercise testing initially worsened, but levels off beyond a maximal LVWT z score of 5.5 (absolute adult LVWT, ~13.5 mm). C, The burden of early phenotypic manifestations increased prominently with LVWT up to a maximal LVWT z score of 8 (absolute adult LVWT, ~16 mm). *Adjusted for age, sex, and within-family correlation.
Early Phenotypes and the Influence of Left Ventricular Wall Thickness

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Ho reported being a founder of and owning shares in Myokardia inc, a start-up company that is developing therapeutics that target the sarcromere. No other disclosures were reported.

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REFERENCES


19. Park SH, Shub C, Nobrega TP, Bailey KR, Seward JB. Two-dimensional echocardiographic calculation of left ventricular mass as recommended by the American Society of Echocardiography: correlation
Invited Commentary

Assessing the Phenotypic Burden of Preclinical Sarcomeric Hypertrophic Cardiomyopathy—New Assessments to Guide Diagnosis and Management

Jil C. Tardiff, MD, PhD

Our understanding of hypertrophic cardiomyopathy (HCM) has advanced in the past 50 years since the original description by Teare and the characterization of phenotypic variability by Frank and Braunwald. Indeed, the central definition of HCM as “unexplained left ventricular hypertrophy (LVH)” has undergone recent modification to address the observation from genotyped cohorts that the degree (and even the presence) of LVH is highly variable. Besides the conundrum of how to incorporate into clinical teaching and training the reality that patients with HCM may not exhibit overt LVH, this nuanced definition raises important questions regarding modern patient care.

The discovery that familial HCM is caused by mutations in genes encoding sarcomeric proteins was a watershed in our understanding of this complex disorder. Not only were entirely new fields of basic mechanistic inquiry established, but the subsequent rapid growth and accessibility of new genetic sequencing techniques has led to a much broader range of clinical phenotypes observed in current practice as cascade sequencing identified preclinical genotype-positive (G+) individuals. The resultant transition from a practice cohort dominated by patients with significant and often dynamic dyspnea on exertion to a practice cohort defined by a gradient of disease symptoms and phenotypes from none to severe has vastly altered the management of HCM. Moreover, strong evidence is accruing that patients carrying sarcomeric gene mutations have more severe outcomes.

This new evidence has brought up issues regarding the timing of the onset of disease, whether to treat before symptom development, and the trajectory of myocardial remodeling over time. While the answers to these questions will guide modern management and the development of novel therapeutics, robust new clinical approaches to characterizing the earliest disease manifestations beyond binary measures of the left ventricular wall thickness (LVWT) in G+ individuals must first be established.

In this issue of JAMA Cardiology, Ho et al address this central question of how to better identify and classify the earliest stages of disease in subclinical (G+/LVH+) HCM cohorts. This cross-sectional multicenter observational study was designed to evaluate the collective burden of phenotypic manifestations in G+ (sarcomeric mutation) carriers (LVH+/LVH−) vs G− control individuals and the relationship between LVWT and other known disease features. Drawing from a consortium of HCM specialty centers across the United States (HCMNet), the authors collected small yet meticulously phenotyped cohorts representing G+/LVH+, G+/LVH−, and G−/LVH− (control) individuals. Phenotypic burden was assigned as the cumulative number of 7 traits that were chosen based on the ability to discriminate between G+/LVH+ vs G+/LVH− (left ventricular end-systolic and end-diastolic dimensions derived from 2-dimensional echocardiography, the ratio of LVWT to diastolic diameter, and serum troponin and N-terminal brain-type natriuretic peptide levels) or between G+/LVH+ vs controls (septal E′ velocity and electrocardiographic evidence of Q waves or ST changes). Primary results demonstrated a significant increase in early phenotypes among G+/LVH− individuals vs controls, particularly marked by smaller left ventricular diameters and further supported by smaller left ventricular volumes on cardiac magnetic resonance imaging and increased ratios of LVWT to diastolic diameter. When LVWT was analyzed as a continuous variable, the above abnormalities tracked in a linear fashion in G+ individuals, yet no single