Assessment of Diastolic Function With Doppler Tissue Imaging to Predict Genotype in Preclinical Hypertrophic Cardiomyopathy

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Background—Unexplained left ventricular hypertrophy (LVH) is considered diagnostic of hypertrophic cardiomyopathy (HCM) but fails to identify all genetically affected individuals. Altered diastolic function has been hypothesized to represent an earlier manifestation of HCM before the development of LVH; however, data regarding the clinical utility of imaging techniques that assess this parameter are limited.

Methods and Results—Echocardiographic studies including Doppler tissue imaging (DTI) were performed in a genotyped HCM population with β-myosin heavy chain (β-MHC) mutations. Genotype (+) individuals with LVH (G+/LVH+; n=18) and genotype (+) individuals without LVH (G+/LVH−; n=18) were compared with normal control subjects (n=36). Left ventricular ejection fraction (EF) was significantly higher in both genotype (+) groups (75±5% and 71±6%, respectively, versus 64±5% in control subjects; P<0.0001). Mean early diastolic myocardial velocities (Ea) were significantly lower in both genotype (+) subgroups, irrespective of LVH (P<0.02). However, there was substantial overlap in Ea velocities between the G+/LVH− and control groups. An Ea velocity of ≥13.5 cm/s had 86% specificity and 75% sensitivity for identifying genotype-positive subjects. The combination of EF ≥68% and Ea velocity <15 cm/s was 100% specific and 44% sensitive in predicting affected genotype.

Conclusions—Abnormalities of diastolic function assessed by Doppler tissue imaging precede the development of LVH in individuals with HCM caused by β-MHC mutations. Although Ea velocity alone was not sufficiently sensitive as a sole diagnostic criterion, the combination of Ea velocity and EF was highly predictive of affected genotype in individuals without overt manifestations of HCM. (Circulation. 2002;105:2992-2997.)

Key Words: hypertrophy ■ cardiomyopathy ■ genetics ■ echocardiography ■ diastole

Hypertrophic cardiomyopathy (HCM) is an autosomal-dominant disorder characterized by unexplained left ventricular hypertrophy (LVH), abnormalities of diastolic function, and increased risk for sudden death.1,2 More than 140 disease-causing mutations have been identified in genes encoding sarcomere proteins, most commonly myosin heavy chain (β-MHC), cardiac myosin binding protein C, cardiac troponin T, and cardiac troponin I.3–5 The echocardiographic finding of LVH not accounted for by other systemic or cardiac disease generally serves as the basis for diagnosing HCM. Nevertheless, the presence or absence of LVH is not an infallible diagnostic criterion and the spectrum of hypertrophy is broad, even among individuals with the same mutation.2,6 Furthermore, because full expression of the HCM phenotype typically does not occur until adolescence or thereafter,4,7,8 establishing the clinical diagnosis of HCM early in life may be particularly challenging.

Impaired diastolic function is common in patients with overt HCM,9 but its role in preclinical HCM is less well established. Conventional echocardiographic methods for assessing diastolic function have relied on transmitral Doppler flow patterns and velocities, but there has been no consistent relationship between these indices and left ventricular (LV) end-diastolic pressure,10,11 the extent of LVH, the presence or absence of LV outflow tract obstruction, symptoms, or exercise capacity.9–13 Doppler tissue imaging (DTI) is a recently validated echocardiographic technique that allows measurement of myocardial velocities in systole and diastole and provides the potential for load-independent assessments of diastolic function.14,15 Indeed, studies incorporating DTI have shown a close correlation between early diastolic myocardial (Ea) velocities and LV filling pressures...
in patients with HCM. Data from a transgenic rabbit model of HCM and preliminary echocardiographic studies on humans suggest that Ea velocities are abnormally low in genotype-positive subjects before the development of LVH. Therefore, we performed a study to assess whether abnormalities of diastolic function may appear before development of LVH and serve as a clinical marker for the presence of genetic mutations in HCM.

Methods

Study Population

Our study population consisted of genotyped individuals from kindreds with HCM caused by \( \beta \)-MHC mutations. Genetically affected subjects with LVH (G+/LVH+, maximal wall thickness \( >12 \) mm) and without LVH (G+/LVH−) were compared with each other and with normal control subjects of similar age (genetically unaffected family members and healthy volunteers). Informed consent was obtained from all participants in accordance with the guidelines of the Institutional Review Board of Brigham and Women’s Hospital. Study subjects were assessed by history, physical examination, ECG, and echocardiography. Individuals were excluded if they were found to have coexistent conditions that may contribute to the development of LVH or diastolic abnormalities (eg, systemic hypertension, coronary artery disease, and valvular heart disease).

Echocardiographic Protocol

Echocardiographic studies were performed with an Agilent Sonos 5500 ultrasound system. Standard 2-dimensional images, M-mode, spectral and color Doppler, and Doppler tissue interrogation were performed. A single observer (C.Y.H.) analyzed the echocardiographic data offline, blinded to knowledge of both genotype and control status. LV septal and posterior wall thickness, cavity dimensions and volumes, mass, and left atrial size were determined from 2-dimensional images according to established criteria. LV ejection fraction (EF) was calculated from LV volumes using the modified Simpson’s method. Peak early (E) and late (A) transmitral velocities, E/A ratio, E-wave deceleration time, and isovolumic relaxation time (IVRT) were measured from spectral Doppler displays. Doppler tissue myocardial velocities were measured in systole (Sa) and early diastole (Ea) and with atrial contraction (Aa) at the lateral, septal, inferior, and anterior corners of the mitral annulus.

Statistical Analysis

ECG findings between the study groups were compared using Fisher’s exact test. Multiple group comparisons of echocardiographic parameters among the 3 study groups were made using ANOVA followed by post hoc \( t \) testing with Bonferroni correction. \( P<0.05 \) was considered statistically significant. Univariate and multivariate logistic regression analyses were used to assess the relative contribution and independence of echocardiographic parameters and age on genotype status. Receiver operating characteristic (ROC) curves were plotted to determine the optimal cutoff values for individual parameters to predict genotype status.

Results

Clinical Characteristics

Subjects were recruited from 5 unrelated HCM kindreds with different \( \beta \)-MHC mutations, Gly768Arg (n = 1), Arg719Trp (n = 1), Arg663His (n = 6), Arg652Gly (n = 6), and Asp906Gly (n = 22). The clinical characteristics of the study subjects are shown in Table 1. The mean ages of the G+/LVH− (n = 18) and control (n = 36) subgroups were not significantly different (24.2 ± 10.2 years and 25.8 ± 9.2 years, respectively).

### Table 1. Clinical and Echocardiographic Characteristics of the Study Group

<table>
<thead>
<tr>
<th></th>
<th>G+/LVH+ (n = 18)</th>
<th>G+/LVH− (n = 18)</th>
<th>Normal Controls (n = 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>35.6 ± 12.6*</td>
<td>24.2 ± 10.2‡</td>
<td>25.8 ± 9.2</td>
</tr>
<tr>
<td>Females/Males</td>
<td>8/10</td>
<td>11/7</td>
<td>15/21</td>
</tr>
<tr>
<td>Mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg719Trp</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Gly768Arg</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Arg663His</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Arg652Gly</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Asp906Gly</td>
<td>9</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>LV end-diastolic diameter, cm</td>
<td>4.3 ± 0.3†</td>
<td>4.7 ± 0.5‡</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td>LV end-systolic diameter, cm</td>
<td>2.2 ± 0.4†</td>
<td>2.6 ± 0.4‡</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>75 ± 5†</td>
<td>71 ± 6‡</td>
<td>64 ± 5</td>
</tr>
<tr>
<td>Left atrium, cm</td>
<td>4.2 ± 0.5†</td>
<td>3.7 ± 0.5‡</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>Septal thickness, mm</td>
<td>17.5 ± 6.8†</td>
<td>9.5 ± 1.5§</td>
<td>9.1 ± 1.5</td>
</tr>
<tr>
<td>Posterior wall thickness, mm</td>
<td>11.1 ± 3.3†</td>
<td>8.5 ± 1.4±</td>
<td>8.2 ± 1.3</td>
</tr>
<tr>
<td>LV mass, g</td>
<td>225.6 ± 85.4†</td>
<td>152.6 ± 39.7‡</td>
<td>140.6 ± 42.4</td>
</tr>
<tr>
<td>Peak E-wave velocity, cm/s</td>
<td>80.1 ± 24.0</td>
<td>85.3 ± 12.0</td>
<td>83.0 ± 18.8</td>
</tr>
<tr>
<td>Peak A-wave velocity, cm/s</td>
<td>59.3 ± 21.6</td>
<td>53.6 ± 10.6</td>
<td>48.9 ± 14.2</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.5 ± 0.4</td>
<td>1.7 ± 0.4</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>E deceleration time, ms</td>
<td>201 ± 86*</td>
<td>177 ± 33</td>
<td>156 ± 23</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>113 ± 17</td>
<td>103 ± 15</td>
<td>101 ± 22</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. *P<0.05 compared with normal controls. †P<0.0001 compared with normal controls. ‡P<0.05 compared with G+/LVH+. §P<0.0001 compared with G+/LVH+. G+/LVH− and control subjects were asymptomatic and taking no cardiac medications and had normal standard echocardiographic studies. There were no significant differences in left atrial size, LV wall thickness, diastolic diameter, or LV mass between the G+/LVH− subgroup and normal control subjects. There were no statistically significant differences in ECG findings between the G+/LVH− subgroup and normal control subjects (Table 2). G+/LVH− individuals were more likely to have ECG abnormalities; however, most (56%) of these changes were nonspecific ST- and T-wave changes. Only 2 of 18 (11%) of G+/LVH− individuals had ECG changes previously implicated as potential early signs of disease expression; both of these individuals had repolarization abnormalities. No G+/LVH− individuals had ECG criteria for LVH, pathological Q waves, or abnormal QRS axis.

G+/LVH+ individuals (n = 18) were asymptomatic and had well-preserved LV systolic function. This subgroup was found to have significantly greater LV mass and maximal wall thickness than the other 2 groups. Of note, LVEF in both of the genotype (+) subgroups was significantly higher than in the control group. The G+/LVH+ subgroup had the highest mean EF (75 ± 5%), significantly greater than the G+/LVH− group (mean EF 71 ± 6%, P<0.05) and normal control subjects (mean EF 64 ± 5%, P<0.0001 compared with both genetically affected subgroups).
Traditional measures of diastolic function, including E/A ratio, E-wave deceleration time, and IVRT, were not significantly different among the 3 groups with the exception of increased E-wave deceleration time in the G+/LVH+ subgroup relative to normal control subjects (201±86 versus 156±23 ms, respectively, P=0.007).

**Doppler Tissue Imaging**

Compared with normal control subjects, the 2 subgroups with β-MHC mutations had evidence of abnormal diastolic function by Doppler tissue imaging. This was manifested as significantly lower Ea velocities at each individual corner of the mitral annulus (anterior, inferior, lateral, and septal) as well as the averaged Ea velocities from these 4 corners, regardless of the presence or absence of LVH (Table 3). Ea velocities were highest in the normal control group (13.5 to 18.2 cm/s) and lowest in the G+/LVH+ group (8.5 to 10.9 cm/s). The velocities of the G+/LVH− group were intermediate (11.6 to 15.8 cm/s). At the individual corners of the mitral annulus, the early diastolic velocities were reduced by 13% to 19% in the G+/LVH− group compared with normal control subjects (P<0.02). Myocardial velocities in systole (Sa) did not differ significantly between the G+/LVH− group and normal control subjects but were reduced by 15% to 21% in the G+/LVH+ group compared with normal control subjects (P<0.005) (Table 3).

**Prediction of Affected Genotype**

Despite statistically significant differences in mean Ea velocities, there was considerable overlap between the individual velocities among the 3 groups (Figure 1). The greatest degree of overlap occurred between the G+/LVH− and control groups. In an analysis of these 2 groups, we found an inverse relationship between Ea velocity and age. As a consequence, a higher Ea velocity was associated with affected genotype in younger individuals (Figure 2). An averaged Ea velocity of 9.9 cm/s in individuals >25 years of age predicted an 80% probability of affected genotype but was nearly 100% predictive in those ≤25 years of age.

**LVH, Ea velocity, EF, and age were all univariate predictors of affected genotype. In G+/LVH− patients, only Ea velocity and LVEF were independently predictive of genetic**
status after adjusting for age, wall thickness, and left atrial size (Table 4). ROC curve analysis identified an optimal cutoff value for averaged Ea velocity of <13.5 cm/s. This velocity was associated with a sensitivity of 75% and specificity of 86% for predicting affected genotype. The optimal cutoff value of LVEF was >68%, associated with a sensitivity of 77% and a specificity of 81%. The combination of EF and Ea velocity was the most specific in predicting genetic status. ROC analysis of the combination of EF and averaged Ea velocity identified an optimal cutoff of LVEF ≥68% and averaged Ea velocity <15 cm/s. This combination yielded a sensitivity of 44% and specificity of 100% in predicting affected genotype. There were no G+/LVH− individuals with a combined EF <68% and averaged Ea velocity ≥15 cm/s and, conversely, no control subjects with an EF ≥68% and averaged Ea velocity <15 cm/s (Figure 3).

**Differences Among Individual MHC Mutations**

We examined the effect of specific β-MHC mutations (Arg663His, n=6; Arg652Gly, n=6; and Asp906Gly, n=22) on echocardiographic parameters in 3 unrelated families. LV mass in hypertrophied individuals was greatest in association with the Arg663His mutation (379.5±83.7 g) and least with Asp906Gly (192.6±33.5 g; P<0.0001). LVEF in nonhypertrophied family members was significantly higher with the Arg663His mutation versus Asp906Gly (80±4% versus 69±4%, P=0.005). There were minor intermutational differences for Ea and Sa velocities among hypertrophied individuals, but no significant differences were found among nonhypertrophied individuals (data not shown).

**Discussion**

We report that Doppler tissue imaging can detect subtle abnormalities of diastolic function in individuals with hypertrophic cardiomyopathy before the development of both LVH and alterations in more traditional echocardiographic parameters of diastolic function. Nevertheless, the range of Ea velocities is broad. Although our data and those previously reported suggest that diastolic dysfunction may be a primary component of preclinical HCM, determination of Ea velocities alone in individuals of unknown genotype is not sufficiently sensitive to supplant genetic analysis for definitive, independent determination of affected status. Using age-specific cutoffs for Ea velocity and incorporating other parameters, such as LVEF, may improve the predictive accuracy of these clinical markers.

Our findings of altered diastolic function in HCM caused by β-MHC mutations relate to those reported by Nagueh et al involving patients with a variety of causal mutations, predominantly in the gene encoding cardiac myosin binding protein C. We limited the scope of our investigation to mutations involving β-MHC to attempt to minimize biological variation associated with different mutations. It is possible that our results reflect intrinsic differences between β-MHC mutations and those involving other components of the sarcomere, although considerable data indicate that diastolic dysfunction is an important component of HCM produced by β-MHC mutations. In addition, the mean age of our G+/LVH− subgroup was 11 years younger than that of the comparable group in the prior study. Thus, our study population likely represents an earlier stage of disease development, accounting for the higher Ea and Sa velocities described here.

Our data were consistent with previous studies demonstrating that parameters of diastolic function, including Ea velocities, tend to change with age and suggest that age modifies...
the relationship between the likelihood of affected genetic status and Ea velocity in HCM. Taken as a whole, our findings indicate that abnormalities of diastolic function become more apparent as genetically affected individuals, with or without LVH, age. Because our G+/LVH− subjects were younger than our G+/LVH+ subgroup and G+/LVH− subjects in prior studies,18 we postulate that abnormalities in systolic function may develop later in life than those in diastolic function.

We found a substantial degree of overlap of Ea velocities between the G+/LVH− group and normal control subjects, corresponding to an intermediate sensitivity and specificity of diastolic tissue Doppler velocities in predicting affected genotype. In contrast, Nagueh et al18 reported that a lateral Ea <14 cm/s had 100% sensitivity and 90% specificity for differentiating genotype-positive individuals without LVH from normal control subjects. Applying this criterion to our population would result in the misclassification of two thirds from normal control subjects. Applying this criterion to our differentiating genotype-positive individuals without LVH would become more apparent as genetically affected individuals, with or without LVH, age. Because our G+/LVH− subjects were younger than our G+/LVH+ subgroup and G+/LVH− subjects in prior studies,18 we postulate that abnormalities in systolic function may develop later in life than those in diastolic function.

In this study, we found reduced systolic myocardial velocities in G+/LVH+ individuals but not in the G+/LVH− subgroup. Thus, our results do not support the hypothesis that impaired systolic function is a primary manifestation of HCM,24,25 suggesting instead that diastolic abnormalities may be an earlier consequence of sarcomere protein mutations. Nevertheless, the time course of the development of impaired systolic tissue Doppler velocities relative to the onset of LVH is unclear. It is possible that impaired systolic myocardial velocities develop before and contribute to the development of LVH.

We found a statistically significant increase in LVEF in both of the genotype-positive groups compared with normal control subjects. The observation that EF was increased in the G+/LVH− group suggests that this finding is not simply the result of a smaller LV cavity size secondary to LVH but may be related to intrinsic physiological changes caused by sarcomere mutations. Although it has been reported that HCM-causing mutations are associated with impaired sarcomere contractility in vitro studies,24,25 other experimental models, particularly those evaluating β-MHC mutations, suggest that a hypercontractile state is conferred by sarcomere protein mutations.26–28 It is conceivable that this hypercontractility may then serve as the fundamental stimulus for the development of myocyte hypertrophy. Recognizing that the absolute increase in EF was modest, our findings of supranormal LVEF in individuals with β-MHC mutations before the development of LVH may support the presence of fundamentally augmented sarcomere function in HCM.

It is intriguing that the combination of a relatively reduced Ea velocity and increased EF was a highly specific marker of affected genetic status in our population, a finding that could assist in the preclinical diagnosis of HCM. In our study, a LVEF ≥68% in association with an Ea velocity <15 cm/s indicated an affected genotype, whereas an EF <68% and Ea velocity ≥15 cm indicated unaffected genetic status with 100% specificity. Determining the strength of this association and whether this finding is specific only for β-MHC mutations, or occurs with other genetic causes of HCM, warrants additional study.

HCM is the most common heritable cardiovascular disease with a prevalence that approaches 1 in 500.29 These data provide additional evidence that abnormal diastolic function is an inherent component of the pathophysiology of HCM rather than merely a consequence of LVH or impaired systolic function. Documenting diastolic abnormalities as an early sign of disease could ultimately prove to be an important clinical variable to establish diagnosis, monitor progression of cardiomyopathy, and assess response to early intervention.

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References


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