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Diastolic Abnormalities as the First Feature of Hypertrophic Cardiomyopathy in Dutch Myosin-Binding Protein C Founder Mutations

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OBJECTIVES To test the hypothesis that carriers of Dutch founder mutations in cardiac myosin-binding protein C (MYBPC3), without left ventricular hypertrophy (LVH) or electrocardiographic abnormalities, have diastolic dysfunction on tissue Doppler imaging (TDI), which can be used for the screening of family members in the hypertrophic cardiomyopathy (HCM) population.

BACKGROUND TDI is a more sensitive technique for the assessment of left ventricular contraction and relaxation abnormalities than is conventional echocardiography.

METHODS Echocardiographic studies including TDI were performed in genotyped hypertrophic cardiomyopathy patients (genotype-positive, G+/LVH+; n = 27), mutation carriers without LVH (G+/LVH−; n = 27), and healthy controls (n = 55). The identified mutations in MYBPC3 in the G+/LVH+ subjects were c.2864_2865delCT (12 subjects), c.2373dupG (n = 8), and p. Arg943X (n = 7). In the G+/LVH− subjects, the following mutations were identified: c.2864_2865delCT (n = 11), c.2373dupG (n = 8), and p. Arg943X (n = 8).

RESULTS Mean TDI-derived systolic and early and late diastolic mitral annular velocities were significantly lower in the G+/LVH+ subjects compared with the other groups. However, there was no difference between controls and G+/LVH− subjects. Mean TDI-derived late mitral annular diastolic velocities were significantly higher in the G+/LVH− subjects compared with controls and G+/LVH+ subjects. Using a cut-off value of mean ± 2 SD, an abnormal late mitral annular diastolic velocity was found in 14 (51%) of G+/LVH− patients. There was no difference among the 3 different mutations.

CONCLUSIONS In contrast to earlier reports, mean mitral annular systolic velocity and early mitral annular diastolic velocity velocities were not reduced in G+/LVH− subjects, and TDI velocities were not sufficiently sensitive for determination of the affected status of an individual subject. Our findings, however, support the theory that diastolic dysfunction is a primary component of pre-clinical HCM. (J Am Coll Cardiol Img 2009;2:58–64) © 2009 by the American College of Cardiology Foundation

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Hypertrophic cardiomyopathy (HCM) is defined by the presence of left ventricular hypertrophy (LVH) in the absence of loading conditions (hypertension, valve disease) sufficient to cause the observed abnormality (1). In most cases, it is a familial disease with an autosomal dominant pattern of inheritance caused by mutations in genes that encode different proteins of the cardiac sarcomere. However, LVH is absent in a significant number of mutation carriers because of the variable penetrance of the mutations and confounding effects of modifier genes, sex, and environmental factors. Overall, LVH is neither very sensitive nor very specific in HCM diagnosis (2–4).

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An alternative approach to the early diagnosis of HCM is genetic testing, which can identify mutation carriers before development of LVH. However, using the current techniques, sarcomeric mutations are identified in 50% to 60% of HCM patients, making screening of family members by genetic testing impossible in up to 50% of HCM families (5).

Experimental data suggest that cardiac myocyte contractile function in HCM is reduced and that the hypertrophy is compensatory (6,7). These data, in conjunction with myocyte disarray, the characteristic hallmark of HCM, led to the hypothesis that tissue Doppler imaging (TDI), with its possibility to identify contraction and relaxation abnormalities, would be more sensitive for the diagnosis of HCM than conventional echocardiography. This principle has been proven by several studies in both animals and patients carrying different sarcomeric mutations (8–10).

The current study was performed to test the hypothesis that TDI can be used for the screening of family members in the Dutch HCM population. The Dutch HCM population is special because the majority of HCM in the Netherlands is caused by 1 of 3 founder mutations in cardiac myosin-binding protein C (MYBPC3); c.2373dupG (11), c.2864_2865delCT, and p. Arg943X.

METHODS

Study population. At our cardiogenetic outpatient clinic, systematic family screenings of genotyped HCM patients are performed. The mutation carriers (G+/LVH–; n = 27) were selected from consecutive genotyped family members without major or minor criteria for the diagnosis of HCM on routine echocardiography or electrocardiography as described by McKenna et al. (12). The control group consisted of 55 healthy age- and gender-matched subjects without cardiovascular disease or diabetes mellitus and with normal left atrial dimension and left ventricular dimension and function. The third group included 27 HCM patients (LV wall thickness ≥15 mm) based on 1 of the Dutch founder mutations. All subjects provided informed written consent. The study complies with the Declaration of Helsinki, and the Erasmus University Medical Center Review Board has approved the study.

2-dimensional echocardiography. Echocardiographic studies were performed with a Sonos 7500 ultrasound system with a S3 transducer or an iE33 system with a S5-1 transducer (Philips Medical Systems, Best, the Netherlands). The acquired data were digitally stored and subsequently analyzed by an observer, who was blinded to the clinical data. From the second harmonic M-mode recordings, the following data were acquired: left atrial dimension, left ventricular septal and posterior wall thickness, and left ventricular end-diastolic and end-systolic dimension. From the Doppler mitral-inflow pattern, early (E) and late (A) left ventricular filling velocities, the E/A ratio, E-velocity deceleration time, and the duration of the A were measured. Pulmonary vein systolic flow, diastolic flow, and flow during atrial contraction were also measured. Left ventricular ejection fraction was assessed on 2-dimensional echocardiography using the biplane Simpson method.

TDI. TDI was performed by placing the sample volume at the side of the medial, lateral, inferior, anterior, posterior, and anteroseptal mitral annulus in the standard apical views. Gain and filter settings were adjusted as needed to eliminate background noise and to allow for a clear tissue signal. To acquire the highest tissue velocities, the angle between the Doppler beam and the longitudinal motion of the investigated structure was adjusted to a minimal level. The mitral annular systolic (Sa) and early (Ea) and late (Aa) diastolic velocities were recorded at end-expiratory at a sweep speed of 100 mm/s and measured using electronic calipers with Enconcert software (Philips Medical Systems). TDI-derived Sa, Ea, and Aa mitral annular velocities from 6 mitral annular regions were measured. The individual average for the 6 measurements was

ABBREVIATIONS AND ACRONYMS

A = late LV filling velocity
Aa = late mitral annular diastolic velocity
E = early LV filling velocity
Ea = early mitral annular diastolic velocity
G = genotype
HCM = hypertrophic cardiomyopathy
LVH = left ventricular hypertrophy
MYBPC3 = cardiac myosin-binding protein C
Sa = mitral annular systolic velocity
TDI = tissue Doppler imaging

Table 1. Subject Characteristics

Characteristics	G+/LVH+ (n = 27)	G+/LVH− (n = 27)	Controls (n = 55)
Age, yrs	47 ± 12*†	37 ± 12	37 ± 10
Percentage male	63*†	33	37
Identified mutation			
2864delCT	12	11	0
2373insG	8	8	0
R943X	7	8	0

Between-groups Games-Howell–corrected p value: *p < 0.01 versus controls; †p < 0.01 versus G+/LVH− subjects.
G = genotype; LVH = left ventricular hypertrophy.

used for analysis. The different mitral annular regions were also compared. The dimensionless ratio of E/Ea was computed at all corners; this parameter is an index that corrects for the influence of left ventricular relaxation on mitral peak E velocity and provides a good estimate of left ventricular filling pressures in HCM (13).

Diastolic function was subsequently graded 0 to 4, as previously described (14). Based on color Doppler and TDI measurements, normal left ventricular diastolic function (stage 0) was defined as a combination of E/A ratio between 0.75 and 1.50, deceleration time between 150 and 220 ms, pulmonary vein systolic flow greater than diastolic flow, duration of the pulmonary vein flow during atrial contraction less than A-wave duration +30 ms, mean Ea >10.0 cm/s, and E/Ea <9. Stage 1 diastolic dysfunction (impaired LV relaxation) was defined as an E/A ratio <0.75 and deceleration time >220 ms. Stage 2 diastolic dysfunction (pseudonormal LV filling) was defined as an E/A ratio between 0.75 and 1.5, with a mean Ea <7 cm/s and E/Ea >15. Stage 3 diastolic dysfunction (reversible restrictive LV filling) was defined as an E/A ratio >1.5 with a deceleration time <150 ms, mean Ea <7 cm/s, and E/Ea >15 reversing to pseudonormal or even impaired relaxation during the Valsalva maneuver. Stage 4 diastolic dysfunction (fixed restrictive) was defined as E/A ratio >1.5 with a deceleration time <150 ms, a mean Ea <7 cm/s, and an E/Ea >15 without change with Valsalva (14–16).

Statistical analyses. All statistics were performed using the SPSS version 14 for Windows (SPSS Inc., Chicago, Illinois). Descriptive data were computed as a mean value ± SD. Variables among the 3 groups were compared by analysis of variance using the Games-Howell corrections for post-hoc analysis. Statistical significance was defined by $p \leq 0.05$. The Welch and Brown-Forsyth test confirmed the standard statistics.

RESULTS

Study population. The subject characteristics are displayed in Table 1. The mean age and the sex of controls and G+/LVH− subjects were similar. In the G+/LVH+ population, the mean age was significantly higher, and there were significantly more males compared with the other 2 groups. Among the G+/LVH− subjects, the distribution of the MYBPC3 mutations was as follows: c.2864_2865delCT in 11, c.2373dupG in 8, and p.Arg943X in 8 subjects. Among the G+/LVH+ subjects, the distribution of the MYBPC3 mutations was as follows: c.2864_2865delCT in 12, c.2373dupG in 8, and p.Arg943X in 7 subjects.

Echocardiographic analysis. All subjects had 2-dimensional and Doppler studies satisfactory for analysis. The results of the echocardiographic analysis are displayed in Table 2.

By definition, mean septal wall thickness was significantly higher in the G+/LVH+ subjects compared with the control group and the G+/LVH− subjects. There were no differences in left ventricular end-diastolic diameter, left ventricular end-systolic diameter, fractional shortening, and left ventricular ejection fraction among the 3 groups. Left atrial dimension was significantly

Table 2. Echocardiographic Characteristics of the Study Population

	G+/LVH+ Subjects (n = 27)	G+/LVH− Subjects (n = 27)	Controls (n = 55)
2D echocardiography			
LVEDD (mm)	46 ± 6	49 ± 5	50 ± 5
LVESD (mm)	28 ± 8	30 ± 5	31 ± 5
FS (%)	39 ± 9	38 ± 7	36 ± 7
EF (%)	61 ± 11	61 ± 5	62 ± 7
IVS (mm)	19 ± 5*†	10 ± 2	9 ± 1
LVPW (mm)	11 ± 2	9 ± 2	9 ± 2
LA (mm)	49 ± 9*†	36 ± 5	32 ± 5
Diastolic dysfunction			
Stage 0	5	27	55
Stage 1	5	0	0
Stage 2	7	0	0
Stage 3	7	0	0
Stage 4	3	0	0

Between groups Games-Howell-corrected p value: *p < 0.01 versus controls; †p < 0.01 versus G+/LVH− subjects.
FS = fractional shortening; G = genotype; IVS = interventricular septal thickness; LA = left atrial diameter; LVEDD = left ventricular end-diastolic diameter; LVESD = left ventriclar end-systolic diameter; LVH = left ventricular hypertrophy; LVPW = left ventricular posterior wall thickness; stages of diastolic dysfunction, stage 0 = normal, stage 1 = abnormal relaxation, stage 2 = pseudonormal, stage 3 = reversible restrictive, and stage 4 = fixed restrictive.

higher in the G+/LVH+ subjects compared with the other 2 groups.

All patients with definite HCM were in sinus rhythm. None had a left ventricular outflow tract gradient >50 mm Hg. Most patients, 16 (60%), were asymptomatic; 11 (40%) were in New York Heart Association functional class II. Medical treatment was used by 12 (44%) of the HCM patients, and consisted of beta-blockers for 6 (22%) patients, a combination of a beta-blocker and diuretics for 2 (7%) patients, and a combination of a beta-blocker, diuretics, and angiotensin-converting enzyme inhibitors for 3 (11%) patients; amiodarone was used by 1 (4%) HCM patient.

Diastolic function was normal (grade 0) in all controls and all G+/LVH− subjects. In the majority of G+/LVH+ subjects, diastolic dysfunction was present.

Mean Sa, Ea, and Aa velocities were significantly lower in the G+/LVH+ subjects compared with controls and G+/LVH− subjects. Mean Sa and Ea were no different between controls and G+/LVH− subjects. However, mean Aa velocities were significantly higher in the G+/LVH− subjects compared with controls and G+/LVH+ subjects (Fig. 1). Using a cut-off value of mean + 2SD (>97 mm/s), an abnormal Aa was found in 14 (51%) of G+/LVH− patients. The G+/LVH+ patients have abnormal regional velocities compared with G+/LVH− subjects and controls (Fig. 2). In the G+/LVH− patients, Aa velocity was abnormal in most of the mitral annular regions. There was no difference among the 3 different mutations.

DISCUSSION

Hypertrophic cardiomyopathy is a primary disorder of cardiac myocytes, characterized by hypertrophy in the absence of increased external load. In most cases, it is an autosomal-dominant disease, and routine echocardiography and electrocardiography are used to screen family members (12). However, LVH is absent in a significant number of mutation carriers. Early and accurate phenotypic diagnosis of affected family members by TDI could provide an opportunity to prevent or modify the clinical manifestations of HCM and would complement genetic testing, which is complicated by allelic and nonallelic heterogeneity. In contrast to earlier reports, in our study population, mean Sa and mean Ea velocities were not reduced in G+/LVH− subjects compared with controls and could not be used to differentiate the G+/LVH− subjects from the

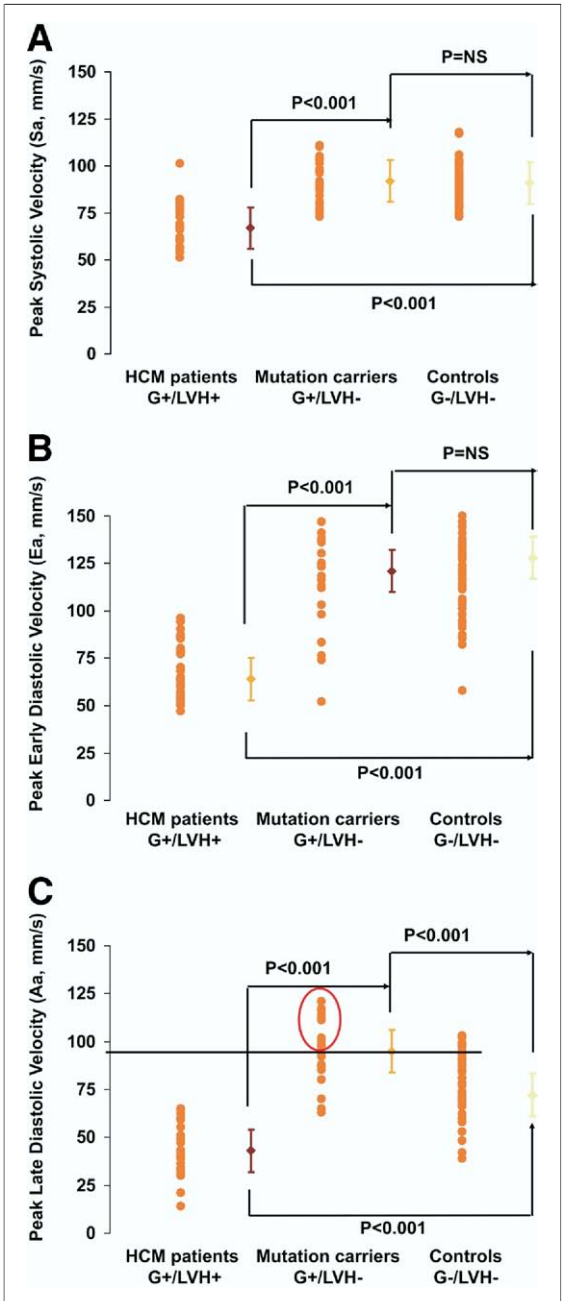

Figure 1. Individual Mean Values of Tissue Doppler Velocity

The individual mean values of the peak early systolic **(A)**, peak early diastolic **(B)**, and peak late diastolic **(C)** components of longitudinal velocity obtained from tissue Doppler imaging, which are averaged from 6 different mitral annular sites (anterior, anteroseptal, inferior, lateral, posterior, and posteroseptal) in the hypertrophic cardiomyopathy (HCM) patients, family members, and healthy controls. Aa = late mitral annular diastolic velocity; Ea = early mitral annular diastolic velocity; G+/LVH+ = genotyped hypertrophic cardiomyopathy patients; G+/LVH− = carriers of Dutch myosin-binding protein C founder mutations without left ventricular hypertrophy; NS = not significant; Sa = mitral annular systolic velocity.

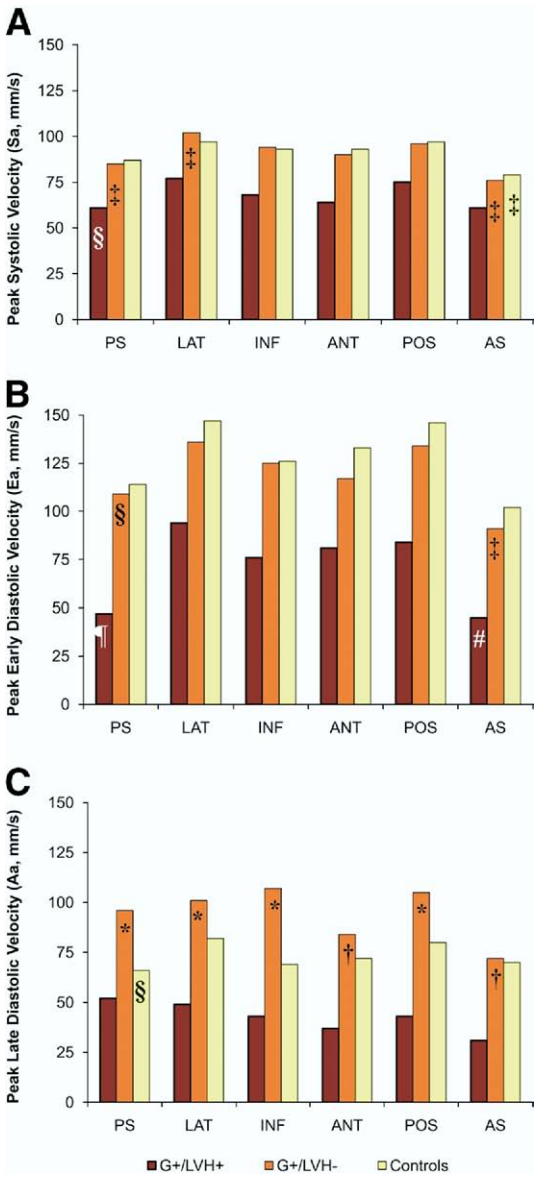

Figure 2. Mean Regional Values of Tissue Doppler Velocity

Mean regional values of the 3 components of tissue Doppler longitudinal velocity from 6 mitral annular sites during systole **(A)**, early diastole **(B)**, and late diastole **(C)** in genotyped hypertrophic cardiomyopathy patients (G+/LVH+), mutation carriers without left ventricular hypertrophy (G+/LVH−), and controls. Mitral annular regions in sequence are as follows: PS = posteroseptal; LAT = lateral; INF = inferior; ANT = anterior; POS = posterior; and AS = anteroseptal. Between-groups Games-Howell–corrected p value: all G+/LVH+ mean values of mitral annular systolic velocity (Sa), early mitral annular diastolic velocity (Ea), and late mitral annular diastolic velocity (Aa), averaged from 6 mitral annular sites and individual sites, are significantly different from other 2 groups ($p < 0.001$). Between mutation carriers and controls: *$p < 0.01$ mutation carriers versus controls; †$p < 0.05$ mutation carriers versus controls. Within-group Games-Howell–corrected p value: ‡$p < 0.01$ versus the other 5 segments; §$p < 0.05$ versus lateral and posterior segments; ¶$p < 0.05$ versus posteroseptal, lateral, inferior, and anterior segments; and #$p < 0.05$ versus lateral, inferior, anterior, and posterior segments.

controls. The reasons for this difference could be the underlying sarcomeric mutation. Unlike previous studies, our study population consisted only of subjects with a truncating mutation in MYBPC3, which is known for its later onset of HCM compared with mutations in the beta-myosin heavy chain (2).

We did, however, find an increased Aa velocity in the majority of G+/LVH− subjects carrying 1 of 3 truncating Dutch founder mutations in MYBPC3. To our knowledge, this is the first study to describe an isolated increase in mean Aa velocity in G+/LVH− subjects. An increase of mean Aa velocity means that the mitral annulus displacement is increased during late diastole. This could be a very early sign of diastolic abnormality, preceding other signs of myocardial contraction and relaxation abnormalities and LVH (1).

Prediction of genetic abnormalities in patients with a clinical diagnosis of HCM and vice versa has been investigated in several studies (17–19). Fifty percent to 60% of HCM patients have abnormalities in the sarcomeric genes (20). Most HCM mutations are found in the beta-myosin heavy chain, which encodes a thick myofilament protein, and in MYBPC3, which encodes an intermediate myofilament protein. Mutations in both genes cause an indistinguishable disease phenotype in which the ventricular septum has a characteristic reverse curve in approximately 40% of cases (20). One of the great benefits of genetic analyses in HCM families is the identification of at-risk family members, which will allow early detection and possible prevention of poor outcome (21).

In the present study, almost all MYBPC3 mutation carriers have an increased left ventricular late diastolic lengthening in the form of high Aa peak velocity on TDI. In contrast to HCM mutations in other sarcomeric genes, which are mostly missense mutations, most MYBPC3 mutations are truncating mutations. Haplo insufficiency is, therefore, thought to be an important disease mechanism in MYBPC3-associated HCM. The 3 described mutations in this study are truncating mutations, thought to lead to a reduction in MYBPC3 protein because of a lack of expression from the mutated allele by the cellular surveillance mechanism of nonsense-mediated decay (22). The regulatory role of MYBPC3 on contraction is still controversial; although it has been shown that removal of the MYBPC3 can increase the velocity of shortening, force output, and force redevelopment in skinned preparation (23–26).

Pohlmann et al. (27) investigated the consequences of removal of MYBPC3 in ventricular myocytes and left atria from MYBPC3 knockout mice compared with wild type. Both sarcomere shortening and Ca^{2+} transient were prolonged in MYBPC3 knockout mice. Isolated left atria of MYBPC3 knockout mice exhibited a marked increase in sensitivity to external Ca^{2+} and low micromolar Ca^{2+}. The main consequence of removal of MYBPC3 in the MYBPC3 knockout mice was a defect in diastolic relaxation and a smaller dynamic range of cell shortening, both of which likely result from the increased myofilament Ca^{2+} sensitivity (27). Therefore, MYBPC3 might function as a restraint on myosin-actin interaction at low Ca^{2+} and short sarcomere length to allow complete relaxation during diastole. When applying these experimental findings to our findings, the late diastolic abnormality can be a very early sign or pre-clinical phenotypic expression of disease in the G+/LVH− subjects.

CONCLUSIONS

Based on the current study, we conclude that TDI velocities are not sufficiently sensitive for determination of affected status of an individual patient. Our findings, however, support the theory that diastolic dysfunction is a primary component of pre-clinical HCM.

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Key Words: tissue Doppler imaging ■ hypertrophic cardiomyopathy ■ myosin-binding protein C.