ORIGINAL ARTICLE

# Myocardial Fibrosis as an Early Manifestation of Hypertrophic Cardiomyopathy

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## ABSTRACT

### BACKGROUND

Myocardial fibrosis is a hallmark of hypertrophic cardiomyopathy and a proposed substrate for arrhythmias and heart failure. In animal models, profibrotic genetic pathways are activated early, before hypertrophic remodeling. Data showing early profibrotic responses to sarcomere-gene mutations in patients with hypertrophic cardiomyopathy are lacking.

### METHODS

We used echocardiography, cardiac magnetic resonance imaging (MRI), and serum biomarkers of collagen metabolism, hemodynamic stress, and myocardial injury to evaluate subjects with hypertrophic cardiomyopathy and a confirmed genotype.

### RESULTS

The study involved 38 subjects with pathogenic sarcomere mutations and overt hypertrophic cardiomyopathy, 39 subjects with mutations but no left ventricular hypertrophy, and 30 controls who did not have mutations. Levels of serum C-terminal propeptide of type I procollagen (PICP) were significantly higher in mutation carriers without left ventricular hypertrophy and in subjects with overt hypertrophic cardiomyopathy than in controls (31% and 69% higher, respectively; P<0.001). The ratio of PICP to C-terminal telopeptide of type I collagen was increased only in subjects with overt hypertrophic cardiomyopathy, suggesting that collagen synthesis exceeds degradation. Cardiac MRI studies showed late gadolinium enhancement, indicating myocardial fibrosis, in 71% of subjects with overt hypertrophic cardiomyopathy but in none of the mutation carriers without left ventricular hypertrophy.

### CONCLUSIONS

Elevated levels of serum PICP indicated increased myocardial collagen synthesis in sarcomere-mutation carriers without overt disease. This profibrotic state preceded the development of left ventricular hypertrophy or fibrosis visible on MRI. (Funded by the National Institutes of Health and others.)

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Hypertrophic cardiomyopathy is caused by mutations in genes encoding sarcomere proteins.[1,2] With a prevalence of approximately 1 case per 500 persons in the general population, hypertrophic cardiomyopathy is the most common monogenic cardiac disorder.[3] The clinical diagnosis depends on the identification of unexplained left ventricular hypertrophy, but this finding is present only in persons with established disease and is typically absent in childhood.[4] In contrast, genetic diagnosis identifies pathogenic sarcomere mutations in persons at any age, including mutation carriers with overt hypertrophic cardiomyopathy and mutation carriers without hypertrophy who are at high risk for the development of disease. Studying such mutation carriers may provide insight into the pathophysiology of hypertrophic cardiomyopathy by revealing subtle, early manifestations of sarcomere mutations that precede the obvious pathologic remodeling of overt disease. For example, left ventricular relaxation is impaired in mutation carriers without left ventricular hypertrophy, indicating that sarcomere mutations directly affect diastolic function.[5-7]

Myocardial fibrosis, a hallmark of hypertrophic cardiomyopathy, is thought to contribute to sudden cardiac death, ventricular tachyarrhythmias, left ventricular dysfunction, and heart failure.[8-12] Histologic evaluation universally reveals increased interstitial and focal myocardial fibrosis in overt disease. In most patients with overt hypertrophic cardiomyopathy, dense focal fibrosis can also be visualized noninvasively with the use of gadolinium-enhanced cardiac magnetic resonance imaging (MRI).[10,13-18] The trigger for increased myocardial fibrosis in hypertrophic cardiomyopathy remains unclear, but it has been attributed both to premature myocyte death caused by stresses imposed directly by sarcomere mutations[19-22] and to later pathologic changes, including intracavitary obstruction, small-vessel disease, and ischemia.[9,12,23] Animal models of hypertrophic cardiomyopathy that recapitulate human disease[24] have recently shed light on the earliest cellular and molecular responses to sarcomere-gene mutations.[25] Cardiac transcriptional profiling in young mice in which hypertrophy has not yet developed shows activation of pathways involved in fibrosis and collagen deposition.[25] These studies indicate that a profibrotic milieu is present early in hearts with hypertrophic cardiomyopathy, even when cardiac histologic findings are normal.

Biomarkers of collagen synthesis and degradation reflect collagen metabolism. The C-terminal propeptide of type I procollagen (PICP) is released in a 1:1 ratio during the synthesis of type I collagen from its precursor, procollagen type I,[26] and serum levels reliably reflect myocardial type I collagen synthesis.[27] Increased serum PICP levels correlate with adverse outcomes in hypertension, heart failure, and myocardial infarction.[28-30]

There have been few studies of these biomarkers in hypertrophic cardiomyopathy, although preliminary studies of nongenotyped patients with overt disease suggest that collagen turnover is increased.[31-34] Data regarding collagen metabolism in patients with early disease are lacking. We therefore measured serum biomarkers of collagen metabolism to assess profibrotic processes in a genotyped population with hypertrophic cardiomyopathy, comparing mutation carriers in whom left ventricular hypertrophy had not yet developed with those who had overt disease and with mutation-negative normal controls.

## METHODS

### STUDY POPULATION

Genotyped patients with hypertrophic cardiomyopathy and their relatives, identified through research studies or clinical evaluation, were recruited and assigned to one of three groups. Mutation carriers with a left ventricular wall thickness of 12 mm or greater (in adults) or a z score of 2 or more (in children)[35] were classified as having overt hypertrophic cardiomyopathy. Healthy mutation carriers who did not meet these criteria were classified as mutation carriers without left ventricular hypertrophy. The criteria we used are more rigorous than those used clinically to diagnose hypertrophic cardiomyopathy.[36] They were chosen to avoid the inclusion of subjects with borderline left ventricular hypertrophy and potentially emerging or mild cardiomyopathy in the group of mutation carriers without left ventricular hypertrophy. Control subjects were healthy, mutation-negative relatives at ages similar to those of mutation carriers without left ventricular hypertrophy.

The study protocol was approved by the institutional review boards of Brigham and Women's Hospital and Children's Hospital Boston, and the

study was conducted in accordance with the protocol. Written informed consent was obtained from all participants or their parents or legal guardians.

Subjects with systemic hypertension (defined by systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of antihypertensive medication), coronary artery disease, valvular heart disease, previous septal myectomy, alcohol septal ablation, electronic ventricular pacing, or atrial fibrillation were excluded. Subjects with other conditions that might influence collagen metabolism (e.g., surgery or trauma within the previous 6 months, known fibrotic or inflammatory disease, or cancer) were also excluded.

### ECHOCARDIOGRAPHY

Transthoracic echocardiograms were obtained with the use of a Vivid 7 ultrasonography system (General Electric Medical Systems). The average of three cardiac cycles was used for measurements of cardiac dimensions, mitral inflow patterns, and the left ventricular ejection fraction (calculated according to Simpson's method).[37] Myocardial velocities during systole and early diastole were measured by means of tissue Doppler imaging at the lateral, septal, anterior, and inferior aspects of the mitral annulus in the apical four-chamber and two-chamber views. Global values of systolic and early diastolic myocardial velocities were calculated as the average of these four measurements to represent myocardial contraction and relaxation, respectively. The ratio of early mitral inflow velocity to global early diastolic velocity approximately represented left ventricular end-diastolic pressure.[38] Measures of left ventricular stiffness were evaluated, including E-wave deceleration time, end-systolic elastance ([0.9 × systolic blood pressure] ÷ left ventricular end-systolic volume), and stiffness (70 ÷ [E-wave deceleration time −20])$^2$ or (E-wave deceleration time ÷ early mitral inflow velocity).[39,40] Images were analyzed by two observers who were unaware of the subjects' clinical and genetic status.

### CARDIAC MRI

Cardiac MRI studies were performed in a subgroup of subjects with overt hypertrophic cardiomyopathy and in mutation carriers without left ventricular hypertrophy, with the use of a 1.5-T cardiac MRI system (HDX Excite II, General Electric) or a 3.0-T system (Magnetom Trio, Siemens). Steady-state free precession of cine images was used to quantify left ventricular function (short-axis stack, slice thickness of 8 mm) and myocardial mass by means of standard criteria.[41] Segmented inversion-recovery fast gradient–echo imaging was used to assess late gadolinium enhancement 10 minutes after the administration of gadolinium diethylenetriamine pentaacetic acid at a dose of 0.15 mmol per kilogram of body weight. The extent of late gadolinium enhancement was quantified by planimetric assessment of all short-axis slices for total volume (the sum of the areas measured, in grams) and as a proportion of the total left ventricular mass (the percentage of late gadolinium enhancement). Late gadolinium enhancement was defined as 2 standard deviations above the mean signal intensity of the distant myocardium.[42] Images were analyzed offline with the use of QMass MR software (Medis) by two observers who were unaware of the subjects' clinical and genetic information.

### MEASUREMENT OF SERUM BIOMARKERS

Blood samples (serum and plasma) were obtained at the time of cardiac imaging, processed within 60 minutes after phlebotomy, and stored at −80°C before analysis of PICP, matrix metalloproteinase 1 (MMP-1), tissue inhibitor of metalloproteinase 1 (TIMP-1), C-terminal telopeptide of type I collagen (CITP), osteopontin, bone-specific alkaline phosphatase, N-terminal propeptide of B-type natriuretic peptide, B-type natriuretic peptide, and cardiac troponin I. All assays were performed with the use of commercially available reagents (see the Methods section in the Supplementary Appendix, available with the full text of this article at NEJM.org) by investigators who were unaware of the subjects' clinical and genetic status.

### STATISTICAL ANALYSIS

To test for differences among the three groups and between subjects with $\beta$-myosin heavy chain (*MYH7*) mutations and those with cardiac myosin-binding protein C (*MYBPC3*) mutations, analysis of variance and logistic regression were performed with clustering to adjust for family relationships, assuming an exchangeable correlation structure. Age-dependent mitral inflow, myocardial velocities measured by tissue Doppler imaging, and biomarker patterns were adjusted for age; biomarkers were also adjusted for sex. Values are expressed as adjusted means ±SE.

Bonferroni-corrected P values of less than 0.017 were considered to indicate statistical significance for multiple comparisons across the three groups. Pearson's correlation was used to evaluate associations between continuous measures. Analyses were performed with the use of SAS software, version 9.1 (SAS Institute).

## RESULTS

### CLINICAL CHARACTERISTICS

Study procedures were performed in 107 subjects. Forty-two different pathogenic mutations[7] (37 *MYH7*, 31 *MYBPC3*, 9 cardiac troponin T [*TNNT2*], and 1 $\alpha$-tropomyosin [*TPM1*]) were identified in 47 families with hypertrophic cardiomyopathy (Table 1 in the Supplementary Appendix).

On the basis of clinical evaluations and genotypes, 38 subjects were classified as mutation carriers with overt hypertrophic cardiomyopathy, 39 as mutation carriers without left ventricular hypertrophy, and 30 as having neither mutations nor hypertrophic cardiomyopathy (control subjects) (Table 1). Mutation carriers without left ventricular hypertrophy and control subjects were of similar age, were asymptomatic, were not receiving cardiac medications, and had normal cardiac dimensions. These subjects had slightly lower blood pressure than controls, and they also had a higher left ventricular ejection fraction and a lower global early diastolic myocardial velocity, as reported previously for such carriers.[6,7]

Subjects with overt hypertrophic cardiomyopathy had a significantly greater left ventricular wall thickness (76% with asymmetric septal hypertrophy), smaller left ventricular cavity, and larger left atrial diameter than mutation carriers without left ventricular hypertrophy and control subjects. Sixteen percent had intracavitary obstruction at rest (peak gradient, ≥30 mm Hg). Symptoms were generally mild in subjects with overt hypertrophic cardiomyopathy (74% had New York Heart Association [NYHA] class I status and 21% had NYHA class II status). Three subjects had NYHA class III symptoms, including two subjects with end-stage hypertrophic cardiomyopathy, defined by a reduced left ventricular ejection fraction (<50%). Sixty-eight percent of the subjects with overt hypertrophic cardiomyopathy were receiving cardioactive medications, including eight subjects receiving angiotensin-converting–enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARBs), or spironolactone.

### IMAGING OF FIBROSIS WITH CARDIAC MRI

Cardiac MRI studies with the administration of gadolinium were performed in 28 subjects with overt hypertrophic cardiomyopathy and 32 mutation carriers without left ventricular hypertrophy. Studies were not performed in 9 subjects with overt hypertrophic cardiomyopathy who had implantable cardioverter–defibrillators and in 5 mutation-positive children without left ventricular hypertrophy whose parents declined. In 3 subjects, gadolinium was not administered. Late gadolinium enhancement was present in 71% of subjects with overt hypertrophic cardiomyopathy but in none of the mutation carriers without left ventricular hypertrophy (Fig. 1). The extent of late gadolinium enhancement in subjects with overt hypertrophic cardiomyopathy ranged from 0 to 55% of the total left ventricular mass (mean, 14.7±16.9%). There were significant correlations between the extent of late gadolinium enhancement in these subjects and the left ventricular mass, ejection fraction, global early diastolic myocardial velocity, and ratio of early mitral inflow velocity to early diastolic velocity (Tables 2 and 3 in the Supplementary Appendix). There were no significant correlations between late gadolinium enhancement and levels of cardiac troponin I, B-type natriuretic peptide, PICP, CITP, MMP-1, or TIMP-1; age; left ventricular hypertrophy; size of the left atrium; or E-wave deceleration time.

### INCREASED MYOCARDIAL COLLAGEN SYNTHESIS

Analyses of serum biomarkers were performed after exclusion of the eight subjects with overt hypertrophic cardiomyopathy who were receiving ACE inhibitors, ARBs, or spironolactone (including the two subjects with end-stage hypertrophic cardiomyopathy), since these factors may influence collagen metabolism. As compared with PICP levels in controls (82.16±3.03 $\mu$g per liter), the PICP levels were significantly elevated in mutation carriers without left ventricular hypertrophy (107.73±4.65 $\mu$g per liter, a 31% elevation) and in subjects with overt hypertrophic cardiomyopathy (138.70±11.63 $\mu$g per liter, a 69% elevation; P<0.001 for both comparisons with controls) (Table 2 and Fig. 1 in the Supplementary Appendix). PICP was the only biomarker that dif-

**Table 1. Baseline Characteristics of the Subjects.***

| Variable | Control (N=30) | Mutation-Positive, LVH-Negative (N=39) | P Value, Control vs. Mutation-Positive, LVH-Negative | Overt Hypertrophic Cardiomyopathy (N=38) | P Value, Mutation-Positive, LVH-Negative vs. Overt Hypertrophic Cardiomyopathy | P Value, Overt Hypertrophic Cardiomyopathy vs. Control |
|---|---|---|---|---|---|---|
| Age — yr | | | 0.93 | | <0.001 | <0.001 |
| Mean | 19.8±1.4 | 20.0±2.2 | | 40.9±2.3 | | |
| Range | 5–44 | 5–53 | | 14–69 | | |
| Sex — no. (%) | | | 0.57 | | 0.14 | 0.08 |
| Male | 14 (47) | 16 (41) | | 23 (61) | | |
| Female | 16 (53) | 23 (59) | | 15 (39) | | |
| Causal gene — no. of subjects | | | | | | |
| *MYH7* | | 19 | | 18† | | |
| *MYBPC3* | | 15 | | 16† | | |
| *TNNT2* | | 5 | | 4 | | |
| *TPM1* | | 0 | | 1 | | |
| Body-surface area — $m^2$ | 1.72±0.03 | 1.58±0.07 | 0.07 | 1.95±0.05 | <0.001 | <0.001 |
| Blood pressure — mm Hg | | | | | | |
| Systolic | 119±3 | 107±4 | 0.014 | 116±2 | 0.05 | 0.41 |
| Diastolic | 72±2 | 66±1 | 0.006 | 72±2 | 0.015 | 1.0 |
| New York Heart Association functional class — % | | | | | | |
| I | 30 (100) | 39 (100) | | 28 (74) | | |
| II | | | | 8 (21) | | |
| III | | | | 2 (5) | | |
| Medication use — no. (%)‡ | | | | | | |
| Any | 0 | 0 | | 26 (68) | | |
| Beta-blocker | | | | 21 (55) | | |
| Calcium-channel blocker | | | | 11 (29) | | |
| Disopyramide | | | | 2 (5) | | |
| Spironolactone | | | | 3 (8) | | |
| ACE inhibitor or ARB | | | | 6 (16) | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Echocardiographic findings | | | | | | |
| Wall thickness — mm | | | | | | |
| Septal | 8.5±0.1 | 8.1±0.3 | 0.13 | 17.6±0.8 | <0.001 | <0.001 |
| Posterior | 8.5±0.5 | 7.7±0.2 | 0.10 | 12.0±0.3 | <0.001 | <0.001 |
| Maximal | NA | NA | | 20.3±5.3 | | |
| Location of maximal wall thickness — no. (%) | | | | | | |
| Septal | | | | 29 (76) | | |
| Anterior | | | | 7 (18) | | |
| Posterior | | | | 1 (3) | | |
| Apical | | | | 1 (3) | | |
| Left ventricular end-diastolic diameter — cm | 4.6±0.1 | 4.5±0.2 | 0.72 | 4.2±0.1 | 0.1 | 0.003 |
| Left ventricular end-systolic diameter — cm | 2.8±0.1 | 2.4±0.1 | 0.06 | 2.4±0.1 | 0.7 | 0.008 |
| Left ventricular ejection fraction — % | 64±3 | 69±1 | <0.001 | 69±2 | 0.8 | <0.001 |
| Left atrial diameter — cm | 3.4±0.2 | 3.1±0.1 | 0.20 | 4.3±0.1 | <0.001 | <0.001 |
| Early mitral inflow velocity — cm/sec§ | 91±8 | 93±5 | 0.8 | 79±5 | 0.03 | 0.3 |
| Ratio of early mitral inflow velocity to late mitral inflow velocity§ | 2.4±0.2 | 1.9±0.1 | 0.08 | 1.7±0.1 | 0.4 | 0.014 |
| Early mitral inflow deceleration time — msec§ | 162±6 | 175±6 | 0.06 | 194±10 | 0.1 | 0.007 |
| Ratio of E-wave deceleration time to early mitral inflow velocity | 8.27±6.19 | 6.31±3.98 | 0.41 | 3.18±1.59 | 0.38 | 0.36 |
| Global early diastolic myocardial velocity — cm/sec§ | 15.6±0.6 | 12.5±0.4 | <0.001 | 10.1±0.5 | <0.001 | <0.001 |
| Ratio of early mitral inflow velocity to early diastolic myocardial velocity§ | 7.0±0.5 | 7.8±0.5 | 0.08 | 8.8±0.5 | 0.2 | 0.008 |

* Plus–minus values are means ±SE. P values of less than 0.017 were considered to indicate statistical significance. ACE denotes angiotensin-converting enzyme, ARB angiotensin-receptor blocker, LVH left ventricular hypertrophy, and NA not applicable.
† One subject with overt hypertrophic cardiomyopathy was a compound heterozygote: *MYBPC3* Arg502Trp and *MYH7* M982Thr.
‡ Some subjects received more than one medication.
§ Values were adjusted for age and family relationship.







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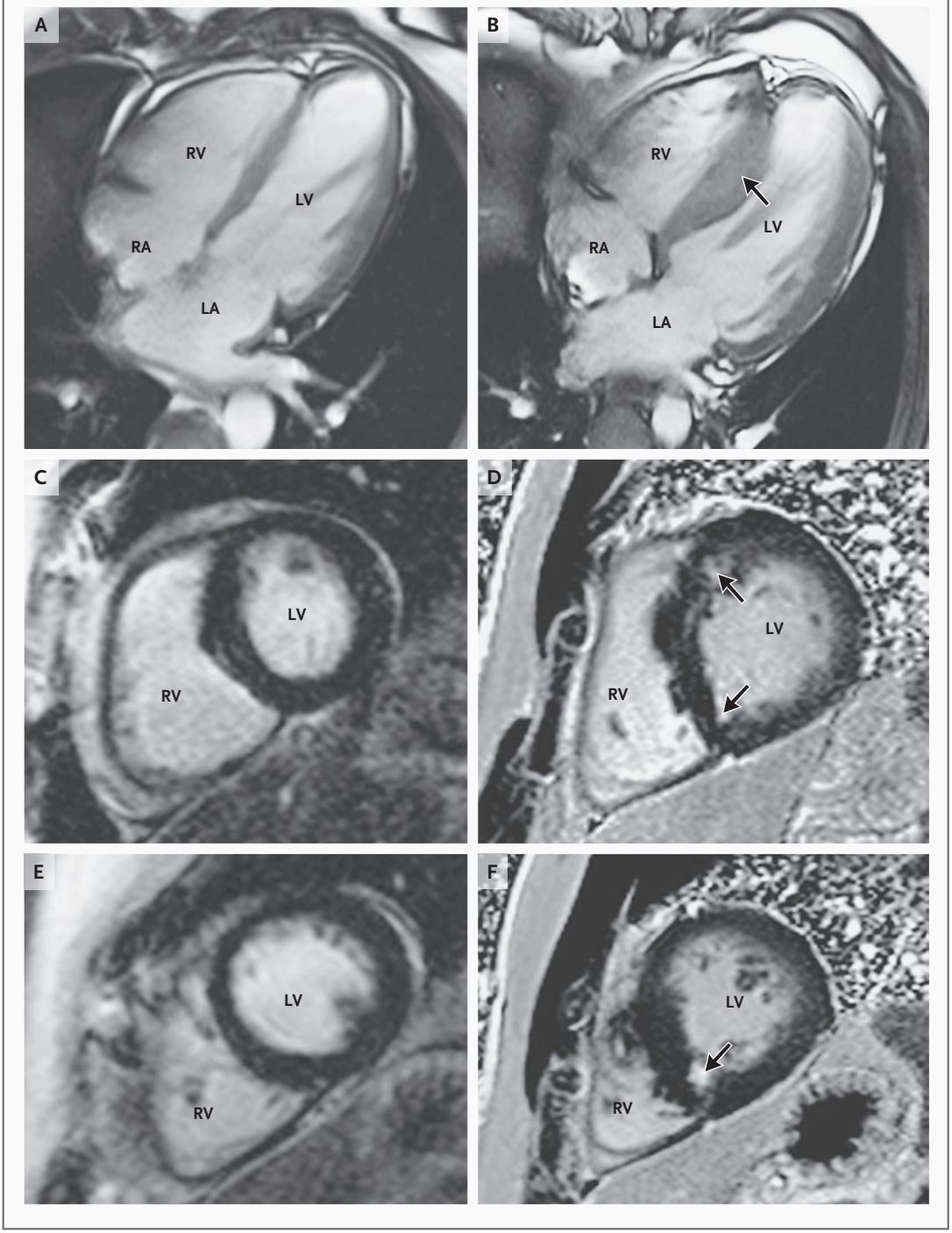


fered between mutation carriers and noncarriers (Table 2). Among all family members with a normal left ventricular wall thickness, the odds of carrying a mutation increased by 50% for every increase in PICP of 10 $\mu$g per liter, after adjustment for age, sex, early diastolic myocardial velocity, and left ventricular ejection fraction (odds ratio, 1.50; 95% confidence interval [CI], 1.18 to 1.91; P=0.001).

Type I collagen is a major component of bone; therefore, PICP levels may reflect bone formation, particularly in growing children and ado-

**Figure 1 (facing page). Gadolinium-Enhanced Cardiac MRI Scans in *MYH7* Mutation Carriers with and Those without Overt Hypertrophic Cardiomyopathy.**

Panels A, C, and E show no morphologic abnormalities suggestive of hypertrophic cardiomyopathy in a carrier of a myosin heavy-chain missense mutation. Left ventricular hypertrophy and late gadolinium enhancement are absent. Panels B, D, and F show typical findings of hypertrophic cardiomyopathy in a relative of the subject in Panels A, C, and E with overt disease caused by the same myosin heavy-chain mutation. These findings include a markedly thickened septum (Panel B, arrow) associated with areas of heterogeneous late gadolinium enhancement, which affects 17% of the total left ventricular mass and involves the anteroseptum (Panel D, arrows) and the inferoseptum (Panels D and F, arrows). LA denotes left atrium, LV left ventricle, RA right atrium, and RV right ventricle.

lescents.[43] To minimize such confounding, we measured levels of bone-specific alkaline phosphatase (a specific marker of bone metabolic activity)[43] and the ratio of PICP to bone-specific alkaline phosphatase to adjust PICP levels for the influence of bone collagen turnover. As compared with levels of bone-specific alkaline phosphatase in controls, levels were 17% lower in mutation carriers without left ventricular hypertrophy (P=0.003) and were 20% lower in subjects with overt hypertrophic cardiomyopathy (P=0.006) (Fig. 1B in the Supplementary Appendix). The ratio of PICP to bone-specific alkaline phosphatase was significantly higher both in mutation carriers without left ventricular hypertrophy and in subjects with overt hypertrophic cardiomyopathy than in controls (77% higher in mutation carriers but no left ventricular hypertrophy, P<0.001) (Fig. 1C in the Supplementary Appendix). These results suggest that elevated PICP levels in sarcomere-mutation carriers reflect increased myocardial collagen synthesis rather than increased bone metabolic activity.

No significant differences in markers of collagen degradation (MMP-1, TIMP-1, and CITP) were identified in isolation. The PICP:CITP ratio reflects the balance between collagen synthesis and degradation.[44] As compared with the PICP:CITP ratio in control subjects, the ratio was unchanged in mutation carriers without left ventricular hypertrophy but significantly higher in subjects with overt hypertrophic cardiomyopathy, suggesting that collagen synthesis exceeds degradation in established disease.

### CLINICAL AND GENETIC ASSOCIATIONS WITH INCREASED PICP LEVELS

No significant correlations were identified between increased PICP levels and levels of B-type natriuretic peptide, N-terminal propeptide of B-type natriuretic peptide, or cardiac troponin I; the degree of left ventricular hypertrophy; left ventricular mass; left atrial size; left ventricular systolic or diastolic function; echocardiographic measurements of left ventricular stiffness; or late gadolinium enhancement on cardiac MRI in any cohort. Among mutation carriers without left ventricular hypertrophy, there were also no significant differences in mean PICP levels between subjects with and those without electrocardiographic (ECG) abnormalities, including Q waves (which were present in 36% of mutation carriers without left ventricular hypertrophy).

We compared subjects who had *MYH7* mutations and those who had *MYBPC3* mutations to assess potential genotype–phenotype correlations in the two most common genetic subtypes of hypertrophic cardiomyopathy (Table 3). PICP levels were 14% higher in subjects without left ventricular hypertrophy who had *MYH7* mutations than in subjects without left ventricular hypertrophy who had *MYBPC3* mutations (P=0.014). Global early myocardial diastolic velocities were 15% lower in subjects without left ventricular hypertrophy who had *MYH7* mutations (P=0.002) than in those who had *MYBPC3* mutations, in whom the velocities were essentially normal. Among subjects with overt hypertrophic cardiomyopathy, PICP levels did not differ significantly between those who had *MYH7* mutations and those who had *MYBPC3* mutations, although early myocardial diastolic velocities were lower in subjects with *MYH7* mutations.

## DISCUSSION

We present data showing increased myocardial type I collagen synthesis in both early and established hypertrophic cardiomyopathy. Elevated serum PICP levels in mutation carriers with normal cardiac morphologic features constitute a potentially useful phenotype for sarcomere mutations and a serologic marker of genetic risk that can be detected before clinical diagnosis. These observations offer insights into the pathophysiology of hypertrophic cardiomyopathy, suggesting that the stimulus for myocardial

**Table 2. Serum Biomarkers of Collagen Metabolism, Hemodynamic Stress, and Myocardial Injury.***

| Variable | Control (N=30) | Mutation-Positive, LVH-Negative (N=39)† | P Value, Control vs. Mutation-Positive, LVH-Negative | Overt Hypertrophic Cardiomyopathy (N=30)‡ | P Value, Mutation-Positive, LVH-Negative vs. Overt Hypertrophic Cardiomyopathy | P Value, Overt Hypertrophic Cardiomyopathy vs. Control |
|---|---|---|---|---|---|---|
| PICP (μg/liter) | 82.16±3.03 | 107.73±4.65 | <0.001 | 138.70±11.63 | 0.03 | <0.001 |
| MMP-1 (ng/ml) | 6.39±0.78 | 12.02±2.60 | 0.04 | 10.26±1.86 | 0.36 | 0.08 |
| TIMP-1 (ng/ml) | 868.33±109.16 | 790.30±36.06 | 0.48 | 785.28±35.64 | 0.66 | 0.46 |
| CITP (μg/liter) | 9.18±1.05 | 9.42±0.87 | 0.87 | 9.66±1.10 | 0.87 | 0.76 |
| Bone-specific alkaline phosphatase (U/ml) | 4.25±0.06 | 3.51±0.25 | 0.003 | 3.42±0.27 | 0.81 | 0.006 |
| PICP: bone-specific alkaline phosphatase | 19.7±0.8 | 34.9±2.5 | <0.001 | 47.7±5.5 | 0.07 | <0.001 |
| PICP:CITP | 12.66±1.30 | 15.54±0.82 | 0.03 | 22.20±1.81 | 0.001 | <0.001 |
| Osteopontin (ng/ml) | 57.84±4.29 | 70.93±7.84 | 0.16 | 76.75±9.93 | 0.66 | 0.10 |
| N-terminal propeptide of B-type natriuretic peptide (pg/ml) | 671.63±70.90 | 772.59±6.45 | 0.19 | 1136.34±101.87 | <0.001 | <0.001 |
| B-type natriuretic peptide (pg/ml) | 21.50±8.96 | 25.63±11.38 | 0.61 | 143.30±35.98 | 0.003 | 0.002 |
| Cardiac troponin I (ng/ml) | <0.001§ | <0.001§ | | 0.045±0.004 | | 0.002 |

* Plus–minus values are means ±SE, adjusted for family relationship, age, and sex. P values of less than 0.017 were considered to indicate statistical significance. P values were adjusted for family relationship, age, and sex. CITP denotes C-terminal telopeptide of collagen type 1, LVH left ventricular hypertrophy, MMP-1 matrix metalloproteinase 1, PICP C-terminal propeptide of procollagen type I, and TIMP-1 tissue inhibitor of metalloproteinase 1.
† Levels of PICP, CITP, osteopontin, MMP-1, and TIMP-1 were not available in one subject who had mutations without LVH.
‡ This category excludes subjects receiving medications that may influence collagen metabolism.
§ The clinical threshold for cardiac troponin I is 0.04 ng per milliliter.

fibrosis is an early manifestation of sarcomere-gene mutations.

In contrast to collagen synthesis, biomarkers of collagen degradation in isolation were not informative regarding genotype or clinical status. However, the PICP:CITP ratio, an index of the dynamic equilibrium between collagen type I synthesis and degradation,[44] was unchanged in mutation carriers without left ventricular hypertrophy but increased in subjects with overt disease. We propose that sarcomere mutations trigger an early increase in collagen synthesis that is initially balanced by degradation, thereby limiting fibrogenesis. In overt hypertrophic cardiomyopathy, synthesis exceeds degradation, resulting in frank myocardial fibrosis. Although our study was not longitudinal, these results are consistent with the natural history of hypertrophic cardiomyopathy and suggest that collagen accumulation increases as disease develops. Indeed, these findings may partially explain why late gadolinium enhancement on cardiac MRI was present in the majority of our subjects with overt hypertrophic cardiomyopathy, as observed in other studies,[10,13,14,16-18] but absent in the mutation carriers without left ventricular hypertrophy, despite elevated PICP levels.

Increased myocardial fibrosis is a hallmark of overt hypertrophic cardiomyopathy and is frequently interpreted as a secondary response to the pathophysiological remodeling of long-standing disease, including ischemia, obstruction, and microvascular abnormalities.[9,12,23] This interpretation is challenged by studies showing early activation of profibrotic genetic pathways in a mouse model of hypertrophic cardiomyopathy. In young mice with a myosin heavy-chain mutation, comprehensive transcriptional profiling revealed increased expression of genes that drive extracellular-matrix formation before fibrosis or left ventricular hypertrophy develops.[25] RNA levels of connective-tissue growth factor, periostin, transforming growth factor $\beta$1 and $\beta$2, fibronectin, and type I collagen were increased despite normal cardiac histologic findings.

Our data translate these findings from mouse models to human disease, providing further evidence that fibrosis is a fundamental, early consequence of sarcomere mutations. On the basis of the elevated PICP levels in mutation carriers without left ventricular hypertrophy, we propose that increased collagen synthesis contributes to the emergence of the pathophysiological changes that characterize overt hypertrophic cardiomyopathy. Analysis of additional biomarkers (collagen type III–derived peptides, other matrix metalloproteinases, and TIMPs) may provide a more comprehensive picture of how sarcomere mutations affect the complex and dynamic nature of fibrillar collagen metabolism.

We could not identify significant correlations between biomarker levels and other clinical variables, including late gadolinium enhancement

**Table 3. Comparison of *MYH7* and *MYBPC3* Mutations.***

| Variable | *MYH7* | *MYBPC3* | P Value |
|---|---|---|---|
| **Overt hypertrophic cardiomyopathy** | | | |
| No. of subjects | 17 | 16 | |
| Age — yr | 36.9±3.5 | 44.7±2.6 | 0.07 |
| Sex — no. (%) | | | 0.64 |
| Male | 11 (65) | 9 (56) | |
| Female | 6 (35) | 7 (44) | |
| Maximal left ventricular wall thickness — mm | 20.5±1.35 | 21.2±1.4 | 0.72 |
| Left ventricular mass on cardiac MRI — g | 186.8±19.3 | 147.2±9.8 | 0.07 |
| Global early diastolic velocity — cm/sec | 6.9±0.5 | 9.3±0.4 | <0.001 |
| Left ventricular ejection fraction — % | 70±2 | 69±2 | 0.79 |
| Late gadolinium enhancement volume — g | 30.8±10.3 | 18.8±7.0 | 0.35 |
| Late gadolinium enhancement — % of total left ventricular mass | 18.9±6.6 | 11.7±4.2 | 0.39 |
| PICP — $\mu$g/liter | 123.01±9.28 | 124.16±1.07 | 0.91 |
| MMP-1 — ng/ml | 8.37±2.23 | 8.70±2.18 | 0.92 |
| **Mutation-positive, LVH-negative** | | | |
| No. of subjects | 19 | 15 | |
| Age — yr | 18.2±3.4 | 21.0±3.6 | 0.57 |
| Sex — no. (%) | | | 0.32 |
| Male | 8 (42) | 4 (27) | |
| Female | 11 (58) | 11 (73) | |
| Global early diastolic velocity — cm/sec | 12.9±0.5 | 15.1±0.5 | 0.002 |
| Left ventricular ejection fraction — % | 71±1 | 69±2 | 0.32 |
| PICP — $\mu$g/liter | 123.51±1.20 | 108.10±5.06 | 0.014 |
| MMP-1 — ng/ml | 11.26±4.29 | 15.47±4.56 | 0.51 |

* Plus–minus values are means ±SE, adjusted for age. P values of less than 0.05 were considered to indicate statistical significance; P values were adjusted for family relationship and age. LVH denotes left ventricular hypertrophy, MMP-1 matrix metalloproteinase 1, MRI magnetic resonance imaging, *MYBPC3* cardiac myosin-binding protein C, *MYH7* cardiac $\beta$-myosin heavy chain, and PICP C-terminal propeptide of procollagen type I.

on cardiac MRI and ECG abnormalities. These results are not surprising, given the limited resolution of late gadolinium enhancement for the detection of focal myocardial fibrosis (theoretical detection limit, approximately 0.2 g of scar; a clinical detection limit of approximately 2.0 g in infarct studies[45,46]) and estimates of the amount of scar (approximately 3% of left ventricular mass) needed to produce changes visible on ECG studies.[47] Moreover, diffuse interstitial fibrosis is common in hypertrophic cardiomyopathy but cannot be visualized by means of cardiac MRI. In contrast, immunoassays detect micrograms of circulating PICP. Serum biomarkers consequently provide a more sensitive index of extracellular-matrix remodeling and may reflect subtle changes in myocardial composition and biochemical features that are not detectable by means of noninvasive cardiac imaging. Notably, we observed that *MYH7* mutation carriers without left ventricular hypertrophy had both higher PICP levels (indicating increased collagen synthesis) and lower early diastolic levels (indicating more impaired relaxation) than *MYBPC3* mutation carriers. Previous studies suggest that mutations in *MYH7* result in an earlier onset of overt hypertrophic cardiomyopathy than do *MYBPC3* mutations.[48,49] Our data provide a potential mechanism for this genotype–phenotype correlation — namely, that *MYH7* mutations, as compared with *MYBPC3* mutations, trigger earlier extracellular-matrix remodeling, more extensive remodeling, or both.

The identification of increased myocardial collagen synthesis in mutation carriers without left ventricular hypertrophy, as with diastolic dysfunction in this population,[5-7] shows that sarcomere-gene mutations have a considerable effect on the heart before the onset of hypertrophy. The detection of a profibrotic myocardial milieu in mutation carriers without left ventricular hypertrophy has intriguing clinical implications. Increased serologic markers of collagen synthesis may identify persons at risk for arrhythmias, sudden death, or heart failure. If so, monitoring levels of these markers may guide new strategies to attenuate disease development or adverse outcomes in hypertrophic cardiomyopathy. We suggest that incorporating genetic testing to identify at-risk mutation carriers, defining features of early disease, and developing therapies to mitigate fibrosis will foster vital new opportunities to change the natural history of hypertrophic cardiomyopathy.

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