Consequences of Pressure Overload on Sarcomere Protein Mutation–Induced Hypertrophic Cardiomyopathy

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Background—Whether ventricular remodeling from hypertrophic cardiomyopathy (HCM), systemic hypertension, or other pathologies arises through a common signaling pathway or through independent molecular mechanisms is unknown. To study this, we assessed cardiac hypertrophy in a mouse model of HCM subjected to increased left ventricular (LV) load.

Methods and Results—Transverse aortic banding of mice with or without an Arg403Gln cardiac myosin heavy chain mutation (αMHC403/+) produced similarly elevated LV pressures (120±30 versus 112±14 mm Hg; P=NS). No mice developed heart failure, and mortality (26% αMHC403/+, 35% wild-type) was comparable. Load-induced hypertrophy was identical in banded 129SvEv αMHC403/+ mice (LV anterior wall [LVAW]=1.28±0.11) and 129SvEv wild-type mice (LVAW=1.29±0.11 mm; P=NS). Genetically outbred Black Swiss (BS) αMHC403/+ mice showed only mildly exaggerated hypertrophy in response to aortic banding (BS αMHC403/+ LVAW=1.30±0.13 mm; BS wild-type LVAW=1.17±0.15 mm; P=0.03), suggesting some effect from a BS genetic locus that modifies hypertrophy induced by the cardiac MHC Arg403Gln mutation. Histopathology and molecular markers of hypertrophy were comparable in all banded 129SvEv or BS mice. Banded αMHC403/+ mice had potential for greater hypertrophy, because cyclosporin A treatment markedly augmented hypertrophy.

Conclusions—The uniform hypertrophic response to increased ventricular load in wild-type and αMHC403/+ mice indicates independent cardiac remodeling pathways and predicts that coexistent hypertension and HCM should not profoundly exacerbate cardiac hypertrophy. In contrast, sarcomere mutation and cyclosporin A–mediated calcineurin inhibition stimulate a shared hypertrophic signaling pathway. Defining distinct signaling pathways that trigger myocyte growth should help to tailor therapies for cardiac hypertrophy. (Circulation. 2003;108:1133-1138.)

Key Words: hypertrophy ■ cardiomyopathy ■ hypertension ■ genetics ■ signal transduction

Hypertrophy is associated with many cardiovascular disorders and is recognized as an independent risk factor for cardiac-related morbidity and mortality.1,2 Left ventricular (LV) hypertrophy arises from diverse factors,3 including mechanical stress, growth factors, cytokines, catecholamines, and primary genetic abnormalities. Arterial hypertension, found in 1 of 4 adult Americans (http://www.americanheart.org/statistics/index.html), is probably the most common cause of secondary cardiac hypertrophy.4 Unexplained ventricular hypertrophy occurs in 1 per 500 individuals in the general population,5 a finding that often indicates hypertrophic cardiomyopathy (HCM), a primary genetic disorder of the myocardium caused by mutation in 1 of 10 sarcomere protein genes.6 Because of the high prevalence of hypertension and HCM, the coexistence of both disorders in an individual patient is not uncommon. Approximately 20% of patients with HCM are reported to have hypertension, although diagnosis of HCM in the setting of hypertension is inherently difficult.7 Several groups have suggested that differences in arterial blood pressure might contribute to the clinical variability of the extent or distribution of ventricular hypertrophy observed among patients with HCM.8 Whether hypertension accentuates or accelerates hypertrophic remodeling or contributes to deterioration of cardiac function in HCM is unknown. Whether sarcomere protein gene mutations and increased blood pressure induce hypertrophy by the same or different pathways is also unknown.

To rigorously address these issues in humans is difficult. We have therefore studied pressure overload in α-myosin heavy chain (MHC)403r+ mice, a genetic model of human HCM. αMHC403r/+ mice have an Arg403Gln mutation in the endogenous α-cardiac MHC gene, a sarcomere protein gene mutation that recapitulates human HCM.9 Previous studies have demonstrated that nonbanded αMHC403r/+ mice develop...
hypertrophy, myocyte disarray, fibrosis, and inducible arrhythmias.10,11 We investigated the combined effects of this sarcomere protein mutation and pressure overload induced by surgical transverse aortic banding of αMHC403/ mice on survival, cardiac morphology, histology, hypertrophy-associated RNA expression, and LV function. Cardiac remodeling was additionally challenged by treatment of banded αMHC403/ mice with the calcineurin-inhibitor cyclosporin A (CsA), an agent known to amplify hypertrophy induced by this sarcomere mutation.12 This model system enabled experimental control of multiple variables, including a defined HCM mutation, background genes, and duration of pressure overload. Surprisingly, the αMHC403/ mutant hearts tolerated pressure overload as well as wild-type hearts, whereas αMHC403/ mutant hearts deteriorated with calcineurin inhibition. These data indicate that independent signaling pathways are involved in hypertrophic remodeling of the myocardium.

Methods

Mice

αMHC403/ mice have been described previously.9,11 The Arg403Gln mutation has been carried in the 129SvEv (designated 129SvEv αMHC403/) and Black Swiss (designated BS αMHC403/) strains for more than 20 generations. Wild-type mice were littermates of the respective αMHC403/ mice. All experimental protocols were reviewed and approved by the Standing Committee on Animals of Harvard Medical Area.

Aortic Banding and Cyclosporin A Treatment

Pressure overload of the left ventricle was induced by thoracic aortic banding of 8-week-old male mice (129SvEv and BS) as described.13 The aorta was ligated (7-0 silk) between the innominate and left common carotid arteries with an overlying 27-gauge needle to produce a discrete stenosis. After ligation, the needle was withdrawn and the pneumothorax was reduced before chest closure and extubation. Sham mice underwent a comparable operation in which the aortic arch was isolated and a band was twined around the aorta but not ligated and subsequently was removed. Mice receiving CsA were injected subcutaneously twice per day with 15 mg of total RNA per gram of body weight.12

Hemodynamics

Mice were anesthetized and ventilated (as described above). The right carotid artery was exposed and ligated distally before insertion of the pressure-volume catheter (Millar Instruments). The catheter was advanced to the LV chamber, and pressure-time loops were recorded using BioBench (National Instruments). Data were analyzed for heart rate, absolute pressures, and the derivatives of pressure loops using Pressure/Volume Analysis Software (PVAN, Millar Instruments).

Echocardiography

Transthoracic echocardiography was performed using a 6- to 15-MHz linear-array probe and a Sonos 4500 ultrasonograph (Hewlett-Packard) as described.11,14 LV fractional shortening was calculated using the formula (LVESD−LVEDD)/LVEDD, where LVEDD indicates LV end-diastolic diameter and LVESD indicates LV end-systolic diameter. LV mass was calculated using the formula LV mass=[(LVEDD+LVPW+LVESD)/2−LVEDD]×1.05, where AW indicates anterior wall and PW indicates posterior wall. Left atrial dimensions were obtained from 2D images in the long-axis view. A single individual performed echocardiography and cardiac measurements without knowledge of genotype or experimental protocol.

Histology, Morphology, and RNA Analysis

Hearts from banded and sham-operated (nonbanded) wild-type and αMHC403/ mice were prepared as described and serially sectioned from ventricular apex to base. Two sections (5 μm each) were retained at the beginning of each 50-μm step. Sections were stained with Masson trichrome stain for collagen as a marker of fibrosis. Assessment of myocyte hypertrophy and disarray was performed at a standardized ×40 and ×200 magnification. Quantification of fibrosis in each heart was then performed using scientific imaging software (IP Labs, version 3.5, Scanalytics Inc), as described.15

RNA expression in total left ventricle RNA, extracted using the TRIzol Reagent (Life Technologies, Invitrogen), was assessed by Northern blot analysis.15 Approximately 2 μg of total RNA per sample were electrophoresed in 1% agarose gels, transferred onto BrightStar-Plus Nylon membranes (Ambion), and hybridized with biotinylated cRNA riboprobes (Ambion).

Statistical Analysis

Differences between groups of wild-type mice and αMHC403/ mice were assessed by unpaired Student’s t tests or ANOVA of continuous variables. Data are expressed as mean±SD. P<0.05 was considered significant.

Results

Survival in Pressure Overload Mice

Sixty-two mice (30 wild-type, 32 αMHC403/ ) underwent aortic banding at age 8 weeks. One wild-type (3%) and 1 mutant mouse (3%) died during the procedure; 4 wild-type (13%) and 7 mutant mice (22%) died within 7 postoperative days. After these acute events, additional banded mice died; however, survival was indistinguishable between banded αMHC403/ and banded wild-type mice (Figure 1; Mantel-Cox log-rank analysis, P=0.89). Survival of sham-operated or nonbanded 30- to 50-week-old BS and 129SvEv wild-type and αMHC403/ mice was 100%.

Left Ventricular Hypertrophy in Pressure Overload Mice

At 30 to 50 weeks after surgery, surviving banded and sham-operated mice underwent serial echocardiography. Maximum LV wall thickness (LVWT) and LV and left atrial dimensions were measured, and LV fractional shortening was calculated (Table 1) in banded mice surviving 15 weeks or longer after surgery (n=23). All banded mice developed concentric LV hypertrophy with identical cardiac morphology in aortic-banded 129SvEv αMHC403/ and aortic-banded wild-type hearts (Table 1) and indistinguishable LVWT

Figure 1. Kaplan-Meier survival curves of banded wild-type and αMHC403/ mice after aortic banding. Two deaths were observed in αMHC403/ mice (■) and 4 deaths in wild-type mice (○) after the 7-day postoperative period.
(129SvEv αMHC403/+ LVWT = 1.29 ± 0.11 mm; 129SvEv wild-type LVWT = 1.28 ± 0.11 mm; \( P = 0.89 \)). LVWT and cardiac morphology of sham-operated mice were identical to nonoperated mice.

Previous studies demonstrated that a polymorphic modifier gene influences the hypertrophic response to the αMHC403/+ mutation.14 To examine whether background genes also altered the response to aortic banding, we compared banded BS wild-type and banded BS αMHC403/+ mice. Nonbanded or sham-operated BS αMHC403/+ mice develop variable degrees of hypertrophy by 30 weeks depending on a polymorphic genetic modifier. Whereas the mean LVWT = 1.01 ± 0.13 mm (Table 1), 50% of BS αMHC403/+ mice have hypertrophy (mean LVWT = 1.12 ± 0.06 mm) but 50% show normal LVWT (<1.0 mm).14 A similar variance of LVWT was observed in banded BS αMHC403/+ mice (SD = 0.13 mm, Table 1). Banded BS wild-type and αMHC403/+ mice demonstrated significantly increased LVWT compared with age- and genotype-matched wild-type mice or sham-operated αMHC403/+ mice (\( P < 0.001 \) for both comparisons; Table 1). Banded BS-αMHC403/+ mice also had greater LVWT than BS wild-type banded mice (LVWT = 1.30 ± 0.13 versus 1.17 ± 0.05 mm; \( P = 0.03 \)).

LV fractional shortening indicated that contractile function was preserved in banded αMHC403/+ and wild-type mice of either genetic background (\( P = \text{NS} \)). Diastolic function was impaired by increased ventricular load, because atrial size increased in nonbanded compared with banded 129SvEv αMHC403/+ hearts (1.63 ± 0.06 versus 1.82 ± 0.27 mm, \( P = 0.05 \)) and in atria from nonbanded compared with banded 129SvEv wild-type hearts (1.76 ± 0.11 versus 1.52 ± 0.02 mm, \( P < 0.001 \); Table 1). Left atrial sizes of banded 129SvEv αMHC403/+ mice were not significantly different from banded wild-type 129SvEv mice. However, banded BS αMHC403/+ mice demonstrated greater left atrial enlargement (1.75 ± 0.07 mm) than banded BS wild-type mice (1.61 ± 0.02 mm; \( P < 0.001 \)).

Serial echocardiography demonstrated no differences in the rates of development of LV hypertrophy in wild-type and mutant mice after aortic banding (Figure 2). Both the initiation and the progression of LV hypertrophy was comparable over a 30-week follow-up period (\( P = \text{NS} \)).

### Treatment With Cyclosporin A

One possible explanation for the observation that banded wild-type and αMHC403/+ produced the same hypertrophic response was that these mice achieve a maximum response and no additional hypertrophy is possible. To assess whether banded αMHC403/+ mice were capable of additional hypertrophic remodeling, we treated banded αMHC403/+ with CsA, which induces no hypertrophic response in wild-type mice but a dramatic hypertrophic response in αMHC403/+ mice12 and within 5 weeks causes 50% mortality in 129SvEv and BS αMHC403/+ mice. Three banded 129SvEv αMHC403/+ mice with maximal load-induced hypertrophy as assessed by serial echocardiograms (10 weeks after banding) were treated with CsA. One mouse died shortly after initiation of CsA treatment; 2 surviving CsA-treated, banded 129SvEv αMHC403/+ mice increased their LVWT within 2 weeks (before CsA

### Table 1. Echocardiographic Characteristics of Wild-Type and αMHC403/+ Mice 30 to 50 Weeks After Aortic Banding

<table>
<thead>
<tr>
<th></th>
<th>Wild-Type</th>
<th></th>
<th></th>
<th>αMHC403/+</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not Banded</td>
<td>Banded</td>
<td>( P^* )</td>
<td>Not Banded</td>
<td>Banded</td>
<td>( P^* )</td>
</tr>
<tr>
<td>129SvEv</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>4</td>
<td></td>
<td>10</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>HR, bpm</td>
<td>550±51</td>
<td>499±24</td>
<td>NS</td>
<td>413±14</td>
<td>489±31</td>
<td>NS</td>
</tr>
<tr>
<td>LVWT, mm</td>
<td>0.88±0.03</td>
<td>1.29±0.11</td>
<td>0.004</td>
<td>1.12±0.08</td>
<td>1.28±0.11</td>
<td>0.01 NS</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>3.13±0.12</td>
<td>2.67±0.19</td>
<td>0.004</td>
<td>2.81±0.25</td>
<td>2.91±0.26</td>
<td>NS</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>1.30±0.14</td>
<td>1.18±0.06</td>
<td>0.01</td>
<td>1.21±0.12</td>
<td>1.41±0.30</td>
<td>NS</td>
</tr>
<tr>
<td>LVFS, %</td>
<td>59±4</td>
<td>56±1</td>
<td>NS</td>
<td>57±3</td>
<td>53±6</td>
<td>NS</td>
</tr>
<tr>
<td>LA, mm</td>
<td>1.52±0.02</td>
<td>1.76±0.11</td>
<td>&lt;0.0001</td>
<td>1.63±0.06</td>
<td>1.82±0.27</td>
<td>0.05 NS</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>90±8</td>
<td>130±8</td>
<td>NS</td>
<td>112±14</td>
<td>146±32</td>
<td>0.05 NS</td>
</tr>
</tbody>
</table>

| Black Swiss    |           |         |         |           |         |         |
| N              | 10        | 7       |         | 7         | 8       |         |
| HR, bpm        | 519±31    | 505±27  | NS      | 423±27    | 516±52  | 0.001 NS|
| LVWT, mm       | 0.88±0.06 | 1.17±0.05| <0.0001 | 1.01±0.13 | 1.30±0.13| <0.0001 |
| LVEDD, mm      | 3.05±0.15 | 2.71±0.28| 0.02    | 2.76±0.49 | 2.32±0.34| NS 0.03 |
| LVESD, mm      | 1.29±0.14 | 0.97±0.28| 0.02    | 1.28±0.29 | 0.88±0.21| 0.01 NS |
| LVFS, %        | 58±3      | 64±9    | NS      | 53±5      | 63±6    | 0.01 NS |
| LA, mm         | 1.51±0.03 | 1.61±0.02| <0.0001 | 1.62±0.08 | 1.75±0.07| 0.02 0.001|
| LV mass, mg    | 86±11     | 115±19  | 0.01    | 91±12     | 111±18  | NS 0.001|
treatment, LVWT=1.13 and 1.3 mm; after CsA treatment, LVWT=1.44 and 1.78 mm). Similarly, 3 long-term banded BS αMHC403 mice with a plateau in the hypertrophic response were treated with CsA. One CsA-treated banded BS αMHC403 mouse died within 2 weeks; 2 surviving CsA-treated, banded BS αMHC403 mice had exaggerated cardiac hypertrophy. Within 2 weeks of CsA treatment, the maximum LVWT of banded BS αMHC403 mice increased from 1.30 and 1.40 mm to 1.48 and 1.51 mm, respectively.

**Table 2. LV Hemodynamics of Banded 129SvEv Wild-Type and 129SvEv αMHC403/− Mice**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild-Type</th>
<th>αMHC403/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>452 ± 156</td>
<td>511 ± 181</td>
</tr>
<tr>
<td>Psys, mm Hg</td>
<td>112.0 ± 13.9</td>
<td>120.3 ± 29.6</td>
</tr>
<tr>
<td>+dP/dtmax, mm Hg/s</td>
<td>5505 ± 1418</td>
<td>8006 ± 1984</td>
</tr>
<tr>
<td>−dP/dtmin, mm Hg/s</td>
<td>−3932 ± 2120</td>
<td>−3397 ± 967</td>
</tr>
<tr>
<td>(+dP/dtmax)/(−dP/dtmin)</td>
<td>1.6 ± 0.5</td>
<td>2.6 ± 1.3</td>
</tr>
</tbody>
</table>

N indicates No. of mice studied; HR, heart rate; Psys, systolic pressure; Pmin, minimum pressure; +dP/dtmax, maximum of first time derivative of a pressure loop; and −dP/dtmin, minimum of first time derivative of a pressure loop.

**Histopathological Changes in Mice With Cardiac Hypertrophy**

Cardiac histopathology in mutant and wild-type mice was assessed 6 to 12 weeks after aortic banding. In nonbanded αMHC403/− mice, mild myocyte hypertrophy and myofiber disarray evident at 15 weeks becomes more pronounced by age 30 to 50 weeks. Cardiac hypereosinophilia and myofiber disarray evident at 15 weeks becomes more pronounced by age 30 to 50 weeks.9,11 Wild-type mice exhibited no significant myocyte hypertrophy, disarray, or fibrosis at any age (Figure 3A, inset). Aortic banding of mutant and wild-type mice caused marked increases in fibrosis throughout the LV (Figure 3). Because the distribution of fibrosis was not uniform (Figure 3A), total LV fibrosis was quantified by summing the fibrosis content in serial (apical to base, n = 15) sections. LV fibrosis was greater in banded than nonbanded wild-type mice (% LV fibrosis: 4.54 ± 1.40 versus 0.24 ± 0.04, respectively; Figure 3B). Banding similarly increased fibrosis in αMHC403/− mice (percent LV fibrosis: 5.91 ± 0.45 versus 4.07 ± 0.16; banded versus nonbanded; P < 0.001). No significant difference (P = 0.19) in LV fibrosis was observed between banded wild-type and banded αMHC403/− mice (Figure 3B), and amounts of fibrosis were comparable in different genetic backgrounds (data not shown).

**RNA Markers of Cardiac Hypertrophy**

Hypertrophy-associated RNAs were assessed by Northern blot analyses using left ventricle and interventricular septum obtained 5 weeks after banding from mutant and wild-type mice (Figure 4, Table 3, and data not shown). Banded BS...
Table 3. Effect of Increased Ventricular Load of RNA Expression in Wild-Type and αMHC<sup>403/403</sup> Mice

<table>
<thead>
<tr>
<th>RNA</th>
<th>Wild-Type Not Banded</th>
<th>Banded</th>
<th>P* Not Banded</th>
<th>Banded</th>
<th>403: Not Banded vs Banded</th>
<th>WT Banded vs 403 Banded</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANF</td>
<td>1 5.6±3</td>
<td>0.03</td>
<td>2.9±2</td>
<td>11.5±7</td>
<td>0.03</td>
<td>NS</td>
</tr>
<tr>
<td>BNP</td>
<td>1 7.4±5</td>
<td>0.04</td>
<td>6.2±2</td>
<td>10.5±5</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>SkA</td>
<td>1 10.5±1</td>
<td>0.01</td>
<td>2.1±1</td>
<td>13.6±5</td>
<td>0.02</td>
<td>NS</td>
</tr>
</tbody>
</table>

More than 4 BS mice were evaluated in each group. RNAs were normalized to 18S mRNA and reported as a fold change compared to levels of ANF, BNP, or α-skeletal actin (Sk Act) mRNAs in wild-type mice (arbitrarily defined as 1). For the P value, 2-tailed t tests were used to compare mRNA levels between wild-type (*) and specified groups.
pressure overload or a sarcomere protein gene mutation; the same amount of fibrosis was observed in banded 129SvEv and BS αMHC<sup>203/+</sup> mice. Because a BS background modifier gene has been previously recognized to alter the hypertrophic response to a sarcomere protein gene missense mutation, this same modifier gene or another likely accounts for the amplified hypertrophic response to the combined stimulus of pressure overload and sarcomere gene protein mutations. By extrapolation, we suggest that modifying genes may cause a modest effect on the hypertrophic phenotype of individuals with both sarcomere protein mutations and hypertension.

The conclusion that pressure overload and sarcomere protein gene mutations lead to hypertrophy by independent pathways has important implications for human HCM. First, investigators have noted that the distribution of hypertrophy differs when produced by pressure overload versus sarcomere protein mutations. Asymmetric septal hypertrophy is the most common morphology of human HCM. Hypertrophy in αMHC<sup>203/+</sup> mice can also be asymmetric. Although the mechanism for this asymmetry remains controversial, one hypothesis has been that differences in regional wall stress influence cardiac remodeling. Pressure overload in both wild-type and αMHC<sup>203/+</sup> mice caused concentric LV hypertrophy. Consistent with the conclusion that pressure overload did not exacerbate the hypertrophic response to a sarcomere protein mutation, changes in ventricular pressure distribution that must accompany aortic banding did not lead to asymmetric or other specific patterns of hypertrophy in αMHC<sup>203/+</sup> mice. We conclude that other factors, both genetic and environmental, must be responsible for the patterning of hypertrophy in individuals with sarcomere protein mutations. Second, the less than additive effect of LV mass observed in mice with hypertrophic cardiomyopathy and hypertension suggests a favorable clinical outcome in terms of both morbidity and mortality in humans with coexistent disease. Patients with coexisting HCM and hypertension should receive only standard therapy for each disorder. Furthermore, substantial cardiac hypertrophy in hypertension is unlikely to reflect unrecognized HCM.

Acknowledgments

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References

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