Ventricular Arrhythmia Vulnerability in Cardiomyopathic Mice With Homozygous Mutant Myosin-Binding Protein C Gene

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Background—Homozygous mutant mice expressing a truncated form of myosin-binding protein C (MyBP-C<sup>t/t</sup>) develop severe dilated cardiomyopathy, whereas the heterozygous mutation (MyBP-C<sup>t/+</sup>) causes mild hypertrophic cardiomyopathy. Adult male MyBP-C<sup>t/t</sup> and MyBP-C<sup>t/+</sup> mice were evaluated for arrhythmia vulnerability with an in vivo electrophysiology study.

Methods and Results—Surface ECGs were obtained for heart rate, rhythm, and conduction intervals. Atrial, atrioventricular, and ventricular conduction parameters and refractoriness were assessed in 22 MyBP-C<sup>t/t</sup>, 10 MyBP-C<sup>t/+</sup>, and 17 wild-type MyBP-C<sup>t/+</sup>/H<sub>11001</sub> mice with endocardial pacing and intracardiac electrogram recording. Arrhythmia induction was attempted with standardized programmed stimulation at baseline and with isoproterenol. Heart rate variability and ambient arrhythmia activity were assessed with telemetric ECG monitors. Quantitative histological characterization was performed on serial sections of excised hearts. MyBP-C<sup>t/t</sup> and MyBP-C<sup>t/+</sup> mice have normal ECG intervals and sinus node, atrial, and ventricular conduction and refractoriness. Ventricular tachycardia was reproducibly inducible in 14 of 22 MyBP-C<sup>t/t</sup> mice (64%) during programmed stimulation, compared with 2 of 10 MyBP-C<sup>t/+</sup> mice (20%) and 0 of 17 wild-type controls (<i>P</i> < 0.001). Ventricular ectopy was present only in MyBP-C<sup>t/t</sup> mice during ambulatory ECG recordings. There were no differences in heart rate variability parameters. Interstitial fibrosis correlated with genotype but did not predict arrhythmia susceptibility within the MyBP-C<sup>t/t</sup> group.

Conclusions—MyBP-C<sup>t/t</sup> mice, despite prominent histopathology and ventricular dysfunction, exhibit normal conduction and refractoriness, yet are vulnerable to ventricular arrhythmias. Somatic influences between genetically identical mutant mice most likely account for variability in arrhythmia susceptibility. A sarcomeric protein gene mutation leads to a dilated cardiomyopathy and ventricular arrhythmia vulnerability phenotype. (Circulation. 2001;104:2734-2739.)

Key Words: arrhythmia ■ cardiomyopathy ■ electrophysiology ■ genetics ■ pathology

Patients with dilated cardiomyopathy (DCM) are at risk for ventricular arrhythmias and sudden death. The clinical phenotypes are broadly heterogeneous, ranging from asymptomatic ventricular dilation to premature sudden death in childhood. Inherited forms account for ~30% of DCM cases, most typically in autosomal dominant or X-linked inheritance patterns. The particular genotype appears to be an important factor in mortality risk stratification and may be consequential in determining arrhythmia frequency and severity. Murine familial hypertrophic cardiomyopathy (FHC) models have been created to allow characterization of the structural and functional consequences of specific mutations in sarcomeric protein genes. In the present series of experiments, a mouse model of DCM resulting from a homozygous mutation in the myosin-binding protein C (MyBP-C<sup>t</sup>) and a model of FHC due to a heterozygous mutation in the same gene (MyBP-C<sup>t/+</sup>) were used to characterize the electrophysiological phenotype and correlate arrhythmia vulnerability with quantitative histopathological changes. The mutation causes production of a truncated MyBP-C protein that becomes incorporated into the sarcomere. The heterozygous form of this mutation accounts for nearly 20% of identified genotypes in human clinical FHC. The exact mechanisms by which a MyBP-C mutation causes FHC or DCM are not yet fully defined, although it has been determined to be an abundant regulatory myofibrillar protein. The cardiac structural and hemodynamic functional properties of homozygous (MyBP-C<sup>t/t</sup>) mice have recently been described, including

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severe histopathology, ventricular dilation, and dysfunction. Heterozygous mice have a less severe structural phenotype but still have distinct ventricular functional abnormalities that appear to progress with greater age. The electrophysiological characteristics of MyBP-C<sup>-/-</sup> mice have not previously been assessed. In the present study, in vivo cardiac electrophysiology studies were performed in MyBP-C<sup>-/-</sup> and MyBP-C<sup>+/+</sup> mutant mice and compared with wild-type (WT) controls to evaluate the cardiac conduction characteristics and vulnerability to ventricular arrhythmias.

**Methods**

**Animals**

Surface resting ECGs and electrophysiology studies were performed on 22 male 30- to 55-week-old homozygous MyBP-C<sup>-/-</sup> mice and compared with those in an age-matched group of 10 adult male MyBP-C<sup>+/+</sup> and 17 male WT control mice. Homozygous MyBP-C<sup>-/-</sup> mice were inbred >5 generations into the 129SvEv background and are genetically equivalent. Mice were housed 3 to 4 per cage and maintained on regular rodent chow with free access to water in a facility with 12-hour light and dark cycles. Animal care protocols conformed to the Association for the Assessment and Accreditation of Laboratory Animal Care and the Harvard Medical School and Children’s Hospital Animal Care and Use Committees.

**Preprocedural Preparation**

The protocols for the in vivo mouse electrophysiology study have been described previously. Each mouse was anesthetized with a combination of ketamine and pentobarbital (0.033 mg/kg IP). Surface ECGs were obtained with 25-gauge subcutaneous electrodes placed in each limb. Heart rate and rhythm were monitored continuously throughout the procedure. A cutoff of the internal jugular vein was performed, and an octapolar 2F electrode catheter (CIBer cath; NuMED, Inc) was placed in the right atrium and ventricle, guided by electrogram tracing to verify placement.

**Electrophysiology Study**

An in vivo electrophysiology study was performed in each mouse to assess atrial and ventricular conduction, refractoriness, and arrhythmia inducibility. As in humans, if programmed electrical stimulation failed to cause an arrhythmia, isoproterenol was administered (1 ng/g IV) and the pacing protocol was repeated to assess the effects of an increased catecholamine state on cardiac conduction and arrhythmia inducibility. The dose was titrated to effect a 25% heart rate increase.

**Ambulatory ECG**

Telemetric ambulatory long-term ECG recordings, analogous to Holter monitoring in humans, were obtained with implantable transmitters. Mice were anesthetized with ketamine hydrochloride and pentobarbital (0.033 mg/g IP each), and a midline incision was made along the spine. An implantable 3.5-g wireless radiofrequency transmitter (DataSciences International) was aseptically inserted into a subcutaneous tissue pocket in the back. The mice were placed in cages overlying a receiver for transmission of ECG signals to a computer for display and analysis, including assessment of heart rate variability parameters with spectral analysis of time and frequency domains. Telemetered ECG tracings were obtained in conscious mice at rest, during quiet awake time, and during graded treadmill exercise testing.

**Heart Rate Variability Protocol**

Experiments were initiated 4 days after recovery from surgical instrumentation. All recordings were performed with the mice in the conscious state between 9:00 AM and 3:00 PM in a constant environment. After a 15-minute control period for heart rate stabilization, the baseline ECG was recorded for 30 to 60 minutes. Digital signal processing of 120-second stable segments of the analog signal was analyzed with custom software using bandpass filtering and a threshold-lockout algorithm. To study frequency-specific contributions of the principal cardiovascular control systems to heart rate fluctuations, selective blockade by specific pharmacological agents was used: atropine (0.5 mg/kg IP) for parasympathetic blockade, propranolol (1 mg/kg IP) for sympathetic blockade, and atropine plus propranolol for combined autonomic blockade. After a 5- to 10-minute equilibration phase after drug administration to allow for heart rate stabilization, ECG recordings were repeated similarly to the baseline state. Pharmacological studies were performed on different days to prevent interference of the drugs.

**Measurements**

The PR, QRS, RR, and QT intervals were measured in 6 surface-lead ECG leads by 2 independent observers, who were both blinded to the animals’ genotype. Intracardiac recordings were obtained during identical pacing and programmed electrical stimulation protocols for all mice by experienced investigators blinded to genotype.

**Histology and Morphology**

Hearts were excised from WT, MyBP-C<sup>-/-</sup>, and MyBP-C<sup>+/+</sup> mice, washed in 37°C Dulbecco’s ×1 PBS, arrested in 50 mmol/L KCl, and fixed in 10% formalin. Fixed hearts were cut transversely and sectioned serially 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’s 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 the proportion of fibrosis in each heart was then performed with scientific imaging software (IP Laboratories, version 3.5, Scanlytics Inc) and high-resolution serial scanning technique. Histological sections from a minimum of 8 anatomic levels through the left ventricle were used for analysis.

**Statistical Analyses**

All continuous variables, such as ECG intervals and cardiac conduction properties, were compared with those of sex- and age-matched control mice, with data presented as the mean±SEM. Statistical analysis between groups included the 2-tailed Student’s t test, Fisher’s exact test, ANOVA with Scheffé subgroup testing when appropriate, and analysis of interobserver variability. Comparisons within groups were done by a paired 2-tailed t test. A value of <0.05 was considered statistically significant.

**Results**

**ECG and Electrophysiological Findings**

The resting heart rates and ECG intervals were similar among control, MyBP-C<sup>+/+</sup>, and MyBP-C<sup>-/-</sup> mice (Table 1). The MyBP-C<sup>-/-</sup> mice had significantly more rightward QRS axes than either the MyBP-C<sup>+/+</sup> or MyBP-C<sup>-/-</sup> mice (P<0.001). All of the MyBP-C<sup>-/-</sup> mice had a frontal-plane QRS axis ≥90°.

Findings from the intracardiac electrophysiology studies demonstrated normal atrial, atrioventricular, and ventricular mean conduction parameters and refractory periods in WT, MyBP-C<sup>+/+</sup>, and MyBP-C<sup>-/-</sup> mice (Table 2). Representative ECG and intracardiac electrogram tracings are illustrated in Figure 1. In addition, with the standardized murine pacing and programmed electrical stimulation protocol, with and without isoproterenol, there were no instances of provoked inducible arrhythmias in any of the WT mice and in only 2 of 10 heterozygous mice (20%). The duration of induced episodes of ventricular tachycardia (VT) was 1 to 3 seconds in 1 MyBP-C<sup>+/+</sup> mouse and 1 to 8 seconds in another. In 1 of the
2 mice, VT was inducible only after administration of isoproterenol. With an identical pacing protocol, the homozygous MyBP-C<sup>Cm</sup> group reproducibly had inducible nonsustained VT (Figure 2), ranging between 2 and 25 seconds, in 14 of 22 mice (64%) ($P<0.001$ versus MyBP-C<sup>Cms</sup> and WT groups). Of the 14 mice with VT, 10 could be induced at baseline with programmed stimulation, whereas in 4 mice, VT could be provoked only after administration of isoproterenol. The mean duration of VT episodes was also longer in the MyBP-C<sup>Cms</sup> group (8.5±5.5 seconds) compared with the MyBP-C<sup>Cms</sup> mice (2.2±3.1 seconds, $P<0.01$). The nonsustained VT was reinduced 3 to 4 times in each mouse to confirm consistency in ventricular arrhythmia vulnerability both before and after the isoproterenol infusion.

Ambulatory ECG and Heart Rate Variability Testing
During the single-lead ECG monitoring in conscious, freely moving mice, there were no differences in heart rate or PR, QRS, or QT intervals. There were also no differences between genotype groups in autonomic control, measured by heart rate variability indices (Table 3). Intrapерitoneal administration of atropine, propranolol, or atropine with propranolol to selectively block limbs of the autonomic nervous system also did not produce any significant differences between genotype groups for heart rate variability indices (data not shown). No spontaneous episodes of sudden death, bradyarrhythmias, or tachyarrhythmias occurred during the monitoring periods in any mice. Isolated ventricular premature beats were frequently observed and ventricular couplets were occasionally seen in MyBP-C<sup>Cms</sup> mice, but not in any of the MyBP-C<sup>Cms</sup> or WT mice.

Quantitative Histopathological Analysis
Myocyte damage as evidenced by interstitial fibrosis was positively correlated with the genotypic class. Regions of fibrosis were identified in the ventricles of each homozygous MyBP-C<sup>Cm</sup> mouse analyzed (Figure 3). Quantitatively, the mean percent fibrosis was 7.94±0.99% in the left ventricles from MyBP-C<sup>Cm</sup> mice ($n=6$), compared with 0.23±0.02% in WT MyBP-C<sup>Cm</sup> mice ($n=4$, $P<0.0001$). The amount of fibrosis present was 30-fold higher than that seen in WT or heterozygous mice, in which no significant interstitial fibrosis or myocyte disarray was apparent. Within the group of homozygous MyBP-C<sup>Cm</sup> mice, however, no direct correlation could be made between the presence or severity of left ventricular interstitial fibrosis and the likelihood of an inducible ventricular arrhythmia ($P=0.47$; MyBP-C<sup>Cm</sup>, VT, versus MyBP-C<sup>Cms</sup>, no VT). Regions of fibrosis varied between ventricular sections, even within serial slices from the same mouse. No dystrophic calcification was observed in any of the left ventricular sections.

Discussion
In this series of in vivo experiments, a truncation mutation in MyBP-C did not lead to manifest abnormalities in cardiac conduction properties or refractoriness. Mice with the homozygous mutation (MyBP-C<sup>Cm</sup>) do have right-axis deviation as well as a significant risk of inducible nonsustained VT during programmed ventricular stimulation. Mice with the heterozygous form of this same mutation (MyBP-C<sup>Cms</sup>) have a mild FHC phenotype that is not evident at younger ages. They also did not have conduction abnormalities, abnormal axes, or significant inducible arrhythmias, with only 2 of 10 having short runs of nonsustained VT from 1 to 8 seconds.

Despite the genetic homogeneity of the MyBP-C<sup>Cm</sup> mice, not all had VT inducible with a standardized pacing and programmed electrical stimulation protocol. It is likely that environmental influences or somatic factors account for the lack of inducibility of uniform ventricular arrhythmia among MyBP-C<sup>Cm</sup> mice. Although the environment is kept similar among all mice and housing conditions are identical, possible somatic influences include anesthesia and surgery-related stressors and variation in level of sedation. The diet was

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**TABLE 1. ECG Data Summary**

<table>
<thead>
<tr>
<th></th>
<th>Sinus Cycle</th>
<th>PR</th>
<th>QRS</th>
<th>QT</th>
<th>QRS Axis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WT MyBP-C&lt;sup&gt;Cm&lt;/sup&gt;/t</strong> ($n=17$)</td>
<td>148.3±20.8</td>
<td>34.5±5.8</td>
<td>18.5±4.2</td>
<td>53.8±21.0</td>
<td>80.5±35.5</td>
</tr>
<tr>
<td><strong>MyBP-C&lt;sup&gt;Cms&lt;/sup&gt;/t</strong> ($n=10$)</td>
<td>142.8±25.5</td>
<td>32.0±2.2</td>
<td>20.3±4.2</td>
<td>59.4±18.1</td>
<td>77.5±22.6</td>
</tr>
<tr>
<td><strong>MyBP-C&lt;sup&gt;Cm&lt;/sup&gt;/t</strong> ($n=22$)</td>
<td>162.9±32</td>
<td>31.4±4.5</td>
<td>19.2±8.9</td>
<td>64.6±7.6</td>
<td>130.0±30.4*</td>
</tr>
</tbody>
</table>

All ECG parameters measured in ms±1 SEM. The QRS axis was significantly more rightward in MyBP-C<sup>Cm</sup> mice than either WT or MyBP-C<sup>Cms</sup> mice.

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**TABLE 2. Electrophysiological Data Summary**

<table>
<thead>
<tr>
<th></th>
<th>SNRT&lt;sub&gt;100&lt;/sub&gt;</th>
<th>CSNRT</th>
<th>AVERP</th>
<th>AVWBCL</th>
<th>AV 2:1</th>
<th>RVERP</th>
<th>VAWBCL</th>
<th>VA 2:1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WT MyBP-C&lt;sup&gt;Cm&lt;/sup&gt;/t</strong> ($n=17$)</td>
<td>183.9±30.9</td>
<td>46.5±34.7</td>
<td>60.7±13</td>
<td>84.0±9.5</td>
<td>64.7±13.5</td>
<td>52.9±9.1</td>
<td>86.7±16.7</td>
<td>70.0±13.1</td>
</tr>
<tr>
<td><strong>MyBP-C&lt;sup&gt;Cms&lt;/sup&gt;/t</strong> ($n=10$)</td>
<td>224.9±28.7</td>
<td>85.6±35.3</td>
<td>61.0±5.5</td>
<td>82.5±9.6</td>
<td>67.5±5.0</td>
<td>49.0±6.5</td>
<td>103.8±26.3</td>
<td>92.5±17.7</td>
</tr>
<tr>
<td><strong>MyBP-C&lt;sup&gt;Cm&lt;/sup&gt;/t</strong> ($n=22$)</td>
<td>205.6±56</td>
<td>74.9±47</td>
<td>63.0±11</td>
<td>78±9</td>
<td>66.3±8.0</td>
<td>57±19</td>
<td>101.9±22</td>
<td>85.0±15.0</td>
</tr>
</tbody>
</table>

Electrophysiological parameters (in ms) are expressed as mean±1 SEM. SNRT<sub>100</sub> indicates sinus node recovery time after atrial pacing at 100 ms for 15 seconds; CSNRT, rate-corrected sinus node recovery time; AVERP, atrioventricular effective refractory period; AVWBCL, AV Wenckebach block cycle length; AV 2:1, AV 2:1 block cycle length; RVERP, right ventricular effective refractory period; VAWBCL, ventriculoatrial retrograde Wenckebach block cycle length; and VA 2:1, ventriculoatrial retrograde 2:1 block cycle length.
standardized, although level of exercise and actual food and water intake are also variable factors.

Vulnerability to ventricular arrhythmia is most likely related, at least in part, to the cardiomyopathic phenotype as an underlying substrate for inducible VT. Although the degree of histopathological fibrosis did not correlate with arrhythmia inducibility, the group of mice with histological abnormalities had a greater frequency of VT, which may be an inherent genetic vulnerability or possibly a secondary finding of abnormal ventricular myocardial structure and functional mechanics.

As in humans, the murine stimulation protocol was designed with the goal of optimizing accuracy, particularly avoiding false-positive results in normal mice. More aggressive electrical programmed stimulation does consistently provoke VT in all mice tested, regardless of genotype. In addition, variability in some of the electrophysiological parameters not directly related to ventricular arrhythmia vulnerability, such as the rate-corrected sinus node recovery time, may have led to a type II statistical error due to insufficient power in animal group sample sizes.

In a mouse FHC model, a missense mutation in the sarcomeric α-myosin heavy chain (Arg403Gln) was shown to manifest electrophysiological abnormalities. Conversely, MyBP-C<sup>−/−</sup> heterozygote mice demonstrated a normal electrophysiological phenotype. This disparity may be related to the milder histological phenotype of the heterozygous MyBP-C mice compared with the heterozygous Arg403Gln mice. In comparison, the frequency of VT in male homozygous MyBP-C mice (64%) is very similar to the VT frequency in male heterozygous Arg403Gln mice (63%).

Electrophysiological phenotype variability is evident in both human DCM and FHC, and clinical disease heterogeneity complicates risk stratification and treatment efficacy. Even in family members with identical mutations causing DCM or FHC, there may be significant inconsistency in the presence and severity of electrophysiological abnormalities. Survival has been clearly linked to specific mutations, irrespective of degree of ventricular hypertrophy and clinical symptomatology. In addition, certain mutations may lead to specific arrhythmia vulnerability. For example, in humans, the Arg663His missense mutation in the β-myosin heavy chain gene has been associated with a higher frequency of atrial fibrillation than in the general FHC population.
Humans with heterozygous mutations in MyBP-C typically have improved survival compared with patients with an Arg403Gln missense mutation in the β-myosin heavy chain gene21,22; it is not certain, however, whether the higher mortality is secondary to malignant arrhythmias or other causative or modifying factors.

Electrophysiological testing and the results of programmed electrical stimulation in animals, as in humans, may not directly correlate with the occurrence of spontaneous cardiac arrhythmias or the mechanism for sudden death.23–25 It may not be possible to relevantly extrapolate the mouse models of DCM and hypertrophic cardiomyopathy and the miniaturization of the in vivo electrophysiology study methodologies to human clinical disease pathophysiology or electrophysiological risk stratification in human hypertrophic cardiomyopathy and DCM.26–28

Mutant MyBP-C<sup>+</sup> mice with a homozygous mutation have a severe DCM, have histological evidence of myocardial damage, and are susceptible to ventricular arrhythmia inducibility on electrophysiological testing. An obvious electrophysiological substrate (such as QT prolongation or heterogeneity of repolarization) is not demonstrated. No histological or electrophysiological differences were detected between MyBP-C<sup>+</sup> mice inducible for VT and those that could not be induced. Variability in the electrophysiological phenotype might relate to genotype-specific risk factors, but the results of the present study in genetically similar mice suggest that environmental influences or somatic factors play an important role in vulnerability to ventricular arrhythmia. Heterozygous mutations in MyBP-C causing human FHC may not be as electrophysiologically dangerous as mutations in the myosin heavy chain gene. In contrast, a homozygous mutation in the same sarcomeric protein gene (MyBP-C) leads to a DCM phenotype with a high frequency of inducible ventricular arrhythmias in mice. This suggests that some particular genotypes may be more susceptible to intrinsic arrhythmia inducibility, rather than arrhythmias being interpreted only as secondary factors of hemodynamic alterations or histological functional tissue damage. It is therefore possible that the particular genotype determines the primary inherent vulnerability to arrhythmia as a consequential determinant of sudden cardiac death and all-cause mortality from hypertrophic cardiomyopathy and DCM.

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References


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