Sarcomere Protein Gene Mutations in Hypertrophic Cardiomyopathy of the Elderly

Hideshi Niimura, MD*; Kristen K. Patton, MD*; William J. McKenna, MD; Johann Souls, MS; Barry J. Maron, MD; J.G. Seidman, PhD; Christine E. Seidman, MD

Background—Hypertrophic cardiomyopathy, a familial myocardial condition caused by sarcomere protein mutations, is usually recognized by early adulthood. Hypertrophic cardiomyopathy of the elderly has similar clinical features but, notably, a later age of onset and noncontributory family history. Causes of elderly-onset hypertrophic cardiomyopathy are unknown.

Methods and Results—Eighteen women and 13 men diagnosed with late-onset hypertrophic cardiomyopathy were studied. Initial symptoms occurred at 59.3 (±12.3) years, and diagnosis was made at 62.8 (±10.8) years. None had family histories of cardiomyopathy. Echocardiography demonstrated maximal left ventricular wall thickness of 19.9±3.8 mm, systolic anterior motion of the mitral valve (58%), and, in 11 individuals, left ventricular outflow tract gradients (average, 63±42.8 mm). Sarcomere protein gene analyses revealed 8 sequence variants in cardiac myosin binding protein-C (1 nonsense, 1 splice acceptor site, and 3 missense), cardiac troponin I (2 missense), and α-cardiac myosin heavy chain (1 missense). Seven variants were not found in over 170 normal chromosomes; 1 variant (cardiac myosin binding protein-C Arg326Gln) also occurred in a healthy adult.

Conclusions—Hypertrophic cardiomyopathy of the elderly can be a genetic disorder caused by dominant sarcomere protein mutations. The distribution of mutations in elderly-onset disease is strikingly different (P<0.00001) from that of familial, early onset hypertrophic cardiomyopathy. Whereas defects in β-cardiac myosin heavy chain, cardiac troponin T, and α-tropomyosin account for >45% of familial hypertrophic cardiomyopathy, none were found here. Rather, mutations in cardiac myosin binding protein-C, troponin I, and α-cardiac myosin heavy chain caused elderly-onset hypertrophic cardiomyopathy. (Circulation. 2002;105:446-451.)

Key Words: cardiomyopathy ■ genes ■ aging ■ hypertrophy

Since early descriptions of hypertrophic cardiomyopathy,1,2 the disease has intrigued clinicians and researchers because of its variable clinical course and heterogeneous pathophysiology.3–5 Defining features of the disease include a nondilated, hypertrophied left ventricle with preserved systolic function, impaired diastolic relaxation, and decreased compliance that is unaccounted for by other cardiac or systemic disease. Pathological examination of tissue reveals characteristic myocyte hypertrophy, disarray, and replacement fibrosis.6 Previously thought to be a rare genetic disorder, recent echocardiographic studies demonstrate the prevalence of hypertrophic cardiomyopathy in the general population to be 1 in 500.7 Clinical analyses established familial hypertrophic cardiomyopathy as a disease of the young adult, with autosomal dominant inheritance.8,9 Cardiac hypertrophy associated with many of the pathophysiological features of hypertrophic cardiomyopathy is also observed in much older individuals,10–12 although age of symptom onset and the absence of known affected relatives are notable clinical differences from younger patients. Uncertainty remains as to whether hypertrophic cardiomyopathy presenting at different ages represents a single disease or distinct entities with overlapping phenotypes.

Over the last decade, molecular genetic approaches have demonstrated that most causes of familial hypertrophic cardiomyopathy are sarcomere protein gene mutations.13,14 More than 150 disease-causing defects in genes encoding 8 sarcomere proteins, β-cardiac myosin heavy chain, cardiac tropinin T and α-tropomyosin, cardiac troponin I, cardiac myosin binding protein-C, and, more rarely, cardiac actin and the essential or regulatory myosin light chains have been reported. The intriguing clinical variability of disease, includ-
ing onset (symptoms and hypertrophy) and prognosis,15–19 is partially explained by this genetic heterogeneity.

We hypothesized that some cardiac hypertrophy of the elderly might also be attributable to sarcomere protein gene mutations. To test this model, 31 individuals diagnosed with hypertrophic cardiomyopathy late in life who had no known affected relatives were studied. Nucleotide sequence analyses of genes encoding α- and β-cardiac myosin heavy chains, cardiac troponin T, α-tropomyosin, cardiac troponin I, and cardiac myosin binding protein-C revealed mutations in a subset of these sarcomere proteins.

Methods

Clinical Evaluations

Informed consent was obtained in accordance with human subject committee guidelines at Brigham and Women’s Hospital, St George’s Hospital Medical School, and Minneapolis Heart Institute Foundation. Because diagnostic criteria for elderly-onset hypertrophic cardiomyopathy are imprecise, subjects were recruited with clinical expression or diagnosis made for the first time after 40 years of age and clinically evaluated as described previously.15,16,18 Diagnostically, subjects were recruited with clinical expression or diagnosis made for the first time after 40 years of age and clinically evaluated as described previously.15,16,18

Clinical Characteristics of Elderly-Onset Hypertrophic Cardiomyopathy

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Subjects</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>MyBP-C</td>
<td>Troponin I</td>
<td>α-MHC</td>
<td></td>
</tr>
<tr>
<td>No. of individuals</td>
<td>31</td>
<td>23</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Male/female, n</td>
<td>13/18</td>
<td>9/14</td>
<td>3/2</td>
<td>1/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Onset of symptom, y</td>
<td>59.5±12.0</td>
<td>60.5±12.1</td>
<td>56.0±13.2</td>
<td>52.5±3.6</td>
<td>74</td>
</tr>
<tr>
<td>Age at diagnosis, y</td>
<td>62.8±10.8</td>
<td>64.2±10.7</td>
<td>60.2±8.9</td>
<td>49.0±9.9</td>
<td>75</td>
</tr>
<tr>
<td>NYHA class</td>
<td>I–III</td>
<td>I–III</td>
<td>I–II</td>
<td>II</td>
<td>II</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>135±19</td>
<td>135±18</td>
<td>129±18</td>
<td>130±0</td>
<td>178±0</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>76±10</td>
<td>73±9</td>
<td>78±8</td>
<td>80±0</td>
<td>100±0</td>
</tr>
<tr>
<td>Abnormal ECG,* n</td>
<td>29</td>
<td>21</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Echocardiography

SAM, n (%) | 18 (58) | 15 (65) | 2 (40) | 0 | 1 (100) |
Left atrial dimension, mm | 41.5±6.3 | 40.8±6.3 | 44.8±5.5 | 36.0±1.4 | 47 |
Max LWTT, mm (range, mm) | 19.9±3.8 (14–31) | 19.3±2.8 (15–26) | 22.7±5.5 (17–31) | 19.0±8.5 (14–25) | 19 |
LVDD, mm | 42.6±8.6 | 43.5±7.8 | 39.2±13.1 | 42.0 | 45 |
LVOT gradient, mm Hg | 36±45 | 43±49 | 8±10 | 25±35 | 54 |

*MyBP-C indicates cardiac myosin-binding protein C; α-MHC, α-cardiac myosin heavy chain; SAM, systolic anterior motion; LVWT, left ventricular wall thickness; LVDD, left ventricular diastolic diameter; and LVOT, left ventricular outflow tract.

Confirmation of Mutations

Sequence variants predicted to alter restriction enzyme sites were independently confirmed by polymerase chain reaction amplification of relevant exons, restriction enzyme digestion, and size fractionation on 3% NuSieve/1% agarose gels. Car0 cardiac binding protein-C Thr59Ala abolished a MseI site, Gln425Stop created a BstI site, Int27ASC (−3)G created an AvrI site, and Arg1002Gln abolished an NruI site. Cardiac troponin I Pro82Ser created a BsrI site, and Asp196Asn abolished a Clal site. Cardiac myosin heavy chain Arg795Gln abolished an Smal site.

Cardiac myosin binding protein-C Arg326Gln was confirmed by oligonucleotide-specific hybridization. Exon 13 was amplified, and DNA was transferred to 2 nylon membranes (Gene Screen Plus), which were individually hybridized with 32P-labeled oligonucleotides corresponding to wild-type and mutant sequences. Nylon membranes were washed in 6×SSC, 0.10% sodium pyrophosphate at 48°C, for 30 minutes, and the hybridization signal was quantified using a PhosphorImager (Molecular Dynamics).

Statistical Analysis

Values are expressed as mean±SD. Differences between 2 groups were compared using the χ² or the Student’s t test for continuous variables.

Results

Eighteen women and 13 men were identified with late-onset cardiac hypertrophy of uncertain etiology (Table). No study subject had family members diagnosed with hypertrophic cardiomyopathy nor medical histories suggestive of this diagnosis. Systolic blood pressure was >160 mm Hg in 6 individuals, and diastolic blood pressure was >90 mm Hg in 3 individuals. Essential hypertension required treatment in 1 of these individuals. The average age when symptoms occurred (most often exertional dyspnea and chest pain) was 59.5 (±12.0) years.
A diagnosis of elderly-onset hypertrophic cardiomyopathy was made on average at age 62.8 \((\pm 10.8)\) years. ECG was abnormal in 92% of individuals. Abnormalities included voltage criteria for left ventricular hypertrophy (43%), non-specific ST-T wave abnormalities (57%), anteroseptal Q waves (14%), rhythm disturbances (14%), and intraventricular conduction delays (7%). Echocardiography demonstrated increased left ventricular hypertrophy (average maximal wall thickness, 19.9\(\pm\)3.8 mm) with no predominant morphological pattern of hypertrophy. Systolic anterior motion of the mitral valve occurred in 58% of individuals, and 11 had left ventricular outflow tract gradients (average, 61\(\pm\)42.8 mm).

DNA sequences encoding \(\alpha\)- and \(\beta\)-cardiac myosin heavy chain, \(\alpha\)-tropomyosin, cardiac troponins T and I, and cardiac myosin binding protein-C were determined from all 31 samples (see Methods section). Sequences of the \(\beta\)-cardiac myosin heavy chain, \(\alpha\)-tropomyosin, and cardiac troponin T genes were normal in all subjects (data not shown). Eight sequence variants identified 3 sarcomere protein genes: cardiac myosin binding protein-C, troponin I, and \(\alpha\)-cardiac myosin heavy chain (Figure 1). All were independently confirmed by restriction enzyme digestion or by oligonucleotide-specific hybridization (see Methods section). Each of these is predicted to alter the structure of each sarcomere protein, with probable functional consequences. Seven of 8 sequence variants were absent from over 170 normal and unrelated control samples; these are considered mutations that cause elderly-onset hypertrophic cardiomyopathy.

### Cardiac Myosin Binding Protein-C Mutations

Four mutations found in individuals with elderly-onset hypertrophic cardiomyopathy occurred in the cardiac myosin binding protein-C gene (Figure 1). Two defects are predicted to truncate cardiac myosin binding protein-C. A C\(\rightarrow\)T transition in exon 16 replaced a glutamine residue with a termination signal (Gln425Stop). A C\(\rightarrow\)G transition altered a highly conserved splice acceptor site 3 bp upstream of exon 28 (Int27ASC-3G). To assess the consequences of Int27ASC-3G on RNA splicing, cardiac myosin binding protein-C cDNAs were isolated and size fractionated. Two fragments (1 normal sized and 1 \(~170\) bp shorter) were identified. Nucleotide sequence analyses of the shorter fragment demonstrated juxtaposition of exons 27 and 29; all exon 28 sequences were absent (data not shown). This mutant transcript encoded only the initial 912 amino acids of cardiac myosin binding protein-C; aberrant splicing then produced a frameshift and premature stop codon.

Two cardiac myosin binding protein-C missense mutations were identified in individuals with elderly-onset hypertrophic cardiomyopathy. An A\(\rightarrow\)G transition (nucleotide 206, exon 3) replaces the normal and conserved hydrophilic polar threonine with a hydrophobic nonpolar residue.
alanine at amino acid residue 59 (Thr59Ala; Figure 2A). A G→A transversion at nucleotide 3037 (exon 30) replaces an arginine that is conserved throughout evolution with glutamine (Arg1002Gln; Figure 2A).

An additional missense codon (G→A transition at nucleotide 1009, exon 13) that replaces a conserved arginine with glutamine at residue 326 (Arg326Gln; Figure 2A) was found in 1 individual with elderly-onset hypertrophic cardiomyopathy and 1 control DNA sample. Clinical evaluations were normal in the 43-year-old individual from whom the control DNA was obtained.

All of the cardiac myosin binding protein-C mutations and the polymorphism are novel (Figure 1). Neither their location nor predicted consequences on protein structure seemed different from mutations previously described to cause familial hypertrophic cardiomyopathy.

Cardiac Troponin I Mutations

Two novel cardiac troponin I missense mutations (Figure 1) were identified that cause elderly-onset hypertrophic cardiomyopathy. A C→T transition in exon 5 replaces proline with serine (Pro82Ser), whereas in another individual, a G→A transition in exon 8 replaces aspartic acid with asparagine (Asp196Asn). Neither Pro82Ser nor Asp196Asn were identified in any control samples. Both proline 82 and aspartic acid 196 are highly conserved in troponin I molecules derived from mammalian, avian, and amphibian species (Figure 2B and data not shown).

α-Cardiac Myosin Heavy Chain Mutation

Because recent studies have demonstrated that normal adult human ventricular myocardium express α-cardiac myosin heavy chain as well as the more abundant β isoform,18 sequences of both isoforms were examined. A G→A transition (exon 20, nucleotide 2384) was identified in the α-cardiac myosin heavy chain gene (Figure 1) that is predicted to substitute glutamine for arginine at residue 795 (Arg795Gln). No control samples contained this sequence variant. Considerable evolutionary conservation of residue 795 is recognized in myosin molecules from a diverse range of species. An arginine is encoded at position 795 in cardiac, skeletal, and smooth muscle myosins from mammals to yeast (Figure 2C). The location of residue 795 on the three-dimensional crystal structure of chicken skeletal myosin21 places this arginine within a conserved protein-binding motif through which myosin heavy chain interacts with essential light chains. Substitution of a hydrophilic glutamine residue for the appropriate basic arginine residue could disrupt this α helical domain and potentially disturb light chain interactions.

Discussion

Hypertrophic cardiomyopathy exhibits a wide spectrum in disease onset, manifestation, and progression. Initial pathological descriptions solidified the diagnosis as a disease of the young,1,2,22 yet even early reports documented its existence in the elderly.10,11,23,24 In 1971, 32% of patients with idiopathic hypertrophic subaortic stenosis referred for catheterization at Massachusetts General Hospital were beyond their sixth decade.25 Several studies of the natural history of elderly patients with hypertrophic cardiomyopathy suggested that, compared with earlier reports of alarming mortality rates, long-term prognosis in elderly patients is more favorable.10,12,26 Total mortality rates and sudden death were significantly higher in younger patients with hypertrophic cardiomyopathy.27 Differences in the cardiac morphology of younger and elderly patients with hypertrophic cardiomyopathy have also been identified. The hearts of younger patients were more often crescent shaped, with reversed septal curvature, compared with the elderly, who had an ovoid cavity contour and normal septal curvature.28 Distinctive differences in the systolic apposition of the mitral valve and septum have been described.29 In older patients, this resulted from a combination of anterior motion of restricted, calcified mitral valve leaflets and posterior excursion of the septum, as opposed to predominant excursion of the mitral valve seen in younger patients.

Despite these clinical differences, our data indicate that like familial hypertrophic cardiomyopathy, ≈20% of elderly-onset, sporadic hypertrophic cardiomyopathy has a genetic cause. We identified 7 sarcomere protein mutations in genes encoding cardiac myosin binding protein-C, cardiac troponin I, and α-cardiac myosin heavy chain that caused symptoms and diagnosis of hypertrophic cardiomyopathy after middle age. An additional sequence variant was identified that could either be a rare polymorphism or a disease-causing mutation. The molecular basis for some elderly-onset hypertrophic cardiomyopathy indicates that this condition, like early onset familial disease, can be a heritable disorder of contractile proteins and defines α-cardiac myosin heavy chain defects as a novel cause of cardiac hypertrophy.

Gene mutations were not identified in 24 study subjects, and the cause of ventricular remodeling in this group remains unknown. Notably, genes encoding myosin light chains, actin, or titin were not analyzed in this study, and, hence, the relevance of these rare causes of familial hypertrophic cardiomyopathy in elderly-onset disease remains unknown. Clinical parameters did not distinguish individuals with and without a sarcomere protein gene mutation (Table 1). Almost one fourth of our study subjects had elevated blood pressure; however, mutations were identified in only 2 of 7 hypertensive individuals. Although this sample size is small, these data do not indicate a substantive role for hypertension as an inciting stimulus for the late expression of a gene mutation. Neither the onset nor severity of symptoms or echocardiographic findings (predominance of eccentric versus concentric hypertrophy, left ventricular wall thickness, or left atrial enlargement) were statistically different in individuals with or without gene mutations; systolic anterior mitral valve motion was more common in subjects without a mutation (65%) than in those with a mutation (38%).

Although these studies define a molecular relationship between early onset, familial hypertrophic cardiomyopathy and disease that characteristically occurs much later, there are notable differences in the sarcomere gene mutations causing each condition. First, none of the nucleotide mutations identified here have been previously reported to cause familial hypertrophic cardiomyopathy; indeed, the amino acid residues affected by late-onset gene defects have not been
mutated in the familial form of this disease. One mutation, Thr59Ala in exon 3 of myosin binding protein-C, is the first defect identified in this exon. Second, the distribution of sarcomere protein disease genes that cause familial hypertrophic cardiomyopathy and hypertrophic cardiomyopathy of the elderly is significantly different (Table). Although missense mutations in the β-myosin heavy chain gene and troponin T are prevalent causes of early onset disease, no mutations in these genes were found in the population studied here (P<0.00001). Collectively, these data may indicate that differences in the biophysical consequences of disease-causing mutations account for variance in clinical expression. Finally, a mutation was discovered in the α-myosin heavy chain that causes late-onset hypertrophic cardiomyopathy. Mutations in this gene have not been previously identified to cause familial hypertrophic cardiomyopathy or any other human pathology. This finding may implicate sarcomere proteins in elderly-onset hypertrophic cardiomyopathy that do not cause early onset, familial disease.

Mutations in β-myosin heavy chain and cardiac troponin T account for ~40% of familial hypertrophic cardiomyopathy. The absence of these mutations in the elderly subjects studied here is perhaps not unsurprising given that β-myosin heavy chain and cardiac troponin T defects cause symptomatic hypertrophy early in life, often in association with clinically important arrhythmias. Approximately 15% to 25% of familial hypertrophic cardiomyopathy is attributable to mutations in the myosin binding protein-C gene; a comparable prevalence (16%) of mutations was found in our elderly cohort (Table). Delayed onset of symptoms and cardiac hypertrophy characterize these familial mutations, with variable prognosis once clinical expression of disease occurs. Each of the 5 cardiac myosin binding protein-C gene mutations described here are novel, although neither their distribution throughout the peptide (exons 3, 14, 16, 30, and intron 27; Figure 2) nor their predicted consequences on structure (3 missense, 1 early termination, and 1 splice site mutation) seem different from previously reported familial mutations. We conclude that mutations in cardiac myosin binding protein-C are frequent causes of both familial disease and hypertrophic cardiomyopathy of the elderly.

Troponin I missense mutations account for <5% of familial hypertrophic cardiomyopathy. Two novel missense mutations (Pro82Ser, exon 5 and Asp196Asn, exon 8) were identified in patients with elderly-onset disease (Figure 2). Both missense mutations are predicted to have dominant-negative activity that may impair the inhibitory and troponin T binding functions of exon 5 residues and the binding to troponin C by exon 8 residues. Although some familial troponin I mutations have been associated with atypical hypertrophic morphologies (particularly apical hypertrophy), these were not observed in individuals with elderly-onset disease.

An α-cardiac myosin heavy chain missense mutation Arg795Gln (Figure 2) also caused elderly-onset hypertrophic cardiomyopathy. The α and β isoforms of cardiac myosin heavy chains are encoded on chromosome 14q12 and expressed in a developmentally regulated program. The α isoform is abundant in both atria and ventricles during embryogenesis; after birth, ventricular chambers predominantly express the β isoform. Historically, these data have been interpreted to imply little function for the α-cardiac myosin heavy chain in postnatal cardiac physiology, but recent studies demonstrating that as much 30% of adult ventricular myosin heavy chain transcripts encode the α isoform challenge this view. Our data provide the first evidence for a human mutation in the α-cardiac myosin heavy chain gene and implicate this protein in cardiac physiology throughout life. We hypothesize that lower abundance of the α versus β isoform accounts for the later onset of hypertrophic cardiomyopathy associated with mutation of the α isoform.

Recognition that cardiac hypertrophy that becomes clinically apparent late in life can be a genetic disorder has important implications for diagnosis and for understanding the mechanisms by which sarcomere dysfunction triggers pathology. We assume that each mutation is a germline defect, because these were identified from peripheral blood samples. Hence, siblings and children of genetically affected individuals are also at risk for developing hypertrophic cardiomyopathy of the elderly. Understanding why some sarcomere defects are quiescent in individuals for many years but produce early onset, often severe disease in others should help to define those factors that modify disease expression. Consideration of the effects of aging, lifestyle, diet, coexisting diseases such as hypertension, and background genes are important areas for these investigations. A clearer understanding of the influence that these factors have on sarcomere mutations may guide the development of specific therapies for hypertrophic cardiomyopathy that is recognized early or late in life.

Acknowledgments

This work was supported by the Howard Hughes Medical Institutes, National Institutes of Health, and Minneapolis Heart Institute. We are indebted to Susan A. Casey, RN, and Barbara McDonough, RN, for their skillful and enthusiastic participation in these studies.

References


Sarcomere Protein Gene Mutations in Hypertrophic Cardiomyopathy of the Elderly
Hideshi Niimura, Kristen K. Patton, William J. McKenna, Johann Soult, Barry J. Maron, J.G. Seidman and Christine E. Seidman

Circulation. 2002;105:446-451
doi: 10.1161/hc0402.102990
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/105/4/446

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/