Nationwide Study on Hypertrophic Cardiomyopathy in Iceland
Evidence of a MYBPC3 Founder Mutation

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Background—The geographic isolation and homogeneous population of Iceland are ideally suited to ascertain clinical and genetic characteristics of hypertrophic cardiomyopathy (HCM) at the population level.

Methods and Results—Medical records and cardiac imaging studies obtained between 1997 and 2010 were reviewed to identify Icelandic patients with HCM. Surviving patients were recruited for clinical and genetic studies. A previously identified Icelandic mutation, MYBPC3 c.927-2A>G, was genotyped, and mutation-negative samples were sequenced for HCM genes and other hypertrophic genes. Record review identified 180 patients with HCM. Genetic analyses of 151 patients defined pathogenic mutations in 101 (67%), including MYBPC3 c.927-2A>G (88 patients, 58%), 4 other MYBPC3 or MYH7 mutations (5 patients, 3.3%), and 2 GLA mutations (8 patients, 5.3%). Haplotype and genetic genealogical data defined MYBPC3 c.927-2A>G as a founder mutation, introduced into the Icelandic population in the 15th century, with a current population prevalence of 0.36%. MYBPC3 c.927-2A>G mutation carriers exhibited phenotypic diversity but were younger at diagnosis (42 versus 49 years; P=0.001) and sustained more adverse events (15% versus 2%; P=0.02) than mutation-negative patients. All-cause mortality for patients with HCM was similar to that of an age-matched Icelandic population (hazard ratio, 0.98; P=0.9). HCM-related mortality (0.78%/y) occurred at a mean age of 68 compared with 81 years for non–HCM-related mortality (P=0.02).

Conclusions—A founder MYBPC3 mutation that arose >550 years ago is the predominant cause of HCM in Iceland. The MYBPC3 c.927-2A>G mutation is associated with low adverse event rates but earlier cardiovascular mortality, illustrating the impact of genotype on outcomes in HCM. (Circulation. 2014;130:1158-1167.)

Key Words: cardiomyopathies • genes • genetics • hypertrophy

Hypertrophic cardiomyopathy (HCM) is a primary myocardial disorder characterized by unexplained left ventricular (LV) hypertrophy in the absence of other predisposing clinical conditions.1 Mutations in the protein components of the sarcomere cause HCM. Hypertrophic remodeling also occurs in disorders that clinically mimic HCM, including Fabry disease (GLA mutations), glycogen cardiomyopathy (PRKAG2 mutations), and Danon disease (LAMP2 mutations).2 More than 1400 different mutations in at least 8 sarcomere protein genes have been reported in HCM.3,4 Even though a majority (=80%) of HCM mutations alter the cardiac β-myosin heavy chain (MYH7) or cardiac myosin binding protein-C (MYBPC3) gene,5 most are “private” and unique to a specific family. The diverse molecular origin of HCM, combined with background genomic variability and lifestyle differences among patients with HCM, has hindered definitive insights into genotype and phenotype relationships.5-6

Clinical Perspective on p 1167

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Previous studies estimate that HCM occurs in =1 in 500 individuals in the United States, but the population prevalence of pathogenic mutations in genes that encode sarcomeric proteins or other hypertrophic molecules is unknown. Capitalizing on the geographic isolation and homogeneous population in Iceland, we undertook population-based clinical and genetic studies of HCM. Iceland, an island with a population of 320,000, has no tertiary HCM referral center, and genetic testing of patients with HCM has not been widely used in clinical practice. Genetic analyses of only 2 Icelandic HCM families have been reported; in both, a MYBPC3 mutation was identified that causes an adenine-to-guanine transition in intron 12 (c.927-2A>G). This mutation is predicted to alter RNA splicing and to result in a frameshift that leads to premature termination and a truncated protein. We performed cascade genetic analyses, initially sequencing patient samples for MYBPC3 c.927-2A>G and then sequencing other genes known to cause HCM and hypertrophic remodeling. Our data identify the prevalence of clinical hypertrophy and HCM in Iceland, define the contribution of gene mutations, and reveal the phenotypic spectrum and associated risk for adverse outcomes of a prevalent MYBPC3 mutation.

**Methods**

**Ethical Considerations**

This study was performed with the approval of the National Bioethics Committee of Iceland, the Icelandic Data Protection Authority, and the Partners Human Research Committee, with informed consent obtained from subjects.

**Subjects**

Patients with a clinical diagnosis of HCM were identified from surveys of electronic medical records and a review of Icelandic echocardiogram databases, including the National University Hospital of Iceland, Akureyri Hospital, and private cardiology clinics. Review of the medical records from January 1997 to November 2010 identified 306 patients with the International Classification of Diseases, 10th Revision codes 142.1 (obstructive HCM), 142.2 (other HCM), and 142.9 (cardiomyopathy, unspecified), and 2197 echocardiography reports identified patients with a maximal LV wall thickness ≥15 mm (Figure 1). All medical records and echocardiogram reports were individually reviewed by participating cardiologists to exclude patients with hypertrophy that was attributable to valvar or hypertensive heart disease. In total, 180 Icelandic patients fulfilled the following clinical criteria for HCM: LV maximal wall thickness ≥15 mm in the absence of another cardiac or systemic disease capable of producing a similar magnitude of hypertrophy.1,9

Surviving patients with HCM (n=150) were invited to participate in clinical and genetic studies. Medical records from enrolled subject were obtained to document evaluations from the initial diagnosis to the most recent visit. Study visits from April 2010 to December 2011 provided additional clinical data, family medical histories, and blood samples for genetic testing. All death certificates, hospital records, and autopsy reports of enrolled subjects were reviewed again in October and November 2012.

The following clinical variables were registered: age at diagnosis; family history of HCM; hypertension; atrial fibrillation (intermittent/persistent); New York Heart Association functional class at diagnosis and at last contact; coronary artery disease (defined as ≥50% narrowing in ≥1 epicardial vessel); medications at study visit; and HCM events, including implanted cardioverter-defibrillator (ICD), appropriate ICD therapy for ventricular tachycardia (VT)/ventricular fibrillation (defined as shock or anti-tachycardia pacing), septal reduction therapy (surgical septal myectomy or alcohol septal ablation), heart failure (documented symptomatic and hemodynamic deterioration),9 cardiac transplantation, stroke, and sudden death (SD; recorded as unexpected nontraumatic premature death within 1 hour after the onset of symptoms, including unwitnessed, unexpected nocturnal death). Risk factors for SD9 were recorded, including (1) a family history of ≥1 HCM-related SD, (2) nonsustained VT (defined as ≥1 runs of ≥6 consecutive ventricular extrasystoles at a rate of >120 bpm), (3) unexplained syncope, (4) abnormal blood pressure response (defined as a failure of the systolic blood pressure to rise by >20 mm Hg from baseline values or a fall of >10 mm Hg from the maximum blood pressure during upright exercise), and (5) massive LV hypertrophy (thickness ≥230 mm). HCM adverse cardiac events were defined as SD, ICD appropriate therapy, heart failure death, stroke death, or cardiac transplantation.

**Echocardiography and Cardiovascular Magnetic Resonance**

Transthoracic echocardiographic studies were performed with commercially available instruments according to the standards at each echocardiography laboratory. Echocardiograms were limited to a few centers with expertise and were not overread. Maximum LV wall thickness represented the largest dimension measured at any site within the LV chamber at end diastole. Left atrial and LV end-diastolic cavity dimensions were assessed by M-mode echocardiography. The peak instantaneous LV outflow gradient was estimated with continuous-wave Doppler ultrasound under basal conditions. End-stage HCM was defined as an ejection fraction <50% and dilated LV (LV end-diastolic cavity dimension ≥58 mm). Cardiac magnetic resonance studies were performed with a clinical scanner (Siemens Magnetom Avanto B17) in 34 patients. Late-gadolinium-enhancement images were acquired.

**Genetic Analyses**

Genetic analyses were performed on 141 patients with HCM (137 surviving and 4 previously consented deceased patients). Genomic DNA was extracted from whole-blood samples and studied using a cascade strategy. Patients with HCM were initially screened for the MYBPC3 c.927-2A>G mutation that was previously reported in 2 Icelandic families.8 Mutation-negative samples (n=61) were studied using targeted capture sequencing of 8 sarcomeric protein genes (ACTC, MYBPC3, MYH7, MYL2, MYL3, TNNI3, TNN2, and TPM1), the lysosomal associated membrane protein-2 gene (LAMP2), and the α-galactosidase A gene (GLA). Because of technical issues, the protein kinase AMP-activated, noncatalytic, γ-2 (PRKAG2) gene was sequenced only in a subset (n=34) of mutation-negative samples.
The MYBPC3 c.927-T>A-G mutation was ascertained by sequencing polymerase chain reaction–amplified MYBPC3 exon 13 and the adjacent 3’ splice site of the intron 12/exon 13 boundaries (forward primer, 5’ TCC CCA GCC CCT CTT CA 3’; reverse primer, 5’ GCC GGA CTC CGC TCT TT 3’) with the 3730XL DNA Analyzer (Applied Biosystems, Carlsbad, CA). DNA sequences were analyzed with DNASTAR Lasergene software (DNASTAR, Madison, WI). Samples negative for the MYBPC3 c.927-T>A-G mutation (n=61) were studied further. Genomic libraries were prepared from 1 to 2 μg of genomic DNA (as previously described) with the use of custom adapters that included a unique 3-base pair (bp) barcode followed by a thymidine residue. The samples were pooled into groups of 19 to 20 libraries (200 ng per library, totaling 4 μg) and hybridized.

DNA was captured for sequencing using 2 strategies. For 34 genomic libraries, we used a filter-based hybridization technique to capture and target-enrich all exons of the 8 sarcomeric protein genes, LAMP2, PKA2G, and GLA, including 10 bp upstream and downstream of exon-intron boundaries. For 27 genomic libraries, we used solution-based hybridization with custom oligonucleotide capture probes (Agilent SureSelect Target Enrichment; Agilent, Santa Rosa, CA) that were designed to cover all 6 sarcomere protein genes (ACTC, MYBPC3, MYH7, TNN1, TNN2, and TPM1) and LAMP2, including 10 bp upstream and downstream of exon-intron boundaries. GLA was also sequenced in these 27 samples by dideoxy sequencing (primers available on request). Details of the capture probe design and solution-base hybridization are provided in the Methods in the online-only Data Supplement.

Sequencing was performed on an Illumina HiSeq2000, producing 50-bp paired-end reads (Illumina, San Diego, CA). All filtered reads were mapped to the human reference genome (hg19) with the use of Novoalign (www.Novocraft.com). Picard HsMetrics was subsequently used to evaluate sequencing quality (www.picard.sourceforge.net). All single-nucleotide variants and small indels were called with the use of the GATK Unified Genotyper. On average, libraries were sequenced to 4 million unique reads per individual, accounting for 20-fold coverage over 96% of targeted bases. We applied standard quality metrics and prioritized non synonymous variants or variants affecting consensus splice sites (2 bp upstream and downstream of exon). All identified variants were visually inspected with the Integrative Genomics Viewer. All variants were reported using Human Genome Variation Society nomenclature (www.hgvs.org/mutnomen).

Variant confirmation was performed by means of polymerase chain reaction amplification followed by traditional dideoxy sequencing (primers available on request). All variants were classified (see Methods in the online-only Data Supplement) as likely pathogenic, variant of unknown significance, likely benign, and benign as indicated by publicly available databases of HCM mutations, previously published mutations, reported functional studies, evolutionary conservation, and bioinformatics analyses of the predicted impact on RNA and protein.

Haplotyping Analysis
Haplotyping analysis was performed by deCODE Genetics. A total of 98 721 Icelanders have been studied with the use of Illumina single-nucleotide polymorphism (SNP) chips, and their genotypes have been long-range phased. Long-range phasing allows accurate imputation of MYBPC3 c.927-T>A-G into the chip-typed individuals on the basis of the genotypes of individuals who have had MYBPC3 sequenced (info=0.999). deCODE Genetics maintains a computerized database of the genealogy of Icelanders. These records include almost all individuals born in Iceland in the last 2 centuries, and for that period, s95% of the parental connections are known. Thirty-seven individuals with the MYBPC3 c.927-T>A-G mutation were genotyped with the use of Illumina SNP chips and had their genotypes long-range phased. The age of the MYBPC3 c.927-T>A-G mutation was estimated by examining the decay of linkage disequilibrium between it and 3155 chip-typed SNPs located between build 36 positions 37.3 Mb and 57.3 Mb on chromosome 11. An initial estimate of the age of the mutation was obtained from the pattern of linkage disequilibrium with each individual SNP based on the formula:

\[ t = \frac{1}{\log(1 - \theta)} \log \left( \frac{x - y}{1 - y} \right) \]

where t is the age of the mutation in generations, \( \theta \) is the recombinaction rate between the mutation and the SNP, x is the frequency of chromosomes carrying both the mutation and the SNP allele that has a higher frequency of the mutation on its background, and y is the frequency of chromosomes carrying the mutation and the other SNP allele. These age estimates were then corrected on the basis of the Luria-Delbrück model, which takes into account the impact of population growth as follows:

\[ t' = t - \log \left( \frac{\theta e^r}{e^r - 1} \right) \]

where r is the rate of past exponential population growth, which we estimate as 0.3. A single estimate for the age of the mutation in generations was then obtained by taking the geometric mean of the age estimates for each SNP and multiplying by 30 to obtain an estimate of the age of the mutation in years.

Statistical Analysis
Descriptive data are expressed as discrete numbers or percentages. Data are displayed as the mean±SD for continuous variables and as proportions for categorical variables. Student t tests were used for continuous variables, with \( \chi^2 \) tests or Fisher exact tests used for categorical variables. Values of P<0.05 were considered significant. Kaplan–Meier curves were compared by use of nonparametric log-rank testing. Expected survival appropriate to age and sex was based on data from Statistics Iceland. The actual and expected hazard ratios were compared by use of nonparametric log-rank testing.

Results
Demographics of HCM in Iceland
The review of medical records between 1997 and 2010 in patients with cardiac hypertrophy identified 180 patients with clinical criteria for HCM (Figure 1). All except 1 of these patients were of Icelandic origin. Because HCM exhibits age-related penetrance, we calculated the prevalence of clinical HCM among 237 500 adults in Iceland at 1 in 1600 individuals.

Longitudinal clinical information on patients with HCM, summarized in Table 1, was obtained for a mean duration of 12.8 years, which spanned the period from initial diagnosis to the most recent contact or death. The age at diagnosis ranged from 4 to 84 years (mean, 45.9±16.9 years), and 117 patients (65%) had a family history of HCM. A family history of HCM-related SD was present in 60 patients (33%). The most recent echocardiographic studies showed maximum LV wall thickness ranging between 15 and 42 mm (mean 21.7±5.2 mm), including 112 patients (62%) with LV wall thickness ≥20 mm and 25 patients (14%) with LV wall thickness ≥30 mm. An LV outflow gradient ≥30 mm Hg at rest was present in 36 patients (20%). Cardiac magnetic resonance imaging studies in 34 patients showed late gadolinium enhancement in 25 (74%). Fifty-one patients (28%) had atrial fibrillation, including 20 (11%) with persistent atrial fibrillation. Coronary arteriograms or computed tomography angiograms performed for clinical indications in 73 patients (41%) revealed clinically significant atherosclerotic coronary artery disease in 13 patients (7.2%). Hypertension was present in 18 patients (10%).

Female patients with HCM (34% of the cohort) were diagnosed at a mean of 7 years later than men (P=0.011) but experienced more heart failure symptoms than men. Sixty percent of female patients were classified as New York Heart Association functional class II or higher compared with 35% of men (P=0.016). Female
Table 1. Clinical Characteristics, Risk Factors, and Outcomes in Icelandic HCM Patients

<table>
<thead>
<tr>
<th></th>
<th>All HCM</th>
<th>Female</th>
<th>Male</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n (%)</td>
<td>180 (100)</td>
<td>62 (34)</td>
<td>118 (66)</td>
<td></td>
</tr>
<tr>
<td>Mean±SD (range) age at diagnosis, y</td>
<td>45.9±16.9 (4–84)</td>
<td>50.4±16.5 (9–84)</td>
<td>43.6±16.8 (4–80)</td>
<td>0.011</td>
</tr>
<tr>
<td>Mean±SD (range) age at final follow-up or death, y</td>
<td>58.7±18.7 (11–92)</td>
<td>64.7±17.7 (11–92)</td>
<td>54.4±18.3 (18–90)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean±SD (range) follow-up, y</td>
<td>12.8±9.4 (0–38)</td>
<td>14.6±10.5 (0–38)</td>
<td>11.8±8.7 (0–34)</td>
<td>0.074</td>
</tr>
<tr>
<td>Familial HCM, n (%)</td>
<td>117 (65)</td>
<td>43 (69)</td>
<td>74 (63)</td>
<td>0.38</td>
</tr>
<tr>
<td>Mean±SD (range) maximal LVWT, mm</td>
<td>21.7±5.2 (15–42)</td>
<td>21.7±5.1 (15–36)</td>
<td>21.8±5.3 (15–42)</td>
<td>0.92</td>
</tr>
<tr>
<td>LV outflow gradient ≥30 mmHg at rest, n (%)</td>
<td>36 (20)</td>
<td>14 (23)</td>
<td>22 (19)</td>
<td>0.53</td>
</tr>
<tr>
<td>Septal therapy, n (%)</td>
<td>4 (2.2)</td>
<td>0</td>
<td>4 (3.4)</td>
<td>0.30</td>
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<tr>
<td>Mean±SD LVEDD, mm</td>
<td>46.7±7.4</td>
<td>43.2±8.2</td>
<td>48.4±6.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean±SD left atrial size, mm</td>
<td>42.6±7.2</td>
<td>40.6±5.9</td>
<td>43.7±7.7</td>
<td>0.013</td>
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<tr>
<td>Atrial fibrillation, n (%)</td>
<td>51 (28)</td>
<td>18 (29)</td>
<td>33 (28)</td>
<td>0.88</td>
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<td>Coronary artery disease, n (%)</td>
<td>13 (7.2)</td>
<td>4 (6.5)</td>
<td>9 (7.6)</td>
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<tr>
<td>Hypertension, n (%)</td>
<td>18 (10)</td>
<td>5 (8.1)</td>
<td>13 (11)</td>
<td>0.52</td>
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<tr>
<td>Stroke, n (%)</td>
<td>14 (7.8)</td>
<td>7 (11)</td>
<td>7 (5.9)</td>
<td>0.24</td>
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<tr>
<td>Initial NYHA class, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>144 (80)</td>
<td>45 (73)</td>
<td>99 (84)</td>
<td></td>
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<tr>
<td>II</td>
<td>35 (19)</td>
<td>17 (27)</td>
<td>18 (15)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1 (0.56)</td>
<td>0</td>
<td>1 (0.85)</td>
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<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
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<td>Final NYHA class, n (%)</td>
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<td></td>
<td></td>
<td>0.016</td>
</tr>
<tr>
<td>I</td>
<td>102 (57)</td>
<td>25 (40)</td>
<td>77 (65)</td>
<td></td>
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<tr>
<td>II</td>
<td>59 (33)</td>
<td>28 (45)</td>
<td>31 (26)</td>
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<td>III</td>
<td>13 (7.2)</td>
<td>6 (10)</td>
<td>7 (5.9)</td>
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</tr>
<tr>
<td>IV</td>
<td>6 (3.3)</td>
<td>3 (4.8)</td>
<td>3 (2.5)</td>
<td></td>
</tr>
<tr>
<td>End-stage (dilated LV, EF &lt;50%), n (%)</td>
<td>11 (6.1)</td>
<td>4 (6.4)</td>
<td>7 (5.9)</td>
<td>1.00</td>
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<tr>
<td>Cardiac transplantation, n (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
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<td>Medications, n (%)</td>
<td></td>
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<tr>
<td>None</td>
<td>41 (23)</td>
<td>8 (13)</td>
<td>33 (28)</td>
<td>0.022</td>
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<td>β-Blockers</td>
<td>114 (63)</td>
<td>42 (68)</td>
<td>72 (61)</td>
<td>0.37</td>
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<tr>
<td>Diuretics</td>
<td>47 (30)</td>
<td>25 (46)</td>
<td>22 (22)</td>
<td>0.0020</td>
</tr>
<tr>
<td>ACE/ARB</td>
<td>37 (21)</td>
<td>15 (24)</td>
<td>22 (19)</td>
<td>0.38</td>
</tr>
<tr>
<td>Coumadin</td>
<td>31 (17)</td>
<td>10 (16)</td>
<td>21 (18)</td>
<td>0.78</td>
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<tr>
<td>Amiodarone</td>
<td>27 (15)</td>
<td>13 (21)</td>
<td>14 (12)</td>
<td>0.10</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>19 (11)</td>
<td>8 (13)</td>
<td>11 (9.3)</td>
<td>0.46</td>
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<tr>
<td>Disopyramide</td>
<td>7 (3.9)</td>
<td>4 (6.5)</td>
<td>3 (2.5)</td>
<td>0.23</td>
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<tr>
<td>Risk factors, n (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Family history of HCM SD</td>
<td>60 (33)</td>
<td>20 (32)</td>
<td>40 (34)</td>
<td>0.82</td>
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<tr>
<td>ABPR</td>
<td>13 (7.2)</td>
<td>4 (6.5)</td>
<td>9 (7.6)</td>
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<tr>
<td>Massive LVH (≥30 mm)</td>
<td>25 (14)</td>
<td>8 (13)</td>
<td>17 (14)</td>
<td>0.78</td>
</tr>
<tr>
<td>NSVT</td>
<td>27 (15)</td>
<td>12 (19)</td>
<td>15 (13)</td>
<td>0.24</td>
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<tr>
<td>Syncope</td>
<td>33 (18)</td>
<td>15 (24)</td>
<td>18 (15)</td>
<td>0.14</td>
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<tr>
<td>ICD implantation</td>
<td>15 (8)</td>
<td>6 (10)</td>
<td>9 (7.6)</td>
<td>0.64</td>
</tr>
<tr>
<td>Adverse cardiac events*</td>
<td>24 (13)</td>
<td>11 (18)</td>
<td>13 (11)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

ABPR indicates abnormal blood pressure response; ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; EF, ejection fraction; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter-defibrillator; LV, left ventricular; LVEDD, left ventricular end-diastolic diameter; LVEDD, left ventricular hypertrophy; LVWT, left ventricular wall thickness; NSVT, nonsustained ventricular tachycardia; NYHA, New York Heart Association; and SD, sudden death.

*SD, ICD therapy, heart failure death, or stroke death.
patients were more often treated with diuretics (P=0.0020), and only 13% of female patients required no medical treatment compared with 28% of male patients (P=0.022).

Thirty-five of the initial 180 patients (19%) with HCM were deceased (mean age at death, 74.3±16.2 years; range, 18–92 years), including 5 patients who died between study initiation and the most recent mortality update (November 2012). At study end, the average age of the 145 surviving patients with HCM was 54.0±17.3 years (range, 11–85 years). HCM-related death occurred at a mean age of 68.3±20.3 years compared with 80.7±6.1 years for non–HCM-related mortality (P=0.022; Figure 2 and Table I in the online-only Data Supplement). Patients with HCM-related mortality were diagnosed an average of 12 years earlier than patients with non–HCM-related mortality (50.4±20.8 versus 62.4±8.3 years; P=0.035). The HCM-related deaths in 18 patients (0.78%/y) included heart failure (n=12), arrhythmic SD (n=4), and embolic stroke (n=2). No patients received cardiac transplantation, but 8 patients survived VT/ventricular fibrillation by virtue of prophylactically implanted ICDs or external defibrillation at a mean age of 44.6±19.5 years. The incidence of VT/ventricular fibrillation was 0.48%/y, and the annual SD mortality rate was 0.17%/y. Non–HCM-related deaths occurred in 17 patients, including 12 resulting from noncardiac causes and 5 caused by coronary artery disease or multiorgan causes.

Genetic Analysis

DNA analyses were performed in 141 patients with HCM, including 137 survivors and 4 consented, deceased patients (Figure 1). The MYBPC3 c.927-2A>G mutation was identified in 76 surviving (55%) and the 4 deceased patients. Using the Icelandic genetic genealogical database, we also imputed the MYBPC3 c.927-2A>G mutation in 8 of 10 patients with HCM. In total, 88 Icelandic patients with HCM carried the MYBPC3 c.927-2A>G mutation (Table 2).

Additional genetic analyses in 61 patients without the MYBPC3 c.927-2A>G mutation revealed variants in sarcomere protein genes and in the GLA gene (Table 2). No pathogenic or likely pathogenic variants were identified in LAMP2 or PRKAG2.

Nine patients presented 8 MYBPC3 or MYH7 variants; of these, 4 were likely pathogenic, 2 were variants of unknown significance, and 2 were likely benign. Two patients shared the likely pathogenic variant MYBPC3 c.506-1G>A and were related within 3 generations, although neither reported a family history of HCM. One patient carried the likely benign variant MYBPC3 c.2686G>A in addition to the variant of unknown significance MYH7 c.2606G>A. Clinical information on patients with HCM with likely pathogenic sarcomere variants or variants of unknown significance (n=7) is provided in Table II in the online-only Data Supplement. Three GLA variants were identified in 10 patients without sarcomere variants: 2 were likely pathogenic, and 1 was likely benign. Clinical evaluations of patients and family members with GLA variants are ongoing.

On the basis of variants classified as pathogenic or likely pathogenic, 67% of Icelandic patients with clinical HCM presented a disease-causing mutation in a sarcomere protein gene (62%) or in GLA (5.3%). Patients diagnosed with HCM before 45 years of age were significantly more likely to carry a sarcomere mutation (50 of 66, 76%) than patients diagnosed later (33 of 71, 47%; P<0.0001).

Familial Clustering

We assessed the deCODE genealogical database to assess the familial relationships of study patients. Among 180 enrolled

### Table 2. Variants Identified Among HCM Patients in Iceland

<table>
<thead>
<tr>
<th>Variant</th>
<th>Patients With Variant, n (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Likely pathogenic</td>
<td>101 (67)</td>
<td>Niimura et al⁸</td>
</tr>
<tr>
<td>MYBPC3 c.927-2A&gt;G</td>
<td>88* (58)</td>
<td>Cardiogenomics⁸</td>
</tr>
<tr>
<td>MYBPC3 c.506-1G&gt;A</td>
<td>2 (1.3)</td>
<td>Novel</td>
</tr>
<tr>
<td>MYBPC3 c.2453G&gt;A</td>
<td>1 (0.7)</td>
<td>Novel</td>
</tr>
<tr>
<td>MYBPC3 c.1639delGT</td>
<td>1 (0.7)</td>
<td>Cardiogenomics</td>
</tr>
<tr>
<td>MYH7 c.2191C&gt;T</td>
<td>1 (0.7)</td>
<td>Novel</td>
</tr>
<tr>
<td>GLA c.695T&gt;C</td>
<td>3 (2.0)</td>
<td>HGMD</td>
</tr>
<tr>
<td>GLA c.966C&gt;A</td>
<td>5 (3.3)</td>
<td>HGMD</td>
</tr>
<tr>
<td>Unknown significance</td>
<td>2 (1.3)</td>
<td>rs202141173, Cardiogenomics²⁰¹⁷</td>
</tr>
<tr>
<td>MYH7 c.2606G&gt;A</td>
<td>1 (0.7)</td>
<td>EHlermann et al²⁰¹⁷</td>
</tr>
<tr>
<td>MYBPC3 c.2873C&gt;T</td>
<td>1 (0.7)</td>
<td>Niimura et al²⁰¹⁷</td>
</tr>
<tr>
<td>Likely benign</td>
<td>5 (3.3)</td>
<td>rs35078470²⁰¹⁴</td>
</tr>
<tr>
<td>MYBPC3 c.2686G&gt;A</td>
<td>2 (1.3)</td>
<td>Cardiogenomics, HGMD</td>
</tr>
<tr>
<td>MYBPC3 c.2497G&gt;A</td>
<td>1 (0.7)</td>
<td>HGMD</td>
</tr>
<tr>
<td>GLA c.937G&gt;T</td>
<td>2 (1.3)</td>
<td>Froissart et al²⁰¹⁷</td>
</tr>
</tbody>
</table>

Mutation and VUS negative, 48* (32)

Cardiogenomics can be found at www.cardiogenomics.org; HGMD can be found at www.hgmd.org. GLA indicates α-galactosidase A gene; HCM, hypertrophic cardiomyopathy; HGMD, Human Gene Mutation Database; MYBPC3, cardiac myosin binding protein-C gene; and MYH7, β-myosin heavy chain gene. *Includes HCM patients studied by imputation.
patients, 99 patients demonstrated relatedness within 3 generations clustered among 28 families, and 81 patients demonstrated no relatedness. When relationships were extended to 5 generations, 113 patients were clustered into 29 families, and 67 patients demonstrated no relatedness. Relationships within 3 generations were identified among 69 of 88 patients with the MYBPC3 c.927-2A>G mutation that clustered into 18 families. When extended to 5 generations, 76 mutation carriers were related and clustered into 12 families. No relatedness was identified among the remaining 12 mutation carriers.

**Founder Effect**

Genetic analyses in 37 patients identified the MYBPC3 c.927-2A>G mutation on a single haplotype spanning 10.7 Mb, indicating a founder mutation. The shared haplotype spans between 45.4 and 56.1 Mb of chromosome 11 (Figure 3) and includes the centromere, a region of limited recombination. The shared haplotype was not observed in any noncarriers of the c.927-2A>G mutation. From the genomic size of this region and expected recombination frequencies and assuming an average generation of 30 years, we estimate that the MYBPC3 c.927-2A>G mutation was introduced into the Icelandic population ≈17.6 generations ago, approximately 1400 ad. Whether the mutation arose as a de novo event in a native Icelander or was introduced into the population by an immigrant is unknown.

On the basis of deCODE genomic genealogical data on 98,721 Icelanders, the shared chromosome 11 haplotype that contains the MYBPC3 c.927-2A>G mutation has a predicted population prevalence of 0.36%. To assess the penetrance of the MYBPC3 mutation, we defined the Icelandic population over the age of 42 years (the mean age at diagnosis for patients studied here) during the study period of 1997 to 2010. Among these 11,660 adult Icelanders, we predicted that 420 individuals carry the MYBPC3 c.927-2A>G mutation, whereas only 88 mutation carriers (80 by sequence analyses and 8 by imputation), corresponding to 21% of the expected number, were clinically identified. Unrecognized HCM in mutation carriers, premature death resulting from HCM, and incomplete penetrance may account for differences between the predicted and observed prevalences of HCM caused by MYBPC3 c.927-2A>G.

**Genotype–Phenotype Correlations**

We compared clinical manifestations and adverse outcomes (Table 3) among patients with the MYBPC3 c.927-2A>G mutation (n=88) and mutation-negative patients with a clinical diagnosis of HCM (n=48). Patients with HCM with other sarcomere variants (pathogenic, likely pathogenic, or variants of unknown significance) were too few (n=7) for meaningful analyses. Compared with mutation-negative HCM patients, patients with the MYBPC3 c.927-2A>G mutation were diagnosed 7 years earlier (P=0.0010; Figure 4A), and male patients presented at slightly younger ages than female patients (Figure I in the online-only Data Supplement). Familial HCM was significantly higher (P<0.0001) among MYBPC3 c.927-2A>G mutation carriers. Prospective familial evaluations led to a diagnosis of HCM in 26 patients with the MYBPC3 c.927-2A>G mutation (30%) and 7 mutation-negative patients (15%; P=0.052). Regardless of mutation status, there was no significant difference in age at diagnosis of HCM among patients identified by prospective familial evaluations or clinical presentations (patients with the MYBPC3 c.927-2A>G mutation, 40.0±15.7 versus 42.4±15 years; mutation-negative patients, 51.6±12 versus 48.5±17.7 years; P≥0.50 for each comparison).

We observed no significant differences in maximum LV wall thickness, LV outflow obstruction, left atrial dimensions, LV end-diastolic cavity dimension, New York Heart Association functional class, or the prevalence of concomitant hypertension, atrial fibrillation, stroke, and end-stage HCM between MYBPC3 c.927-2A>G carriers and mutation-negative patients. However, risk factors for adverse cardiac events were increased among patients with the MYBPC3 c.927-2A>G mutation, although not all reached statistical significance. Compared with mutation-negative patients, mutation-positive patients had more nonsustained VT (n=20, 23%), massive hypertrophy (n=16, 18%), and abnormal blood pressure responses (n=9, 10%). Consistent with an increased risk, adverse cardiac events occurred more frequently in patients with HCM with the MYBPC3 c.927-2A>G mutation than in mutation-negative patients (P=0.010; Figure 4B). Importantly, the risk for adverse events extended to family members; compared with mutation-negative patients, carriers of the MYBPC3 c.927-2A>G mutation demonstrated more frequent HCM SD in family members (n=38, 43%). Among 12 MYBPC3 c.927-2A>G mutation carriers (14%) with an ICD, 7 (58%) experienced appropriate shocks. Among mutation-negative patients, 3 (6.3%) received an ICD, and I experienced an appropriate shock. The hazard ratio for all-cause mortality (HCM and non-HCM deaths) in patients with the MYBPC3 c.927-2A>G mutation, compared
Table 3. Clinical Manifestations of HCM Caused by MYBPC3 c.927-2A>G or of Unknown Origin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MYBPC3 c.927-2A&gt;G</th>
<th>Mutation-Negative</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>88*</td>
<td>48*</td>
<td></td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>56 (64)</td>
<td>36 (75)</td>
<td>0.18</td>
</tr>
<tr>
<td>Means±SD (range) age at diagnosis, y</td>
<td>41.7±15.1 (6–72)</td>
<td>48.9±17.0 (4–74)</td>
<td>0.0012†</td>
</tr>
<tr>
<td>Familial HCM, n (%)</td>
<td>75 (85)</td>
<td>22 (46)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diagnosed through prior family screening, n (%)</td>
<td>26 (30)</td>
<td>7 (15)</td>
<td>0.052</td>
</tr>
<tr>
<td>Means±SD (range) maximum LVWT, mm</td>
<td>21.7±5.4 (15–36)</td>
<td>20.9±4.7 (15–37)</td>
<td>0.37</td>
</tr>
<tr>
<td>LV outflow gradient ≥30 mm Hg at rest, n (%)</td>
<td>16 (18)</td>
<td>11 (23)</td>
<td>0.51</td>
</tr>
<tr>
<td>Septal therapy, n (%)</td>
<td>3 (3.4)</td>
<td>1 (2.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>Means±SD (range) left atrial diameter, mm</td>
<td>42.7±8.3 (4–74)</td>
<td>42.5±6.6 (4–74)</td>
<td>0.87</td>
</tr>
<tr>
<td>Coronary artery disease, n (%)</td>
<td>4 (4.5)</td>
<td>5 (10)</td>
<td>0.28</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>7 (8.0)</td>
<td>6 (13)</td>
<td>0.39</td>
</tr>
<tr>
<td>Atrial fibrillation, n (%)</td>
<td>19 (22)</td>
<td>13 (27)</td>
<td>0.47</td>
</tr>
<tr>
<td>Stroke, n (%)</td>
<td>5 (5.7)</td>
<td>2 (4.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Initial NYHA class, n (%)</td>
<td></td>
<td></td>
<td>0.77</td>
</tr>
<tr>
<td>I</td>
<td>75 (85)</td>
<td>40 (83)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>13 (15)</td>
<td>8 (17)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Final NYHA class, n (%)</td>
<td></td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td>I</td>
<td>50 (57)</td>
<td>31 (65)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>32 (36)</td>
<td>13 (27)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>4 (4.5)</td>
<td>4 (8.3)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>2 (2.3)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>End-stage (dilated LV, EF &lt;50%), n (%)</td>
<td>6 (6.8)</td>
<td>3 (6.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>Cardiac transplantation, n (%)</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>Risk factors, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of HCM SD</td>
<td>38 (43)</td>
<td>12 (25)</td>
<td>0.036</td>
</tr>
<tr>
<td>ABPR</td>
<td>9 (10)</td>
<td>3 (6.3)</td>
<td>0.44</td>
</tr>
<tr>
<td>Massive LVH (≥30 mm)</td>
<td>16 (18)</td>
<td>3 (6.3)</td>
<td>0.055</td>
</tr>
<tr>
<td>NSVT</td>
<td>20 (23)</td>
<td>3 (6.3)</td>
<td>0.014</td>
</tr>
<tr>
<td>Syncope</td>
<td>18 (21)</td>
<td>9 (19)</td>
<td>0.81</td>
</tr>
<tr>
<td>ICD implanted</td>
<td>12 (14)</td>
<td>3 (6.3)</td>
<td>0.19</td>
</tr>
<tr>
<td>Appropriate ICD therapy</td>
<td>7 (58)</td>
<td>1 (33)</td>
<td>0.57</td>
</tr>
<tr>
<td>SD</td>
<td>2 (2.3)</td>
<td>0</td>
<td>0.54</td>
</tr>
<tr>
<td>Adverse cardiac event‡</td>
<td>13 (15)</td>
<td>1 (2.1)</td>
<td>0.020</td>
</tr>
</tbody>
</table>

ABPR indicates abnormal blood pressure response; EF, ejection fraction; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter-defibrillator; LV, left ventricular; LVEDD, left ventricular end-diastolic diameter; LVH, left ventricular hypertrophy; LWWT, left ventricular wall thickness; MYBPC3, cardiac myosin binding protein-C gene; NSVT, nonsustained ventricular tachycardia; NYHA, New York Heart Association; and SD, sudden death.

*Includes HCM patients studied by imputation. Mutation-negative includes 2 clinical HCM patients without the MYBPC3 c.927-2A>G mutation based on imputation.
†Log-rank P value is shown (see Kaplan–Meier analyses).
‡ SD, ICD therapy, heart failure death, and stroke death.

Discussion

The genetic architecture of HCM in Iceland is characterized by a high prevalence of pathogenic mutations, occurring in 67% of patients. More than half of HCM is caused by a founder mutation, MYBPC3 c.927-2A>G, and 3.3% reflects another mutation in MYBPC3 or MYH7. No HCM mutations were found in genes encoding thin filament proteins, PRKAG2 or LAMP2. Genetic analyses demonstrated misclassification of 5.3% of patients with HCM who had GLA mutations and cardiac Fabry disease, therein improving their clinical management.

The high detection rate of gene mutations in Icelandic patients likely reflects the occurrence of the founding MYBPC3 mutation and the familial relatedness of the Icelandic population. Review of cardiovascular imaging and medical records defined HCM in <10% of patients with cardiac hypertrophy. Because our criteria for HCM consisted of LV wall thickness ≥15 mm, patients with HCM with modest hypertrophy, as can occur with cardiac troponin T mutations,27,28 were excluded. In addition, Iceland’s remote, rugged terrain and harsh environment may have produced negative effects on deleterious HCM mutations and may have contributed to the lower population prevalence of HCM in Iceland (1:1600) compared with the United States (1:500).7

The founder mutation in the Icelandic population, similar to previously described founding mutations in Finland,29 the Netherlands,30 India,31,32 Japan,33 South Africa,34 Spain,34 France,35 and the United States,36 affects the MYBPC3 gene. Unlike deleterious HCM mutations, founder MYBPC3 mutations have a minimal effect on overall survival, particularly during the reproductive years of life. Genetic genealogical data on the MYBPC3 c.927-2A>G mutation also support its neutral selection. Haplotype data date this mutation to the late 15th century and inheritance through 17.6 generations of Icelanders. Although we assume neutral selection, the influence of mild negative selection cannot be excluded because Iceland experienced massive population changes throughout the 15th through 18th centuries as a result of plagues, volcanic eruptions, and famine. Rapid population growth during the 19th century may have offset potential negative effects. Studies of other non-HCM founder mutations suggest that penetrance and phenotype change with time, possibly as a result of environmental factors.18,37

The identification of a predominant founder mutation in Iceland provided a rare opportunity to assess longitudinal clinical relationships between genotype and phenotype. HCM in Iceland is a chronic and manageable disease with minimal impact on life expectancy. Similar to other study results,5,38 clinical HCM was recognized in more men than women, and female patients with HCM were diagnosed later in life and were more symptomatic at follow-up than male patients. The mean life expectancy of Icelanders is among the highest in the world, currently 80.8 years for men and 83.9 years for women.19 Approximately 50% of the mortality in patients with HCM was HCM related (0.78%/y) and occurred at a mean age of 68 compared with 81 years for non–HCM-related mortality (P=0.022). Patients with non–HCM-related mortality were older at

with an age- and sex-matched Icelandic general population, was 1.24 (95% confidence interval, 0.79–1.94; P=0.35) compared with 0.98 (95% confidence interval, 0.72–1.33; P=0.89) for the whole HCM cohort (Figure 5).
diagnosis compared with patients with HCM-related mortality, a finding in concordance with prior data. HCM mortality was commonly due to heart failure, often at an advanced age. Five elderly patients with HCM (84.8±4.4 years of age) died of diastolic heart failure after reaching normal life expectancy, underscoring that normal longevity can be achieved in HCM.

In contrast to these overall favorable characteristics among all Icelandic patients with HCM, the clinical course associated with the MYPC3 c.927-2A>G mutation was poorer. Compared with mutation-negative HCM, patients with the MYPC3 c.927-2A>G mutation were diagnosed at a younger age as a result of clinical presentation rather than through family screening. Patients with the MYPC3 c.927-2A>G mutation also had higher rates of adverse cardiac events, including serious arrhythmias, appropriate ICD therapies, and SD. These data have important medical implications for the estimated 1150 carriers of the MYBPC3 c.927-2A>G mutation in Iceland who are at risk for HCM and these adverse events. Thus, cascade gene-based testing in Iceland is ongoing.

Our study design had several sources of potential bias. First, this was fundamentally a study of survivors; we did not include affected individuals who died, possibly of HCM before its clinical presentation, which may have led to an underestimation of morbidity and mortality. Second, clinical data were collected retrospectively. Finally, genetic analyses of the patients with the MYBPC3 c.927-2A>G mutation were limited to the identification of this mutation; therefore, we cannot exclude the possibility that compound mutations contributed to clinical phenotypes in these patients. Although compound HCM mutations typically produce a more severe disease, these are uncommon and typically are identified in <5% of patients with HCM.

Despite these limitations, our study provides the first estimate of the population prevalence of HCM diagnosed by clinical criteria and genetic testing. These findings demonstrate that the MYBPC3 c.927-2A>G mutation increases the risk for adverse outcomes in HCM.

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We thank Drs Uggi Agnarsson, Thorarinn Gudnason, and Hjortur Oddsson and the cardiologists at Landspitali–The National University Hospital of Iceland in Landspitali for clinical contributions; Thor Aspelund for statistical assistance; Drs Daniel Herman and Lien Lam for technical advice and assistance; and Dr Arni Kristinsson.

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Figure 4. Kaplan–Meier analysis comparing (A) age at diagnosis of hypertrophic cardiomyopathy (HCM) in MYBPC3 c.927-2A>G patients and mutation-negative patients and (B) the age of first adverse cardiac event (sudden cardiac death, implantable cardioverter-defibrillator therapy, heart failure death, or stroke death) for MYBPC3 c.927-2A>G and mutation-negative patients.

Figure 5. Kaplan–Meier curves describing all-cause mortality (deaths resulting from any cause) and successful resuscitation or appropriate implantable cardioverter-defibrillator therapy for ventricular tachycardia/fibrillation for (A) all hypertrophic cardiomyopathy (HCM) patients (n=180) and (B) all MYBPC3 c.927-2A>G patients (n=88) compared with the expected survival in the Icelandic general population after adjustment for age and sex. HR indicates hazard ratio.

Disclosures
Drs J.G. Seidman and C.E. Seidman are founders of and own shares in Myokardia Inc, a startup company that is developing therapeutics targeting the sarcomere. The other authors report no conflicts.
References


**CLINICAL PERSPECTIVE**

We performed a clinical and genetic study of hypertrophic cardiomyopathy (HCM) on the whole population of Iceland. Our data identify the prevalence of clinical hypertrophy and HCM in Iceland, define the contribution of gene mutations, and reveal the phenotypic spectrum and associated risk for adverse outcomes of a prevalent MYBPC3 mutation. The genetic architecture of HCM in Iceland is characterized by a high prevalence of disease-causing mutations in sarcomere protein genes and GLA, occurring in 67% of patients. More than half of HCM is caused by MYBPC3 c.927-2A>G, whereas only 3.3% of patients had another mutation in MYBPC3 or MYH7. Mutations in GLA were identified in 5.3% of patients, unmasking cardiac Fabry disease. Haplotype and genetic genealogical data define MYBPC3 c.927-2A>G as a founder mutation, introduced in the late 15th century and inherited through 17.6 generations of Icelanders, with an estimated current population prevalence of 0.36%. HCM in Iceland has overall favorable characteristics with minimal impact on life expectancy. MYBPC3 c.927-2A>G mutation carriers exhibited phenotypic diversity but were younger at diagnosis and sustained more adverse cardiac events than mutation-negative patients. These data have important medical implications for the estimated 1150 carriers of the MYBPC3 c.927-2A>G mutation in Iceland who are at risk for HCM. Harnessing clinical criteria and genetic technologies, our study establishes the first estimate of the population prevalence of HCM. The identification of a predominant founder mutation provides a rare opportunity to assess longitudinal clinical relationships between genotype and phenotype.
Nationwide Study on Hypertrophic Cardiomyopathy in Iceland: Evidence of a MYBPC3 Founder Mutation
Berglind Adalsteinsdottir, Polakit Teekakirikul, Barry J. Maron, Michael A. Burke, Daniel F. Gudbjartsson, Hilma Holm, Kari Stefansson, Steven R. DePalma, Erica Mazaika, Barbara McDonough, Ragnar Danielsen, Jonathan G. Seidman, Christine E. Seidman and Gunnar T. Gunnarsson

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SUPPLEMENTAL MATERIAL
Supplemental Methods

Genetic Analyses

*Capture probe design*

Capture probes (biotinylated RNA library baits) were designed using eArray, a publicly available web-based probe design application ([https://earray.chem.agilent.com](https://earray.chem.agilent.com)) (Agilent Technologies, CA). All custom probes were synthesized and PCR-amplified using universal primers connected to the probes, then amplified and biotin-conjugated by *in vitro* transcription (Agilent Technologies, CA).

*Solution-based hybridization*

We performed solution-based hybridization and target enrichment according to the manufacturer’s protocol with some modifications (Agilent Technologies, CA). Each pool of DNA for target enrichment was prepared by adding human Cot-1 DNA, salmon sperm DNA, and blocking oligonucleotides. Separately, a unit of oligo capture library (ELID# 0382021) was combined with RNAse block and hybridization buffer. Subsequently, each pool was added to the oligo capture library and incubated for 24 hours at 65°C. After the hybridization, the captured subgenomic DNA was selected using streptavidin-coated magnetic beads (Invitrogen, CA) and eluted with the MinElute PCR purification kit (Qiagen, CA). The subgenomic targets were enriched by PCR amplification and sequenced.

*Variant Classification*

Variants were mainly categorized into likely pathogenic, variant of unknown significance (VUS), likely benign and benign variants. The likely pathogenic variants include nonsense variants, frameshift variants, *de novo* variants in proband with *de novo*
disease, variants with prior functional evidence of pathogenicity, variants affecting consensus splices sites (two base pairs upstream and downstream of exon/intron boundary) or missense variants segregating with disease (LOD of 1.5). Although some missense variants have insufficient disease segregation data, they are classified as likely pathogenic if their frequency in ethnically matched chromosomes is less than 0.2%. A group of VUS includes missense variants for which control studies are limited or data is conflicting. Likely benign variants include missense variants present in the general population or ethnically matched population at a minimal allele frequency of between 1-3%, and intronic variants outside splice consensus with a minimal allele frequency of less than 1%. Benign variants include silent variants, missense variants present in the general population or ethnically matched population at a minimal allele frequency of more than 3%, and intronic variants outside splice consensus with a minimal allele frequency of more than 1%. The totally benign variants were not shown but are available upon request.
**Supplemental Tables**

**Supplemental Table I.** Clinical Characteristics of Patients with Other Likely Pathogenic Variants and Variants of Unknown Significance.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Predicted effect</th>
<th>Variant</th>
<th>Gender</th>
<th>Family history of HCM</th>
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AF indicates atrial fibrillation; ABPR, abnormal blood pressure response; CAD, coronary artery disease; F, female; HTN, hypertension; ICD, implantable cardioverter defibrillator; LP, likely pathogenic variant; LV, left ventricle; M, male; MYBPC3, cardiac myosin binding protein-C gene; MYH7, β-myosin heavy chain gene; N/A, not applicable; NYHA, New York Heart Association; VUS, variant of unknown significance.
Supplemental Table II. Adverse Events and Causes of Death

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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>48</td>
<td>M</td>
<td>72</td>
<td>N/A</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>20</td>
<td>2</td>
<td>4.7</td>
<td>40 Multiple CVD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AF indicates atrial fibrillation; AG, myosin binding protein-C mutation c. 927-2A>G; AMI, acute myocardial Infarction; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; EF, ejection fraction; F, female; HF, heart failure; ICD, implantable cardioverter defibrillator; LA, left atrium; LV, left ventricle; LVOT, left ventricular outflow tract; M, male; MN, mutation-negative; N/A, not applicable; NYHA, New York Heart Association; SD, sudden death; VF, ventricular fibrillation; VT, ventricular tachycardia. * LV outflow gradient before septal therapy; † patient died 3 years later, due to HF; ‡ patient died 2 years later, due to embolic stroke.
Supplemental Figures

Supplemental Figure I. Kaplan-Meier analysis comparing age at diagnosis of HCM in female MYBPC3 c.927-2A>G patients and male MYBPC3 c.927-2A>G patients. Male patients were diagnosed at an earlier age than female patients, although the difference was not statistically significant (p=0.26).
Supplemental Figure I

Clinical Diagnosis of HCM (%)

Age (Years)

Male
Female

P-value = 0.26