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Myozenin 2 Is a Novel Gene for Human Hypertrophic Cardiomyopathy

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Abstract

Hypertrophic cardiomyopathy (HCM) is a genetic disorder caused by mutations in sarcomeric proteins (excluding phenocopy). The causal genes in approximately one-third of the cases remain unknown. We identified a family comprised of 6 clinically affected members. The phenotype was characterized by early onset of symptoms, pronounced cardiac hypertrophy, and cardiac arrhythmias. We excluded *MYH7, MYBPC3, TNNT2*, and *ACTC1* as the causal gene either by direct sequencing or by haplotype analysis. To map the putative candidate sarcomeric gene, we perforbold locus-specific haplotyping to detect cosegregation of the locus haplotype with the phenotype, followed by mutation screening. We genotyped 5 short-tandem-repeat markers that spanned a 4.4-centimorgan region on 4q26-q27 locus and encompassed myozenin 2 (*MYOZ2*), a Z-disk protein. The maximum logarithm of odds score was 2.03 (P=0.005). All affected members shared a common haplotype, implicating *MYOZ2* as the causal gene. To detect the causal mutation, we sequenced all exons and exon–intron boundaries of *MYOZ2* in 10 family members and identified a T→C missense mutation corresponding to S48P substitution, which cosegregated with inheritance of HCM (N=6). It was absent in 4 clinically normal family members and in 658 additional normal individuals. To determine frequency of the *MYOZ2* mutations in HCM, we sequenced *MYOZ2* in 516 HCM probands and detected another missense mutation (I246M). It was absent in 2 normal family members and 517 controls. Both mutations affect highly conserved amino acids. We conclude *MYOZ2* is a novel causal gene for human HCM.

Keywords

mutation; gene; hypertrophic cardiomyopathy; calsarcin 1

Hypertrophic cardiomyopathy (HCM) is a genetic disease characterized by unexplained cardiac hypertrophy, myocyte disarray, and interstitial fibrosis (reviewed previously[1]). It is a

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Disclosures

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major cause of sudden cardiac death in the young and diastolic heart failure.[1] Molecular genetic basis of HCM has been partially elucidated. More than a dozen genes, all encoding sarcomeric proteins (except for phenocopy) have been identified.[2,3] We describe *MYOZ2*, encoding myozenin 2 (calsarcin 1), as a novel gene for human HCM.

Materials and Methods

The study protocol was approved by the Institutional Review Board and was in accordance with the Human Subjects' Committee guidelines (University of Texas Health Science Center, Houston). We collected blood samples from the consenting individuals and extracted genomic DNA by a conventional method. The participants underwent detailed history taking, physical examination, 12-lead electrocardiography, and transthoracic echocardiography. Additional procedures were performed as clinically indicated. HCM was diagnosed based on the presence of unexplained left ventricular wall thickness of ≥13 mm on the echocardiogram.

We identified a black family (designated as family 109) comprising 6 affected individuals, including a pair of dizygotic twins (Figure 1). The index case presented at 12 years of age with symptoms of diastolic heart failure and episodes of syncope (supplemental Table I available in the online data supplement available at http://circres.ahajournals.org.). She had severe left ventricular hypertrophy with a septal thickness of 3.2 cm, atrial fibrillation, and runs of ventricular tachycardia. The clinical characteristics of the affected family members are shown in the supplemental Table I.

We screened the candidate genes based on their frequencies as causes of HCM and novelty.[1] We excluded mutations in *MYH7*, *MYBPC3*, TNNC1, and *TNNT2* by direct sequencing. We used a locus-specific haplotyping to screen the less common candidates *TNNI3, TPM1, ACTC1, TTN*, and *MYOZ2*. We genotyped 5 to 6 short-tandem-repeat DNA markers at approximately 1-centimorgan (cM) intervals for each locus using fluorescently labeled primers and PCR (Tables II and III in the online data supplement, available at http://circres.ahajournals.org). The PCR fragments were separated by capillary electrophoresis on an ABI 3130xl Genetic Analyzer and analyzed using the GeneMapper v3.7 (Applied Biosystems).

We excluded *ACTC1* by showing lack of cosegregation. In contrast, all 6 affected members shared a common haplotype for the *MYOZ2* locus on 4q26-q27, whereas 4 clinically normal family members did not (Figure 1 and supplemental Figure I). Two asymptomatic family members (II-4 and III-8) declined to participate. The findings strongly implicated *MYOZ2* as the putative causal genes. The remaining genes were not analyzed further.

The maximum logarithm of odds (LOD) score was 2.03 at markers D4S2303 and D4S1573, the closest markers to *MYOZ2*, assuming a disease allele frequency of 0.001 and penetrance of 0.99 in a dominant disease model (Figure 2 and supplemental Table IV). The LOD score is equivalent to a probability value of 0.005 adjusted for multiple testing of markers. We amplified and sequenced all 6 exons and exon–intron boundaries of *MYOZ2* in 10 family members using the Big Dye Terminator Reaction in an ABI 3130xl Genetic Analyzer (supplemental Table II). Each sequence was analyzed for the presence of variants and compared with the GenBank sequence (NC_000004). We identified a heterozygous T→C missense (S48P) mutation at nucleotide position 15 072 (Figure 2). The mutation was present in all 6 affected members and absent in 4 clinically normal family members (Figure 1). The dizygotic twin brothers with the S48P mutation exhibited different degrees of asymmetric septal hypertrophy, which could reflect the effects of modifier genes and environmental factors (supplemental Table I). The locus comprises 30 genes including 9 encoding hypothetical proteins. None encodes a sarcomeric protein other than MYOZ2 or a known protein for HCM phenocopy. Nevertheless,

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the possibility of linkage disequilibrium with the actual causal mutation cannot be excluded with certainty.

To exclude the possibility of a rare polymorphism, we designed a 5′ nuclease assay and screened 658 normal individuals (asymptomatic with normal ECGs and echocardiograms), including 253 blacks by allelic discrimination on an ABI PRISM 7900HT SDS. The S48P variant was absent in 1316 normal chromosomes. Comparison of MYOZ2 protein sequence across species identified the serine 48 as a completely conserved amino acid (Figure 2).

To determine the frequency of *MYOZ2* mutation in HCM, we screened all exons and exon–intron boundaries of *MYOZ2* in 516 probands by direct sequencing. We detected another heterozygous missense A→G mutation at nucleotide 50 278 in a white proband who had 2 deceased siblings with HCM. The mutation changed amino acid isoleucine 246, a conserved amino acid, to methionine (Figure 2). Two offspring of the proband (54 and 33 years of age) were asymptomatic and had normal physical examination, ECGs, and echocardiograms. They did not carry the mutation. The mutation was also absent in 517 normal individuals (405 whites).

Results and Discussion

We detected several synonymous and intronic variants in *MYOZ2* gene, which are shown in supplemental Table IV.

Under certain circumstances, the haplotype-sharing approach, restricted to the candidate loci, could facilitate mapping of the candidate genes in small families possessing classes of proteins that are known to cause the phenotype. Accordingly, it is applicable to genetic studies of primary cardiomyopathies caused by mutations in sarcomeric, cytoskeletal, and desmosomal proteins, particularly in small families, in which the conventional genome-wide linkage mapping may not offer sufficient power to map the causal gene.

Studies are ongoing to delineate the molecular mechanism(s) by which *MYOZ2* mutations cause HCM. Myozenins are Z-disk proteins exclusive to striated muscles. They bridge Ca^{+2}/calmodulin-regulated protein phosphatase calcineurin to α-actinin.[4] MYOZ2 is specific to cardiac and slow-skeletal muscle fibers.[5] All affected family members had normal skeletal muscle strength on physical examination. However, possibility of sub-clinical abnormalities was not excluded. MYOZ2 is a negative regulator of calcineurin functions.[5] Germline deletion of *Myoz2* in mice leads to activation of calcineurin, nuclear localization of NFAT, expression of cardiac hypertrophic genes, and enhanced cardiac hypertrophic response.[5] We speculate *MYOZ2* mutations cause HCM by activating the calcineurin pathway. The mutations, however, are not located within the known binding domains for calcineurin between amino acids 217 and 240.[4] Similarly, the mutations are outside the α-actinin–binding domain of MYOZ2 between amino acids 153 to 200. The mutations could affect calcineurin signaling and/or binding to α-actinin by changing the secondary structure of MYOZ2. Alternative possibilities include interactions with proteins and/or altered mechanosignaling through the Z-disk, which was implicated in the pathogenesis of HCM caused by T-cap telethonin (TCAP), a Z-disk protein.[6]

Identification of *MYOZ2* mutations highlights the diversity of molecular genetics and pathogenesis of HCM. Whether treatment with calcineurin inhibitors could prevent, attenuate, or reverse the phenotype in the subset of HCM caused by *MYOZ2* mutations remains to be tested. Calcineurin inhibitors have been shown to impart deleterious effects on cardiac structure and function in α-MyHC-403 mice.[7] Thus, it may be necessary to define different subsets of HCM at genetic and molecular levels to deliver gene-specific treatment for patients with HCM.

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In summary, we have identified 2 mutations in *MYOZ2* in patients with HCM. The haplotype and the S48P mutation cosegregated with inheritance of HCM. We also identified a second mutation (I246M) in a proband with HCM. Mutations were absent in ethnically matched controls and in the Single-Nucleotide Polymorphism database. They involved highly conserved amino acids. We conclude *MYOZ2* mutations are uncommon causes of HCM (1:250). Studies are necessary to delineate the molecular mechanisms involved.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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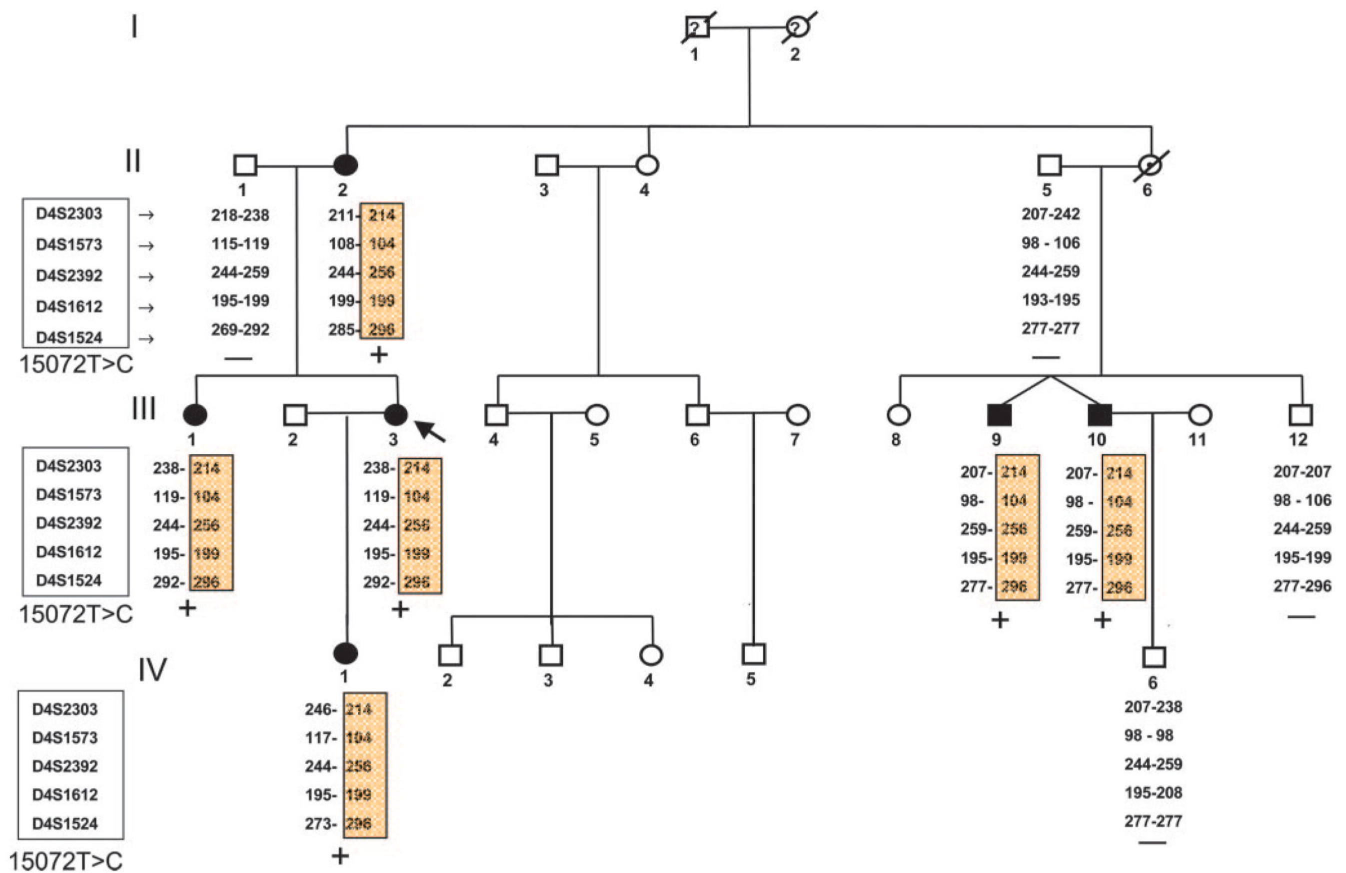

Figure 1.

The pedigree of family 109. Arrow points to the proband. ● (female) and ■ (male) indicate clinically affected individuals. Symbols with dots and slashes indicate obligate carriers and deceased individuals. Short-tandem-repeat DNA markers genotyped are shown to the left and aligned with the corresponding genotypes for each individual. The shared haplotype among the affected individuals is shaded. The + and − symbols indicate the presence or absence of the 15 072 T→C mutation.

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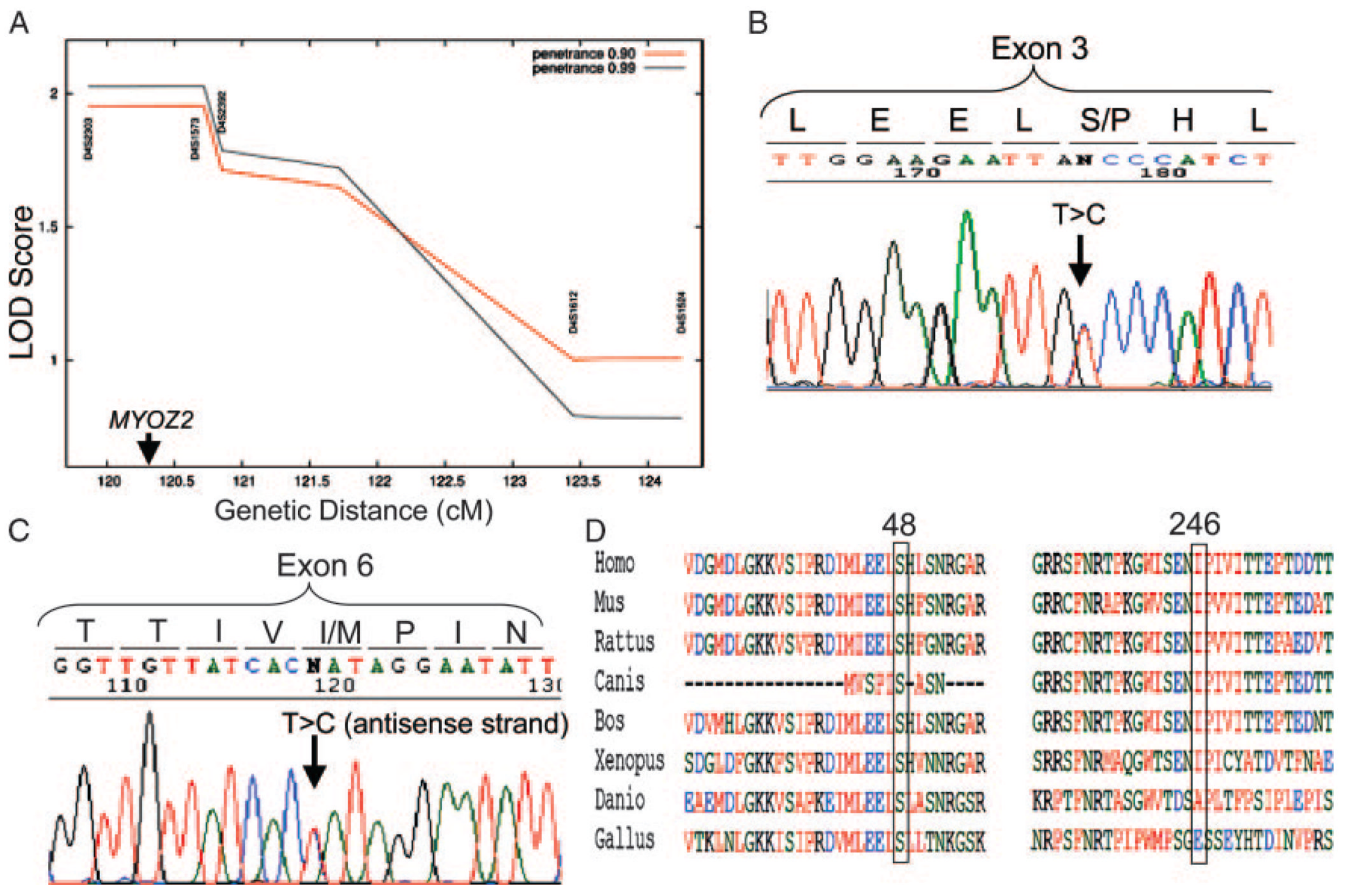

Figure 2.

Multipoint LOD score, detection of S48P and I246M mutations, and cross-species sequence conservation. A, Calculated LOD scores at the 4q26 locus. B and C, Partial sequence of *MYOZ2* exons 3 and 6 encompassing the heterozygous T→C and A→G mutations, respectively. The corresponding amino acid readouts are also shown. D, Multiple sequence alignment of MYOZ2 protein at and near the sites of the mutations.[8] Amino acids S48 and I246 are shown in boxes. S48 was conserved among *Homo sapiens* (Homo), *Mus musculus* (Mus), *Rattus norvegicus* (Rattus), *Canis familiaris* (Canis), *Bos taurus* (Bos), *Xenopus tropicalis* (Xenopus), *Danio rerio* (Danio), and *Gallus gallus* (Gallus). Similarly, the I246 is conserved, except in *D rerio* and *G gallus*.