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Mutations in Alpha-Actinin-2 Cause Hypertrophic Cardiomyopathy

A Genome-Wide Analysis

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Objectives This study describes a genome-wide linkage analysis of a large family with clinically heterogeneous hypertrophic cardiomyopathy (HCM).

Background Familial HCM is a disorder characterized by genetic heterogeneity. In as many as 50% of HCM cases, the genetic cause remains unknown, suggesting that other genes may be involved.

Methods Clinical evaluation, including clinical history, physical examination, electrocardiography, and 2-dimensional echocardiography, was performed, and blood was collected from family members (n = 23) for deoxyribonucleic acid analysis. The family was genotyped with markers from the 10-cM AB PRISM Human Linkage mapping set (Applied Biosystems, Foster City, California), and 2-point linkage analysis was performed.

Results Affected family members showed marked clinical diversity, ranging from asymptomatic individuals to those with syncope, heart failure, and premature sudden death. The disease locus for this family was mapped to chromosome 1q42.2-q43, near the marker D1S2850 (logarithm of odds ratio = 2.82, $\theta = 0$). A missense mutation, Ala119Thr, in the alpha-actinin-2 (*ACTN2*) gene was identified that segregated with disease in the family. An additional 297 HCM probands were screened for mutations in the *ACTN2* gene using high-resolution melt analysis. Three causative *ACTN2* mutations, Thr495Met, Glu583Ala, and Glu628Gly, were identified in an additional 4 families (total 1.7%) with HCM.

Conclusions This is the first genome-wide linkage analysis that shows mutations in *ACTN2* cause HCM. Mutations in genes encoding Z-disk proteins account for a small but significant proportion of genotyped HCM families. (J Am Coll Cardiol 2010;55:1127–35) © 2010 by the American College of Cardiology Foundation

Familial hypertrophic cardiomyopathy (HCM) is the most common genetic myocardial disease with a prevalence of 0.2% in the adult population (1). It is clinically characterized by left ventricular hypertrophy (LVH) in the absence of other loading conditions (2). Genetic studies have shown that HCM is caused by >450 different mutations in at least 13 genes encoding sarcomere or sarcomere-related proteins (3). Screening of the most common sarcomere disease genes identifies a mutation in approximately 50% of HCM cases (4). In the remainder of HCM cases, a number of possibilities exist in defining such cases, including mutations in as yet unknown genes, as well as diseases that may mimic HCM, such as glycogen storage diseases and Fabry disease (5,6). Family-based linkage studies and candidate gene screening techniques are 2 methods used in the identification of new disease genes in HCM.

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The Z-disc region of the cardiac sarcomere is a critical region that represents the interface between the contractile apparatus and the cytoskeleton (7). Although HCM has been defined as a disease of the sarcomere, recent studies using candidate gene approaches have identified several deoxyribonucleic acid (DNA) variants in sarcomere-associated genes to be linked with HCM (8). One of the Z-disc proteins

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Abbreviations and Acronyms

ACTN2 = alpha-actinin-2
HCM = hypertrophic cardiomyopathy
ICD = implantable cardioverter-defibrillator
LOD = logarithm of odds ratio
LVH = left ventricular hypertrophy
PCR = polymerase chain reaction
SR = spectrin-like repeat

of critical biological importance is alpha-actinin-2 (ACTN2), the major component of the Z-disc. The alpha-actinins belong to the spectrin superfamily (reviewed in Sjoblom et al. [9]) and compose 4 members of which ACTN2 is the major cardiac muscle isoform (10). ACTN2 is an important component of the sarcomere Z-disc in which its primary function is to anchor and cross-link actin filaments (7). Binding studies have shown that ACTN2 interacts with a number of other proteins and plays a key role in thin filament organization and the interaction between the sarcomere cytoskeleton and the muscle membrane (9).

This study describes the clinical and genetic investigation of a large, 3-generation, Australian family with clinically heterogeneous HCM and subsequent screening of an additional 297 unrelated HCM probands, which collectively implicates *ACTN2* as a causative gene in HCM.

Methods

Clinical evaluation of subjects. Patients with HCM referred to the HCM Clinic at Royal Prince Alfred Hospital in Sydney, Australia, were included in this study. Individuals were evaluated by using clinical history, physical examination, electrocardiography, and echocardiography, as previously described (11,12). Diagnostic criteria for HCM was defined in adults by a maximal left ventricular wall thickness of ≥13 mm on echocardiography, in the absence of other loading conditions. Within the context of a family history, individuals were considered affected based on the echocardiography criteria (see previous text). Electrocardiographic changes that were considered of clinical significance included abnormal Q waves (>0.04 s or depth >25% of an R-wave), LVH (voltage criteria), and marked repolarization changes (e.g., T-wave inversion in at least 2 leads). The disease status of deceased individuals was based on review of medical records. Individuals younger than 20 years, without evidence of LVH or electrocardiographic abnormalities were considered to be of unknown disease status for the purposes of linkage analysis. All studies were performed in strict accordance with the Sydney South West Area Health Service human ethics standards.

Genotyping. Genomic DNA was extracted from whole blood. The coding regions and intron/exon boundaries of 10 HCM genes (*MYH7*, *MYBPC3*, *MYL2*, *MYL3*, *TNNT2*, *TNNI3*, *TPM1*, *ACTC*, *PLN*, and *CSRP3*) were sequenced to determine whether the family carried a diseasing-causing DNA sequence variant in a known gene.

A genome-wide scan was performed at the Australian Genome Research Facility (Victoria, Australia) using 400 microsatellite markers from the 10 cM AB PRISM human linkage mapping set (Applied Biosystems, Foster City, California). All available individuals were genotyped regardless of their clinical status, and genotypes were determined without knowledge of clinical details. Fine mapping was performed at candidate loci suggestive of linkage, using an additional 26 markers from the 5 cM AB PRISM human linkage mapping set.

Linkage analysis. Two-point linkage analysis was performed using MLINK/LINKAGE software package (version 5.1). Logarithm of odds ratio (LOD) scores were calculated for each marker assuming an autosomal-dominant mode of inheritance for disease, a penetrance of 95%, a disease allele frequency of 0.001, no phenocopies, and equal allele frequencies for genotyped markers. Haplotypes were constructed assuming a minimum number of recombinations.

Candidate gene screening. All coding regions and intron/exon boundaries of *ACTN2* were sequenced (Ensembl ENST00000366578). Primer information and polymerase chain reaction conditions are available (Online Supplementary Data 1). An additional HCM cohort of unrelated probands (n = 297) were screened for mutations in *ACTN2* using high-resolution melt analysis, as previously described (13,14). Primers were designed for high-resolution melt analysis using the Lightscanner Design Software (version 1.0.R.84, Idaho Technology, Inc., Salt Lake City, Utah). Genomic DNA (10 ng) was amplified in 10 μl PCR reactions containing 2.5× high-resolution melt analysis Mastermix (TrendBio, Melbourne, Victoria, Australia) and 10 pmol of each primer. The PCR reactions were overlaid with 10 μl of mineral oil (Sigma-Aldrich, St. Louis, Missouri) and amplified under appropriate conditions. All PCR reactions were performed in 96-well BLK/WHT PCR plates (Bio-Rad Laboratories, Hercules, California). The PCR products were then analyzed using the 96-well LightScanner (Idaho Technology, Inc.) and LightScanner Software (version 2.0). Samples with abnormal melt profiles were sequenced to identify DNA variants.

Screening of the cardiac ryanodine receptor (*RyR2*) involved a previously developed targeted approach of mutational hot spots that account for >90% of reported mutations (15). Specifically, 37 of the 106 exons in the *RyR2* gene were screened (i.e., exons 7 to 9, 13 to 16, 43 to 50, and 83 to 106).

Direct DNA sequencing, in addition to restriction digests or allele specific primers, was used to confirm identified mutations and to determine allele frequencies of DNA variants in 260 non-HCM ethnicity-matched control samples. Intronic sequence variants were evaluated for altering RNA splicing using NetGene2 Server.

Results

Clinical characterization of family EI. A total of 23 individuals from a 3-generation Australian family were in-

cluded in the study (Fig. 1A). The clinical characteristics of the family highlight marked clinical heterogeneity and are summarized in Table 1. Individual III-2 was the first to be diagnosed with HCM after experiencing a resuscitated cardiac arrest during pregnancy. At the time, she had mild apical hypertrophy and an abnormal electrocardiogram indicating LVH. She had an implantable cardioverter-defibrillator (ICD) inserted and has subsequently progressed to severe heart failure. Individuals II-1, III-4, III-6, and III-9 were subsequently diagnosed with HCM as a result of clinical screening of the family. At the time of clinical screening, individual II-1 was being treated for atrial fibrillation and was found to have concentric hypertrophy involving both the left and right ventricles. III-6 was found to have moderate asymmetrical septal LVH, nonsustained ventricular arrhythmias and received an ICD. III-9 had apical hypertrophy with evidence of apical trabeculations and additional mild right ventricular hypertrophy. She also received an ICD that has subsequently delivered appropriate shocks on 2 occasions in response to ventricular tachycardia during a follow-up period of 3 years. Individual III-4 died suddenly at 36 years of age (DNA not available). At post-mortem, he was reported to have asymmetrical septal hypertrophy and histopathological features consistent with HCM, including myocyte hypertrophy, myofiber disarray, and interstitial fibrosis. An echocardiogram obtained 1 year before his death showed an asymmetrical septal wall thickness of 16 mm.

Individual II-3 had previous mild LVH with borderline LVH changes on electrocardiography and at last assessment had evidence of left ventricular wall thinning at age 74 years. His daughter, III-11, had been previously diagnosed with an arrhythmogenic cardiomyopathy and had received an ICD. As a result, II-3 was classed as affected for the purposes of the study. Individuals II-5, III-3, III-8, III-10, and IV-7 were clinically normal and classified as unaffected. Individuals IV-1, IV-2, IV-3, IV-4, and IV-5 did not demonstrate any clinical evidence of HCM on echocardiography or electrocardiography; however, given their age (ranging from 10 to 19 years at last clinical evaluation), they were deemed to be of unknown clinical status. Individual IV-6 has not had clinical testing.

Genome-wide linkage analysis. To exclude the possibility that family EI carried a known HCM mutation in a sarcomere gene, DNA from individual II-1 underwent mutation screening of 10 genes known to cause HCM. No mutation was found, and as a result, a genome-wide linkage analysis was performed. Two-point linkage analysis of 400 markers identified suggestive linkage of 3 loci (1q42.2-q43 [LOD = 1.57, θ = 0.05], 4q31.21 [LOD = 1.32, θ = 0.05], and 17p13.1-17p12 [LOD = 1.65, θ = 0.05]) (Online Supplementary Data 2). Additional markers within these regions were genotyped and maximum LOD scores remained unchanged at the 4q31.21 and 17p13.1 loci, whereas a maximum LOD score of 2.82 at $\theta = 0$ for marker D1S2850 on chromosome 1 was achieved. This LOD score at marker D1S2850 was equivalent to the theoretical maximum LOD score achievable for this family, based on pre-test power calculations using a hypothetical marker that completely segregated with disease. Changing allele frequencies of the polymorphic markers and setting the penetrance at 50% did not significantly alter the LOD scores. Haplotypes were constructed using 11 adjacent markers to define the maximum limits of the disease locus. The genotypes for individual III-4 were inferred using the genotypes of both his parents and children. A recombination event in individual III-2 limits the linkage region to marker D1S2800 on the centromeric side. The recombination event in individual III-10 at marker D1S2670 limits the critical region on the telomeric side (Fig. 1). This resulted in a 5.7-Mb region flanked by markers D1S2800 and D1S2670.

Candidate gene screening. The 5.7-Mb disease region contained 38 genes, of which 8 encoded hypothetical proteins, 9 were pseudogenes, and 19 were considered unlikely candidate genes based primarily on their known biological function and the expression pattern of the encoded proteins. Two genes were selected as potential HCM disease genes based on their known cardiac expression profile and function (Table 2). The ryanodine receptor (*RyR2)* and *ACTN2* are both expressed in the heart. *RyR2* encodes the cardiac ryanodine receptor, a key component in the regulation of calcium cycling in the heart. *ACTN2* encodes ACTN2. Targeted analysis of the *RyR2* gene revealed no disease-causing mutations, although it should be noted that the targeted approach has only been used previously in cases of unexplained sudden death where the diagnosis of HCM had been excluded (15). The coding regions and intron/exon boundaries of the *ACTN2* gene were sequenced in individual II-1, and a G to A missense mutation in exon 3 was identified that resulted in an Ala119Thr substitution. This variant was found in all affected family members and was not present in 260 control samples (520 alleles). The Ala119Thr variant is located in a region that is highly conserved among interspecies orthologues and between the 4 human α-actinin paralogues. Overexpression of the Ala119Thr variant in stably transfected myoblast cells resulted in a significant increase in RNA markers of hypertrophy, supporting the causative nature of the variant (Online Supplementary Data 3).

To determine the prevalence of *ACTN2* mutations in HCM, we screened an additional 297 HCM probands using high-resolution melt analysis and identified 14 novel and 12 known variants (Table 3). Five variants in the *ACTN2* coding region were absent in control samples and in 296 HCM probands. Co-inheritance screening eliminated 2 variants, Arg328Gln and Ala644Thr, because they were identified in unaffected family members. Three variants, Thr495Met, Glu583Ala, and Glu628Gly, were considered disease-causing mutations in 4 families (Fig. 1B). The pathogenic mutations were located in highly conserved regions among interspecies orthologues and paralogues (Fig. 1C) and located in important functional domains (Fig. 2A).

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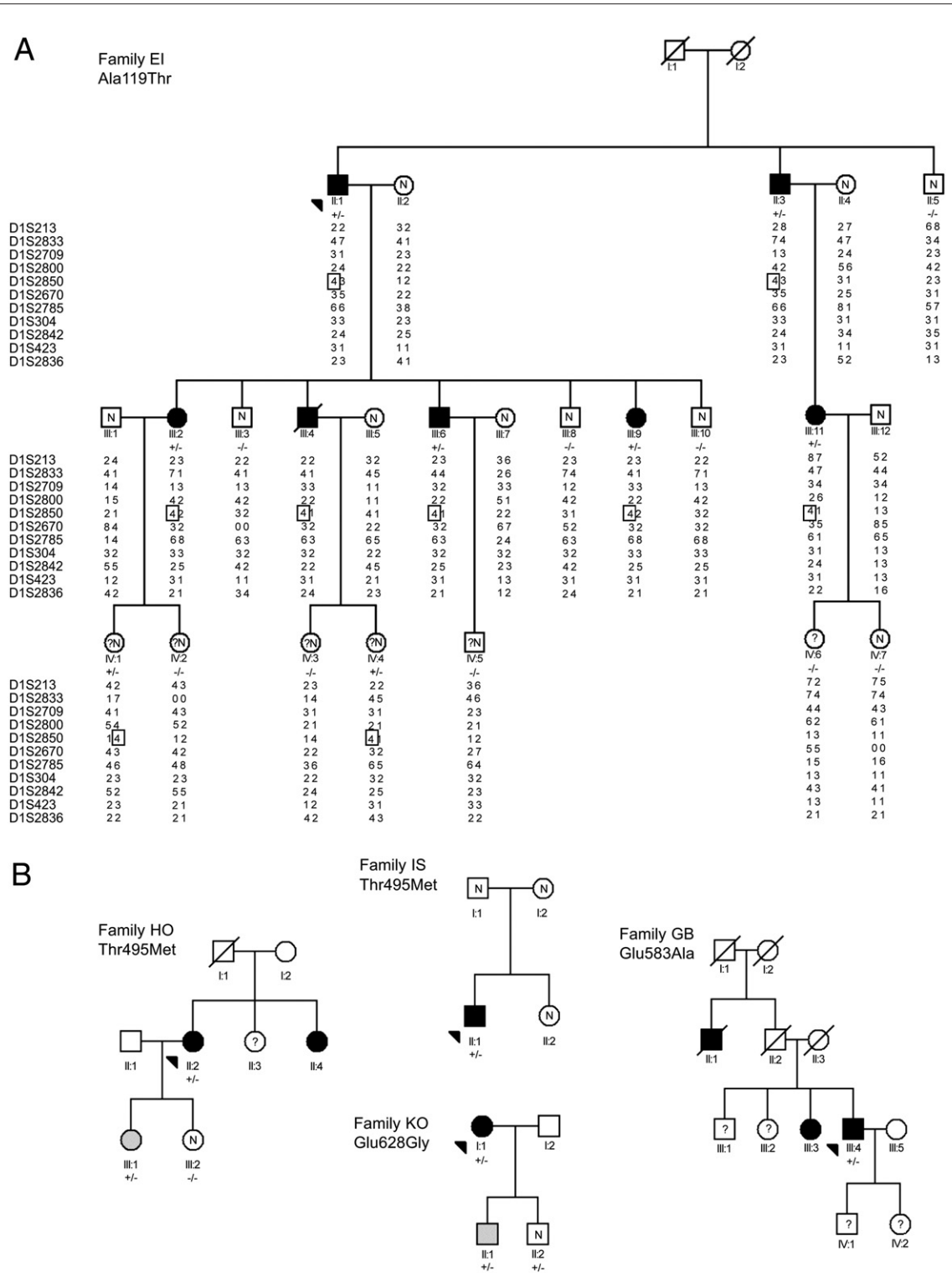

Figure 1 **Pedigrees of HCM Families With *ACTN2* Mutations**

(A) Pedigree including the results of a haplotype analysis of the hypertrophic cardiomyopathy (HCM) family in the linkage analysis. **Squares** = males; **circles** = females; **line through a symbol** = deceased individual; **solid symbols** = clinically affected individuals; **open symbols with "N"** = clinically unaffected individuals; **open symbols with "N?"** = clinically normal individuals with an unknown disease state; **arrow** = proband. The microsatellite alleles contributing to the haplotype of all affected individuals are marked with **boxes.** +/− = presence of the heterozygous Ala119Thr mutation; −/− = no mutation identified. **(B)** Pedigrees of HCM families HO, IS, GB, and KO. **Symbols** represented as described for **A**. +/− = presence of a heterozygous mutation as marked; −/− = no mutation identified. *Continued on the next page.*

C

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Ala119Thr     104 -DYIASKGVKLVSIGTEEIVDGNVKMTLGMIWTIILRFAIQDISVEETSAKEGLLLWCQRK-  164
Human               DYIASKGVKLVSIGAEEIVDGNVKMTLGMIWTIILRFAIQDISVEETSAKEGLLLWCQRK
Chimpanzee          DYIASKGVKLVSIGAEEIVDGNVKMTLGMIWTIILRFAIQDISVEETSAKEGLLLWCQRK
Dog                 DYIASKGVKLVSIGAEEIVDGNVKMTLGMIWTIILRFAIQDISVEETSAKEGLLLWCQRK
Bull                DYIASKGVKLVSIGAEEIVDGNVKMTLGMIWTIILRFAIQDISVEETSAKEGLLLWCQRK
Mouse               DYIASKGVKLVSIGAEEIVDGNVKMTLGMIWTIILRFAIQDISVEETSAKEGLLLWCQRK
Rat                 DYIASKGVKLVSIGAEEIVDGNVKMTLGMIWTIILRFAIQDISVEETSAKEGLLLWCQRK
Chicken             DYIASKGVKLVSIGAEEIVDGNVKMTLGMIWTIILRFAIQDISVEETSAKEGLLLWCQRK
Zebrafish           DFI+TKGVKLVSIGAEEIVDGNVKMTLGMIWTIILRFAIQDISVEETSAKEGLLLWCQRK

Thr495Met     486 -ICDQWDRLGMLTQKRREALE- 505 --- 573 -QSIMAIQNEVEKVIQSYNIRI- 593 --- 617 -LVPIRDQSLQEELARQHA
Glu583Ala         -ICDQWDRLGTLTQKRREALE- 505 --- 573 -QSIMAIQNEVAKVIQSYNIRI- 593 --- 617 -LVPIRDQSLQEELARQHA
Glu628Gly         -ICDQWDRLGTLTQKRREALE- 505 --- 573 -QSIMAIQNEVEKVIQSYNIRI- 593 --- 617 -LVPIRDQSLQGELARQHA
Human             -ICDQWDRLGTLTQKRREALE- 505 --- 573 -QSIMAIQNEVEKVIQSYNIRI- 593 --- 617 -LVPIRDQSLQEELARQHA
Chimpanzee        -ICDQWDRLGTLTQKRREALE- 505 --- 573 -QSIMAIQNEVEKVIQSYNIRI- 593 --- 617 -LVPIRDQSLQEELARQHA
Dog               -ICDQWDRLGTLTQKRREALE- 505 --- 573 -QSIMAIQNEVEKVIQSYNIRI- 593 --- 617 -LVPIRDQSLQEELARQHA
Cow               -ICDQWDRLGTLTQKRREALE- 508 --- 573 -QSILAIQNEVEKVIQSYSIRI- 593 --- 617 -LVPIRDQSLQEELARQHA
Mouse             -ICDQWDRLGTLTQKRREALE- 508 --- 573 -QSILAIQNEVEKVIQSYSIRI- 593 --- 617 -LVPVRDQSLQEELARQHA
Rat               -ICDQWDRLGTLTQKRREALE- 508 --- 573 -QSILAIQNEVEKVIQSYSIRI- 593 --- 617 -LVPVRDQSLQEELARQHA
Chicken           -ICDQWDSLGTLTQKRREALE- 508 --- 573 -QAILSIQNEVEKVIQSYSMRI- 593 --- 617 -LVPQRDQSLQEELARQHA
Zebrafish         -ICDLWDHLGTLTQRRREALE- 508 --- 573 -QAILGIQQEVLKIFQSYGIRG- 593 --- 617 -LVPQRDSALQEELARQHA
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Figure 1 Continued

(C) Conservation of the amino acid changes in *ACTN2* pathogenic mutations across species.

The clinical characteristics of the 4 families are summarized in Table 4. The Thr495Met variant was identified in 2 unrelated individuals, HO and IS, and has been previously reported to cause HCM (8). The families are from different ethnic backgrounds (i.e., European and South American). In addition, single-nucleotide polymorphism analysis in the *ACTN2* gene indicated the mutations in these 2 individuals from families HO and IS were from different founders, collectively supporting the pathogenic role of the Thr495Met mutation. HO (II-2) has a family history of HCM with 1 affected sibling. One of her daughters HO (III-1), age 15 years, was found to also carry the Thr495Met

Table 1 Clinical Features in Family EI

Individual	Sex	Ala119Thr	Current Age (yrs)	Age at Echo (yrs)	LVmax (mm)	PW (mm)	LVESD (mm)	LVEDD (mm)	FS (%)	ICD	ECG	Comments
II-1	M	+/−	75	75	15	19	36	42	14	No	Atrial fibrillation	Progression to severe HF
II-3	M	+/−	74	70	12	11	39	55	29	No	T-wave changes	Progression to LV wall thinning
III-2	F	+/−	48	44	9	8	34	47	27	Yes	NA	Resuscitated cardiac arrest, progression to severe HF
III-4	M	NA	Died 36	35	18	NA	NA	NA	NA	No	NA	Sudden death; myocyte hypertrophy, disarray, fibrosis
III-6	M	+/−	44	NA	22	NA	NA	NA	NA	Yes	LVH voltage criteria	
III-9	F	+/−	39	36	15	8	22	35	31	Yes	LVH voltage criteria	Apical hypertrophy and trabeculations, RV hypertrophy
III-11	F	+/−	51	50	13	10	27	43	37	Yes	ST-T changes	Midseptal and lateral LVH
IV-1	F	+/−	19	NA	10	NA	NA	NA	NA	No	NA	
IV-4	F	+/−	10	10	6	6	26	43	38	No	Normal	
II-5	M	−/−	68	67	12	11	40	57	30	No	Normal	
III-3	M	−/−	47	45	6	7	NA	NA	NA	No	Normal	
III-8	M	−/−	41	38	9	9	32	55	42	No	Normal	
III-10	M	−/−	35	33	10	10	35	54	35	No	Normal	
IV-2	F	−/−	18	NA	8	8	NA	NA	NA	No	Normal	
IV-3	F	−/−	11	11	8	9	30	47	37	No	Normal	
IV-5	M	−/−	18	16	8	6	NA	NA	NA	No	Normal	
IV-6	F	−/−	31	NA	NA	NA	NA	NA	NA	No	NA	
IV-7	F	−/−	26	25	8	8	26	44	41	No	Normal	

ECG = electrocardiogram; Echo = echocardiography; FS = fractional shortening; HF = heart failure; ICD = implantable cardioverter-defibrillator; LV = left ventricular; LVEDD = left ventricular end-diastolic diameter; LVESD = left ventricular end-systolic diameter; LVH = left ventricular hypertrophy; LVmax = maximal left ventricular wall thickness; NA = not available; PW = posterior wall thickness; RV = right ventricular.

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Table 2 Genes Residing in 5.7-Mb Region of Linkage

Gene	Encoded Protein	Main Organ Site of Expression	Known Disease Association
TARBP1	TAR (HIV-1) RNA binding protein-1	Intestine, testis	HIV-1 disease
IRF2BP2	Interferon regulatory factor-2 binding protein-2	NA	
TOMM20	Translocase of outer mitochondrial membrane-20 homolog	Mitochondrial, heart	
SNORA14B	Small nucleolar RNA, H/ACA box 14B	NA	
RBM34	RNA binding motif protein-34	NA	
ARID4B	AT rich interactive domain-4B (RBP1-like)	Respiratory epithelium, testis	
GGPS1	Geranylgeranyl diphosphate synthase-1	NA	
TBCE	Tubulin folding cofactor E	Spleen, Purkinje cells, heart	Sanjad-Sakati syndrome
B3GALNT2	β-1,3-*N*-acetylgalactosaminyltransferase-2	NA	
GNG4	Guanine nucleotide binding protein (G protein), gamma-4	NA	
LYST	Lysosomal trafficking regulator	NA	Chediak-Higashi syndrome
NID1	Nidogen-1	Spleen, lymph node	
GPR137B	G-protein–coupled receptor-137B	Hepatocytes, adrenal cortex	
ERO1LB	ERO1-like beta	NA	
EDARADD	EDAR-associated death domain	Pancreas, thyroid	Anhidrotic ectodermal dysplasia
LGALS8	Lectin, galactoside binding, soluble-8	NA	
HEATR1	HEAT repeat containing-1	NA	
ACTN2	**Actinin, alpha-2**	**Heart, glands, neuronal**	**Dilated cardiomyopathy**
MTR	5-Methyltetrahydrofolate-homocysteine methyltransferase	NA	Methylcobalamin deficiency G
RYR2	**Ryanodine receptor-2**	**Heart**	**CPVT, ARVD**
ZP4	Zona pellucida glycoprotein-4	Epithelia	

Bold text indicates 2 main candidate genes. Genes are ordered from centromere to telomere. Hypothetical and pseudogenes are not shown. Main site of expression is according to the Human Protein Atlas Database (http://www.proteinatlas.org/index.php). Known disease association is according to the Online Mendelian Inheritance in Man (OMIM) database.

ARVD = arrhythmogenic right ventricular dysplasia; CPVT = catecholaminergic polymorphic ventricular tachycardia; HIV-1 = human immunodeficiency virus type 1; NA = not available; RNA = ribonucleic acid.

variant and has localized thickening of the intraventricular septal wall, indicating early HCM (Fig. 1B). The second individual IS (II-1) is a male age 20 years with severe hypertrophy. Clinical examination of his parents and sister found them to be unaffected. A genetic result could not be obtained because the family declined to be tested (Fig. 1B). The Glu583Ala variant was identified in GB (III-4), a male age 65 years with mild hypertrophy and a family history of HCM. No further information was available (Fig. 1B). The Glu628Gly variant was identified in KO (I-1), a female age 44 years with moderate hypertrophy. Her 2 sons, KO (II-1) and KO (II-2), also carried the Glu628Gly variant. Clinical screening of KO (II-1) showed mild asymmetrical septal hypertrophy with borderline voltage criteria for LVH. KO (II-2) was clinically normal; however, he is 15 years of age and most likely is in the presymptomatic phase of disease (Fig. 1B).

Discussion

This study presents the first genome-wide linkage analysis that identifies *ACTN2* as a causative gene in HCM. After excluding mutations in known sarcomere genes, the maximum peak obtained after 2-point linkage analysis showed suggestive linkage to chromosome 1q42.2-43. Two key candidate genes under this linkage peak, *RyR2* and *ACTN2*, were investigated. Mutation analysis of the *ACTN2* gene identified a heterozygous missense mutation Ala119Thr that cosegregated with disease in the EI family. Further *ACTN2* gene screening in an HCM population identified an additional 3 variants in 4 fami-

Table 3 *ACTN2* Variants Identified in Australian HCM Population Studied

Variant	Frequency in HCM Cohort (%)	Reference
IVS2+33 A>G	0.3	Novel
Ala119Thr	**0.3**	**Novel**
Asn126Asn	4.7	rs1341863
IVS4+73 A>G	0.3	rs1341862
Glu161Glu	9.8	Novel
IVS5+10 C>T	0.3	Novel
IVS7+34 G>A	23.9	rs819640
Val235Val	0.3	rs2288599
IVS8+23 G>C	34.7	rs2288600
IVS9-48 G>C	0.3	Novel
IVS9-42 C>T	0.3	Novel
IVS9+41 C>T	1.7	Novel
IVS10-42 C>T>G	16.5	rs7527525
IVS10-8 G>C	9.4	rs2288601
Arg328Gln	0.3	Novel
Phe447Phe	0.3	rs34785693
Thr495Met	**0.7**	**Reported (8)**
IVS13+48 T>C	5.7	Novel
IVS13+62 A>G	1.3	rs10925211
IVS15delTTG-28	1.0	Novel
Glu583Ala	**0.3**	**Novel**
Glu628Gly	**0.3**	**Novel**
Ala644Thr	0.3	Novel
Thr713Thr	0.3	rs34975493
IVS18-30 G>A	1.3	Novel
IVS20+34 A>G	2.7	rs2282366
Ser870Ser	33.3	rs12063382

Bold type indicates causative mutations.

HCM = hypertrophic cardiomyopathy.

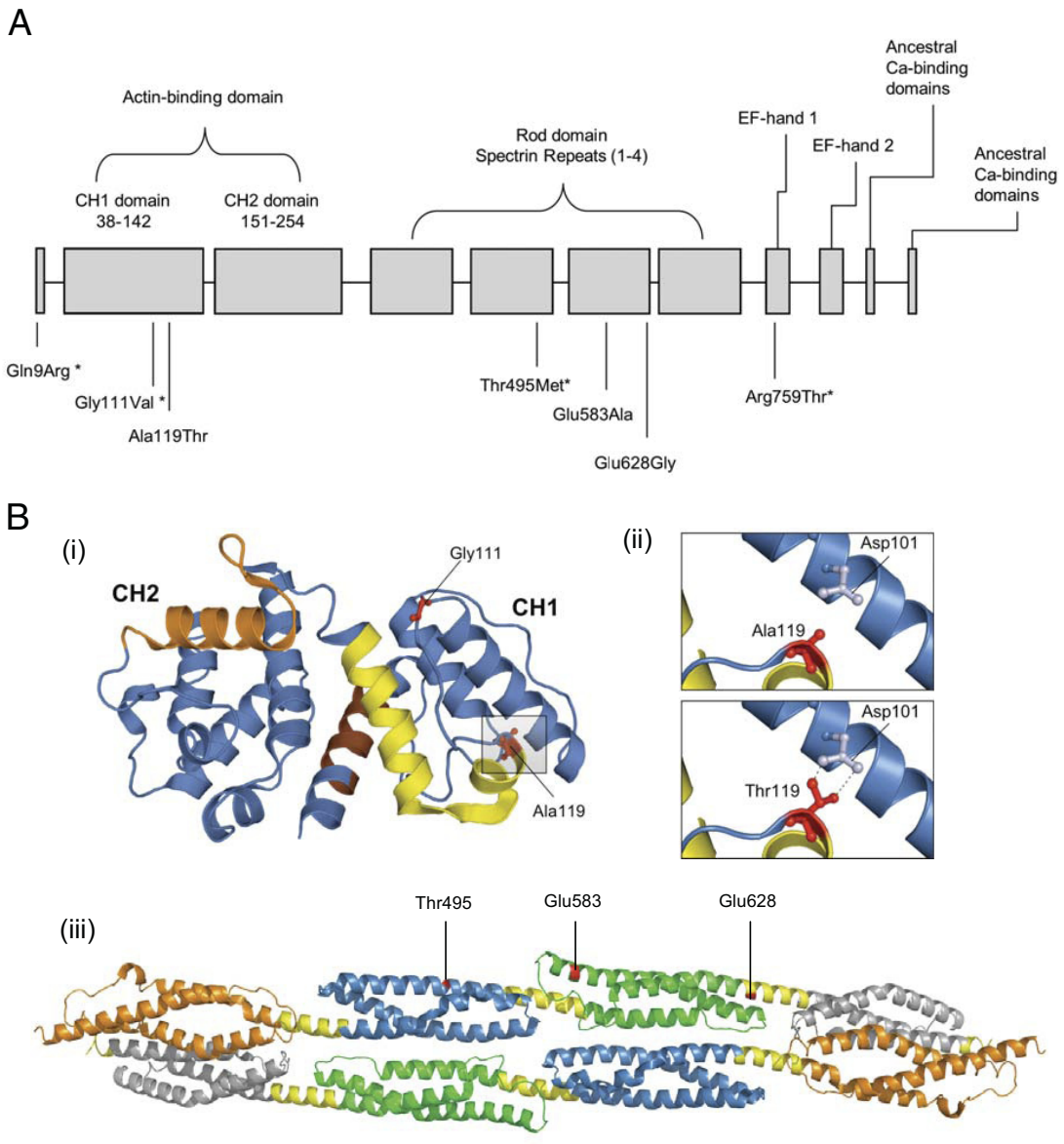

Figure 2 ***ACTN2* Mutations in HCM**

(A) Location of *ACTN2* mutations identified to date. **Asterisks** indicate *ACTN2* mutations identified in previous studies. **(B)** Predictive protein modeling (using PyMOL Molecular Viewer). **(i)** Actin-binding domains CH1 and CH2 of ACTN3 (Protein Data Bank entry code 1wku), with the Ala119Thr mutation marked. **(ii)** The possibility of steric hindrance when Ala is substituted with a Thr at residue 119. **(iii)** The spectrin repeat rod domain of ACTN2 (Protein Data Bank entry code 1hcl), with the positions of the mutations identified marked in **red**. HCM = hypertrophic cardiomyopathy.

lies. All 4 *ACTN2* variants were considered to be disease-causing mutations based on established criteria. Mutations in *ACTN2* are an important cause of HCM and support the notion that disruption of Z-disc proteins can lead to HCM.

Clinical heterogeneity is a feature of HCM and is illustrated in the diversity of pathologies, clinical presentations, and clinical outcomes in family EI. The cardiac hypertrophy observed in affected patients was generally mild in severity, with the distribution of hypertrophy involving the septum in some individuals, but also apical, concentric, and right ventricular hypertrophy in others. Despite this generally mild hypertrophic phenotype, the clinical outcomes in affected individuals have been significant, including sudden cardiac death, a resuscitated cardiac arrest during pregnancy, and a number of patients progressing from a hypertrophic to a dilated phenotype with associated severe heart failure. Such clinical heterogeneity reinforces the importance of thorough clinical evaluation of family members, regular follow-up of at-

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Table 4 Summary of the Clinical Features in Australian HCM Families With *ACTN2* Mutations

Individual	Sex	Mutation	Current Age (yrs)	Age at Echo (yrs)	IVS (mm)	PW (mm)	LVESD (mm)	LVEDD (mm)	FS (%)	ECG
HO:1	F	Thr495Met	49	48	18	8	NA	NA	NA	NA
HO:2	F	Thr495Met	15	11	11	8	NA	NA	NA	Abnormal ECG
IS:1	M	Thr495Met	20	16	36	9	21	34	37	LVH voltage criteria
GB:1	M	Glu583Ala	65	64	15	12	37	54	30	LVH voltage criteria
KO:1	F	Glu628Gly	44	44	17	11	21	37	43	LVH voltage criteria
KO:2	M	Glu628Gly	16	16	12	10	27	47	43	Borderline LVH
KO:3	M	Glu628Gly	13	13	9	9	25	46	46	Normal

IVS = intraventricular septal wall thickness; other abbreviations as in Tables 1 and 3.

risk individuals, and the importance of a genetic diagnosis to complement the clinical findings. This diversity of phenotypes may reflect a distinction between *ACTN2*-mediated disease and HCM caused by genes directly involved in sarcomere function. Specifically, the role of *ACTN2* in the Z-disc may affect both sarcomere and cytoskeletal function, leading to a combination of hypertrophic and dilated phenotypes.

Few studies have investigated the role of *ACTN2* in familial cardiomyopathies. One study identified an *ACTN2* mutation in an individual with dilated cardiomyopathy (16). The affected individual had severe disease and died at age 7 years. The Gln9Arg mutation was located in the actin-binding domain region and was found to prevent ACTN2 binding to the muscle LIM protein. More recently, a candidate gene approach identified 3 *ACTN2* mutations in 3 HCM probands (1.3% of the HCM cohort tested) (8). A feature identified in the study was the predisposition to developing a sigmoidal type of septal hypertrophy in patients with Z-disc–associated HCM. Two families in our current study were found to carry 1 of the variants (Thr495Met) identified in the previous study. However, our current families did not display sigmoidal morphology, but rather the diverse hypertrophic and, in some cases, progression to a dilated phenotype. Possible explanations for this diversity may relate to the location and functional significance of the *ACTN2* mutations identified, as well as the role of secondary genetic or environmental modifying factors (17).

The mutations identified in the current study span a number of important functional domains within the structure of ACTN2. The alpha-actinin is the major actin cross-linking protein in muscle cells and is composed of an amino terminal actin-binding domain containing 2 calponin homology domains (CH1 and CH2), followed by a rod domain consisting of 4 spectrin-like repeats (SRs) (SR1 to SR4) and a calmodulin-like domain at the carboxy terminal (Fig. 2) (18,19). ACTN2 exists as a functional antiparallel homodimer (20) with an actin-binding domain at either end that allows the cross-linking of actin filaments. The Ala119Thr mutation identified in family EI is positioned at the start of the second actin-binding site in CH1 (Fig. 2A). Replacing the alanine at position 119 with the larger threonine residue in this structure suggests a possible steric hindrance between 2 juxtaposed helices in CH1, which may cause a structural change affecting the actin-binding interface and thereby alter the actin-binding affinity of ACTN2 (Fig. 2B). The 3 additional *ACTN2* mutations identified in our HCM cohort map to the 2 central SRs of the rod domain; specifically, Thr495Met is in SR2 and Glu583Ala and Glu628Gly are in SR3 (Fig. 2B). The SRs are important interaction sites for structural and signaling proteins such as muscle LIM protein (21), titin (22), and myozenin (23). Collectively, the location of the identified *ACTN2* mutations may, at least in part, explain some of the clinical heterogeneity in the HCM families described here. Further functional and structural studies are required, including the determination of the crystal structure of ACTN2, to further understand how these mutations in *ACTN2* lead to cardiomyopathic disease.

The identification of *ACTN2* as a causative disease gene in HCM, based on both genome-wide and candidate gene approaches, highlights the importance of trying to identify a genetic cause in those patients with HCM in whom screening results of known sarcomere genes are negative. Knowledge of the causative gene mutation has significant clinical implications both in terms of diagnosis and in identifying family members at risk of developing disease. The families described in the current study highlight the vast clinical heterogeneity seen in affected individuals, spanning from mild symptoms to severe heart failure and sudden death. ACTN2 is one of a collection of Z-disc proteins that have been shown to be associated with HCM. Mutations in titin, telethonin, muscle LIM, and myozenin have been found to be associated with HCM in a small number of cases (24–26). These studies have largely been based on targeted candidate gene approaches, raising some issues of true pathogenicity. The current study shows for the first time that by using a genome-wide linkage approach, a Z-disc gene, *ACTN2*, causes HCM and provides further evidence of the importance of both sarcomere and sarcomere-associated proteins in the pathogenesis of HCM.

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Key Words: α-actinin-2 ■ gene mutations ■ hypertrophic cardiomyopathy.

APPENDIX

For Supplementary Data 1 to 3, please see the online version of this article.