Original Article

Burden of Recurrent and Ancestral Mutations in Families With Hypertrophic Cardiomyopathy

Samantha Barratt Ross, BMedSci; Richard D. Bagnall, PhD; Jodie Ingles, GradDipGenCouns, PhD, MPH; J. Peter Van Tintelen, MD, PhD; Christopher Semsarian, MBBS, PhD, MPH

Background—Hypertrophic cardiomyopathy is a genetically heterogeneous myocardial disease with >1000 causal variants identified. Nonunique variants account for disease in many families. We sought to characterize nonunique variants in Australian families and determine whether they arise from common ancestral mutations or recurrent mutation events.

Methods and Results—Genetic test results of 467 index patients from apparently unrelated families with hypertrophic cardiomyopathy were evaluated. Causal variants were found in 185 of 467 (40%) families. Nonunique variants accounted for 122 of 185 (66%) families. The most common single genetic cause of hypertrophic cardiomyopathy is the recurrent *MYBPC3* (myosin-binding protein-C) variant c.1504C>T, p.Arg502Trp, which was found in 13 of 185 (7%) families with a causal variant identified. Thirteen variants in *MYBPC3* and *MYH7* (myosin heavy chain 7) were each identified >3 times and accounted for 78 of 185 (42%) hypertrophic cardiomyopathy families with a causal variant. Haplotype analysis of these 13 variants was performed on 126 individuals from 70 Australian families, and 11 variants arose through recurrent mutation events. Two variants, *MYBPC3* c.1928-2A>G and *MYH7* c.2681A>G, p.Glu894Gly, were found on 1 haplotype in 6 families each, supportive of a single mutation event inherited from a common ancestor.

Conclusions—The majority of families with a causal variant identified have a nonunique variant. Discovery of the genetic origins of human disease forms a fundamental basis for improved understanding of disease pathogenesis and phenotype development. **(*Circ Cardiovasc Genet*. 2017;10:e001671. DOI: 10.1161/CIRCGENETICS.116.001671.)**

Key Words: cardiomyopathy, hypertrophic ■ genetics ■ haplotypes ■ mutation ■ phenotype

Hypertrophic cardiomyopathy (HCM) is a common inherited myocardial disease characterized by unexplained hypertrophy of the left ventricle.[1–3] Most patients with HCM have a relatively benign clinical course, whereas others experience major adverse events, including heart failure and sudden cardiac death.[4,5] HCM is primarily associated with an autosomal dominant inheritance pattern, and a pathogenic variant is identified overall in 30% to 50% of patients.[6–8] Over 1000 causal variants have been described in at least 8 sarcomere or sarcomere-related genes although >80% of variants are found in 2 genes: *MYBPC3* (myosin-binding protein-C) and *MYH7* (myosin heavy chain 7).[9,10] Historically, causal variants were described as largely family-specific or private; however, wider access to genetic testing has inevitably led to a decline in the number of novel variants reported.[8]

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See Clinical Perspective

A major recent advance in genetic testing has been the increased stringency of variant interpretation criteria. The American College of Medical Genetics and Genomics recently released a guidelines document to better delineate evidence required to assign causation to rare variants.[11] Presence of a variant in additional unrelated cases with the same phenotype is now one of the key criteria that can help to determine whether a rare variant is pathogenic. This is especially important because most families are not sufficiently powered to perform informative segregation studies. Therefore, an effort to catalogue and describe families has enormous potential to provide additional supportive information, impacting on clinical care of families worldwide.

A common haplotype among families with HCM carrying an identical variant implies that a single mutation event occurred in a common ancestor, whereas recurrent variants tend to occur on different haplotypes. Overall, relatively few founder HCM variants have been reported, and they are predominantly in *MYBPC3*, which account for a large proportion of patients in some countries (Table 1). For example, nearly one fourth of HCM cases in The Netherlands share a haplotype with a c.2373dupG variant in *MYBPC3*.[12] In contrast,

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From the Agnes Ginges Centre for Molecular Cardiology, Centenary Institute, Newtown, New South Wales, Australia (S.B.R., R.D.B., J.I., C.S.); Sydney Medical School, University of Sydney, New South Wales, Australia (S.B.R., R.D.B., J.I., C.S.); Department of Cardiology, Royal Prince Alfred Hospital, Sydney, New South Wales, Australia (J.I., C.S.); and Department of Clinical Genetics, Academic Medical Centre, University of Amsterdam, The Netherlands (J.P.V.T.).

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Correspondence to Christopher Semsarian, MBBS, PhD, MPH, Agnes Ginges Centre for Molecular Cardiology, Centenary Institute, Locked Bag 6, Newtown, New South Wales 2042, Australia. E-mail c.semsarian@centenary.org.au

Table 1. Founder Variants Causing Hypertrophic Cardiomyopathy

Gene	Variant	Country
MYBPC3	c.2373dupG (p.Trp792Valfs*41)	The Netherlands[12]
MYBPC3	c.2864_2865delCT (p.Pro955Argfs*95)	The Netherlands[13]
MYBPC3	c.2827C>T (p.Arg943Ter)	The Netherlands[13]
MYBPC3	c.1504C>T (p.Arg502Trp)	England[14]
MYBPC3	c.3181C>T (p.Gln1061Ter)	Finland[15]
MYBPC3	c.1928-2A>G	France[16]
MYBPC3	c.2308+1G>A	Spain[17]
MYBPC3	c.309_321dup (p.Pro108Alafs*9)	Spain[18]
MYBPC3	c.3330+2T>G	Amish America[19]
MYBPC3	c.821+1G>A	Germany[20]
MYBPC3	c.3697C>T (p.Gln1233Ter)	Turkey[20]
MYBPC3	c.1775delT (p.Val592fs)	Japan[21]
MYBPC3	c.3628-34_3628-10del	India[22]
MYH7	c.1207C>T (p.Arg403Trp)	South Africa[23]
MYH7	c.2389G>A (p.Ala797Thr)	South Africa[23]
TNNT2	c.274C>T (p.Arg92Trp)	South Africa[23]
TPM1	c.523G>A (p.Asp175Asn)	Finland[15]

the most common genetic cause of HCM elsewhere is the c.1504C>T, p.Arg502Trp variant in *MYBPC3*, which is found on multiple different haplotypes.[24]

To date, no common ancestral variants have been reported in Australian patients with HCM. The Australian population of 24 million is of multiethnic origin, with historical migration from many regions, including Europe, the United Kingdom, Asia-Pacific, and North America. Population data report that ≈1 in 4 Australians were born overseas, illustrating the degree of multiculturalism present. We report the occurrence of variants found in multiple Australian families with HCM and determine whether they arise from common ancestral mutations or recurrent mutation events.

Methods

Participants

We recruited an index patient from apparently unrelated families with HCM who attended the Genetic Heart Diseases Clinic, Royal Prince Alfred Hospital, Sydney, and had undergone research-based or clinical genetic testing. Family members with available DNA were included. Participants were clinically assessed, including clinical history, physical examination, and by investigations, including electrocardiography and echocardiography. Family history was collected by a cardiac genetic counselor as per clinic practice. The diagnostic criterion for HCM was a maximum left ventricular wall thickness of ≥15 mm in the absence of loading conditions that could otherwise explain the extent of hypertrophy.[25] Dutch probands with HCM and their available family members, who had variants in common with Australian HCM families, were included. Studies were approved by the Sydney Local Health District Review Committee and University Medical Center Utrecht, University Medical Center Groningen, and the Academic Medical Center Amsterdam institutional review committee, and all subjects gave informed consent.

Genetic Analyses and Variant Classification

DNA was isolated from peripheral blood using the QIAamp DNA Mini Kit (Qiagen, Germantown, MD) or from buccal swabs using the Isohelix DNA isolation kit (Isohelix, Kent, United Kingdom) according to the manufacturer's protocol. Variants in 8 established HCM genes (*MYBPC3*, *MYH7*, *TNNT2*, *TNNI3*, *TPM1*, *MYL2*, *MYL3*, and *ACTC1*) with a minor allele frequency of ≤0.02% in the Exome Aggregation Consortium data set (http://exac.broadinstitute.org/) were classified for pathogenicity. Variants were classified using in-house criteria (see ClinVar, Agnes Ginges Centre for Molecular Cardiology variant assessment and assertion criteria; https://submit.ncbi.nlm.nih.gov/ft/byid/djgybgii/mdi-5363_505375_agnesginges_variantassess_clinvar.pdf). Key determinants of pathogenicity included rarity (minor allele frequency of ≤0.02%) or absence from the Exome Aggregation Consortium data set, previous reports of the variant in ≥2 additional unrelated patients with HCM (http://ncbi.nlm.nih.gov/pubmed and http://clinvar.com/), segregation with affected relatives where possible, as well as any supportive experimental data. Overall, agreement among in silico tools and conservation scores was considered a single low-level supportive criterion (Combined Annotation Dependent Depletion; Sorting Intolerant From Tolerant, http://sift-dna.org/; Polymorphism Phenotyping Ver2 http://genetics.bwh.harvard.edu/pph2/; PolyPhen-HCM [http://genetics.bwh.harvard.edu/hcm/).

Where indicated, segregation analysis was performed on family members on a research basis and in combination with clinical cascade genetic testing where requested. DNA containing variants of interest was polymerase chain reaction amplified and excess primers and deoxynucleotide triphosphates removed with exonuclease I and antarctic phosphatase, respectively. Polymerase chain reaction products were Sanger sequenced (Macrogen, Seoul, Republic of Korea), and variants were identified by manual inspection of chromatograms using Sequencher v5.1 software (Gene Codes Corp, MI).

Haplotype Analysis

Haplotypes were constructed using polymorphic dinucleotide repeat markers within and flanking *MYBPC3* (D11S1344, MYBPC3-CA, and D11S4109) or *MYH7* (D14S990, MYOI, MYOII, and D14S972; Table I in the Data Supplement). Microsatellites were polymerase chain reaction amplified using standard conditions with a fluorescently labeled primer at 95°C for 2 minutes, followed by 30 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 1 minute. Polymerase chain reaction products mixed with GeneScan500 LIZ size standard (Life Technologies) were separated on an ABI 3730xl DNA analyzer, and alleles were sized using Peak Scanner software v1.0 (Life Technologies). To establish the phase of alleles, haplotypes were directly determined with cosegregation analysis of family members. For probands without available family members, haplotypes were inferred if the available allele and causal mutation combinations matched a directly determined disease-associated haplotype.

Polymorphism Information Content

Polymorphisms are highly informative for haplotype studies if any individual chosen at random is likely to be heterozygous for the marker. Polymorphism information content (PIC) is a statistical measure of the expected fraction of informative offspring. PIC values range from 0 to 1; a marker with only 1 allele has a PIC value of 0 and a marker with infinite alleles has a PIC value of 1. PIC values of dinucleotide repeat markers (Table I in the Data Supplement) were calculated from the allele frequencies observed in 70 apparently unrelated index patients with HCM using the formula of Hildebrand et al[26] $\mathrm{PIC} = 1 - \sum_{i=1}^{n} p_i^2 - (\sum_{i=1}^{n} p_i^2)^2 + \sum_{i=1}^{n} p_i^4$, where p_i is the frequency of the marker allele a_i and n is the number of different alleles.

Results

Genetic Testing

Genetic testing was performed on 467 unselected and apparently unrelated families with HCM. A likely pathogenic or

pathogenic (LP/P) variant, that is, causal variant was found in 185 (40%) families, with the majority in *MYBPC3* (n=107) or *MYH7* (n=58; Figure 1). There were 95 distinct LP/P variants and most were unique to 1 family (n=63; 66%). However, when considering only LP/P variants, more than half of all families had a nonunique variant (n=122; 66%; Table II in the Data Supplement). The most common LP/P variants were in *MYBPC3*: c.1504C>T, p.Arg502Trp (13 families); c.2373dupG, p.Trp792Valfs*41 (8 families); and c.2864_2865delCT, p.Pro955Argfs*95 (7 families). Thirteen LP/P variants alone accounted for 78 (42%) families, and all have been reported multiple times in non-Australian families with HCM (Table 2).

Haplotype Analysis

We performed haplotype analysis on 126 individuals from 70 Australian families with HCM (median number of individuals haplotyped per family=1, range=1–7) to determine whether nonunique variants represent multiple independent mutation events or single ancestral mutations. We focused on the 13 variants found in >3 families (Table II and Figures I and II in the Data Supplement). The common *MYBPC3* c.1504C>T variant causing p.Arg502Trp was found on 3 haplotypes in 13 families. There were 2 insertion or deletion (indel) variants and both were highly recurrent, being found on 5 different haplotypes in 8 families (*MYBPC3* c.2373dupG) and 5 different haplotypes in 7 families (*MYBPC3* c.2864_2865delCT; Figure 2B and 2C). In contrast to the recurrent variants, 2 variants were found on 1 haplotype in 6 families each, supportive of a single mutation inherited from a common ancestor: *MYBPC3* c.1928-2A>G; *MYH7* c.2681A>G (Figure 2A and 2D). All 12 families with ancestral mutations were of European descent.

To determine the origins of the 13 most common Australian HCM variants, we performed haplotype analysis on 14 individuals from 5 Dutch families (median number of individuals haplotyped per family=3, range=2–4). The recurrent *MYBPC3* c.2373dupG variant is a founder mutation in The Netherlands, however, has occurred multiple times to cause HCM in Australian families. Two Australian patients shared the same *MYBPC3* haplotype as a Dutch patient with this founder mutation, suggesting a shared ancestry, and both Australian patients were of European descent (Figure 2B). We next compared the haplotypes of 3 Dutch and 7 Australian families with the *MYBPC3* c.2864_2865delCT variant. The 3 Dutch families carried 2 different haplotypes and neither matched the haplotypes of Australian families, further demonstrating that c.2864_2865delCT is a recurrent mutation (Figure 2C). Finally, the single haplotype found in 6 Australian families with the c.1928-2A>G variant was compared with that in a Dutch family with this variant, and the alleles at markers D11S1344 and MYBPC3-CA were shared, whereas the D11S4109 allele size was 181 bp in Australian families and 177 bp in the Dutch family (Figure 2A).

Mutation Mechanisms

Seven of the 9 (78%) recurrent single nucleotide variants were C to T transitions at a CpG dinucleotide (Table 2), whereas neither of the 2 ancestral variants were C to T transitions at a CpG dinucleotide. To determine whether recurrent transition single-nucleotide variants occur more often than expected at CpG sites, the number of CpG transitions at unique and recurrent sites were compared. Twenty-four of 48 (50%) unique single-nucleotide variants were C to T transitions at a CpG dinucleotide compared with 7 of 9 recurrent variants, and this was not significant (P=0.153).

Genotype–Phenotype Associations

Table 3 shows the clinical and demographic characteristics of Australian probands with common ancestral and recurrent variants. Whereas the total number of probands in each genotype was relatively small, probands with ancestral variants were diagnosed at a similar age and had a similar maximum left ventricular wall thickness as those with recurrent variants and included families with a history of sudden cardiac death. None of the comparisons of clinical features reached statistical significance.

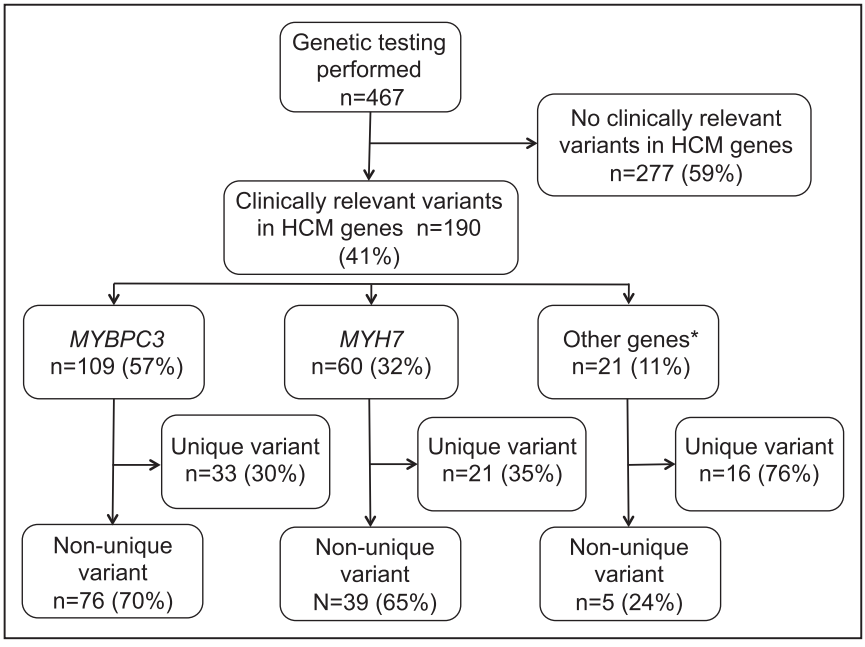

Figure 1. Genetic testing results of Australian families with hypertrophic cardiomyopathy. **TNNT2*, *TNNI3*, *TPM1*, *MYL2*, *MYL3* and *ACTC1*. HCM indicates hypertrophic cardiomyopathy; *MYBPC3*, myosin-binding protein-C; and *MYH7*, myosin heavy chain 7.

Table 2. Common Clinically Relevant Variants in Australian Families With Hypertrophic Cardiomyopathy

Gene	cDNA	Consequence	Australian Families	Australian Haplotypes (Families)	ClinVar Families, Individuals (ClinVar ID)	ExAC Allele Count	Flanking Sequence	CpG Site
MYBPC3	c.1504C>T	p.Arg502Trp	13	2 (13)	55, 117 (42540)	3	gaacc(G>A)gtatt	Yes
MYBPC3	c.2373dupG	p.Trp792Valfs*41	8	5 (8)	47, 137 (42619)	1	tccca(C>CC)tgtac	No
MYBPC3	c.2864_2865delCT	p.Pro955Argfs*95	7	5 (7)	17, 47 (42663)	0	gtaac(AG>.)gggct	No
MYBPC3	c.1624G>C	Splice donor	6	3 (3)	31, 51 (8608)	2	ctcac(C>G)ctgca	No
MYBPC3	c.1928-2A>G	Splice acceptor	6	1 (6)	24, 52 (51755)	0	ggttc(T>C)gcaga	No
MYBPC3	c.927-9G>A	Splice acceptor	6	2 (3)	37, 91 (42807)	0	tgtcc(C>T)gcagt	Yes
MYBPC3	c.1624+4A>T	Splice donor	5	2 (5)	11, 25 (51726)	1	caggc(T>A)caccc	No
MYBPC3	c.772G>A	Splice donor	5	3 (5)	32, 70 (42792)	3	ctcac(C>T)gtgga	Yes
MYH7	c.2681A>G	p.Glu894Gly	6	1 (6)	10, 24 (42922)	0	cttgt(T>C)cctga	No
MYH7	c.4135G>A	p.Ala1379Thr	4	4 (4)	6, 9 (42993)	0	aatgg(C>T)gtccg	Yes
MYH7	c.2389G>A	p.Ala797Thr	4	3 (3)	20, 61 (42901)	4	tctgg(C>T)gagca	Yes
MYH7	c.1988G>A	p.Arg663His	4	3 (3)	33, 83 (42875)	2	tggag(C>T)gcaag	Yes
MYH7	c.1816G>A	p.Val606Met	4	4 (4)	13, 38, (14091)	0	gccca(C>T)gacag	Yes

ExAC indicates Exome aggregate consortium.

Discussion

Discovery of the genetic origins of human disease forms a fundamental basis for improved understanding of disease pathogenesis and phenotype development. In this study, an LP/P variant was identified in 40% of Australian families with HCM. The majority of families with a causal variant identified have an LP/P variant in common with another family. Two ancestral variants and 11 recurrent variants account for 17% of all Australian families with HCM and are prevalent among non-Australian patients. Highly recurrent variants

A *MYBPC3* c.1928-2A>G

Patient ID	25	26	27	28	29	30	D5
Haplotype	**1**	**1**	**1**	**1**	**1**	**1**	**2**
D11S1344	289	289	289	289	289	289	289
MYBPC3-CA	383	383	383	383	383	383	383
c.1928-2A>G	+	+	+	+	+	+	+
D11S4109	181	181	181	181	181	181	177

B *MYBPC3* c.2373dupG

Patient ID	31	34	33	37	35	36	32	38	D1
Haplotype	**1**	**1**	**2***	**2**	**3**	**4**	**5***	**5**	**5**
D11S1344	287	287	297	297	285	297	287	287	287
MYBPC3-CA	379	379	379	379	379	381	379	379	379
c.2373dupG	+	+	+	+	+	+	+	+	+
D11S4109	163	163	177	NR	163	177	165	165	165

C *MYBPC3* c.2864_2865delCT

Patient ID	41	42	43	40	44	45	39	D2	D3	D4
Haplotype	**1**	**1**	**2**	**2**	**3**	**4***	**5***	**6***	**6***	**7**
D11S1344	295	295	297	297	289/295	295	297	295	295	283/297
MYBPC3-CA	379	379	385	385	379/383	383	379	379	379	383
c.2864_2865delCT	+	+	+	+	+	+	+	+	+	+
D11S4109	165	165	173	173	171/181	183	181	179	179	171/179

D *MYH7* c.2681A>G

Patient ID	65	66	67	68	69	70
Haplotype	**1***	**1**	**1**	**1***	**1***	**1**
D14S990	127	127	127	127	127	127
MYOI	313	313	313	313	313	313
c.2681A>G	+	+	+	+	+	+
MYOII	147	147	147	147	147	147
D14S972	205	205	205	205	205	205

Figure 2. Haplotype analysis of ancestral mutations and recurrent indels. **A–D**, Allele sizes in base pairs. For inferred haplotypes, both alleles are shown if the haplotype could not be resolved. *Directly determined haplotype from family cosegregation analysis. + indicates causal variant present; D1–D5, Dutch patients; and NR, not recorded.

Table 3. Clinical and Demographic Characteristics of Probands With Ancestral and Recurrent Variants

	MYBPC3 Variant cDNA Position						*MYH7* Variant cDNA Position						
Characteristic	c.1504	c.1624	c.1624*	c.1928†	c.2373	c.2864_5	c.772	c.927-9	c.1816	c.1988	c.2681†	c.4135	c.2389
No. of probands	11	5	5	5	7	7	5	4	4	4	6	4	3
European (%)	10 (91)	4 (80)	5 (100)	5 (100)	7 (100)	7 (100)	5 (100)	2 (50)	4 (100)	2 (50)	6 (100)	4 (100)	3 (100)
Male (%)	8 (73)	4 (80)	5 (100)	4 (80)	4 (57)	6 (86)	3 (60)	4 (100)	1 (25)	3 (75)	2 (33)	1 (25)	1 (33)
Family history HCM (%)	8 (73)	2 (40)	3 (60)	3 (60)	5 (71)	3 (43)	3 (60)	2 (50)	1 (25)	3 (75)	4 (66)	2 (50)	2 (66)
Family history SCD (%)	1 (13)	0 (0)	1 (20)	2 (40)	3 (43)	1 (14)	1 (20)	1 (25)	2 (50)	1 (25)	1 (17)	1 (25)	0 (0)
Age at diagnosis (y±SD)	44±12	35±17	36±12	44±27	38±17	42±15	35±15	34±16	39±18	24±15	33±5	41±17	29±13
Max LVWT (mm±SD)	22±7	23±5	26±7	22±5	21±5	30±5	24±6	18±4	24±7	33±7	26±7	22±3	23±5
ICD (%)	5 (45)	2 (40)	3 (60)	5 (100)	4 (57)	5 (71)	2 (40)	1 (25)	3 (75)	4 (100)	4 (66)	3 (50)	1 (33)
SCDE (%)	0 (0)	1 (20)	1 (20)	2 (40)	3 (42)	1 (14)	1 (20)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)

HCM indicates hypertrophic cardiomyopathy; ICD, implantable cardioverter defibrillator; LVWT, left ventricular wall thickness; SCD, sudden cardiac death; and SCDE, sudden cardiac death event.

*c.1624G>C; None of the comparisons of clinical parameters reached statistical significance.

†Ancestral mutation.

found on multiple different haplotypes tend to occur at CpG dinucleotides or lead to small insertions or deletions. Despite the tendency of Australian families with HCM to share a small number of nonunique LP/P variants, most variants are unique. The origins and variety of LP/P variants in HCM pose a challenge for classification of pathogenicity, and both local and global networks that share genetic and phenotype data will be helpful to define clinically relevant variants.

Our genetic test yield of 40% is in line with other recent large unselected cohorts of HCM.[8,27] The yield is lower compared with historical studies, which may reflect an increased stringency of variant classification criteria, our improved appreciation of rare variation in the general population and more widespread uptake of genetic testing in individuals with less severe HCM. Additionally, this study is limited to variants identified in 8 established causal HCM genes. The most common single genetic cause of HCM in Australian patients is the c.1504C>T, p.Arg502Trp variant in *MYBPC3*, which was found on 3 different haplotypes in 13 (2.8%) families. Because 2 haplotypes differ by only 2 bp at marker D11S4109, the c.1504C>T variant may have arisen from 3 independent mutation events or from 2 mutation events with a new haplotype formed either by a D11S4109 allele size change or a recombination event. Nevertheless, the prevalence and recurrence of the c.1504C>T variant in our cohort is consistent with other published cohorts of primarily European patients with HCM.[24] Australia has a multicultural population, with only 54% of Australians declaring in the 2011 census that both parents were born in Australia, and historically, immigration from European countries has been high.[28]

We found 2 variants, *MYBPC3* c.1928-2A>G and *MYH7* c.2681A>G, on the same haplotype in 6 families of European ancestry, and both likely represent ancestral mutations that originate from European ancestors. The c.1928-2A>G ancestral variant in *MYBPC3* was haplotyped in a Dutch patient with this variant, and the alleles at D11S1344 and an intragenic marker matched the Australian haplotype, whereas the D11S4109 allele was different. Therefore, we were unable to establish whether the Australian and Dutch patients share a common ancestral mutation on a haplotype that has undergone a recombination event or if these variants represent 2 independent mutations.

The identification of the *MYH7* c.2681A>G variant on the same haplotype in 6 seemingly unrelated Australian families was unexpected given the low number of reported founder variants in *MYH7*. To date, the only founder variants in *MYH7* that have been reported are *MYH7* p.Ala797Thr and *MYH7* p.Arg403Trp, which have both been identified in a South African cohort.[23] When compared with disease-causing variants in *MYBPC3*, *MYH7* variants seem more likely to cause earlier disease onset and greater disease severity, which may contribute to this trend.[24] The *MYH7* c.2681A>G variant is well described in HCM and has been reported in at least 6 additional families in North America, the United Kingdom, and Canada.[29–31] We were unable to determine if the original mutation event occurred in a common Australian ancestor or was introduced to Australia through migration.

The c.2373dupG variant in *MYBPC3* is a founder variant in The Netherlands[12] and was found in 8 Australian families on multiple haplotypes. Two Australian families with European ancestry shared the Dutch founder haplotype, implying that they have a common ancestor. A further 2 Australian families have this variant on a haplotype that differs by only 1 dinucleotide repeat at marker D11S4109 and may represent an additional transmission of the Dutch founder mutation. Delayed disease onset and incomplete penetrance may explain why founder variants that cause HCM, such as *MYBPC3* c.2373dupG, escape negative selection pressures to cause disease across many generations.[12,16,18]

There were 11 common recurrent variants, 7 of which are C to T transitions at a CpG dinucleotide. The cytosine of a CpG dinucleotide is often methylated and can spontaneously deaminate, producing a T:G mismatch, which can lead to a C to T transition mutation.[32] All of the 7 common C to T transitions found in Australian patients have collectively been reported

in ClinVar database in 469 individuals from 196 families, not accounting for potential overlap of reported patients. The remaining 2 common recurrent variants in Australian patients lead to a short insertion or deletion. Local DNA sequence context can indicate possible mechanisms leading to short insertions or deletions, such as tandem duplication of adjacent sequence or DNA replication slippage errors at homopolymer nucleotide runs.[33,34] No such sequences were apparent flanking the indels, and the mechanism leading to the recurrent indels remains to be determined.

The clinical significance of establishing whether a variant is unique, recurrent, or a common ancestral mutation is important. These findings assist in determining the pathogenicity of variants specific to a phenotype, such as HCM. Correlation of the variant with phenotype features, such as disease penetrance and severity of disease, remains poorly understood in HCM. Given the small number of probands with HCM within each mutation group (Table 3), no definitive conclusions could be drawn comparing clinical features, such as age at diagnosis, severity of left ventricular hypertrophy, or sudden death events. Larger studies focused on a single variant, such as the c.1504C>T variant in the *MYBPC3* gene, have elucidated more data on disease penetrance and severity.[18,24]

This study highlights the importance of sharing genetic test results to facilitate variant classification and explore possible genotype–phenotype relationships. The majority of families with a causal variant identified carry a nonunique variant that has been identified in patients around the world. The low mutation yield reported here and by others[8] emphasizes that we have much to learn about the genetic causes of HCM. The increasing use of next-generation sequencing techniques, such as whole-exome and whole-genome sequencing, will help to advance this area. Our study further highlights the importance of readily available and accessible genetic and phenotype data in human genetic diseases, such as HCM. Both international and national initiatives are important. A local network of genetic testing providers who share genotype and phenotype data, such as the Australian Cardiac Genetic Testing Network,[35] is a useful adjunct to the multidisciplinary approach of establishing the pathogenicity of variants in the local community. Global networks of genetic testing providers promoting public reporting of curated variants, such as ClinVar, will be invaluable for interpretation of clinically relevant variants that are rare among families with HCM and other inherited diseases.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Hypertrophic cardiomyopathy (HCM) is a clinically and genetically heterogeneous disease in which mutations in sarcomere genes cause disease. Genetic testing in HCM has had its greatest effect on cascade testing in families, as well as defining HCM phenocopies, such as glycogen storage diseases. Establishing whether a gene variant in HCM is unique, recurrent, or a common ancestral mutation is, therefore, of major clinical significance and impacts directly on the care of patients with HCM. Our findings will assist in determining the pathogenicity of variants specific to the HCM phenotype. Correlation of the variant with phenotype features, such as disease penetrance and severity of disease, remains poorly understood in HCM and so attempts to provide a more detailed landscape of genetic variants and family-based clinical information in HCM is important. This study also highlights the importance of sharing genetic test results at a global level to facilitate more precise variant classification and explore genotype–phenotype relationships.

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Burden of Recurrent and Ancestral Mutations in Families With Hypertrophic Cardiomyopathy

Samantha Barratt Ross, Richard D. Bagnall, Jodie Ingles, J. Peter Van Tintelen and Christopher Semsarian

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SUPPLEMENTARY MATERIAL

Table 1. Dinucleotide repeat markers

Marker	Repeat start (hg19)	Primer sequence (forward)	Primer sequence (reverse)	Observed allele size range (bp)	Observed allele number	PIC
D11S1344	chr11: 46166860	AGTGAGCCCTGAACTTCTGC	CCACAGCGCCTGGCTTGTAC	285 - 301	9	0.77
MYBPC3-CA	chr11:47361706	ATGGTTTCGATCTCCTGATC	GGGGAACATCAGTGTCTTGC	385 - 397	4	0.51
D11S4109	chr11:47601406	CTGGGAGTTAGGAGACCTGG	CTTGAAGATCCCTCACAGAC	151 - 187	16	0.88
D14S990	chr14:23586268	TTGGTCATGGAAACAAGAGG	AGTTGCACTGTGACTGGGTG	113 - 129	9	0.80
MYOI	chr14:23904574	AACCTCCTCGTTCACAGACG	TAGGAAGGGATTCCTCTTGG	311 - 323	7	0.60
MYOII	chr14:23892656	AACATCCTCTAACCCTACCC	ATGCCATGTCTATCTGTGCC	137 - 153	8	0.77
D14S972	chr14:24347553	GTTAACGCATAACAGCCAAG	TGACTGCCTCCATGATTTCC	203 - 215	7	0.57

PIC - polymorphism information content

Table 2. Distinct variants in Australian families with Hypertrophic Cardiomyopathy

#	Gene	cDNA	Consequence	Classification	Families	Family members with variant (phenotype positive)	ClinVar families (ClinVar ID)	ExAC allele count
1	*MYBPC3*	c.1504C>T	p.Arg502Trp	Pathogenic	13	34 (21)	55 (42540)	3
2	*MYBPC3*	c.2373dupG	p.Trp792Valfs*41	Pathogenic	8	20 (17)	47 (42619)	1
3	*MYBPC3*	c.2864_2865delCT	p.Pro955Argfs*95	Pathogenic	7	10 (8)	17 (42663)	0
4	*MYBPC3*	c.1624G>C	Splice donor	Pathogenic	6	10 (10)	31 (8608)	2
5	*MYBPC3*	c.1928-2A>G	Splice acceptor	Pathogenic	6	8 (6)	24 (51755)	0
6	*MYBPC3*	c.927-9G>A	Splice acceptor	Pathogenic	6	7 (7)	37 (42807)	0
7	*MYBPC3*	c.1624+4A>T	Splice donor	Pathogenic	5	7 (6)	11 (51726)	1
8	*MYBPC3*	c.772G>A	Splice donor	Pathogenic	5	4 (4)	32 (42792)	0
9	*MYBPC3*	c.2905C>T	p.Gln969Ter	Pathogenic	3	6 (4)	5 (42669)	0
10	*MYBPC3*	c.3697C>T	p.Gln1233Ter	Pathogenic	3	7 (6)	10 (42735)	1
11	*MYBPC3*	c.2429G>A	p.Arg810His	Likely pathogenic	2	3 (3)	10 (42620)	4
12	*MYBPC3*	c.25+1G>A	Splice donor	Pathogenic	2	2 (2)	0	0
13	*MYBPC3*	c.3190+5G>A	Splice donor	Pathogenic	2	2 (2)	4 (155808)	2
14	*MYBPC3*	c.3192dupC	p.Lys1065Glnfs*12	Pathogenic	2	3 (3)	4 (42693)	0
15	*MYBPC3*	c.3624delC	p.Lys1209Serfs*28	Pathogenic	2	2 (2)	4 (42727)	0
16	*MYBPC3*	c.655G>C	p.Val219Leu	Pathogenic	2	4 (3)	9 (42784)	0
17	*MYBPC3*	c.913_914delTT	p.Phe305Profs*27	Pathogenic	2	2 (2)	6 (42801)	0
18	*MYBPC3*	c.1153_1168delGTGGAACTGGCTGACC	p.Val385Metfs	Pathogenic	1	2 (2)	1 (222706)	0
19	*MYBPC3*	c.1302C>A	p.Tyr434Ter	Pathogenic	1	1 (1)	1 (217827)	0
20	*MYBPC3*	c.1359delT	p.Val454Cysfs*12	Pathogenic	1	3 (3)	1 (217835)	0
21	*MYBPC3*	c.1458-1G>A	Splice acceptor	Pathogenic	1	1 (1)	1 (42532)	0
22	*MYBPC3*	c.1483C>G	p.Arg495Gly	Likely pathogenic	1	1 (1)	4 (42537)	0
23	*MYBPC3*	c.1484G>A	p.Arg495Gln	Pathogenic	1	4 (4)	12 (164113)	1
24	*MYBPC3*	c.1505G>A	p.Arg502Gln	Pathogenic	1	1 (1)	7 (42541)	0
25	*MYBPC3*	c.162delG	p.Lys54Asnfs*13	Pathogenic	1	2 (2)	1 (219730)	0
26	*MYBPC3*	c.177_187delAGAGGGCACAC	p.Glu60Alafs*49	Pathogenic	1	2(2)	7 (42565)	0
27	*MYBPC3*	c.1838dupA	p.Asp613Glufs*25	Pathogenic	1	1 (1)	1 (1838dupA)	0
28	*MYBPC3*	c.2079_2082dupCCCA	p.Ala695Profs*14	Pathogenic	1	1 (1)	0	0
29	*MYBPC3*	c.2096delC	p.Pro699Glnfs	Pathogenic	1	1 (1)	4 (42596)	0

30	*MYBPC3*	c.2267delC	p.Pro756Leufs*66	Pathogenic	1	1 (1)	1 (181078)	0
31	*MYBPC3*	c.2308+1G>A	Splice donor	Pathogenic	1	1 (1)	1 (42610)	0
32	*MYBPC3*	c.2308G>A	p.Asp770Asn	Likely pathogenic	1	1 (1)	6 (36604)	1
33	*MYBPC3*	c.2309-2A>G	Splice acceptor	Pathogenic	1	2 (2)	11 (42613)	0
34	*MYBPC3*	c.2413+1G>A	Splice donor	Pathogenic	1	2 (1)	0	0
35	*MYBPC3*	c.2524dupT	p.Tyr842Leufs	Pathogenic	1	1 (1)	2 (42632)	0
36	*MYBPC3*	c.2533C>A	p.Arg845Ser	Pathogenic	1	2 (2)	0	0
37	*MYBPC3*	c.2558delG	p.Gly853Alafs*26	Likely pathogenic	1	1 (1)	3 (42641)	0
38	*MYBPC3*	c.2604-2605delTCinsA	p.Ser871Alafs*8	Pathogenic	1	1 (1)	2 (177870)	0
39	*MYBPC3*	c.2735delG	p.Gly912Alafs*12	Pathogenic	1	5 (3)	0	0
40	*MYBPC3*	c.2738-2A>T	Splice acceptor	Pathogenic	1	1 (1)	0	0
41	*MYBPC3*	c.2780_2781delCA	p.Thr927Ilefs*123	Pathogenic	1	2 (2)	2 (177660)	0
42	*MYBPC3*	c.2827C>T	p.Arg943Ter	Pathogenic	1	1 (1)	12 (37039)	2
43	*MYBPC3*	c.3408C>A	p.Tyr1136Ter	Pathogenic	1	1 (1)	1 (36611)	0
44	*MYBPC3*	c.3476_3477insATTT	p.Phe1159Leufs*11	Pathogenic	1	3 (2)	0	0
45	*MYBPC3*	c.3490+1G>A	Splice donor	Pathogenic	1	2 (1)	1 (42715)	0
46	*MYBPC3*	c.3490+1G>T	Splice donor	Pathogenic	1	1 (1)	1 (181008)	0
47	*MYBPC3*	c.3712_3713delCT	p.Leu1238Glyfs*3	Pathogenic	1	2 (2)	1 (217836)	0
48	*MYBPC3*	c.613C>T	p.Gln205Ter	Pathogenic	1	3 (2)	1 (42774)	0
49	*MYH7*	c.2681A>G	p.Glu894Gly	Likely pathogenic	6	16 (13)	10 (42922)	0
50	*MYH7*	c.4135G>A	p.Ala1379Thr	Pathogenic	4	9 (7)	6 (42993)	0
51	*MYH7*	c.2389G>A	p.Ala797Thr	Likely pathogenic	4	3 (3)	20 (42901)	4
52	*MYH7*	c.1988G>A	p.Arg663His	Pathogenic	4	6 (6)	33 (42875)	2
53	*MYH7*	c.1816G>A	p.Val606Met	Pathogenic	4	5 (5)	13 (14091)	0
54	*MYH7*	c.2167C>T	p.Arg723Cys	Pathogenic	3	5 (4)	12 (14095)	3
55	*MYH7*	c.2221G>T	p.Gly741Trp	Pathogenic	3	3 (3)	8 (177665)	0
56	*MYH7*	c.438G>T	p.Lys146Asn	Pathogenic	3	4 (4)	3 (43013)	0
57	*MYH7*	c.1954A>G	p.Arg652Gly	Likely pathogenic	2	3 (3)	3 (177626)	1
58	*MYH7*	c.2156G>A	p.Arg719Gln	Pathogenic	2	4 (3)	15 (14107)	0
59	*MYH7*	c.2609G>A	p.Arg870His	Pathogenic	2	2 (2)	5 (14120)	1
60	*MYH7*	c.2207T>C	p.Ile736Thr	Pathogenic	2	3 (2)	8 (175481)	0
61	*MYH7*	c.428G>A	p.Arg143Gln	Likely pathogenic	1	1 (1)	6 (43006)	1
62	*MYH7*	c.427C>T	p.Arg143Trp	Likely pathogenic	1	1 (1)	3 (164401)	6

63	*MYH7*	c.611G>A	p.Arg204His	Likely pathogenic	1	1 (1)	5 (43095)	0
64	*MYH7*	c.1208G>A	p.Arg403Gln	Pathogenic	1	2 (2)	16 (14087)	0
65	*MYH7*	c.1207C>T	p.Arg403Trp	Pathogenic	1	1 (1)	6 (14102)	0
66	*MYH7*	c.1324C>T	p.Arg442Cys	Pathogenic	1	1 (1)	3 (177897)	1
67	*MYH7*	c.1357C>T	p.Arg453Cys	Pathogenic	1	2 (2)	25 (14089)	0
68	*MYH7*	c.2155C>T	p.Arg719Trp	Pathogenic	1	1 (1)	8 (14104)	0
69	*MYH7*	c.2087A>G	p.Asn696Ser	Likely pathogenic	1	1 (1)	1 (181173)	0
70	*MYH7*	c.715G>A	p.Asp239Asn	Likely pathogenic	1	3 (3)	7 (43100)	0
71	*MYH7*	c.4066G>A	p.Glu1356Lys	Likely pathogenic	1	1 (1)	1 (164294)	3
72	*MYH7*	c.2770G>A	p.Glu924Lys	Pathogenic	1	4 (4)	5 (14092)	0
73	*MYH7*	c.1727A>G	p.His576Arg	Likely pathogenic	1	1 (1)	4 (177625)	1
74	*MYH7*	c.1370T>C	p.Ile457Thr	Likely pathogenic	1	1 (1)	3 (42840)	1
75	*MYH7*	c.2722C>G	p.Leu908Val	Pathogenic	1	1 (1)	21 (14097)	0
76	*MYH7*	c.1051A>G	p.Lys351Glu	Likely pathogenic	1	1 (1)	1 (181335)	0
77	*MYH7*	c.2539A>G	p.Lys847Glu	Likely pathogenic	1	1 (1)	6 (177757)	0
78	*MYH7*	c.1478T>A	p.Met493Lys	Likely pathogenic	1	1 (1)	1 (192308)	0
79	*MYH7*	c.2093T>C	p.Val698Ala	Pathogenic	1	7 (5)	2 (42878)	0
80	*TNNT2*	c.487_489delGAG	p.Glu163del	Pathogenic	3	3 (3)	13 (43648)	0
81	*TNNT2*	c.275G>A	p.Arg92Gln	Pathogenic	2	4 (4)	5 (12409)	0
82	*TNNT2*	c.236T>A	p.Ile79Asn	Pathogenic	1	7 (5)	5 (12408)	2
83	*TNNT2*	c.274C>T	p.Arg92Trp	Pathogenic	1	1 (1)	5 (43627)	1
84	*TNNT2*	c.311C>T	p.Ala104Val	Likely Pathogenic	1	1 (1)	2 (177633)	1
85	*TNNT2*	c.833G>A	p.Arg278Pro	Likely Pathogenic	1	2 (1)	1 (177635)	0
86	*TNNI3*	c.485G>A	p.Arg162Gln	Likely Pathogenic	2	6 (2)	11 (43389)	3
87	*TNNI3*	c.433G>A	p.Arg145Trp	Pathogenic	1	1 (1)	5 (12426)	1
88	*TNNI3*	c.485G>C	p.Arg162Pro	Likely pathogenic	1	1 (1)	1 (43390)	0
89	*TNNI3*	c.509G>A	p.Arg170Gln	Pathogenic	1	1 (1)	8 (165516)	0
90	*TNNI3*	c.593T>C	p.Leu198Pro	Likely pathogenic	1	1 (1)	0	0
91	*TNNI3*	c.611G>A	p.Arg204His	Pathogenic	1	3 (3)	2 (177679)	0
92	*TPM1*	c.548C>T	p.Ala183Val	Likely Pathogenic	1	3 (3)	2 (43424)	0
93	*TPM1*	c.574G>A	p.Glu192Lys	Likely Pathogenic	1	1 (1)	14 (31882)	0
94	*MYL3*	c.461G>A	p.Arg154His	Likely Pathogenic	1	1 (1)	3 (14062)	0
95	*ACTC1*	c.968C>T	p.Ala323Val	Likely Pathogenic	1	3 (3)	1 (180771)	0

Figure 1. Haplotype analysis of recurrent mutations at CpG dinucleotides. Tables show allele sizes in base pairs. For inferred haplotypes, both alleles are shown if the haplotype could not be resolved. *Directly determined haplotype from family co-segregation analysis; (+) causal variant present.

Figure 2. Haplotype analysis of recurrent mutations *MYBPC3* c.1504C>T, *MYBPC3* c.1624G>C and *MYBPC3* c.1624+4A>T. Tables show allele sizes in base pairs. For inferred haplotypes, both alleles are shown if the haplotype could not be resolved. *Directly determined haplotype from family co-segregation analysis; (+) causal variant present.

Supplementary Figure 1

MYBPC3 c.772G>A

Patient ID	46	47	48	49	50
Haplotype	**1**	**1**	**2**	**2**	**3**
D11S1344	289	289	297	297	291/297
MYBPC3-CA	383	383	379	379	383
c.772G>A	+	+	+	+	+
D11S4109	171	171	175	175	167/179

MYBPC3 c.927-9G>A

Patient ID	54	55	56
Haplotype	**1**	**1**	**2**
D11S1344	297	297	295/297
MYBPC3-CA	379	379	383
c.927-9G>A	+	+	+
D11S4109	183	183	173/175

MYH7 c.1816G>A

Patient ID	57	58	59	60
Haplotype	**1**	**2**	**3**	**4**
D14S990	119/121	119	115/127	113/123
MYOI	313/323	311/313	313/317	317/319
c.1816G>A	+	+	+	+
MYOII	139/149	145/153	145/147	151/153
D14S972	205/209	205/209	205/213	205

MYH7 c.1988G>A

Patient ID	61	62	63
Haplotype	**1**	**2**	**3***
D14S990	113/115	117/125	123
MYOI	313/317	311/313	315
c.1988G>A	+	+	+
MYOII	147/149	147/151	145
D14S972	205	207/215	205

MYH7 c.2389G>A

Patient ID	76	77	78
Haplotype	**1**	**2**	**3**
D14S990	117/119	119	117/119
MYOI	311/315	313	317/321
c.2389G>A	+	+	+
MYOII	147/151	147/151	137/147
D14S972	205/207	205/209	211

MYH7 c.4135G>A

Patient ID	71	72	73	74
Haplotype	**1**	**2**	**3***	**4**
D14S990	113/127	119	125	119/129
MYOI	313/321	313	313	313/317
c.4135G>A	+	+	+	+
MYOII	137/147	151/153	147	141/147
D14S972	205/209	205/213	213	203/209

Supplementary Figure 2

MYBPC3 c.1504C>T

Patient ID	1	2	3	4	5	6	7	8	9	10	11	12	13
Haplotype	**1***	**1**	**1***	**1***	**1***	**1**	**1***	**2**	**2**	**2**	**2***	**3***	**3**
D11S1344	289	289	289	289	289	289	289	289	289	289	289	293	293
MYBPC3-CA	383	383	383	383	383	383	383	383	383	383	383	383	383
c.1504C>T	+	+	+	+	+	+	+	+	+	+	+	+	+
D11S4109	179	179	179	179	179	179	179	177	177	177	177	177	177

MYBPC3 c.1624G>C

Patient ID	20	22	23
Haplotype	**1**	**2***	**3***
D11S1344	295/297	293	295
MYBPC3-CA	383	383	383
c.1624G>C	+	+	+
D11S4109	171/181	181	177

MYBPC3 c.1624+4A>T

Patient ID	15	16	14	17	18
Haplotype	**1**	**1**	**2**	**2**	**2***
D11S1344	295/299	295/301	297	297	297
MYBPC3-CA	379/385	379/385	379	379	379
c.1624+4A>T	+	+	+	+	+
D11S4109	151/177	151/175	151	151	151