

The Origins of Hypertrophic Cardiomyopathy–Causing Mutations in Two South African Subpopulations: A Unique Profile of Both Independent and Founder Events

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Summary

Hypertrophic cardiomyopathy (HCM) is an autosomal dominantly inherited disease of the cardiac sarcomere, caused by numerous mutations in genes encoding protein components of this structure. Mutation carriers are at risk of sudden cardiac death, mostly as adolescents or young adults. The reproductive disadvantage incurred may explain both the global occurrence of diverse independent HCM-associated mutations and the rare reports of founder effects within populations. We have investigated whether this holds true for two South African subpopulations, one of mixed ancestry and one of northern-European descent. Previously, we had detected three novel mutations—Ala797Thr in the β -myosin heavy-chain gene (β MHC), Arg92Trp in the cardiac troponin T gene ($cTnT$), and Arg645His in the myosin-binding protein C gene ($MyBPC$)—and two documented β MHC mutations (Arg403Trp and Arg249Gln). Here we report three additional novel mutations—Gln499Lys in β MHC and Val896Met and Δ c756 in $MyBPC$ —and the documented β MHC Arg719Gln mutation. Seven of the nine HCM-causing mutations arose independently; no conclusions can be drawn for the remaining two. However, the β MHC Arg403Trp and Ala797Thr and $cTnT$ Arg92Trp mutations were detected in another one, eight, and four probands, respectively, and haplotype analysis in families carrying these recurring mutations inferred their origin from three common ancestors. The milder phenotype of the β MHC mutations may account for the presence of these founder effects, whereas population dynamics alone may have overridden the reproductive disadvantage incurred by the more lethal, $cTnT$ Arg92Trp mutation.

Introduction

Hypertrophic cardiomyopathy (HCM), an autosomal dominantly inherited primary cardiac disease, historically has been defined as a rare cardiomyopathy of unknown cause, characterized by hypertrophy of the ventricular muscle, in the absence of other predisposing clinical conditions (Teare 1958). Studies initiated during the past decade have, to some extent, changed this view of the disease. It is now known that HCM is a disease of the sarcomere, caused by ≥ 100 different mutations in seven genes encoding proteins constituting the cardiac contractile unit (reviewed in Bonne et al. 1998). Recent clinical observational studies performed in the general population have suggested that, far from being a rare genetic disorder, the prevalence of HCM is close to 1/500 individuals (Maron et al. 1995). Furthermore, although genotype-phenotype correlations have emphasized the varied clinical spectrum that long has been associated with this disease, they have also shown that ventricular hypertrophy is not an inevitable consequence of the presence of an HCM-causing mutation (Bonne et al. 1998). However, it appears that mutation carriers do remain at risk of sudden cardiac death, as adolescents or young adults, whatever the other clinical manifestations of their condition (Moolman et al. 1997).

Mutations that are associated with significant mortality, especially at a young age, confer a reproductive disadvantage. Consequently, within populations, recurring identical disease-causing mutations are more likely to have arisen independently, rather than to reflect a common ancestor (de la Chapelle et al. 1993). An investigation of the origin of eight different HCM-causing mutations in the β -myosin heavy-chain gene (β MHC) revealed that they had arisen independently in ≥ 12 of the 14 families studied (Watkins et al. 1993b). This finding led Watkins et al. (1993b) to predict that the prevalence of HCM-causing mutations would be comparable in all populations globally and that founder mutations would be rare. The continuing identification of diverse disease-associated mutations in various sarcomeric pro-

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tein-encoding genes in populations in North America, Europe, Asia, Australia, and South Africa (Bonne et al. 1998, and references therein) supports this reasoning.

Indeed, reports of evidence of founder effects in familial HCM are rare and often incidental to the main investigation. The first such report, which appeared in 1993, described two small families of Portuguese descent harboring the β MHC Gly584Arg mutation (Watkins et al. 1993b). Five years later, truncating mutations in myosin-binding protein C (*MyBPC*), which were likely to be identical by descent, were detected in Japanese families, with three kindreds bearing the InsG791 mutation and two bearing the Int12ASA-2G mutation (Niimura et al. 1998). In the same year, it was suggested that the Asp175Asn mutation in the α -tropomyosin gene (α TM), borne by four Finnish families, might have arisen from a common ancestor (Jääskeläinen et al. 1998).

We have undertaken investigations of the molecular spectrum of HCM-causing mutations in the seven implicated genes (reviewed in Bonne et al. 1998) in two subpopulations living in the Western and Eastern Cape provinces of South Africa—namely, persons of mixed ancestry and those of white descent. The history and population dynamics of these two groups, from the 17th century to the present, is probably responsible for the presence of several well-documented founder effects, particularly in persons of Afrikaans descent (Kotze et al. 1991; Brink et al. 1995; De Jager et al. 1996; Warnich et al. 1996). The Afrikaners are a subgroup of Afrikaans-speaking South Africans of northern-European origin, who have retained a cultural identity largely through intermarriage and who show minor differences, in allelic-gene frequencies, from the English-speaking white subpopulation (Botha and Pritchard 1972). The mixed-ancestry and white subgroups represent an ideal model with which to investigate whether the HCM-causing mutations that they carry generally arose independently and fairly recently or whether founder effects are present in a particular population context. A comparison of the HCM-associated mutational spectrum of the particular South African subpopulations studied versus that in other countries would indicate whether the mutations either have an independent origin or possibly emanate from Europe. In the case of identical mutations being detected in apparently unrelated families, haplotype analysis would ascertain whether they were of independent or founder origin, on the basis of the chromosomal background in which they are embedded.

Previously, we had reported three β MHC mutations—namely, the novel Ala797Thr (Moolman et al. 1995), the Arg249Gln (Posen et al. 1995), and the Arg403Trp (Moolman et al. 1993; Posen et al. 1995) mutations—as well as novel mutations in *cTnT* (Arg92Trp [Moolman et al. 1997]) and *MyBPC* (Arg645His [Moolman-Smook et al. 1998]).

Table 1**HCM-Causing Mutations Present in Two South African subpopulations**

Gene, Mutation, and Source	Ethnic Group	Reference
<i>βMHC:</i>		
Arg249Gln: ^a		
Ped108	Mixed ancestry	Posen et al (1995)
Arg403Trp: ^b		
Ped106	Mixed ancestry	Posen et al. (1995)
SB1140	Mixed ancestry	Present study
Ala797Thr:		
Ped101	White	Moolman et al. (1995)
Ped104	Mixed ancestry	Present study
Ped110	Mixed ancestry	Present study
Ped124	White	Present study
Ped131	White	Present study
Ped138	White	Present study
SB902	White	Present study
SB983	Mixed ancestry	Present study
SB955	Mixed ancestry	Present study
Glu499Lys:		
SB1112	White	Present study
Arg719Gln: ^c		
SB1159	White	Present study
<i>cTnT:</i>		
Arg92Trp:		
Ped100	Mixed ancestry	Moolman et al. (1997)
Ped109	Mixed ancestry	Moolman et al. (1997)
Ped137	Mixed ancestry	Present study
Ped139	Mixed ancestry	Present study
Ped142	Mixed ancestry	Present study
SB385	Mixed ancestry	Present study
<i>MyBPC:</i>		
Arg654His:		
Ped136	Mixed ancestry	Moolman-Smook et al. (1998)
Δ c756:		
SB752	Mixed ancestry	Present study
Val896Met:		
SB852	White	Present study

^a Also described, by Watkins et al. (1992), in a Canadian family.

^b Also described, by Dausse et al. (1993), in a French family and, by Al-Mahdawi et al. (1994), in two Polish families.

^c Also described, by Consevage et al. (1994), in a Hispanic family.

In the present study, we describe detection of a further three β MHC mutations, two of which are novel, and detection of two previously unreported *MyBPC* mutations and show that the β MHC Arg403Trp mutation arose independently in South Africa and France. Thus, it appears that a high proportion of the South African HCM-causing mutations are unique to South Africa, confirming the expectation that, in particular subpopulations, HCM is caused by a high proportion of relatively new mutations. However, both unlike the mutation profile most often seen in other countries and contrary to generally held views, which have been discussed by Wat-

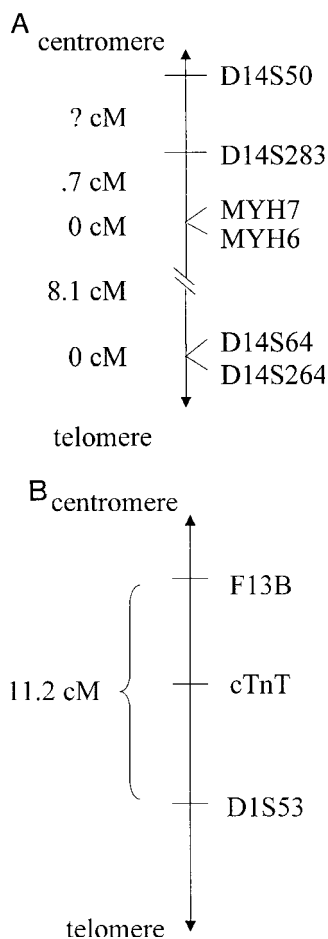


Figure 1 A, Genetic map of the region flanking the β MHC locus on chromosome 14q11-12. The order of genetic markers, as well as the genetic distances between them, used in haplotype construction in families harboring the β MHC Arg403Trp and the Ala797Thr mutations are shown, according to GeneMap '99. MYH7 = β MHC; MYH6 = α MHC. B, Genetic map of the region flanking the c TnT locus on chromosome 1q3. The order of genetic markers, as well as the genetic distances between them, used in haplotype construction in families harboring the c TnT Arg92Trp mutation are shown, as mapped by Watkins et al. (1993a).

kins et al. (1993b), we also report the existence of strong founder-gene effects in both of the subpopulations studied. Both the occurrence of founder-gene effects and the history of the South African subpopulations in which they were detected are discussed, together with the significance that these results have for molecular diagnosis.

Patients and Methods

Patient Study Group and Clinical Examination

The study was performed with the approval of the University of Stellenbosch ethics committee, with informed consent obtained either from subjects or, in the

case of minors participating in the study, from parents. A panel of apparently unrelated HCM probands (denoted by the prefix "SB"), of mixed ancestry or white descent, who reside in the Western and Eastern Cape provinces of South Africa, had previously been established, all of whom were screened for reported and novel mutations. After identification of a disease-causing mutation in a proband, relatives were traced and pedigrees were established, whenever feasible. The pedigrees extended in this manner were given a pedigree number (denoted by the prefix "Ped").

As a result of continuing prospective studies, at the time of the present study the panel consisted of 40 HCM-affected probands, including those 6 in whom an HCM-causing mutation had been detected in earlier studies; these were the probands of Ped108 with the Arg249Gln (Posen et al. 1995), Ped106 with the Arg403Trp (Posen et al. 1995), and Ped101 with the Ala797Thr (Moolman et al. 1995) mutations in β MHC, the probands of Ped100 and Ped109 with the c TnT Arg92Trp mutation (Moolman et al. 1997), and the proband of Ped136 with the MyBPC Arg654His mutation (Moolman-Smook et al. 1998). Mutations detected in the present study, those previously detected in South African families, relevant proband or extended-pedigree identification codes, and either the individual's or the family's ethnicity are shown in table 1.

Clinical examination was performed as described elsewhere (Posen et al. 1995). In brief, echocardiographic diagnosis of HCM was made in the presence of a maximal ventricular-wall thickness ≥ 13 mm, in the absence of contributing confounding factors. In children, diagnosis of HCM was made with reference to age-adjusted tables (Feigenbaum 1981, pp. 551–552). Electrocardiographic diagnosis of HCM was based on the presence of either left-ventricular hypertrophy or abnormal Q waves. Other echocardiographic or electrocardiographic changes previously described in familial HCM were noted for each patient.

Source of DNA and DNA Extraction

Peripheral blood for genotypic analysis was collected from all probands and family members and from healthy, unaffected, unrelated control individuals of mixed ancestry or white descent (100 individuals per control panel) entered in the study, and DNA was extracted as described elsewhere (Corfield et al. 1993). Dr. Lucy Carrier supplied DNA from French family 730 (Dausse et al. 1993).

Mutation Detection

Members of the HCM-affected panel were screened for previously reported and novel mutations in exons of β MHC (exons 3–10 and 12–23), MyBPC (exons 8, 18,

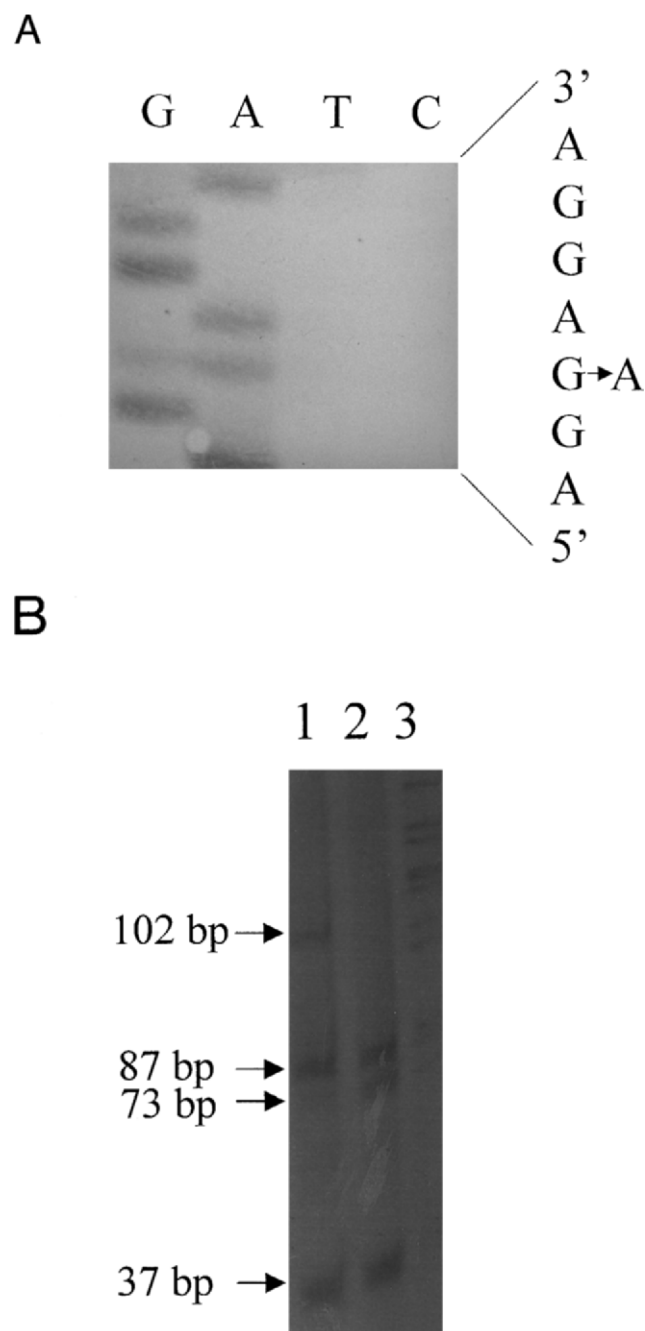


Figure 2 Identification of the Glu499Lys mutation in exon 15 of β MHC. **A**, Partial sequence of the coding strand of exon 15 of β MHC in an individual heterozygous for the Glu499Lys mutation, showing the g→a transition, which results in the amino acid substitution of Lys for Glu at codon 499. **B**, Genotyping the Glu499Lys mutation by ASREA. Products of *MnlI* digestion of the 243-bp PCR-amplified product of exon 15 were separated on a 12% polyacrylamide gel. Internal *MnlI* sites generated 87-, 73-, 37-, 16-, and 15-bp fragments in unaffected individuals (lane 2). Loss of one of these sites in an HCM-affected heterozygous individual resulted in an additional, undigested 102-bp fragment (lane 1). Lane 3, Lambda *PstI* molecular-size marker. The 16- and 15-bp fragments were not resolved on this gel.

24–28, and 34), *cTnT* (exons 8, 9, 11, and 14–16), α TM (exons 2b, 3–5, 6b, 7, 8, and 9ab), cardiac troponin I (*cTnI*) (exons 7 and 8), myosin essential light chain (*MELC*) (exon 4), and myosin regulatory light chain (*MRLC*) (exons 2 and 5) (Vikstrom and Leinwand 1996; DNA Mutation Database), by PCR-SSCP analysis, and allele-specific restriction-enzyme analysis (ASREA) when applicable. Amplified exons in which mobility shifts were identified by PCR-SSCP analysis were bidirectionally sequenced as described elsewhere (Posen et al. 1995).

In the case of the β MHC Glu499Lys mutation, exon 15 of the gene was PCR-amplified by use of custom-synthesized primers (DNA Laboratory, Cape Town)—MYH15F (5'-CACCCACTTTCTGACTGC-3') and MYH15R (5'-GAATTCAGGTGGTAAGGCC-3')—designed from published gene sequences (Jaenicke et al. 1990). In the case of variants Δ c756 and Val896Met, exons 24 and 27 (exon numbering is according to Niimura et al. 1998), respectively, of *MyBPC* were amplified by use of the relevant published primer sequences (Carrier et al. 1997). PCR amplification was performed in a standard reaction mixture (Moolman et al. 1993), during 30 cycles of the following cycling profile: denaturation at 95°C (30 s), annealing at either 52°C (for Glu499Lys) or 55°C (for Δ c756 and Val896Met) (30 s), and extension at 72°C (30 s), by a GeneE thermocycler (Techne). The sequence changes causing the β MHC Glu499Lys and *MyBPC* Val896Met mutations were verified by ASREA. The β MHC Glu499Lys nucleotide substitution abolished a *MnlI* site, whereas the *MyBPC* Val896Met mutation created an additional *NlaIII* site, in the respective PCR-amplified products. These ASREA tests—or, in the case of *MyBPC* Δ c756, PCR-SSCP analysis—were used to genotype 100 unrelated, unaffected control samples of the relevant ethnic group, for the presence of these mutations.

Previously reported mutations—namely, β MHC Arg719Gln (Conseville et al. 1994), additional β MHC Arg403Trp (Moolman et al. 1993) and Ala797Thr (Moolman et al. 1995) and *cTnT* Arg92Trp—mutation carriers (Moolman et al. 1997)—identified in HCM-affected probands and family members in the present study, were detected by ASREA, as described elsewhere.

Genotyping Markers at the β MHC and cTnT Loci

Members of Ped106, the French family 730, and individual SB1140 were genotyped for the β MHC Arg403Trp mutation, an intragenic marker in the 5' UTR region of β MHC (*MYH7* 5'UTR), and with β MHC-flanking markers at the *D14S50*, *MYH6*, and *D14S64* loci, covering a distance of ≥ 8.8 cM (fig. 1A) (GeneMap '99). In addition to these markers, members of Ped101,

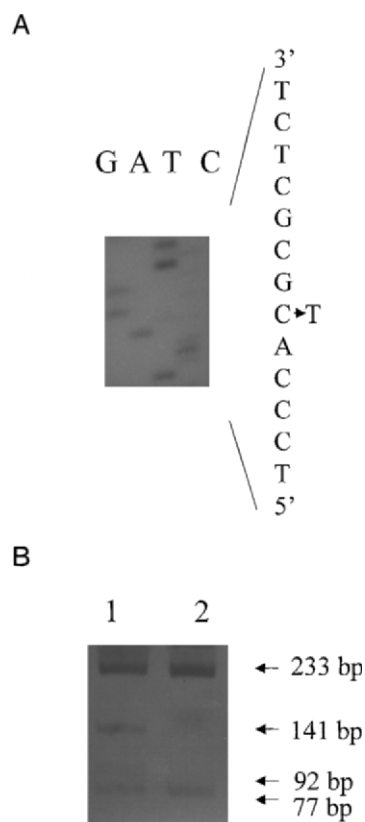


Figure 3 Identification of the Val896Met mutation in exon 27 of *MyBPC*. *A*, Partial sequence of the noncoding strand of exon 27 in an individual heterozygous for the Val896Met mutation, showing the *c*→*t* transition (*g*→*a*, coding strand) that results in the amino acid substitution of Met for Val at codon 896. *B*, Genotyping of the Val896Met mutation, by ASREA. Products of *Nla*III digestion of the 310-bp PCR-amplification product of exon 27 were separated on a 12% polyacrylamide gel. An internal *Nla*III site generated fragments of 233 and 77 bp in an unaffected individual (lane 2). The creation of an *Nla*III site in an HCM-affected heterozygous individual resulted in additional, 141- and 92-bp fragments (lane 1).

Ped104, Ped110, Ped124, Ped131, and Ped138 and individuals SB902, SB983, and SB995, all carrying the β *MHC* Ala797Thr mutation (table 1), were genotyped at two other loci—*D14S283* and *D14S264*. Members of Ped100, Ped109, Ped137, Ped139, and Ped142 and individual SB385, all carrying the *cTnT* Arg92Trp mutation (table 1), were genotyped with *cTnT*-flanking markers at the *F13B* and *D1S53* loci, spanning a distance of 11.2 cM (Watkins et al. 1993a), and with an exon 9 *cTnT* intragenic RFLP marker (*TnTex9*) (Thierfelder et al. 1994) (fig. 1B).

The short tandem-repeat markers at the *D14S50*, *D14S283*, *MYH7 5'UTR*, *MYH6*, *D14S64*, *D14S264*, *F13B*, and *D1S53* loci were analyzed by PCR-based assays, according to standard methods (Brink et al. 1995). The *TnTex9* marker was genotyped by ASREA after

TaqI digestion of the PCR-amplified product of exons 8 and 9, by use of a published primer sequence (Thierfelder et al. 1994). Forty members of each of the two control panels were genotyped at each marker locus analyzed in the family studies, to determine the frequency of alleles in the two subpopulations.

Haplotype and "Assumed" Haplotype Construction

Most-likely haplotypes were constructed with reference to gene and marker order on the respective chromosomes, in a family context. In the case of probands without a traced family, evidence of identity by descent was accepted if the alleles present allowed construction of a haplotype (designated as the "assumed haplotype") identical with the disease-associated haplotype.

Results

Identification of Novel and Reported Mutations in the South African HCM-Affected Panel

During screening of the panel of HCM-affected probands, a novel guanine-to-adenine (*g*→*a*) transition was detected in exon 15 of β *MHC* in one individual (SB1112) (fig. 2A). This modification results in the substitution of lysine for the conserved wild-type glutamine at amino acid residue 499 in the protein (Glu499Lys), fortuitously resulting in the loss of an *Mn*II restriction-enzyme site, allowing mutation screening by ASREA (fig. 2B). Although it was not possible to extend the study to the proband's family, the mutation was not present in 100 white control individuals.

A further two, apparently novel mutations were also detected in *MyBPC*, one in each of two probands (SB752 and SB852) in the HCM panel. One of these mutations was a *g*→*a* transition in *MyBPC* exon 27 in SB852 (fig. 3A), which would cause the substitution of a methionine for a valine at residue 896 (Val896Met) and which, since it created an *Nla*III restriction-enzyme site (fig. 3B), could be detected by ASREA. The second nucleotide variation, detected in SB752, involves the deletion of a cytosine at the first base position in codon 796 in *MyBPC* exon 24 (Δ c756) (fig. 4A); this results in both a shift in open reading frame 3' to codon 796 and the predicted incorporation of 65 aberrant amino acid residues before premature truncation of the protein. This deletion did not create or abolish a restriction-enzyme site, and screening for the mutation was subsequently based on PCR-SSCP analysis (fig. 4B). It was not possible to perform extended family studies in the case of the two probands with these sequence variations. However, the sequence variations were not present in 100 control individuals of the appropriate subpopulation groups screened by ASREA or PCR-SSCP analysis.

In addition to the three novel mutations reported

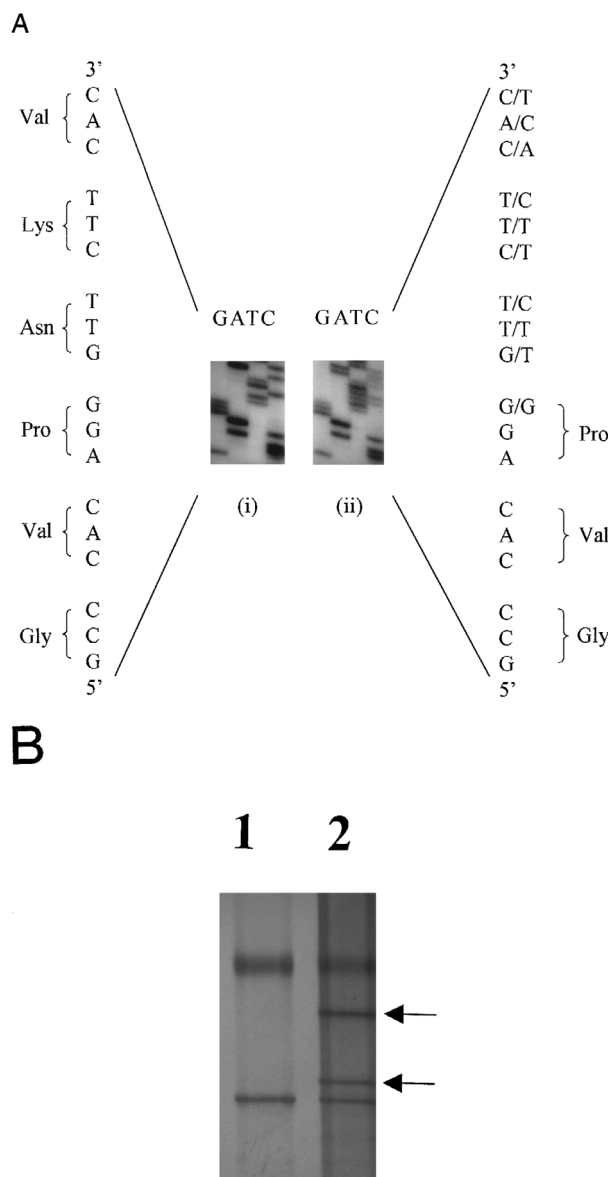


Figure 4 Identification of the cytosine deletion in codon 796 in exon 24 of *MyBPC*. *A*, Partial sequence of the noncoding strand of exon 24 of *MyBPC* in an unaffected individual (i) and a heterozygous HCM-affected individual (ii), showing the deletion of a g (c, coding strand) at nucleotide position 2298 (first position of codon 796), which results in a shift in reading frame. *B*, PCR-SSCP analysis of amplified products of exon 24 of *MyBPC*, separated on a 10% polyacrylamide gel containing 5% glycerol. The arrows indicate the mobility shifts caused by the Δc mutation at nucleotide 2298 in a heterozygous HCM-affected individual (lane 2). The single-stranded conformers of an unaffected individual are seen in lane 1.

above, three documented β MHC mutations were detected by ASREA in the present study—the Arg403Trp substitution in an additional proband (SB1140), the Ala797Thr substitution in eight more probands, and the Arg719Gln substitution in a single proband of the

HCM-affected panel. The previously reported *cTnT* Arg92Trp mutation was detected in a further four probands.

Thus, if the five mutations previously identified in six probands are included, a total of nine different mutations have been found in 23 of the individuals constituting the panel of 40 HCM-affected patients (table 1). In the remaining 17 probands, none of the ≥ 100 previously reported mutations were present (Bonne et al. 1998), nor were any novel mutations detected in the screening of selected exons of the seven candidate sarcomeric protein-encoding genes. Of these genetically undiagnosed individuals, 60% were white and the remainder were of mixed ancestry.

Origins of the β MHC Arg403Trp Mutation

The β MHC Arg403Trp mutation (Dausse et al. 1993; Posen et al. 1995) was embedded in two distinct haplotypes in South African Ped106 and French family 730, indicating their independent origins (fig. 5). However, individual SB1140 shared alleles—and, therefore, an assumed disease-associated haplotype—with Ped106, implying the existence of a common ancestor (fig. 5). The validity of this assumption was supported by the fact that the allele occurring at each of the markers from which the disease-associated haplotype was constructed was not the one carried most commonly in the control mixed-ancestry population (fig. 6, legend). The disease-associated haplotype extended from *D14S50* to *D14S64*, a distance of ≥ 8.8 cM, with no evidence of recombination in the extended Ped106 (data not shown).

Origin of the β MHC Ala797Thr Mutation

In the six pedigrees—Ped101, Ped104, Ped110, Ped124, Ped131, and Ped138—that harbored β MHC Ala797Thr, a common core haplotype encompassing the β MHC and α MHC loci segregated with the disease-causing mutation, suggesting descent from a shared ancestor (fig. 6). Furthermore, the two parents shown in the Ped101 subset (fig. 6) both carried the Ala797Thr mutation, embedded in the disease-associated haplotype, suggesting that they may be related. Within the families, the shared haplotype generally extended ≥ 8.8 cM across the region flanking β MHC and α MHC, with evidence of recombination both between *MYH7* 5'UTR and the proximal *D14S283* marker, in Ped138, and between *MYH6* and the distal *D14S64* marker, in Ped104 (fig. 6). These events reduced the extent of the conserved haplotype to ≤ 8.8 cM, which, because of both the unavailability of mapped markers between *MYH6* and *D14S64* and the small genetic distance between *MYH7* and *D14S283* (fig. 1A), could not be substantially narrowed further. The alleles at the marker loci genotyped in individuals SB902, SB983, and SB995 were also com-

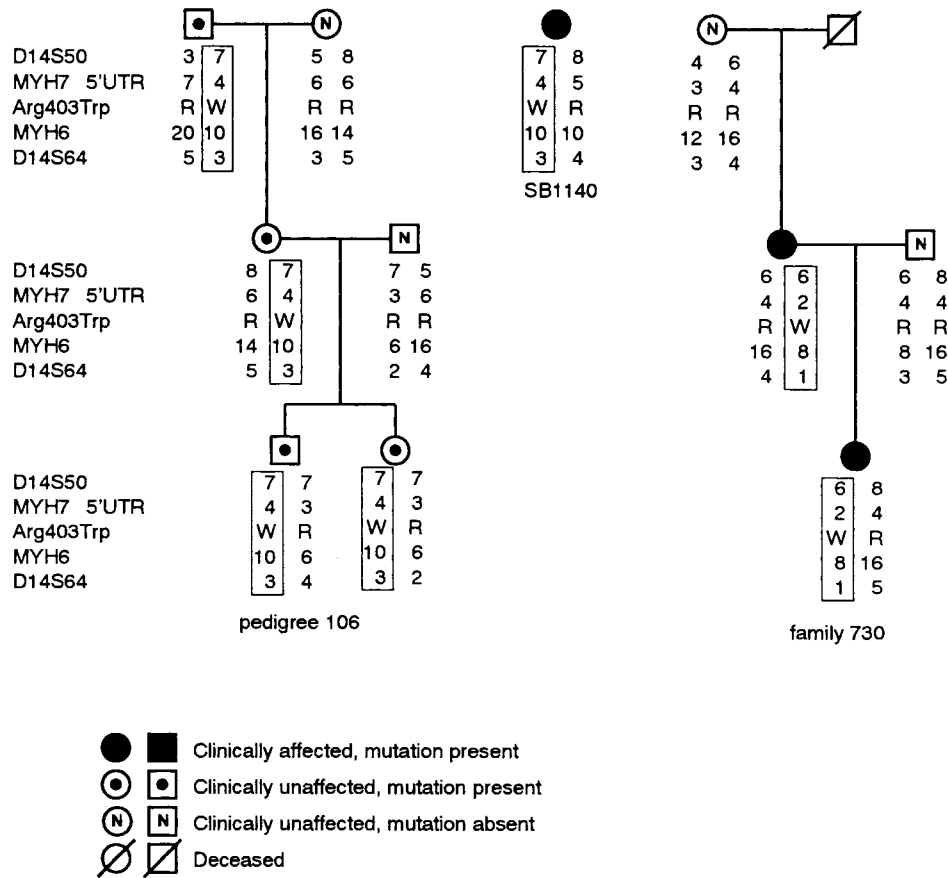


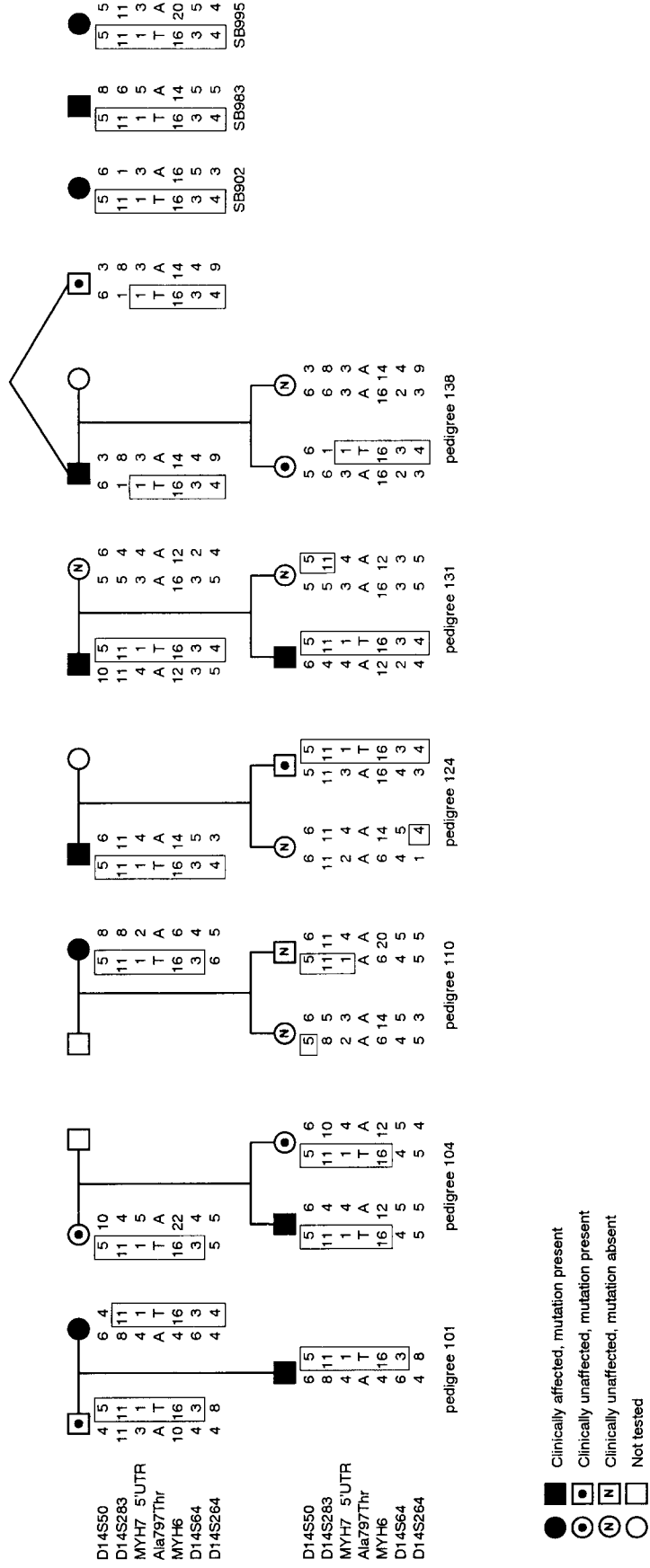
Figure 5 Haplotypes across the β and α MHC loci associated with the β MHC Arg403Trp mutation. The disease-associated haplotypes in a representative subset of South African Ped106 and the French family 730, as well as the assumed haplotype of individual SB1140, bearing the β MHC Arg403Trp mutation are boxed. In the married-in individuals, the haplotypes were inferred on the basis of those of their children. The marker loci used in haplotype construction, covering a distance ≥ 8.8 cM, are indicated to the left of the subsets, in centromeric-telomeric order, from top to bottom. The frequencies of the alleles of the markers genotyped in the control mixed-ancestry subpopulation are given in the legend to figure 6. Note that in the Ped106 subset shown there were no clinically HCM-affected mutation carriers; however, in the extended pedigree there were numerous affected individuals (Posen et al. 1995). MYH7 = β MHC; MYH6 = α MHC; R = arginine; W = tryptophan.

patible with that of the common extended ancestral disease-associated haplotype that was ≥ 8.8 cM (fig. 6). The existence of a common ancestor was supported by the fact that the allele at the markers from which the disease-associated haplotype was formed was generally not the one carried most commonly in either of the control subpopulation groups (fig. 6, legend). The only exception was at the *D14S50* locus in an individual in Ped101, in whom a recombination event had occurred. Five of the families or probands carrying the β MHC Ala797Thr mutation were members of the white subpopulation, and four were of mixed ancestry (table 1).

Origin of the cTnT Arg92Trp Mutation

In the five families that carried the *cTnT* Arg92Trp mutation and that could be traced (i.e., Ped100, Ped109, Ped137, Ped139, and Ped142), a common disease-as-

sociated haplotype, stretching, without recombination, between marker loci *D1S53* and *F13B* (a distance of ~ 11.2 cM) in the extended pedigrees, segregated with the mutation (fig. 7). Since genotyping was not continued beyond these markers, the size of the conserved fragment is unknown and may indeed extend further. In addition, individual SB385 possessed, at the markers genotyped, alleles that were compatible with the disease-associated haplotype. The data suggest that, in these families and this individual, all of which are members of the mixed-ancestry subpopulation, the mutation is identical by descent. The validity of this assumption was supported by the fact that the allele occurring at each of the markers from which the disease-associated haplotype was constructed was not the one carried most commonly in the control mixed-ancestry population, except at the biallelic *TnTex9* marker (fig. 7, legend).



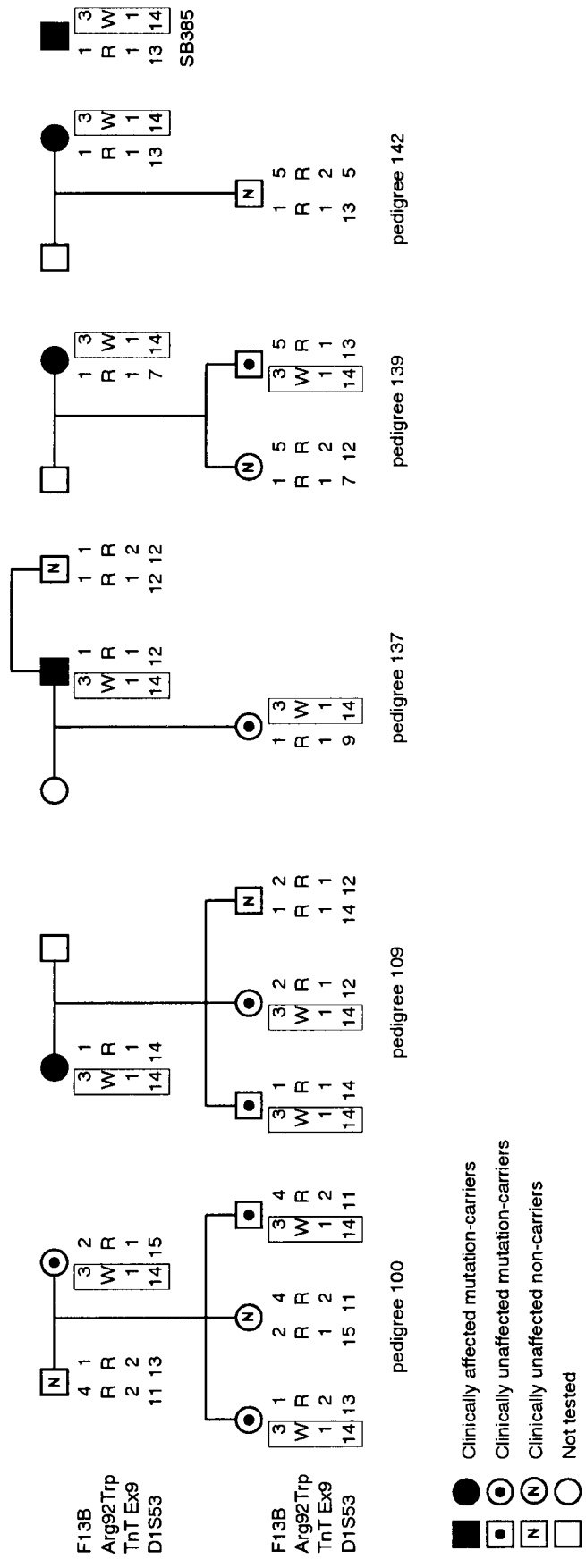


Figure 7 Haplotypes across the *cTnT* locus associated with the Arg92Trp mutation. The disease-associated haplotype in representative subsets of families Ped100, Ped109, Ped137, Ped139, and Ped142, as well as the assumed haplotype of individual SB385, bearing the Arg92Trp mutation are boxed. In the married-in individual, the genotype at each marker locus is shown; a haplotype was not constructed, since the phase was unknown. The marker loci used in haplotype construction, covering a distance of 11.2 cM, are indicated to the left of the subsets, in centromeric-telomeric order, from top to bottom. The frequencies of the alleles at the markers genotyped in the control mixed-ancestry subpopulation were as follows, listed in the order of marker-allele number: *F13B*—1 (.28), 2 (.23), 3 (.14), 4 (.08), and 5 (.27); *TnT*Ex9—1 (.70) and 2 (.30); and *D1S53*—1 (.05), 2 (.09), 4 (.02), 5 (.02), 7 (.05), 8 (.02), 9 (.11), 10 (.12), 11 (.09), 12 (.06), 13 (.09), 14 (.05), 15 (.14), 16 (.02), and 17 (.07). Note that, although, in the Ped100 subset shown, there were no HCM-affected mutation carriers, in the extended pedigree there were numerous affected individuals (Moolman et al. 1997). R = arginine; W = tryptophan.

Discussion

The Spectrum and Possible Effects of HCM-Causing Mutations in South Africa

Our investigations into the molecular causes of HCM in a panel of 40 apparently unrelated South African patients revealed that previously reported mutations, occurring worldwide, were apparently rare in this country. Of these mutations, only the β MHC Arg719Gln (Conseville et al. 1994) and Arg249Gln (Watkins et al. 1992; Posen et al. 1995) substitutions were detected in two separate probands of the South African HCM panel. Additionally, the β MHC Arg403Trp mutation, which originally had been described as occurring in South Africa (Moolman et al. 1993), subsequently was reported in French and Polish families (Dausse et al. 1993; Al-Mahdawi et al. 1994). Other than the aforementioned β MHC mutations, none of the ≥ 100 other mutations detected globally (Bonne et al. 1998, FHC database) in β MHC, *cTnT*, *MyBPC*, α TM, MELC, MRLC, or *cTnI* were present in the South African subpopulations screened. The α -cardiac actin gene, which recently had been implicated as another cause of HCM (Mogensen et al. 1999), was not screened in the present investigation.

We previously had described three novel mutations in the South African population—namely, β MHC (Ala797Thr) (Moolman et al. 1995), *cTnT* (Arg92Trp) (Moolman et al. 1997), and *MyBPC* (Arg654His) (Moolman-Smook et al. 1998). Here, we have presented evidence of three additional novel disease-causing mutations identified in HCM-affected individuals in the Western and Eastern Cape provinces of South Africa, one in β MHC and two in *MyBPC*.

The β MHC Glu499Lys substitution occurred at a residue conserved in myosin proteins, across a range of species—including human, rabbit, mouse, pig, hamster, and rat—and in a region that probably forms an interface with the reactive sulfhydryls (Rayment et al. 1995). The Δ c756 *MyBPC* deletion, which occurs in a region encoding the Ig-like module-C5 domain of the protein, results in a reading frameshift, with predicted production of an aberrant and prematurely terminated molecule. The resultant loss of the carboxy-terminal residues of *MyBPC* is likely to affect the ability of the protein both to be incorporated into the A-band of the sarcomere and to bind to myosin and titin (Freiburg and Gautel 1996). Considering the Val896Met substitution, structurally similar nonpolar amino acid residues with aliphatic hydrocarbon side chains occur at position 896 in *MyBPC*, across isoforms. Valine, which has a branched side chain, occurs in human cardiac *MyBPC* at residue 896 and differs, by a single methyl group, from the isoleucine residue found in human fast and slow, chicken cardiac,

and rat *MyBPC* isoforms at this position. It can be surmised that replacement of this valine by methionine, with its longer, unbranched side chain containing a sulfur group, will affect the conserved fibronectin type 3 structure of *MyBPC* module C7 (Gautel et al. 1995). The latter domain, along with modules C8–C10, is necessary for incorporation of the protein into the A-band of the sarcomere (Freiburg and Gautel 1996).

It was not possible to follow the segregation of either these described variants or the disease, in the families of the three probands. However, their occurrence in conserved positions and/or regions postulated to be functionally important protein domains of β MHC and *MyBPC*, as well as their absence in control panels of both subpopulation groups studied, implicates them as disease-causing mutations.

Independent Origin of the South African Mutations

In the 17th century, French Huguenots settled in the Western Cape region of South Africa (Botha and Beighton 1983), so the possibility existed that the β MHC Arg403Trp mutation in Ped106 and French family 730 were identical by descent. The mild phenotypic expression associated with this mutation on both continents (Dausse et al. 1993; Posen et al. 1995) also suggests that it might have spread by migration, since reproductive fitness might be unaffected in families harboring it. However, a comparison of the haplotypes associated with the mutation in these two kindreds provides evidence in favor of their independent origin (fig. 5). The β MHC Arg403Trp mutation has also been reported in Polish families (Al-Mahdawi et al. 1994), and the β MHC Arg249Gln and Arg719Gln mutations identified in the South African HCM-affected panel also occur in Canadian and Hispanic families, respectively (Watkins et al. 1992; Conseville et al. 1994). We do not know whether the South African versions of these mutations are identical by state or identical by descent, to their counterparts in the other populations. It is possible that French immigrants to Canada and South Africa may share common ancestry for the Arg249Gln mutation. However, since immigrants from Poland and Spain have not made a major contribution to the South African gene pool, it is likely that Arg403Trp and Arg719Gln are identical by state, rather than identical by descent, to those in these two countries.

Six of the nine HCM-causing mutations detected in South Africa are novel and, when the β MHC Arg403Trp data presented here also are considered, at least one of the other three also has an independent origin, whereas the source of the other two mutations could not be investigated. No other of the disease-associated mutations reported worldwide occurs in the South African groups studied. These data support the proposal that the profile

of HCM-causing mutations will be unique in different geographic areas and that it is the result of numerous nascent mutations.

Founder Effects in South African HCM-Affected Families

In addition to the evidence supporting the independent occurrence of several HCM-causing mutations in probands originating from the Western and Eastern Cape provinces, three of the mutations were found in more than one apparently unrelated affected individual. The β MHC Arg403Trp and Ala797Thr mutations were found in two and nine probands, respectively, and the *cTnT* Arg92Trp mutation was found in six apparently unrelated affected individuals. Haplotype analysis across the β MHC and *cTnT* loci in relevant families and individuals (table 1) revealed that all the individuals who carried the same mutation also shared either a disease-associated haplotype or a haplotype that was assumed to be disease associated (figs. 5–7). The haplotype was conserved over a region of ≤ 8.8 cM of the β MHC locus in persons harboring the Ala797Thr mutation and over a region of ≥ 11.2 cM of the *cTnT* locus in individuals carrying Arg92Trp. These data are consistent with the possibility that each of the three recurring mutations is identical by descent—because of the existence, in each case, of a common ancestor—rather than being identical by state.

This report is the first that substantiates the existence of strong founder effects as the cause of HCM in generally homogeneous population groups. Jääskeläinen et al. (1998) have suggested that the occurrence of the α TM Asp175Asn mutation in four unrelated Finnish kindreds might reflect the presence of a common ancestor. This finding would not be unexpected, since the genetically homogeneous population of Finland is known for its “Finnish disease heritage” (de la Chapelle 1993, p. 857). However, the proposal was based only on the fact that all the HCM-affected relatives shared the same allele at an α TM intragenic marker, and the frequency of this allele in this homogeneous population was not given. In the two Cape subpopulations studied, the likely existence of two founder individuals for the β MHC Ala797Thr and *cTnT* Arg92Trp mutations was based on extended haplotype data.

The presently reported exception to the general finding of the independent nature of HCM-causing mutations is not unanticipated. Founder-gene effects are not uncommon in South Africa, especially among the Afrikaner subgroup of the white population (Botha and Beighton 1983; Kotze et al. 1991; Brink et al. 1995; De Jager et al. 1996; Warnich et al. 1996). These effects are probably due to bottlenecks in gene transmission, caused by rapid expansion of the white population in the West-

ern Cape after the initial European colonization (Botha and Beighton 1983). During the period 1691–1791, the colony’s population increased from 1,000 to 13,028 (Botha and Beighton 1983), mainly because of natural expansion, since only 766 new European immigrant surnames were tabulated during this period (Theal 1922; Cameron and Spies 1992, pp. 11–139). At the same time, a group of ethnic admixture originated by intermingling of the European immigrants, mostly with the resident Khoi-khoi and San peoples (the Khoisan) but also with native black Africans and, later, with slaves transported from Malaysia and other East Indian countries (Cameron and Spies 1992, pp. 11–139). This group has become a distinct genetic entity, herein referred to as “mixed ancestry” (often referred to as “colored”), which has largely been maintained by intercultural matings (Botha and Pritchard 1972; May and Du Toit 1989). The history of the white and mixed-ancestry subpopulations of the Western Cape also explains the presence of the β MHC Ala797Thr mutation in both subpopulations. After the initial colonization of the Western Cape, changing economic circumstances resulted in the migration of individuals to the Eastern Cape (Botha and Beighton 1983).

The ethnicity of the three ancestors in whom the founder mutations arose cannot be ascertained; however, since our data suggest that it is unlikely that the mutations originated in Europe, it can be assumed that they arose in Africa. Since HCM-causing mutation screening has not been performed in the Khoisan or black African subpopulations, the latter’s contribution to the pool of disease-associated alleles cannot be established. It is possible that the β MHC Ala797Thr mutation arose in the white population, postcolonization, and thereafter was introduced into the mixed-ancestry group. However, both the mild phenotype generally accompanying this mutation (authors’ unpublished observations) and the disease-associated haplotype of ≤ 8.8 cM, allow speculation that this is an older mutation, which arose in the indigenous Khoisan people, whose presence in the Western Cape predated migration of black Africans from north of the Cape Province (Cameron and Spies 1992, pp. 11–139). Its presence in families of white descent would reflect the blurring of ethnic distinctions, because of earlier admixture among the subpopulations living in the Western Cape. In contrast, the reduced life expectancy previously reported to be associated with the *cTnT* Arg92Trp mutation (Moolman et al. 1997), together with the extensive conserved haplotype in which it is embedded in so many families, suggests a recent mutational event. Since this variant has been detected only in persons of mixed ancestry, it can be speculated that it arose in an ancestor of this subgroup and then, despite its malignant phenotype, increased in frequency during a time of rapid population expansion. The decreased

survival associated with this *cTnT* mutation was most pronounced in male family members (Moolman et al. 1997). Therefore, it is possible that inheritance of the mutant allele was either through female carriers in the generally large families seen to this day in this subpopulation group or through those male carriers who did survive to reproductive age. Rationally, pronounced founder effects are not expected to be associated with mutations resulting in significant mortality during reproductive years (de la Chapelle 1993; Watkins et al. 1993b). We propose that the population dynamics seen in the South African subpopulations studied here have overridden the reproductive disadvantage conferred by these mutations.

The prevalence of founder effects in a population may have an impact on the success of any molecular diagnosis program. In the Western and Eastern Cape provinces, the β MHC Ala797Thr mutation alone accounts for ~25% of the HCM cases in the panel, whereas the *cTnT* Arg92Trp is the causative mutation in 15%, and the β MHC Arg403Trp in 5%, of affected individuals. Three other β MHC mutations—namely, Arg249Gln, Arg719Gln, and Glu499Lys—collectively account for another 7.5% of disease cases. Therefore, in the South African subpopulations studied here, five β MHC mutations are collectively responsible for ~37.5% of HCM. This finding is very different from the profiles seen in most North American and European referral centers, where numerous “private” β MHC mutations are reported to account for ~30%–40% of HCM. Consequently, we have adopted a mutation-screening program in which any patient referred for molecular diagnosis is first screened for these three founder mutations, by ASREA, and more extensive mutation screening is performed only in their absence. Such a focused molecular diagnosis program saves both time and money.

Moreover, the presence of large cohorts of affected individuals who are members of relatively homogeneous subpopulations bearing the same β MHC Ala797Thr or *cTnT* Arg92Trp mutations will make it possible to develop a clearer picture of the true genotype-phenotype correlations. This is especially relevant, since it is increasingly being realized that the clinical expression of a disease is context dependent and is influenced not only by the presence of a recognized major-locus mutation but also by the genetic and environmental background in which it occurs (Bonne et al. 1998). In a previous study based on two families (Ped100 and Ped109), the *cTnT* Arg92Trp mutation was shown to be associated with only moderate hypertrophy but with a high risk of sudden cardiac death (Moolman et al. 1997). A similar clinical picture was evident in the additional families bearing this mutation (our unpublished observations). However, although the initial report of the β MHC

Ala797Thr mutation seen in one nuclear family suggested a poor prognosis (Moolman et al. 1995), continuing investigations have suggested a milder clinical disease course (authors' unpublished observations). It is anticipated that further extensive genotype-phenotype correlations will facilitate more-accurate prognostication and thereby improve patient management and counseling of this clinically insidious condition.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

DNA Mutation Database, <http://www.angis.org.au/Databases/Heart/heartbreak.html> (for familial HCM)

GeneMap '99, <http://www.ncbi.nlm.nih.gov/genemap/map.cgi?CHR=14>

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/omim> (for HCM [MIM 192600])

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