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Independent Origin of Identical β Cardiac Myosin Heavy-Chain Mutations in Hypertrophic Cardiomyopathy

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Summary

The origins of the β cardiac myosin heavy-chain (MHC) gene missense mutations that cause familial hypertrophic cardiomyopathy (FHC) in 14 families have been evaluated. Of eight different mutations, four were present in single families, while four occurred in two or more families. To investigate the origins of the four shared mutations, we defined the β cardiac MHC haplotypes of each of the mutation-bearing chromosomes by determining the alleles present at three intragenic polymorphic loci. Two of the mutations (Arg453Cys and Val606Met) have arisen independently in each of three families, being found on different chromosomal backgrounds. A third mutation (Gly584Arg) is associated with identical haplotypes in two families with Portuguese ancestors, suggesting a founder effect. Haplotype analysis was uninformative for the fourth mutation (Arg403Gln). Thus, FHC-causing mutations have arisen independently in at least 12 of the 14 families studied, suggesting that the majority have arisen relatively recently as new mutations. This finding predicts the prevalence of disease-causing β cardiac MHC mutations to be comparable in all population groups.

Introduction

Familial hypertrophic cardiomyopathy (FHC) is a primary heart muscle disorder usually inherited as an autosomal dominant trait (Maron et al. 1987; McKenna 1989). Molecular genetic studies have demonstrated that up to half of all cases of FHC result from missense mutations in the β cardiac myosin heavy-chain (MHC) gene (Watkins et al. 1992*a*). De novo occurrence of similar mutations in the β cardiac MHC gene can also result in sporadic cases of hypertrophic cardiomyopathy (Watkins et al. 1992*c*).

Nine missense mutations have been identified in individuals with FHC, all affecting conserved residues in the amino-terminal half of the peptide (Geisterfer-Lowrance et al. 1990; Rosenzweig et al. 1991; Epstein et al. 1992; Watkins et al. 1992*a*, 1992*c*). Of these, eight mutations are represented among the families described here (Watkins et al. 1992*a*, 1992*c*). Four of these mutations have been found in two or more apparently unrelated families. Two explanations could account for the finding of identical mutations in different families: this might reflect either a founder effect or recurrent identical mutation occurring independently. Were a founder effect to be present (i.e., apparently unrelated families descend from an unidentified common ancestor), then the disease-causing mutation would be found on the same chromosomal background in each family (Orkin and Kazazian 1984). To test this hypothesis, we have studied the β cardiac MHC haplotypes associated with the disease-causing mutation in each family affected by one of the four shared mutations. Haplotypes were constructed by typing the alleles present at three intragenic polymorphisms that span the mutations in question. These analyses reveal the presence of identical muta-

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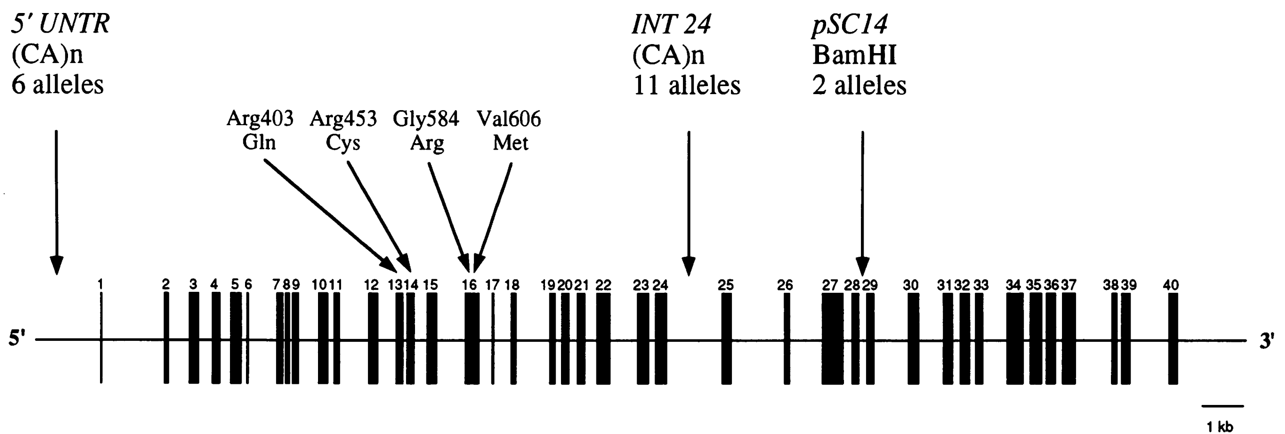

Figure 1 Location of polymorphisms and missense mutations within the β cardiac MHC gene. The four missense mutations that were found in multiple families are shown, designated by the normal amino acid residue, its position, and the residue substituted by the mutation.

tions on different chromosomal backgrounds, indicating an independent origin in each of three families for two of the mutations.

Material and Methods

Analysis of β Cardiac MHC Polymorphisms

Two of the polymorphisms analyzed were dinucleotide repeats within the β cardiac MHC gene: one in the 5′ untranslated region, designated "*5′UNTR*" and described elsewhere (Polymeropoulos et al. 1991), and one in intron 24, designated "*INT-24*" and amplified by the following primers (generating products of around 210 bp): INT-24F, 5′-GATATGCCATGTCTATCTGTGC-3′; and INT-24R, 5′-TGATCATACCAGTGAGATTAGATAC-3′. Alleles were typed by amplification by PCR with end-labeling of the forward primer, followed by PAGE, as described elsewhere (Chou et al. 1992). The third polymorphism was the *Bam*HI RFLP recognized by probe *pSC14* (Solomon et al. 1990*a*). The polymorphic site was mapped to intron 28, allowing typing by *Bam*HI digestion of a 248-bp PCR product amplified by the following primers: pSC14F, 5′-GAGACCCAGCGTTCTGTCAACGACC-3′; and pSC14R, 5′-CCTGGGGAAGCACCATTCTAGATCA-3′.

Haplotypes of Mutation-bearing Chromosomes

Genotypic analyses were performed on nuclear families from each kindred, to determine which alleles segregated with disease at the polymorphisms defined above. To ensure correct identification of particular alleles, DNA samples from individuals with a defined genotype were run on each gel, and, for confirmation, representative individuals from each family were run side by side on the same gel.

Results

The four mutations that have been identified in more than one family have been described elsewhere: Arg453Cys and Val606Met have each been found in three families, and Arg403Gln and Gly584Arg are each present in two families. Haplotypes have been determined for the mutation-bearing chromosome in each of the 10 families, by identifying alleles present at three intragenic polymorphic loci (figs. 1 and 2).

Arg453Cys

Families B (Solomon et al. 1990*b*) and E (Watkins et al. 1992*b*) have been described before as sharing the same missense mutation but with affected members of family B also bearing an a/b hybrid gene on the same chromosome (Watkins et al. 1992*a*). Affected members of family B have a unique allele identified by Southern analysis with probe *pSC14*, because of the rearrangement of the MHC genes on the disease-related chromosome (Tanigawa et al. 1990; table 1, allele 3). Family E is of British descent, while family B is of northern European and Native American ancestry. The Arg453Cys missense mutation was subsequently found also in family AE from Japan, whose affected members have typical features of FHC. Analyses of the three β MHC polymorphisms were fully informative, such that the alleles present on the affected chromosome in each family could be determined (table 1). Each family has a distinct

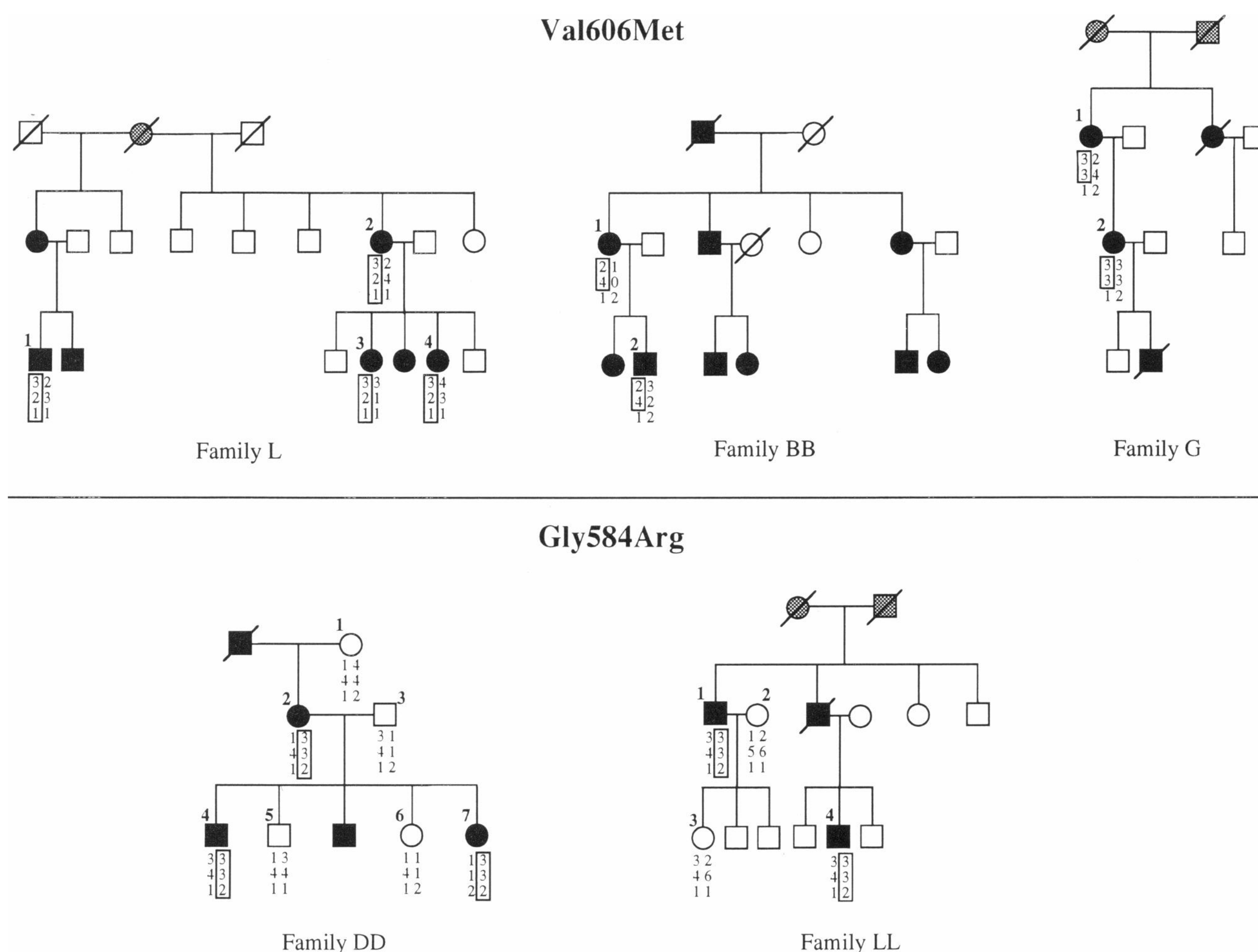

Figure 2 Pedigrees of families with the Val606Met and Gly584Arg mutations. An individual's identification number is shown above his or her pedigree symbol. The alleles at the *5'UNTR*, *INT-24*, and *pSC14* polymorphisms are shown for representative individuals. The haplotype that segregates with the FHC-causing mutation is boxed. In the upper panel, three families are shown to carry the Val606Met mutation on different chromosomal backgrounds; in the lower panel, two families with the Gly584Arg mutation share an identical haplotype. Pedigrees of families with the Arg403Gln mutation (Geisterfer-Lowrance et al. 1990) and Arg 453Cys mutation (Solomon et al. 1990*b;* Watkins et al. 1992*b*) have been published elsewhere.

combination of alleles on the affected chromosome, indicating that they did not originate from a common ancestor.

Val606Met

Three apparently unrelated families have been identified with this mutation: families G and L have British ancestors; family BB ancestors emigrated to the United States from eastern Europe. Although analysis of inheritance of alleles at the *pSC14* polymorphism was not informative for family G, the dinucleotide repeat polymorphisms were fully informative for the three families (figs. 2 and 3 and table 1). As with the Arg453Cys mutation, the disease gene in each family is associated with a different haplotype. Thus this mutation also has occurred independently in each kindred.

Arg403Gln

Because of the small size of family SS (pedigree not shown), it was not possible to determine which of the

Table 1

β MHC Haplotypes of Families with Identical Mutations

Mutation and Family	No. of		
	5'UNTR	*INT-24*	*pSC14*
Arg453Cys:			
E	2	4	1
AE	2	5	1
B	3	3	3[a]
Val606Met:			
L	3	2	1
BB	2	4	1/2[b]
G	3	3	1/2[b]
Arg403Gln:			
A	3	4	1
SS	1/3[b]	1/4[b]	1/2[b]
Gly584Arg:			
DD	3	3	2
LL	3	3	2

[a] A novel allele due to the presence of a hybrid gene (Tanigawa et al. 1990; see text).

[b] Haplotyping was not sufficiently informative to allocate a single allele to the mutation-bearing chromosome.

alleles present at each polymorphism should be assigned to the affected chromosome. The haplotype of the disease chromosome in family A (Geisterfer-Lowrance et al. 1990), a large family of French-Canadian descent, identifies alleles that are carried by one or other of the chromosomes in family SS, a family of British origin (table 1). Thus the possibility exists that the disease chromosome shares the same haplotype in both families.

Gly584Arg

Families DD and LL with the Gly584Arg mutation are both of Portuguese descent. Moreover, both carry identical β MHC haplotypes on the mutation-bearing chromosome (figs. 2 and 3 and table 1). While this is highly suggestive of a founder effect, the frequency of this particular haplotype among the Portuguese population is unknown, and so the likelihood of a random coincidence of the two haplotypes cannot be assessed.

Discussion

Of the 14 families in which we have documented FHC-causing β cardiac MHC mutations, 4 have unique mutations (Watkins et al. 1992*a*). Further, two of these mutations have occurred de novo in the generation under study (Watkins et al. 1992*c*). The data presented here demonstrate that, of the shared mutations, two (Arg453Cys and Val606Met) are present in unrelated families with different haplotypes, excluding a founder effect. Therefore the β cardiac MHC mutations have arisen independently in at least 12 of the 14 families studied, indicating that the majority of FHC is attributable to new mutation in recent generations.

This finding is in keeping with the negative selective pressure predicted for an autosomal dominant disease gene that is associated with significant mortality before reproductive age (Maron et al. 1987; McKenna 1989). The only alternative explanation for the different haplotypes would be that the mutations are ancient and precede either recombination between the mutation and

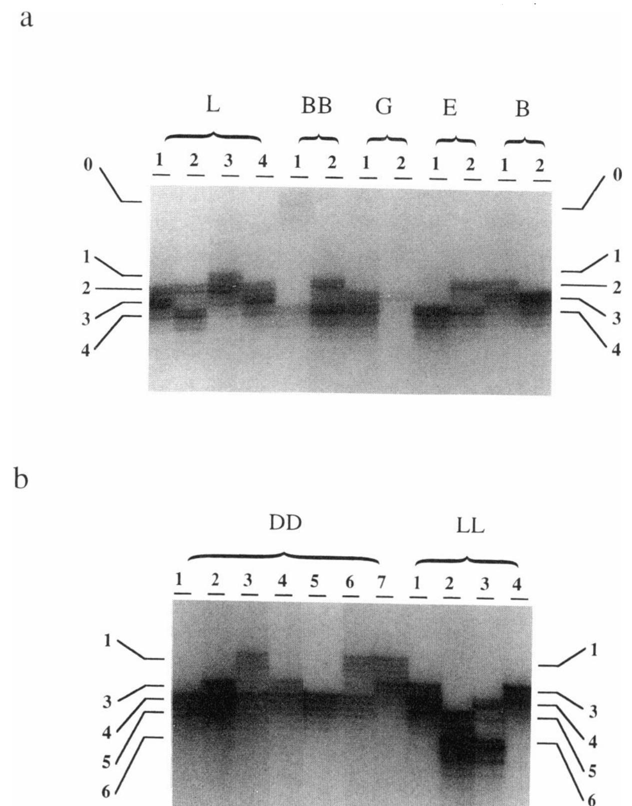

Figure 3 Alleles at the *INT-24* polymorphism on PAGE. Lanes are identified by an individual's identification number. The different alleles present (numbered 0–6) are identified, with consistent numbering in the two gels. *a,* Different alleles segregating with the Val606Met mutation. All individuals on the gel carry a mutation; hence the Val606Met mutation segregates with allele 2 in L, allele 4 in BB, and allele 3 in G. Members of families E and B with the Arg453Cys mutation are shown for comparison. *b,* Inheritance of alleles in families DD and LL, which indicates that in both families the mutation is carried on a chromosome bearing allele 3.

the polymorphism or mutation to a new allele at the polymorphism. This possibility is effectively excluded by the negative selective pressure, the proximity of the polymorphic markers on both sides of the mutations (<10 kb), and the low mutation rate at these polymorphisms (no mutations detected in >200 meioses examined; authors' unpublished data). The existence of identical mutations on different chromosomal haplotypes has been noted in other dominant disorders, e.g., retinitis pigmentosa (Dryja et al. 1991) and hyperkalemic periodic paralysis (Wang et al. 1993). However, in disorders such as these, which are not associated with significant negative selective pressure, this finding may reflect either recurrent mutation or that the disease-causing mutations preceded mutation at the polymorphic dinucleotide repeat locus.

Estimates based on linkage analysis of the proportion of FHC attributable to β cardiac MHC mutations in different geographic and ethnic populations have generated conflicting findings (Hejtmancik et al. 1991; Schwartz et al. 1992). One possible explanation would be that the prevalence of β cardiac MHC mutations varies by population. While such population differences could arise from a pronounced founder effect, our data suggest that the majority of FHC-causing mutations have arisen relatively recently, as the result of new mutations. We predict the prevalence of disease-causing β cardiac MHC mutations to be roughly equal in all population groups. This prediction is supported by the identification of β cardiac MHC mutations in ethnic groups other than those in which the first mutations were found (e. g., family AE from Japan).

Given that the β cardiac MHC coding sequence comprises 6,008 nucleotide pairs, the occurrence of identical mutations within this small sample is evidently not a random event. Two potential mechanisms may contribute to an excess of mutations at certain residues within this gene. The first would be that there is a predilection for mutation at certain nucleotides that are inherently more prone to mutagenesis and therefore act as hot spots for mutation. Seven of the nine β cardiac MHC mutations, including both that have occurred recurrently, can be accounted for by C→T transitions at CpG dinucleotides. CpG dinucleotides, although underrepresented in the human genome, are the site of >30% of all known disease-related point mutations; of these, 90% are accounted for by C→T transitions (Cooper and Youssoufian 1988; Green et al. 1990). However, there are many more CpG dinucleotide pairs in the β cardiac MHC gene than are involved in the documented FHC-causing mutations. It is likely, therefore, that a second mechanism is also involved—i.e., that of selection by phenotype. Of all the missense mutations that may occur in this gene, only mutation at certain sites may produce the clinical phenotype of FHC. Presumably, mutations at some sites are silent, while mutations at other sites may be lethal. Mutations within the critical sites of actin binding or ATPase activity in the nematode MHC gene, *unc 54*, produce complete loss of muscle function (Bejsovec and Anderson 1990).

The ability to make a genetic diagnosis of hypertrophic cardiomyopathy in affected families and individuals is of clinical benefit. Children and young adults may be diagnosed preclinically (Rosenzweig et al. 1991), at a time when they do not yet manifest features of cardiac hypertrophy but are at risk of sudden death (Maron et al. 1987; McKenna 1989). Identification of the precise mutation responsible appears to provide important data regarding life expectancy (Epstein et al. 1992; Watkins et al. 1992*a*). These findings imply that genetic screening of individuals with hypertrophic cardiomyopathy will be desired. Data available to date suggest that the variety of disease-causing β cardiac MHC missense mutations is large, without any one mutation accounting for a significant proportion of total cases. Further, it is now apparent that the majority of FHC has arisen because of an independent mutation, with few examples of founder effect mutations. We conclude that, even within a given population, effective genetic screening will require systematic screening of the β cardiac MHC gene, rather than attempts to detect known mutations.

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