Multiple disease genes cause hypertrophic cardiomyopathy

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The clinical features of hypertrophic cardiomyopathy (HCM) are heterogeneous. There is considerable variability in both the morphological manifestations of ventricular hypertrophy^{1,2} and the prognosis of affected individuals.³⁻⁵ This variation is often most apparent between unrelated families, but intrafamilial heterogeneity may also be quite pronounced.⁶ Sporadic cases widen clinical heterogeneity even further.⁷⁻⁹ Despite this, the clinical features of unexplained ventricular hypertrophy and the histological findings of extensive myocyte disarray with interstitial fibrosis¹⁰ were regarded as sufficiently specific to define a disease.^{11,12} Other phenotypes with some features that overlap with those of "typical" hypertrophic cardiomyopathy have been more difficult to classify clinically. These include apical hypertrophic cardiomyopathy,^{13,14} hypertrophic cardiomyopathy of the elderly,¹⁵ and families with myocyte disarray and sudden death but without hypertrophy.¹⁶

A more fundamental classification of these clinical entities is becoming possible with the insights provided by molecular genetics. Analyses in familial HCM show that genetic heterogeneity exceeds clinical heterogeneity—that is, hypertrophic cardiomyopathy is genetically not one disease but many. At least four separate disease genes can cause "classic" HCM, indicating greater complexity than might have been expected on clinical grounds. Whether such a genetic classification will be more useful than a clinical one will depend on the extent to which knowledge of the underlying molecular defect helps to predict clinical

outcome. In addition knowledge of the underlying molecular defect will assist diagnosis and may provide important insights into the disease process and the potential for intervention.

Genetic analyses

Because familial hypertrophic cardiomyopathy is inherited as an autosomal dominant mendelian trait, all affected members of a given family must have an identical gene defect. The defect need not be the same in unrelated families with familial HCM, however; either a different mutation within the same gene or a mutation in another gene could be responsible.

MISSENSE MUTATIONS IN THE β CARDIAC MHC GENE ARE A MAJOR CAUSE OF HYPERTROPHIC CARDIOMYOPATHY

The β cardiac myosin heavy chain (MHC) gene was first implicated as a possible disease gene for HCM by linkage analysis in a single large pedigree in which the disease gene was mapped to chromosome 14 q1.¹⁷ A point mutation in the β MHC gene that resulted in the substitution of a single amino acid in the myosin polypeptide was subsequently identified in all affected members of this family.¹⁸ Screening of the β cardiac MHC gene in unrelated individuals with familial HCM showed a missense mutation in this gene in up to 50% of families.¹⁹ Numerous different missense mutations have been identified (table), all are single nucleotide substitutions resulting in the change of a single amino acid in the

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β cardiac MHC mutations identified in FHC

Amino acid*	Nucleotide*	Exon	Change in charge	Survival†	Restriction‡ site	Reference
Thr124Ile	C457T	5	0			25
Arg249Gln	G832A	9	-1	Intermediate	- EcoRI	19,20
Gly256Glu	G853A	9	-1			28
Arg403Gln	G1294A	13	-1	Poor	+ DdeI	18,19,22,26
Arg453Cys	C1443T	14	-1	Poor		19,21,31
Phe513Cys	T1624G	15	0	Good		30
Gly584Arg	G1836C	16	+1		- MspI	19
Val606Met	G1902A	16	0	Good	+ NlaIII	19
Lys615Asn	G1931C	16	-1			24
Gly716Arg	G2232A	19	+1		+ SfiI	30
Arg719Trp	C2241T	19	-1	Poor		30
Arg723Cys	C2253T	20	-1		+ BcgI	23
Gly741Arg	G2307C	20	0			28
Asp778Gly	A2419G	21	+2			29
Leu908Val	C2808G	23	0	Good	- PvuII	22,27
Glu924Lys	G2856A	23	+2			19,23
Glu949Lys	G2931A	23	+2		- DdeI	19

*Mutations are denoted by the normal residue, its position (reference 49), and the mutant residue.

†Mutations for which there are sufficient survival data have been classified into categories of good, intermediate, and poor survival of affected individuals.

‡Changes in restriction enzyme site are denoted as + for gain in site and - for loss of site.

globular head or head-rod junction region of the MHC.¹⁸⁻³¹

All of the mutations share certain features that are characteristic of disease-causing missense mutations (as opposed to neutral polymorphisms): there is perfect cosegregation of the presence or absence of the mutation with the disease status of adult individuals; the mutations have been found only in individuals affected with familial HCM and never in unaffected individuals; and the amino acid substitutions affect residues that have been conserved throughout vertebrate evolution, implying particular functional importance. Although these characteristics of the mutations strongly suggest that they are disease causing, they do not exclude the possibility that the β MHC mutations are merely polymorphisms linked to an adjacent gene that is itself responsible for the disease. Thus the most conclusive evidence has been the demonstration of newly arising, or de novo, β cardiac MHC mutations in individuals with sporadic HCM.²³ Where a normal copy of the β cardiac MHC gene in an unaffected parent acquires a new mutation in an offspring who develops HCM, this cannot be attributed to chance association.

The finding of myosin mutations in sporadic HCM is one instance where genetic analyses have clarified the clinical classification of the disease—by showing that familial and sporadic forms of HCM are different parts of a single disease process. In some instances the newly arising β MHC mutations that cause sporadic HCM will be transmitted as familial HCM.²³ This finding has implications for genetic counselling of individuals with sporadic HCM and for assessing their prognosis in the absence of a family history.

OTHER DISEASE GENES FOR FAMILIAL HCM

Linkage analyses with markers in the region of the β cardiac MHC gene identified families in which HCM is not linked to this locus.³²⁻³⁴ These findings showed genetic heterogeneity, implying the presence of one or more additional disease gene loci for familial HCM. Direct screening suggests that mutations in the β cardiac MHC gene account for less than half of all cases of HCM,^{19,35} thus leaving much of the disease unaccounted for. The identification of other disease genes is now an important goal—that is, necessary both for clinical applications and for understanding the pathogenesis of myocardial hypertrophy. So far the chromosomal locations of three further disease loci for familial HCM have been identified by genome-wide searches using linkage analysis.

CMH2—A DISEASE LOCUS ON CHROMOSOME 1q3

A large family was identified with clinical and histological features typical of familial HCM; however, HCM was not caused by a β cardiac MHC mutation.³⁶ Linkage analyses were performed with highly polymorphic short tandem repeat (STR) sequences, which allow a systematic screening of all chromosomes to look for a polymorphism transmitted with the disease gene. In this way the disease gene in this family was mapped to chromosome 1q3 (fig 1). This new locus was designated CMH2 (cardiomyopathy, hypertrophic, 2) in keeping with HGM 10 nomenclature.³⁷

Subsequent linkage analyses in nine smaller families in which HCM was not linked to the β MHC gene showed that the disease was linked to the CMH2 locus in two of these families but not in the remaining seven, thus indicating the existence of further familial HCM loci.

Figure 1 Diagrams of the four chromosomes containing known disease gene loci for hypertrophic cardiomyopathy. The disease loci (designated CMH1-4) and relevant genes are identified by arrows (where the gene itself is known) or by square brackets (showing the range of possible map locations). Positions of four other contractile protein genes, previously considered candidate genes for CMH2 on chromosome 1, are also shown by square brackets.

CMH3—A DISEASE LOCUS ON CHROMOSOME 15q2
Of the families that were not linked to either the β cardiac MHC gene or CMH2, one was of sufficient size for a further genome-wide search using linkage analysis.³⁸ Affected members of this family show a phenotype in which hypertrophy was milder than in typical HCM families with β cardiac MHC gene mutations. Penetrance is incomplete in this kindred, with some members without demonstrable hypertrophy passing the condition to offspring. Linkage analysis mapped the disease gene in this family to chromosome 15q2 (fig 1). Of the remaining families known not to be linked to either CMH1 or CMH2, two were informative for linkage analyses with chromosome 15q2 markers. Multipoint linkage analyses showed that one of these families was linked to CMH3, while the other was unlinked. This last unlinked family again implied the existence of at least one further disease locus for familial HCM.

CMH4—A DISEASE LOCUS ON CHROMOSOME 11
A fourth disease locus was identified by similar analyses in a single large French family with typical autosomal dominant HCM.³⁹ In this family the disease gene was mapped to a region of chromosome 11 (fig 1).

The proportion of HCM that can be attributed to each disease locus is not yet known; nor is it known how many more disease loci there are. The data available suggest that each locus contributes a minority of the total disease prevalence. For example, of five families screened by linkage since the mapping of these disease loci, one was linked to each of CMH1 and CMH2, one mapped to the region of CMH4, the fourth was uninformative, and the fifth appeared unlinked to each of the known loci (H Watkins, H Yeon, and L Thierfelder, unpublished data). Workers expect that more than one disease locus remains to be mapped; this illustrates the remarkable genetic heterogeneity in this condition.

Many autosomal disorders that were regarded as a single clinical entity have been shown to be caused by mutations in more than one gene^{40 41} but few show the extent of genetic heterogeneity now documented in HCM. Perhaps this reflects the relatively crude techniques available to evaluate the phenotype of myocardial hypertrophy that do not distinguish phenocopies (unrelated conditions that mimic each other) as well as they might in other systems. Alternatively, in HCM mutations in related genes may have similar effects because they act through a common pathogenic pathway.

IDENTIFICATION OF THE DISEASE GENES AT CMH2 AND CMH3: MUTATIONS IN CARDIAC TROPONIN T AND IN α TROPOMYOSIN CAUSE HCM

If a family of related genes underlies the various disease loci then genes for cardiac muscle contractile protein would be the presumed candidates for the CMH loci. Many components of the thick filaments of striated muscle (for example, myosin light chains) and thin filaments of striated muscle (for examples iso-

forms of actin, troponin, or tropomyosin) interact directly or indirectly with the myosin heavy chains and might be involved in a common disease pathway.

Four genes for sarcomeric proteins had been mapped to regions of chromosome 1 that include the CMH2 locus (fig 1): genes encoding α skeletal actin, slow-twitch troponin I, the non-muscle isoform of α tropomyosin, and the human homologue of myosin binding protein-H. These candidate genes at the CMH2 locus have been investigated by the identification of intragenic STR markers to allow precise linkage mapping. Recombination between CMH2 and the genes encoding skeletal actin and non-muscle tropomyosin excluded these as HCM-causing genes at CMH2. Though there was no recombination between the slow-twitch troponin I gene or the myosin-binding protein H gene and CMH2, analyses of coding sequences did not show abnormalities in affected individuals (H Watkins and C MacRae, unpublished results).

A similar approach was then used to study the α tropomyosin gene as a candidate gene for HCM linked to the CMH3 locus on chromosome 15 on the basis of mapping data in the mouse. Two missense mutations (Asp175Asn and Glu180Gly) in the α tropomyosin gene were shown to cause HCM in the two families previously linked to chromosome 15q2.⁴² These two missense mutations occur in exon 5, which encodes part of a putative binding domain for troponin T. These findings supported the model that HCM is a disease of the sarcomere and suggested other thin filament protein genes as candidates for HCM linked to chromosome 1; in particular, cardiac troponin T was specifically implicated.

The previously unmapped gene for cardiac troponin T was mapped to chromosome 1q by polymerase chain reaction (PCR) amplification of somatic cell hybrids and then shown to be tightly linked to the CMH2 locus. Mutations in this gene were shown to cause HCM in affected members from three unrelated families.⁴² Two were missense mutations in exons 8 and 9 (Ile79Asn and Arg92Gln, respectively) and the other was a splice site mutation in intron 15 that is predicted to produce a truncated cardiac troponin T peptide. This mutation in cardiac troponin T is analogous to that found in *Drosophila melanogaster upheld*², a splice donor mutation that is functionally null because it encodes an unstable peptide. Thus, although most HCM-causing mutations are missense, the splice donor mutation in cardiac troponin T may be functionally null. This suggests that abnormal stoichiometry of sarcomeric proteins can cause cardiac hypertrophy and raises the possibility that an imbalance of sarcomeric proteins can cause secondary forms of cardiac hypertrophy.

Clinical applications

PRECLINICAL DIAGNOSIS AND MUTATION SCREENING

Because clinical diagnosis of HCM is often

Figure 2 Kaplan-Meier curves showing the survival of affected individuals with different β cardiac myosin missense mutations. Where available, data have been amalgamated from unrelated families with identical mutations.

difficult a genetically based diagnosis would offer many advantages. In particular, children who have inherited the disease gene may be at risk of sudden death before the onset of detectable myocardial hypertrophy. Unfortunately, genetic heterogeneity makes preclinical diagnosis more difficult in HCM than in conditions caused by a single or predominant mutation (for example Huntington's chorea⁴³). To date, genetic diagnosis has only been available for members of families with HCM resulting from β MHC mutations. The recent identification of mutations in cardiac troponin T and α tropomyosin causing HCM, however, will broaden the scope of such genetic testing.

Genetic diagnosis based on identification of β MHC mutations is itself complex because of the large variety of mutations causing disease. Some of the mutations have been identified in several families (for example Arg403Gln, Val606Met, and Arg719Trp (table), but many others have only been found in single kindreds. Even within groups of families sharing identical mutations, haplotype analysis has shown instances of independent origin of the mutations rather than a founder effect.³¹ This implies that most mutations that cause HCM have arisen in recent generations, indicating a relatively high new mutation rate for this gene. Thus effective detection requires systematic screening that will detect both known and novel mutations. Several screening techniques have been used to analyse β cardiac MHC DNA or cDNA sequences for point mutations, including ribonuclease protection assays,^{19-21 23 35} single-strand conformational polymorphism,^{22 24 28 29} and chemical cleavage of heteroduplexes.³⁰

In the initial screening of the β cardiac MHC gene the entire coding sequence was screened but no mutations were identified in

the coiled rod region.¹⁹ Subsequent searches for mutations have largely ignored the rod region on the assumption that missense mutations in this region cannot produce the HCM phenotype. Some support for this strategy is provided by linkage data on families in which no mutations were found on screening, showing no evidence of missed mutations.³⁵ This implies that, if they occur at all, mutations in the rod causing disease are very infrequent. A single deletion mutation in the terminal portion of the rod has been reported,⁴⁴ but without evidence of segregation with disease. Southern blot analyses in 20 probands with familial HCM did not find other examples of deletion mutations.⁴⁵

The feasibility of preclinical diagnosis has been shown in several kindreds.^{19 22 26} The value of preclinical diagnosis will depend on the success of therapeutic interventions in modifying the disease process or its complications; this potential may be greatest in those individuals without established hypertrophy in whom HCM is diagnosed preclinically. Moreover, trials of treatments can now be performed in homogeneous groups of patients. Preliminary evaluation of interventions should be possible in transgenic mice that carry mutated myosin heavy chain genes; these models would allow testing of both conventional pharmacological treatments and possible genetic manipulations.

Also important will be the degree to which the presence of a particular β cardiac MHC mutation predicts the outcome for any individual, especially the risk of sudden death. Does the presence of a β cardiac MHC mutation in a child necessarily indicate that important disease will follow? While evaluation of the extent of correlation between phenotype and genotype is undertaken, the implications of preclinical screening programs within families with HCM will require careful evaluation.

PROGNOSTIC IMPLICATIONS OF SPECIFIC MYOSIN MUTATIONS

The finding of multiple different mutations within the β cardiac myosin gene raises the possibility that it is this allelic heterogeneity that determines the wide variation in severity of HCM in unrelated families. The influence of a specific mutation can be determined by studying individuals from unrelated families—such that differences in outcome are more likely to relate to the specific mutation that they have in common rather than other genetic or environmental influences. Different branches of single large pedigrees are also adequate for these analyses because in a dominant disease the only genetic material that all affected members will have in common will be the region of the genome carrying the mutation causing the disease.

Phenotype/genotype analyses have not shown clear differences between clinical variables such as the echocardiographic features of hypertrophy,⁴⁶ but there were important differences in the survival of affected individuals. Kaplan-Meier analyses (fig 2) have identified some mutations associated

with particularly poor prognosis (for example, Arg403Gln, Arg453Cys, and Arg719Trp), with a high incidence of sudden death and significantly shorter survival than is seen with other mutations.^{19 22 30}

Certain mutations seem to have an intermediate effect on survival (for example, Arg249Gln,¹⁹) while three mutations have been reported to have a particularly good prognosis with near normal survival: Val606Met,¹⁹ Leu908Val,²² and Phe513Cys.³⁰ These three mutations differ from others in that they do not involve a net change in charge and so would be predicted to cause less severe disturbance to the structure and function of the myosin polypeptide. It is notable that some of the affected members of three unrelated families with the conservative Val606Met mutation had pronounced ventricular hypertrophy but still tended to survive longer than individuals with other mutations.¹⁹ These data suggest that the specific myosin mutation is a major determinant of survival and that knowledge of the precise defect may contribute significantly to the identification of those at increased risk of sudden death.

The findings with the three non-charge change mutations suggest that conservative substitutions may in general produce less severe disease. In addition, however, the location of the mutation within the globular head of the myosin polypeptide as well as the change in charge would be expected to determine the impact of each mutation. The tertiary structure of the head region of a myosin polypeptide has recently been established by x ray crystallography.⁴⁷ This allows identification of the location of each β cardiac MHC mutation relative to the functional domains and prediction of changes in polypeptide folding and shape in response to particular amino acid substitutions. One striking finding is that the Arg403Gln mutation, which produces the most severe phenotype, is situated in a critical position relative to the cleft involved in actin binding.⁴⁸ This finding was not predicted from the primary structure and so illustrates the potential value of this new data for determining the effects of the mutations causing HCM.

CLINICAL FEATURES OF INDIVIDUALS WITH MUTATIONS IN CARDIAC TROPONIN T AND TROPOMYOSIN

In a disease with three known disease genes, each of which is associated with multiple different mutations, the potential for phenotypic heterogeneity is considerable. For practical purposes it will be important to determine whether it is the disease locus itself or the specific mutation within it that most influences the natural history of hypertrophic cardiomyopathy in a given family.

With the β MHC gene locus the range of life expectancies for different mutations is as wide as is seen in the disease as a whole; no clinical features have been identified that distinguish the subgroup of families whose disease is accounted for by mutations in this

gene.^{19 46} Preliminary analyses of the other loci also suggest that specific mutations determine outcome and that classification by disease gene alone may not identify clinically distinct subsets. The families selected for the linkage analyses that ultimately identified the troponin T and α tropomyosin disease genes were chosen for their large size, rather than any phenotypic characteristics. Of the families now shown to have mutations in these genes, only one shows features that make it distinct from the typical range of HCM. One of the families shown to have an α tropomyosin missense mutation has significantly less myocardial hypertrophy than other familial HCM families, with many confirmed gene carriers with normal cross sectional echocardiograms.^{38 42} In contrast, affected members of a family with a different α tropomyosin missense mutation have typical hypertrophy. Presumably, then this aspect of the phenotype relates to the particular mutation and is not a general characteristic of α tropomyosin mutations.

INVOLVEMENT OF CMH LOCI IN VARIANTS OF HCM

No family with features of a clinically identifiable variant of HCM has yet been linked to any of the known HCM loci. Two families with apical HCM¹³ and one with disarray without hypertrophy¹⁶ have been examined by linkage analysis and do not seem to map to any of the CMH loci (H Watkins and L Thierfelder, unpublished data). Similarly, DNA from individuals with these phenotypes and also from individuals with Noonan's syndrome has been screened and no β cardiac MHC mutations found (H Watkins, unpublished data). Together these findings may indicate that such disease entities are genetically distinct from HCM and are the result of mutations in disease genes that are not involved in the pathogenesis of HCM itself.

Conclusions

The genetics of HCM are revealing a more complex picture than might have been expected on clinical grounds. Three disease genes have now been identified in which mutations result in the same clinical disease entity. These genes encode functionally related components of the cardiac sarcomere: β cardiac myosin heavy chain, α tropomyosin, and cardiac troponin T. Further disease genes for HCM exist and are likely also to encode components of the cardiac contractile apparatus. Within each disease gene a variety of mutations is anticipated, resulting in a range of clinical manifestations. Clinical application of genetic diagnosis is likely to remain complex, yet a genetic classification may help to identify subgroups with a different natural history of the disease. Preclinical diagnosis and evaluation of prognosis in association with mutation type, currently limited to those families with mutations in the β cardiac MHC gene, can now be extended to the α tropomyosin and cardiac troponin T genes.

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