Hypertrophic Cardiomyopathy: Genetic Advances And Their Clinical Implications

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Introduction

In 1958 Teare described marked asymmetrical cardiac hypertrophy in eight unrelated young adults who died suddenly (Teare 1958). This report stimulated the characterization of a syndrome, now termed hypertrophic cardiomyopathy (HCM). However, diagnostic criteria, like many aspects of this condition, continue to arouse controversy.

As methods for evaluating cardiac structure and function have improved, so diagnostic criteria have changed. In the 1960s, diagnosis was based on clinical features and a subaortic gradient at cardiac catheterization. The advent of M mode, followed by cross sectional echocardiography in the 1970s and 80s, led to new and broader diagnostic criteria. In a minority of patients, hypertrophy was seen to be concentric rather than asymmetric, and anatomical criteria were broadened, defining HCM as a hypertrophied non-dilated left ventricle in the absence of known cardiac or systemic disease that itself produced left ventricular hypertrophy. Histologically, myocyte disarray and myofibrillar disorganization have also been considered as an aid to diagnosis however, while present in hearts with HCM, neither feature is pathognomonic (Becker et al 1982).

There is as yet no single clinical, anatomical or functional criterion which can define a heart as being affected with HCM. With time, the range of features encompassed by the disease widens, and one must begin to consider where the boundaries lie. A recent description of a man with a family history of HCM who died suddenly highlights the problem. His heart was macroscopically normal but showed extensive myocyte disarray (Maron et al 1990). There is uncertainty as to which criteria, histological or anatomical, should be used to define whether this condition is HCM. Indeed, questions have been raised as to whether HCM is a single disease, or whether it represents a common end point of a number of distinct pathological processes.

Much of the confusion relating to this disorder can be attributed to a lack understanding of its aetiology and pathophysiology. Advances in genetic and molecular biology are at last rectifying this situation. This article shall describe these advances and discuss their current and potential clinical implications.

Identification of the candidate gene

The genetics of HCM were alluded to in Teare's original report; the sudden death of a brother of one of the cases, suggesting a familial element to the condition (Teare 1958). A subsequent study of two generations of this family, showed that asymmetrical hypertrophy was inherited in a manner consistent with an autosomal dominant trait (Hollman et al 1960). Large echocardiographic studies have since indicated that HCM is genetically transmitted in 55% of cases with the remaining 45% being apparently sporadic (Maron et al 1984; Greaves et al 1987).

In genetic disorders such as the thalassaemias, protein abnormalities were recognised before the genetic defect was characterized. In others, such as cystic fibrosis and HCM, the protein abnormality was not known and identification of the candidate gene relied on defining the chromosomal location of the defect. This in turn depended on linkage analysis, a study of the cosegregation of the disease trait with a DNA polymorphism of known chromosomal location, within a family pedigree. The characterization of familial cases thus allows analysis of the genetic defect, thence the polypeptide defect, and an understanding of pathophysiological mechanisms.

Linkage analysis in a large French Canadian family with familial HCM (FHCM) tested a panel

of probes on all chromosomes. Eventually one was found, identifying a locus on the long arm of chromosome 14, that consegregated with the disease (Jarcho et al 1989). The alpha and beta cardiac myosin heavy chain (MHC) genes had previously been assigned to this location (Saez et al 1987), and further studies with a cardiac beta MHC gene probe suggested linkage of HCM and the cardiac MHC genes (Solomon et al. 1990a).

Subsequent work has shown that disease is linked to MHC genes in only some pedigrees with a history of HCM. In half of the small numbers of families studied to date, disease is linked to a locus on chromosome 14q (Solomon et al 1990b; Elstein et al 1992; Epstein et al 1992; Schwartz et al 1992). Linkage with the MHC genes in at least 50% of familial cases, has led to speculation that abnormalities of other myofibrillary proteins may play a role in the development of this disease in the remaining families. Schwartz et al (1992) have excluded linkage with the cardiac actin genes in eight families studied. A different sarcomeric actin or other proteins necessary for myofibrillary organization and function may however still prove to be involved.

The defect in linked families may be in either the MHC genes, their regulator regions, or nearby but unrelated genes.

Early evidence for involvement of the beta MHC gene itself relates to the observation that the gene is expressed in skeletal, as well as cardiac muscle (Saez et al 1987). If mutations alter function in cardiac myocytes, one might also expect abnormalities of skeletal muscle in patients with FHCM. A small study has raised the possibility of subclinical myopathy in a subgroup of patients with HCM (Caforio et al 1989). One might speculate that the underlying defect in the patients with subclinical skeletal myopathy lay in the beta MHC gene. The patients without skeletal myopathy may be part of the group in whom no linkage has been demonstrated between HCM and the beta MHC gene, and in whom the abnormality may be in a cardiac specific protein.

The first reports of defects at the genetic level appeared in 1990. Tanigawa et al (1990) studied a family in which a locus at 14q11-12 cosegregated with HCM. The alpha and beta cardiac MHC genes of affected and unaffected individuals were compared using restriction enzyme maps. Affected individuals were found to have a partial duplication of these genes.

Molecular genetic analysis in a second family revealed a point mutation, rather than a duplication, in the cardiac MHC genes of affected individuals (Geisterfer-Lowrance et al 1990). A restriction fragment length polymorphism, derived from exon 13 of the beta MHC gene, was present only in individuals affected with HCM. The nucleotide sequence of this exon was identical in affected and unaffected individuals, except for a single nucleotide change converting an arginine to a glutamate residue at position 403.

It is possible that these characterized mutations are incidental findings, being linked to, but not causative of, disease phenotype, while nearby but uncharacterized mutations are responsible for disease pathogenesis. Evolutionary evidence, however, suggests a causative role for the mutations described.

A mutation is more likely to be causative than associative if it leads to an alteration in protein function. The functional importance of particular amino acids is suggested by their conservation through evolution. Fifteen different MHC polypeptides have been sequenced in a range of species that includes man, chicken and amoeba. Arginine residue 403, identified in the work by Geisterfer-Lowrance et al, is contained in a region of six highly conserved amino acids, and is invariant in all 15 polypeptides. A mutation altering an amino acid residue that has been conserved for over 600 million years may well lead to altered function and hence play a role in disease pathogenesis.

That the beta MHC mutations so far characterized are causally linked with FHCM is, however, not yet proven. Even if these mutations do play a role in disease pathogenesis, the following observations suggest that other genetic or environmental factors are also involved.

Firstly, beta MHC is the predominant form of

myosin in both the right and left ventricles of the human adult heart, and yet the lesion of HCM is predominantly in the left ventricle, and usually localized to the septum. Other factors must be responsible for this differential expression.

Secondly, Epstein et al (1992) studied a family in whom a missene mutation, in codon 908 of the beta MHC gene, was strongly linked with disease phenotype. Of the 31 individuals aged over 17 with this mutation, echocardiographic criteria for HCM were fulfilled in ony 19. The situation is clearly not one of autosomal dominant inheritance with 100% penetrance; other factors in addition influence disease expression.

Mutant MHC genes may play a role in disease pathogenesis in 50% of cases of FHCM. The role of these genes in the sporadic cases of HCM remains uncertain.

The characterization of genetic lesions in HCM has depended upon linkage analysis, a technique which can only be used to study pedigrees. The identification of specific mutations in affected individuals from such kindreds, raises the possibility of analysing either genomic or complementary DNA from sporadic cases, to determine whether the same genetic lesions are present in this group of patients. Sporadic occurrence might result from mutations in myocardial precursor cells; the mutant phenotype would then not be transmitted through the germline. Such analysis has not, however, been carried out to date.

From genotype to phenotype

Symptoms are a consequence of the expression of the genotype rather than the genotype itself; an understanding of the genesis of the phenotype will help elucidate the pathophysiological mechanisms underlying this disorder.

There are several models for the autosomal dominant phenotype produced by mutations in the cardiac MHC genes.

Firstly, myosin gene mutations may act in a recessive fashion, even though they are inherited as an autosomal dominant trait, with mutations of the normal allele being required for expression. Mutations in both alleles may result in myosin that is quantitatively or qualitatively abnormal and thus cause myocyte dysfunction. Such an inheritance pattern has been described for retinoblastoma (Cavenee et al 1983). This theory might explain the variable age of onset of disease phenotype in kindreds, for the second mutation may occur at any age. One might extrapolate that individuals with sporadic HCM have developed two de novo mutations. Given the reduced statistical likelihood of this developing, one might expect sporadic cases to present at a later age than familial ones. This, however, is not the case. Another difficulty with this theory is that mutationally altered MHC genes, unlike retiniblastoma genes lack any apparent selective advantage.

Secondly, the mutant MHC gene may result in a low level of functionally active MHC protein, the consequent imbalance between functionally active myosin and other myofibrillary components leading to impaired myofibril assembly and function. The autosomal dominant effects of such myosin gene dosage abnormalities are seen in drosophila (Beall et al 1989).

Thirdly, the gene product of the mutant allele, either mRNA or polypeptide may impair transcription, splicing, translation, protein assembly or function of the gene product of the normal allele. Such dominant effects have been demonstrated in the nematode Caenorhabditis elegans in which MHC mutant polypeptide interferes with the function of the residual normal heavy chain (Dibb et al 1985).

Given that myofibril organization is the same in patients with HCM and a missense mutation at position 403, those with HCM and undefined genetic lesions and in control patients (Vybiral et al 1992), it has been proposed that subtle functional abnormalities of mutant proteins are responsible for clinical HCM. Assuming that the mutant genes are expressed in cardiac myocytes, that the mutations characterized are at least in part responsible for disease pathogenesis, and indeed that genetic heterogeneity may explain phenotypic heterogeneity, how may mutations in the myosin proteins affect function?

The structure and function of the MHC protein has been studied in detail and three functionally important subdomains, the ATP, actin and myosin light chain binding sites, identified (Keihart 1990). The duplication mutation (Tanigawa et al 1990) and the eight point mutations (Geisterfer-Lowrance et al 1990; Watkins et al 1992) characterized in the beta MHC gene, affect the globular head region of the MHC protein, and the junction between the head and the tail. None of the mutationally altered sites lie within recognised functionally important subdomains. Nevertheless, evolutionary conservation of these sites does suggest a functional role. Mutations might alter myosin contractility and thus the stress strain relationship within the myocyte, hypertrophy occurring as a response.

Considerable work needs to be done before the significance of the mutations described to date, in the development of the disease phenotype, can be defined. Transgenic animals may provide some insights. The tailoring of beta MHC genes with those specific mutations found in human pedigrees and the transfection of these genes with appropriate regulatory elements into experimental animals at an early stage in development may help define relationships between genotype and phenotype.

Clinical implications of genetic research

Defining the genetic basis of a disease has a number of potential clinical consequences. Included amongst these are genetic counselling, screening at a presymptomatic stage allowing the introduction of treatments to alter disease natural history, and the development of more rational treatments based on an enhanced understanding of disease pathophysiology.

Methods of screening

A number of methods are available to screen for HCM: clinical, electrocardiographic, echocardiographic and, recently, the possibility of genetic. Sensitivity, specificity, complications and cost are important factors to consider with any screening technique. Of the first three options, echocardiography is the most sensitive and specific. It is, however, fraught with problems. Firstly, there is great heterogeneity in echocardiographic findings in this disease, and diagnostic criteria are constantly changing. Secondly, the interindividual variation in time course of development of morphological abnormalities, and chronological relationship between the development of these abnormalities and the onset of symptoms, makes HCM difficult to screen for with imaging techniques.

Genetic screening would allow earlier, even prenatal diagnosis and thus more time to intervene before the onset of symptoms. This method of screening, however, raises a number of problems.

The first concerns the nature of the population to be screened. Genetic screening would currently be possible in only 25% of cases of HCM, in whom the disease was both familial and linked to the beta MHC gene. Screening for the remaining cases of FHCM, and the sporadic cases of HCM, will clearly not be possible until the genetic defects have been defined in these cases.

The feasibility of screening the general population, if and when such defects are characterized, is questionable. The number of gene probes that would be needed raises technical problems. The range of mutations linked with FHC to date is large. As research advances, it is likely that more will be described. Even assuming that sporadic cases are the result of mutations similar to those responsible for familial cases, one can envisage the need for a large number of genetic probes to screen an individual for HCM with any degree of sensitivity. In addition the low prevalence of HCM in the population, estimated at 20 per 100,000 (Codd et al 1989), means that pick up rate would be low, raising cost benefit issues.

Rather than screening the general population, one might elect to screen a subgroup. This group could include individuals at a high risk of developing HCM, such as those with a family history, or those at high risk of complications if they do to develop HCM. Athletes with HCM would fall into this category, being at a high risk of sudden death (Maron et al 1980).

A second problem relates to the fact that not all mutations have the same positive predictive value for disease development. Penetrance of dif-

ferent mutations has been reported to vary from 60% to 100% (Epstein et al 1992). Sensitivity of genetic screening would thus vary depending on the particular mutation.

Aims of screening

The identification, by genetic screening, of individuals at a presymptomatic stage would allow the early introduction of treatments to alter disease natural history.

A devastating event in disease natural history is sudden death. Sudden death in patients with HCM has an incidence in adults of 2% per year, and 6% per year in children (McKenna et al 1981). It may be the first manifestation of disease, and a number of management options have been employed in an attempt to reduce its incidence.

The first alters the patient's lifestyle, limiting the amount of vigorous exercise undertaken. The rationale for this is that HCM is the commonest cause of sudden death amongst young athletes during exercise (Maron et al 1980). However, the majority of patients with HCM who die suddenly are not exercising strenuously at the time of death (Maron et al 1982). This suggests the existence of factors besides exercise that precipitate sudden death, and although individuals with HCM are advised not to exercise strenuously, other approaches are also necessary to reduce the risk of sudden death.

The use of amiodarone to reduce the incidence of sudden death in a subgroup of patients is supported by evidence from a trial with historical controls (McKenna et al 1985), but the case is not proven. Indeed, in some reports it has been associated with a higher incidence of sudden deaths (Fananapazir et al 1991). Other treatments such as beta blockers, and calcium channel blockers (Maron et al 1987), and automatic implantable defibrillators (Odemuyiwa et al 1991), have also been used but evidence of a reduction in sudden death is lacking. With such poor evidence that current treatment improves prognosis in HCM, the aims of screening are brought into question.

Genetic screening may, however, have researcl implications in the development of treatments al tering natural history. Those trials conducted tc date have used patients diagnosed either clinically after the onset of symptoms or echocardiographically after the development of morphological abnormalities. Genetic screening would allow for trials comparing the effects of treatment on natural history before the development of clinical or echocardiographic evidence of disease.

Many of the treatment options described have important drawbacks. Automatic defibrillators are expensive, while lifelong amiodarone treatment is associated with serious side effects. One might wish to use these treatments only in subgroups of HCM patients at very high risk of sudden death. A number of strategies are available for risk stratification.

High risk of sudden death has been predicted by non-sustained ventricular tachycardia on Holter monitoring in adults (McKenna et al 1981). In childhood and adolescence an unfavourable family history appears the best predictive factor (McKenna et al 1981) however this cannot be used in the 50% of individuals with sporadic HCM.

The possibility of risk stratification on the basis of genetic lesions is now raised. Studies of other genetic disorders has demonstrated that different mutations within the same gene can cause different phenotypes. Certain mutations in the dystrophin gene lead to Duchenne muscular dystrophy, whereas others produce the milder variant, Becker muscular dystrophy (Kunkel 1986). In FHCM, survival of affected individuals appears to be influenced by the particular mutation (Watkins et al 1992). The number of families studied to date is very small, but the suggestion is that those mutations resulting in a change in charge carry a worse prognosis than neutral mutations. One might speculate that changes in intracellular charge influence ion fluxes across myocyte membranes, and that this underlies the mechanism of sudden death. Alternatively, it may be that the charge change is incidental, and that the site of the mutation is the important prognostic determinant. However, until more families are studied, and the link between specific mutations and prognosis is more clearly

defined, such information cannot be used in clinical practice.

Conclusion

Recent advances in molecular biology have raised the possibilities of genetic screening, to identify individuals at an early stage, and genetic prognosis prediction. This, however, is currently limited to a minority of patients. Screening in these individuals should be carried out only as part of research trials studying the natural history of the disease and the effects of treatment. Even if considered desirable, the possibility of genetic screening of the general population is many years away. In the more foreseeable future, elucidation of the genetics of certain types of HCM will help unravel the pathophysiology of this disorder, and allow for the development of more rational treatments. Of wider interest, understanding the mechanisms by which specific mutations produce the cardiac abnormalities of HCM may provide insight into normal myocyte growth and function and how these are altered in other cardiac diseases.

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