GENETIC TESTING FOR HYPERTROPHIC CARDIOMYOPATHY

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INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a disease that has grown more fascinating as new diagnostic and therapeutic tools have emerged over the last 50 years. In the early 20th century, HCM was a pathological entity. Subaortic obstruction was described at the outset but was only documented in patients after the advent of cardiac catheterization; it was first coined as "idiopathic hypertrophic subaortic stenosis" (IHSS) until the breakthrough of echocardiography revealed the broader phenotype. The nature of the outflow tract gradient as either dynamic or true mechanical obstruction was for years the subject of hot debate.

Today HCM is considered a primary disease of the myocardium characterized by cardiac hypertrophy in the absence of a discernible clinical cause, such as hypertension or valvular heart disease.1 Typically, the left ventricle is hyperdynamic and has a small cavity size. The prevalence of HCM is at least 1:500 in the younger population,2 although it is likely to be higher in older individuals because of age-dependent expression of the disease. Early descriptions recognized sudden cardiac death (SCD) as a major clinical manifestation of HCM. While it remains the primary concern of physicians and patients, the development of internal defibrillators has provided a means to deal with this tragic event.

The first series of surgeries for HCM were performed and refined about 40 years ago. The Moro procedure for septal myectomy became the standard surgical therapy for obstructive HCM. Advances in percutaneous techniques led to percutaneous septa! ablation, commonly performed by injecting alcohol into the main septal branches of the left anterior descending coronary artery. Its widespread use has led to ongoing debates about the superiority of surgical myectomy versus alcohol septal ablation, yet this debate remains unsettled due to the lack of large-scale randomized studies.

While a familial form of HCM was recognized in the mid-20th century, the molecular genetic basis of HCM was revealed only in the last two decades with the development and application of modern molecular genetic techniques. Today, one of the most common questions asked by both physicians and patients centers around the availability and utility of genetic testing to diagnose and predict the risk of SCD.

DIAGNOSIS

Cardiac hypertrophy is the quintessential feature of HCM and the basis for its clinical diagnosis. The most commonly used diagnostic modalities are electrocardiogram (ECG) and echocardiogram. ECG is sensitive bur less specific, except in familial cases wherein any ECG abnormality in at-risk family members suggests HCM. The diagnosis is usually established on an echocardiogram showing a left ventricular wall thickness of 13 mm or greater. Hypertrophy is typically concentric and asymmetric in approximately two-thirds of the cases, with the predominant involvement of the incerventricular septum. GlobaJ cardiac function is preserved, but regional myocardial function usually shows impaired function that could be detected early - prior co the development of cardiac hypertrophy - through tissue Doppler imaging.3

Pathological features of HCM include myocyte hypertrophy, myocyte disarray, and interstitial fibrosis. Cardiac myocyte disarray is the pathological hallmark of HCM, while myocyte hypertrophy and interstitial fibrosis contribute ro diastolic heart failure and cardiac arrhythmias.

FALLACIES OF THE CLINICAL DIAGNOSIS

A clinical diagnosis of HCM - based on the detection of "primary" cardiac hypertrophy on an echocardiogram - is neither completely sensitive nor free of false positives. The common reasons for this imperfect sensitivity are due to incomplete penetrance and variable expressivity. Incomplete penerrance means that not all individuals with the disease-causing mutations show the clinical phenotype. Young individuals who carry the disease-causing mutation may not show clinical HCM but may develop the disease at an older age. Variable expressiviry means char individuals with the disease-causing mutation show a variable degree of the phenotype. Therefore, some individuals with the mutation could exhibit minimal or mild hypertrophy and would nor be diagnosed as having HCM.

The specificity of the echocardiographic diagnosis is compounded by the presence of phenocopy, which refers to conditions that phenotypically mimic HCM but are mostly storage diseases and not true HCM.4 Conditions that mimic HCM include isolated cardiac Fabry disease, various glycogen and

lysosomal storage disorders, mitochondrial disorders, and ochers. The presence of subtle non-cardiac phenotype such as skeletal myopachy, neurological disorders, and involvement of multiple organs could point to phenocopy scares. While the distinction could prove difficult on che echocardiogram alone, it is important since che pathogenesis and treatment of the two conditions differ significamly.5 The prevalence of HCM phenocopy is estimated to comprise 10-15% of all cases clinically diagnosed as HCM.4

Hypertension is another confounding factor for the accurate clinical diagnosis of HCM. Conventionally, the presence of hypertension excludes the diagnosis of HCM. Given char hypertension is a common disease, however, a subset of patients with HCM could have concomitant hypertension. Another diagnostic challenge is the distinction between physiological hypertrophy in athletes and HCM. The clinical significance of this distinction is emphasized by rhe face chat HCM is rhe most common cause of SCD in competitive achleres.6 These limitations in conjunction with recent advances in the molecular genetics of HCM have raised considerable interest in generic testing. Commercial services have become available and have sec in motion the rouci ne application of generic testing. Thus, it has become imperative for clinical cardiologists to familiarize themselves with the utility and limitations of the genetic-based diagnosis and risk stratification.

MOLECULAR GENETICS

HCM is familial with an aucosomal dominant mode of inheritance in approximately half of the cases. Generic linkage studies in che 1980s and early 1990s led co mapping of several chromosomal loci and identification of the first causal mutation (R403Q) in MYH7, encoding the (3-myosin heavy chain (MyHC).[7] Since then over a dozen causal genes and more than 400 mutations have been identified (Table l). The causal genes encode sarcomeric proteins; hence, HCM is considered a disease of sarcomeric proteins.

The prevalence of the causal genes varies in different populations. Overall, the known genes and mutations account for approximately two-thirds of all cases. The most common causal genes are MYH7 and MYBPC3, the latter encoding myosin binding protein-C (MyBP-C). Each accounts for approximately 25-30% of the cases. Mutations in TNNT2, TNNI3 and TPM1, which encode cardiac troponin T, cardiac troponin I and CY-tropomyosin, respectively, account for approximately 10% of the cases. Consequently, these five genes collectively account for approximately two-thirds of all HCM cases, and mutations in the remainder are quite uncommon (Table I), as are double mutations.[8]

The prevalence of each causal mutation is very low, each being responsible for <1% of the cases ("private mutations"). The vast majority of mutations are point or missense mutations. Insertion/deletion or splice junction mutations are more common in che MYBPC3 than in ochers. Not all variants in che sarcomeric genes are causal mutations. Many could be simple polymorphisms. Thus, when finding a variant in a sporadic case, establishing the causality requires additional studies.

Table 1. Causal Genes for HCM.

Gene	Gene Symbol	Prevalence
13-Myosin heavy chain	MYH7	- 25-30%
Myosin binding protein-C	MYBPC3	- 25-30%
Cardiac troponin T	TNNT2	- 2-5%
Cardiac troponin I	TNNI	- 2-5 %
a-tropomyosin	TPM1	- 2-5%
Myozenin 2 (calsarcin 1)	MYOZ2	1:250
Cardiac a-actin	ACTA	<1%
Titin	TTN	<1%
Essential myosin light chain	MYL3	<1%
Regulatory myosin light chain	MYL2	<1%
Tcap (Telethonin or titin-cap)	TCAP	Rare
Phospholamban	PLN	Rare
Caveolin 3	CAV3	Association
a-Myosin heavy chain	MYH6	Association
Cardiac myosin light peptide kinase	MYLK2	Association
Cardiac troponin C	TNNCI	Association

GENETIC TESTING

Causal mutations and prediction of the **clinical** outcomes: Do the causal mutations predict the risk of SCD in patients with HCM? This is a commonly asked question by clinicians and patients alike. The answer is provisionally yes, but only partially. HCM is a classic single-gene disorder, which means a mutation in a single gene is sufficient to cause the phenotype. Thus, causal genes and mutations are pre-requisites for the development of HCM and exert significant effect on phenorypic expression. The group data suggests MYH7 mutations as compared ro MYBPC3 mutations are usually associated with an earlier onset of disease, severe hypertrophy, and an increased risk of SCD. Mutations in the component of the chin filaments are commonly associated with a mild degree of cardiac hypertrophy but an increased risk of SCD. However,

Risk Factors for SCD in Patients with HCM

- Prior episode of aborted SCD (an indication for implantation of a defibrillator)
- History of SCD in more than one family member
- Causal mutations, including double mutations
- Modifier genes
- Recurrent syncope
- Extensive cardiac hypertrophy
- Sustained and repetitive non-sustained ventricular tachycardia
- Severe outflow tract gradient (overall mortality)
- Severe interstitial fibrosis and myocyte disarray
- Early onset of clinical manifestations
- Abnonnal blood pressure response to exercise, exercise induced hypotension
- Presence of myocardial ischemia in children

Table 2. Risk Factors for SCD in Patients with HCM

It is important to note that there is considerable variability, no phenotype is specific co a mutation, and no mutation is always benign or malignant.

Clinical **utility** of genetic testing: The interest in genetic testing is primarily based on its potential to render an accurate diagnosis and prognostication. With regard to the diagnostic utility, the best scenario is the familial setting whereby the causal mutation in some but not all family members is known. Accordingly, one could with reasonable certainty identify the mutation carriers and hence the risk of HCM from the non-carriers, i.e., normal individuals. Pre-clinical diagnosis could provide an opportunity for interventions to prevent the evolving phenotype, as has been shown in an experimental model.[9] Unfortunately, this is rarely the case as the causal mutation is usually unknown in the family. Nonetheless, in families with five or more affected individuals, one could initially map the causal genes, identify the causal mutation, and then screen the family members.

Routine genetic testing for an accurate diagnosis is hampered by several difficulties. First, there are more than 400 known causal mutations and over a dozen causal genes. Second, the prevalence of each particular causal mutation is low since almost all are private. Hence, a genetic screening approach cannot be restricted to known mutations since the pre-test probability of detection is very low and there is no common mutation co test. Third, the screening technique has co provide more than 80% sensitivity and almost near-complete specificity. Currently, direct sequencing is the only desirable screening method but requires sequencing of large segments of genomic DNA, which is expensive and labor intensive. Fourth, not all genes for HCM are known. Thus, even in the best scenario, the causal mutation could be identified in about two-thirds of the cases. An alternative approach is to restrict murarion screening co the fuur-to-five most common genes, which could lead to identification of the causal mutation in about half of the cases. The approach is currently available through commercial sources and in the research laboratories.

The impetus to use genetic testing for prognostication is based on identification of the so-called "malignant" mutations in families who had a high incidence of SCD in the affected members. Likewise, it is tempting ro posrulare that implanting internal defibrillators in chose with "malignant" mutations could reduce the risk of SCD. Despite rhe theoretical benefits, the clinical utility of genetic testing for risk stratification has not been established. Given rhe high degree of inter-individual variability of phenorypic expression of HCM, even in chose wirh identical mutations, generic testing alone is insufficient to detect the risk of SCD. A comprehensive approach that utilizes the information content of the causal mutation, modifier genes, clinical phenotype, and environmental factors is necessary for appropriate risk stratification and interventions to reduce the risk of SCD.

Determining the risk of SCD in HCM: In addition to the causal mutations, several genetic and non-genetic factors contribute to the expression of the phenotype and the risk of SCD (Table 2). Among the genetic factors, modifier genes and double mutations are important determinants of the risk. The modifier genes, by definition, contribuce to expression of the phenotype by either enhancing or suppressing its expression. Modifier genes are neither necessary nor sufficient to cause HCM. However, rhey affect the degree of expression of the disease.[10-11] The contribution of the modifier genes could be quire considerable, and in a homozygous stare for che modifying allele ir can surpass the effect of the causal mutarionP Double mutations, while uncommon, are expected to increase the risk of SCD.[8,13]

Several clinical factors have been associated with the risk of SCD, including a prior episode of aborted SCD, a strong family history of SCD, frequent syncope, severe cardiac hypertrophy, and ochers as listed in Table 2. The potential impact of left ventricular outflow tract gradient on the risk of SCD is unclear, but it is associated with increased overall morbidity, and surgical myeccomy appears to reduce the risk of SCD. Heavy physical exercise is expected to worsen the phenotype; however, there is insufficient data in humans. Patients with HCM are advised to avoid heavy physical exercise since HCM is the most

common cause of SCD in competitive athletes, and death often occurs during or shortly after exercise.[6]

CONCLUSION

Advances in the molecular genetics of HCM have set the stage for translational studies to determine the clinical utility of genetic diagnosis and prognostication. Limited genetic testing is currently available and is likely to become routine as efficiency improves and DNA sequencing techniques become more cost effective. Molecular genetic studies establishing the heterogeneity of HCM could lead to gene-specific therapy.

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