Chapter 4

Hypertrophic Cardiomyopathy and Sudden Cardiac Death: Molecular Genetics Aspects

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Introduction

Sudden cardiac death (SCD) is commonly linked to heart diseases of structural and non-structural origin. Among the nonstructural are cardiac channelopathies, such as long QT, short QT, and Brugada syndromes, and catecholaminergic polymorphic ventricular tachycardia. Structural causes of SCD include dilated and hypertrophic cardiomyopathies and arrhythmogenic right ventricular cardiomyopathy. This chapter summarizes the genetic aspects of SCD related to hypertrophic cardiomyopathy (HCM).

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Hypertrophic Cardiomyopathy: Basic Principles

In 1958 Donald Teare described an “asymmetrical hypertrophy or benign tumor of the heart” in eight patients with a disproportionate inter ventricular septum (Teare, 1958).

Hypertrophic Cardiomyopathy (HCM OMIM, # 192600, www.ncbi.nlm.nih.gov) is currently the accepted denomination for this clinical entity, defined as the idiopathic left ventricular hypertrophy (LVH) of unknown cause; i.e. not secondary to other clinical manifestations that may cause LVH such as hypertension, cardiac valvular disease, physical exercise. While in the physiological hypertrophy the interventricular septum (IVS) thickness disappears when the risk factors are controlled, in HCM the LVH remains therein.

HCM is one of the most common inherited cardiac pathologies (approximate incidence, 1/500), and is responsible for many cases of SCD in young adults and a major cause of morbidity and mortality among the elderly. Dyspnoea, chest pain, and palpitations are the most common symptoms of HCM, while syncope is less frequent but should be considered seriously because frequently anticipates episodes of SCD (Kofflard et al., 2003, Nienaber et al., 1990, Robinson et al., 1990).

The same concern should be taken about supraventricular arrhythmias and atrial fibrillation, that are commonly associated with an adverse outcome (Robinson et al., 1990). In the presence of clinical symptoms, HCM may be suspected by systolic murmur on auscultation associated with obstruction to left ventricular outflow, or by an abnormal electrocardiogram (ECG). The diagnosis should be confirmed by the observation of a ventricular interseptum thickness > 15 mm.

Depending on the location of the thickening three morphological types are recognised: septal asymmetric, symmetrical or concentric, and apical HCM.

HCM and SCD

A meta-analysis of large cohort studies indicated that the rate of SCD should be <1% per year in patients who fulfilled the diagnostic criteria for HCM (Elliott et al., 2006).
One of the main objectives of research is to identify markers that could be associated with a poor prognosis, to improve quality of life and survival through prophylactic therapies in patients at risk. Because pharmacologic therapy has not been demonstrated to provide protection from SCD, implantable cardioverter defibrillator (ICD) remains the most effective strategy to prolong life expectancy by terminating ventricular tachyarrhythmias. A recent report from the American College of Cardiology Foundation/American Heart Association (ACCF/AHA) established a guideline for SCD risk stratification and recommendations for the placement of ICD in HCM patients (Gersh et al., 2011).

For SCD risk stratification, the ACCF/AHA has established the following main recommendations:

1. All patients with HCM should be initially evaluated to determine the existence of a personal history for ventricular fibrillation (VF), sustained ventricular tachycardia (VT), previous SCD events, unexplained syncope, documented nonsustained VT (defined as 3 or more beats at ≥ 120 bpm on Holter ECG), a maximal IVS ≥ 30 mm, and a family history of SCD events (Cecchi et al., 1989, Elliott et al., 1999, Elliott et al., 2000, Fananapazir et al., 1992, Maki et al., 1998, Maron et al., 1981, Maron et al., 2007, Maron, 2010a, Maron, 2010b, McKenna et al., 1981, Monserrat et al., 2003, Spirito et al., 2009).

Risk stratification for SCD every 12-24 months is considered reasonable for those patients without an ICD but who would otherwise be eligible if risk factors are identified.

2. The following might be considered in selected patients with HCM for whom a borderline risk was obtained based on the previous conventional risk factors: cardiac magnetic resonance (CMR) imaging with late gadolinium enhancement (LGE), marked left ventricular outflow tract obstruction (LOVTO), and/or multiple mutations (Adabag et al., 2008, Efthimiadis et al., 2009, Elliott et al., 2006, Maki et al., 1998, Maron et al., 2003, Moon et al., 2003).

3. Invasive electrophysiologic testing should not be performed as routine SCD-risk stratification.
Table 1. ACCF/AHA recommendations for ICD placement

<table>
<thead>
<tr>
<th>RECOMMENDED</th>
<th>Prior cardiac arrest</th>
</tr>
</thead>
<tbody>
<tr>
<td>RECOMMENDED</td>
<td>Sustained VT</td>
</tr>
<tr>
<td>REASONABLE</td>
<td>LV thickness ≥ 30 mm</td>
</tr>
<tr>
<td>REASONABLE</td>
<td>Recent unexplained syncope</td>
</tr>
<tr>
<td>REASONABLE</td>
<td>SCD in ≥ 1 relatives</td>
</tr>
<tr>
<td>USEFUL</td>
<td>Non sustained VT + other SCD risk factors</td>
</tr>
<tr>
<td>USEFUL</td>
<td>Abnormal blood pressure + other SCD risk factors</td>
</tr>
<tr>
<td>UNCERTAIN</td>
<td>Non sustained VT / No other SCD risk factors</td>
</tr>
<tr>
<td>UNCERTAIN</td>
<td>Abnormal blood pressure / No other SCD risk factors</td>
</tr>
<tr>
<td>NOT RECOMMENDED</td>
<td>None of the previous</td>
</tr>
</tbody>
</table>

ICD, implantable cardioverter-defibrillator; VT, ventricular tachycardia; LV, left ventricular; SCD, sudden cardiac death.

Based on the SCD risk criteria, several recommendation levels for ICD placement were established (Maron et al., 2000, Maron et al., 2007, Maron et al., 2008, Maron et al., 2010) (Table 1):

1. ICD is recommended for HCM patients with prior documented cardiac arrest, VF, or hemodynamically significant VT (Cecchi et al., 1989, Elliott et al., 1999, Fananapazir et al., 1992, Maron et al., 2007).
2. ICD implant is reasonable in patients with one or more unexplained syncopal episodes, a maximum left ventricle wall thickness ≥ 30 mm, or who have at least one relative who died suddenly and the death was presumably caused by HCM (Bos et al., 2010, Elliott et al., 2000, Elliott et al., 2001, Olivotto et al., 2003, Sorajja et al., 2006, Spirito et al., 2000, Spirito et al., 2009).
3. An ICD can be useful in patients who are <30 years old and have nonsustained VT in the presence of other SCD risk factors, or in HCM patients who had other risk factors and showed an abnormal blood pressure response with exercise (Frenneaux et al., 1990, Maki et al., 1998, Maron, 2010, Monserrat et al., 2003, Olivotto et al., 1999, Sadoul et al., 1997).
4. For children with HCM and unexplained syncope, massive hypertrophy, or family history of SCD, it is reasonable to implant an ICD after taking into account the long-term complications.

5. In patients with isolated bursts of nonsustained VT or with an abnormal blood pressure response with exercise but no other SCD risk factors, the usefulness of an ICD is uncertain (Maki et al., 1998, Maron, 2010, Olivotto et al., 1999, Sadoul et al., 1997).

6. Finally, it is potentially harmful the ICD placement as a routine strategy in patients without an indication of increased risk for SCD, in patients who are mutation carriers but have no clinical manifestation of HCM, or to permit HCM patients to participate in competitive athletics.

Hypertrophic Cardiomyopathy: Genetic Aspects

Many HCM patients have a family history of the disease, that is transmitted as a mendelian dominant disorder linked to mutations in several genes that encode proteins involved in the contractility of cardiac sarcomere (Maron et al., 1995). Although most of the patients could be classified as familial cases when first degree relatives are properly examined, it has been estimated that up to 10% would be sporadic, with no family history of the disease (Marian et al., 1995). HCM is characterized by a wide heterogeneity of symptoms and echocardiographic finding, ranging from a severe hypertrophy at a young age to a mild hypertrophy (or even no hypertrophy at all) at old age. This heterogeneity is also seen among individuals from the same family, who carry the same causative mutation (Brito et al., 2003).

The clinical complexity that characterizes MCH could be primarily explained by its genetic heterogeneity, with more than 20 well characterized genes and many different mutations at each gene (Arad et al., 2002, Epstein et al., 1992). More than half of the mutations have been found in the genes encoding the cardiac beta-myosin heavy chain (MYH7) and the myosin-binding protein C3 (MYBPC3). Mutations in other genes (TNNT2, MYL2, MYL3, TNNI3, TPM1, ....) were found in <5% of the cases (table 2).
Table 2. Genes most frequently mutated in hypertrophic cardiomyopathy

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Protein</th>
<th>% HCM *</th>
<th>Sarcomeric function</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYH7</td>
<td>14q12</td>
<td>Cardiac beta myosin heavy chain (β-MHC)</td>
<td>25%</td>
<td>motor</td>
</tr>
<tr>
<td>MYBPC3</td>
<td>11q11</td>
<td>Myosin binding protein C3 (mybpc3)</td>
<td>30%</td>
<td>structural</td>
</tr>
<tr>
<td>TNNT2</td>
<td>1q32</td>
<td>Cardiac Troponin T (cTnT)</td>
<td>5%</td>
<td>regulatory</td>
</tr>
<tr>
<td>TNNI3</td>
<td>19q13</td>
<td>Casdiac Troponin I (cTnI)</td>
<td>5%</td>
<td>regulatory</td>
</tr>
<tr>
<td>TPM 1</td>
<td>15q22.1</td>
<td>α-tropomiosin (α-TM)</td>
<td>1%</td>
<td>regulatory</td>
</tr>
<tr>
<td>MYL2</td>
<td>12q23-p24</td>
<td>Myosin regulatory light chain (MLC1)</td>
<td>&lt;1%</td>
<td>motor</td>
</tr>
<tr>
<td>MYL3</td>
<td>3p21.3-p21.2</td>
<td>Myosin essential light chain (MLC2)</td>
<td>1%</td>
<td>motor</td>
</tr>
<tr>
<td>ACTC</td>
<td>15q11-q14</td>
<td>Cardiac α-actinin</td>
<td>&lt;1%</td>
<td>structural</td>
</tr>
<tr>
<td>TTN</td>
<td>2q31</td>
<td>Titin</td>
<td>&lt;1%</td>
<td>structural</td>
</tr>
<tr>
<td>TCAP</td>
<td>17q12</td>
<td>Teletonin</td>
<td>&lt;1%</td>
<td>structural</td>
</tr>
<tr>
<td>CSRP3</td>
<td>11p15.1</td>
<td>LIM protein</td>
<td>&lt;1%</td>
<td>structural</td>
</tr>
<tr>
<td>MYH6</td>
<td>14q12</td>
<td>Alpha-myosin heavy chain (α-MHC)</td>
<td>&lt;1%</td>
<td>structural</td>
</tr>
<tr>
<td>MYLK2</td>
<td>7p22.3</td>
<td>myosin light chain kinase 2</td>
<td>&lt;1%</td>
<td>regulatory</td>
</tr>
<tr>
<td>TNNC1</td>
<td>3p21.3-p14.3</td>
<td>Cardiac troponin C (cTnC)</td>
<td>&lt;1%</td>
<td>regulatory</td>
</tr>
<tr>
<td>CAV3</td>
<td>3p 25.3</td>
<td>Caveolin 3</td>
<td>&lt;1%</td>
<td>regulatory</td>
</tr>
<tr>
<td>PLN</td>
<td>6q22.31</td>
<td>Phospholambam</td>
<td>&lt;1%</td>
<td>regulatory</td>
</tr>
</tbody>
</table>

* % of index cases with mutations at this gene.

Patients with more than one mutation in the same or different genes have been reported.

Mutations in the HCM genes result in alterations in the architecture and contractile capacity of the cardiac sarcomere (Rayment et al., 1995). A striking aspect is that although the mutation exists throughout the cardiac tissue the hypertrophy only manifests at the left ventricle. Two molecular pathogenic mechanisms have been proposed: in the toxic peptide model the mutant protein is incorporated into the sarcomere and would have a "toxic" effect; in
the haploinsufficiency model the mutation results in a reduction of the amount of normal protein which weakens the sarcomere contractile capacity. It is likely that the two models are valid, and for some genes/mutations a toxic peptide effect explains the disease while the haploinsufficiency is the mechanism for others.

A major breakthrough for understanding the pathophysiological mechanisms of hypertrophy has been the creation of transgenic animals that incorporate mutations in the sarcomeric genes. For example, mice with mutations gln-403 in the alpha tropomyosin or gln-92 in the troponin C genes (two mutations found in patients with HCM) developed a disease characteristic phenotype, but rarely showed the fibrillar disorganization at the extent of hypertrophy found in humans (Geisterfer-Lowrance et al., 1990, Oberst et al., 1998).

To date, HCM patients have been sequenced for specific genes to search for causative mutations, at a considerable cost due to the large size of most the genes (Coto et al., 2012). The development of Next Generation Sequencing (NGS) technologies facilitates the genetic screening by analyzing the whole sequence of many genes at the same time, and at a reasonable cost. Once a mutation is found in a patient, the corresponding nucleotide change can be determined in the relatives to provide a genetic counseling. The genetic testing is fundamental to identify at risk individuals who do not have symptoms but are mutation carriers, and would thus benefit from life style recommendations to prevent adverse effects (i.e. avoid sport activities that could increase the risk for SCD). In addition, at risk healthy individuals who did not carry the familial mutation could be excluded from follow up. (Ho, 2010)

**Genotype-Phenotype Correlation: Benign vs. Malignant Mutations?**

A major goal of the HCM research has been to find markers of poor outcome, to facilitate the identification and intervention on patients at high risk for SCD (Hagege et al., 1998, Marian, 2009a, Marian, 2009b). As indicated above, a family history of SCD, an extreme LVH (wall thickness of ≥30 mm), and the presence of unexplained syncope or non-sustained ventricular tachycardia at exercise test or 24 h ambulatory Holter are considered markers for SCD (Gersh et al., 2011). However, none of the existing risk factors alone are reliable predictors of SCD-risk in patients with HCM. The genetic research
has tried to fill this gap by investigating the relationship between disease progression and survival and the mutated gene and the type of mutation at each gene.

Studies aimed to establish a relationship between the genotype and the phenotype are limited by several facts. First of all, most of the mutations have been found in few patients, frequently from a single family ("private" mutations). It was thus impossible to collect a sufficiently large number of mutation carriers to obtain statistically significant results from follow up studies. Exceptions are those mutations found in many different families ("recurrent" mutations). For instance, MYH7 R403QQ and R453C were associated with decreased survival compared to the V606M mutation (Watkins et al., 1992, Watkins et al., 1995).

Table 3. Sarcomeric mutations found in a cohort of 200 HCM index patients from the region of Asturias (Northern Spain). In bold, mutations related with SCD (index cases and/or relatives)

<table>
<thead>
<tr>
<th>MYBPC3 Mutations</th>
<th>Patients (n=32)</th>
<th>MYH7 Mutations</th>
<th>Patients (n=25)</th>
<th>Other Genes</th>
<th>Patients (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A216T</td>
<td>2</td>
<td>S4L</td>
<td>1</td>
<td>TNNT2-R92Q</td>
<td>2</td>
</tr>
<tr>
<td>Y237C</td>
<td>1</td>
<td>A100T</td>
<td>1</td>
<td>TNNT2-R278C</td>
<td>5</td>
</tr>
<tr>
<td>G263X</td>
<td>4</td>
<td>R143Q</td>
<td>1</td>
<td>TPM1-D175N</td>
<td>1</td>
</tr>
<tr>
<td>A328fs</td>
<td>1</td>
<td>F247L</td>
<td>2</td>
<td>TNNI3-R136Q</td>
<td>1</td>
</tr>
<tr>
<td>Q404fs</td>
<td>1</td>
<td>R403Q</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E441K</td>
<td>2</td>
<td>R453C</td>
<td>1</td>
<td>TNNC</td>
<td>0</td>
</tr>
<tr>
<td>R495W</td>
<td>2</td>
<td>K542R</td>
<td>1</td>
<td>ACTC1</td>
<td>0</td>
</tr>
<tr>
<td>G531R</td>
<td>1</td>
<td>A583V</td>
<td>1</td>
<td>MYL2</td>
<td>0</td>
</tr>
<tr>
<td>G532fs</td>
<td>1</td>
<td>R663H</td>
<td>1</td>
<td>MYL3</td>
<td>0</td>
</tr>
<tr>
<td>E542Q</td>
<td>4</td>
<td>R652G</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C566R</td>
<td>1</td>
<td>R723G</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R891fs</td>
<td>1</td>
<td>R787C</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R726C</td>
<td>1</td>
<td>V822M</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V771M</td>
<td>2</td>
<td>R870H</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M844fs</td>
<td>2</td>
<td>P828S</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1022S</td>
<td>1</td>
<td>K1459N</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A627V</td>
<td>1</td>
<td>E1555K</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W1115I</td>
<td>1</td>
<td>E1829G</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1248R</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Int21-2A&gt;G</td>
<td>1</td>
<td></td>
<td></td>
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</tbody>
</table>
Based on these seminar studies the terms "benign" and "malignant" mutations were coined. However, the concept has been questioned by mutations that behave as malignant in some families but were benign in others, and by the heterogeneous phenotype associated with some mutations in the same family (Landstrom et al., 2010).

The severity of the hypertrophy and/or the risk of adverse events could also depend on the mutated gene. Most of the studies based on large cohorts of HCM patients/families concluded that compared to MYBPC3, MYH7 mutations were associated with severe hypertrophy and higher penetrance (ie, increased probability of developing symptoms and hypertrophy at an early age). However, the fact that some MYBPC3 mutations were more “malignant” than some MYH7 mutations questioned the ideal scenario of an almost “perfect” genotype-phenotype correlation.

Our own experience as a reference center summarizes the state of the art in the genetics of HCM, and the difficulties of translate these findings to the clinical practice. We sequenced the coding exons (and at least 20 nucleotides of intron flanking nucleotides) of the most frequently mutated genes (MYH7, MYBPC3, TNNT2, TNNI3, TPM1, ACTC1, MYL2, MYL3, TNNC1) in a large cohort of unrelated HCM patients (index cases) from Spain (Garcia-Castro et al., 2003, Garcia-Castro et al., 2005, Garcia-Castro et al., 2007, Garcia-Castro et al., 2009). Table 3 summarizes the mutational spectrum of HCM in our population. The main findings were:

1. Forty percent of the patients had relatives who also had HCM or suffered SCD, and were thus considered as familial cases. Twenty percent had a family history of SCD, but only 2% had themselves suffered SCD and received an ICD. Here, it is important to consider that the familial history is primarily stated by the patient, and for some of the apparently sporadic cases no first degree relatives (including both parents) were echocardiographically evaluated to exclude the presence of asymptomatic HCM individuals. The frequency of actually familial cases would therefore be underestimated in most of the studies.

2. A mutation was found in 30% of the patients: 61% were found in MYH7 or MYBPC3, and <5% in TNNT2, TPM1, and TNNI3. A total of 65% were new mutations; i.e. not included in the databases of HCM mutations (such as www.HGMD.org).

3. 75% of the patients with MYH7 mutations had a family history of the disease, compared to 45% of the patients with mutations in MYBPC3.
and 15% of the cases in which no mutation was found. Therefore, the family history is important to define the likelihood of finding mutations and to guide the genetic screening (gene/genes that could be first analyzed).

4. We found no differences in the onset age or the degree of hypertrophy between patients with mutations in MYH7 and MYBPC3. In the patients with mutations in MYH7 the average thickness of the septum was 22 mm (± 6), compared to 21 mm (± 5) among carriers of mutations in MYBPC3.

**MYBPC3 Mutations**

The MYBPC3 has been associated with a more benign LVH compared to the MYH7 gene, in terms of septum size, risk of SCD, and onset age. The latter would mean that a significant number of MYBPC3 mutation carriers would remain asymptomatic at ages at which most of the MYH7 mutations carriers had disease symptoms. It was also more likely that a MYH7 mutation carrier showed a family history of HCM compared to MYBPC3 mutation carriers. Christiaans et al. conducted a large scale analysis on Dutch patients, with 235 carriers of 14 different mutations evaluated for the presence of LVH and SCD risk factors (Christiaans et al., 2010). Disease penetrance at the age of 65 years was incomplete for all types of MYBPC3 mutations, and was higher in males than in females (30% vs. 13% at 50 years, respectively). Eleven percent of the mutation carriers could be at risk for SCD. The main conclusion of this study (and others) was that cardiologic evaluation on the presence of HCM and risk factors for SCD are justified until advanced age among MYBPC3 mutation carriers.

**Case 1. MYBPC3 M844fs**

The index case was a 59 years old female with dyspnea and fatigue as presentation symptoms. She had an IVS of 17 mm, and a posterior wall (PW) of 18 mm. Two relatives had died suddenly at ages 39 and 55, without previous manifestation of HCM. All the available family members at risk were evaluated with an echocardiogram, and three showed LVH. The main sarcomeric genes were sequenced in the index case, and an insertion of two nucleotides (ins GA) in exon 26 of MYBPC3 was found: p.M844fs. The
geneic testing was offered to the relatives and a total of 8 mutation carriers were identified. These included the index case and the 3 with LVH. Two of these mutation carriers (40 and 35 years old male) were sons of SCD cases. They had IVS = 21 and 19 mm and received an ICD, a decision based on the facts that they manifested LVH, had suffered syncope, and their parents had died suddenly.

There were two individuals for whom a lifestyle recommendation was taken based on the genetic study. A 22 years old male was asymptomatic but showed an IVS of 17 mm. He was involved in aerobic sports, and was thus impossible to conclude whether the hypertrophy was of genetic origin or secondary to exercise. Because the genetic testing showed he was a mutation carrier, the cardiologist recommended to limit the extent of exercise to avoid heart stresses that could increase the risk of SCD. A 25 years old female was asymptomatic, without LVH. However, she was a mutation carrier and a follow up was recommended to control for SCD risk factors prior to implant an ICD. In this family, several at risk individuals were asymptomatic and negative for the MYBPC3 mutation. These individuals benefited from the genetic testing because a follow up was not necessary.

**MYH7 Mutations**

The majority of MYH7 mutations are missense amino acid changes (single nucleotide changes leading to substitution of one amino acid by another). In contrast, insertion/deletion and nonsense mutations that lead to frameshifting and premature stop codons are common in MYBPC3. Most of the MYH7 mutation carriers have LVH by the second decade of life, at ages at which a significant amount of MYBPC3 mutation carriers have normal IVS and remain asymptomatic. Based on these and other findings, MYH7 has been considered as a malignant gene compared to MYBPC3. A recent report that found a higher frequency of MYH7 mutation carriers among cardiac failure patients supported this view (Garcia-Pavia et al., 2011). These authors sequenced 10 HCM-related genes (MYH7, MYBPC3, TNNT2, TNNI3, TPM1, TNNC1, MYL3, MYL2, ACTC, LDB3) in 26 patients (age 40.4 ± 14.5 years; 46% male) transplanted for end-stage HCM. Pathogenic mutations were found in 13 patients (50%): 6 in MYH7, 3 in MYBPC3, 2 in MYL2, 1 in TNNI3, and 1 MYL3. Three patients were homozygous for a mutation.
Case 2. **MYH7 A583V**

The index case was a 36 years old male who presented to the Cardiology Department for dyspnea and syncope. He showed an IVS of 23 mm. His uncle (mother’s brother) died suddenly at the age of 45 years while running. His mother was asymptomatic at the age of 80 years, but had an IVS of 18 mm in the absence of any condition that could explain the hypertrophy. He was thus classified as an HCM patient, with a likely positive family history of the disease.

After sequencing the sarcomeric genes, we found a single nucleotide change in the **MYH7** gene that would result in Ala 583 to Val change. This change was not reported as a mutation or a polymorphism, and we did not find it among 250 healthy population controls. An informatic bioanalysis classified this variant as possibly damaging, and we thus considered likely this was the mutation responsible for the disease in the index patient and other affected relatives. To determine the segregation of Val 583 with the disease we genotyped all the available relatives. Two patient’s brothers aged 38 and 34 years old were asymptomatic but showed IVS of 17 and 16 mm, respectively. The two (and the mother) were mutation carriers. The Val 583 could thus be considered a mutation of variable penetrance, with no symptoms at elderly age in some mutation carriers. However, the fact that a patient’s uncle died suddenly while he was running suggested that this mutation could result in a malignant phenotype under some circumstances. We thus recommended a cardiologic follow up of the mutation carriers, avoiding sport activities that could increase the risk of SCD, and proceeding to implant ICD in case they manifested risk factors for SCD.

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**TNNI3 Mutations**

Troponin I (TnI) constitutes, with troponin C and troponin T, the troponin complex of the thin filaments of cardiac muscle. The troponin complex regulates cardiac muscle contraction through a calcium-sensitive mechanism. In particular, in the absence of calcium TnI binds to actin and inhibits the ATPase activity. Mutations in the TnI gene (**TNNI3**) have been found in <5% of MCH cases (Kimura et al., 1997). In a cohort of 748 consecutive families with hypertrophic cardiomyopathy, Mogensen et al. found 13 different mutations in 23 families (3%), and identified a total of 100 mutations carriers (Mogensen et al., 2004). Disease penetrance was 48%, with onset ages from
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the second to eighth decades of life, and a heterogeneous phenotypic spectrum. This included elderly asymptomatic mutation carriers, offspring of clinically unaffected mutation carriers who were resuscitated from cardiac arrest, and individuals with sudden death as their initial presentation. Some TNNI3 mutations were particularly frequent in specific populations. For instance, the Lys183 del was found in 5% of 130 unrelated Japanese HCM cases (Kokado et al., 2000). This mutation was associated with SCD at any age and dilated cardiomyopathy-like features in those aged >40 years. In a screening of 1,040 Dutch index cases with cardiomyopathy (HCM and restrictive cardiomyopathy), Van den Wijngaard et al. found 14 different TNNI3 mutations in 30 families (3%) (van den Wijngaard et al., 2011). The majority of mutations were found among HCM cases, with an early onset (mean age 36.5 years), and a severe clinical presentation. The conclusion of these and other studies was that the clinical expression of TNNI3 mutations was very heterogeneous both within and between families. The genetic counseling based on TNNI3 could be important to identify asymptomatic mutation carriers that could be at high risk for adverse effects.

Case 3. TNNI3 R136Q

The index case was a 61 years old woman with dyspnea as presentation symptom, and a septum of 29 mm. She had VF and an ICD. A son had died suddenly at the age of 41 years while running, without previous symptoms of LVH. We found a new TNNI3 mutation in the index case, R141>Q. A total of five patient’s offspring were screened, and 4 mutation carriers identified (Figure 1). The fifth was an asymptomatic 45 years old male with an IVS of 16 mm, diagnosed prior to the genetic study. Because he was involved in sports, it was impossible to conclude whether the hypertrophy was secondary to the exercise or could be caused by a putative mutation. Further sequencing showed he was non mutation carrier. The four mutation carriers had IVS 17-21 mm, and three had risk factors for SCD and received an ICD. The mutation status was also determined in a total of nine individuals from the third generation, aged 12 to 19 years old. All were asymptomatic and without signs of LVH. Five were mutation carriers and a follow up was thus recommended (Figure 1).
IVS=interventricular septum;  
VF=ventricular fibrillation;  
NSVT=non sustained ventricular tachycardia.

Figure 1. Family tree and main characteristics of the individuals with a TNNI3 mutation (R141>Q). Filled symbols indicate individuals with an implantable cardioverter defibrillator (ICD).

**TNNT2 Mutations**

HCM caused by mutations in the cardiac troponin T gene (TNNT2) has been associated with a high risk of SCD in spite of mild LVH. Because SCD is frequently the first disease manifestation among TNNT2 mutation carriers, the genetic testing should be particularly relevant for asymptomatic individuals from TNNT2 families. However, data about the natural history of TNNT2 mutations was limited by the small size of cohorts and a lack of data on disease expression among the relatives of index cases. Recently, Pasquale et al. performed a large scale study on 92 TNNT2 mutation carriers from 20 families (65% had a history of SCD) (Pasquale et al., 2012). A total of 75 patients were available for a follow-up study (mean 9.9 ± 5.2 years) and 22% received an ICD. LVH was rare in children with TNNT2 mutations and was absent in the minority of adults (most with an abnormal ECG). The annual rate of cardiovascular death, transplant, and ICD discharge was 1.6% (0.016 person/year) and the rate of SCD 0.93% (0.0093 person/year). In particular, two mutations (Ile79Asn and Arg92Gln) appeared to be associated with a higher rate of juvenile sudden death compared to other mutations (i.e. Glu163del and IVS15+1 G>A). The main conclusion of this study was that in
spite of adverse family histories, the rate of cardiovascular death among TNNT2 mutations carriers was similar to that reported in unselected large referral HCM cohorts (approx. 1% per annum) (Elliot et al., 2006, Pasquale et al., 2012).

Case 4. TNNT2 R278>C

The index case was a woman who presented to the Cardiology Department at the age of 35 years for dyspnea. At the initial study she had no ventricular fibrillation or arrhythmia (Holter), and the echocardiography showed an interventricular septum of 18 mm and a posterior wall thickness of 11 mm. She was normotensive and had no disease that could justify the hypertrophy. Based on these findings HCM was diagnosed. When asked about other affected in her family she stated that a paternal uncle had died suddenly at the age of 27 years. The patient's father had died in a car accident at the age of 50 years, without symptoms of LVH. The mother was healthy with normal electro and echocardiographic values. During the follow up the patient had VF and received an ICD. The patient was a carrier (heterozygous) of the R278>C mutation in exon 16 of TNNT2 (c.C832T). This is a common mutation, with a large number of carriers reported in the literature and associated to a malignant phenotype (Moolman et al., 1997, Watkins et al., 1992, Watkins et al., 1995). The patient had three children aged 18, 21, and 25 years, without symptoms of HCM and normal ECG and echocardiograms. One was a mutation carrier and a follow up was recommended to detect risk markers for SCD.

**TPM1 Mutations**

Tropomyosins are actin-binding proteins that regulate muscle contraction. Mutations in the gene encoding alpha-tropomyosin gene (TPM1) are a rare cause of HCM, accounting for < 3% of cases. Like TNNT2 mutations, TPM1 mutations have been associated with relatively mild / subclinical hypertrophy, but a high incidence of SCD. The genetic testing could thus be particularly important in families with TPM1 mutations.
Case 5. *TPM1* D175N

The index case was a female diagnosed at the age of 41 years (dyspnea, angina) with a severe hypertrophy (32 mm), and a family history of HCM but not SCD. She had a *TPM1* mutation, D175N. Her son (33 years) and brother (53 years) were also mutation carriers and showed IVS of 27 and 20 mm, respectively.

The *TPM1* D175N is one of the best characterized HCM mutations, in part because it is worldwide distributed with a high prevalence in some regions. It has been found in approximately 6.5% of the HCM cases from central and western Finland (Jaaskelainen et al., 2012).

Hedman et al. performed programmed ventricular stimulation in 21 adult subjects with this mutation, and induced polymorphic ventricular tachycardia or ventricular fibrillation in 7 (33%) (Hedman et al., 2004). Severe LVH, family history of SCD, syncope or presyncope, and fall in systolic blood pressure during exercise (all markers for SCD) were more common among inducible compared to non-inducible subjects. It could, thus, be concluded that in patients with the *TPM1* Asn 175 mutation the susceptibility to ventricular arrhythmias was related to the HCM phenotype.

**Multiple Mutations**

HCM patients with multiple mutations have been reported, including cases homozygous and compound heterozygous for mutations at the same and different genes (Garcia-Castro et al., 2005, Ingles et al., 2005, Maron et al., 2012, Richard et al., 1999, Richard et al., 2000, Van Driest et al., 2004). The presence of several mutations has been associated with a more severe phenotype and an adverse prognosis even in the absence of conventional risk markers for SCD (Kelly et al., 2009). This conclusion was also supported by a database of 3 HCM centers that identified 18 probands with 2 mutations in cardiac sarcomere genes (Maron et al., 2012). A total of 7 (39%) showed a severe disease progression or adverse cardiovascular events, including 3 patients (ages 31, 37, and 57 years) who suffered SCD arrest in the absence of conventional risk factors.
Case 6. MYBPC3 A627>V, Double Mutant

A 47-year-old patient with a severe LVH (septum = 28 mm; posterior wall = 17 mm) diagnosed at the age of 16 years (dyspnea, fatigue, and angina after moderate physical exercise) was homozygous for MYBPC3 Ala 627>Val. His father died suddenly at the age of 75 years without previous symptoms of HCM, and his mother (mutation carrier) was 78 years old and clinically asymptomatic, with an IVS of 16 mm. The mutation was also found in one of patient’s sister (53 years old) and nephew (31 years old), both clinically asymptomatic and with interventricular septums of 18 mm and <15 mm, respectively. The clinical findings in this family was in agreement with a gene dosage effect for MYBPC3 mutations, with the homozygous individual showing a more severe phenotype compared to heterozygous mutation carriers (Garcia-Castro et al., 2005, Nanni et al., 2003, Richard et al., 2003).

The Future of Genetics in HCM and SCD

A current limitation of the genetic studies is the inability to identify mutations in a high percentage of patients, mainly cases without a family history of the disease. The search for mutations is restricted to specific regions of genes that have been associated with HCM. However, some patients may have mutations in regions not commonly studied, such as introns (gene sequence that is not translated into amino acids of the protein but may affect RNA processing and normal protein synthesis) and promoters (the region that regulates gene expression and the amount of protein by binding of transcription factors). The already discovered HCM genes could represent the tip of the iceberg, with many genes remaining to be identified. Mutations in these genes could be common among HCM cases but associated with a reduced penetrance (making thus difficult to establish a familial segregation), or could be restricted to a few families. Together, these genes to be discovered might explain a significant percentage of the HCM cases.

Finally, common variants (polymorphisms) in several genes involved in cardiovascular physiology may contribute to modulate the degree of hypertrophy and the risk of adverse events. As an example, we found an association between the severity of HCM and a functional polymorphism in the angiotensin type 1 receptor (Coto et al., 2010). These modifier genotypes could also in part explain the phenotypic differences between carriers of a particular mutation. Hundred of gene variants could act as disease modifiers.
affecting the extent of the hypertrophy, clinical manifestations, and risk of SCD events. All this gap in our knowledge of HCM will be filled in coming years with the full genome sequencing of large cohorts of patients.

**Conclusion**

As indicated above, the usefulness of genetic screening for the "management" of HCM patients is controversial. Several studies have shown that patients who have a mutation would have a more severe phenotype with younger age at diagnosis and greater likelihood of family history than those without a recognized mutation (Garcia-Castro et al., 2009, Van Driest et al., 2005). The presence of a mutation would confer a risk-rate of adverse events of 4.3 (confidence interval 1.5% -12.5%), a value greater than the age, the degree of outflow obstruction, or the presence of atrial fibrillation (Olivotto et al., 2008). Compared with those in which no mutations were found, these patients also have a higher risk of progressing to symptoms of grade III / IV of the New York Heart Association scale and death from cardiac causes.

However, guidelines for the diagnosis and treatment of HCM have judged as potentially harmful the ICD placement as a routine strategy based solely in the presence of a mutation, and in the absence of an indication of increased risk for SCD. The genetic screening in families with an identified causative mutation would help to identify mutation carriers, who would be followed up for the development of clinical manifestations, and non-mutation carriers who are not at risk for disease development.

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