GENETIC ASPECTS OF HYPERTROPHIC CARDIOMYOPATHY

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ABSTRACT

Being prevalent as high as 0.2% (or 1 in 500) in the general population, Hypertrophic Cardiomyopathy (HCM) is a disease marked by phenotypic and genotypic heterogeneity and is the most prevalent heritable cardiovascular disease. The heterogeneous nature of HCM can be explained by environmental influences such as exercise, modifier genes, or the presence of compound or double disease causing mutations in affected individuals. Variability in clinical presentation ranges from minimal or no symptoms to more serious complications, including heart failure and sudden cardiac death. As the first cardiac disorder in which a genetic basis was identified, HCM has acted as a paradigm for the study of an inherited cardiac disorder. So far 20 sarcomere-related and myofilament-related genes have been identified and within these genes about 1400 different mutations have been found. All the genes have large allelic heterogeneity. The molecular diagnosis of HCM allows the detection of subjects carrying a mutation of genes that cause the disease, even before the development of symptoms. This review focuses on the current knowledge about HCM- highlighting the genetic and allelic heterogeneity underlying HCM and pointing out the methods used for molecular testing of HCM.

Keywords: Hypertrophic Cardiomyopathy, Review, Genetic Aspects, Mutations, Genetic Testing

1. INTRODUCTION

Cardiomyopathies are a clinically heterogeneous group of heart muscle disorders and frequently lead to progressive heart failure-related disability or cardiovascular death (Maron & Roberts, 1979; Maron et al., 1996). According to the morphological and functional phenotype; cardiomyopathies can be divided in hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy, restrictive left ventricular cardiomyopathy, arrhythmogenic cardiomyopathy and left ventricular non-compaction (Maron & Roberts, 1979; Maron et al., 1996). However differences exist in the classification schema of major cardiac organizations. HCM is the most common and phenotypically variable familial form of cardiomyopathy that occurs mainly due to mutations in genes encoding for the cardiac contractile apparatus (Efthimiadis et al., 2014; Khouzam & Naidu, 2014). The molecular diagnosis of HCM allows the detection of subjects carrying a mutation of genes that cause the disease, even before the development of symptoms (Bos et al., 2009). This review outlines the current knowledge about HCM, highlighting the genetic and allelic heterogeneity underlying HCM, and pointing out the methods used for molecular diagnosis of HCM.

2. CLINICAL DESCRIPTION OF HYPERTROPHIC CARDIOMYOPATHY

HCM is clinically heterogeneous, with inter- and intrafamilial variability ranging from benign forms (Marian, 2000) to malignant forms with a high risk of cardiac failure or sudden cardiac death (Maron, 2002). Clinical manifestations range from being completely asymptomatic to progressive heart failure and sudden cardiac Death (SCD). The latter is often the first manifestation of the disease in the young and the most common cause of SCD in competitive athletes (Maron et al., 1996). About 2/3 of individuals with HCM have asymmetric septal hypertrophy. About 25% of individuals demonstrate an obstruction to the outflow of blood from the left ventricle during rest. Patients with HCM are commonly seen to exhibit variable symptoms. Symptoms may include shortness of breath (dyspnea), temporary loss of consciousness (syncope) and rapid heart rate (palpitations). Pathological features of HCM are also diverse and include myocyte hypertrophy and disarray, interstitial fibrosis and thickening of the media of intramural coronary arteries (Maron & Roberts, 1979). The age at onset of HCM can
range from infancy to old age; manifestations usually do not appear before adolescence in carriers of a pathogenic variant (Maron et al., 2003). Many cases are suspected from clinical history and physical examination and traditionally diagnosed using cardiac imaging modalities, such as echocardiography and cardiac magnetic resonance imaging (Cirino & Ho, 1993).

3. MOLECULAR GENETICS: GENES AND MUTATIONS

The first reported genetic linkage study of HCM was in a large French-Canadian family in 1989 (Jarcho et al., 1989). Since then, during the last two decades, major advances have been made in understanding the molecular basis for HCM. Although reduced penetrance and clinical variability are common, HCM mainly follows autosomal-dominant inheritance pattern. So far 20 sarcomere-related and myofilament-related genes have been identified and within these genes about 1400 (Roma-Rodrigues & Fernandes, 2014) different mutations have been found (Yingchoncharoensakul & Tang, 2014). Collectively, the findings have established HCM as a disease of sarcomeric protein: primarily, the thick filaments, to a lesser extent, the thin filaments and uncommonly, the Z disk proteins (A. J. Marian, 2010). The 10 sarcomeric related proteins are the β-myosin heavy chain (MYH7) (Geisterfer-Lowrance et al., 1990), the myosin ventricular essential light chain 1 (MYL3) (Poetter et al., 1996), the myosin ventricular regulatory light chain 2 (MYL2) (Flavigny et al., 1998), the cardiac α-actin (ACTC) (Mogensen et al., 1999), α-tropomyosin (TPM1) (Thierfelder et al., 1994), the cardiac troponin I (TNNI3) (Kimura et al., 1997), cardiac troponin I (TNNI3) (Kimura et al., 2002), cardiac troponin C (TNNC1) (Hoffmann et al., 2001), the cardiac myosin binding protein C (MYBPC3) (Watkins et al., 1995) and titin (TTN) (Hoffmann et al., 2001) (Table 1). Linkage studies and candidate-gene approaches have demonstrated that about half of the patients have mutations in one of six disease genes- MYBPC3, TNNT2, TPM1, MYH7, MYL2 and MYL (Kimura et al., 1997). The nature of individual mutations is also highly variable, including missense, frame-shift and nonsense mutations, as well as a few small in-frame deletions or insertions and very rare large deletions. Mutations are usually located along the full length of a gene with no predominant location (Charron et al., 2010).

Mutations in the gene of MYH7 and MYBPC3 together account for about 50% of cases (Marian, 2010; Niimura et al., 2002; Watkins et al., 1995; Watkins et al., 1992). MYH7 is the first gene identified to cause HCM. Mutations found in MYH7 are mostly point mutations leading to the substitution of one amino acid by another (missense mutations). These mutations are happened to occur mainly in the region encoding the globular head region of the protein, which interacts with the actin filament (Marian, 2010; Roberts, 2002). Mutations in MYBPC3 are mainly insertion and deletion mutations leading to a frame shift or premature truncation of the protein and affect the protein binding site. TNNT2, TNNI3, TPM1 and ACTC1 mutations are mostly missense mutations. Among these TNNT2 accounts for 5-10% of HCM and the rest are insignificant in causing HCM (Fowle et al., 2010).

4. GENOTYPE – PHENOTYPE CORRELATION

Researchers have always attempted to correlate genotypes to particular clinical phenotypic expressions. But several limitations stand in establishing genotype-phenotype correlation, such as small size and number of families with identical mutations, variable penetrance and expressivity, low frequency of each mutation, and the effect of modifier genes and non-genetic factors (Roberts, 2002). In general, it is clear that no particular clinical phenotype is mutation-specific and mutations exhibit highly variable clinical, electrocardiographic, and echocardiographic manifestations (A. Marian & Roberts, 2001). Considering the limitations, genotype-phenotype correlation studies have been performed for a limited number of mutations in the MYH7, TNNT2, TPM1 and MYBPC3 genes (Charron et al., 1998; Epstein et al., 1992; Tesson et al., 1998; Thierfelder et al., 1994; Watkins et al., 1995).

The first study of genotype-phenotype correlation was published by Watkins et al. (Watkins et al., 1992) in 1992 in which they found 7 different missense mutations in head or head-rod junction of MYH7 gene. Their analyses confirmed that the Val606Met (V606M;exon14) mutation was associated with longer survival than was the Arg453Cys (R453C;exon14) and Arg403Gln (R403Q;exon 13) mutations (Watkins et al., 1992). Along with R403Q and R453C mutations, Gly716Arg (G716R; exon19) and Arg719Trp (R719W; exon 19) mutations are reported to alternate the charge of the encoded amino acid and thus associated particularly with a high incidence of SCD and considered “malignant” mutations in comparison with other HCM-causing mutations (Anan et al., 1994; Bos et al., 2009; Van Driest et al., 2002). Glu930Lys and Arg349Gln are reported to have intermediate severity with a life span of about 45 years (Roberts, 2002). In contrast, 6 particular missense mutation in MYH7 (N232S, G256E, F513C, V606M, R719Q, and L908V), as well as the S179F mutation in troponin I (TNNT2) and the D175N mutation in α-tropomyosin (TPM1), have been designated as benign mutations with no increased risk of SCD (Anan et al., 1994; Fananapazir & Epstein, 1994; Marian & Roberts, 1998; Roberts & Sigwart, 2001; Van Driest et al., 2002; Watkins et al., 1992).

In 1995, Niimura et al. determined the clinical consequences of 12 novel mutations in the MYBPC3 gene (Niimura et al., 1998). All the mutations exhibited reduced penetrance until the fifth decade of life, whereas hypertrophic cardiomyopathies caused by mutations in other genes were almost completely penetrated by the second or third decade (Niimura et al., 1998). They concluded that MYBPC3 mutations account for the milder forms of hypertrophic cardiomyopathy and survival of patients with cardiac myosin binding protein C mutations was better than that observed with cardiac troponin T mutations or malignant cardiac β-myosin heavy-chain mutations (Niimura et al., 1998).

Huges S. (Hughes, 2004) illustrated that mutations in troponinT cause only mild or subclinical hypertrophy yet studies of families bearing this mutation reveal extensive myocyte disarray (Varnava et al., 2001) and a poor prognosis with a high risk of SCD in adolescence or early adulthood.
Despite minimal hypertrophy

...sequencing (NGS) techniques

...eliminated the

...2009

...entered the mainstream of the health care arena from

...testing help in assessing the prognosis. In younger family members who do not have clinical or echocardiographic evidence of hypertrophy, it would be an invaluable tool for determining those at risk and would provide a window of opportunity to apply treatment or to prevent the phenotype from developing. Furthermore, genetic testing will help to develop the necessary database that will ultimately be used to routinely screen for mutations (Roberts, 2002).

Mutations in the α-tropomyosin gene are also associated with variable clinical phenotypes. Some mutations are characterized by low penetrance, variable hypertrophy and a near normal life expectancy (Coviello et al., 1997), whereas others are associated with a more aggressive clinical course (Karibe et al., 2001). Thierfelder L et al. (Thierfelder et al., 1994) in 1994 demonstrate that missense mutations (Asp175Asn; Glu180Gly) in the α-tropomyosin gene cause HCM.

It is clearly understood from the observation of genotype-phenotype studies that though certain mutations are predictive of SCD, the clinical features don’t reliably predict SCD.

5. Genetic Testing of HCM

Major breakthroughs in the molecular genetics of HCM have made genetic testing now available in clinical practice, also have raised new questions about its implications, potential benefits, and the organization of the procedure (P Charron et al., 2002). Genetic testing of HCM relies on complete sequencing of the gene coding regions (Golbus et al., 2012). Because of the significant variability in phenotypic presentation, age of onset and the course of disease associated with genetic heterogeneity, it is difficult to suggest a common guideline which would be applicable in clinical practice (Roberts, 2002). Despite these limitations, genetic testing would provide important information in families with a history of HCM. The principle role of genetic testing is in diagnosis (Bos et al., 2009). Screening of family members is universally recommended (i.e., to identify those at risk for developing disease who do not have LV hypertrophy) (Ackerman et al., 2011). The likelihood of obtaining a positive test is about 50%, as all genes causing HCM have not yet been identified and are absent from testing panels (Ackerman et al., 2011; Andersen et al., 2009; Erdmann et al., 2003). Depending on the type of identified mutation, genetic testing help in assessing the prognosis. In younger family

<table>
<thead>
<tr>
<th>Gene</th>
<th>Symbol</th>
<th>Frequency</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-myosin heavy chain</td>
<td>MYH7</td>
<td>30-50% (Kaskietal., 2009; Perrotetal., 2005)</td>
<td>14q12</td>
</tr>
<tr>
<td>Myosin binding protein C</td>
<td>MYBPC3</td>
<td>30-40% (Kaskietal., 2009)</td>
<td>11p11.2</td>
</tr>
<tr>
<td>Cardiac tropinin</td>
<td>TNN2</td>
<td>&lt;5% (Kaskietal., 2009)</td>
<td>1q22</td>
</tr>
<tr>
<td>Cardiac troponin</td>
<td>TNN3</td>
<td>&lt;5% (Kaskietal., 2009)</td>
<td>19q13.4</td>
</tr>
<tr>
<td>Cardiac troponinC</td>
<td>TNNC1</td>
<td>&lt;1% (Kimura etal., 1997)</td>
<td>3p21.1</td>
</tr>
<tr>
<td>α-tropomyosin</td>
<td>TPM1</td>
<td>1-5% (Richardetal., 2003)</td>
<td>15q22.1</td>
</tr>
<tr>
<td>Tnβin</td>
<td>TIN</td>
<td>&lt;1% (Satohetal., 1999)</td>
<td>2q31</td>
</tr>
<tr>
<td>α-Actin</td>
<td>ACTC</td>
<td>&lt;1% (Mogensenetal., 1999)</td>
<td>15q14</td>
</tr>
<tr>
<td>Myosin ventricular essential light chain 1</td>
<td>MYL3</td>
<td>&lt;5% (Kaskietal., 2009)</td>
<td>3p21</td>
</tr>
<tr>
<td>Myosin ventricular regulatory light chain 2</td>
<td>MYL2</td>
<td>&lt;5% (Richardetal., 2003)</td>
<td>12q4.11</td>
</tr>
</tbody>
</table>

(Kimura et al., 1997; Moolman et al., 1997). The Arg92Trp (R92W:exon 9) mutation in TNNT2 has been associated with a high incidence of SCD in spite of minimal hypertrophy (Ackerman et al., 2002). A recently identified novel missense mutation Lys273Glu in the cardiac troponinT gene is reported to be associated with a high degree of penetrance, a high incidence of SCD and a partial transition to the dilated phase of HCM (Fujino et al., 2002).

Until recently, genetic testing for HCM was confined to few research laboratories in order to understand the genetic basis of the disease. Now, genetic testing has been possible because of the advances in DNA sequencing techniques. Currently, four techniques are being used- Sanger sequencing, high resolution melting, mutation detection using DNA arrays and next-generation sequencing (NGS) techniques (Rom-Rodrigues & Fernandes, 2014). In 2003, molecular genetic testing entered the mainstream of the health care arena from the research field with automated DNA Sanger Sequencing (Maron et al., 2012). The necessity for electrophoretic separation and also the large sizes of main HCM genes made Sanger Sequencing labor intensive and expensive (Bos et al., 2009; Hert et al., 2008; Maron et al., 2012; Santos et al., 2011). Nevertheless, Sanger Sequencing is a gold standard validation technique in parallel with novel techniques (Mardis, 2011; Santos et al., 2012).

The limitations of Sanger Sequencing accelerate the use of DNA microarrays as an alternative technique. In contrast with automated Sanger sequencing, high throughput techniques such as iPLEX mass spectrometry genotyping (MSG) and high resolution melting (HRM) allow rapid and cost-effective testing for a large number of different mutations simultaneously. MSG involves multiplex primary PCR using outer primers that flank HCM mutation sites followed by a homogeneous mass extend (hME) reaction with multiple single inner primers that together generate fragments of different mass specific for each genotype (iPLEX). On the other hand, HRM relies on the differential melting properties of sequences that vary in at least one nucleotide which makes it a very sensitive technique. For this reason it is used to identify novel mutations within samples (Santos et al., 2011).

NGS technologies have now completely eliminated the limitations of Sanger Sequencing (Need et al., 2012). By virtue of sequencing clonally amplified DNA templates or single DNA molecules in a massively parallel fashion in a
flow cell, NGS provides both qualitative and quantitative sequence data (Voelkerding et al., 2010). Most institutional and commercial laboratories screen frequently mutated sarcomere genes–MYH7, MYBPC3, TNNT2, MYL2, TPM1, TNN3, MYL2, MYL3, ACTC, CSRP3, TNNC1 and TCAP (Bos et al., 2009; A. J. Marian, 2010; Roma-Rodrigues & Fernandes, 2014; Van Langen, 2010), Harvard Partners, Corelagen, PGiHealth, GeneDX and Transgenomic-FAMILION these companies are currently offering genetic testing for these genes (Bos et al., 2009; Maron et al., 2012). At the cardiogenetics clinic of the Erasmus Medical Centre in Rotterdam, the molecular analysis of HCM involves initial screening for the three Dutch founder mutations in the MYBPC3 gene. In the absence of the founder mutations, subsequently the MYBPC3 and the MYH7, TNNT2, TNN3, MYL2, MYL3, ACTC and TPM1 are sequenced (Michels et al., 2007).

All the high-throughput techniques in current days allowed simultaneously testing of more samples and inclusion of established HCM-causative genes, phenocopy associated genes and other genes with lesser evidence of pathogenicity. But the high-throughput combination of qualitative and quantitative sequence information generated by NGS has positioned the technique as the method of choice for large-scale complex genetic analyses including whole genome and transcriptome sequencing (Voelkerding et al., 2010). As a result, NGS has had a fundamental and broad impact on many facets of biomedical research. In contrast, the dissemination of NGS into the clinical diagnostic realm is in its early stages. Though NGS is powerful and can be envisioned to have multiple applications in clinical diagnostics, the technology is currently complex. Successful adoption of NGS into the clinical laboratory will require expertise in both molecular biology techniques and bioinformatics.

6. GENETIC COUNSELING OF HCM

Genetic counseling is an essential component of any genetic testing process and aims to provide patients and their families with the resources necessary to understand, cope with and make decisions about genetic disease (Cowan et al., 2008). Genetic testing is associated with a variable degree of complexity and psychological implications (P Charron et al., 2002). Here, genetic counseling plays the role of a helpful adjunct to assist with the diagnosis and management of the diseases (Cowan et al., 2008; Hanson & Hershberger, 2001; Hershberger, Cowan, Morales, & Siegfried, 2009; Yu et al., 1998). In USA and Canada, genetic counseling is traditionally carried out by board certified, masters-trained counselors in collaboration with physicians (Cowan et al., 2008). The variable penetrance in HCM, unpredictability of SCD and genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). Europace, 13(8), 1077-1109.

7. CONCLUSION

New HCM-related mutations are continuously being revealed and much more are still to be discovered (Roma-Rodrigues & Fernandes, 2014). And because of the large genetic heterogeneity of the disease, new strategies of genetic testing is needed to be developed. This demand for molecular genetic testing will certainly increase patient care and influence family-planning decisions (Bonne et al., 1998). Matter of hope that the continued investment in the full spectrum of genetic research, from identification of human mutation to analyses of mechanism, has great promise for advancing the science and treatment of Hypertrophic Cardiomyopathy (Seidman & Seidman, 2001).

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9. REFERENCES


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