

Pathological Mechanisms of Sarcomere Mutations in the Disease Hypertrophic Cardiomyopathy

A Review

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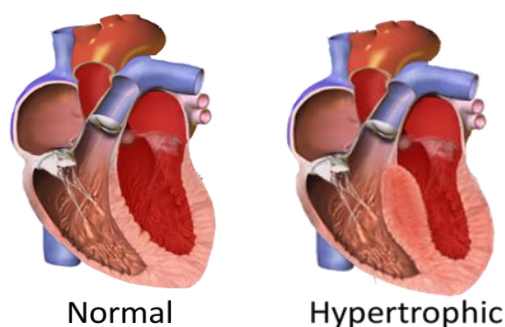
1. Abstract

Hypertrophic cardiomyopathy is a heart disease that is characterized by an enlarged heart muscle. Mutations to sarcomere proteins in the muscle fibers give rise to the disease, and this review aims to compile the mechanisms by which the mutations cause the disease phenotype. β -myosin heavy chain mutants affect the thick filament structure and contraction velocity of the muscle. Mutations to the myosin-binding protein C produces truncated proteins with decreased expression in the cells. Troponin T mutants cause myofibrillar disarray, alters affinity to α -tropomyosin, and are linked to a higher risk of sudden death. Troponin I is an unpredictable mutant that needs to be further researched but is thought to cause regulatory problems. Mutations to α -tropomyosin and the regulatory myosin light chain both affect the Ca^{2+} -affinity of the proteins and leads to contractile problems. Hypercontractility as a result of the mutations seems to be the primary cause of the disease. Hypertrophic cardiomyopathy is linked to sudden death, and factors such as a family history of sudden death, multiple simultaneous mutations, unexplained syncope, non-sustained ventricular tachycardia, abnormal blood pressure response and extreme hypertrophy (>30 mm) heightens the risk of a sudden death. An increased knowledge about the disease will aid in the mission to better the treatments for the affected, but further investigation of pathological pathways needs to be performed.

Keywords: Hypertrophic Cardiomyopathy, Mutations, Proteins, Sarcomere, Sudden Cardiac Death.

2. Introduction

Hypertrophic cardiomyopathy (HCM) is a relatively common cardiac disease that is generally defined by an enlarged and thickened heart muscle (Fig 1), which is often most prevalent around the left ventricle (reviewed by Maron, 1997 and Maron, 2002). Symptoms of the disease cover a broad spectrum of severity, and the affected can range from asymptomatic to having severe diastolic problems and cardiac output obstruction leading to heart failure (Spirito et al., 1989; Maron et al., 2018). It has long been established that the hypertrophy is a response to functional defects of the sarcomere in the myocytes (Bonne et al., 1998). The sarcomere defect is thought to cause the muscle to be in a hypocontractile or hypercontractile state which in turn leads to a changed cardiac function, which is compensated for by the hypertrophic response of the heart muscle (Bonne et al., 1998). A normal cardiac muscle fiber contains myofibrils with many sarcomeres. The contractile unit of the sarcomere consists of myosin (thick filament) and actin (thin filament) and other collaborating proteins. During contraction (initiated by Ca^{2+} binding to actin), the thick and thin filament slide by each other.



*Figure 1. Hypertrophic obstructive cardiomyopathy.
The difference between a diseased and normal heart.
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HCM is estimated to be prevalent in about 1 out of 500 in the population, which makes it one of the most common heart diseases (Maron et al., 1995). The prognosis of HCM is comparatively benign as even with its high prevalence, it sports a low annual mortality rate of 1% (Kofflard et al., 1993). HCM is linked to sudden cardiac death and is the main cause for this (Maron et al., 1982; Maron & Maron, 2013). However, sudden cardiac death can also happen as a result of mutations to ion channels (reviewed by Abriel et al., 2015). Sudden cardiac death predominantly affects the young population and well-trained athletes, and most

have experienced no or very mild symptoms before the sudden death (Maron & Maron, 2013).

Hypertrophic cardiomyopathy is a genetic disease that is inherited in an autosomal dominant pattern (Maron et al., 1984). This means that an offspring who has an affected parent has a 50% chance of inheriting the disease. HCM is caused by mutations to the various genes encoding sarcomere proteins (Richard et al., 2003). Mutations of genes that affect the β -myosin heavy chain protein and the myosin-binding protein C of cardiac muscle cells are the most common ones, though various other mutations are also known (Richard et al., 2003). This review will summarize some of the mutations and present the pathways that lead to cardiac hypertrophy. A link between these mutations that cause HCM and sudden cardiac death will also be explored along with factors that increase the risk for sudden death to happen to the affected.

3. Genetic Alterations Underlying Hypertrophic Cardiomyopathy

So far, researchers have found that mutations of several different sarcomere protein genes contribute to hypertrophic cardiomyopathy (reviewed by Maron & Maron, 2013). Along with those, several Z-disc proteins have also been linked to hypertrophic cardiomyopathy, broadening the causative genes to the sarcomere and its linking structures (Satoh et al., 1999; Alcalai et al., 2008). Together with the mutations in sarcomeric proteins, other diseases and syndromes are also known to cause hypertrophic cardiomyopathy as an additional effect. A description of the mutations of sarcomere proteins and the pathway from mutation to hypertrophic cardiomyopathy are presented below. All proteins and mentioned substitutions are summarized in Table 1.

Table 1. The sarcomere genes and their respective proteins, with mentioned missense, deletion and insertion mutations. For example, Thr124Ile means that the amino acid threonine has been changed to amino acid isoleucine at position 124.

Gene	Protein	Mutations
MYH7	β -myosin heavy chain	Thr124Ile
		Tyr162Cys
		Gly256Glu
		Arg403Gln
		Arg453Cys
		Val606Met
		Arg719Trp
		Gly741Arg
		Arg870His
		Leu908Val
MYBPC3	Myosin-binding protein C	DelCys698
		InsGly791
TNNT2	Troponin T	Ile79Asn
		Arg92Leu
		Arg92Gln
		Arg92Trp
		Arg94Leu
		Ala104Val

		Phe110Ile
		Glu163Lys
TNNT3	Troponin I	Arg141Gln
		Arg145Trp
		Arg145Gly
		Arg145Gln
		Ala157Val
		Arg186Gln
TPM1	α -Tropomyosin	Val95Ala
		Asp175Asn
		Glu180Gly
MYL2	Regulatory myosin light chain	Ala13Thr
		Phe18Leu
		Glu22Lys
		Asn47Lys
		Arg58Gln
		Pro95Ala

3.1. Mutations That Affect the β -Myosin Heavy Chain Protein

The first mutation that linked altered sarcomere proteins to hypertrophic cardiomyopathy was discovered in the β -myosin heavy chain gene MYH7 by Geisterfer-Lowrance et al. (1990).

The research team studied two unrelated families who both expressed the disease, and separately found a mutation in exon 13 of the gene where a point mutation had substituted an arginine residue at position 403 to a glutamine residue (Geisterfer-Lowrance et al., 1990). The Arg-403 residue is highly conserved and has been unaltered for at least 600 million years, which contributed to the drastic change to the disease phenotype. The mutation was also hypothesized to markedly change the structure in the mutated region, as the charge of the region was altered from positive to neutral (Geisterfer-Lowrance et al., 1990). Furthermore, the malignancy of this specific Arg403Gln mutation was a discussed topic after its discovery. In a study by Watkins et al. (1992) which included the mutation, it had an immensely poor prognosis where mortality of the affected neared 50% in deaths related to the disease.

Including sudden deaths, that number exceeded the 50 mark. On the other hand, in a study by Fananapazir & Epstein (1994) a Korean family with the Arg403Gln mutation that expressed

hypertrophic cardiomyopathy all had a 100% survival rate at 50 years old, completely contradicting the previous study and the malign prognosis. Landstrom & Ackerman (2010) took both studies into account and speculated that epigenetic and genomic factors may have a bigger role than previously expected on the phenotypic expression of the disease. Still, many mutations fall into “benign” or “malign” labels based on their statistical record on expression and mortality.

After the initial discovery, many more mutations of the MYH7 gene were found. Several studies have pointed out mutations to the β -myosin heavy chain gene to be one of the 2 most common causes of hypertrophic cardiomyopathy with a frequency of 25-44 % in the affected, with the other being MYBPC3 (Richard et al., 2003; Alcalai et al., 2008; Bos et al., 2009). The β -myosin heavy chain protein is the primary protein of the thick filament of the sarcomere, and thus an important member of the functional contractile unit (Fig 2). One can therefore assume that a disrupted or altered β -myosin heavy chain protein will affect the contraction of the cardiomyocyte. Indeed, while measuring the rate by which the β -myosin heavy chain translocated actin, all MYH7 mutations in the study negatively affected the translocation rate compared to the controls in varying degrees (Cuda et al., 1997). The seven MYH7 mutations in the study were Leu908Val, Arg870His, Val606Met, Arg403Gln, Gly256Glu, Tyr162Cys and Thr124Ile, where Arg403Gln and Tyr162Cys had the most reduced motility (Cuda et al., 1997). This study also supports the shown heterogeneity of the disease, as mutations caused a variety of different expressions. An earlier study by Lankford et al. (1995) additionally demonstrated contractile deviations of some β -myosin mutations. The Arg403Gln mutant had a lowered force:stiffness ratio and reduced motility, while the Gly741Arg mutant had a reduced power output. Meanwhile, the Gly256Glu mutation had no significant deviation from the muscle of those in the control subjects. Many of the MYH7 mutations affected the ATP, actin, and light chain binding sites of the heavy chain (Richard et al., 2003).

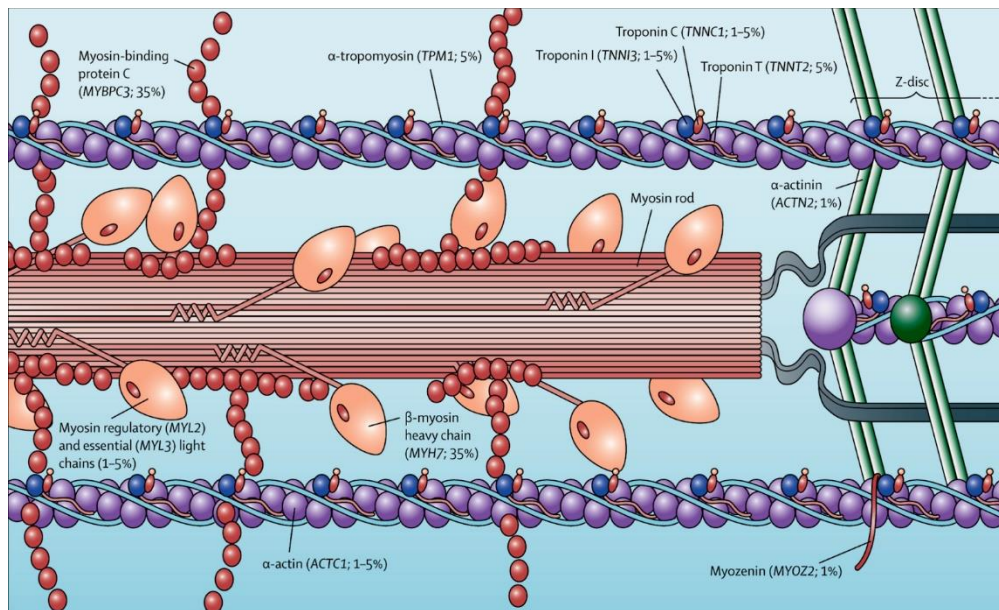


Figure 2. Some proteins and genes of the cardiac sarcomere that make up the cardiac contractile unit. In parentheses are rough frequencies of overall HCM cases caused by respective parts. Used with permission from Elsevier (from Maron & Maron, 2013).

Recently, new studies have established that MYH7 mutations induce hypercontractility, that is, excessive contraction. The MYH7 mutation caused prolonged and delayed relaxation of the fibers and increased force of muscle twitches (Cohn et al., 2019). This also caused extreme stress for the muscle fibers, as it induced oxidative stress and elevated cytotoxicity (Cohn et al., 2019).

In general, the three mutations Gly256Glu, Val606Met and Leu908Val are considered benign with a relatively safe prognosis (Marian & Roberts, 1998). In most cases, few expressed the disease phenotype, the risk for sudden cardiac death was relatively low and for the Gly256Glu and Leu908Val mutations, the hypertrophy was light (Epstein et al., 1992; Fananapazir & Epstein, 1994). Other notable mutations have malign prognoses of varying extent. Apart from the one Korean family already mentioned, most lineages with the Arg403Gln mutation experiences grim fates with high risk of sudden death while young (Watkins et al., 1992; Marian & Roberts, 1998). The Arg453Cys and Arg719Trp mutations were also linked to premature death due to HCM, and the hearts of the individuals with the latter mutation were characterized by severe hypertrophy (Watkins et al., 1992; Anan et al., 1994). According to Woo et al. (2003) mutations that affect the actin binding site and the rod of the protein often had a poor prognosis. In the study by Richard et al. (2003), HCM-affected families with a poor prognosis most often had a mutation of the MYH7 gene.

3.2. Mutations That Affect the Myosin-Binding Protein C

The role of the myosin binding protein C (encoded by the gene MYBPC3) is to bind to the previously mentioned β -myosin heavy chain (Fig 2) (Niimura et al., 1998). It regulates the interaction between the myosin and actin chains, and in addition to providing structural support to the sarcomere, it also seems to somewhat affect the contraction by altering attachment rates (Weisberg & Winegrad, 1996; Niimura et al., 1998). The mutations of the MYBPC3 gene comprises around 18-42% of the total hypertrophic cardiomyopathy cases, and MYH7 and MYBPC3 mutations are often said to together account for around 70% of the cases (Erdmann et al., 2003; Richard et al., 2003).

Compared to the mutations that affect the β -myosin heavy chain which have mostly been reported to be missense mutations, myosin-binding protein C mutations have a reported range from missense mutations, nonsense mutations, splice mutations and insertions and deletions leading to a frameshift of the gene and transcribed mRNA (Niimura et al., 1998; Richard et al., 2003). In a study with 57 unrelated individuals with MYBPC3 mutations, 44% turned out to have a missense mutation, while 38% had a mutation that involved a deletion, insertion, or a splicing mutation (Page et al., 2012). While many of the MYH7 mutations had a malign prognosis, many of the MYBPC3 mutations instead have a milder prognosis. Penetration of the disease is not at all guaranteed, and when the disease is expressed phenotypically it is often developed later in life (Charron et al., 1998; Page et al., 2012). The hypertrophy and symptoms could be considered mild, and if death occurred it was mainly sudden and risk of death due to complications of the disease was quite low (Charron et al., 1998; Niimura et al., 1998; Page et al., 2012). When the prognoses of MYBPC3 mutation patients were studied, 90% of them had a good or intermediate prognosis, better than any of the other sarcomere protein mutations (Richard et al., 2003).

One special type of mutation in the MYBPC3 gene is one that causes a premature stop codon in transcribed RNA, leading to a truncated polypeptide of the myosin-binding protein C (Carrier et al., 1997). For example, InsGly791 and DelCys698 (insertion and deletion, respectively) both caused a frameshift that caused the premature termination of translation and ended in a truncated protein where the important C-terminal was either completely gone or severely mutated (Niimura et al., 1998). Until recently, it was unclear how the truncated protein affects the sarcomere, but aside from the subsequent hypertrophy, the altered protein

was expected to influence sarcomere structure and contraction regulation as several phosphorylation sites are lost (Niimura et al., 1998). People affected by a truncation mutation of the myosin binding protein C gene had an earlier onset of hypertrophic cardiomyopathy expression, and often needed more intense treatments than missense/in-frame mutations (Erdmann et al., 2001). Approximately 2 in 3 mutations of the MYBPC3 gene that causes HCM are truncations (Marston et al., 2009).

Modern research shed a bit of light of the pathology of HCM-related truncation mutations, special for the myosin-binding protein C. Mutations of the other sarcomere proteins have a “poison peptide”-effect meaning that their defects interfere with or affect the efficiency of how the sarcomere normally works, however evidence suggests that MYBPC3 mutations instead results in haploinsufficiency (Marston et al., 2009). Haploinsufficiency describes a phenomenon where the single normal allele cannot produce a functioning protein in combination with a mutant allele in heterozygotes, or at least decreases the amount of functioning proteins. Indeed, Marston et al. (2009) found that HCM patients with MYBPC3 mutations had a significantly lower content of myosin-binding protein C compared to non-HCM individuals. They suggest that it is likely that some of the truncated proteins are degraded and thus lowers the expression. This phenomenon was interestingly enough not only restricted to truncation mutations, as even missense mutations also produced lower amounts of the protein, perhaps due to premature proteolysis of the mutants (Cohn et al., 2009). It is highly expected that the reduction of the MYBPC proteins has a contractile effect and the haploinsufficiency must be a big part in the development of the hypertrophic cardiomyopathy disease, with this explanation it is not surprising that patients with the truncation type of HCM-mutations have an earlier onset either.

3.3. Mutations That Affect the Troponin T Protein

Cardiac troponin T (encoded by gene TNNT2) is a regulatory protein that has an important role in the heart muscle contraction through its interactions with other proteins (reviewed by Perry, 1998). Troponin T forms the troponin complex together with troponin I and troponin C, and then interacts with tropomyosin and actin, other sarcomere proteins (reviewed by Perry, 1998). This interaction allows troponin T to fill a structural role and position the troponin complex along the actin protein, and together with tropomyosin it regulates heart muscle contraction via calcium ion sensitivity (reviewed by Perry, 1998). Though mutations that affect this protein are not as common as the aforementioned MYH7 and MYBPC3, it still

causes around 5-7% of overall hypertrophic cardiomyopathy cases (Richard et al., 2003; Alcalai et al., 2008).

In an experiment by Marian et al. (1997), the mutation Arg92Gln was induced in troponin T to study its effect on the myocyte's contraction. Compared to a normal non-mutant troponin T after 3 days, the normal shortening of the myocyte of the mutated troponin T was reduced by 45%, while the rate of said shortening had been reduced by 39% (Marian et al., 1997). This indicates that the mutation Arg92Gln had a significant effect on myocyte and sarcomere motility, and that other mutations may also have this effect. A few years later, Palm et al. (2001) sought to answer the question of by which mechanism the mutations cause the disease and contractile problems. Like the previous study, they introduced several different mutations to TNNT2 in different locations between the 79-179 regions. It was thus found that a region between residues 92-110 affected the α -tropomyosin mediated responsibilities of troponin T and the affinity to α -tropomyosin by influencing the helical stability of the protein (Palm et al., 2001). Mutations Arg92Leu, Arg92Gln, Arg92Trp, Arg94Leu, Ala104Val, and Phe110Ile induced difficulties in the binding of the tropomyosin to actin by making the α -helices in troponin T "too stable" and stiff, while the mutations Ile79Asn and Glu163Lys did not (Palm et al., 2001). Those three mutations outside the 92-110 region still caused HCM in the carriers, but in another way than disrupting the interaction between troponin T and α -tropomyosin. Mutations that do reside inside the region seemingly cause muscle fibril disarray and negatively affected force generation together with contractile abilities (Palm et al., 2001). This explains why Lin et al. (1996) a few years earlier could not find any change in affinity of troponin T for α -tropomyosin while studying the link between Ile79Asn and hypertrophic cardiomyopathy. However, it was found that the Ile79Asn mutation on TNNT2 increased the motility as the sliding rate of the thin filament was enhanced (Lin et al., 1996). This provides another mechanism by which the disease can be induced in the heart, as increased contraction can lead to the heart going into a hypercontractile state, leading to the compensatory hypertrophy.

Generally, individuals with troponin T mutations seem to have less severe symptoms and phenotype of the heart than individuals with MYH7 mutations (Watkins et al., 1995). Notably, the hypertrophy was lighter and less thick (Watkins et al., 1995; Moolman et al., 1997). However, the families that had histories of hypertrophic cardiomyopathy due to troponin T mutations had a considerable higher risk of sudden cardiac death (Watkins et al.,

1995; Moolman et al., 1997). The life prognosis for many troponin T mutations is poor, and Watkins et al. (1995) reported a life expectancy of around 35 years for the affected. The people who died because of sudden cardiac death were very young, with a mean age of 17 years (Moolman et al., 1997). It is speculated that the high prevalence of sudden death linked to the troponin T mutations may have a part in the low frequency of overall troponin T HCM cases (Richard et al., 2003).

3.4. Mutations That Affect the Troponin I Protein

Troponin I, encoded by the *TNNI3* gene, has an inhibitory role in the troponin complex. It binds to troponin C in the complex and through binding to actin, it can prevent myosin from binding to the actin, preventing contraction. Mutations that affect the troponin I protein are rare with a prevalence of 3% in HCM cases, making research of it scarce (Mogensen et al., 2004; Millat et al., 2010). Most of the mutations that causes the disease seem to be localized in one of three regions; the actin binding site, the troponin C binding site or the C-terminal, all localized in the highly conserved 7th or 8th exon (Kimura et al., 1997; Mogensen et al., 2004; Millat et al., 2010; Curila et al., 2012). The hypertrophy can thus be suspected to have been caused by disruptions in the binding of troponin I to the troponin complex or the binding to actin, but further research and studies would have to support this. Kimura et al. (1997) also speculate that the mutations may cause contractile dysregulation in the sarcomere and myocyte, leading to hypercontractility.

Arginine substitutions have been reported across multiple studies and seem to be the most commonly mutated amino acid residue in *TNNI3* (Kimura et al., 1997; Mogensen et al., 2004; Millat et al., 2010; Curila et al., 2012). The mutation Arg141Gln was found in two separate studies, and in both studies it was found twice in two unrelated families, indicating that this mutation may be a common one among the *TNNI3* mutations (Curila et al., 2012; Mogensen et al., 2004). Arginine at position 145 is indicated to be a hotspot for substitutions as Arg145Trp, Arg145Gly and Arg145Gln have all been found to cause troponin I mutants in 3 studies (Kimura et al., 1997; Mogensen et al., 2004; Millat et al., 2010).

HCM-causing mutations of the *TNNI3* gene are very heterogeneous, that is, disease expression is both malignant and benign in almost equal tendencies and symptoms of the disease vary (Mogensen et al., 2004). Penetrance of the disease is not complete, as in Mogensen and his team's study (2004) only 48 individuals of 100 mutant carriers expressed

the disease phenotype (48%). Individuals with an Ala157Val respective Arg186Gln mutation showed symptoms of systolic dysfunction and later heart failure, along with disarray in the myofibrils (Mogensen et al., 2004). People with the Arg145Trp mutation varied extraordinarily in their symptoms. In the same family, one individual died from complications of severe hypertrophy, while another family member had only ever shown some irregularity in screenings (Mogensen et al., 2004). The story was the same for other families and family members, and points to the unpredictability of the troponin I mutations. Some individuals had sudden deaths, but TNNI3 is not considered high risk in that regard (Mogensen et al., 2004). All in all, no pattern of expression could be put on the TNNI3 mutations because of the great diversity in disease expression. Troponin I mutants are perhaps the most unpredictable and heterogenous of the sarcomere protein mutants, which contributes to the difficulty in studying them. It also suggests that other factors may contribute to the disease expression, which will need to be further studied.

3.5. Mutations That Affect the α -Tropomyosin Protein

The contraction and relaxation of the heart muscle cells is partially regulated through α -tropomyosin. Encoded by the TPM1 gene, the α -tropomyosin protein binds to troponin T and mediates interaction between actin and myosin depending on the calcium ion levels in the cell. A mutation that affects α -tropomyosin can be considered very rare, as it makes up barely 2.5% of overall HCM cases (reviewed by Alcalai et al., 2008).

Like many other sarcomere mutations, TPM1 mutants were expected to impair or affect regular contractile abilities of the sarcomere. Bottinelli et al. (1998) sought to find an answer to this by studying the α -tropomyosin mutation Asp175Asn that was a known mutation that caused HCM in carriers. It was found that the α -tropomyosin in the hypertrophic cardiomyopathy patients had acquired a higher sensitivity for the calcium ions (Bottinelli et al., 1998). The mutation had occurred in the Ca^{2+} -mediated troponin T-binding region in amino acid residues that have been particularly conserved throughout history. This was thought to cause increased contractility of the heart, meaning that it brings on a hypercontractile state in the heart muscle which in turn induces the compensatory hypertrophy of the disease (Bottinelli et al., 1998). At the time, causes of HCM were thought to be mostly because of hypocontractility, or impairment of the contractile unit, but it was now beginning to become clear that enhancement of the sarcomere contractions could also induce the disease by several mutations and perhaps even be the primary cause of the disease.

The effects of α -tropomyosin mutations were further investigated by Karibe et al. (2001), this time studying the pathology and phenotype of the Val95Ala mutation in a family. Normally, the valine residue involved in the mutation is very conserved (Karibe et al., 2001). The disease phenotype was mild in this family with a hypertrophy thickness of 16 mm on average, but the family was largely plagued by sudden deaths (11 of the 26 members). Even in this study the α -tropomyosin had an increased affinity to Ca^{2+} , by 40-50%, and atypical contraction regulation leading to a decreased myosin cycle and filament sliding speed (Karibe et al., 2001). The region that the mutation affects is said to be for actin filament activation and stabilization. The thin filament has several conformation states, and the region is important for stabilizing the final activation state (Karibe et al., 2001). Issues with the activation could lead to complications with normal contraction. Additionally, the α -tropomyosin mutant Glu180Gly has proven to be more flexible while wild-type α -tropomyosin is usually stiff and rigid for the sake of stability (Li et al., 2012). This in turn causes reduced affinity for actin while the calcium affinity is up, which is another source of contractile dysregulation. Whether it is the small but notably decreased motility of the contraction or the irregularity of the regulation of the contraction that in the end caused the compensatory hypertrophy is unknown, and it is not impossible that both together could have a cooperating effect on the disease phenotype.

In general, mutations to α -tropomyosin seem to increase the affinity to calcium which leads to a chain reaction that causes the HCM disease phenotype. Mutations that cause a decreased affinity to calcium have instead proven to lead to another cardiac disease, dilated cardiomyopathy (Robertson et al., 2007).

3.6. Mutations That Affect the Regulatory Myosin Light Chain Protein

The regulatory myosin light chain encoded by the gene MYL2 is a Ca^{2+} -binding component of the thick filament of the sarcomere, and HCM mutations that affect this protein are rare at only 2% of overall cases (Alcalai et al., 2008). It binds to the neck of the myosin chain and thus regulates contraction kinetics and function through being phosphorylated.

Although the studies that have investigated MYL2 mutations are few, the effect on calcium-binding, phosphorylation and the secondary structure of the protein have been explored. Four known HCM-causing mutations on MYL2 (Ala13Thr, Phe18Leu, Glu22Lys & Pro95Ala) are located near a phosphorylation site, while the two mutations Glu22Lys and Arg58Gln are in

the immediate area of a calcium-binding loop (Szczesna et al., 2001). A sixth mutation, Asn47Lys, have been found in the actual Ca^{2+} -loop (Szczesna-Cordary et al., 2004). The calcium binding to the myosin regulatory light chain was altered for all the mutations on different levels. For Ala13Thr, Phe18Leu and Pro95Ala, the affinity to calcium was decreased 3 times compared to the wild type, whereas it had decreased 17 times for the Glu22Lys mutation (Szczesna et al., 2001). For Arg58Gln and Asn47Lys, the calcium-binding had been completely impeded (Szczesna et al., 2001; Szczesna-Cordary et al., 2004). Interestingly, phosphorylation of the mutants increased calcium affinity while simultaneously lowering the affinity for calcium in the wild-type protein (Szczesna et al., 2001). While phosphorylated, Arg58Gln mutants regained their ability to bind calcium, while the Glu22Lys mutants proved unable to be phosphorylated (Szczesna et al., 2001).

While the works that have been able to advance the explanation on the underlying mechanisms of why these changes cause the characteristics of the hypertrophic cardiomyopathy disease are few so far and do not give a straight answer, it can be assumed that the interaction between the myosin heavy chain and the regulatory myosin light chain is affected. Current research indicates that MYL2 mutations reduce rigidity and tension in the light chain which structurally affects the myosin cross bridge, which is suspected to lead to inefficient contractions in turn bringing on the disease phenotype (Zhang, in press). The reverse effect of phosphorylation between mutant and wild type MYL2 will also cause a disconnect between the intended outcome and the actual outcome of contraction regulation. While the exact steps of what happens when mutant MYL2 is present are unclear, it can be presumed that the normal contraction properties of the thick filament are affected in some way, presumably leading to hypercontraction and hypertrophy.

4. Sudden Cardiac Death

One of the most devastating potential effects that accompanies hypertrophic cardiomyopathy is the risk of sudden cardiac death, where an individual suddenly and inexplicably dies because of cardiac arrest. Sudden death in HCM patients is most prevalent among teenagers and young adults but can occur in all age groups (Maron et al., 2000). Young athletes also seem to suffer from HCM-related sudden deaths during or after physical exertion (Maron et al., 1996). All in all, sudden cardiac death is the most common way of death in the HCM-affected population, even as the annual rate of sudden death is less than 1% (Maron et al., 2000). However, some groups within the HCM-population are at a substantially higher risk of sudden death. Most of the deceased were also characterized as asymptomatic or had very mild symptoms prior to the death event, adding to the conundrum of the phenomenon (Maron et al., 2000). Because of this and that the deaths are sudden; researchers have long been striving toward identifying plausible answers as to why these deaths happen and to identify risk factors that raise the risk of sudden death for those affected by hypertrophic cardiomyopathy.

4.1. How Sudden Cardiac Death Happens

Sudden cardiac death was linked to hypertrophic cardiomyopathy and studied long before the disease was connected to mutations of the sarcomere. The cardiac impairment behind the death is brought on by cardiac arrhythmias caused by ventricular fibrillation (quivering) or ventricular tachycardia (fast heart rate) (reviewed by John et al., 2012). These arrhythmias were often instigated by premature ventricular contractions, where the arrhythmia quickly followed (O'Mahony et al., 2012).

Many mechanisms can explain why these arrhythmias occur and cause sudden cardiac death. One such explanation is the myofibrillar disarray as a consequence of the hypertrophy of the disease. The disarray caused abnormal conduction in the affected and changed conduction pathways or fragmented them, possibly due to the altered size, shape and diameter of the fibrils (Saumarez et al., 1992). These altered conduction paths can cause an irregular rhythm because of delayed conduction, increased velocity or altered refractory periods. Myofibrillar disarray is often seen as one aspect of the disease, and perhaps not always studied as the generating factor of death. In a few studies, the disarray has however been shown to be present in sudden cardiac deaths in up to 30% of individuals (reviewed by Finocchiaro et al., 2021). During an examination of myofibrillar disarray in postmortem HCM hearts, the

disorganized fibers also had disorganized gap junctions (Sepp et al., 1996). The gap junctions, which transports action potentials between cells and indirectly synchronizes contraction, had lost their specific localization, and instead globalized across the cell surface (Sepp et al., 1996). While the study did not examine the effect on live hearts, it is not far-fetched to believe that the disorganization of the gap junctions would change the electrical properties of the muscle cells and the propagation of potentials, leading to aberrant heart rhythms. Additionally, the cell structure-upholding desmosomes had also globalized across the cell, possibly explaining the disarray of the muscle fibers (Sepp et al., 1996).

Another mechanism takes calcium ion sensitivity into consideration. Several of the mentioned mutated genes increased the calcium sensitivity of the protein and filament. In transgenic mice with heightened Ca^{2+} sensitivity as a result of troponin mutations the chance of ventricular tachycardia being developed increased the higher the calcium sensitivity got (Baudenbacher et al., 2008). The increased sensitivity caused a shortening of the effective refractory period, uneven continuation of action potentials and the uneven dispersion of ventricular activation in fast beating hearts, all contributing to making the heart muscle more prone to tachycardia and sudden death (Baudenbacher et al., 2008). As the heart beats faster during and immediately after exercise, it explains why young athletes with underlying disease are so often affected, because the arrhythmia is more easily triggered during those conditions. This is also a good explanation for a higher risk of sudden death in those mutations that do not cause myofibrillar disarray, as some mutations are instead more prone to directly cause arrhythmias.

Hypertrophic cardiomyopathy is responsible for about 36% of sudden cardiac deaths (Maron et al., 2009). Other sudden deaths occur due to mutations that instead affect ion channels (called channelopathies) that causes abnormal ECG patterns and for example long QT-syndrome, which increase the risk of irregular heartbeats (reviewed by Abriel et al., 2015). Recently, channelopathies have been found to be the reason for many former unexplained sudden deaths, and they are suspected to be more common than previously thought. Initially they were found to cause 4% of sudden cardiac deaths, but now several studies have reported a range of 4-30% of deaths (Maron et al., 2009; Napolitano et al., 2012). The rest of the causes of sudden cardiac death are other cardiomyopathies, coronary artery problems, or myocarditis among others (Maron et al., 2009).

4.2. Factors Behind a Heightened Risk of Sudden Death

Some risk factors lie in the genetics of the disease. The most fatal of the gene mutants mentioned is the troponin T mutant, which almost universally comes with a poor prognosis (Watkins et al., 1995; Moolman et al., 1997; Marian & Roberts, 1998). It can perhaps also be called the most deceitful, as the phenotype of the disease caused by the mutant is very mild compared to most other mutants with light symptoms before the death events (Watkins et al., 1995; Moolman et al., 1997). Unfortunately, many of the affected by this specific mutant does not live past the age of 35 (Watkins et al., 1995). Together with most of the troponin T mutations, many β -myosin heavy chain mutations also come with a heightened risk of sudden cardiac death. Together with the most reported mutation that is Arg403Gln, Arg453Cys and Arg719Trp are also considered high risk of sudden death (Watkins et al., 1992; Anan et al., 1994). Compared to the troponin T mutants though, the heavy chain mutants often come with a more noticeable phenotype and more severe symptoms. The other gene mutants are not overall considered to be of substantially higher risk of sudden death than normally. Having said that, an emerging genetic risk factor for sudden death is the presence of multiple sarcomere mutations in the same or different genes (Maron et al., 2012). HCM-patients with a pair of mutations in the myosin-binding protein C gene, a mutant generally considered comparatively harmless, had had sudden deaths or were on the list for a heart transplant (Maron et al., 2012).

A very accurate indicator of an increased risk is a prior family history of sudden cardiac death (Bos et al., 2012). This is particularly notable when proactive work has been done in HCM family members where an implantable cardioverter-defibrillator (ICD) has been inserted to assist in detecting arrhythmias and shocking the heart to correct the rhythm. Of a group of patients who had been inserted with an ICD because of a family history of sudden cardiac death, 10% had afterwards received a discharge from the defibrillator which without it may have otherwise led to death (Bos et al., 2012). Elliott et al. (2000) found that a family history also was significant when paired with symptoms of syncope, loss of consciousness. Since then, unexplained syncope has been found to be an independent risk for sudden death in HCM patients. In particular, the risk for sudden cardiac death increased 5-fold in the aftermath of an unexplained syncope event (Spirito et al., 2009).

Another major risk factor is non-sustained ventricular tachycardia, defined as fast ventricular beats (≥ 120 beats/min) with a duration of under 30 seconds (Monserrat et al., 2003). In

Monserrat et al's (2003) group of HCM patients, 19.6% of overall patients showed non-sustained ventricular tachycardia. In a follow-up, 12.5% of those with non-sustained ventricular tachycardia had died suddenly (Monserrat et al., 2003). The study also found that non-sustained ventricular tachycardia was more so associated with a higher risk of sudden cardiac death in young people than that of older people over 30 years of age.

Multiple studies have found an abnormal blood pressure response during exercise to be a major risk factor for sudden death (Sadoul et al., 1997; Olivotto et al., 1999; Elliott et al., 2000). The abnormal response was defined by an inability of the blood pressure to rise during physical activity (a rise of <20 mm Hg), or even an unexpected decrease in blood pressure during the exercise (Sadoul et al., 1997; Olivotto et al., 1999). In three separate studies, the frequency of abnormal blood pressure response in HCM patients was found to range from 22-38%, and it was a significant marker for increased sudden death risk in people under 40-50 years of age (Sadoul et al., 1997; Olivotto et al., 1999; Elliott et al., 2000). In an earlier study by Frenneaux et al. (1990), it is explained that those with an abnormal response had normal stroke volume output of blood but a significant decrease in vascular resistance, and it was hypothesized that the fault may lie in the baroreceptors. Nevertheless, abnormalities in the blood pressure response may explain the many sudden deaths seen in young athletes during and after exercise.

Lastly, an extreme wall thickness (severe hypertrophy) is a significant marker for a heightened risk of sudden cardiac death (Spirito et al., 2000). When a group of HCM patients were divided into subgroups based on wall thickness (<15 mm, 16-19 mm, 20-24 mm, 25-29 mm, >30 mm) the risk for sudden death rose in proportion to more severe wall thickness (Spirito et al., 2000). Of those with the most severe hypertrophy (>30 mm), 16.3% died suddenly (Spirito et al., 2000).

HCM patients with several inherent risk factors are at a higher risk of experiencing sudden deaths (Fig 3) (Elliott et al., 2000). To have more than three risk factors at the same time is rare, or perhaps too much for the body to withstand. Furthermore, having 3 risk factors comes with a poor prognosis, and even 1 or 2 risk factors increases the risk of suffering a sudden cardiac death markedly. Risk factors and screening for risk factors is an important key to identify those at highest risk and preventing sudden death, for example with the implantation of an ICD to counter unexpected arrhythmias.

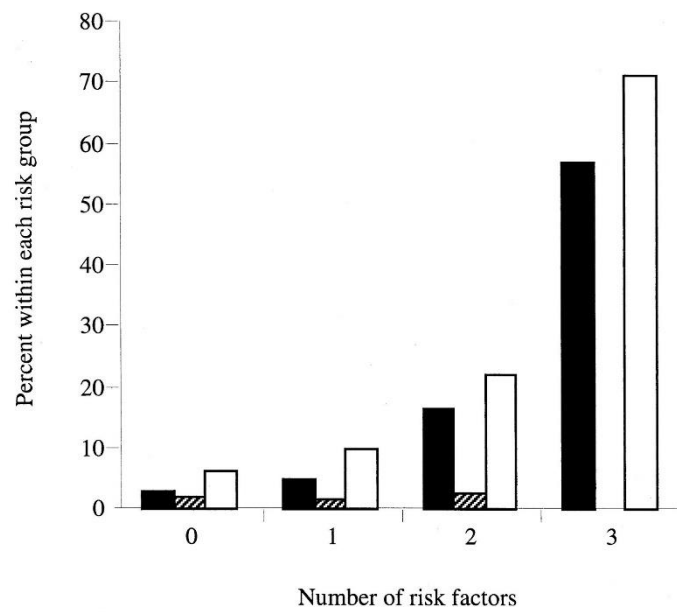


Figure 3. Groups based on number of risk factors and the percentage of deaths within the groups. Black bars represent sudden deaths while white bars represent all deaths. Striped bars represents patients who had heart failures or went through heart transplants. Individuals with 3 risk factors have an almost 3-fold increased risk of sudden death compared to those with 2 factors. With permission from Elsevier (from Elliott et al., 2000).

5. Societal Aspects

As a familial disease, hypertrophic cardiomyopathy does not only affect the individual but also whole families and lineages. Given that the prevalence of hypertrophic cardiomyopathy is 1 in 500 in a specified population, the number of individuals in Sweden can be estimated to be around 20 000 individuals. The disease is estimated to be the most common heart disease, but even then, a large number of unrecorded cases or “hidden statistics” are expected because of the heterogeneity of the disease and the large number of asymptomatic and/or undiagnosed individuals (Maron, 2004). Because of the high prevalence, research of the disease is severely needed as mysteries remain unanswered. While parts of the puzzle are answered and hypercontractility of the heart brings on the hypertrophy as a compensation, further investigation is needed to explain what brings the disease from that point to the expressed phenotype. It may even open the door for new potential treatments. Even ongoing research about the sarcomere proteins are important for risk evaluation and their part in the expression of the disease. It may also be beneficial to research the effects of various mutations that affect the genes to provide some statistics on what may happen to other individuals and use it as a ground for counseling after genetic screenings. This review has compiled various mechanisms by which the disease is caused, which may point to some expected outcomes of some aspects of the disease and help to understand what further work needs to be done to better understand this disease and potential treatments to the affected.

Risk factors for sudden death are also important to understand and explore to prevent future deaths. Risk factors can give an indication of which groups of people are at a higher risk percentage regarding sudden deaths and can provide guidelines of which individuals may need further intervention in the form of ICDs. The number of risk factors can thus stipulate the individuals who need to be prioritized in that regard. This review has presented several major risk factors of sudden cardiac death that should be taken into consideration when evaluating risk groups.

6. Concluding Remarks

This literature review has explored HCM sarcomere protein mutants and how they lead to the hypertrophic disease phenotype, together with how the disease is linked to sudden cardiac death and the risk factors that heightens the risk for the affected to experience sudden deaths. Mutations of the β -myosin heavy chain protein mainly affects the filaments' contraction velocity and filament structure which induces hypercontraction. Myosin-binding protein C mutants instead cause truncated proteins which may be non-functional and less expressed. Troponin T mutants caused myofibrillar disarray or affected the affinity to α -tropomyosin, which led to contractile problems. Troponin I mutants are hypothesized to cause regulatory issues regarding contraction, but further research is needed on these unpredictable mutants. Mutations to α -tropomyosin and the regulatory myosin light chain both affected the Ca^{2+} affinity of the proteins which leads to an altered contraction and the expression of the disease. In general, most of the mutations in the end cause the heart to go into a hypercontractile state which leads to the enlarged muscle of the disease. The compensatory hypertrophy may be induced to ensure sufficient pumping of blood in the heart due to impaired normal contraction.

Sudden cardiac death is an event often linked to hypertrophic cardiomyopathy and is caused by ventricular fibrillation or tachycardia because of myofibrillar disarray or increased calcium sensitivity. Factors in HCM carriers that heightens the risk of sudden death include (1) a family history of sudden death, (2) troponin T and β -myosin heavy chain mutations, (3) multiple sarcomere mutations, (4) unexplained syncope, (5) non-sustained ventricular tachycardia, (6) an abnormal blood pressure response and (7) severe hypertrophy (>30 mm). Possession of multiple risk factors also increases the risk of sudden cardiac death and is an indicator of which groups that should be prioritized in preventive work.

Hypertrophic cardiomyopathy is a complex disease that is characterized by its many aspects and expressions. This review has compiled information about the mutations but also shown where more research is needed. Exactly how hypercontractility causes compensatory hypertrophy is something that could be further investigated and analyzed in depth. An increased understanding of the disease and the various mutations will lead to better treatments for the affected may in the future better our comprehension to prevent sudden deaths.

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Abbreviations

(in order of appearance)

HCM	—	Hypertrophic cardiomyopathy
MYH7	—	β -myosin heavy chain
MYBPC3	—	Myosin-binding protein C
TNNT2	—	Troponin T
TNNI3	—	Troponin I
TPM1	—	α -tropomyosin
MYL2	—	Regulatory myosin light chain
ICD	—	Implantable cardioverter defibrillator