Cardiac Myosin Binding Protein C: At the Heart of Hypertrophic Cardiomyopathy in Humans and Domestic Cats

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Summary

Hypertrophic cardiomyopathy (HCM) is a disease of the myocardium that is estimated to affect one in 500 individuals (Marian & Roberts, 2001; Maron, 2002). Additionally, it is the most common form of cardiovascular disease in the domestic cat (Kittleson et al., 1999). Mutations in the cardiac myosin binding protein C (MYBPC3) gene are the most common genetic cause of HCM in humans (Richard et al., 2003) and are currently the only gene with identified mutations in cats (Meurs et al., 2005; Meurs et al., 2007). The function of the protein encoded by the MYBPC3 gene is not well understood, but further research into this area will hopefully provide more information about the manifestation of the disease and will allow for the identification of prospective treatments. With continued investigation, more can be learned about normal cardiac function, which could be applied in understanding HCM as well as other cardiovascular diseases.

Introduction

Cardiovascular disease is a public health problem that affects millions of individuals world-wide. Cardiomyopathies represent a major cause of cardiovascular morbidity and death, usually as a result of heart failure, arrhythmias, or sudden death during strenuous physical activity (Richard et al., 2006; Seidman & Seidman, 2001). They are defined as disease of the myocardium, the thick muscular wall of the heart (Seidman & Seidman, 2001). There are four main classifications of cardiomyopathy, but the two most prevalent forms are dilated, which results in an increase in cardiac chamber volumes, and hypertrophic, which results in an increase in ventricular wall thickness (Richard et al., 2006; Seidman & Seidman, 2001). The role of genetics in the etiology of these diseases has been the focus of extensive research in the medical field for the past fifteen years, which has resulted in a fundamental change in conventional knowledge surrounding cardiac function and the pathogenesis of these disorders.

Hypertrophic cardiomyopathy (HCM) is a myocardial disease that is associated with cardiac dysfunction due to asymmetrical thickening of the left ventricular wall and myocyte disarray (Richard et al., 2003; Richard et al., 2006; Seidman & Seidman, 2001). In humans, HCM has an estimated prevalence of one in 500, and it is believed to be the most common genetic form of cardiovascular disease (Marian & Roberts, 2001; Maron, 2002). Additionally, it has been estimated that the disease is inherited as a familial disease in at least 60% of cases (Marian & Roberts, 2001). Familial HCM is characterized by an autosomal dominant mode of disease inheritance and is both genetically and clinically heterogeneous (Charron et al., 1998; Richard et al., 2003).

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Causative mutations for HCM have been identified in thirteen genes encoding sarcomeric proteins (Richard et al., 2006), including the ß cardiac myosin heavy chain (*MYH7*), cardiac troponin T (*TNNT2*), and cardiac myosin binding protein C (MYBPC*3*) genes (Richard et al., 2003). Comprehensive studies by Richard et al. (2003) and Van Driest et al. (2005) both determined that mutations in MYBPC3 were the most common cause of HCM, accounting for 42% and 20% of mutations in the respective study populations.

HCM is also commonly observed in veterinary medicine and has been identified as the most common form of cardiac disease in domestic cats (Kittleson et al., 1999). Feline HCM is heritable and has been noted to be present more frequently in specific breeds, such as the Maine Coon and the Ragdoll (Kittleson et al, 1999; Meurs et al., 2005; Meurs et al., 2007). Clinical and pathological analysis have demonstrated that heritable HCM in cats closely resembles familial HCM in humans, and it has thus been proposed that cats may serve as a natural animal model of the disease (Fox et al., 1995; Kittleson et al., 1999). Recent studies have identified causative mutations in the feline MYBPC3 gene in Maine Coon and Ragdoll colonies with familial HCM. This suggests that changes in the cardiac myosin binding protein C due to mutations in the MYPBC3 gene may be an underlying cause of HCM in both humans and cats.

Hypertrophic Cardiomyopathy

Hypertrophic Cardiomyopathy has been associated with distinctive structural and cellular abnormalities within the heart. As previously mentioned, the principal anatomical feature of HCM is left ventricular hypertrophy, which is generally asymmetric and often involves the interventricular septum (Richard et al., 2003; Richard et al., 2006; Seidman & Seidman, 2001). In humans, left ventricular wall thickness is generally less than 12 mm, but can range from 13 mm to 60 mm in individuals with HCM (Maron, 2002). As a consequence of this abnormal ventricular morphology, the volumes of the left ventricular chambers are diminished considerably (Seidman & Seidman, 2001).

At the cellular level, HCM is associated with an increase in the size of cardiac muscle cells (myocytes), which ultimately causes an increase in cardiac mass (Seidman & Seidman, 2001). In addition, the enlarged, irregularly shaped myocytes can result in an overall disruption of myocardial cell architecture throughout the ventricle, collectively termed myocyte disarray (Seidman & Seidman, 2001). Interstitial fibrosis, in which more cardiac fibroblasts and extracellular matrix are present than in the healthy heart, is also common and has been associated with increased cardiac stiffness (Seidman & Seidman, 2001). In conjunction with the decrease in ventricular volume, this alteration in the heart's anatomy can impair relaxation during ventricular diastole, which hinders atrial emptying of the blood (Seidman & Seidman, 2001).

Within the medical community, HCM is recognized as heterogeneous in clinical expression, natural history, and prognosis (Maron, 2002; Richard et al., 2006). Hence, clinical manifestations of HCM are very diverse, with some affected individuals living a normal life span and experiencing no symptoms of the disease. Others, however, will experience dyspnea (difficulty breathing), syncope (fainting), angina (chest pain), arrhythmias, life-threatening heart failure, and sudden death (Seidman & Seidman, 2001;

Van Driest et al., 2005; Richard et al., 2006). Presentation of disease phenotype can occur at virtually any age, from infancy to greater than 90 years of age (Lever et al., 1989; Maron et al., 1982; Maron, 2002; Niimura et al., 2002). Clinically, HCM is usually identified through echocardiographic examination, but diagnosis of HCM will only be made in the absence of any other cardiac or systemic disease that could cause similar symptoms (Maron, 2002; Seidman & Seidman, 2001).

Clinical genetic testing for HCM is not widely available and is complicated by the involvement of numerous genes and a diverse array of mutations in the disease genotype (Van Driest, 2005). The best approach to genetic testing may be to first target the two genes associated with the greatest number of mutations, MYH7 and MYBPC3 (Richard et al., 2003; Van Driest et al., 2005). Further research into the possibility of gene-specific phenotypes in HCM patients may be beneficial prior to beginning widespread genetic testing, however, since research thus far has produced conflicting reports about whether progression of the disease varies depending on which gene carries the mutation (Anderson et al., 2004; Charron et al., 1998; Richard et al., 2006; Van Driest, 2005).

Due to the phonotypical heterogeneity of the disease, treatment strategies for HCM should be evaluated in terms of specific subgroups within the hypertrophic cardiomyopathy clinical spectrum. For patients at high risk of sudden death, the most effective treatment is the implantable cardioverter-defibrillator (Maron, 2002). Drug therapies such as ß-blockers, calcium channel blockers, antiarrhythmic agents, and diuretics, are generally utilized with patients showing signs of progressive heart failure, although heart transplantation is sometimes necessary (Maron, 2002). It is important to note, however, that except for organ transplant surgery, these treatments do not halt the progression of the disease. Ideally, more effective treatments will arise as a result of further investigation into the genetic and pathological basis of the disease.

Cardiac Myosin Binding Protein C

Mutations in the gene encoding the sarcomeric protein cardiac myosin binding protein C, MYBPC3, are recognized as a major cause of hypertrophic cardiomyopathy in humans and domestic cats (Kittleson et al., 1999; Meurs et al., 2005; Meurs et al., 2007; Richard et al., 2003; Van Driest et al., 2005). As a consequence, cMyBP-C has been the focal point of extensive research, which has provided valuable information about the gene, the resulting protein, and their significance in the manifestation of HCM.

Gene Characteristics

MYBPC3 encodes the cardiac isoform of myosin binding protein-C, which is found in the A bands of nearly all vertebrate striated muscle (Harris et al., 2002; Sadayappan, 2006). Unlike the genes for other myosin binding protein-C isoforms, however, MYBPC3 is almost exclusively expressed in heart muscle (Harris et al., 2002). The MYBPC3 gene is located on chromosome 11p11.2, as was initially determined by Gautel et al. (1995) through fluorescent *in situ* hybridization (FISH). The physical mapping of MYBPC3 to this location was one of the first indications that it may be associated with hypertrophic cardiomyopathy, which had previously been linked to mutations in this region (Gautel et al., 1995). According to AceView, the gene covers 21.3 kb on the reverse DNA strand. It contains 34 introns and transcription produces nine different mRNA (National Center for Biotechnology Information, 2007). Six of the introns are alternatively spliced, while the remaining three are unspliced forms (NCBI, 2007). Interestingly, only six of the nine mRNA variants are translated into proteins, suggesting that three of the mRNAs are non-coding (NCBI, 2007).

Protein Structure and Function

Cardiac myosin binding protein C (cMyBP-C) is a member of the intracellular immunoglobulin (Ig) superfamily and is comprised of Ig and fibronectin type III repeating domains labeled C0-C10 (see Figure 1) (Gautel et al., 1995; Kulikovskaya, 2007; Sadayappan, 2006). It contains myosin and titin binding sites near the C-terminus and actin binding sites at the protein's N-terminus (Kulikovskaya et al., 2003; Winegrad, 1999). In contrast to the skeletal muscle isoforms of myosin binding protein C, cMyBP-C contains an extra Ig domain at the N-terminus, a proline rich 28 residue insertion within the C5 domain, and three potential phosphorylation sites between the C1 and C2 domains (Gautel et al., 1995; Oakley et al., 2004; Sadayappan et al., 2006). These phosphorylation sites consist of serine residues that act as substrates for differential phosphorylation by the enzymes cAMP-dependent PKA, PKC, and Ca^{2+}-calmodulin-activated kinase (Gautel et al., 1995; Sadayappan et al., 2006).

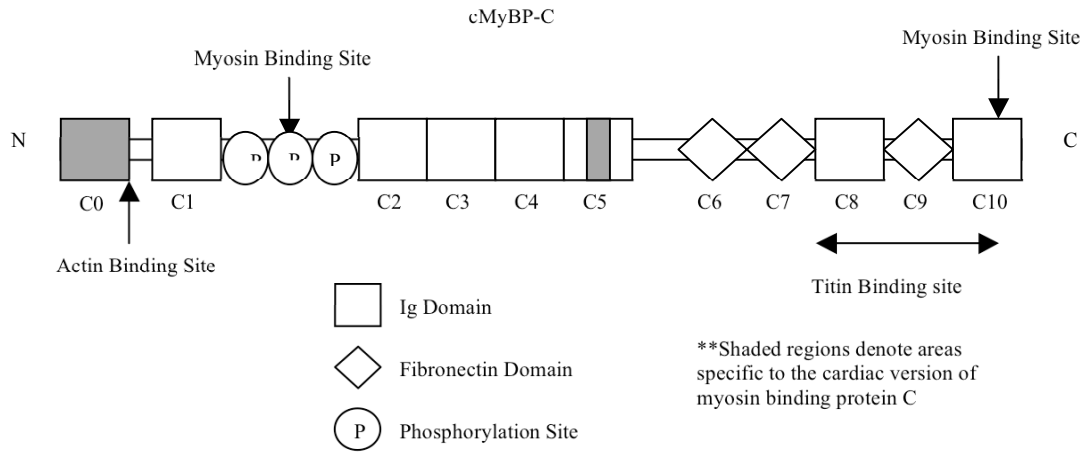

Figure 1. Diagram of cardiac myosin binding protein C. Diagram of cMyBP-C structure, indicating domains and sites of interaction with actin, myosin, and titin. Location of the three phosphorylation sites is also shown. Cardiac specific regions are shaded. Modified from Kulikovskaya et al., 2007 & Sarikas et al., 2005.

cMyBP-C is localized to the A-region of arcomeric thick filaments in the myocardium, where there are believed to be three molecules of cMyBP-C approximately every 43 nm (Granzier, 2006; Harris, 2002; Kulikovskaya, 2007; Sadayappan, 2006; Yank, 1998). cMyBP-C is believed to play a role in cardiac regulation and structure, but its specific functions are not well understood. Studies involving knockout mice lacking cMyBP-c provided evidence that the protein is not required for cardiac sarcomere development; however, it is still necessary for normal cardiac function since its absence resulted in severe cardiac hypertrophy and impaired contractile function (Harris et al., 2002). This supports earlier findings that indicated that cMyBP-C might be involved in cardiac contractility (Gautel et al., 1995).

The functions of cMyBP-C may be regulated by Ca^{2+}-regulated kinase phosphorylation, which has been shown to alter thick filament structure and stability (McClellan et al., 2001; Kulikovskaya et al., 2003; Kulikovskaya et al., 2007). Phosphorylation results in a more stable thick filament, with well-ordered myosin heads and increased resistance to damage when subjected to a strong force (Kulikovskaya et al., 2003; Kulikovskaya et al., 2007). Additionally, cMyBP-C prevents binding of the N-terminus to myosin and increases the likelihood that it will bind to actin thin filaments (Kulikovskaya et al., 2003; Kulikovskaya et al., 2007; Sadayappan et al., 2006). These changes are believed to affect thick filament organization and sarcomeric integrity (Sadayappan et al., 2006). Interestingly, a study by Sadayappan et al. (2006) indicated that in maintaining thick filament organization and structure, phosphorylation of cMyBP-C plays a critical role in cardio-protection.

In addition to affecting structure, phosphorylation and dephosphorylation of cMyBP-C are suspected to regulate cardiac contraction by modulating sensitivity to Ca^{2+} and ß-adrenergic stimulation (Gautel et al., 1995; Kulikovskaya et al., 2003, Kulikovskaya et al., 2007; Sadayappan, 2006). Switching the binding of the N-terminus of cMyBP-C between actin and myosin may be another way in which phosphorylation and dephosphorylation can regulate cardiac contractility (Kulikovskaya et al., 2003). This is consistent with the model for muscle contraction, which is initiated by entry of calcium into the sarcomere. This causes sliding of the thick and thin filaments, which contain myosin and actin, respectively (Seidman & Seidman, 2001). More research is required, however, to gain a more complete understanding of the mechanisms by which cMyBP-C may carry out each of these functions, which are clearly essential to cardiac stability.

Genetic Mutations and Molecular Changes Associated with HCM

According to Richard et al. (2006), there are approximately 150 identified mutations in the MYBPC3 gene that cause HCM in humans, and they are located along the entire span of the gene. About 70% of the mutations are nonsense mutations that lead to premature termination codons, splice site mutations, small deletions or insertions, or frameshift mutations (Richard et al., 2006). The remaining mutations are believed to be missense mutations, which have an amino acid change within the protein sequence (Richard et al., 2006). Frameshift mutations appear to be the most common mutations found in individuals with HCM (Richard et al., 2003; Sarikas et al., 2005) and are suspected to lead to happloinsufficiency. This could either be due to the production of a truncated form of the protein or degradation of the mutant mRNA transcript (Richard et al., 2003; Richard et al., 2006).

Studies of gene disruption in model organisms have provided valuable insight into the processes by which mutations in the MYBPC3 gene can lead to the HCM phenotype. In a 1998 study of a mouse model of HCM, researchers created transgenic mice expressing a murine isoform of cMyBP-C that lacked both the titin and myosin binding domains (Yang et al., 1998). The mutant protein was found to be stable, but was not incorporated efficiently into the sarcomere. In addition, expression of the mutant protein lead to decreased levels of normal cMyBP-C, which caused sarcomeric disorganization and reduced power output within cardiac muscle fibers. More recently, investigation of rat hearts demonstrated that loss of cMyBP-C makes the thick filament more susceptible to fragmentation when exposed to a strong force than intact filaments. Forces such as this may exist in parts of the heart during normal contraction, which suggests that lacking cMyBP-C can accelerate thick filament degradation (Kulikovskaya et al., 2007).

Scientists' understanding of the molecular basis for HCM was further complicated by a study in which truncated proteins resembling those produced by mutations identified in HCM patients were expressed in neonatal rat cardiomyocytes (Sarikas et al., 2005). The truncated cMyBP-C appeared to be rapidly eliminated by the ubiquitin-proteasome system for intracellular degradation, rather than being incorporated into the sarcomere. In addition, the mutant cMyBP-C was found to impair the ubiquitin-proteasome system's ability to degrade other molecules, which was hypothesized to be a contributing factor in the pathogenesis of HCM (Sarikas et al., 2005). In conjunction with research in humans, these genetic studies indicate that the mechanisms by which mutations in MYBPC3 cause HCM are very complex, suggesting that further research is required to elucidate the multiple factors contributing to the manifestation of the disease.

Feline Models of HCM: MYBPC3 Mutations

In 2005, a research team lead by Dr. Kathryn Meurs discovered the first spontaneously occurring mutation causing heritable HCM in a non-human species. The mutation was found in a family of Maine Coon cats that had been previously diagnosed with the disease. HCM in this colony of cats was shown to closely resemble the human form by Kittleson et al. in 1999. They found that, consistent with HCM in humans, these cats presented varying phenotypes, from mild to severe (Kittleson et al., 1999). These results lead to the proposal that affected cats are a valuable, naturally occurring animal model for studying the cellular, molecular, and anatomical aspects of HCM and may be an important resource for studying possible therapeutic treatments of the condition (Kittleson et al., 1999).

In the study by Meurs et al. (2005), affected Maine Coon cats were determined to have a causative mutation in exon 3 of the feline ortholog of the MYBPC3 gene. The mutation, a single base pair change of G to C, modified the amino acid sequence of the cMyBP-C protein, which was predicted to result in an alteration in protein structure. This abnormal conformation may explain the sarcomeric disorganization and increased left ventricular wall thickness (range = 6-9 mm, normal = 3-5mm) observed in these cats. Furthermore, affected cats were found to have decreased levels of the cMyBP-C protein in their myocardium in comparison to control cats ($p < 0.001$). This may indicate that the mutated proteins are degraded due to their instability and their inability to integrate into the sarcomere, supporting the previously mentioned findings by Sarikas et al. (2005).

More recently, a second mutation in the feline MYBPC3 gene was identified in a similar population of Ragdoll cats with HCM (Meurs et al., 2007). The mutation caused similar phenotypes, but was in a different domain than the Maine Coon mutation. This suggests that the two

mutations were independent and not the result of a common founder. The mutations resulted in a change from arginine to tryptophan in the protein's amino acid sequence due to a single base pair change from C to T in codon 820, yielding an abnormal protein product. These discoveries in feline models of HCM provided more evidence for the importance of the MYBPC3 gene in cardiac structure and function and illustrate that it is a key component of hypertrophic cardiomyopathy in cats as well as humans.

Conclusion

The study of cardiac myosin binding protein C and associated mutations in the MYBPC3 gene has provided a more comprehensive understanding of hypertrophic cardiomyopathy in humans and domestic felines. Our knowledge about this disease as a whole, however, remains quite limited, necessitating investment in further research. Additional research should focus on identifying the complete spectrum of polymorphisms in the MYBPC3 gene that do not cause HCM for genetic comparison with mutations in these allelic variants. Elucidating the specific functions of cMyBP-C is also fundamental to the appreciation of the molecular basis for HCM. Furthermore, continued study of the naturally occurring animal model for the disease, the domestic cat, may also prove essential to gaining a complete understanding of disease pathogenesis and the development of prospective treatments. Finally, with continued research, more knowledge can be gained about normal cardiac function. This, in turn, could be applied to understanding cardiovascular disease to develop alternative treatments for these devastating diseases.

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