University of Nevada, Reno

Hypertrophic Cardiomyopathy: A Review of Clinical and Molecular Characteristics and Effects and A Clinical Case Study

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by

NATALIE E. FREITAS

Dr. Josh Baker, Ph.D. in Biochemistry, Biophysics, and Molecular Biology
Thesis Advisor

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Josh Baker, Ph.D., Thesis Advisor

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Tamara Valentine, Ph.D., Director, Honors Program

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Abstract

Hypertrophic cardiomyopathy (HCM) is an autosomal-dominant disease of the myocardium characterized by left ventricular hypertrophy and myofibrillar disarray. HCM is considered the most common cause of sudden cardiac death in young athletes. Mutations of the myosin-binding protein C (cMyBP-C) have been targeted as one of the most prevalent causes of this disease, and the deactivating effects of such mutant forms on the quality control ubiquitin-proteasome system (UPS) contribute to cardiac dysfunction. The E334K mutant cMyBP-C has been shown to destabilize its protein and lead to the impairment of the UPS, resulting in the clinical arrhythmias and cardiac dysfunction observed in HCM patients. A case study has been incorporated to illustrate the clinical manifestations of apical hypertrophic cardiomyopathy (ApHCM). ApHCM is a rare morphological variant of HCM characterized by nonobstructive hypertrophy localized at the cardiac apex.

Acknowledgements

Special thanks to my mentors Tenille Smith, MSIV and Searus Mandegary, MSIV for helping me through the development and compilation of this case study. I would also like to thank Dr. Josh Baker for his guidance throughout the research component of this thesis and Gina Sella for facilitating mentor assistance. Also many thanks to Mark Taylor, Jessica Chen, and Justin Yeung for providing their theses as examples to reference.

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Overview of Disease

Introduction

HCM is a disease characterized by the enlargement of cardiac muscle cells that causes thickening of the myocardium. Thickened heart muscle can reduce the efficiency of cardiac function by obstructing blood flow, decreasing ejection fraction, causing mitral valve regurgitation, and disrupting the electrical signals throughout the heart (1). HCM is an inherited disease most widely caused by mutations in sarcomeric proteins which function in heart muscle contraction (2). Severity of the symptoms associated with HCM are highly variable as some people show no signs or health issues related to the disease while others present arrhythmias, obstructed blood flow, and heart failure (3). As a common cause of sudden cardiac arrest in young people and the leading cause of heart-related sudden death in young adults, HCM and the molecular mechanisms by which it is caused present a highly investigated topic of current research (4). This review presents a comprehensive overview of the clinical and genetic characteristics, diagnosis, and treatment of HCM, as well as an inspection of current studies, theories, and therapeutic targets related to the pathogenesis of this disease.

Clinical Features

The main feature of HCM is an excessive thickening of the heart muscle, or myocardium. Hypertrophy of the myocardium can occur evenly throughout the left ventricle, a condition classified as concentric HCM. More commonly seen is asymmetric HCM, which is the thickening at the septum, or the muscular wall that separates the left

and right chambers of the heart (5). Thickening is seen in ventricular septal measurement, which can range anywhere from 1.3–6.0+ cm as compared to the normal range of 0.08–1.2 cm (4). Both types of HCM cause a narrowing that can block or reduce blood flowing out from the left ventricle to the aorta, a condition called "outflow tract obstruction" (5). In order to overcome this narrowing or blockage, as well as compensate for the reduced end-diastolic volume and ejection fraction of the heart caused by the thickening and stiffness of the left ventricle, the heart must pump harder to deliver sufficient amounts of oxygen-rich blood to the rest of the body. This increased force results in higher blood pressure and other health issues observed in this disease. The narrowing of the left ventricle can also disrupt the proper function of the mitral valve, as it can strike the thickened septum and cause blood to leak back into the left atrium (5). Another prominent feature of HCM is the cellular changes observed in cardiac cells, which not only enlarge, but also undergo organizational changes to assume irregular conformations in a condition referred to as myocardial disarray (5). This disarray disrupts the normal patterns of electrical signaling throughout the heart and causes various types of abnormal heart rhythms, or arrhythmias (6).

Pathophysiology

The molecular events that lead to the clinical phenotype of HCM are unclear, although several potential pathways have been proposed and investigated. Impaired myofibrillar contractile function due to genetic mutations encoding sarcomeric proteins accounts for the hypertrophy and diastolic dysfunction in many HCM cases (7). Other suggested mechanisms include altered energy homeostasis, changes in calcium cycling

and sensitivity, and increased myocardial fibrosis (7). As the sarcomere is responsible for generating and transmitting contractile forces of the heart, mutations in sarcomeric proteins which disrupt normal sarcomeric function can cause inefficient energy utilization (7). This inefficient energy usage compromises the ability of cardiomyocytes to maintain critical homeostatic functions, which can result in ion imbalances and consequent arrhythmias (7). Cardiac arrhythmogenesis is also regulated by specific channel proteins whose level of expression is regulated by quality control systems within the cell such as the ubiquitin proteasome system (UPS) (8). The impairment of the UPS can cause accumulation of cardiac channel proteins and thus changes in intracellular ion concentrations. Perturbation of calcium fluxes resulting from either increase in ion channel expression or accumulation in Ca^{2+} handling proteins can cause significant increases in calcium transients and longer action potential durations (APD), which are features observed in HCM cases (7,8). The activation and proliferation of fibroblasts is also observed in HCM patients and is correlated with impairment of cardiac relaxation (7). Although the connection between HCM-associated sarcomeric mutations and this proliferation of nonmyocytes has yet to be elucidated, increased expression in profibrotic molecules such as collagen and elastin seem to play a role in inducing fibroblast growth (7).

Symptoms

The symptoms and onset of HCM is case dependent, as there is no particular symptom or period of occurrence unique to the disease (4). The severity of manifestation of HCM ranges from almost no health-related problems to the development of life-

shortening heart conditions such as sudden cardiac arrest, or sudden cardiac death (5). Sudden cardiac death, most commonly caused by ventricular fibrillation, is among the most devastating consequences of HCM and has the highest occurrence in preadolescent and adolescent youth, especially during extreme exertion (6). However, most people diagnosed with HCM are at low risk of sudden cardiac death and present, to varying degrees, numerous symptoms associated with ventricular tachycardia (fast but regular heart rhythm) and heart failure, which is defined as weaker than normal contractile forces of the heart (3,5,6). The most commonly presented of these symptoms is dyspnea, or shortness of breath, which is caused by elevated diastolic pressure that reverts back into pulmonary circulation (6). Elevated pressure gradients across the left ventricular outflow tract also can result in dizziness, which is exacerbated by high levels of exertion (6). Another cause of dizziness, as well as the cause of palpitations experienced by HCM patients is arrhythmias, such as sinus pauses, atrial fibrillation, atrial flutter, supraventricular tachycardia, and premature atrial and ventricular beats (6). Also common is syncope, or fainting or passing out, which results from arrhythmias or inadequate cardiac output upon exertion (6). Syncope is more commonly seen in children and young adults having reduced left ventricular chamber size due to hypertrophy (6). Presyncope can also be experienced by HCM patients and is characterized by "greying-out" spells which can be relieved upon lying down (6). Both syncope and presyncope are indications of patients who present high risk of sudden death (6). Angina, or chest pain, may also occur due to subendocardial ischemia resulting from ventricular hypertrophy (6). A less common symptom of HCM is congestive heart failure, which can also result from subendocardial ischemia or diastolic dysfunction (6).

Epidemiology

HCM is estimated to affect one in every 500 Americans and is considered most prevalent in young adults, serving as the leading cause of sudden cardiac death in people under 30 (5). Most HCM-related sudden cardiovascular deaths occur in adolescents and young adults, especially competitive athletes, and the risk of sudden death in children is as high as 6% per year (6). However, expression of HCM is not confined to young adults and athletes, as cases of HCM have been observed in people of all ages (1). HCM most commonly presents in the second or third decade of life, and the average age for diagnosis is 35 (4,9). This disease has not been correlated to any specific trends, yet some studies suggest that HCM may be slightly more prevalent in males (9). According to the echocardiographic analysis of one study conducted by the Coronary Artery Risk Development in (Young) Adults (CARDIA) Study, prevalence of HCM in men and women was 0.26:0.09% and in blacks to whites was 0.24:0.10% (10). However, no widely accepted ethnic or gender trends have been reported as unique to HCM.

Genetics

HCM is the most common form of Mendelian–inherited heart disease and is transmitted as an autosomal-dominant trait with incomplete penetrance in the majority of cases (2,7). Fifty percent of HCM cases can be attributed to specific disease-causing mutations, although it is still a rather heterogeneous disease, with the most common forms being linked to more than 500 mutations on 19 different genes encoding sarcomeric proteins (2). For three quarters of all clinical cases of HCM, the underlying mutation has been identified as alterations in either of the two genes MYH7 and

MYBPC3, which encode β-myosin heavy chain and cardiac myosin-binding protein C, respectively (2,7). In contrast, the third most predominant gene mutation occurring in HCM cases is on the gene encoding cardiac troponin T (TNNT2), which accounts for less than 10% of HCM cases (7). MYBPC3 presents a popular target of investigation in current research, as at least 185 HCM-associated mutations have been identified on this protein (2). Although these include several missense mutations, approximately 61% of known MYBPC3 mutations are nonsense or frameshift mutations, which can in many cases cause premature termination in the transcription of mRNA and thus produce C-terminal truncated MYBPC's lacking binding sites essential for proper function (2). HCM patients are generally heterozygotes, and the high rate of phenotypic variability suggests the presence of other factors and disease modifiers such as environment, gene polymorphisms, posttranslational modification, and epigenetics as having an influence on the penetrance of this disease (2). Due to the heterogeneity of HCM and the number of unidentified gene mutations and regulatory DNA sequences associated with the disease, genetic testing for HCM presents clear limitations in diagnostic methods and clinical utility (7). Furthermore, in regards to the HCM-associated mutations that have been identified, the pathogenic pathways by which these mutations cause the disease remain largely unclear and controversial (7).

Diagnosis

HCM may first be suspected upon presentation of symptoms, detection of a heart murmur, or an abnormal electrocardiogram (ECG/EKG) (1,4). An ECG records electrical signals from the heart and can detect abnormal electrophysiology due to heart thickening

and myocardial disarray (4). However, in a minority of cases, an ECG of HCM patient may be normal, or ECG abnormalities can be attributed to other heart conditions (1). For this reason, an echocardiogram (ECHO) is currently used as a more conclusive method to diagnose HCM. An echocardiogram uses sound waves to produce an image of the heart in order to examine muscle thickness, movement, and blood flow, and thus identify abnormalities in the heart muscle and valves (1). Types of echocardiograms include transthoracic echocardiograms, in which an ultrasound beam is aimed through one's chest to the heart, and transesophageal echocardiograms, in which a flexible transducer is guided into one's esophagus in order to obtain a more detailed image of the heart (1). In cases where echocardiogram images are inconclusive, cardiac magnetic resonance imaging (MRI) is performed to produce higher resolution images of the heart by use of magnetic fields and radio waves (4). In order to record and detect abnormal heart rhythms over an extended period of time, a holter monitor is used and functions as a portable ECG that records a continuous electrocardiogram of the heart over the course of one to two days (1). Other effects of HCM, such as high blood pressure inside the heart can be measured using various techniques including cardiac catheterization, in which a catheter is threaded into heart chambers through which dye is injected in order to produce angiograms of the heart and blood vessels (1). Screening for HCM through these multiple methods is recommended regularly for people with first-degree relatives with HCM (1). An ECG or ECHO should be given each year to such people until they reach adulthood and stop competitive athletics, after which time screening can be conducted less frequently (1).

Treatment

In order to relieve symptoms and prevent sudden cardiac death of those at risk, various treatment options including drugs, surgery and other methods to destroy obstructive heart tissue or regulate heart rhythms are offered. Current drug treatment includes various medications selected to relax the heart and allow it to pump more efficiently. Such drugs commonly prescribed include: beta blockers, such as atenolol or metoprolol used to relieve chest pain and palpitation; calcium channel blockers, such as verapamil which improves filling of the heart and lowers heart rate and blood pressure; and anti-arrhythmic drugs, such as Amiodarone and Disopyramide used when arrhythmia is detected (4). Specific complications may require the use of the following additional drugs: anticoagulants for patients with persistent atrial fibrillation, diuretics for development of fluid retention, and antibiotics in cases in which there is an increased risk of bacterial infection (1). In cases in which medication does not relieve symptoms, surgical options are next considered. Such surgical options include septal myectomy, in which a portion of the thickened, overgrown septum is removed in order to improve blood flow and reduce mitral valve regurgitation (4). Also available is septal ablation, or septal alcohol ablation, by which portions of thickened myocardium are destroyed by alcohol injection through a catheter. However, this presents potential disruption of the heart's electrical system, which would then require implantation of a pacemaker (1). A pacemaker is a small electronic device that is inserted beneath the skin and sends constant electrical signals to the heart to regulate heartbeat. A similar device also used to monitor one's heartbeat is a implantable cardiodiverter-defibrillator (ICD) (1). This device is capable of sensing potentially lethal arrhythmias and inducing electrical shocks to

terminate such arrhythmias and restore normal heart rhythm. ICD implantation is offered to patients identified as being high risk for sudden cardiac death (4). Pulmonary vein ablation or pulmonary vein antrum isolation (PVAI) is also offered as an alternative treatment for atrial fibrillation for those with HCM and disconnects pathways of abnormal rhythms in impulse firing pulmonary veins by ablation (4).

Body of Review

Although mutations encoding sarcomeric proteins have been determined as the most prevalent cause of HCM, over 500 such mutations in 19 different sarcomeric genes have been identified in typical forms of HCM, and the exact pathogenesis of this heterogeneous disease remains unclear (2). However, most of these mutations have been located in or associated with the function of the MYH7 and MYBPC3 genes, which encode β-myosin heavy chain and cardiac myosin-binding protein C (cMyBP-C), respectively (2). cMyBP-C is a component of the thick filaments of the sarcomere, and mutations in this protein are widely recognized as causes of HCM (2). Specific mechanisms by which MYBP3 mutations cause the disease are controversial and extensively investigated in current clinical studies. One proposed molecular mechanism involves the hypothesized effects of carboxyl terminal truncated cMyBP-C proteins and there altered accumulation and/or incorporation into the sarcomere (11). Studies such as those conducted by Flavigny et al., in which localization of several different immunolabeled COOH-truncated cMyBP-C proteins is observed in the A-band of the sarcomere suggest that truncated MYBPC3 mutations are incorporated into the sarcomere and act as poison polypeptides that disrupt myofibrillar architecture and result in altered

contractility and other symptoms of HCM (11). A different mechanism, such as that proposed by Mathias Gruen and Mathias Guatel attributes disruption in the regulatory function of MyBP-C to interactions with β-myosin S2 mutations (12). cMyBP-C is positioned transversely in the A-band of the sarcomere and interacts with the myosin rod at the S2 fragment to regulate contractility and maintain structural integrity of the sarcomere by phosphorylation (12). Thus, familial hypertrophic cardiomyopathy representative mutations in β-myosin S2 may alter interactions with cMyBP-C and result in complications associated with this disease (12).

In addition to impaired myofibrillar contractile function, MYBPC3 mutations have also been suggested to influence the cardiac arrhythmogenesis commonly seen in HCM. Being that the generation and transduction of electrical signals throughout the heart is affected by ion flux within cardiomyocytes, ion channel proteins play an integral role in regulating cardiac arrhythmogenesis (8). As ion channel protein levels are in turn regulated by the ubiquitin-proteasome system (UPS), impairment of this system by mutant forms of cMyBP-C such as E334K can result in accumulation of cardiac channel proteins and lead to electrophysiological dysfunction (8). Accumulation of other proteins including calcium handling proteins and pro-apoptotic proteins is also caused by the suppression of the UPS by unstable E334K mutant cMyBP-C (8). This effect results in a higher concentration of calcium transients associated with cardiac apoptosis, which can be attenuated by treatment with calcium antagonist azelnidipine (8). Other potential treatments include cMyBP-C replacement by gene therapy and suppression of endogenous mutation at pre-mRNA levels (8). Although RNA-targeting therapies could be well suited for treating HCM, further development of analytical techniques to better

elucidate the structure and function of mutant cMyBP-C is necessary and warrants further investigation.

Conclusion

Hypertrophic cardiomyopathy is a very common cardiac disorder that exhibits remarkable clinical and genetic heterogeneity. The severity of symptoms characteristic of this disease can range from little to no expression, to sudden cardiovascular death. Diagnostic tests are used to reveal thickening of the heart muscle, irregular blood flow, and abnormal electrical signals, and treatment involves removal of overgrown myocardium or implantation of a pacemaker or defibrillator. The spectrum of genes that are involved in the development of HCM has expanded, but the most predominant of these genes are those that encode cMy-BPC. Studies of genetically engineered models of mutants of such genes have increased the understanding of some potential mechanisms of the pathophysiology of this disease, although much still remains unclear. The current repertoire of identified HCM genes has allowed gene-based diagnosis, and further investigation of specific mechanisms of such genes, such as the impairment of the ubiquitin-proteasome system for example, will lead to therapeutic targets and provide new benefits for HCM patients.

Literature Review:
Quality control ubiquitin-proteasome system impairment by E334K mutant cMyBP-C instability is associated with hypertrophic cardiomyopathy

Introduction

cMyBP-C mutations are of the most common genetic causes associated with HCM (8). cMyBP-C is a component of the thick filaments of the sarcomere and regulation of cMyBP-C by phosphorylation may alter thick filament structure and affect thick-thin filament interaction (13). cMyBP-C is therefore a key regulator of contraction and relaxation of the heart, yet mechanisms by which mutations in the MYBPC3 gene encoding cMyBP-C lead to HCM remain elusive. However, Western blot analysis has consistently shown significantly lower levels of C-terminal truncated and other mutant cMyBP-C proteins compared to wild type expression levels, suggesting that mutant proteins are instable and qualitatively degraded by some quality control mechanism within the cell (14). The three potential quality control systems that regulate expression of cMyBP-C mutations include nonsense-mediated mRNA decay (NMD), UPS, and autophagy (2). Impairment of the UPS caused by the instability of the missense MYBPC3 mutation E334K may contribute to accumulation of both ion channels and Ca^{2+} handling proteins and play an important role in the progression of cardiac dysfunction (8). The hypothesized proteasome inhibition by E334K cMyBP-C has been investigated by measuring the expression levels and effects of proteins that accumulate as a result of impaired degradation through the UPS. Such proteins include pro-apoptotic proteins and other cardiac ion channel and Ca^{2+} handling proteins; the overexpression of which can lead to prolongation of action potential duration (APD), elevated intracellular Ca^{2+}

concentration, and cardiac cell death. These effects are closely associated with the arrhythmias and electrophysiological dysfunctions characteristic of HCM (1).

Results

UPS impairment associated with E334K mutant cMyBP-C protein expression

Different mutations identified in Japanese HCM patients using denaturing HPLC and sequencing were investigated in COS-7 and neonatal rat cardiac myocytes (NRCM) in a study conducted by Bahrudin et al. Expression of five novel MYBPC3 mutations including E334K were identified and protein stability and proteasome activity in cells expressing such mutations were examined through various affinity chromatography techniques. Western blot analysis of protein extracts from COS-7 cells co-transfected for 48hr with selected mutant MYBPC3 and enhanced green fluorescent protein (EGFP) plasmids was performed. Figure 1a depicts a Western blot and quantitative densitrometric scan of target myc-MyBP-Cs. This procedure and analysis was duplicated for COS-7 cells co-transfected with either wild-type (Wt) or E334K and EGFP constructs in absence or after treatment with MG132 (Fig. 1b), a proteasome inhibitor. Expression of E334K mutant protein was observed to be remarkably reduced in COS-7 cells in comparison to Wt cMyBP-C protein. E334K mutant protein levels were seen to increase by pretreatment with proteasome inhibitor MG132 (15).

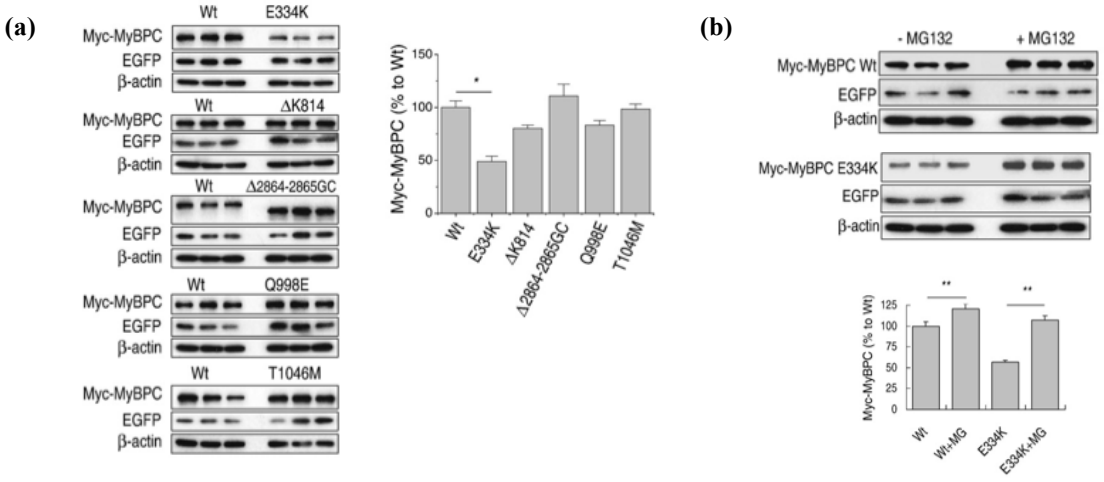

Figure 1. cMYBP-C protein levels in COS-7 cells. (a) Western blot analysis (left) of protein extracts of COS-7 cells co-transfected with MYBPC3 and EGFP plasmid; right, quantitative densitrometric scan of corresponding blot. (b)Representative Western blot (top) and densitrometric scan (bottom) of protein extracts pretreated with MG132. Quantification of EGFP was used to control minor differences in transfection efficiency and β-actin served as a control for protein loading. Figure adapted from reference 3.

Stability of the E334K mutant cMyBP-C protein was investigated by measuring the rate of its degradation within COS-7 expressing cells using pulse-chain analysis. Wt and E334K cMyBP-C transfected COS-7 cells were pulse-labeled with [^{35}S]methionine, and the cMyBP-C protein density was determined at specific time intervals by use of anti-myc immunoprecipitates subjected to autoradiography. Decay curves constructed from representative autoradiographs such as the one displayed in Figure 2 reveal the fast degradation process of E334K mutant cMyBP-C protein. From the analysis conducted by Bahrudin et al. represented in Figure 2, the half-life of E334K mutant protein was observed to be 2.01 ± 0.26 h, which is significantly faster than the half-life of the Wt (9.55 ± 2.059 h). The other cMyBP-C mutants were observed to have the same half-life as that of the Wt. Pretreatment with MG132 was found to prolong the half-life of E334K mutant protein to 10.46 ± 2.68 h (3). Additional correlative analysis from

immunoprecipitation experiments shows that the polyubiquitination, which targets proteins for the proteolytic process of the UPS, of E334K mutant protein is significantly higher than that of the Wt (2,15).

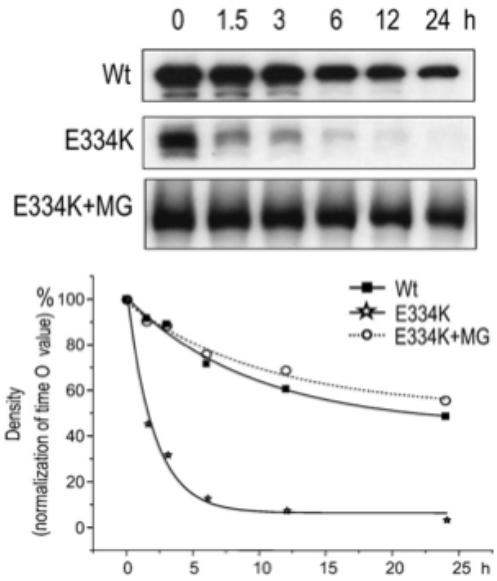

Fig. 2. Degradation and half-life determination of E334K mutant cMyBP-C protein. Wt and E334K mutant protein expressing COS-7 cells pulse-labeled with [^{35}S]methionine and chased at indicated time increments. The anti-myc immunoprecipitates were detected using autoradiography. The autoradiograph (top) and corresponding decay curve (bottom) are shown. Figure adapted from reference 3.

E334K is a missense mutation in MYBPC3 caused by an amino acid charge change of +2, as the positively charged lysine (K) is substituted for the negatively charged glutamic acid (E) at position 334. Affects on the stability of the cMyBP-C protein were investigated by substituting differently charged amino acids at position 334 and measuring protein expression with anti-myc immunoprecipitates in Western blot analysis. Stability of the protein was restored by replacement of glutamic acid (E) with aspartic acid (D), another negatively charged amino acid. However, replacement with uncharged glutamine (Q) and nonpolar glycine (G) only partially restored the expression level of the protein (Fig. 3a) (3). Position 334 is located in exon 12, which encodes the MyBP-C motif, a phosphorylation dependent regulatory region that controls actin and

myosin-S2 binding and thus contraction and relaxation of cardiac muscle (2). Phosphorylation of E334K compared to Wt cMyBP-C in transfected COS-7 cells was examined by immunoblotting with anti-phosphoserine or anti-myc antibody to measure phosphorylated E334K mutant cMyBP-C protein compared to the Wt (Fig. 3b). There was no observed difference in phosphorylation between E334K mutant and Wt cMyBP-C (15).

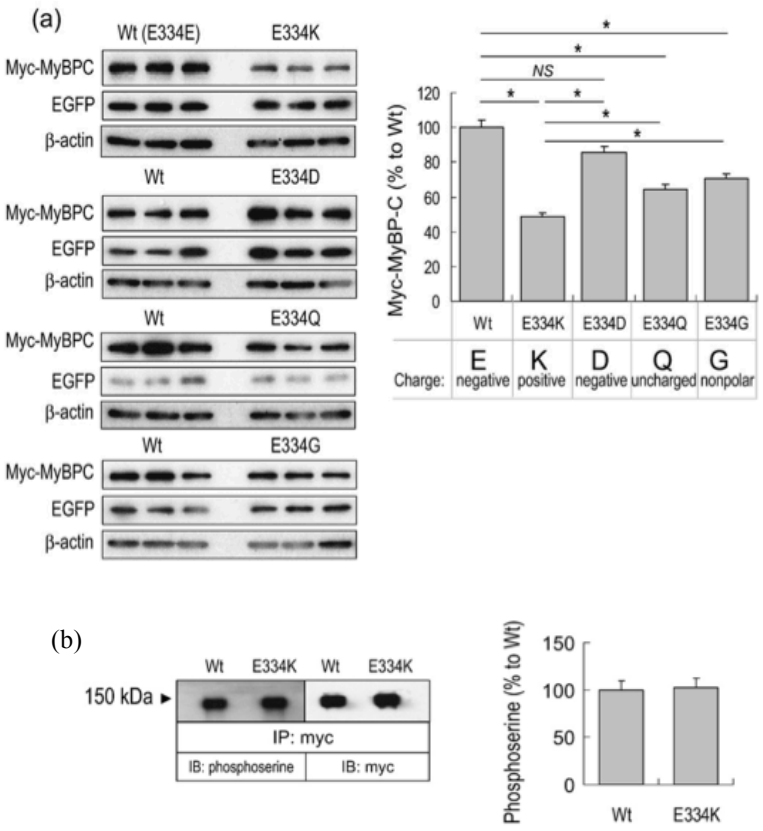

Fig. 3. Protein stability and phosphorylation of missense mutation at position 334 of cMyBP-C. (a) COS-7 cells were co-transfected with Wt, E334K, and three other constructs encoding cMyBP-C with different charged amino acids at position 334 (E334D, E334Q, and E334G) for 48 h and protein extracts were used for Western blotting (left) and densitrometric scanning (right). Minor differences in transfection efficiency were controlled for by the use of EGFP, and β-actin was used as the control for protein loading. (b) Cell lysates from cells transfected with either Wt or E334K mutant were blotted with anti-myc immunoprecipitates and subjected to anti-phosphoserine or

anti-myc antibody. Representative blots (left) and quantitative densitrometric scan (right) are shown. Figure adapted from reference 3.

Impairment of UPS associated with expression of unstable E334K mutant cMyBP-C protein was determined by measuring the 20S proteasome (the core catalytic unit within the UPS) activity from purified proteasomes isolated either from Wt or mutant cMyBP-C transfectants of COS-7 cells. Proteasome or chymotrypsin-like activity was indicated by the detection of fluorescence of free 7-amino-4-methylcoumarin (AMC) produced from specific digestion of substrate peptide. From this analysis, as shown in Figure 4a, reduced proteasome 20S activity was observed in E334K expressing cells as compared to Wt. To further investigate proteasome activity, levels of pro and anti-apoptotic proteins were measured in either Wt or E334K mutant cMyBP-C stable transfectants of COS-7 cells (Fig. 4b). Western blot analysis with anti-myc and other antibodies for the chosen pro or anti-apoptotic proteins detected the corresponding protein levels and revealed greater accumulation of pro-apoptotic proteins in E334K transfectants compared to Wt expressing cells (15,16).

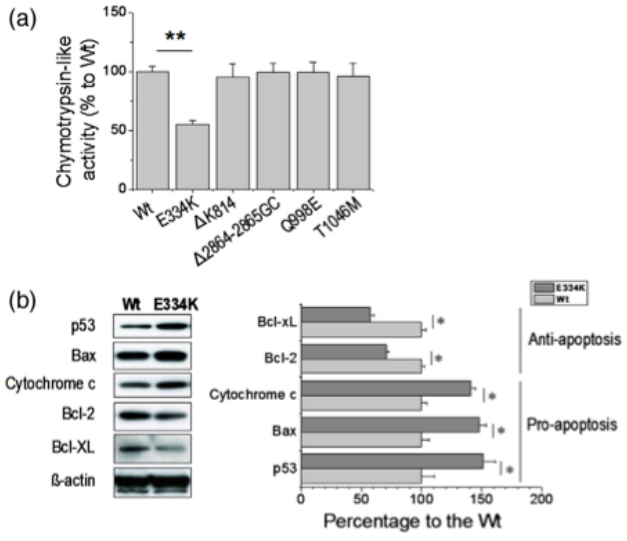

Fig. 4. Impaired proteasome activity associated with expression of E334K mutant cMyBP-C protein. (a) 20S proteasome activity in isolated proteasomes of Wt or cMyBP-C mutant transfectants of COS-7 cells measured by fluorescence detection of free 7-amino-4-methylcoumarin (AMC) liberated from digestion of substrate peptide Suc-Leu-Leu-Val-Tyr-AMC. (b) Western blot analysis of pro and anti-apoptotic proteins in Wt or E334K expressing COS-7 cells (left) and quantitative densitrometric scan (right) are shown. Figure adapted from reference 3.

Accumulation of cardiac proteins caused by suppressed UPS activity

Studies such as those conducted by Bahrudin et al. further investigate suppressed proteasome activity and the implications of UPS impairment using cardiomyocytes from a HL-1 cardiac muscle cell line and primary cultured NRCMs. Levels of specific proteins as well as the electrophysiological activity of these cell models overexpressing either Wt of E334K cMyBP-C were analyzed using immunoprecipitation and electrophysiological recording techniques. Lower cMyBP-C protein levels, higher levels of polyubiquitination of these proteins, and suppressed chymotrypsin-like activity observed in E334K expressing COS-7 cells presented in Figures 1 and 4 were confirmed through similar experiments using HL-1 cells. Further experimentation led to the discovery of suppressed trypsin-like and caspase-like activities in E334K expressing HL-1 cells and NRCMs. Consequences of such impaired proteasome activity were explored by measuring resulting levels of ion channel proteins and Ca^{2+} handling proteins. Expression levels of such proteins, including Kv1.5, $Na_v1.5$, $Ca_v3.2$, Hcn4, $Ca_v1.2$, Serca, RyR2, and Ncx were measured in NRCM and HL-1 cells expressing either Wt or E334K cMyBP-C (Fig. 5a). Significantly increased levels of these proteins in E334K expressing cardiac cells compared to cells expressing the Wt protein were observed, while no such trend was observed in the mRNA levels of these proteins in E334K and Wt cardiac cells. Chase experiments revealed the prolonged decay of these ion channels proteins and Ca^{2+} handling proteins in E334K expressing cells compared to the decay of such proteins in the Wt (Fig. 5b). Despite higher levels and prolonged decay of these proteins, Western blot analysis of accumulated $K_v1.5$ and $Ca_v1.2$ showed localization of these channel

proteins in the cell membrane, indicating that these proteins do not become sequestered in nonfunctional compartments when not degraded (Fig. 5c) (1).

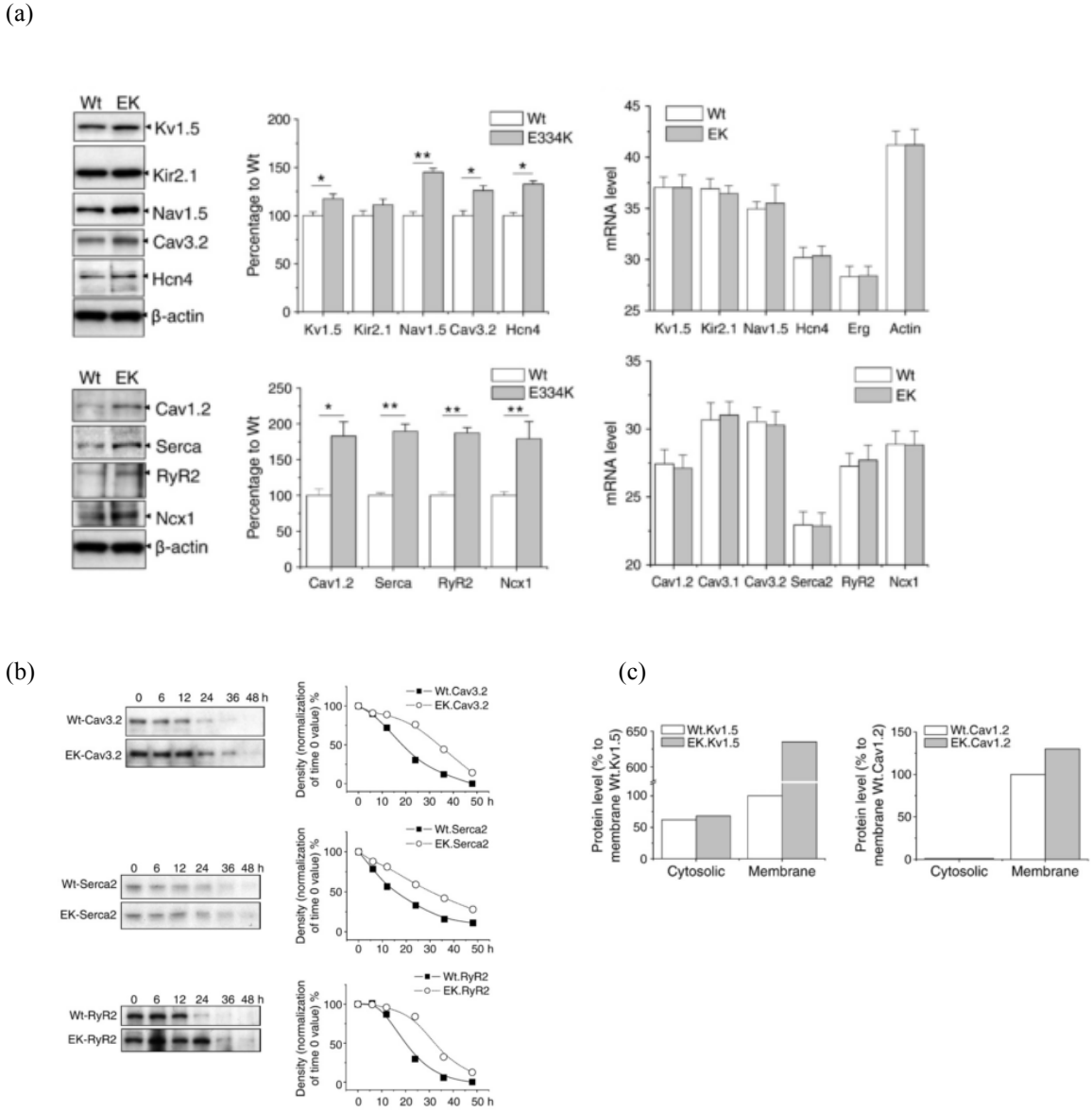

Fig. 5. Accumulation of cardiac channel and calcium handling proteins in E334K expressing cells. (a) Western blot analysis using β-actin as a control for protein loading (left), quantitative densitrometric scan (middle), and quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) with mRNAs of indicated proteins in E334K and Wt cells are shown. (b) Chase analysis of ion channel proteins at the indicated time

intervals after treatment with transcription inhibitor cycloheximide with representative blots (left) and time-course decays (right) are shown. (c) Quantitative densitrometric scan of representative blot (not shown) of cytosolic and membrane fractions of $K_v1.5$ and $Ca_v1.2$ in E334K and Wt expressing HL-1 cells. Na^+/K^+ ATPase and tubulin from whole-cell lysates were used as protein marker and loading control. Figure adapted from reference 1.

The electrophysiological effects of ion channel and Ca^{2+} handling protein accumulation in cardiac cells were investigated by examining the action potential duration (APD) and calcium transients in E334K and Wt expressing HL-1 cells. Using the whole-cell patch-clamp technique, the APD of isolated HL-1 cells was measured. Figure 6a shows the increase in APD at 90% repolarization of HL-1 cells expressing E334K compared to the Wt. Furthermore, some E334K mutant expressing HL-1 cells exhibited afterdepolarizations (ADs), which are abnormal depolarizations that may interrupt the phases of the cardiac action potential. Calcium transients in HL-1 cells were also recorded by exciting E334K mutant or Wt MYBPC3 and pMAX-EGFP cotransfected cells at 365 ± 10 nm and recording fluorescence images at 405 ± 10 nm and 480 ± 10 nm every 10 ms. After subtracting background fluorescence, the ratios of these images were calculated and used to determine resting and peak levels as well as amplitudes of calcium transients (Fig. 6b). Such measurement levels were observed to be higher in E334K cMyBPC expressing cells as compared to Wt (1).

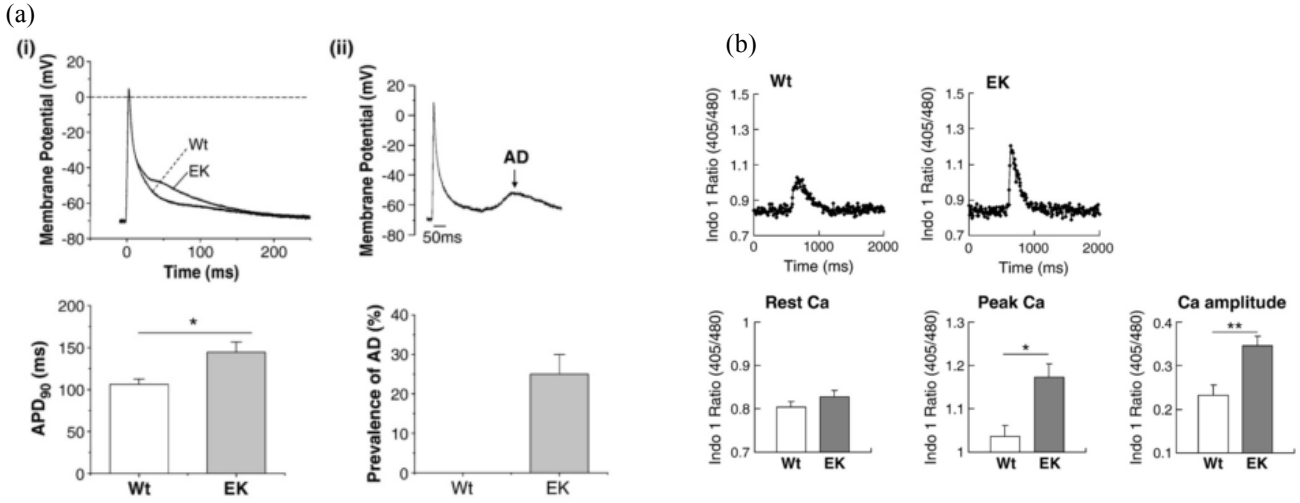

Fig. 6. Action potential and calcium transients in cardiomyocytes expressing E334K cMyBPC. (a) (i) Traces of the action potential of HL-1 cells expressing either E334K or Wt cMyBPC (top) and the bar graph representing the APD of those cells (bottom) are shown. (ii) Presence of ADs in E334K expressing HL-1 cells was traced (top) and quantified in a bar graph (bottom). (b) Representative traces of calcium transients were measured in both Wt and E334K expressing HL-1 cells (top), and the component resting Ca^{2+} levels, peak Ca^{2+} levels, and Ca^{2+} amplitudes were quantified in bar graphs (bottom). Figure adapted from reference 1.

Intracellular calcium concentration serves an important role in regulating the electrophysiological function of cardiomyocytes and has been linked to specific cell death modalities (17). Calcium transients and APDs, as well as apoptosis regulating protein levels associated with E334K mutant protein were further investigated by examining the effects of the Ca^{2+}antagonist, azelnidipine. By pretreating both Wt and E334K NRCMs and HL-1 cells with 1 μM azelnidipine for 24 hours and then measuring the calcium transients and APDs as described in Figure 6, azelnidipine was observed to both lower calcium transients (Fig. 7a) and shorten APD (Fig. 7b) in both cell models to levels and duration times comparable to Wt expressing cells. Immunoprecipitation of proteins within Wt and E334K cells showed a decrease in ratio of pro-apoptotic to anti-apoptotic proteins when the cardiac cells were treated with azelnidipine (Fig. 7c), which

results in a decrease in the prevalence of cardiac cell death. Additional chase and immunofluorescence experiments showed that azelnidipine neither affected the time-dependent decay of E334K mutant proteins nor affected the accumulation of the mutant protein or it's incorporation into the sarcomere, as indicated by the punctuate florescence observed in both the E334K expressing cells and the E334K expressing cells treated with azelnidipine (Fig. 7d) (8).

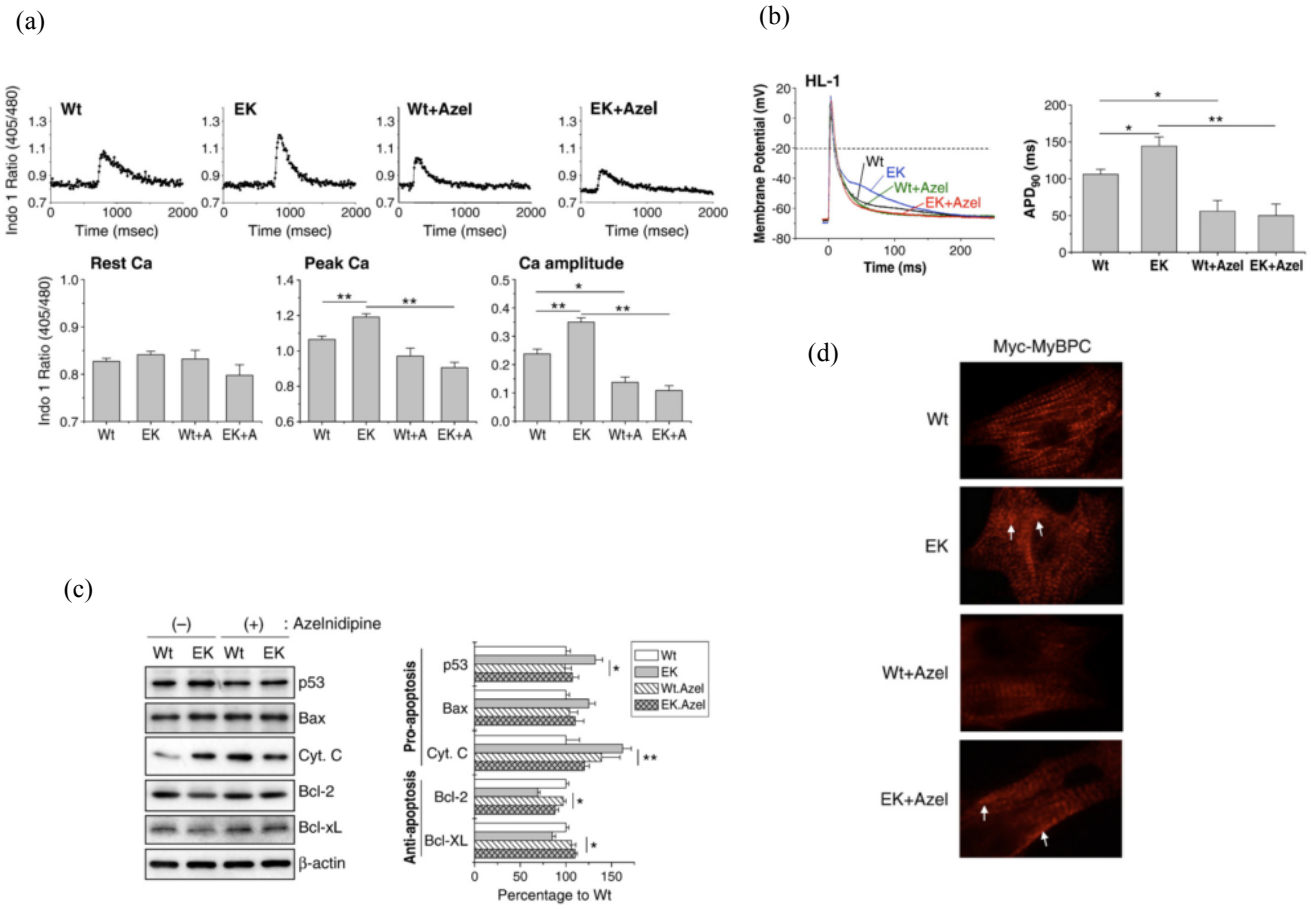

Fig. 7. Effects of azelnidipine on E334K cMyBPC protein. (a) Normalization of calcium transients in E334K expressing HL-1 cells treated with azelnidipine indicated by the representative traces (top) and bar graphs (bottom) of rest, peak, and amplitude calcium transients. (b) Decrease in APD of azelnidipine treated E334K expressing HL-1 cells shown by the action potential traces (left) and corresponding bar graph of APD (right). (c) Western blot analysis (left) and bar graph (right) showing levels of indicated pro and anti-apoptotic proteins measured in HL-1 cells expressing Wt or E334K cMyBPC in the

presence and absence of azelnidipine treatment. (d) Immunofluorescence of either Wt or E334K mutant cMyBPC in NCRMs with and without azelnidipine treatment. Arrows indicate punctuate fluorescence that possibly correlates to E334K protein accumulation. Figure adapted from reference 1.

Discussion

Evidence for UPS impairment by mutant cMyBP-C proteins has been documented in various studies. The stability of cMyBP-C protein is amino acid charge dependent (Fig. 3) and the E334K missense mutation has been found to cause instability of cMyBP-C. The phosphorylative function of the MyBP-C motif of the cMyBP-C protein encoded for at position 334 is unaffected in the E334K mutant form and the protein structure of this mutant is unknown. However, control of mutant E334K expression by UPS is indicated by reduced levels of E334K mutant protein in transfected COS-7 cells and the restored expression of this mutant by treatment with proteasome inhibitor MG132 (Fig. 1). The significantly shorter half-life and higher level of polyubiquination observed in E334K mutant as compared to the Wt also suggest the instability of E334K mutant and its degradation by UPS (Fig. 2). As a consequence of its encounter with instable E334K mutant cMyBP-C, the UPS is impaired. UPS impairment is evinced by the lesser concentration of peptides resultant of 20S proteasome activity in E334K expressing cells than in Wt expressing cells (Fig. 4) (3). Lower trypsin-like and caspase-like activities observed in HL-1 and NRCMS expressing E334K also indicate suppressed proteasome activity (1). Such reduced proteasome activity results in accumulation of proteins including ion channel and Ca^{2+} handling proteins, yet proper localization of these accumulated proteins within the membrane is retained (Fig. 5). The accumulation and prolonged decay of these proteins in E334K expressing NRCMs and HL-1 cells may

account for the higher peaks and amplitudes of Ca^{2+} transients observed in these cell models. Increased concentration of intracellular Ca^{2+} may in turn account for the prolonged APDs and presence of ADs observed in E334K expressing cells (Fig. 6). ADs in E334K mutant cMyBP-C expressing cells resulting from prolonged APDs and increased Ca^{2+} transients may cause arrhythmias in cardiac myocytes (1). Furthermore, increased ratios of pro-apoptotic to anti-apoptotic proteins and thus increased cell apoptosis in E334K mutant expressing HL-1 and COS-7 cells were observed (Fig. 4). Apoptosis of cardio myocytes related to suppressed proteasome activity and increased intracellular Ca^{2+} is observed in failing hearts and has been postulated to result in fibrosis and consequent arrhythmias (1). This theorized effect as well as observed influences of UPS impairment by instable E334K mutant protein are diagrammed in Figure 8. Taken together, prolongation of APDs, occurrence of ADs, higher calcium transients, and enhanced apoptosis observed in E334K mutant cMyBP-C transfected cardiomyocytes are all abnormalities observed in left ventricular hypertrophy and thus these electrophysiological effects of E334K expression serve as possible explanations for the arrhythmogenicity characteristic in E334K cMyBP-C carrying HCM patients (1).

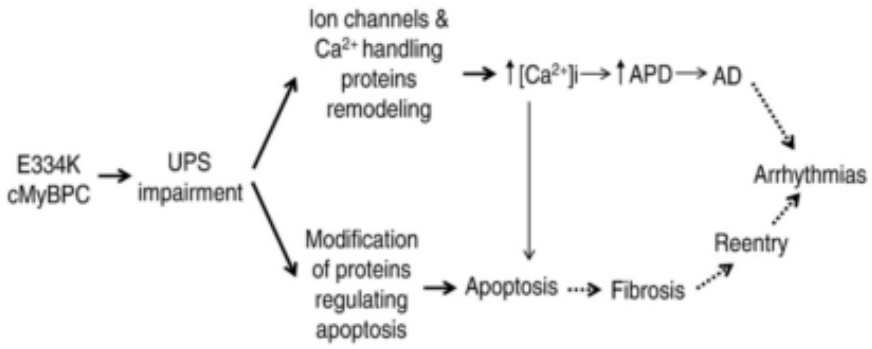

Fig. 8. Proposed mechanisms for electrophysiological dysfunction associated with UPS impairment by E334K cMyBP-C expression. Figure taken from reference 1.

The Ca^{2+} antagonist azelnidipine was found to suppress Ca^{2+} transients and attenuate the apoptosis of cardiac myocytes expressing E334K cMyBP-C (Fig. 7). This finding suggests the presence of a mechanism of increased apoptosis related to increased intracellular Ca^{2+}, and effects of azelnidipine in normalizing elevated intracellular $Ca^{2+}$and APD serves as a potential treatment in abating cardiac apoptosis. However, Ca^{2+} antagonist azelnidipine affects neither the degradation of E334K cMyBP-C nor its incorporation or accumulation into the sarcomere, and thus UPS impairment by instable E334K and consequent apoptosis may still persist (3). Potential apoptotic models, such as the mitochondrial death pathway induced by sarcoplasmic reticulum Ca^{2+} overload, as well as pathways dependent on proteasome inhibition and enhancer-binding proteins such as C/EBP homologous protein have been hypothesized, yet further studies to investigate these possibilities are required (18,19). Furthermore, it was found that substitution of glutamic acid for differently charged residues at position 334 corresponds to instability of cMyBP-C, yet lack of information on the crystal structure of E334K mutant cMyBP-C hinders further analysis of the effect of E334K mutation on its protein structure and function. Determination of the structure of mutant E334K and the way in which it is incorporated into the sarcomere, as well as the specific mechanism by which it impairs the UPS warrants further investigation (3). Additional perspectives report the importance of β-adrenergic receptors (β-ARs) in the cardiac dysfunction of HCM associated with cMyBP-C mutations. Studies have shown that overactivity of β-ARs leads to suppressed signaling by increased activity of inhibitory G protein, which negatively affects growth and function of cardiac myocytes. Evidence of increased β-adrenergic signaling in cardiomyocytes of patients with chronic heart failure has been presented, yet

understanding this possible link between β-ARs, UPS impairment by E334K mutant cMyBP-C, and HCM calls for further experimentation and analysis (20). Ultimately, elucidation of the precise structure and pathophysiology of this E334K mutant cMyBP-C presents possible therapeutic targets invaluable for treating the clinical arrhythmias and cardiac dysfunction caused by E3334K induced impairment of UPS in HCM patients.

Clinical Case Study

The following case study concerns an otherwise healthy, partially vaccinated 19-year-old, Japanese American female who was referred to a cardiologist upon presenting an abnormal EKG performed for a sports physical. The purpose of this case study is to investigate this patient's cardiac function and elucidate the cause of her electrophysiological dysfunction.

Addie Mori

Chief Complaint

Addie Mori is a 19-year-old, Japanese American female. Upon presenting an abnormal pre-participation EKG for sports at UNR, Addie was brought to the hospital the following day for further evaluation.

History of Present Illness

Addie had never had an EKG done prior to this physical exam. She has never experienced any chest pain, discomfort, or heart palpitations to prompt such procedure. She has had syncope 3 times within the past 5 years, all occurring shortly after moderate exercise. She reports waking up after each episode and feeling perfectly normal. She otherwise doesn't feel limited in any way.

Past Medical History

Addie was born overseas on a U.S. base. After receiving three rounds (2, 4 and 6 month) of vaccinations, her mother opted to not vaccinate her any further. Addie contracted pertussis at 4 months old and a series of ear infections after her mother stopped breast-feeding her around 20 months old. Beyond having her adenoids removed

at the age of 2 and her wisdom teeth removed at the age of 17, Addie has had no other surgeries or hospitalizations. Addie has had regularly occurring headaches throughout her youth, which her mother attributes to her forceps facilitated birth. When Addie began to participate in organized sports in her early teen years, she would on occasion complain of shortness of breath. She was tested for exercise-induced asthma with negative results.

Social History

Addie is an active adolescent and has participated in sports throughout middle school and high school. Addie played a semester of collegiate soccer last fall and then transferred to UNR this spring. She was receiving a physical to try out for the UNR soccer team when her abnormal EKG was detected. Addie follows a considerably healthy diet and reports no known food allergies. She has temporarily adopted a vegan diet within the last month for a school assignment. Addie is well liked by her peers and teammates, excels in academics, and leads a balanced life. She denies the use of any drugs or alcohol and is currently not on any medications. Addie has a boyfriend of a couple weeks but states to be sexually inactive at the present.

Family History

Addie's mother is very active with no medical problems at age 51. Her father is age 52 and has a record of abnormal EKGs. He states to have an enlarged left ventricle and has been told to have an "athletic heart." Reports from 2010 shows left ventricular wall thickness of 1.3cm, yet he has refused further investigative procedures. Addie's father denies ever passing out and only experiences shortness of breath and minor chest discomfort on occasion. Both of Addie's paternal grandparents had bypass surgery. Addie's little sister was born with a benign pediatric heart murmur of which she grew out

by the age of five. Her other little sister presents no symptoms of abnormal cardiac function. There is no history of early or sudden cardiac death.

Review of Systems

General: Maintains an average body weight. Sleeps well and reports sufficient and sustained energy throughout daily activities. Minor fatigue due to strenuous exercise.

HEENT: Reports recent vision problems such as trouble seeing the white board in class. Regular dull headaches. No auditory problems or oral lesions. Last dental visit within the past year.

Pulmonary: Occasional shortness of breath during exercise as stated in PMH.

Cardiac: No notable chest pain. See HPI.

Gastro: No nausea, indigestion, or difficulty swallowing. Reports regular stools.

Heme.: No bruising or hemorrhaging. Mother suspects anemia due to lack of red meat in diet.

Endo.: Denies mood swings. No symptoms of thyroid problems.

Musculoskeletal: Post workout stiffness. No joint swelling. Minor pain in right ankle from former sprain.

Neuro.: Dizziness before syncope. See HPI.

Derm.: No rashes or lesions. Minimal acne.

Uro/Genito.: No past UTI's or irritative/obstructive symptoms. Regular light menstruation every month. LMP: 4/16/14. Sexually inactive.

Physical Exam

BP 110/73 | Pulse 52 | O2 100% | Ht 5'5" (165.1 cm) | Wt 141lb (63.957kg) | BMI 23.46kg/m2

CONSTITUTIONAL: She is oriented to person, place, and time and moves all extremities. She has no acute distress and is not agitated, anxious, or depressed. She appears well-developed and well-nourished.

HEAD: Normocephalic and atraumatic.

EYES: Cojunctivae are normal and clear, PERRLA

THROAT: No gross abnormalities in oral mucosa, palate or teeth, MMM

NECK: FROM. Supple. Shows no venous distension. Full active range of motion. Thyroid is normal size. No carotid bruit.

CARDIOVASCULAR: Slightly slower rate, regular rhythm and normal S1 and S2 sounds. Exam reveals no gallop and no friction rub. No murmur heard. Radial and pedal pulses bilaterally 2+. No cyanosis. Cap refill less than 2 sec. No peripheral edema. No JVD. Non-displaced PMI.

PULMONARY/CHEST: Effort normal and BS normal. No crackles or wheezes. No accessory muscle use.

ABDOMINAL: Soft. Bowel sounds are normal. Exhibits no distension. No organomegaly, masses, or tenderness.

MUSCULOSKELETAL: Full active range of motion. Exhibits no edema or no tenderness. Normal muscle strength with no kyphoscoliosis.

GU: Deferred.

BREASTS: Deferred.

NEUROLOGICAL: A/O x3. Speech clear, appropriate, and cooperative. Cranial nerves 2-12 fully intact. Sensation intact. Gait is normal. No focal neurological deficit.

SKIN: Warm and dry with no dermatitis or xanthomas.

Laboratory and Imaging Results

Electrocardiography:

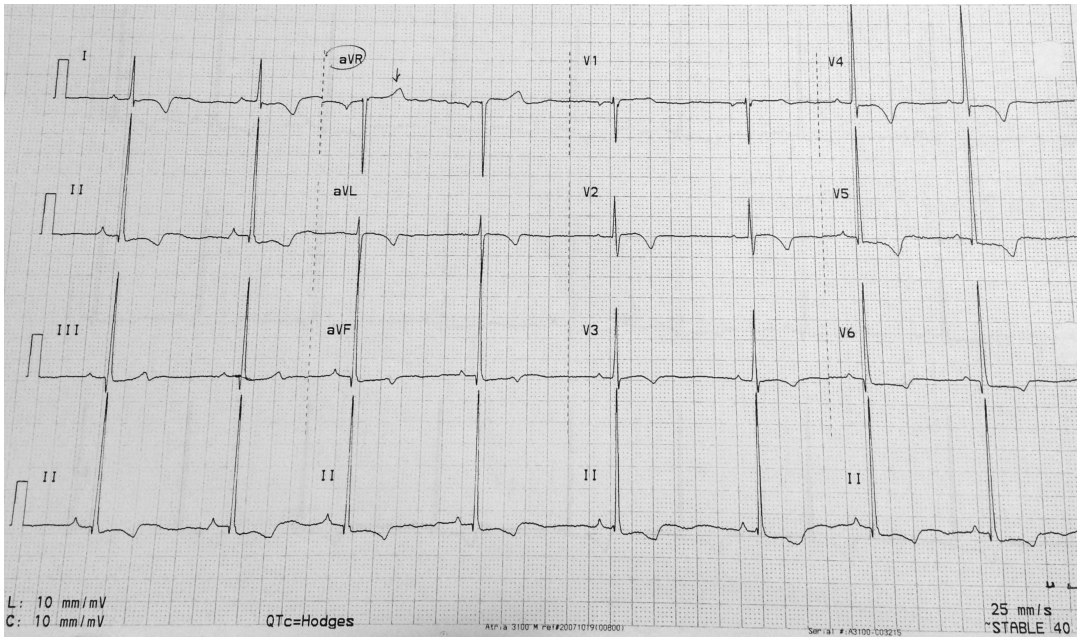

Figure 9: 12-lead EKG of patient- Sinus Bradycardia. Biphasic morphology of P wave indicates left atrial hypertrophy. Positive deflections of leads I and aVF indicate normal

axis alignment. Increased QRS amplitude in V5 suggests left ventricular hypertrophy. Deep anterolateral T-wave inversion reveals abnormal left ventricular repolarization. Image obtained from MyHealth at Stanford - Test Results [Internet]. [cited 2014 Apr 21]. (21)

Echocardiography:

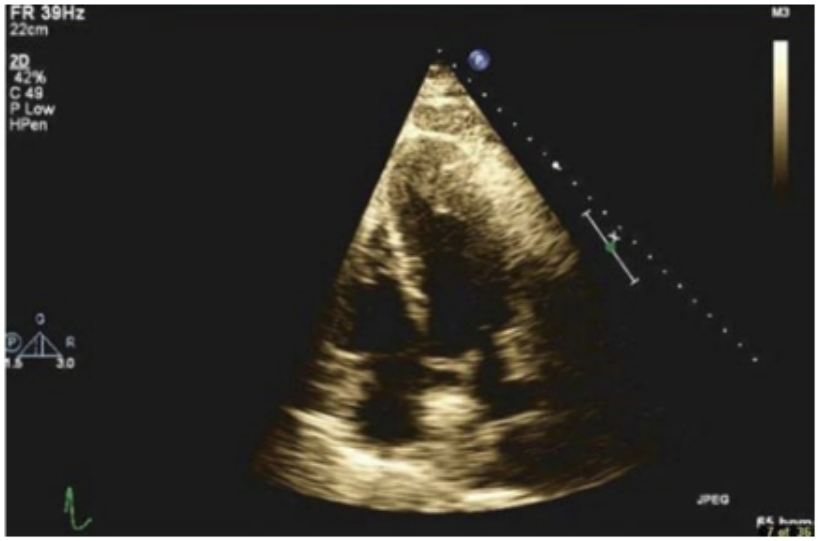

Figure 10: 2-D echocardiogram of patient- Mild LVH with wall thickness of 1.5 cm mid apical septum. Normal left ventricular end-diastolic dimension of 4.1cm (normal <5.5cm). LA enlargement with LA volume of 40 ml/m^2 (normal <28ml/m^2). Mild mitral regurgitation. Image obtained from Yusuf SW, Bathina JD, Banchs J, Mouhayar EN, Daher IN. Apical hypertrophic cardiomyopathy. World J. Cardiol. [Internet]. 2011 Jul 26 [cited 2014 Apr 24];3(7):256–9. (22)

Laboratory Panels:

CBC	**Standard Range**	**Values**
WBC	4.0 - 11.0 K/uL	6.2
RBC	3.80 - 5.20 MIL/uL	4.69
Hemoglobin	11.7 - 15.7 g/dL	13.1
Hematocrit	35.0 - 47.0 %	37.8
MCV	82.0 - 98.0 fL	80.7
MCH	27.0 - 34.0 pg	26.9
MCHC	32.0 - 36.0 g/dL	34.5
RDW	11.5 - 14.5 %	14.8
Platelet count	150 - 400 K/uL	237
NEUT, %	40-74%	50.7
LYM, %	14-46%	39.7
MONO, %	4-13%	7.6
EOS, %	0-7%	1.0
BASO, %	0-3%	1.0
NEUT, ABS	1.8 - 8.0 K/uL	3.14
LYM, ABS	1.5 - 6.5 K/uL	2.46
MONO, ABS	0 - 0.4 K/uL	0.47
EOS, ABS	0 - 0.2 K/uL	0.06
BASOS, ABS	0 - 0.25 K/uL	0.06
Glucose	70-105mg/dL	70
Renal Function		
BUN	5-20 mg/dL	13
Creatinine, serum	0.5-0.8 mg/dL	0.76
Electrolytes		
Chloride, Serum	96 - 108 mEq/L	101
Calcium, Serum	8.8 - 10.8 mg/dL	10.0
Sodium, serum	133 – 145 mEq/L	138
Potassium, serum	3.3 - 5.1 mEq/L	4.5
CO2	20-28 mEq/L	27
Lipid Panel		
Cholesterol, Total	<170 mg/dL	153
Triglyceride, Ser/Plas	<150 mg/dL	72
HDL Cholesterol	>40 mg/dL	73
LDL (Calculated)	<130 mg/dL	66

Cholesterol/HDL Ratio	<5 ratio	2.1
Non-HDL Chol, Calc	<160 mg/dL	80
LDL/HDL Ratio	<3 ratio	0.9
High Sensitivity CRP	<3.0 mg/L	<0.2
Troponin		
Troponin I	0.0-0.4mg/ml	0.32
NT-proBNP	<300 pg/mL	539

Cardiac MRI:

Left Ventricle- Normal wall motion and systolic function. LVEF is 69%. There is mild apical hypertrophy present at apex with maximal wall thickness measuring 15mm at the anterior apical septum. No evidence of LV outflow tract obstruction.

Right Ventricle- Normal wall motion, size, and function.

Atria- Slight enlargement of left atrium with end diastolic diameter measuring 42mm (normal <40mm). Right atrium is unremarkable.

Valves- Mild mitral valve regurgitation. Pulmonic, aortic, and tricuspid valves are unremarkable.

Quantitative Assessment:

Right Ventricle:

1. End diastolic volume, absolute: 148.6 mL; corrected (EDVI): 86.4 mL/m^2. (normal 79.1±29.9ml)

2. End systolic volume, absolute: 62.0 mL; corrected (ESVI): 36.1 mL/m^2. (normal 32.6±19.7ml)

3. Stroke volume, absolute: 86.6 mL; corrected (SVI): 50.4 mL/m^2. (normal 60-100mL)

4. Cardiac output: 3.7 L/min; corrected (CI): 2.2 L/min/m^2. (normal 2.5-4.0 L/min/m^2)

5. RV ejection fraction: 58.3%. (normal >49%)

Left Ventricle:

1. End diastolic volume, absolute: 150.4 mL; corrected (EDVI): 73.2 mL/m^2. (normal <78 mL/m^2)

2. End systolic volume, absolute: 46.3 mL; corrected (ESVI): 26.9 mL/m^2. (normal 16-143mL)

3. Stroke volume: 104.1 mL; corrected (SVI): 46.3 mL/m^2. (normal 33-47 mL/m^2)

4. Cardiac output: 4.5 L/min; corrected (CI): 2.6 L/min/m^2. (normal 2.5-4.0 L/min/m^2)

5. LV ejection fraction: 69.2%. (>56%)

6. End diastolic mass: 152.0 g (normal range: 66-150g)

All reference values come from MyHealth at Stanford - Test Results [Internet]. [cited 2014 Apr 21].

Genetic Screening:

Genetic screening of the sarcomere tested negative for any suggestive mutations.

Diagnosis and Supporting Argument

Addie's condition was presented with an abnormal EKG and several noted episodes of exercise-induced syncope within the past five years. A history of cardiac disorders is present in the paternal side, with both of Addie's paternal grandparents having bypass surgery and her father presenting abnormal EKGs and mild left ventricular hypertrophy. The maternal family history has no known cardiac complaints. There is no history of early or sudden cardiac death. Review of Addie's 12-lead EKG shows sinus rhythm with voltage criteria for left ventricular hypertrophy and increased QRS amplitude in V5, biphasic P waves, and deep anterolateral T wave inversion. 2-D echocardiography featured left ventricular hypertrophy with maximal wall thickness of 1.5cm at the mid apical septum. Laboratory studies include an N-terminal proBNP abnormal level at 539 pg/ml. Cardiac magnetic resonance imaging confirmed left ventricular hypertrophy with normal wall motion and systolic function and no left ventricular outflow tract obstruction.

Based on her abnormal EKG, propensity to syncope, and imaging of left ventricular hypertrophy, the differential diagnosis may include: apical hypertrophic cardiomyopathy (ApHCM), hypertrophic obstructive cardiomyopathy (HOCM), athletic heart syndrome, or aortic stenosis. Imaging techniques as well as cardiac catheterization presented no evidence of left ventricular outflow tract obstruction characteristic of

HOCM. Furthermore, no heart murmur associated with HOCM and aortic stenosis was detected upon physical examination, and maximal left ventricular wall thickness was measured at the mid apical septum rather than the more proximal end of the septum, which causes narrowing of the outflow tract (23). Cardiac magnetic resonance imaging revealed exaggerated thickening at the apex, which may be consistent with antero-lateral T wave inversion and reveal evidence of ApHCM rather than HOCM. Left ventricular hypertrophy in athletic heart syndrome can reach 14-16mm in wall thickness and is a potential diagnosis of Addie's left ventricular hypertrophy given her participation in competitive athletics. However, echocardiographic assessment shows heterogeneous left ventricular hypertrophy particularly in the apical region and no enlarged left ventricular end-diastolic cavity common in athletic heart syndrome. These findings favor ApHCM over athletic heart syndrome (24). Aortic stenosis was ruled out based on assessment of aortic valve structure and pulmonary pressures measured by electrocardiography and cardiac magnetic resonance imaging. These image results yield no evidence of any narrowing of the aortic valve. Vasovagal syndrome might be considered as a possible factor causing syncope but does not account for the other abnormal cardiac findings and thus does not suffice as primary diagnoses. Diagnostic findings, mainly inverted T waves and left ventricular hypertrophy predominantly at the apex strongly suggest ApHCM as the source of the patient's symptoms.

Treatment

Apical hypertrophic cardiomyopathy is not a curable disease and treatment is often a multidisciplinary approach aimed to promote heart relaxation and/or avoid abnormal heart rhythms depending on the symptoms presented. As Addie fails to present any angina or tachycardia, use of beta blockers to alleviate such symptoms was not considered. Based on AHA classifying guidelines for patients with HCM, Addie lacks most clearly defined risk factors such as outflow tract obstruction, ventricular wall thickness greater than 3cm, and family history of sudden cardiac death (25). Nonetheless, family history of left ventricular hypertrophy in her father as well as past episodes of syncope related with exertion are of concern. The implications of these concerns were discussed at length, including the recommendation for avoidance of high-intensity burst-type activities as well as the utility of an implantable cardioverter defibrillator (ICD) for prevention of sudden cardiac death. The possibility of further review after 6 weeks of de-training was offered as a diagnostic test to evaluate if there is any regression of Addie's current cardiac hypertrophy. Addie agreed to undergo this complete de-training, as she is currently in the off-season for her collegiate sport. Screenings of all first-degree relatives both genetic and with echocardiograms and EKGs is advised. It was recommended that Addie's two sisters be screened now and then on a once-yearly basis until the age of 18 or until cessation of competitive exercise.

Prognosis

The most devastating complication of HCM is sudden cardiac death and usually occurs without warning largely in asymptomatic or mildly symptomatic young patients (26). Apical hypertrophic cardiomyopathy is a relatively rare form of HCM that currently presents benign prognosis in terms of cardiac mortality. The risk of cardiac complications varies, and one third of ApHCM patients may develop potentially life-threatening complications such as arrhythmias or stroke. However, roughly half of ApHCM patients remain asymptomatic and the long-term outcome for these people is generally good (27). As Addie presents minimal symptoms, she can expect to have a normal life expectancy free of any significant limiting symptoms.

Follow up

Addie will return in approximately 6 weeks for evaluation and discussion of results of de-training. Further action will depend on results of this diagnostic test. In the case of regression, Addie's diagnosis may be reconsidered to favor athletically-induced cardiac hypertrophy rather than ApHCM. Addie would continue on with her normal lifestyle yet with recommended avoidance of high-intensity burst-type activities, and she would receive annual clinical evaluations unless reoccurrence of symptoms should prompt re-evaluation.

No regression will further support ApHCM as the diagnosis. Such case will warrant extensive discussion with Addie and her family regarding discontinuance of competitive sports, as intense physical exertion is linked to the risk of sudden cardiac death in patients with HCM. Lower risk activities within heart rate targets would be recommended. ICD therapy and its life saving benefits as well as potential complications

would be discussed with Addie and her family and encouraged as a treatment in preventing sudden cardiac death.

In either case, establishment with a cardiologist and annual clinical evaluations will be advised. Clinic and patient support networks to deal with the social and emotional trauma associated with this new diagnosis will be offered.

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