University of Nevada, Reno

Clinical and molecular overview of Hypertrophic Cardiomyopathy through JPH-2 mutations and presentation in a clinical case study

A thesis submitted in partial fulfillment
of the requirements of the degree

Biology, Bachelor of Science

By

Justin Yeung

Josh Baker, Ph.D., Thesis Advisor

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Justin Yeung

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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 a disease which presents a distinct morphological change in cardiac tissue. Pathology of the disease widely ranges from being largely asymptomatic to the sudden onset of cardiac death. The disease's etiology stems from genetic mutations in various components of the sarcomere such as the myosin binding regions. One particular mutation affecting calcium handling in the sarcoplasmic reticulum, JPH-2, manifests as a possible mechanism for the onset of sudden death and histological features consistent with myocardial disarray. This thesis aims to understand junctional complex gene's, JPH-2, maintenance of the dyadic cleft and how mutants variants result in pathogenesis of HCM.

Hypertrophic Cardiomyopathy's clinical presentation is highlighted in the subsequent case study, of Paco Sanchez. The case study seeks to emphasis the use of laboratory diagnostics and treatment of HCM. The social effects of HCM and treatment beyond the use of medicine are of importance in handling the onset of such disease.

Acknowledgements

The formation of this thesis would not have been possible without the guidance and advisement of my mentors and fellow colleagues. Their constructive criticism and input were invaluable in completion of this project. I would like to give my gratitude to Dr. Josh Baker for establishing a timeline and overseeing this entire process. Dr. Baker's expertise on this disease and thoroughness when revising this thesis has been crucial in its development. Deepa Dandge, MSIV played a pivotal role in overseeing the formation of my case study. Her clinical knowledge supplemented in forming a realistic and consistent patient. I am also grateful for the input that my colleagues, Jessica Kim and Susan Alaei, have given me in this thesis development.

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Chapter I: Overview of Hypertrophic Cardiomyopathy

Introduction

Hypertrophic cardiomyopathy is a disease characterized by the enlargement of the myocardium, the thick middlemost layer of cardiac tissue. This enlargement is the cause of a wide range of abnormal pathologies of the heart, manifesting into a large variety of symptoms including irregular heart rhythms, valvular abnormalities (particularly in the mitral valve), shortness of breath, or chest pains. However, Hypertrophic Cardiomyopathy (HCM) is notorious for being one of the leading causes of sudden cardiac death, especially in the demographic of physically active individuals; sudden cardiac death is often ascribed many similar physiological factors to HCM, such as asynchronous heart beats and enlargement of cardiac tissue.

Although the scope of the disease is statistically minute (affecting 0.05% of the patient population), a strong correlation within the epidemiology of HCM can be seen. A strong genetic basis is observed within 25% of nuclear relatives signifying dominant phenotypic expression within family pedigrees (Bope, 2013). Several genetic defects within a family of cardiac microfilament genes are responsible for the causation of HCM; however penetrance is variable leading to asymptomatic individuals and those with more extreme physiological conditions. The focus of many studies goes into the mutations of sacromeric component genes involving myosin heavy-chains and troponin such as the MYHY and MYBPC3 genes.

Treatment of HCM is limited to invasive procedures involving surgery to ablate overgrowth of tissue or to maintain the electrochemical integrity. More so, clinical presentation is hard to detect as the variable nature of HCM symptoms can lead to

diagnoses of other cardiac or metabolic diseases. Thus, the use of familial screening and echocardiogram testing is pertinent due to the nature of HCM.

Clinical Presentation:

HCM manifests itself in a variety of cardiac conditions stemming from the abnormal increase of myocardial tissue in the heart. Most commonly, the left ventricle shows hypertrophy of the myocardium (the left ventricle is notably the area of the heart in which the myocardium of a physiologically normal heart is the thickest) or the septum which divides the left and right ventricles. This enlargement in the left ventricle or septum ranges from 50 - 60mm in thickness, generally focused within the apex of the heart. The increase in myocardial tissue in some individuals has effects upon the systolic and diastolic functions of the heart. Due to misalignment of contractile subunits of the cardiac tissue, outflow from the left ventricle to the left atrium is more difficult for patients with HCM (Shah, 2012). Increase in pressures of greater than 30mmHg within the ventricle, creates stress upon contraction and mitral valve regurgitation. Since the pressure within the left ventricle is larger, the contraction creates stress which forces the heart to increase oxygen demand (Bonow, 2012).

Ventricular hypertrophy's effect in the mitral valves is also documented in the observation of abnormal valvular motion. During systole, the high pressure gradient within the left ventricle causes blood outflow at a much higher velocity causing a hydrodynamic event known as the Venturi effect. As explained by the Venturi effect, fluid flowing into a constricted system from a system with a larger cross sectional area experiences a decrease in pressure; this change in pressure when blood is flowing from a contracted ventricle to the atria is hypothesized to cause the mitral leaflets to move

towards the septum at an approximate 90^{o} from the hydrodynamic forces (Bonow, 2012). This is often observed using Doppler imaging, (as in Figure 1.) and is labeled Systolic anterior motion; such imaging often can explain other abnormalities such as mitral regurgitation. Another theory contributing to abnormal valvular movement is abnormal positioning of the papillary muscles which connect the valves to the walls of the ventricle (Bope, 2013). Over 25% of patients with HCM have ventricular or septal obstructions causing problems associated with the pressure gradient within the ventricles (Cirino, 2011). Ventricular obstructions caused by an abnormal gradient are an important facet in the clinical presentation of HCM; however its manifestation is not necessarily indicative of increased risk of sudden cardiac death (sans risk of progressive heart failure).

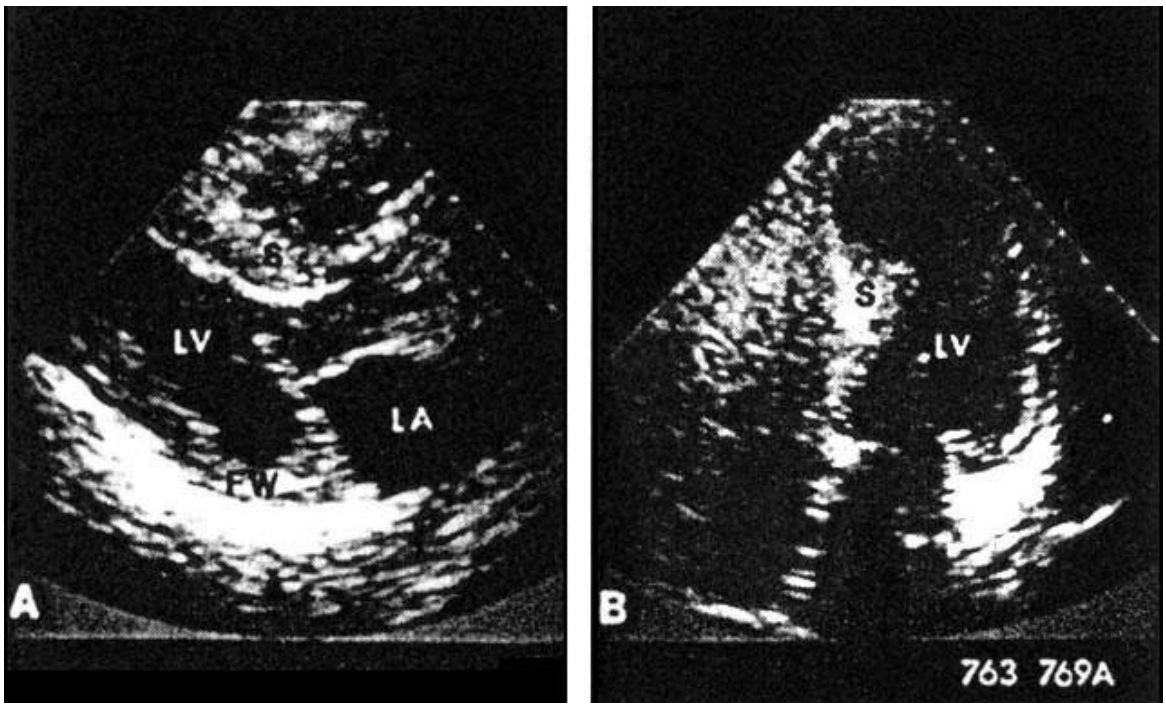

Figure 1. Doppler imaging of patient with Hypertrophic Cardiomyopathy. In image A, SAM can be indicated by the abnormal mitral valve moving towards the septum (labeled S above LV). Septal wall thickness can also be seen in Image B. Colored Doppler imagery may reveal systolic gradients used to help diagnose HCM (Shah, 2012).

Patients with HCM often manifest symptoms associated with atrial fibrillation such as angina, syncope, or postural hypotension. Indication of atrial fibrillation is generally detected in an electrocardiogram by the presence of the P waves in the PQRS complex. Complaints of chest pain as well as shortness of breath are correlated to the diastolic function of the obstructed ventricle developing large intercavity pressure. Irregular beating of the heart can cause those with HCM, to have bouts of unconsciousness due to hypotension.

Atrial fibrillation, withstanding other complications of HCM, is capable of inducing a multitude of fatal conditions, such as heart failure or formation of thromboembolisms due to impaired blood flow. Ventricular arrhythmias have been correlated to increased incidence of sudden cardiac death by about 10 - 20% due to the frequency of atrial/ventricular fibrillation in symptomatic individuals (Elliot et al, 2000). The mechanism for the onset of sudden cardiac death via HCM is currently unknown; however studies have indicated that myocardial disarray is a major culprit in the onset of cardiac death.

Genetics/Pathophysiology

Although the overall pathophysiology of HCM is not well understood, the genomics of the disease has been catalogued to mutations within 14 sacromeric genes. These genes include sequences which encode for myosin heavy chains, actin, and etc. Over 900 different mutations within the 14 sequences have been known to cause hypertrophic cardiomyopathy. HCM mutations are often carried down in a dominant phenotypic fashion with variable expressivity. Individuals can often exhibit variable

penetrance as a number of those with HCM are often asymptomatic; in contrast some develop serious cardiac complications associated with arrhythmia.

The variance of symptoms often leads to questions on the mechanisms of how HCM can clinically manifest itself in some patients but not others. This is especially true in cases of sudden cardiac death; many patients often do not realize they have genetic mutations causing Hypertrophic Cardiomyopathy until fatal incidence of heart failure. The difficulty of diagnosing HCM in patients with cardiac problems adds to the difficulty in linking asymptomatic versus symptomatic presentations to specific gender or ethnic populations. Although the phenotypic expression of HCM has no definite correlation within populations, the genotypic presence of the mutations causing HCM is transmitted in an autosomal dominant fashion.

This facet of many sacromeric mutations causing HCM can provide clinical insight in establishing a management plan. Based on information provided by GeneReviews on Familial Hypertrophic Cardiomyopathy, analyzing the pedigree of an individual of symptomatic HCM shows a 90% correlation their progeny developing variable symptomology (Cirino, 2011). Variance of symptoms can also be a result of a wide variety of different mutations which may independently cause HCM; these mutations affect different parts of the sacromere and may have slightly different pathologies associated with some.

The most common mutations in symptomatic HCM are caused by missense mutations within the myosin heavy chains coding for the head and neck regions or the myosin binding proteins. Of the many HCM- causing mutations, MYH7 and MYBPC3 are responsible for 80% of HCM cases (Cirino, 2011). MYH7 encodes for the myosin

heavy ß-chain, and has the function of maintaining the slow twitch of cardiac muscle fibers (Quiat *et al*, 2011). Expression of this protein product is normally localized in the ventricle of humans as well as the atria of some failing hearts. Mutations occurring within the gene MYH7 afflict the amino head and hinge of the coded protein (OMIM: 160760). Analysis of MYH7 mutations has shown that missense mutations code for amino acid substitutions, specifically the Arginine to Glutamine mutation on amino acid location 403, evident in Figure 2. Such mutations are hypothesized to cause lower sliding velocity during myosin and actin interaction and therefore lead to overall all abnormal contractions (Kelly & Strauss, 1994). In contrast some mutations such as the D778G MYH7 mutants, have been observed to increase actin sliding by approximately 30-50%; the theoretical mechanism of how such mutants cause abnormal cardiac function is consequence of faster actin myosin cross bridging which breaks down ATP ($ADP + P_I$) faster than the myocyte can produce (Reverra et al., 2007). This build up of ADP and inorganic phosphates is shown to impair relaxation rates of diastole.

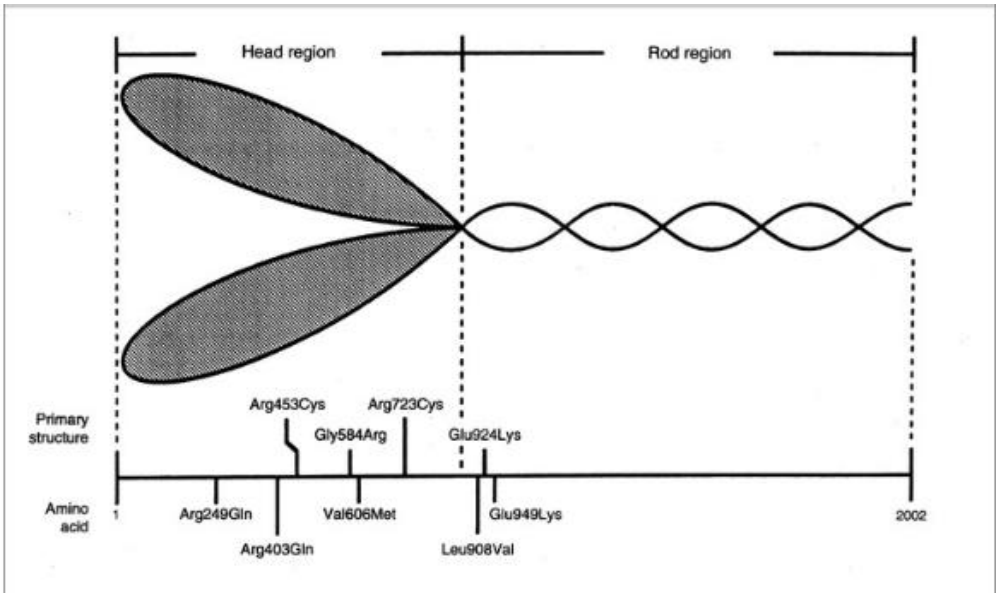

Figure 2. Mutations known to cause Hypertrophic Cardiomyopathy. These mutations correlate with the Head and Rod region of ß Myosin Heavy Chain. Many MYH7 mutations corresponding to different parts of the Myoin heavy chain dimer, can cause clinical manifestations of abnormal cardiac function. Arg403Gln, is one specific mutation known to have higher incidence of sudden cardiac death associated with ventricular arrhythmia. Many other missense mutations occur along the head region of myosin heavy chain or the head/rod intersect. (Kelly & Strauss, 1994)

MYBPC3 encodes for cardiac binding proteins, more specifically Cardiac Binding Protein C; such proteins play roles in the contraction by the ATP dependent process of myosin binding or response to sympathetic activity (OMIM: 600958). Various mutations, such as missense, deletions and insertions in the sequence (located on 11p11.2), cause truncations in the protein product leading to Hypertrophic Cardiomyopathy. Mutations in this gene also cause sacromeric disorganization as well as decreased force generation. As seen in Figure 3, proper binding of the myosin head as well as proper sliding of actin/myosin is ideal in cardiac function; mutations in either the ß myosin heavy chain or the cardiac binding protein, (MYH7 and MYBPC3, respectively) can cause pathology hallmark of HCM.

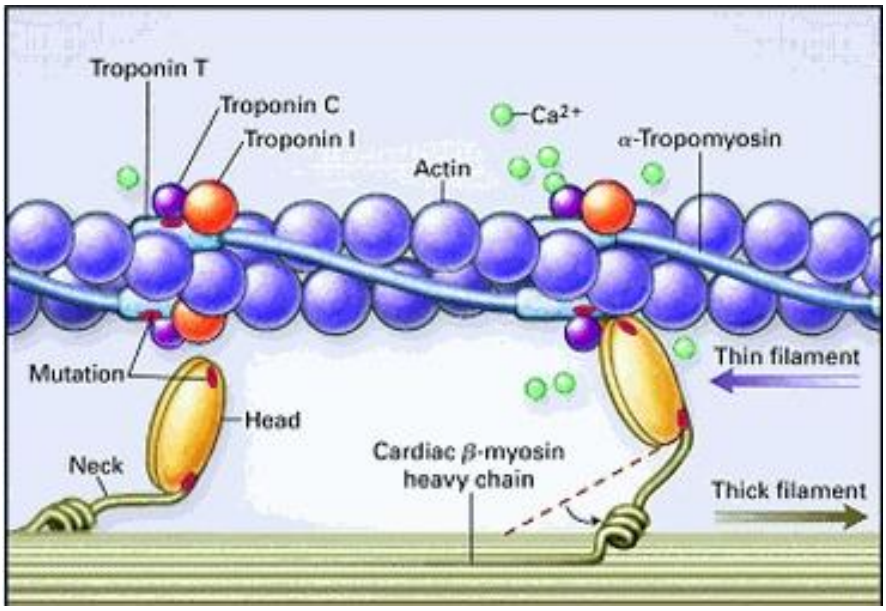

Figure 3. Contraction by interactions of thin and thick filaments as a result of calcium mediated binding events. ATPase activity triggers the binding of actin to the head; the binding of the myosin head to actin causes sliding events which leads to contraction. Mutations in the myosin protein complex have been shown to increase or decrease sliding velocities causing abnormal pathology. (Cirino, 2011)

Although mutations in the myosin-actin complex have a large contribution in the overall symptomology of HCM, mutations in the calcium channels of the sacromere are found to contribute to the large incidence of sudden cardiac death. JPH2, proteins which mediate calcium release in the sacromere, have been found to contribute to this increased incidence. By impairing synchronny events of calcium influx into the cells, mutations in JPH2 can cause ventricular arrythimias which have the probability of leading to sudden cardiac death (Landstrom, 2007). The mechanism of how JPH2 mutant may trigger sudden cardiac death due to abnormal calcium handling, is focused on rearrangement of the 'dyadic cleft' which facillates cross talk of calcium channels. The topic of HCM related sudden cardiac death as a consequence of dyadic cleft remodeling from JPH2 mutation will be explored in Chapter II. Considering the quiescent nature of various

HCM-causing mutant phenotypes, analysis of pathophysiology of different mutations can play a large role in modifying treatment management for an infamously asymptomatic disease.

Treatment

Treatment for HCM is limited to addressing the symptoms which can arise due to ventricular obstructions and or arrythimia. Antiarrythmics can be prescribed to stabilize heart rate and reduce the risk that is posed by the higher incidence of atrial fibrillation. Such drugs include the use of Beta-blockers or calcium channel blockers. Since atrial fibrillation offers a higher risk of the development of thromboembolisms due to impaired blood flow, it is pertinent to prescribe anticoagulants such as heparin or warfarin to manage risk.

Due to the enlarged myocardium, the method of treatment should address symptoms that arise from impaired flow/pressure gradient within the ventricles. Symptoms such as chest pain or ischemia can be alleviated by prescribing beta blockers to reduce physical exertion of the heart. Other treatments such as diuretics to maintain blood volume can be prescribed to also reduce the stress of contraction, although issues have to be addressed about hypotension and inherent risk of syncope in individuals with HCM.

More drastically, many invasive surgical procedures are used to treat severe cases of HCM from those with dysfunction in the physiology of the heart. Often times, procedures involve removing portions of the septum (septal myectomy) to relieve the ventricle of obstructions. Patients showing a left ventricle pressure gradient of over 50mmHg are often good candidates for this procedure as there is a relatively low risk

(<1%) operative risk with a high survival rates post procedure. Often times a section of 3-10 g of septal tissue is removed and is sufficient in alleviating symptoms caused by severe HCM. Another form of myectomy can be performed by using high percentage alcohol injected into a coronary artery around the proximity of the obstruction to induce necrosis of the tissue.

Chapter II: Sudden Cardiac Death in HCM as a Consequence of Architectural Changes in Dyadic Cleft due to JPH-2 Mutation

The majority of clinical manifestations of HCM are often consequence of physical obstructions due to hypertrophy of myocardial tissue in septum, left ventricle or the apex of the heart. Enlargement of tissue cause patients to experience chest pain, increased oxygen demand in cardiac tissue, and abnormal valvular movements. However, a hallmark clinical manifestation of HCM is within the increased incidence of Sudden Cardiac Death (SCD) in patients; a 3 - 6% mortality rate caused by sudden cardiac death is observed within the HCM patient population. Although this is a relatively low mortality rate, diagnosis of SCD due to pre-existing HCM is often made postpartum. In the study of HCM, the disease is often attributed to mutations in proteins coding for microfilaments of cardiac muscle tissue. Mutations in genes encoding for myosin binding protein (MYH7 and MYBPC3) are observed in approximately 40% of the patient demographic. However such mutations do not sufficiently explain the etiology of HCM-onset SCD. Studies suggest mutations in calcium channel are more directly related to the increased risk of sudden death in HCM patients (Blayney, 2009). More specifically, mutations in the junctophilin-2 complex can manifest arrhythmic cardiac events leading to sudden death in those with HCM. Decreased expression of functional Junctophilin-2 remodels the normal geometry of the dyadic cleft.

Calcium ions play a paramount role in the contraction mechanism of muscle tissue, from binding to troponin C to allow for actin/myosin binding as well as cell depolarization events of muscle tissue. In cardiac tissue, calcium influx events occur with synchrony via L-type calcium channels (LTCC). Disruptions in the calcium influx

can cause complications, which ultimately manifest as arrhythmic events. Studies link the onset of arrhythmia in the ventricle to increased sensitivity to calcium ions. The increased sensitivity may lead to shorter refraction periods before the initiation of another action potential to contract the myofilaments. Abnormalities in calcium channel proteins may also induce problems associated with an increased risk of abnormal depolarization events. The ability for proteolytic enzymes to degrade faulty channels is decreased as calcium influx is altered; this provides a positive feedback in which symptoms relating to the electrophysiological effects of such abnormalities continue to worsen (O'Mahoney, 2012).

Role of Coupled Receptors and Calcium Handling in Sacromere

Since calcium influx is known to affect the synchrony of contractile rhythm of the heart, mutations affecting calcium channel proteins can give rise to dyssynchrony events leading to serious complications such as abnormal ventricular rhythms. A mediator of proper calcium influx, the junctophilin membrane complex, helps couple the Ca^{2+} influx of LTCC and ryanodine receptors (RyR) (Landstrom, 2012). This coupling of the two receptors maintains the geometry of the dyadic cleft; when coupled (LTCC and RyR) the influx of calcium helps develop action potentials for proper cardiac contraction. LTCC is found in the T-tubules of the sacrolemma; T-tubules are the invagination of the muscle membrane (sacrolemma), which helps propagate the influx of calcium through the interior of the cell. An extensive network of T-tubules is strewn throughout the sacrolemma to ensure that the membrane depolarization initiates a uniform action potential. When the membrane of the sacrolemma is depolarized, LTCC releases a small amount of calcium ions which cascades the RyR receptors on the sacroplasmic reticulum

to release a large amount of calcium in its lumen, into the junction (dyadic cleft) initiating contraction of cardiac tissue (Blayney, 2009). Figure 1 diagrams the subcellular organization and function of the couplon made by LTCC and RyR. For proper propagation of contraction, the couplon of the RyR and LTCC must exist in a proper ratio (varies per species) as well as provide synchrony in the release of calcium into the cystol of the sacromere during systole (Heinzel, 2011). Failure of synchrony leads to decreased force of contraction as well as reduction in filament overlap in the areas where contraction is delayed.

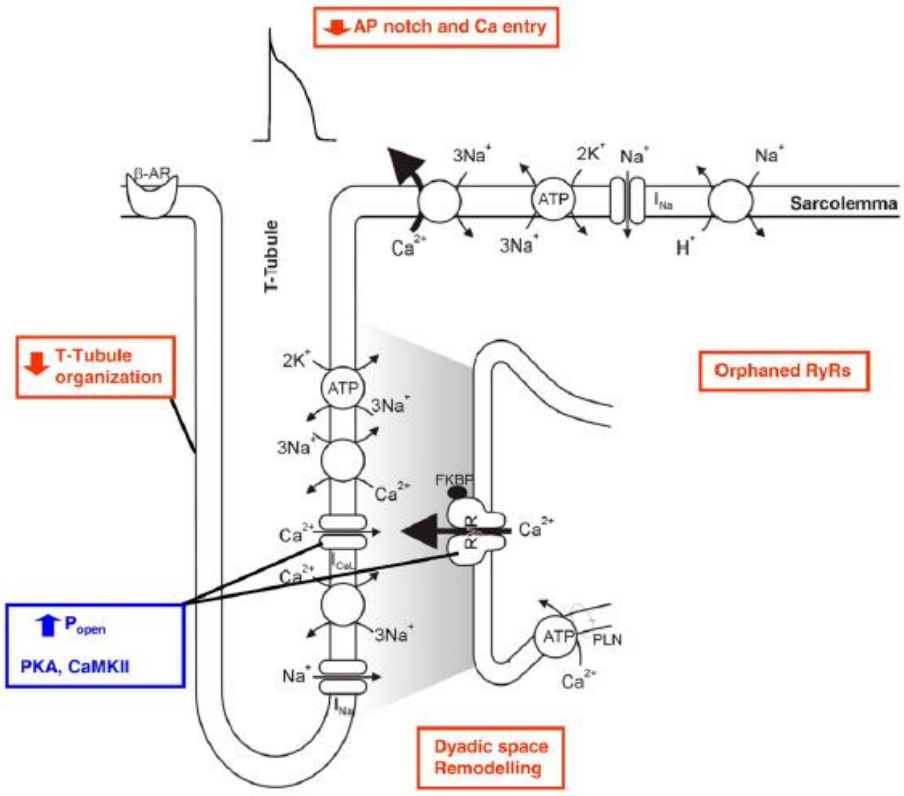

Figure 4. Organization of LTCC and RyR proteins. Couplons of proper dyadic geometry allows for function of Dyadic Space in Ca^{2+} Handling**.** L-type calcium channels are strewn across the sacrolemma, in particular within the T-tubules, paired with RyR proteins on the sacroplasmic reticulum. The overall positioning allows LTCC to initiate a Ca^{2+} spark to signal to RyR to release a larger influx of Ca^{2+} ions into the

cytosol; the area in between which mediates the crosstalk between the two channels called the dyadic cleft is shaded in grey. Boxes in red and blue indicate conditions which would decrease or increase, respectively, the efficiency of local Ca^{2+} release from the sacroplasmic reticulum.

Although there are a large number of mechanisms which can affect Ca^{2+} synchrony, change in the architecture of the dyadic space will be the focus of the investigation, in particular as a result of mutations in the expression JPH-2. In the observation of the histology of cardiac myocytes, remodeling of the dyadic cleft is often present in hypertrophied myocytes. The dyadic cleft spans the radius of 0.05µm between the T-Tubule and the sacroplasmic reticulum where the coupled calcium channels are located (Fan, 2009).

The geometry of this space is important in localizing LTCC's controlled triggering of RyR's intracellular calcium release. The density of the LTCC's along the T-tubule in the couplon is important in carrying out calcium signaling along the dyadic cleft; hence the dyad geometry must accommodate for proper alignment of the LTCC and RyR channels to initiate effective signaling cascade (Fan, 2009). On the molecular basis of an individual myocyte, as shown in Figure 2, the significance of the dyadic cleft is evident in localizing the initiation of $Ca2^{+}$ 'spark'; conversely, the mechanism of how LTCC and RyR couple to cascade throughout the entire contraction of heart is not currently a well understood mechanism.

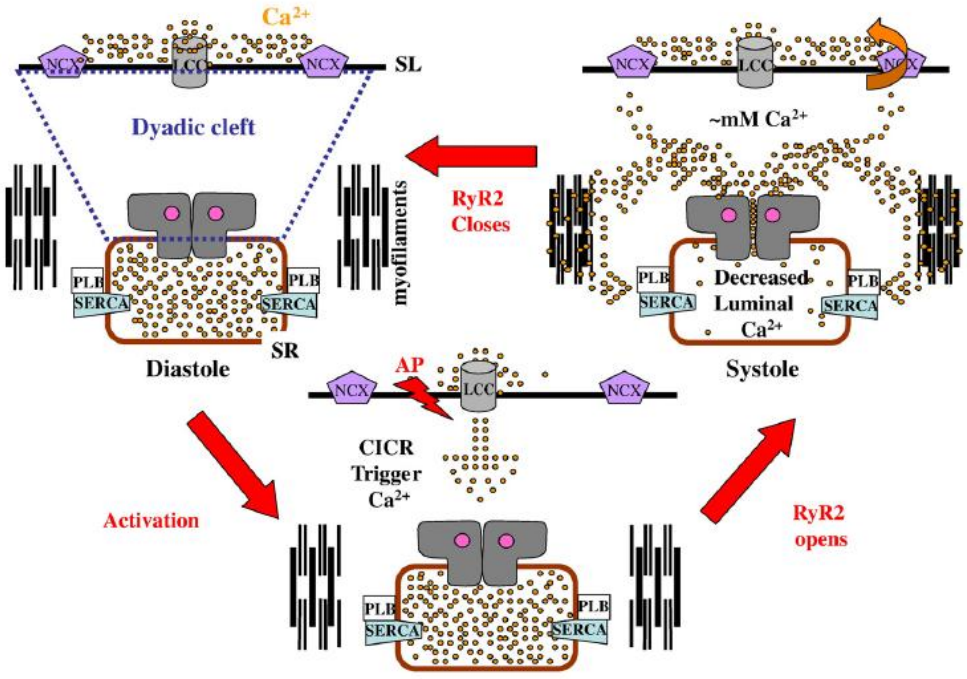

Figure 5. Mechanism of Interaction between LTCC and RyR mediated by the Dyadic Cleft. The geometry of the dyadic cleft has its importance in localizing the Ca^{2+} trigger from LTCC, allowing RyR to release the ionic contents within the lumen of the sacroplasmic reticulum. The diagrammed subcellular event occurs throughout the heart to initiate contraction, although its exact mechanism of how this event propagates is unknown. (Blayney, 2009)

The importance of maintaining the geometry in the dyadic space can be seen in the pathological effects of remodeling of the receptor coupling as a result of Hypertrophic Cardiomyopathy. Relevant to abnormalities in the dyadic cleft due to HCM, are mutations downregulating the expression of junctophilin membrane complex proteins. Junctophilins (also called junctional complexes) help mediate the localization of Ca^{2+} signaling between the sacrolemma and the sacroplasmic reticulum. Junctophilins typically are membrane spanning proteins with a hydrophobic C-terminus as well as several functional domains which have affinity to the sacrolemma (OMIM: 605267). A series of domains in junctophilins called the multiple membrane occupation and

recognition nexus (MORN) are believed to reconcile the calcium 'crosstalk' between the two membrane calcium channels (Landstrom, 2007). Mutations in genes that express junctophilins often lead to pathogenesis involving dyssynchrony events linked to hyperplasia of cardiac tissue.

Junctophilin-2 Mutations Linked to Rearranged Dyadic Cleft

One type of junctophilin, Junctophilin 2 (JPH-2), is responsible for approximating calcium signaling of the T-tubule and sacroplasmic reticulum. The level of expression of JPH-2 has a pivotal role in the onset of its own type of hypertrophic cardiomyopathy (hypertrophic cardiomyopathy type 17); this type is characterized by altered localization of the sacroplasmic reticulum as well as decreased Ca^{2+} released from the lumen of the SR. These observations can be related to the lack of physical approximation between the LTCC and RyR calcium channels; the change in density of LTCC causes an impaired Ca^{2+} spark to trigger release of a delayed or insufficient respons by the RyR to release lumenal Ca^{2+} in the SR.

Three different missense mutations in the exons coding for JPH-2 have been catalogued to introduce the onset of HCM (type 17): S101R, Y141H, S165F. S101R and Y141H mutation involves the MORN domain IV, and substitutes a hydrophilic amino acid for a basic (slightly hydrophobic) amino acid (Figure 3). S101R mutation in particular, replaces a serine for an arginine amino acid in a protein domain responsible for sacrolemma affinity. A similar mechanism, instead substituting a hydrophilic amino acid for a hydrophobic amino acid is also characteristic of the S165 mutation, however S165 indirectly inhibits a PKC phosphorylation site by removing the necessary hydroxyl group present in serine (Landstrom, 2007).

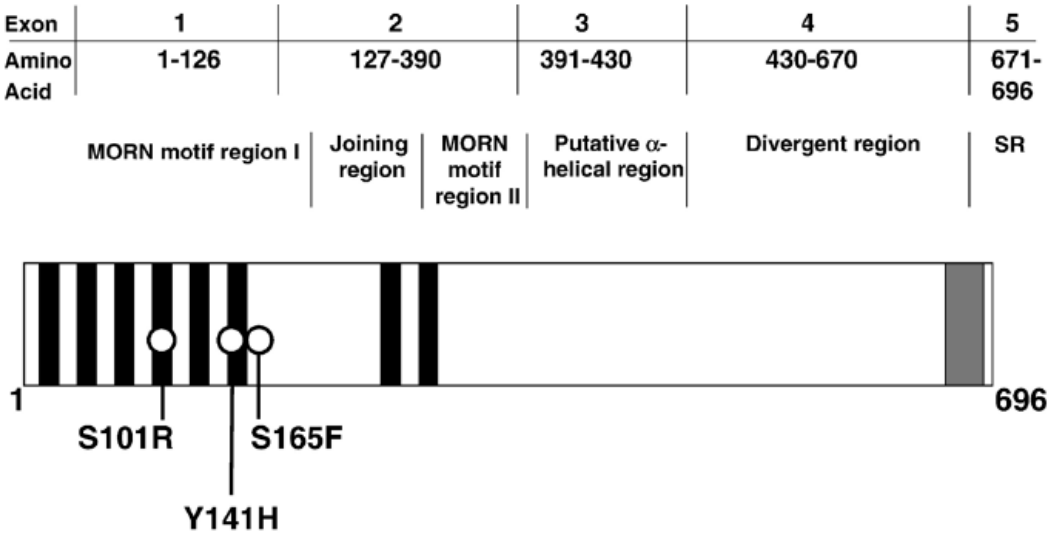

Figure 6. HCM associated mutations and functional domains on primary structure of JPH-2. 3 mutations, S101R, Y141H, and S165, occur within the MORN (membrane occupation and recognition nexus) Motif 1 region (on locations 1-126) is hypothesized to give proper localization between the dyadic cleft, in particular the T-tubules, since JPH-2 spans the sacrolemma. (Landstrom, 2007)

Experiments have linked the decreased expression of JPH-2 to decrease in Ca^{2+} in the intracellular junctions. Mutations in junctional membrane complexes can cause LTCC to not be in the proper position to trigger a response to the RyR receptors. This is evidenced in studies examining transient calcium levels in post-myocardial infarction mice (Landstrom, 2007) The observed current through LTCC was normal, opposed to the intracellular calcium concentrations which were present in decreased levels. In the event of under expressed JPH-2, a deficiency in the local positioning of the LTCC relative to the RyR leads in failure of Ryanodine receptors to release luminal SR Ca^{2+} stores into the cytoplasm. Failure of the dyadic space to mediate the couplon's interaction, can lead to larger physiological conditionals quintessential to the symptoms of HCM. Delayed action potential responses throughout the cardiac tissue cause

dysynchronous contraction in systole; dsynchronny within ventricular myocytes can lead to ventricular arrhythmia, hallmark of sudden cardiac death (Heinzel, 2011). In addition, delayed action potentials reduce the overall force of contraction as a direct result of decreased summation in total synchronous sacromeric contraction. Inefficient contraction involving overlaps in thin and thick filaments due to failures in the calcium pump can present the hallmark pathophysiology in hypertrophic cardiomyopathy. Series of dyssynchronous contraction events exacerbate myocardial stress and the formation of myocardial scar tissue (myocardial fibrosis), affecting the parallel alignment of myocardial tissue, as seen in Figure 4. The outcome of myocardial disarray can be seen as an indirect influence of propagation of uncoupled excitation due to a remodeled dyadic geometry.

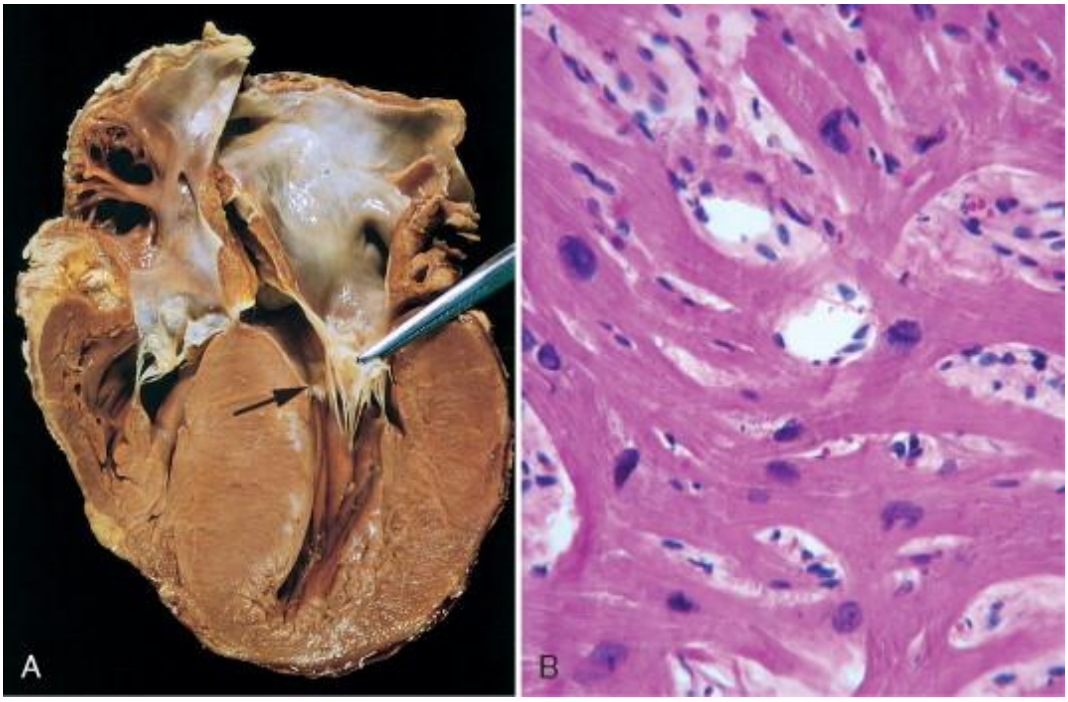

Figure 7. Gross appearance and histology of myocardial disarray in HCM. The image on the left shows hallmark signs of hypertrophied cardiac tissue, in particular the septum which is enlarged and gives a characteristic bent shape of the ventricular lumen.

Upon histological examination, myocytes are not aligned in normal parallel fashion, possibly due to asynchronous contractile events causing deformation of tissue. (Kumar *et al*, 2013)

Conclusion

Junctophilin-2 plays in an important role in coupling proteins in the dyadic space to ensure the initiation of coupled excitation between LTCC and RyR receptors. The effects of mutated JPH-2 are sufficient in incurring the pathogenesis seen in hypertrophic cardiomyopathy. A type of HCM has been classified to describe HCM onset by a junctophilin-2 mutation, HCM-type 17. Due to junctophilin-2's interaction in facilitating proper localization of Ca^{2+}, the pathogenesis of sudden cardiac death in type 17 HCM is related to the occurrence of improper calcium handling by the dyadic space (OMIM: 613873). The incidence of ventricular arrhythmia due to calcium asynchrony gives insight in screening for risk of sudden cardiac death based on genetic screening for JPH-2. By understanding the mechanism of how junctophilin mutations affect the overall pathology of HCM compared to myofilament mutations, a different approach in risk assessment can be taken for multiple forms of HCM.

Case Study

Chief Complaint:

Patient's reason for visit is due to increasing chest pain and shortness of breath after soccer practice.

History of Present Illness:

Paco, a 6 year old Hispanic male, presented with complaints of chest pain and shortness of breath. The patient's symptoms are often exacerbated by physical activities; Paco recalls several times after soccer practice where he feels uncomfortable in the chest, as if "squeezed" around torso. Patient reports duration of pain from 'a couple seconds' to 5 minutes; given on a pain scale of 1-10, the patient reports a 5-6 during episodes of chest pain.. Paco, who is always under his mother's supervision during these games, has no reports of fainting after activity, although he did complain of slight dizziness and fatigue after a game 3 weeks prior to visit. Symptoms are reported by patient to have gotten progressively worse since his first episode. In addition, Paco has recently been observed to develop a slight cough during his sleep. The mother states Paco has experienced such symptoms for a period of 4 months, around the same time he joined Little League Soccer. He is currently not taking any medications to alleviate the symptoms.

Past Medical History:

Before the onset of symptoms, Paco has been a relatively healthy individual. Although Paco was born 2 weeks early, he has shown normal development throughout childhood. His only admittance to the hospital was for a falling injury which required the

wound be stitched. Besides his symptoms of chest pain and shortness of breath, Paco seems to be a relatively healthy child. Since both of Paco's parents work tight schedules around the night, Paco's diet often consists of a high sodium fast food such as 'McDonald's' and etc. Paco seems slightly overweight for his age, hence why his parents enrolled him in sports a couple months ago. Paco is not currently on any medication nor does he have any known drug allergies. He also had a history of slight animal allergy when the family owned a dog. Paco has no known other environmental allergies. The patient is not exposed to tobacco smoke. He has been living in the same house since his birth, which was built 2 years prior. His immunizations are also up to date.

Family History:

The patient is an only child of a couple in their early 30's. The father and his extended family have had a history of higher than normal cholesterol levels as well as hypertension. The paternal grandfather, who is still living, has been previously diagnosed with dysmetabolic syndrome. The father's brother died at the age of 49 due to heart failure from poorly managed case of coronary heart disease.

The mother is seen to be in relatively good health, with no known medical conditions. The mother was sent to foster care where she was consequently adopted at a very young age (her sources say at ~2yo); the mother does not have any information of her nuclear/extended family's medical condition.

Social History:

Paco is a 1st grade student at a private school his parents have enrolled him in. He shows no learning disability and is excelling in his subjects; much of his time is spent reading books and playing soccer. Socially, Paco shows no difficulty in communicating openly with his peers. He is well-liked by much of his peers in class and his soccer team and shows no sign of social anxiety problems. Although Paco lives with both his parents, he spends many nights under the supervision of a babysitter. No abnormal behavior has been reported by the babysitter although she did report to the mother of slight episodes of coughing during his sleep. Paco does not live in a household with a history of smoking or drug abuse. There are no signs of abuse from the parents. Questions regarding the sexual history of Paco were not asked.

Review of Systems:

General: Denies weight loss, fevers, bed wetting. Positive for coughing while sleeping [obstructive sleep apnea]. Fatigue and chronic chest pain as stated in HPI.

HEENT: Last eye exam within 1 year, no auditory problems, no oral lesions. Last dental visit within 1 year. Denies change in voice.

Pulmonary: Cough present during sleep S.O.B upon exertion as stated in HPI.

Cardiac: Chest pain and dyspnea upon exertion.See HPI

Gastro.: No nausea, vomiting, difficulty swallowing. Denies diarrhea, irregular passing of stools, and abnormal stool color.

Genito/Uro.: No history of sexual activity, no pain, blood, abnormal coloration during urination.

Heme.: Denies bruising or hemorrhagic problems

Endo.: No thyroid problems, or hot/cold intolerance

Vasc.: No known information

Neuro.: See HPI [Dizziness]

Derm.: Denies rash and skin lesions

Psych.: No history of depression or mood swings.

Physical Examination:

Vitals:	Weight: 59lbs	Height: 4'1"	BMI: 17.3
	BP: 104/71	HR: 95	O_2: 98%

General: Child with no signs of physical distress.

Head: NC/AT

Eyes: PERRLA

Ears: Normal external ears, canal and tympanic membrane.

Nose: Symmetric with no signs of obstruction or inflammation.

Mouth: Normal Dentition and pharynx. No oral lesions or vesicles

Neck: Thyroid is normal, Lymph nodes are normal, neck is supple. JVD 1cm above sternal angle; a-wave is slightly more prominent.

Lymphatic: Adenopathy not present in cervical, supraclavicular, axillary, and inguinal areas.

Lungs: Lung acoustics clear bilaterally. Percussion is resonant.

Heart: Systolic ejection murmur 2/6 at lower left sternal border, increase in intensity after Valsava maneuver. S3 sound present

Abdomen: Normal bowel sounds, soft and non-tender.

Rectal/GU:-no need to check-

Back: No spinal deformities or tenderness of back

Extremities: Touch and sensation are intact.

Skin: No rash or lesions present

Musculoskeletal: Muscle tone and bulk is normal. Strength is intact. No sign of tenderness.

Neuro: Affect is normal and Cranial nerves are intact

Labs/Radiology

Electrocardiography:

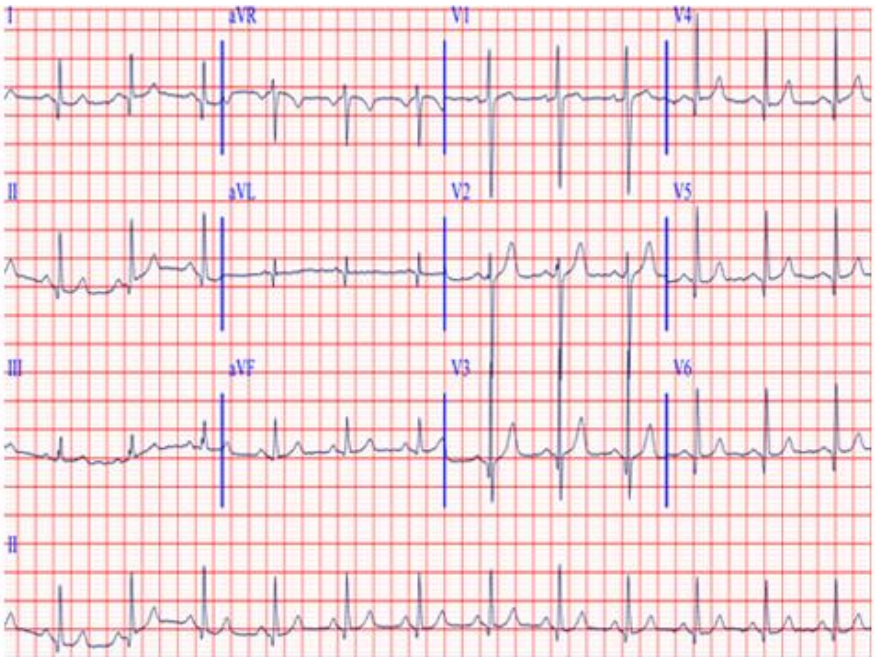

Figure 8. ECG of Paco Sanchez- 'Dagger-like' Q waves in lateral/ inferior leads I, II, IV, V3 and V6. Prominent R wave in V1. Findings show septal hypertrophy. Image courtesy of Medscape (Case study of 15 year old athlete)

Echocardiography:

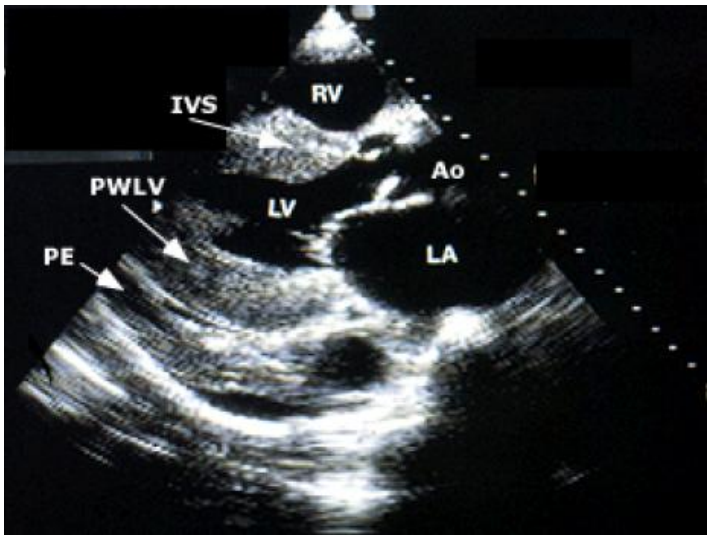

Figure 9. Parasternal Long axis view of 2-D echo of patient's heart. Septal (IVS) shows significant hypertrophy. Posterior left ventricle wall also shows slight hypertrophy. Wall thickness shown to be ~12mm in left ventricle.

Laboratory Panels:

Table 1. Lab Results for Paco Sanchez

CBC	**Reference Range**	**Units**	**Values**
RBC	3.00 - 5.00	10^6/µL	4.3
Hemoglobin	12.5 - 15.0	g/dL	13.1
Hematocrit	31.0 - 43.0	%	37
MCH	27.0 - 32.0	pg	30.5
MCHC	32.0 - 36.7	g/dL	35.4
MCV	77 - 103	cu µm	86.04
RDW	11.5 - 14.5		12.3
Platelets	351±85	10^3/µL	285
WBC	4000-11000	cells/mm^3	7500
Neutrophil, segmented	32-62	%	49.5
Neutrophil, band	0-11	%	3.6

	Lymphocyte	28-48	%	39.4
	Monocyte	3-6	%	4.6
	Eosinophil	0-3	%	2.7
	Basophil	0-1	%	0.2
	Neutrophil, [Absolute]	2.0 - 8.0	10^3/µL	3.98
	Lymphocyte,[Absolute]	0.6 - 5.5	10^3/µL	2.95
	Monocyte,[Absolute]	0.24-0.88	10^3/µL	0.345
	Eosinophil,[Absolute]	0.00-0.54	10^3/µL	0.2
	Basophil,[Absolute]	0.00-0.06	10^3/µL	0.015
Glucose		70-105	mg/dL	86
Renal Function				
	BUN	5-20	mg/dL	16.3
	Creatinine, serum	0.5-0.8	mg/dL	0.6
Electrolytes				
	Chloride, Serum	96 - 108	mEq/L	99
	Calcium, Serum	8.8 - 10.8	mg/dL	9.5
	Sodium, serum	133 - 145	mEq/L	139
	Potassium, serum	3.3 - 5.1	mEq/L	5.1
	CO2	20-28	mEq/L	23
Liver Function Test				
	Albumin	3.8 - 5.4	g/dL	4.2
	ALT	4 - 40	U/L	8
	ALP	20-150	U/L	32
	AST	8-20	U/L	15
	Bilirubin	0.3-1.0	mg/dL	0.64
Lipid Panel, fasting				
	Cholesterol	<200	mg/dL	160
	LDL	50-190	mg/dL	91
	HDL	30-70	mg/dL	56
Pro-BNP		0-125	pg/mL	253
Alpha-Galactosidase		0.074-0.457	U/L	0.263

Reference Values from:

[1] Gomella, L G, Haist, S A. *Clinician's Pocket Reference, 11th edition.* Garden Grove, California: McGraw-Hill; 2007.

[2]GBMC Health Care Laboratory Test Directory: http://www.specialtylabs.com/clients/gbmc/default.asp

Transvascular Endomyocardial Biopsy:

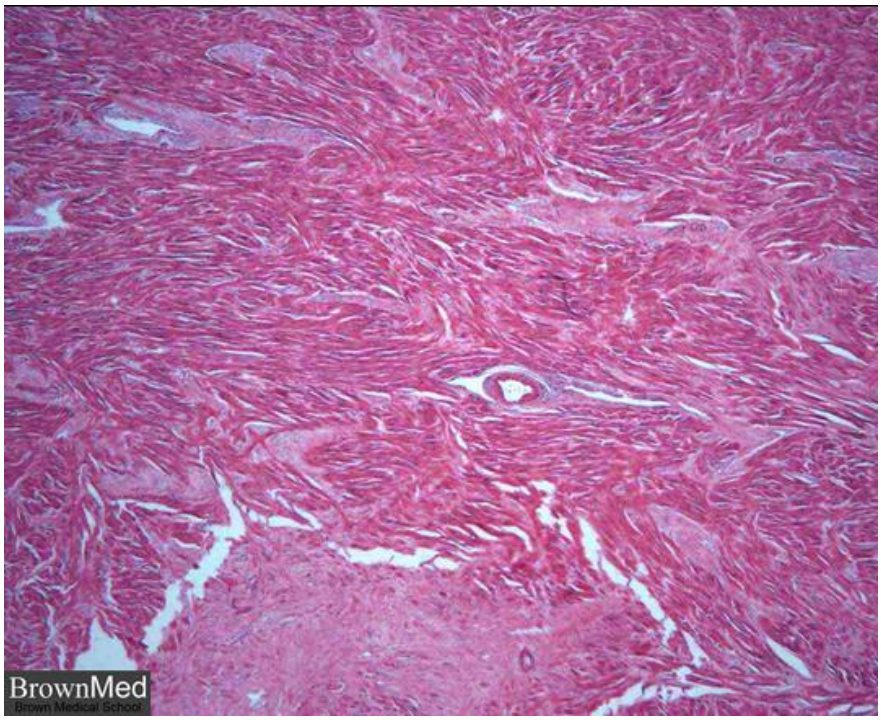

Figure 10. Histology of patient's myocardium. Biopsy of tissue showing myocardial disarray in the septum of the patient. Interstitial fibrosis may also be seen in the image.

Genetic Screening

Genetic screening for mutations in the sacromere tested positive for mutations in the MYBPC3 gene and JPH-2. A missense mutation, S101R, was detected in JPH2 coding region.

Assessment and Differential

Paco's illness was presented with a pre-syncopic episode, dyspnea and stable angina upon exertion; these symptoms have been progressing for a period of 4 months in conjunction with Paco's participation in sports. A history of metabolic disorders is present in the paternal side, including the death of Paco's uncle through cardiovascular

complications involving coronary artery disease; the maternal family history is unknown although the mother. Upon physical exam, Paco exhibited a systolic murmur at the lower left sternal border, graded at 2/6; murmur sounds were intensified during when the Valsava Maneuver was applied. Labs showed slightly elevated B-type natriuretic peptide; electrocardiography featured prominent 'dagger-like' Q-waves in lateral and inferior leads. 2-D echocardiography revealed hypertrophy of septal tissue and left ventricular wall at ~12mm. Transvascular endomyocardial biopsy showed disarray of the myocardial cells in the septum as well as fibrosis of the cardiac interstitium.

Based on the presentation of symptoms, shortness of breath, chest pain, propensity to syncope, the imaging of hypertrophied septal/ventricular cardiac tissue, and electrocardiographic abnormalities, the differential diagnoses may include: Hypertrophic Obstructive Cardiomyopathy, Athlete's heart, Apical Hypertrophic Cardiomyopathy, Aortic Stenosis, Fabry's Disease. Based on 2-D echocardiogram imaging and electrocardiogram readings strongly suggest Hypertrophic Obstructive Cardiomyopathy as the source of the patient's symptoms. Presentation of wall thickness at ~12mm puts the patient within the 'grey zone' diagnostic area between Hypertrophic Cardiomyopathy and Athlete's heart, [normally at 13-15mm, age must be taken into account]. In addition to echo, and EKG imaging, biopsy of myocardial tissue also showed disarray in the cells of the septum; although it is often found in patients with HCM, it is not pathognomic of the disease. The presence of pathological Q-waves (dagger-like) signifies the depolarization of an asymmetric septum; EKG readings in athlete's heart are often characterized by non-pathological readings like sinus bradycaradia and an enlarged QRS voltage. The location of the hypertrophied tissue at the septum and the posterior left

ventricular wall ruled out a non-obstructive, apical variant of myocardial hypertrophy. The lack of inverted T-waves in the precordial leads, sufficient ruled out Apical Hypertrophic Cardiomyopathy. Aortic stenosis from congenital deformations of the aortic valve was also ruled out; murmur sounds after Valsava maneuver, normally decreased/unchanged in valvular obstructions, intensified correlating with hypertrophied tissue. Fabry's disease was out due to its X-linked recessive inheritance; since the mother is asymptomatic of Fabry's disease, it is unlikely the patient is afflicted with this genetic disorder. Fabry disease also results in lack of alpha galactosidase enzymatic activity as well as presence of renal failure; both of these facets were negative in the blood test.

Treatment

Due to the uncurable nature of Hypertrophic Cardiomyopathy and the increased risk of sudden cardiac death, Paco's treatment requires a multi-faceted approach. The patient's young age requires risk management into the future to prevent the progression of the disease. Paco is strongly advised to withdraw from sport programs to attenuate the exacerbation of symptoms. Antiarrythmics and beta blockers are employed to treat symptoms of dyspnea and assists with ventricular relaxation. Referral to a cardiologist is necessary to monitor Paco's progress and determine further course of action. The patient's mother and father are prompted to be evaluated by a geneticist for key genotypes that may cause HCM, due to its asymptomatic nature and dominant phenotypic inheritance pattern.

Rx:

1. Verapamil

80 mg PO q6- 8Hrs

2. Metoprolol tartrate

25 mg PO bid

3. Disopyramide

260 mg PO per day; 100mg prepared syrup bid

Cardiologist Considerations:

1. Progression of symptoms should consider the implantable cardioverter device
2. Modification to prescription regimen if needed

Genetic Screenings:

1. Genotypic screening for mutation in: *MYH7, MYBPC3, JPH-2, TNNT2*
2. First degree relative screening:
    - Screening of father due to cardiac complications in the family
    - Screen of mother due to loss of pedigree; genetic disorders in family are unknown

References:

Cirino AL, Ho C. Familial Hypertrophic Cardiomyopathy Overview. 2008 Aug 5 [Updated 2011 May 17]. In: Pagon RA, Bird TD, Dolan CR, et al., editors. GeneReviews™ [Internet]. Seattle (WA): University of Washington, Seattle; 1993-. URL: http://www.ncbi.nlm.nih.gov/books/NBK1768/

Bonow, Robert O. "Bonow: Braunwald's Heart Disease - A Textbook of Cardiovascular Medicine, 9th ed.". 9th ed. Philadelphia, PA: Elsevier, 2012. 1582-94. Print.

Bope, Edward T. "Bope & Kellerman: Conn's Current Therapy 2013, 1st ed.". 1st ed. Phildeplphia, PA: Elsevier, 2013. 447-449. Print.

Sandy N Shah. "Hypertrophic Cardiomyopathy Overview" Medscape Reference Apr 11, 2012. World Wide Web URL: http://emedicine.medscape.com/article/152913-overview

Elliott *et al*. Sudden death in hypertrophic cardiomyopathy: identification of high risk patients. J Am Coll Cardiol. 2000; 36:2212–8.

Online Mendelian Inheritance in Man, OMIM®. Johns Hopkins University, Baltimore, MD. MIM Number: { 160760}: {02/03/2012}: . World Wide Web URL:http://omim.org/

Online Mendelian Inheritance in Man, OMIM®. Johns Hopkins University, Baltimore, MD. MIM Number: { 600958}: {02/03/2012}: . World Wide Web URL:http://omim.org/

D. Quiat *et al.*, Concerted regulation of myofiber-specific gene expression and muscle performance by the transcriptional repressor Sox6. *Proc Natl Acad Sci U S A* **108**, 10196 (Jun, 2011).

Kelly DP, Strauss AW. "Inherited cardiomyopathies. New England Journal of Medicine .1994; 330 (13):913–919

A. P. Landstrom *et al.*, Mutations in JPH2-encoded junctophilin-2 associated with hypertrophic cardiomyopathy in humans. *J Mol Cell Cardiol* 42, 1026 (Jun, 2007).

M. Revera *et al.*, Troponin T and beta-myosin mutations have distinct cardiac functional effects in hypertrophic cardiomyopathy patients without hypertrophy. *Cardiovasc Res***77**, 687 (Mar, 2008).

L. M. Blayney, F. A. Lai, Ryanodine receptor-mediated arrhythmias and sudden cardiac death. *Pharmacol Ther* 123, 151 (Aug, 2009).

J. Fan, Z. Yu, A univariate model of calcium release in the dyadic cleft of cardiac myocytes. *Conf Proc IEEE Eng Med Biol Soc* 2009, 4499 (2009).

F. R. Heinzel, N. MacQuaide, L. Biesmans, K. Sipido, Dyssynchrony of Ca2+ release from the sarcoplasmic reticulum as subcellular mechanism of cardiac contractile dysfunction. *J Mol Cell Cardiol* 50, 390 (Mar, 2011).

A. P. Landstrom *et al.*, Junctophilin-2 expression silencing causes cardiocyte hypertrophy and abnormal intracellular calcium-handling. *Circ Heart Fail* 4, 214 (Mar, 2011).

A. P. Landstrom *et al.*, Mutations in JPH2-encoded junctophilin-2 associated with hypertrophic cardiomyopathy in humans. *J Mol Cell Cardiol* 42, 1026 (Jun, 2007).

Online Mendelian Inheritance in Man, OMIM®. Johns Hopkins University, Baltimore, MD. MIM Number: { 605267}: {02/03/2012}: . World Wide Web URL:http://omim.org/

Online Mendelian Inheritance in Man, OMIM®. Johns Hopkins University, Baltimore, MD. MIM Number: {613873}: {02/03/2012}: . World Wide Web URL:http://omim.org/

C. O'Mahony, P. Elliott, W. McKenna, Sudden Cardiac Death in Hypertrophic Cardiomyopathy. *Circ Arrhythm Electrophysiol*, (Sep, 2012).

Kumar, V *et al*. Kumar: Robbins Basic Pathology, 9th ed. 9th ed. Phildelphia, PA. Elsevier: Saunders, 2013 Print