

Hypertrophic Cardiomyopathy: How do Mutations Lead to Disease?

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Abstract

Hypertrophic cardiomyopathy (HCM) is the most common monogenic genetic cardiac disease, with an estimated prevalence of 1:500 in the general population. Clinically, HCM is characterized by hypertrophy of the left ventricle (LV) walls, especially the septum, usually asymmetric, in the absence of any cardiac or systemic disease that leads to a secondary hypertrophy. The clinical course of the disease has a large inter- and intrafamilial heterogeneity, ranging from mild symptoms of heart failure late in life to the onset of sudden cardiac death at a young age and is caused by a mutation in one of the genes that encode a protein from the sarcomere, Z-disc or intracellular calcium modulators. Although many genes and mutations are already known to cause HCM, the molecular pathways that lead to the phenotype are still unclear. This review focus on the molecular mechanisms of HCM, the pathways from mutation to clinical phenotype and how the disease's genotype correlates with phenotype.

Introduction

Hypertrophic cardiomyopathy (HCM) is the most common monogenic genetic cardiac disease with an estimated prevalence of 1:500¹. HCM is characterized by hypertrophy of the left ventricle (LV), especially the septum, usually asymmetric, in the absence of any cardiac or systemic disease that leads to a secondary hypertrophy. The clinical course has a large inter and intrafamilial heterogeneity, ranging from mild symptoms of heart failure late in life to the onset of sudden cardiac death at a young age. Besides cardiac hypertrophy, there is marked histological disarray of myofilaments and fibrosis of varying degrees². The disease is an important cause of disability and death in patients of all ages, although sudden and unexpected death in young individuals is perhaps the most devastating component of its natural history. This review will focus on the molecular mechanisms of HCM, the pathways from mutation to clinical phenotype and how the disease's genotype correlates with phenotype. Moreover, we will show recent data on the genetic profile of HCM patients on in the Brazilian population^{1,3}.

Keywords

Cardiomyopathy, Hypertrophic; Phenotype; Genotype; Genes; Sarcomers.

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Genetics of HCM

The disease is caused by a mutation in one of the genes that encode a protein from the sarcomere, Z-disc or intracellular calcium modulators (Table 1). For sarcomeric genes, identified mutations are in their majority single nucleotide substitutions and for most genes the mutant protein is thought to incorporate into the sarcomere exerting a poisoned peptide effect (negative dominant mutations). So far, the only exception is the cardiac binding protein C (*MYBPC3*) gene, in which many mutations are deletions or insertions that lead to a frameshift and most of the mutations lead to a truncated protein with no function, thus the disease appears to be caused by a haploinsufficiency mechanism in this case⁴. For other related genes (Z-disk and calcium modulators), the specific mechanism is yet to be elucidated. Most of the mutations are single substitutions, but it is not known whether the disease is caused by negative dominance or haploinsufficiency.

Genotype x phenotype association

The understanding of the molecular ways from mutations that lead to the disease opens the prospect for the identification of both molecular and clinical genotype-phenotype associations. This could potentially lead not only to better predictive disease models, but also to target therapeutics. In fact, several authors have described suggestive genotype-phenotype associations in several populations.

Presence or absence of an identified mutation

Mutation detection in one of the sarcomeric genes is associated with increased severity of the disease. One study revealed that the ratios of intraventricular septum thickness to posterior wall thickness were significantly higher in the patients with *MYBPC3*, *MYH7*, or *TPM1* mutations than in patients without sarcomeric gene mutations⁵. On the other hand, another group observed that patients with apical hypertrophy and a positive genotype were more likely to have a positive family history for HCM than patients with a negative genotype. In all other aspects, there were no differences between the positive versus negative genotype patients⁶. Another group described that the morphology of hypertrophy has an impact on the positivity of genetic testing. They compared the sigmoid, reverse curve, apical, and neutral subtypes of hypertrophy and found out that in the reverse curve subtype, a mutation in one of the myofilament genes can be identified in almost 80% of the patients, while in the sigmoid type, less than 10% of the patients harbor a sarcomere mutation. They suggest that these data can be used to help in the decision to proceed to genetic testing⁷. In the Brazilian population, the authors found a correlation

Table 1 - Genes implicated in hypertrophic cardiomyopathy and their frequency in HCM patients

Gene	Protein	Chromosome	Frequency
Myofilament genes			
<i>TTN</i>	Titin	2	<1%
<i>MYH7</i>	β -myosin heavy chain	14	15-25%
<i>MYH6</i>	α -myosin heavy chain	14	<1%
<i>MYL2</i>	Myosin regulatory light chain	12	<2%
<i>MYL3</i>	Myosin essential light chain	3	<1%
<i>MYBPC3</i>	Myosin binding protein C	11	15-25%
<i>TNNT2</i>	Troponin T	1	<5%
<i>TNNI3</i>	Troponin I	19	<5%
<i>TPM1</i>	α -tropomyosin	15	<5%
<i>ACTC</i>	Alpha actin cardiac	15	<1%
<i>TNNC1</i>	Troponin C	3	<1%
Z-Disc genes			
<i>LBD3</i>	LIM domain-binding protein 3	10	1-5%
<i>CSRFP3</i>	Muscular LIM protein	17	<1%
<i>TCAP</i>	Telethonin	17	<1%
<i>VCL</i>	Vinculin/Meta-vinculin	10	<1%
<i>ACTN2</i>	α -actin	1	<1%
<i>MYOZ2</i>	Myozenin	4	<1%
<i>NEXN</i>	Nexilin	1	<1%
Calcium handling genes			
<i>JPH2</i>	Junctophilin-2	20	<1%
<i>PLN</i>	Phospholamban	6	<1%

between the presence of an identified mutation with higher frequency of non-sustained ventricular tachycardia, younger age and the patients were younger at diagnosis when compared with patients without an identified mutation in the *MYH7*, *MYBPC3* and *TNNT2* genes⁸.

Mutated gene

There was an initial consensus that mutations in *MYH7* had variable clinical features with some malignant alterations^{1,9}, mutations in the *MYBPC3* gene were associated with a more benign prognosis^{10,11} and mutations in the *TNNT2* gene^{12,13} were associated with higher rates of sudden death with little or no hypertrophy. For *MYH7*, there is still a great variability between patients, ranging from asymptomatic to very severe phenotypes.

However, for the other two genes firstly described and later for the other genes related to HCM, it is not possible yet to make an assumption based on the mutated gene. A study performed by Wang et al¹⁴ compared patients with mutations in the *MYH7* and *MYBPC3* genes and concluded that patients with mutations in the *MYH7* gene develop the disease at a younger age, have a higher rate of familiar sudden death, higher proportion of atrial fibrillation and needed more surgical

intervention than the second group. Another group performed an analysis in 389 unrelated patients with and without *MYH7* mutations. They observed that those with mutations in the *MYH7* gene were also younger at time of diagnosis, had more hypertrophy and had more frequently undergone myectomy, but they found no difference in family history of sudden death between groups¹⁵.

Yet, another group found a higher mean IVS diameter in carriers of a *MYBPC3* mutation compared to *MYH7*-positive patients¹⁶. A recent study with Brazilian HCM patients has shown that mutations in the *MYH7* gene are more often associated with higher left atrium size and higher frequency of atrial fibrillation when compared with mutations in the *MYBPC3* gene, suggesting a worse prognosis in these patients⁸.

Type of mutations

The association of specific mutations with the clinical phenotype of HCM is still very controversial. Since the discovery of the causative genes for the disease, many authors tried to label the mutations as "malignant" or "benign", according to the patient's phenotype. However, for most of the associations, there is an exception or subsequently, researchers

fail to replicate the data in another group of patients. In fact, this topic was the subject of a debate between the authors Ho¹⁷ and Ackerman¹⁸, and they both concluded that it is still hard to make prognosis based on a single mutation.

There is some agreement among specialists regarding the severity of some mutations, although the clinical presentation may vary. For example, the R723G in the *MYH7* gene mutation was first described in 2000 by Enjuto et al¹⁹, with a high rate of sudden death and progressive heart failure, both around the 50th decade of life. In 2006, a Chinese group detected a family with the same mutation and among them, only 1 man had syncope history; other 5 died of severe heart failure and the mean age at death was 66 years. No sudden death was described¹⁴. This suggests that the phenotype becomes more malignant with age, being benign in younger patients, but the phenotype presentation was not the same. On the other hand, for other mutations there is no clear picture of even how severe the phenotype can be. The Val606Met mutation in the *MYH7* gene was first described as a benign mutation, with patients harboring this alteration having near-normal survival²⁰. Later on, several researchers described the same mutation as being malignant, with a higher risk of sudden death²¹⁻²³.

We have to agree with Ho¹⁷ and Landstrom e Ackerman¹⁸ that it is not possible yet to determine prognosis based on the mutation. We believe that there are so many different mutations that can lead to the HCM in so many different genes that it is hard to find a high number of patients with the same mutation to compare and establish the phenotype end-point with some precision. Even if we did, there have been several descriptions in the literature about the effect of modifier genes' modulation on the phenotype; thus, even in patients with the same mutation, the phenotype can, and most probably will, differ.

Number of mutations

Some patients harbor more than one pathogenic mutation and the literature so far estimates that it occurs in 3-5% of the patients²⁴⁻²⁶. According to Kelly and Semsarian²⁷, the clinical features in patients with more than one mutation are more severe, with greater risk of sudden death and LV hypertrophy. In their review, they compared the published data from patients with homozygous^{28,29}, compound heterozygous^{30,31} and double heterozygous mutations^{25,32} and in most of the cases, the phenotype is more severe with the age at diagnosis, including pediatric patients. Another recently published study described a clinical profile of triple sarcomeric mutations³³. They found 4 patients within a 488 unrelated cohort with a triple mutation (0.8%) and concluded that this scenario is rare, but it causes an increased risk of end-stage progression and ventricular arrhythmias. However, more studies are still necessary to completely understand how the different mutation combinations act to create the final phenotype, as the possible combinations are countless.

Modifier Genes

Most probably there are modifier genes that can modulate the phenotype, making genotype-based prognosis even

harder. For example, some variants of the angiotensin II type 2 receptor gene were significantly associated with hypertrophy degree in patients with HCM, regardless of their blood pressure levels³⁴. The angiotensin-converting enzyme has also been implicated as phenotype modulator. Researchers correlated a gene polymorphism with QT dispersion, which reflects the regional differences in centrocular repolarization and is used as a marker of collagen content (in patients with the DD genotype, QT dispersion is increased)³⁵.

Another group studied in HCM patients, the presence of resistin, a novel cytokine associated with inflammation suspected to induce hypertrophy in rats. They analyzed both resistin levels and a -410C>G polymorphism in the gene promoter and found that both resistin levels and the polymorphism may be associated with cardiac hypertrophy in the studied group³⁶.

Other studies evaluated a calmodulin (*CALM3*) gene polymorphism and observed that -34T>A *CALM3* polymorphism is a modifier gene for HCM, probably affecting the expression of *CALM3* due to its key role in Ca²⁺ homeostasis, acting as an intracellular sensor for Ca²⁺ ions³⁷. Also, the role of sex hormone receptor genes in left ventricular hypertrophy in HCM male patients was also investigated. It is described that a lower number of (CAG)_n repeats in the androgen receptor were associated with higher left ventricular wall thickness (LVWT) in males. Males carrying the A allele at rs6915267 SNP from the promoter region of the estrogen receptor 1 gene had an 11% decrease in mean LVWT when compared with homozygous GG males³⁸. Since there are so many different polymorphisms and genes involved with the modulation effect of HCM, this can at least in part explain the great phenotypic variability seen in the disease.

Expression unbalance

Nearly all patients with HCM are heterozygous for mutations in the affected gene, thus it is assumed that half of the proteins are defective. However, Trpathi et al³⁹ showed in their study that there is an imbalance between wild-type and mutated myofilaments in patients with mutations in the *MYH7* and *MYBPC3* genes. It has also been reported that the fraction of mutated protein correlates with disease severity and it is usually dependent of the specific mutation. The authors demonstrated in their study that the expression of mutated *MYH7*-mRNA was similar among patients of different generations and also from unrelated patients and that this difference depends on the mutation. For instance, the V606M mutation in the *MYH7* gene leads to skipping of exon 16, so it is likely to have a nonsense-mediated decay. On the other hand, the mechanisms underlying regulation of those mutations are not fully understood, but it is known that some mutations influence splicing efficiency, so it is speculated that some of them can have a more efficient splicing than the wild-type transcript, leading to a greater rate of mutated proteins in the tissue. They also report in their study that the abundance of mutated protein is related to a severe long-term disease progression and conspicuous effects on the contractile apparatus function. This seems to be an interesting discovery, but it needs to be replicated in other studies.

Animal models

The creation of suitable animal models of HCM offers considerable insight into the disease progression over the animal's lifetime. With this purpose, several animal models of the disease were created over time. In 1996, two distinct groups created mice models of HCM with a *MYH7* mutation that helped studies of the natural history of the disease^{40,41}.

In 1999, a different group created another lineage of mice with HCM with an alpha-cardiac myosin heavy chain mutation. They discovered that heterozygous mice had the classic features of HCM, but homozygous animals died soon after birth due to fatal dilated cardiomyopathy. The group also noticed that the incorporation of the mutant protein increased rapidly after birth and speculated that this variable incorporation may be responsible for focal myocyte death in HCM⁴².

More recently, some animal models have been used for pharmacological assays. For instance, researchers showed that sarcomeric protein mutations activate signaling pathways that promote the transcription of proliferative and proinflammatory signals in non-myocyte cells, mainly via Tgf- β , in a mouse model. They administered Tgf- β neutralizing antibodies and observed that non-myocyte proliferation and fibrosis were abolished. Also, the chronic administration of losartan, an angiotensin II type 1 receptor antagonist, prevented the emergence of hypertrophy, non-myocyte proliferation and fibrosis in mutation-positive mice. In animals with established hypertrophy, the drug did not reverse the phenotype, but decreased the non-myocyte proliferation⁴³. The study was successful in establishing that Tgf- β is a pivotal mechanism for fibrosis development and opened doors to the treatment of mutation carriers.

The murine heart is constituted mainly of α -myosin, while the human heart consists mainly of β -myosin. The rabbit could be a better model because the myosin composition is similar to that of the human heart. Thus, a transgenic rabbit model with the *MYH7* R400Q mutation was successfully created⁴⁴. Also, a naturally occurring HCM mutation in Maine coon cats mimics the hereditary aspects, natural history, and pathological characteristics of human HCM. This animal model has been a valuable tool for studying the gross, cellular, and molecular pathophysiology of the disease⁴⁵.

A transgenic mouse model was created with varying amounts of a mutated *MYBPC3* gene lacking the myosin and titin binding domains. They found out that the peptide was stable, but did not effectively incorporate into the sarcomere. The expression of transgenic protein leads to a decrease in the levels of endogenous protein⁴⁶. The same group produced another transgenic model with a *MYBPC3* gene lacking only the myosin binding site and little protein was found in the mice's hearts⁴⁷. The authors believe that these experiments can help understand the genetic mechanisms of disease. According to them, both the null allele and poison peptide hypothesis are relevant to the phenotype and their relative importance may vary considerably depending upon the particular *MYBPC3* mutation.

Another very important animal model for HCM is the zebrafish. The fish model is a powerful tool to study several diseases and is particularly important in heart development,

due to some characteristics such as the external development of the heart, allowing non-invasive analysis of the heart during its formation. Also, initially, zebrafish embryos do not rely on the cardiovascular system for oxygen, so the mutants can survive and continue development for several days. Finally, although the zebrafish heart is simpler in structure, the essential genes responsible for heart development are conserved^{48,49}. One group developed a *TNNT2* human mutation model in zebrafish to assess its impact during heart development. They found that the mutation led to the same phenotype observed in mutated humans: sarcomere disarray and massive induction of myocardial hypertrophic pathways. Also, they observed that the embryonic hearts, in opposition to the adult heart, developed cardiomyocyte hyperplasia. They concluded that sarcomeric mutations can have an impact on cardiomyocyte biology much earlier than previously thought and with distinct effects from the adult heart, even though they have the same transcriptional responses⁵⁰.

Molecular mechanisms of HCM

Although many genes and mutations are already known to cause HCM, which was extensively proven through animal models, the molecular pathways that lead to the phenotype are still unclear. The first proposed hypothesis was that incorporation of a mutated protein would lead to a depressed contractile function and this could trigger a compensatory hypertrophy^{51,52}. However, according to some authors, this proposal was proven inconsistent with laboratory and clinical evidence.

In their review, Asharafian et al⁵³ listed three arguments against this hypothesis: 1) Initially, the experiments with mutant proteins revealed a decreased function, especially characterized by a reduction in protein motility. However, with the development of more advanced assays, it was realized that in some mutations there is actually an increase in motility, suggesting a gain of function of the mutated protein. Thus, decreased function cannot be the only stimulus for hypertrophy. Corroborating these data, mutation carriers who did not develop hypertrophy have an increased motility in the cardiac tissue that can be visualized in the echocardiogram; 2) the hypertrophy present in patients with HCM is asymmetric, very different from the concentric hypertrophy that is seen in hearts with increased load, such as in hypertension. 3) HCM patients usually develop hypertrophy after puberty and it does not appear to progress much afterwards. Also, some patients only develop hypertrophy in adulthood. Thus, these patterns cannot be explained by a compensatory mechanism, considering that the mutation is present since heart development.

Despite all convincing arguments that there is no clinical dysfunction preceding hypertrophy development, these do not address the main question, whether there is sarcomere dysfunction due to structural problems or, alternatively, if there are other epistatic mechanisms responsible for hypertrophy development. In this scenario, decreased or increased motility are not well-established surrogates for sarcomere function, and even if they are, any of these two could be the result of an structural disassemble of the molecular contraction

apparatus; the asymmetry of hypertrophy is probably related to the interaction between physical and molecular forces within the organ (see below); and it is not clear whether there is no progression in hypertrophy during the life span of HCM patients (that is certainly not the case for other associated phenotypes, such as fibrosis).

Many studies focus on Ca^{2+} sensitivity, which is altered in the hypertrophic heart, as a mechanism leading to HCM phenotypes. There is increasing evidence that mutations in different genes of myofilament proteins increase Ca^{2+} sensitivity and the consequent defect on the homeostasis, such as intracellular Ca^{2+} handling, sarcoplasmic reticulum Ca^{2+} reuptake and phosphorylation of some proteins, likely contribute to several of the aspects of the disease^{54,55}. Some studies have shown that greater myofibrillar sensitivity to Ca^{2+} is enough to increase the probability of arrhythmias. In mice, it was demonstrated that with greater Ca^{2+} sensitization, the shape of the ventricular action potentials was changed, resulting in shorter effective refractory periods, greater beat-to-beat variability of action potential duration and increased dispersion of ventricular conduction velocities at high heart rates. It is believed that this is sufficient to create an arrhythmogenic substrate⁵⁶.

This association was reinforced by the study of transgenic mice with a troponin T mutation, in which the burden of ventricular arrhythmias had a correlation with the degree of Ca^{2+} sensitivity. Finally, the myofilament Ca^{2+} sensitizer EMD 57033 exacerbates arrhythmias in this model, while blebbistatin, which reverses Ca^{2+} sensitization, almost entirely eliminated the arrhythmias⁵⁶. It is not clear, however, if these observed alterations are causative of hypertrophy or, alternatively, an epiphenomenon of the entire process. They could modulate the clinical phenotype and explain a great deal of interindividual variability, but cannot be considered sufficient conditions for the development of myocyte hypertrophy and myofibrillar disarray.

In conclusion changes in calcium homeostasis are associated with arrhythmic phenotypes in human and animal models of HCM. Nonetheless, the mechanism that leads to hypertrophy, probably the process initiator, remains unclear.

In 2002 Ashrafian et al⁵³ stated that the unifying dysfunction in HCM was the increased energy demand due to an inefficient sarcomeric ATP utilization and, based on that, created another hypothesis called “the energy depletion hypothesis” proposing that increased demand compromises the capacity of the cardiomyocyte to maintain energy levels in subcellular compartments.

In 2008, Belus et al⁵⁷ studied the contraction and relaxation mechanics of single myofibrils in patients carrying the R403Q mutation in the *MYH7* gene and compared with normal healthy hearts, finding out that the tension generation and relaxation following Ca^{2+} increase and decrease were much faster in the mutated myofibril, but at a higher energy cost, corroborating the idea of inefficient ATP utilization for tension generation. This hypothesis can also provide an explanation for the asymmetric hypertrophy, because if the myocardial wall tension and the energy demand are not uniform throughout the entire organ, extra energy requirement can be more damaging in specific myocardial regions.

More recently, some authors described the so-called 4th stage model that can help understand how the mutation can act leading to disease, not only in HCM but also in dilated cardiomyopathy and some skeletal muscle diseases^{58,59}. This hypothesis refers to a fourth stage in addition on the well-known 3-stage regulation model. In his review, Lehrer⁵⁸ explains that understanding how Ca^{2+} and myosin are involved in muscle contraction is largely phenomenological and the molecular basis is not yet elucidated. In the regular 3-stage model⁶¹, tropomyosin bound to actin equilibrates between 3 stages, the relative occupancy, which are affected by the Ca^{2+} concentration and strong binding myosin heads. The described stages are the “blocked stage”, in which the thin filament is unable to bind myosin, the “closed state” in which the thin filament can only bind to relatively weak myosin and the “open stage”, in which myosin can both bind and undergo isomerization to a more strongly bound, rigor-like conformation. Nonetheless, according to Lehrer, there are several unexplained phenomena in muscle regulation (e.g. increased Ca^{2+} binding caused by strong-binding myosin heads and residual active force at low Ca^{2+} in the case of HCM) that could involve an additional active stage in the absence of Ca^{2+} . This new proposal consider that there are two open stages, one in the presence of Ca^{2+} and another myosin-induced open stage in the absence of Ca^{2+} (Figure 1). This additional state could help understand how a mutation in any of the sarcomeric proteins could cause changes in Ca^{2+} sensitivity in HCM and DC.

The authors believe that myocyte dysfunction results in hypertrophy. However, once again, the mechanism is not clear. The main question regarding HCM is still exactly how a mutation can lead to the diverse clinical and anatomical phenotypes, especially hypertrophy. We believe that energy dysfunction and altered calcium sensitivity can be as well the consequence of sarcomere dysfunction, rather than the primary cause of hypertrophy.

Global expression analysis

Understanding the molecular basis of disease development goes beyond the identification of different causative mutations. This identification should lead to the characterization and understanding of true landscape changes in several gene-networks. Recent works are focusing on the whole transcriptome, searching for expression differences between healthy and affected individuals. This way, it is possible to understand how a disease affects the whole signaling cascade. In relation to HCM, one study compared global gene expression that identified 29 transcription factors distinctly expressed between wild-type and pre-hypertrophic hearts of mice⁶¹.

Another study was performed using a non-HCM hypertrophy transgenic mouse model through *Gαq* protein silencing to understand the signaling pathways activation in cardiac hypertrophy. It was found that *Gαq* mediates a hypertrophic response in cardiac myocytes, leading to heart failure⁶², so the authors used this model to compare the whole transcriptome of male non-transgenic and transgenic mouse cardiac tissue using next-generation sequencing systems

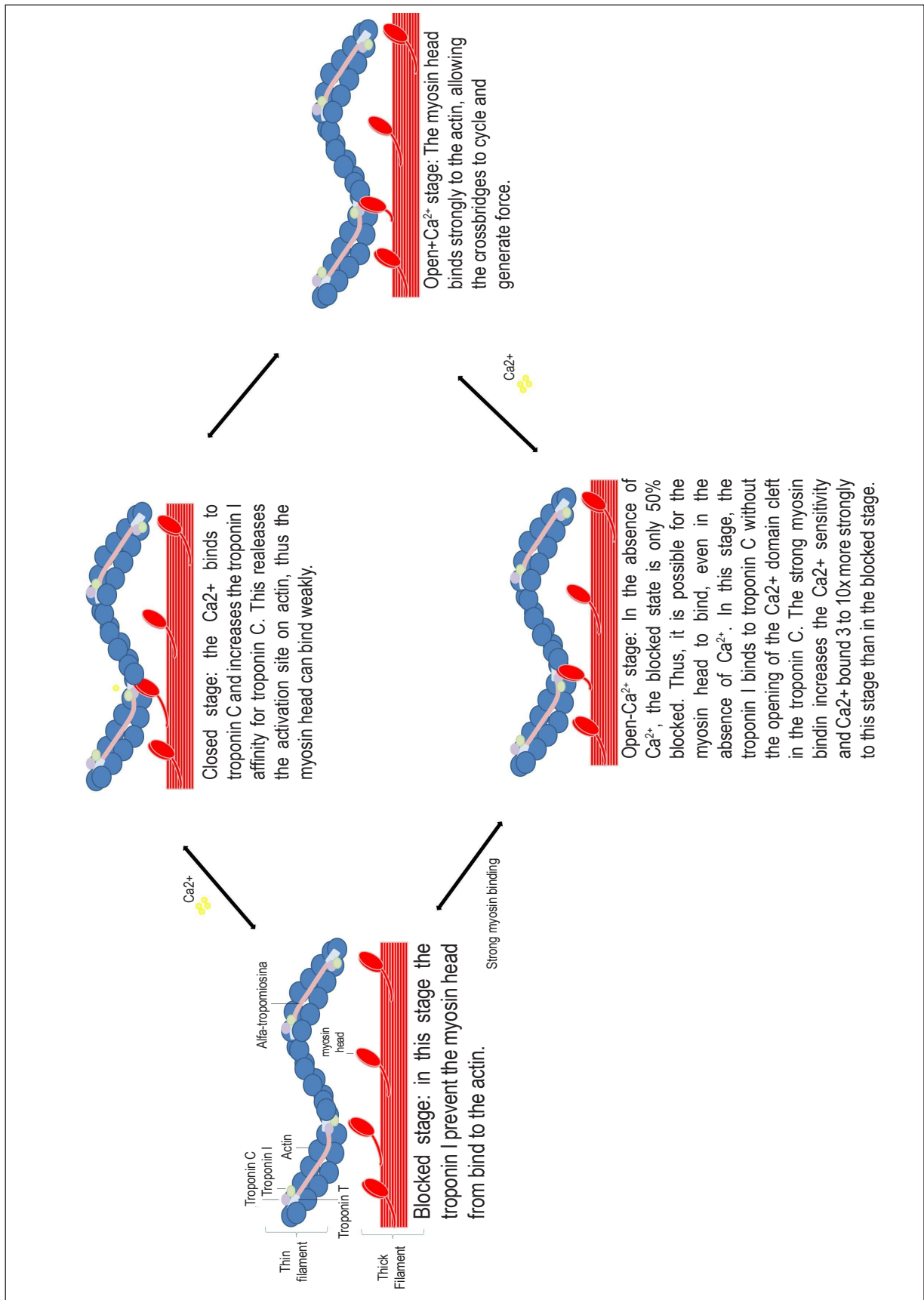


Figure 1 - schematic figure from the 4th stage model.

against the same analysis using expression arrays. The *Gαq* transgenic mouse exhibited cardiac hypertrophy, ventricular enlargement, diminished ejection performance, and increased expression of fetal cardiac genes when compared to the non-transgenic mouse. Also, they concluded that this new technology is better than traditional array-based experiments.

Cost effectiveness of a cascade screening program for HCM

Despite the doubts regarding the pathological mechanism of the disease, it is clear that the genetic diagnosis has its space in clinical practice, especially in cascade screening. The cascade screening is defined as an approach to the family of a patient affected by a genetic disease and a predictive DNA test offer to those at potential risk, based on information about a confirmed pathogenic genetic mutation⁶³. This approach has been used successfully in several genetic diseases such as familial hypercholesterolemia⁶⁴, cystic fibrosis⁶⁵ and long QT syndrome⁶³. One study analyzing specifically the cost-effectiveness of HCM genetic screening showed that this approach is cost effective⁶⁶, as relatives with a negative genetic result would be dismissed from further clinical evaluations and at-risk individuals could be diagnosed early, avoiding expensive and unnecessary clinical screening. The genetic diagnosis of relatives at risk of developing the disease is particularly important, because HCM can be asymptomatic and have sudden death as its first manifestation⁶⁷. Cascade screening has been used in HCM with positive results⁶⁷ and is recommended by the European Cardiology Society⁶⁸. Relatives with a positive DNA result must be referred to a cardiologist and counseled to avoid risk factors, discouraged to pursue athletic careers and encouraged to acquire healthy life habits.

The downside of this approach is the potential psychological impact on both the asymptomatic affected individuals, especially the young ones, and the non-affected relatives. In the first case, the discovery can generate anxiety and depression and in the second case, relatives can undergo survivor's guilt. Also, the patients with an unclear clinical result that receive a negative genetic result can experience frustration towards the result's uncertainty, as the disease cannot be confirmed or excluded in this scenario⁶³. Genetic counseling

is fundamental and must be careful and as informative as possible and the patient must have a doctor's appointment available.

Conclusion

The constant development of new technologies may help elucidate the mechanisms of this complex and intriguing disease. The technologies of next generation sequencing will provide great amounts of data and soon we will be able to sequence the patient's entire genome, which could help us understand how the mutation interacts with modifier genes to create the phenotype. This technology can be also applied to RNA sequencing and we will be able to obtain the patient's entire transcriptome profile, helping us visualize the impact of the mutation on the signaling cascade.

Comparative expression of networks of genes associated with cardiomyopathies can give us an insight of how those genes interact with each other. For now we can say that there still a lot of work to do until we have some definitive answers about HCM, but genetic screening of patients brings several benefits to the patients and the physicians and should be used in medical practice.

Author contributions

Conception and design of the research and Critical revision of the manuscript for intellectual content: Pereira AC; Acquisition of data, Analysis and interpretation of the data and Writing of the manuscript: Marsiglia JDC.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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References

1. Marian AJ, Mares AJ Jr, Kelly DP, Yu QT, Abchee AB, Hill R, et al. Sudden cardiac death in hypertrophic cardiomyopathy: variability in phenotypic expression of beta-myosin heavy chain mutations. *Eur Heart J*. 1995;16(3):368-76.
2. Maron BJ. Hypertrophic cardiomyopathy: a systematic review. *JAMA*. 2002;287(10):1308-20.
3. Marsiglia JD, Batitucci Mdo C, Paula F, Barbirato C, Arteaga E, Araujo AQ. [Study of mutations causing hypertrophic cardiomyopathy in a group of patients from Espirito Santo, Brazil]. *Arq Bras Cardiol*. 2010;94(1):10-7.
4. Marston S, Copeland O, Jacques A, Livesey K, Tsang V, McKenna WJ, et al. Evidence from human myectomy samples that MYBPC3 mutations cause hypertrophic cardiomyopathy through haploinsufficiency. *Circ Res*. 2009;105(3):219-22.
5. Otsuka H, Arimura T, Abe T, Kawai H, Aizawa Y, Kubo T, et al. Prevalence and distribution of sarcomeric gene mutations in Japanese patients with familial hypertrophic cardiomyopathy. *Circ J*. 2012;76(2):453-61.
6. Gruner C, Care M, Siminovitch K, Moravsky G, Wigle ED, Woo A, et al. Sarcomere protein gene mutations in patients with apical hypertrophic cardiomyopathy. *Circ Cardiovasc Genet*. 2011;4(3):288-95.
7. Binder J, Ommen SR, Gersh BJ, Van Driest SL, Tajik AJ, Nishimura RA, et al. Echocardiography-guided genetic testing in hypertrophic cardiomyopathy: septal morphological features predict the presence of myofibrillar mutations. *Mayo Clin Proc*. 2006;81(4):459-67.
8. Marsiglia JD, Credidio FL, de Oliveira TG, Reis RF, Antunes M de O, de Araujo AQ, et al. Screening of MYH7, MYBPC3, and TNNT2 genes in Brazilian patients with hypertrophic cardiomyopathy. *Am Heart J*. 2013;166(4):775-82.

9. al-Mahdawi S, Chamberlain S, Chojnowska L, Michalak E, Nihoyannopoulos P, Ryan M, et al. The electrocardiogram is a more sensitive indicator than echocardiography of hypertrophic cardiomyopathy in families with a mutation in the MYH7 gene. *Br Heart J*. 1994;72(2):105-11.
10. Charron P, Dubourg O, Desnos M, Bannaceur M, Carrier L, Camproux AC, et al. Clinical features and prognostic implications of familial hypertrophic cardiomyopathy related to the cardiac myosin-binding protein C gene. *Circulation*. 1998;97(22):2230-6.
11. Niimura H, Patton KK, McKenna WJ, Soultis J, Maron BJ, Seidman JG, et al. Sarcomere protein gene mutations in hypertrophic cardiomyopathy of the elderly. *Circulation*. 2002;105(4):446-51.
12. Watkins H, McKenna WJ, Thierfelder L, Suk HJ, Anan R, O'Donoghue A, et al. Mutations in the genes for cardiac troponin T and alpha-tropomyosin in hypertrophic cardiomyopathy. *N Engl J Med*. 1995;332(16):1058-64.
13. Moolman JC, Corfield VA, Posen B, Ngumbela K, Seidman C, Brink PA, et al. Sudden death due to troponin T mutations. *J Am Coll Cardiol*. 1997;29(3):549-55.
14. Wang S, Zou Y, Fu C, Xu X, Wang J, Song L, et al. Worse prognosis with gene mutations of beta-myosin heavy chain than myosin-binding protein C in Chinese patients with hypertrophic cardiomyopathy. *Clin Cardiol*. 2008;31(3):114-8.
15. Van Driest SL, Jaeger MA, Ommen SR, Will ML, Gersh BJ, Tajik AJ, et al. Comprehensive analysis of the beta-myosin heavy chain gene in 389 unrelated patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2004;44(3):602-10.
16. Waldmuller S, Erdmann J, Binner P, Gelbrich G, Pankuweit S, Geier C, et al; German Competence Network Heart Failure. Novel correlations between the genotype and the phenotype of hypertrophic and dilated cardiomyopathy: results from the German Competence Network Heart Failure. *Eur J Heart Fail*. 2012;13(11):1185-92.
17. Ho CY. Genetics and clinical destiny: improving care in hypertrophic cardiomyopathy. *Circulation*. 2010;122(23):2430-40.
18. Landstrom AP, Ackerman MJ. Mutation type is not clinically useful in predicting prognosis in hypertrophic cardiomyopathy. *Circulation*. 2010;122(23):2441-9.
19. Enjuto M, Francino A, Navarro-Lopez F, Viles D, Pare JC, Ballesta AM. Malignant hypertrophic cardiomyopathy caused by the Arg723Gly mutation in beta-myosin heavy chain gene. *J Mol Cell Cardiol*. 2000;32(12):2307-13.
20. Watkins H, Rosenzweig A, Hwang DS, Levi T, McKenna W, Seidman CE, et al. Characteristics and prognostic implications of myosin missense mutations in familial hypertrophic cardiomyopathy. *N Engl J Med*. 1992;326(17):1108-14.
21. Havndrup O, Bundgaard H, Andersen PS, Larsen LA, Vuust J, Kjeldsen K, et al. The Val606Met mutation in the cardiac beta-myosin heavy chain gene in patients with familial hypertrophic cardiomyopathy is associated with a high risk of sudden death at young age. *Am J Cardiol*. 2001;87(11):1315-7.
22. Semsarian C, Yu B, Ryce C, Lawrence C, Washington H, Trent RJ. Sudden cardiac death in familial hypertrophic cardiomyopathy: are "benign" mutations really benign? *Pathology*. 1997;29(3):305-8.
23. Nakajima-Taniguchi C, Azuma J, Nagata S, Kishimoto T, Yamauchi-Takahara K. A missense mutation in the beta-myosin heavy chain gene in a Japanese patient with hypertrophic cardiomyopathy. *Jpn Circ J*. 1995;59(12):833-7.
24. Van Driest SL, Vasile VC, Ommen SR, Will ML, Tajik AJ, Gersh BJ, et al. Myosin binding protein C mutations and compound heterozygosity in hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2004;44(9):1903-10.
25. Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C, et al. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation*. 2003;107(17):2227-32. Erratum in *Circulation*. 2004;109(25):3258.
26. Ingles J, Doolan A, Chiu C, Seidman J, Seidman C, Semsarian C. Compound and double mutations in patients with hypertrophic cardiomyopathy: implications for genetic testing and counselling. *J Med Genet*. 2005;42(10):e59.
27. Kelly M, Semsarian C. Multiple mutations in genetic cardiovascular disease: a marker of disease severity? *Circ Cardiovasc Genet*. 2009;2(2):182-90.
28. Nishi H, Kimura A, Harada H, Adachi K, Koga Y, Sasazuki T, et al. Possible gene dose effect of a mutant cardiac beta-myosin heavy chain gene on the clinical expression of familial hypertrophic cardiomyopathy. *Biochem Biophys Res Commun*. 1994;200(1):549-56.
29. Nanni L, Pieroni M, Chimenti C, Simonati B, Zimbello R, Maseri A, et al. Hypertrophic cardiomyopathy: two homozygous cases with "typical" hypertrophic cardiomyopathy and three new mutations in cases with progression to dilated cardiomyopathy. *Biochem Biophys Res Commun*. 2003;309(2):391-8.
30. Nishi H, Kimura A, Harada H, Koga Y, Adachi K, Matsuyama K, et al. A myosin missense mutation, not a null allele, causes familial hypertrophic cardiomyopathy. *Circulation*. 1995;91(12):2911-5.
31. Jeschke B, Uhl K, Weist B, Schroder D, Meitinger T, Dohlemann C, et al. A high risk phenotype of hypertrophic cardiomyopathy associated with a compound genotype of two mutated beta-myosin heavy chain genes. *Hum Genet*. 1998;102(3):299-304.
32. Richard P, Isnard R, Carrier L, Dubourg O, Donatien Y, Mathieu B, et al. Double heterozygosity for mutations in the beta-myosin heavy chain and in the cardiac myosin binding protein C genes in a family with hypertrophic cardiomyopathy. *J Med Genet*. 1999;36(7):542-5.
33. Girolami F, Ho CY, Semsarian C, Baldi M, Will ML, Baldini K, et al. Clinical features and outcome of hypertrophic cardiomyopathy associated with triple sarcomere protein gene mutations. *J Am Coll Cardiol*. 2010;55(14):1444-53.
34. Carstens N, van der Merwe L, Revera M, Heradien M, Goosen A, Brink PA, et al. Genetic variation in angiotensin II type 2 receptor gene influences extent of left ventricular hypertrophy in hypertrophic cardiomyopathy independent of blood pressure. *J Renin Angiotensin Aldosterone Syst*. 2011;12(3):274-80.
35. Kaya CT, Gurlek A, Altin T, Kilickap M, Karabulut HG, Turhan S, et al. The relationship between angiotensin converting enzyme gene I/D polymorphism and QT dispersion in patients with hypertrophic cardiomyopathy. *J Renin Angiotensin Aldosterone Syst*. 2010;11(3):192-7.
36. Hussain S, Asghar M, Javed Q. Resistin gene promoter region polymorphism and the risk of hypertrophic cardiomyopathy in patients. *Transl Res*. 2010;155(3):142-7.
37. Friedrich FW, Bausero P, Sun Y, Treszl A, Kramer E, Juhr D, et al; EUROGENE Heart Failure Project. A new polymorphism in human calmodulin III gene promoter is a potential modifier gene for familial hypertrophic cardiomyopathy. *Eur Heart J*. 2009;30(13):1648-55.
38. Lind JM, Chiu C, Ingles J, Yeates L, Humphries SE, Heather AK, et al. Sex hormone receptor gene variation associated with phenotype in male hypertrophic cardiomyopathy patients. *J Mol Cell Cardiol*. 2008;45(2):217-22.
39. Tripathi S, Schultz I, Becker E, Montag J, Borchert B, Francino A, et al. Unequal allelic expression of wild-type and mutated beta-myosin in familial hypertrophic cardiomyopathy. *Basic Res Cardiol*. 2011;106(6):1041-55.
40. Vikstrom KL, Factor SM, Leinwand LA. Mice expressing mutant myosin heavy chains are a model for familial hypertrophic cardiomyopathy. *Mol Med*. 1996;2(5):556-67.
41. Geisterfer-Lowrance AA, Christe M, Conner DA, Ingwall JS, Schoen FJ, Seidman CE, et al. A mouse model of familial hypertrophic cardiomyopathy. *Science*. 1996;272(5262):731-4.
42. Fatkin D, Christe ME, Aristizabal O, McConnell BK, Srinivasan S, Schoen FJ, et al. Neonatal cardiomyopathy in mice homozygous for the Arg403Gln mutation in the alpha cardiac myosin heavy chain gene. *J Clin Invest*. 1999;103(1):147-53.
43. Teekakirikul P, Eminaga S, Toka O, Alcalai R, Wang L, Wakimoto H, et al. Cardiac fibrosis in mice with hypertrophic cardiomyopathy is mediated by non-myocyte proliferation and requires Tgf-beta. *J Clin Invest*. 2010;120(10):3520-9.

44. Marian AJ, Wu Y, Lim DS, McCluggage M, Youker K, Yu QT, et al. A transgenic rabbit model for human hypertrophic cardiomyopathy. *J Clin Invest*. 1999;104(12):1683-92.
45. Kittleson MD, Meurs KM, Munro MJ, Kittleson JA, Liu SK, Pion PD, et al. Familial hypertrophic cardiomyopathy in Maine coon cats: an animal model of human disease. *Circulation*. 1999;99(24):3172-80.
46. Yang Q, Sanbe A, Osinska H, Hewett TE, Klevitsky R, Robbins J. A mouse model of myosin binding protein C human familial hypertrophic cardiomyopathy. *J Clin Invest*. 1998;102(7):1292-300.
47. Yang Q, Sanbe A, Osinska H, Hewett TE, Klevitsky R, Robbins J. In vivo modeling of myosin binding protein C familial hypertrophic cardiomyopathy. *Circ Res*. 1999;85(9):841-7.
48. Beis D, Stainier DY. In vivo cell biology: following the zebrafish trend. *Trends Cell Biol*. 2006;16(2):105-12.
49. Liu J, Stainier DY. Zebrafish in the study of early cardiac development. *Circ Res*. 2012;110(6):870-4.
50. Becker JR, Deo RC, Werdich AA, Panakova D, Coy S, MacRae CA. Human cardiomyopathy mutations induce myocyte hyperplasia and activate hypertrophic pathways during cardiogenesis in zebrafish. *Dis Model Mech*. 2011;4(3):400-10.
51. Lankford EB, Epstein ND, Fananapazir L, Sweeney HL. Abnormal contractile properties of muscle fibers expressing beta-myosin heavy chain gene mutations in patients with hypertrophic cardiomyopathy. *J Clin Invest*. 1995;95(3):1409-14.
52. Watkins H, Seidman CE, Seidman JC, Feng HS, Sweeney HL. Expression and functional assessment of a truncated cardiac troponin T that causes hypertrophic cardiomyopathy: evidence for a dominant negative action. *J Clin Invest*. 1996;98(11):2456-61.
53. Ashrafian H, Redwood C, Blair E, Watkins H. Hypertrophic cardiomyopathy: a paradigm for myocardial energy depletion. *Trends Genet*. 2003;19(5):263-8.
54. Robinson P, Mirza M, Knott A, Abdulrazzak H, Willott R, Marston S, et al. Alterations in thin filament regulation induced by a human cardiac troponin T mutant that causes dilated cardiomyopathy are distinct from those induced by troponin T mutants that cause hypertrophic cardiomyopathy. *J Biol Chem*. 2002;277(43):40710-6.
55. Guinto PJ, Haim TE, Dowell-Martino CC, Sibinga N, Tardiff JC. Temporal and mutation-specific alterations in Ca²⁺ homeostasis differentially determine the progression of cTnT-related cardiomyopathies in murine models. *Am J Physiol Heart Circ Physiol*. 2009;297(2):H614-26.
56. Baudenbacher F, Schober T, Pinto JR, Sidorov VY, Hilliard F, Solaro RJ, et al. Myofilament Ca²⁺ sensitization causes susceptibility to cardiac arrhythmia in mice. *J Clin Invest*. 2008;118(12):3893-903.
57. Belus A, Piroddi N, Scellini B, Tesi C, Amati GD, Girolami F, et al. The familial hypertrophic cardiomyopathy-associated myosin mutation R403Q accelerates tension generation and relaxation of human cardiac myofibrils. *J Physiol*. 2008;586(Pt 15):3639-44.
58. Lehrer SS. The 3-state model of muscle regulation revisited: is a fourth state involved? *J Muscle Res Cell Motil*. 2011;32(3):203-8.
59. Ly S, Lehrer SS. Long-range effects of familial hypertrophic cardiomyopathy mutations E180G and D175N on the properties of tropomyosin. *Biochemistry*. 2012;51(32):6413-20.
60. McKillop DF, Geeves MA. Regulation of the interaction between actin and myosin subfragment 1: evidence for three states of the thin filament. *Biophys J*. 1993;65(2):693-701.
61. Kim JB, Porreca GJ, Song L, Greenway SC, Gorham JM, Church GM, et al. Polony multiplex analysis of gene expression (PMAGE) in mouse hypertrophic cardiomyopathy. *Science*. 2007;316(5830):1481-4.
62. Fan C, Jiang YP, Lu Z, Martin DW, Kelly DJ, Zuckerman JM, et al. A transgenic mouse model of heart failure using inducible Galpha q. *J Biol Chem*. 2005;280(48):40337-46.
63. Smart A. Impediments to DNA testing and cascade screening for hypertrophic cardiomyopathy and Long QT syndrome: a qualitative study of patient experiences. *J Genet Couns*. 2010;19(6):630-9.
64. Nherera L, Marks D, Minhas R, Thorogood M, Humphries SE. Probabilistic cost-effectiveness analysis of cascade screening for familial hypercholesterolaemia using alternative diagnostic and identification strategies. *Heart*. 2011;97(14):1175-81.
65. Sorensen PL, Gane LW, Yarborough M, Hagerman RJ, Tassone F. Newborn screening and cascade testing for FMR1 mutations. *Am J Med Genet A*. 2012;161A(1):59-69.
66. Wordsworth S, Leal J, Blair E, Legood R, Thomson K, Seller A, et al. DNA testing for hypertrophic cardiomyopathy: a cost-effectiveness model. *Eur Heart J*. 2010;31(8):926-35.
67. Miller EM, Wang Y, Ware SM. Uptake of cardiac screening and genetic testing among hypertrophic and dilated cardiomyopathy families. *J Genet Couns*. 2012;22(2):258-67.
68. Charron P, Arad M, Arbustini E, Basso C, Bilinska Z, Elliott P, et al; European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. Genetic counselling and testing in cardiomyopathies: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J*. 2010;31(22):2715-26.

