Molecular Genetic Basis of Hypertrophic Cardiomyopathy

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Abstract

Hypertrophic cardiomyopathy (HCM) is a genetic disease of the myocardium characterized by a hypertrophic left ventricle with a preserved or increased ejection fraction. Cardiac hypertrophy is often asymmetric, which is associated with left ventricular outflow tract obstruction. Myocyte hypertrophy, disarray and myocardial fibrosis constitute the histological features of HCM. HCM is a relatively benign disease but an important cause of sudden cardiac death in the young and heart failure in the elderly.

Pathogenic variants (PVs) in genes encoding protein constituents of the sarcomeres are the main causes of HCM. PVs exhibit a gradient of effect sizes, as reflected in their penetrance and variable phenotypic expression of HCM. *MYH7* and *MYBPC3*, encoding β-myosin heavy chain and myosin binding protein C, respectively, are the two most common causal genes and responsible for ~40% of all HCM cases but a higher percentage of HCM in large families. PVs in genes encoding protein components of the thin filaments are responsible for ~5% of the HCM cases. Whereas pathogenicity of the genetic variants in large families has been firmly established, ascertainment causality of the PVs in small families and sporadic cases is challenging. In the latter category PVs are best considered as probabilistic determinants of HCM.

Deciphering the genetic basis of HCM has enabled routine genetic testing and has partially elucidated the underpinning mechanism of HCM as increased number of the myosin molecules that are strongly bound to actin. The discoveries have led to the development of mavacamten that targets binding of the myosin molecule to actin filaments and imparts beneficial clinical effects. In the coming years the yield of the genetic testing is expected to be improved and the so-called "missing causal gene" be identified. The advances are also expected to enable development of additional specific therapies and editing of the mutations in HCM.

Subject Terms:

Genetics; Heart Failure; Hypertrophy

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Keywords

Hypertrophy; Genetics; Mutation; Sudden Cardiac Death; Heart Failure; Myosin heavy chain; Sarcomere; diastolic heart failure

INTRODUCTION

The main objective is to discuss the genetic basis of hypertrophic cardiomyopathy (HCM) from a prospective of deterministic and probabilistic approaches in discerning the pathogenicity of the genetic variants (GVs). The second objective is to highlight impact of the genetic discoveries in providing fundamental insights into the pathogenesis and hence, enabling specific treatment of patients with HCM. Other aspects of HCM are briefly discussed in order to provide a context for the genetic discoveries. For a conventional review, the readers are referred to a recent review co-authored by Dr. Braunwald, whose seminal characterization of the clinical and hemodynamic aspects of HCM has formed the essence of our current understanding of this intriguing disease.[1]

Henri Liouville, a French pathologist has been credited for describing the first case of HCM, a case likely with left ventricular outflow tract obstruction (LVOTO) in 1869 (described in[2]). In the same year, Frederick Miescher isolated DNA, which he described as "nuclein". (described in[3]) In the next few decades, HCM was merely a pathological entity, detected at autopsy. In 1949, William Evans recognized the familial occurrence of HCM and referred to the disease as "familial cardiomegaly"[4]. A few years later, Donald Teare described the familial nature of asymmetric septal hypertrophy, which he described as muscular hamartoma[5]. An autosomal dominant mode of inheritance of HCM was illustrated by J.A.P. Paré and colleagues in a large French-Canadian family in 1961.[6] About 30 years later, Christine and Jonathan Seidman identified the first mutation in the gene encoding the myosin heavy chain 7 (MYH7) protein in the family that was described by Paré.[7]. The seminal discovery paved the way for partial delineation of the molecular genetic basis of HCM. The advances ushered in the era of molecular genetic testing and laid the foundation for design of specific therapies for HCM.[8]

DEFINITION

HCM is defined based on its phenotypic features, which are cardiac hypertrophy, a non-dilated left ventricle, and a preserved or an enhanced left ventricular ejection fraction (LVEF). Cardiac hypertrophy in an adult is defined as a maximum left ventricular end diastolic wall thickness of >13 mm on an echocardiogram or other imaging techniques. In children, a Z-score of greater 2 of maximum wall thickness, matched for sex and age, is used to detect cardiac hypertrophy. A maximum wall thickness of 15 mm in adults is used to define HCM, which offers a greater specificity at the cost of under-detection.

Expression of cardiac hypertrophy is age-dependent, evolving gradually during the second to fourth decades of life.[9, 10] Cardiac hypertrophy may be regional, involving only a segment of the left ventricle, rendering it difficult to detect on imaging modalities. The presence of confounding factors, such as concomitant hypertension, valvular diseases, athletes' heart,

and the phenocopy conditions further compound the accurate diagnosis of HCM. Phenotypic features, such as the asymmetric nature of hypertrophy with a predominant involvement of the interventricular septum, a small left ventricular cavity size, and LVOTO could enhance an accurate diagnosis. In a subset of patients, hypertrophy is predominantly or exclusively restricted to left ventricular apex, which is referred to as apical HCM. It has the characteristic electrocardiographic findings notable for a deep T wave inversion in the precordial leads.

EPIDEMIOLOGY

The estimated prevalence of HCM in the general adult population, diagnosed based on the presence of cardiac hypertrophy, is between 1:300 to 1:600.[11, 12] Incorporation of more sensitive diagnostic tools, family information, and the genetic data increases the population prevalence of HCM to ~1:250 individuals in the general population[13, 14]. HCM, as an autosomal disease, does not show a predilection toward a specific ethnic backgrounds, sex, or geographic locations, albeit sex and ethnic backgrounds influence its phenotypic expression.[15, 1617, 18]

MOLECULAR GENETIC BASIS OF HCM

Classically, HCM is a quintessential single gene disorder with an almost exclusive autosomal dominant mode of inheritance. Genetic linkage and co-segregation analysis in large families, exhibiting an autosomal dominant model of inheritance, have led to partial elucidation of the molecular genetic basis of HCM. Christine and Jonathan Seidman identified the p.Arg403Glu (p.R403Q) mutation in the *MYH7* gene, encoding sarcomere protein MYH7, as the first mutation for familial HCM.[7] Subsequently, over a dozen causal genes were identified, which led to a near complete elucidation of the molecular genetic basis of HCM in large families.[19–21] In about half of the cases, either the family size is too small or the disease is sporadic. The molecular genetic etiology of HCM in the sporadic cases and small families is less well-defined, as unequivocal ascertainment of causality in such cases is exceedingly challenging. To set the stage and gain some insights into the challenges encountered in full delineation of the genetic basis of HCM, a brief discussion on the genome-wide spectrum, population frequencies, and functions of the GVs is presented. An expanded discussion of these topic is provided in a recent review.[22]

A primer on the spectrum of the GVs in the human genome

Each human genome, comprised of $\sim 3.2 \times 10^9$ base pairs, differs in approximately 4 to 5 million nucleotides from the reference genome.[23–25] The variation is mainly the consequence of the error rate of the DNA replication machinery that is estimated to be $\sim 1.3 \times 10^{-8}$ per nucleotide.[26–28] Consequently, each genome introduces approximately 60 *de novo* variants (absent in the parents' genomes) to the population pool.[26–29] Common variants are typically shared across the populations; however, the vast majority of the rare variants are restricted to specific populations.[2530]

GVs exhibit a continuum of size variation, ranging from a single base to the whole-chromosome (Table 1). Variants affecting a single nucleotide, referred to as single nucleotide

variants (SNVs) or single nucleotide polymorphism (SNP), are the most common variants, totally about 4 million in each genome. Small insertion/deletion (indels) variants are the second most common variants in the human genome.

Functional characteristics of the GVs

The wide spectrum of the variants in the genomes of apparently healthy individuals demonstrates the challenging nature of ascertaining their functional significance and clinical pathogenicity.[23, 24] *In silico* prediction of pathogenicity is based on the anticipated effects of the GVs on the structure and function of the genome and the involved proteins, such as the effects on polarity, charge, and hydropathy of the involved amino acid, secondary and tertiary structures of the mRNAs and proteins, as well as the catalytic activities of the involved proteins. (reviewed in[31]) Likewise, evolutionary conservation of the involved codon and the population frequency of the variant denote provisional functionality, requiring validation based on the clinical and experimental evidence. However, experimental data, while valuable, are subject to the experimental conditions, which often are not reflective of the *in vivo* biology in human, as functional and clinical significance of the GVs is often influenced by the milieu in which they occur. Likewise, population-level functional data, although valuable, often are not fully applicable at the individual level, necessitating the focus on the individual in assessing function and pathogenicity of the GVs.

At the genetic level, the loss-of-function (LoF) variants are predicted to impair protein-coding function of the involved genes. Accordingly, non-sense, gain-of-stop codon, canonical splice site, and frame-shift indel variants, which lead to expression of unstable premature truncated proteins, are predicted to be LoF variants. A LoF variant might also delete an entire exon or the gene itself. Throughout the text LoF is used to denote the predicted function, unless the LoF variant is experimentally documented. A gain-of-function (GoF) variant is defined biologically, as it affords the protein a new function, such as interaction with a new protein, or enhances its enzymatic activity.

Categorization of the GVs for clinical applications

Clinical significance of the GVs to some extent is reflective of their functional significance, albeit not all functional variants impart clinically discernible effects. This is amply evident in the personal genome sequencing data showing the presence of several hundred predicted (p)LoF variants in each genome in the absence of a discernible related clinical phenotype.[23, 24][25] On the other hand, all clinically significant variants are expected to be functional, even though their functions might not be evidence, when the clinical phenotype is detected.

Clinical impacts of the functional variants are also determined by the tolerance of the involved gene to the LoF variants, which varies across the genes. The intolerance reflects a gradual elimination of the most deleterious variants from the genome by natural selection. Accordingly, a LoF variants in a gene that exhibits a strong natural selection against LoF variants, i.e., the gene that is intolerant to the LoF variants, is expected to impart larger clinical effects than a LoF variant in a gene that is tolerant to LoF variants. Consequently, LoF variants in genes that are intolerant to LoF variants are rare in the general population and when present, they are typically pathogenic. In contrast, LoF in genes that are tolerant to

the LoF variants, are often found in the general population with no discernible phenotype. Thus, information on the pathogenicity of LoF is partially embedded in the population frequency of a LoF variant.

Clinically, the GVs are categorized into pathogenic variants (PVs), likely (L)PVs, variants of unknown significance, likely benign variants and benign variants (Table 2). In addition, a subset of GVs affects function or level of the involved protein without a clear clinical significance. The focus in clinical genetics is on the PVs, defined as variants for which there is strong evidence for involvement in the pathogenesis of the disease, and to a lesser extent on LPVs. At the broader genetic parlance, any change in the nucleotide sequence is considered a mutation. At the clinical genetic level, however, a mutation is defined as a variant with a population frequency of 1%. Given the plethora of the rare variants in each genome and to imply a clinical significance, the term mutation should be reserved only for a subset of the PVs for which there is a well-established robust and unequivocal evidence of causality in the phenotype.

PVs and the clinical phenotype

Expression of the clinical phenotype of HCM is the consequence of non-linear and stochastic interactions among multiple genetic and non-genetic factors. PVs are the key determinants of the phenotype but their penetrance varies according to their effect sizes and the genetic backgrounds of the individuals. Penetrance of the PVs is partially determined by the impact of the PVs on the structure and function of the genes and the encoded proteins with those totally disrupting the protein function exhibiting the highest penetrance. In addition, the penetrance is expected to be higher for PVs that are located in the proteins that are proximal to the phenotype, i.e., directly related to the pathogenesis of the phenotype, as opposed to PVs in proteins that are distant or indirectly related to the phenotype. Moreover, functional variants in other genes and proteins related to the phenotype are expected to influence penetrance of the main PVs and modify phenotypic expression of the phenotype. Hence, the latter group of the functional variants are referred to as the modifier variants (MVs).[32–34] The MVs are neither sufficient nor necessary to cause HCM but in aggregate impart a large influence on its phenotypic expression. Consequently, in the classic single gene HCM, the phenotype is also a polygenic trait, influenced by a large number of functional variants. The collective effect sizes of the MVs and non-genetic factors increase inversely with the effect size of the main PV. Accordingly, when the effect size of the main PV is large, the contribution of the MVs and non-genetic factors is relatively modest and vice versa.

Deterministic vs. Probabilistic approach to understanding the genetic basis of HCM

The conventional deterministic approach to genetic studies of HCM, as a quintessential single gene disease, posits that the presence of the single PV or mutation is necessary and sufficient to cause HCM, albeit with a variable penetrance and expressivity. The contributions of the deterministic approach to elucidation of the genetic basis of HCM in large families, exhibiting an autosomal dominant mode of inheritance, are irrefutable. The approach has led to identification of the most common causal genes in familial HCM. The deterministic approach to some extent, however, is oblivious to the gradient of effect sizes of

the PVs and the continuity of the phenotypic expression at the molecular and clinical levels. Consequently, the deterministic approach cannot be successfully applied to decipher the genetic basis of HCM in small families wherein co-segregation and linkage cannot be established; and to sporadic cases, wherein the causality of a given PV cannot be ascertained unambiguously. Consequently, the genetic basis of HCM has remained obscure in about half of the cases.

The probabilistic approach surmises that PVs in genes encoding proteins directly or indirectly involved in the biological networks pertinent to the phenotype contribute to the pathogenesis of the HCM, albeit the extent of contributions varies according to the effect size of the variants. It also conjectures that there is a genetic hierarchy in the pathogenesis of the phenotype. Accordingly, PVs in genes whose encoded proteins are involved in biological pathways directly relevant to the pathogenesis of HCM have a higher probability of exerting larger effect sizes than those residing in genes that are indirectly related to the phenotype. Similarly, PVs in genes that are expressed at high levels in cardiac myocytes, the primary cell type involved in HCM, are expected to impart larger effects on the phenotype than those that are expressed at low levels. Moreover, PVs in genes that are highly intolerant to the LoF or missense variants are expected to make larger contributions to the phenotype than those in genes that are somewhat tolerant to LoF or missense variants.

The probabilistic approach considers the phenotype in HCM as a polygenic trait secondary to contributions, large and small, of multiple PVs (Figure 1). Consequently, it shifts the focus from identification of a single PV to multiple PVs in inducing the phenotype in HCM, particularly in small families and the sporadic cases. The approach is predominantly pertinent to the PVs that do not exhibit a high penetrance and therefore, do not show robust evidence of co-segregation and genetic linkage. Given the complexity of the clinical phenotype, a probabilistic approach is preferable in discerning determinants of the phenotype in HCM. According to this notion, PVs in genes responsible for the classic single gene HCM, such as those in *MYH7* and *MYBPC3*, are expected to be enriched in the sporadic cases with HCM. Further extension of this notion posits that such PVs are also expected to be enriched in individuals who exhibit an exaggerated hypertrophic response to external stimuli, such as pressure overload (aortic stenosis or systemic arterial hypertension) or physical exercise (professional athletes). Finally, the probabilistic stipulates that the PVs involving pathways that are relevant to the phenotype, directly or indirectly, account for the so-called "missing causal genes", as discussed later. The clinical impact of the approach is amply evident in clinical genetic testing, which thus-far has had a relatively low yield by focusing in identifying the PVs in genes already known to cause HCM.[35] The main caveat with the probabilistic approach is to the risk of over-interpretation and improper clinical conclusions. The proper utilization of the approach requires a clear understanding of the molecular genetics, biology and pathogenesis of HCM.

Specific genes implicated as causes of HCM

In keeping with the complexity of the clinical phenotype, a large number of genes are involved in the pathogenesis of HCM. Genetic data, from the very early on, identified HCM mainly as a disease of the sarcomeres. Sarcomeres are orderly structures in the striated

muscles, which are comprised of thick and thin filaments, whose highly regulated ATP-dependents interaction generate the force of muscle contraction (Figure 2). Several specific genes, according to the biological functions of the encoded proteins, are briefly discussed.

Genes encoding thick filament proteins of the sarcomere: The main constituents of the thick filaments involved in HCM are MYH7 and MYBPC3, which are responsible for force generation.

MYH7: The gene codes for MYH7 or β-MYH, which comprises about 1/3 of the sarcomere proteins. It is predominantly expressed in cardiac myocytes and to a lesser extent in slow fibers of the skeletal muscles (https://www.proteinatlas.org/). The protein has 1,935 amino acid and is ~ 220 kDa in molecular weight. Structurally, it has distinct domains including a globular head, which is the sites of ATP binding and hydrolysis as well as binding to the actin molecule in the thin filament. It contains a lever arm or a hinge region, which is followed by the α-helical coil-coil rod domain. The latter comprises the majority of the amino acids in the protein. MYH7 is the main muscle motor molecule and essential for muscle contraction. It forms a hexameric complex composed of two MYH7, 2 myosin light chain 2 (MYL2), and 2 myosin light chain 3 (MYL3). The hexameric complex interacts with actin through the actin-binding domains, and hydrolyzes ATP to ADP and inorganic phosphate to generate the force of contraction.

Several hundred PVs and LPVs in the *MYH7* gene, primarily missense variants and a few nonsense and indels variants, have been identified in patients with HCM (https://www.ncbi.nlm.nih.gov/clinvar/). The population frequency of each mutation is low, as each is found in less than 1% of the HCM cases. The founder mutations, which are uncommon in the *MYH7* gene, have a higher population frequency in specific populations. Overall, PVs in the *MYH7* gene are found in approximately 20% of patients with HCM cases but aa higher number of large families with HCM. *MYH7* shows selection against LoF variants, reflected in the loss-of-function observed/expected upper bound fraction (LOEUF) index of 0.57 in the gnomAd database (a low LOEUF score indicates strong selection toward LoF variants). Given its key biological functions in cardiac myocytes and its well-established role in HCM, PVs in the *MYH7* gene rank among the highest in the hierarchy of the genetic causes of HCM.

MYBPC3: The gene codes for myosin binding protein C3, which is associated with the thick and thin filaments of the sarcomere in cardiac myocytes (Figure 2). The protein has 1,274 amino acids and a molecular weight of ~ 140 kDa. MYBPC3 binds to MYH7 and cardiac actin and regulates acto-myosin interactions. It undergoes extensive post-translational modifications (PTMs), including phosphorylation by PKA, PKC, GSK3β, and other kinases at multiple sites, which collectively modulates its interactions with other sarcomere proteins and hence, muscle contraction.[36]

Heterozygous mutations in the *MYBPC3* gene are found in about 20% of the HCM cases and in a higher number of large HCM families.[37][38, 39] A notable feature of mutations in the *MYBPC3* gene is over-representation of the truncating mutations, such as indels, splicing, and nonsense mutations, as opposed to other well-established causal genes, whereby the

missense mutations prevail.[38, 39] The truncating mutations typically activate the nonsense-mediated decay (NMD) and the ubiquitin-proteasome system (UPS), resulting in haplo-insufficiency of the MYBPC3 protein.[40]

The population frequencies of specific *MYBPC3* mutations are rare with the exception of the founder mutations, which are responsible for a significant number of HCM cases in the European countries and in Japan.[41–43] A common 25-bp deletion in intron 32 of *MYBPC3* gene has been associated with cardiomyopathies, including HCM in the South Asian population.[44] This 25-bp variant is in linkage disequilibrium with an intronic variant, which introduces a cryptic splice acceptor site in intron 13, resulting in inclusion of 50 intronic nucleotide in the transcript, and subsequent premature termination of the MYBPC3 protein.[45] The *MYBPC3* gene is tolerant to missense and LoF variants (LOUEF:0.96). Despite its well-established role in HCM, PVs in the *MYBPC3* have to be analyzed further to implicate their causal role in HCM.

MYL2 and MYL3: Genes encoding MYL2 and MYL3 were amongst the first set of genes identified as causes of HCM.[46] The proteins are part of the myosin hexamer and interact with the hinge region of the MYH7 protein. Upon phosphorylation by myosin light chain kinase (MLCK), they regulate cardiac contractility. Both proteins have well-established roles in muscle contraction and are highly expressed in cardiac myocytes. *MYL2* and *MYL3* are uncommon causes of HCM, together being responsible for <1% of the HCM cases. The *MYL3* and MYL2 genes are largely tolerant to missense and LoF variants. Therefore, pathogenicity of the variants identified in the *MYL2* and *MYL3* genes has to be assessed for the presence of co-segregation, whenever feasible, and in the context of the population frequency of the variants as well as the functional data. Overall, given their relatively high levels of expression in cardiac myocytes and the existing genetic data, PVs in these genes are plausible candidates to cause HCM.

TTN: The gene encodes the giant sarcomere protein titin (TTN), which expands from M to Z lines, i.e., half of the sarcomere length. It is almost exclusively expressed in cardiac myocytes and skeletal muscles. The gene is intolerant to LoF (LOEUF: 0.35) but tolerant to missense variants. Truncating variants in the TTN gene are major causes of familial and sporadic dilated cardiomyopathy (DCM) but are rare in HCM.[47] Overall, PVs in the *TTN* gene rank relatively low as causes of HCM.

MYH6: Myosin heavy chain 6 protein, encoded by the *MYH6* gene, comprises less than 10% of the total MYH protein, with MYH7 being the main isoform in the human cardiac ventricular myocytes. Despite being the minor myosin isoform, its transcripts are abundant in the human cardiac myocytes. The gene is mostly tolerant to missense and LoF variants (LOEUF:0.86). Variants in the *MYH6* have been reported in HCM cases.[48, 49] However, robust genetic evidence to implicate PVs in the *MYH6* gene as causes of HCM are lacking. Given that MYH6 comprises a small fraction of the total MYH pool in cardiac myocytes, heterozygous PVs in the *MYH6* gene, by affecting only a very small fraction of the sarcomere MYH protein (likely <5%), are not expected to cause HCM.

Genes encoding thin filament proteins of the sarcomere: The thin filaments in cardiac myocytes are comprised of a polymer of ACTC1 and the troponin/tropomyosin complex. The troponin/tropomyosin complex is formed by cardiac troponin C (TNNC1), which is the calcium sensing unit of the complex, cardiac troponin I (TNNI3), which is the inhibitory component, and cardiac troponin T (TNNT2), which anchor the troponin complex to tropomyosin (TPM1). The latter places the troponin/tropomyosin complex on ACTC1, forming the thin filaments (Figure 2).

TNNT2: The gene codes for TNNT2, which constitute about 5% of the sarcomere proteins. The *TNNT2* gene was among the first genes whose causality in HCM was established through genetic linkage.[21] The finding along with identification of mutations in other major sarcomere proteins established HCM mainly as a disease of sarcomere.[21] A large number of PVs and LPVs in the *TNNT2* gene, mostly missense variants, have been identified in patients with HCM. The *TNNT2* gene is tolerant to LoF variants (LOEUF:0.97). In view of its key role in cardiac myocyte function, and robust evidence for the causal role of this gene in HCM, PVs in the *TNNT2* gene should be assessed further to imply a causal role in the pathogenesis of HCM.

TNNI3, TNNC1, and TPM1: The genes encode thin filament proteins TNNI3, TNNC1, and TPM1, respectively. Mutations in the *TNNI3*, *TNNC1*, and *TPM1* genes are uncommon causes of HCM (<5% of the HCM cases). The three genes are partially intolerant to missense and LoF variants and in view of their functions and well-established role in HCM, PVs in these genes are plausible candidates to cause HCM.

ACTC1: The gene encodes ACTC1, which is among the least tolerant genes to missense and LoF variants. ACTC1 is a major component of the thin filaments and together with troponin/tropomyosin complex interact with the MYH7 protein to generate force of muscle contraction. Mutations in the *ACTC1* gene are rare causes of HCM.[50] Given the highly conserved nature of ACTC1 and its major role in cardiac myocyte contraction, PVs in the *ACTC1* gene benefit from a high probability of exerting large effect sizes and causing HCM (LOEUF:0.48).

Genes encoding the protein constituents of the Z lines (or Z discs or Z bands): The Z lines anchor the end of the thin filaments, providing mechanical stability for contraction and relaxation of the filaments and transmission of the force of contraction to neighboring units (Figure 2). The Z lines in cardiac muscle are comprised of a large number of proteins, including α actinin 2 (ACTN2), myozenin 2 (MYOZ2), cysteine and glycine-rich protein 3 (CSRP3), titin-cap (TCAP), and Four and half LIM domain 1 (FHL1), to name a few.

MYOZ2: The encode protein MYOZ2 is exclusively expressed in cardiac myocytes and skeletal muscles, where it interacts with ACTN2, and others and presumably regulates myofibrillogenesis and calcium-dependent signaling pathways. The gene is tolerant to LoF and missense variants. PV and LPVs in the *MYOZ2* have been implicated in sporadic as well as familial HCM.[51, 52] Overall, PVs in *MYOZ2* are expected to exert moderate effects sizes on the phenotypic expression of HCM, as the causal or the modifier variants.

ACTN2: The gene is predominantly expressed in cardiac and skeletal muscle, where it binds to the thin filament protein actin and regulates sarcomere function. The gene is very intolerant to LoF variants (LOEUF:0.24). PVs in the *ACTN2* gene have been associated with skeletal myopathy as well as various forms of cardiomyopathies, including sporadic HCM.[53, 54]

CSRP3: The encoded protein is a small multi-functional protein referred to as CSRP3, which was formerly known as muscle lim protein (MLP). The protein is highly expressed in cardiac and skeletal myocytes and interacts with a number of sarcomere and cytoskeletal proteins as well as cardiac transcription factors. Mutations in the *CSRP3* gene are implicated in HCM with the strongest evidence emerging from linkage analysis in a large family.[55] The gene is, however, tolerant to missense and LoF variants. Therefore, PVs in this gene alone are not expected to cause HCM, but act in concert with PVs in other genes relevant to the pathogenesis of HCM.

TCAP: Rare variants in the *TCAP* gene, which encodes titin-cap, have been reported in patients with HCM.[56] The protein regulates assembly of the TTN protein in cardiac and skeletal muscle. The gene is tolerant to missense but tolerant to LoF variants (LOEUF:0.95). This gene, like the *TTN* gene, does not appear to be a major cause of HCM.

FHL1: The *FHL1* gene is located on the X chromosome and encodes the FHL1 protein, which is expressed predominantly in skeletal muscle, cardiac myocytes, smooth muscle cells, and to a lesser extent in other cell types. The gene is highly intolerant to the LoF (LOEUF:0.28) and to a lesser degree to missense variants. PVs in the *FHL1* gene have been predominantly associated with skeletal myopathy but also have been reported in sporadic cases with HCM.[57] In addition, *FHL1* is considered a modifier gene for cardiac hypertrophy in HCM.[58, 59] Overall, PVs in *FHL1* gene are expected to exert modest effect sizes on the expression of HCM and are unlikely to cause HCM in the absence of skeletal myopathy.

Genes coding for proteins located at the M line (M band): Whereas the Z lines largely anchor the thin filaments, the M lines, located in the middle of the sarcomere, are considered anchors for the thick filaments. In addition to the structural proteins, several other proteins with enzymatic functions are localized to the M lines. Notable examples of proteins that are localized to the M lines are obscurin (OBSCN), myomesin 2 (MYOM2), and tripartite motif containing 63 (TRIM63).

OBSCN: The gene codes for obscurin, which is a large protein predominantly expressed in the skeletal muscle and to a lesser extent in cardiac and tongue muscles. It interacts with TTN and MYBPC3 and also has signaling functions and kinase activity. The gene is somewhat tolerant to LoF (LOEUF:0.87) and tolerant to missense variants. A few PVs in the *OBSCN* gene have been associated with various forms of cardiomyopathies, including HCM.[60]

TRIM63: The gene encodes TRIM63 or muscle ring finger 1 (MuRF1) protein, which is predominantly expressed in cardiac and skeletal myocytes. The protein is an E3 ubiquitin ligase, which tags the thick filament proteins for degradation by the UPS. The gene is

tolerant to LoF and missense variants. LoF variants in the *TRIM63* gene have been associated with HCM in small families and sporadic cases.[61] The mechanisms involve impaired ubiquitination of MYH7 and MYBPC3 and activation of the MTOR-S6K pathways.[61] Likewise, homozygous LoF in the *TRIM63* gene have been associated with an autosomal -recessive form of familial HCM.[62] Overall, the data points to the modest to moderate effect sizes of the PVs in the *TRIM63* gene in HCM.

MYOM2: The encoded protein cross links TTN and MYH7 at the M line and is implicated in sarcomerogenesis. The gene is predominantly expressed in cardiac and skeletal muscle. Variants in the *MYOM2* gene have been associated with HCM.[63]

Gene regulating calcium homeostasis in cardiac muscle: Intracellular Ca^{2+} concentration regulates acto-myosin interaction and generation of the force of muscle generation.

JPH2: The encoded protein junctophilin 2 is a major component of the junctional membrane complex involved in calcium homeostasis and excitation-contraction coupling. Expression of this gene is enriched in cardiac, skeletal, and smooth muscles, the latter in various organs, including colon and urinary bladder. The gene is intolerant to LoF (LOEUF:0.14) and missense variants. PVs are enriched in patients with sporadic HCM, as compared to the general population.[64] PVs in the *JPH2* gene are expected to exert moderate effect sizes on the phenotypic expression of HCM.

PLN: The gene encode phospholamban, which is predominantly expressed in cardiac myocytes where it functions as a regulator of Ca^{2+} homeostasis through inhibiting ATP2A2 (SERCA2a) activity. Consequently, it is a negative regulator of cardiac contraction. PVs in the PLN gene are best known to cause DCM. A rare truncating variant has been reported in HCM.[65, 66]

Genes encoding sarcomere-associated proteins: Several other genes with indirect role in regulating sarcomere structure and function are also implicated as causes of HCM.

FLNC: *FLNC* is one of the latest genes implicated in HCM but most prominently in DCM.[67, 68] The gene is highly intolerant to LoF and to a lesser extent to missense variants. Its expression is enriched in cardiac myocytes and more so in the skeletal muscle. The protein interacts and cross-links with actin and is presumed to regulate organization of the cytoskeletal protein in response to stress. FLNC gene is intolerant to LoF variants (LOEUF:0.25). *FLNC* variants have been identified in sporadic cases and in small families with HCM.[67, 68]

ALPK3: The gene, which codes for α kinase 3, is most abundantly expressed in cardiac and skeletal muscle and implicated in myocyte differentiation. The gene is tolerant to missense and LoF variants. PVs variants in this gene, including homozygous truncating variants, have been reported in patients with HCM alone or in conjunction with other PVs.[69, 70]

CAV3: The gene codes for caveolin 3, which is a component of caveolae of the cytoplasmic membrane. The gene is expressed in cardiac and skeletal muscle, where the encoded protein interacts with G-protein alpha subunits, the dystrophin complex, and the T-tubule system. CAV3 is implicated in regulating myogenic differentiation, endocytosis, mechanosensing, signaling, and mitochondrial homeostasis, among others. The gene is tolerant to missense and LoF variants. PVs in the *CAV3* gene are associated with skeletal myopathy and long QT syndrome 9 and various forms of cardiomyopathies, including HCM.[71]

CRYAB: The gene encodes crystalline αB, which is a member of small heat shock proteins, with chaperon-like activity. The gene is expressed in multiple cell types but is enriched in cardiac and skeletal muscles. The gene is tolerant to LoF and missense variants. PVs in the *CRYAB* gene have been reported mostly in patients with DCM and in a few patients with sporadic HCM. Overall, PVs and LPVs are not expected to emerge as major contributors to the phenotypic expression of HCM.

Common and founder mutations in HCM

With the exception of a few founder and population-specific mutations, the population frequency of each specific mutation in HCM is less than 1%.[35] The p.Arg502Trp and p.Val762Asp in the *MYBPC3*, the latter in a Japanese population, have been reported at a relatively high frequencies in the HCM populations, ranging from 1 to 4%[35, 72, 73]. Likewise, the c.927-2A>G founder mutation in the *MYBPC3* gene is found in about 50% of the patients with HCM in Iceland.[41] Several other population-specific founder mutations, mostly in the genes coding for sarcomere proteins have been reported.[74–76]

Digenic and oligogenic HCM

HCM in large families exhibits an autosomal dominant pattern of inheritance and is an archetypical single gene disorders, whereby the presence of a single PV, typically with a large effect size, is sufficient to cause the disease. The severity of the phenotype, however, is determined not only by the main PV but also by additional PVs with smaller effect sizes, i.e., the MVs. On the other end of the spectrum is the sporadic form of HCM, which often is the consequence of a *de novo* PVs and on occasion aggregation of multiple low penetrance PVs, each exerting modest to moderate effect sizes and collectively leading to the clinical phenotype of HCM. Approximately, 5% of the HCM cases are estimated to be digenic or oligemic in etiology.[35, 70, 77, 78] The presence of multiple PVs is typically associated with more pronounced phenotype, such as severe cardiac hypertrophy or LVOTO[35, 70, 77, 78]. The prevalence of the digenic and oligogenic HCM and the impact of multiple PVs on the clinical manifestations of HCM remain to be fully understood.

Missing causal genes

The PVs responsible for HCM have been identified in less than half of the cases and small families with HCM but in almost all large families. The difficulty in ascertaining the remaining causal genes pertains to the modest to moderate effect sizes of the responsible PVs and consequently, incomplete penetrance, or the *de novo* nature of the variants. In the latter situations, unambiguous ascertainment of causality is exceedingly challenging, resulting in the so-called "missing causal gene" in HCM.[79]

To identify the "missing causal genes" it is necessary to shift from a deterministic, which assumes PVs with large effect sizes are the causes of HCM, to a probabilistic approach, which posits multiple PVs with moderate and modest effect sizes collectively are responsible for HCM in the sporadic cases and in small families. In the probabilistic approach, all PVs in genes encoding proteins involved in the networks pertaining to the HCM phenotype are expected to contribute to expression of the phenotype in HCM. However, detection of the PVs in the pathways that pertain to the HCM phenotype alone, is insufficient to conclude a causal role, as the effects are often context-dependent. Therefore, the findings should be analyzed in the context of HCM phenotype, its pathogenesis, and myocardial biology, including gene expression. Identification of the so-called "missing causal genes" in HCM would require genome-wide analysis to detect the candidates and more importantly, to gain a clear understanding of the biological functions of the encoded proteins.

GENETIC TESTING

Genetic testing, typically by whole exome sequencing (WES), is routinely performed in patients with HCM.[35] The objective is to find the PV and LPVs, which would provide insights into the pathogenesis of HCM, and ultimately, to prevention of the evolving phenotype, and attenuation or reversal of the established disease. The most informative yield of genetic testing is in the familial setting, whereby co-segregation of the variant of interest with the phenotype could be ascertained to deduce causality. Cascade screening of the family members at risk could lead to identification of those who carry the PVs. Such individuals undergo frequent clinical evaluations to detect the disease early, and to intervene, as clinically indicated.[80, 81] Moreover, identification of the family members who have not inherited the culprit PV(s) would abrogate unnecessary frequent clinical evaluations. Moreover, genetic testing could lead to identification of the phenocopy conditions, including storage diseases and the athletes' hearts. Approximately 3% of the individuals who have undergone genetic testing for HCM are found to have PVs in genes known to cause phenocopy conditions.[35] The distinction is clinically important as the treatment of HCM from the phenocopy conditions differ, particularly with the availability of specific therapies.

Despite its informativity, the current yield of genetic testing in HCM, by WES is rather disappointing, as only in ~ 30% to 50% of probands with HCM, a candidate PV is identified.[35] There are a number of reasons, in addition the current deterministic approach, that lead to a relatively low yield of genetic testing in HCM. (reviewed in[22]) Another shortcoming is the current approach to variant detection, including by WES, which is based on the Willie Sutton idiom, as the existing data show that the majority of the discovered mutations reside in the coding region. Nevertheless, the approach excludes the vast majority of the variants in the genome. The whole genome sequencing (WGS) approach, on the other hand, requires extensive computational analysis of a complex set of data, which is challenging. In addition, the current techniques are based on short-read sequencing, which results in imperfect variants detection and do not provide a robust platform for detection of large indels and structural variations. A number of alternative approaches, such as long-read sequencing and single molecule sequencing, have been developed. However, none is ready for clinical genetic testing, partly because of a relatively high error rate.[82]

Genetic testing has modest utility in predicting the phenotypic outcomes, partly because the outcomes are the consequence of stochastic and non-linear interactions among multiple determinants. The effect size of a specific culprit PV in HCM diminishes with the remoteness of the phenotype to the genotype, because of the contributions of other competing determinants. Accordingly, a PV is expected to exert a greater effect on the transcript level of the involved gene than on the risk of heart failure or sudden cardiac death (SCD), as the latter are also affected by a much larger number of genetic and non-genetic factors. The "mutation load", however, seems to be an important determinant of severity of the phenotype in HCM, as the presence of multiple PVs in genes with key roles in cardiac myocyte biology.[35, 70, 77, 78]

The main challenge in genetic testing is in interpretation of the genetic findings in terms of their clinical significance. This is particularly the case in small families and sporadic cases, as each genome contains a large number of PVs and LPVs. To extract robust and accurate information from the genetic data, it is important to garner knowledge not only about how to discern the PVs, but also in understanding the role of the involved proteins in cardiac muscle biology and function as well as the pathogenesis of the HCM, while keeping in mind the complexities of the clinical phenotype. Finally, genetic counseling and guidance on exercise and physical activities are routinely incorporated in the care of patients with HCM.

GENETIC-BASED INSIGHTS INTO THE PATHIOGENESIS OF HCM

Familial HCM is mainly an autosomal dominant disease, and hence, only one copy of the gene carries the causal variant. By definition the primary defect in HCM is the causal mutation or the PVs. For simplicity, the focus is on a single PV in providing the impetus for the initiation of a cascade of events that leads to the pathological phenotypes, such as myocyte hypertrophy, disarray, and fibrosis, and the clinical phenotypes, such as heart failure with preserved ejection fraction (HFpEF), arrhythmias, and SCD. Nonetheless, the mechanisms discussed below are also applicable when multiple PVs are operant, whereby, each provides an impetus, often overlapping with another, for the instigation of the phenotype.

The mutation operates mainly by changing the amino acid sequence or reducing expression level of the encoded protein. The former mechanism is referred to as a dominant-negative (or poison-peptide) effect and the latter to haplo-insufficiency (Figure 3). A dominant-negative mechanism is more common in HCM in keeping with the majority of the known mutations being missense mutation. A haplo-insufficiency mechanism is more common in HCM caused by mutations in the *MYBPC3* gene, as the majority of mutations in this gene are frameshift and stop codon mutations.[38, 39, 83, 84].

Effects of the mutations on protein expression:

The missense mutations are typically incorporated into the expressed full-length protein. However, the mutation could affect mRNA transcription rate, stability, and its translation efficiency, leading to a modest reduction in the level of the mutant protein.[85, 86] Transcripts containing a premature termination codon are detected by the surveillance machinery and degraded by the NMD pathway.[87] Similarly, the elongated transcripts are surveyed by the

non-stop decay pathway, are released from the ribosomes to prevent translation, and are degraded by the exosomes.[88] Moreover, the No-Go mRNA decay pathway stops the ribosomal translation of the mRNA followed by its degradation by endonucleolytic cleavage near the stalled site.[89] The net effect of such quality surveillance mechanisms is prevention or reduction in the expression levels of the truncated proteins. Those transcripts escaping the NMD are translated as truncated or elongated proteins, which are typically degraded by the protein quality control mechanisms.[90] There is, however, partial allelic compensation with the wild type allele compensating for the fraction of the lost transcripts. Despite, these surveillance mechanisms, if and whenever a truncated or an elongated protein is stably expressed, it functions through a dominant -negative mechanism.

The mutation could also affect protein folding and its tertiary structure, which typically result in degradation of the misfolded protein by the UPS. (reviewed in[91]) However, accumulation of the misfolded protein could lead to endoplasmic reticulum stress and activation of the unfolded protein response, and aggregation myopathy.

Effects of the mutations on protein and sarcomere structure and function:

The mutant protein, whenever stably expressed, exhibits impaired biological functions, such as ATPase activity, its interactions with its binding partners (interactome), and/or incorporation into sarcomeres. Filaments incorporating the mutant sarcomere protein often exhibit impaired calcium-dependent acto-myosin cross-bridge cycling, altered Ca^{2+} sensitivity of the troponin complex, and impaired force generation (Figure 3).

Impaired acto-myosin cross-bridge cycling has emerged as the key mechanisms in the pathogenesis of HCM. (reviewed in[92]) In brief, binding of the ATP molecule to the globular head of MYH7 leads to dissociation of MYH7 from the ACTC1 molecule, whereas subsequent hydrolysis of ATP to ADP and inorganic phosphate by the MYH7 results in association of the two molecules (Figure 4). The release of the inorganic phosphate induces conformation changes in the MYH7 protein, flexion of the myosin lever arm, and the consequent displacement of the actin filament by about 10 nm, which generates the force of muscle contraction. (reviewed in[92]) It is estimated that under physiological conditions during diastole, about 10% of the myosin molecules are bound to the actin filaments, which increase to about 30% during systole.[92] The actin-bound myosin molecules have a high ATP turnover rate and produce the force of muscle contraction.[93] (Figure 4) Approximately 50 to 60% of the myosin molecules are unbound to actin, have a very low ATP turnover rate, and are considered to be in a super-relaxed state.[94] The remaining 30 to 40% of myosin molecules are weakly associated with the actin molecule and exhibit an intermediary ATP turnover rate. A prevailing hypothesis in the pathogenesis of HCM posits that the causal mutations shift the number of the myosin molecules that are in the super-relaxed state toward the actin-bound state.[95, 96] The shift is regulated by the intra-cellular Ca^{2+} concentration, cytosolic ADP/ATP content, and phosphorylation states of the sarcomere proteins, such as MYL2, MYL3, and MYBPC3. The ensuing increase in the number of actin-bound myosin molecules in HCM results in increased myocyte contractility and ATP utilization, which instigates a cascade of the secondary molecular events that induce the histological, morphological, and the clinical phenotype of HCM.

In accord with the diversity of the mutations and genes involved, a diverse array of molecular and cellular functional defects is implicated in the pathogenesis of HCM.[97–102] Likewise, there are differences in the functional phenotypes induced by the PVs in different genes. For example, mutations in the *MHY7* gene exhibit more pronounced effects on the myofibrillar ATPase activity and a higher energy cost for generation of muscle tension those located in the *MYBPC3* gene.[97, 98] The increased cost of tension generation in the presence of mutant sarcomere protein results in a reduced ratio of cardiac phosphocreatine to ATP in the heart and myocardial bioenergetic deficiency in patients with HCM.[103, 104]

Differential Ca^{2+} sensitivity of myofibril function is also implicated in the pathogenesis of HCM caused by mutations in genes encoding thick and thick filament proteins. Specifically, mutations in the thin filament proteins, in contrast to those in the thick filament proteins, increase Ca^{2+} sensitivity of myofibrillar ATPase activity, and enhance maximum force generation[100, 102, 105–107]. Moreover, expression levels of the mutant transcripts and Ca^{2+} sensitivity of maximal force generation also vary at the cellular level.[86] Finally, there is considerable molecular heterogeneity, including differences in gene expression, among myocardial regions, which could influence the effects of the mutant protein on regional phenotypic expression in HCM.[108, 109] Collectively, the data suggest the presence of mosaic molecular and cellular functional defects among different myocytes and myocardial regions in HCM

Functional defects observed at the molecular and cellular levels seem to occur early and precede the development of cardiac hypertrophy.[98, 110101]. Development and progression of cardiac hypertrophy, is also associated with secondary modifications of the sarcomere proteins, including phosphorylation of TNNI3 and MYBPC3, which are known to affect sarcomere functions[106, 111]. Functional changes collectively induce biochemical, bioenergetics, or mechanical stress and lead to expression of stress-responsive genes and activation of stress-sensing signaling pathways, which mediate the subsequent downstream phenotypes (Figure 3).

Pathogenesis of the histological phenotype in HCM:

Three main histological features of HCM include myocyte hypertrophy, myocyte disarray, and myocardial fibrosis. The prevailing hypothesis posits that all three main histological phenotypes are largely the consequences of activation of the intermediary molecular pathways, ranging from stress-sensing signaling pathways to expression of trophic and mitotic factors followed by secondary gene expression, partially similar to those activated in other forms of myocardial stress and injury.[112] The extent of the histological changes often reflects the severity of the disease and is a marker for poor clinical outcomes, including risk of heart failure, cardiac arrhythmias, and SCD[113, 114].

Cardiac myocyte hypertrophy is the consequence of increased expression of trophic factors and activation of the signaling pathways involved in cardiac hypertrophy, such as the MAPK and calcineurin pathways, among others.[52, 61, 115–118] Likewise, myocardial fibrosis, which is common in HCM, results from increased expression and secretion of the mitotic factors, such as TGFβ1 from cardiac myocytes and activation of the corresponding downstream pro-fibrotic cascades in cardiac myocytes as well as fibroblasts.[116, 119] Myocyte disarray, the

pathological hallmark of HCM, is conventionally thought to result from altered myocardial architecture. Recently, however, cell-to-cell heterogeneity in gene expression, including in the expression levels of the mutant protein, and consequently, cell-to-cell functional differences among cardiac myocytes, have been advocated as a mechanism for disorganized architecture and orientation in the myocardium.[86, 109]

Pathogenesis of the clinical phenotype in HCM:

The clinical phenotype of HCM is the consequence of cardiac myocyte hypertrophy and myocardial fibrosis in the background of abnormal gene expression, including those affecting Ca^{2+} homeostasis and myocyte relaxation. Notable clinical features of HCM are cardiac hypertrophy, asymmetric septal hypertrophy, enhanced LVEF, LVOTO, HFpEF, cardiac arrhythmias, and SCD. These phenotypes are affected not only by the primary defect but also by the secondary changes that occur in the myocardium, such as PTMs of the sarcomere and the related proteins, as well as by non-genetic and external factors.

Cardiac hypertrophy is primary due to myocyte hypertrophy, as discussed earlier. Predilection of cardiac hypertrophy toward the interventricular septum, apical hypertrophy, and other forms of localized hypertrophy, a characteristic feature of HCM, likely reflects differential gene expression among myocardial regions, which is partly a consequence of differential regional wall stress, although direct evidence in support of such mechanisms in HCM is scant.[108, 109]

A notable physiological feature of HCM is an enhanced left ventricular global systolic function, as measured by the LVEF, or a hyperdynamic left ventricle, despite reduced regional myocardial strain and tissue Doppler velocities.[80, 81, 120–122]. The phenotype likely reflects the increased number of myosin molecules that are in bound state with the thin filament actin during cardiac cycle, as discussed earlier (Figure 4). Increased Ca^{2+} sensitivity of the myofilament filaments is also a major determinant of cardiac systolic function. A number of other secondary changes, such as phosphorylation of the sarcomere proteins or other PTMs are also known to affect cardiac function. These changes are extensive and involve almost all sarcomere proteins and are beyond the scope of the present review. Myocardial relaxation is commonly impaired in HCM and left ventricular compliance is reduced, indicating diastolic dysfunction, which is particularly accentuated during exercise, providing an explanation for exercise intolerance and exertional dyspnea. Impaired relaxation is a major determinant of HFpEF, which manifests in a subset of patients with HCM, particularly in those with a long-standing history of severe LVOTO. Impaired myocardial relaxation and HFpEF in part reflect the increased number of aacto-myosin complex, decreased sensitivity of the acto-myosin dissociation to cytosolic ATP, and the secondary molecular changes in the myocardium, such as impaired Ca^{2+} uptake during diastole as well as PTMs of the sarcomere proteins. Likewise, histological changes in the myocardium, such as fibrosis, also contribute to diastolic dysfunction and HFpEF.

An intriguing physiological phenotype of HCM is the LVOTO, which is present at rest in approximately a third of the patients and could be provoked in another third with maneuvers, such as Valsalva, or infusion of inotropic agents, such as dobutamine. LVOTO is considered a consequence of encroachment of the hypertrophied septum toward the outflow tract, which

along with the hyperdynamic ejection resulting in systolic anterior motion of the mitral valve leaflets, causing an obstruction to blood flow through the LVOT. Consequently, the presumed mechanisms for the expression of LVOTO in a subset of patients with HCM are regional differences in gene expression, wall stress due to hemodynamic forces, and likely the developmental anatomical variations of the interventricular septum and mitral leaflets.

Cardiac arrhythmias are common in HCM and on occasional lead to syncope and SCD. Non-sustained ventricular tachycardia (NSVT) is present in about a quarter of patients with HCM but sustained ventricular tachycardia and ventricular fibrillation are uncommon. [123, 124] Supraventricular arrhythmias, including atrial fibrillation, are also relatively common and present in about 20% of patients with HCM, particularly in those with LVOT obstruction.[125–127]. Cardiac arrhythmias in HCM are likely the consequences of a combination of changes in the biophysical characteristics of ion channels, secondary to PTMs in cardiac myocytes harboring the mutant sarcomere protein, altered myocardial architecture; such as fibrosis, and dysregulated gene expression. (reviewed in[128])

Genotype-Phenotype correlation in HCM:

A large number of genotype-phenotype correlation studies have been described, albeit mostly in small data sets, which are prone to spurious results. As a general principle, PVs with large effect sizes would be expected to influence the clinical expression of HCM more than those with small effect sizes. Besides this principle and as discussed earlier, the effect of the PV on the distant phenotypes, such as the clinical phenotype of HFpEF and SCD is relatively modest. This modest effect is in part because of the multiplicity of the factors that are involved in influencing the clinical phenotype, which through competition diluting the influence of the PVs.

The majority of patients with HCM, particularly those without LVOTO, experience a normal life span free of significant limitations or symptoms, consistent with the modest effect of the PVs on survival.[129, 130] Approximately, 5 to10% of patients with HCM, particularly those with LVOTO develop cardiac systolic dysfunction and heart failure with reduced ejection fraction (HFrEF).[131] The role of the underlying PVs in susceptibility of patients to HFrEF is unclear.

There are sex-dependent differences in the phenotypic expression of HCM, as women exhibit HCM at an older age but are at a higher risk of heart failure than men.[15, 16][17, 18] However, sex-dependent differences in the clinical expression of HCM is not related to the underlying genetic causes, as HCM is typically not an X-liked disorder. The differences are likely secondary to effects of the sex hormones on the phenotype.

The most dreadful complication of HCM is SCD, which occurs in 0.5% to 2% per year in adults with HCM.[124, 132, 133] The major risk factors for SCD include a family history of SCD, unexplained syncope, ventricular tachycardiac, including frequent NSVT, severe cardiac hypertrophy and myocardial fibrosis.[124] Although a number of initial studies suggested genotype-dependent differences in the risk of SCD in HCM, there is no consistent evidence to suggest the underlying PVs are major determinants of the risk of SCD in HCM.

GENETIC-BASED INSIGHTS INTO CLINICAL MANAGEMENT OF PATIENTS WITH HCM

The important components in the management of patients with HCM are evaluation for the risk of SCD, HFpEF, LVOTO, atrial fibrillation, and thromboembolic complications. Despite the extensive genetic heterogeneity of HCM, a similar pharmacological and surgical approaches are used in treatment of patients with HCM. Beta-adrenergic receptor blockers are the mainstay of pharmacological therapy of symptomatic patients with HCM. The L-type calcium channel blockers, such as verapamil and diltiazem are also used in symptomatic patients, including in those with LVOTO. Disopyramide, a negative inotropic agent, is used, in conjunction with beta blockers, to reduced LVOTO,[134] Diuretics are used carefully, when there is evidence of increased left ventricular end diastolic pressure, pulmonary congestion, and elevated B-type natriuretic peptide levels. Patients with atrial fibrillation require cardioversion or rate control and anticoagulation, the latter, using a direct oral anticoagulant. Those at high risk for SCD typically undergo implantation of a cardioverter/defibrillator (ICD), which is highly effective in aborting malignant rhythm and hence, by inference, SCD.[135] Surgical myectomy (marrow's procedure) and alcohol septal ablation are highly effective in alleviating LVOTO, improving symptoms, and alleviating HFpEF, regardless of the genotype.[136]

One of the fundamental contributions of genetic discovery has been partial elucidation of the molecular pathogenesis of HCM, which has led to the development of specific molecules that target the underpinning mechanisms. Mavacamten, which targets the acto-myosin interaction, imparts a dose-dependent reduction in the MYH7 ATPase activity and hence, left ventricular contractility.[8] The drug has been advanced to the phase III clinical trials and the initial results have been favorable.[137, 138] Treatment with mavacamten improved NYHA functional class and peak oxygen consumption and reduced outflow tract gradient in patients with HCM and LVOTO.[137] Likewise, it has beneficial effects on plasma levels of cardiac biomarkers in patients with non-obstructive form of HCM.[138] The effects of this drug on hard clinical endpoints such as mortality, SCD, or hospitalization because of heart failure remain to be determined. The main concern with the use of mavacamten is its negative inotropic effect, which is its main mechanism of action, and hence, the risk of inducing HFrEF. When used judiciously, mavacamten reduces LVOTO without a significant drop in LVEF.[137] The clinical use of this drug, once approved, would require individualized dose titration and close monitoring to avoid HFrEF.

There have been a number of other experimental therapies for HCM, including angiotensin II receptor blockade, 3-hydroxy-3-methyglutaryl-coenzyme A reductase inhibitors (statins), mineralocorticoid receptor blockers, and a N-acetylcysteine, none, however, has imparted notable beneficial effects in HCM patients, despite the evidence for their beneficial effects in model organisms.[116–119, 139–146] Likewise, gene therapies approaches are only at the experimental phase and are not expected to advance to clinical use in the foreseeable future.

PERSPECTIVE

Advances in the sequencing technologies, such as the development of more accurate long-read WGS approaches are expected to shift the genetic testing approach from WES to WGS and hence, enhance detection of the PVs in HCM. The shift along with interpretation of the PVs as probabilistic determinants of the phenotype in HCM are expected to elucidate a comprehensive landscape of the genetic basis of HCM and resolve the current enigma of the so-called "missing causal genes". The frequencies of the newly discovered genes and PVs in the HCM populations are expected to be low, indicating the presence of extensive genetic diversity of HCM. Likewise, the majority of the newly discovered PVs and genes are expected to exert modest to moderate effect sizes. Consequently, the phenotype in HCM will be recognized as a continuum, in one end being an archetypical single gene disorder caused by a major PV with a large effect size and on the opposite end of the spectrum an oligogenic or even polygenic trait caused by the cumulative effects of multiple PVs, each exerting a modest effect size and modifying the phenotype.

Clinical applications of genetic discoveries are expected to remain largely limited to cascade screening and the distinction of HCM from the phenocopy conditions, such as storage diseases. Genotype-phenotype correlation studies are not expected to yield consistent and robust associations, because of the numerosity of the determinants and remoteness of the clinical phenotypes to the genotype.

The model organisms, including iPSC-CMs, despite their shortcomings, would be expected to continue providing insights in to the molecular pathogenesis of HCM. Given the diversity of the mechanisms involved, one might surmise that a single therapeutic approach will not applicable to all HCM cases. Consequently, the focus is expected to shift to gene- and base-editing approaches, which are currently are at the early stages of development, inefficient, and have considerable off target effects. The success in the clinical applications of gene and base-editing will depend on fundamental discoveries to enhance the efficiency of the approach and abrogate the off-target effects. The progress, which is unavoidable, will enable the clinician-scientists to gain deep insights into molecular genetics, pathogenesis, and clinical aspects of HCM and practice Sir William Osler conviction that "the good physician treats the disease; the great physician treats the patient who has the disease"

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NONSTANDARD ABBREVIATIONS AND ACRONYMS

ACTC1   Cardiac α actin 1

ACTN2   α actinin 2

ALPK3   α kinase 3

CAV3   Caveolin 3

CNV	Copy number variant
CRYAB	crystalline αB
CSRP3	Cysteine and glycine-rich protein 3
DCM	Dilated cardiomyopathy
EF	Ejection fraction
FHL1	Four and a half LIM domain 1
FLNC	Filamin C
GoF	Gain-of-function
GV	Genetic variant
HCM	Hypertrophic cardiomyopathy
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
INDEL	Insertion/deletion (typically involving < 50 nucleotides)
JPH2	Junctophilin 2
LOEUF	Loss-of-function observed/expected upper bound fraction
LoF	Loss-of-function
LPV	Likely pathogenic variant
LVEF	Left ventricular ejection fraction
LVOTO	left ventricular outflow tract obstruction
MV	Modifier variant
MYBPC3	Myosin binding protein C3
MYH7	Myosin heavy chain 7
MYL2	Myosin light chain 2
MYL3	Myosin light chain 3
MYOM2	Myomesin 2
MYOZ2	Myozenin 2
NMD	Non-sense mediated decay
nsSNV	Non-synonymous SNV
OBSCN	Obscurin

PLN	Phospholamban
PTM	Post-translational modification
PV	Pathogenic variant
SCD	Sudden cardiac death
SNV/SNP	Single nucleotide variant/single nucleotide polymorphism
SV	Structural variant
SynSNV	Synonymous SNV
TCAP	Titin capping protein
TNNC1	Cardiac troponin C
TNNI3	Cardiac troponin I
TNNT2	Cardiac troponin T
TPM1	α tropomyosin
TRIM63	Tripartite motif containing 63
TTN	Titin
UPS	Ubiquitin-proteasome system
WES	Whole exome sequencing
WGS	Whole genome sequencing

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The author apologizes to the authors whose valuable work was not cited, simply because of the vast number of such articles in the literature. For genetic variants the readers are referred to https://www.ncbi.nlm.nih.gov/clinvar/, for expression data in all genes discussed in the manuscript to https://www.proteinatlas.org/) and for data on gene tolerance to predicted loss-of-function variants to https://gnomad.broadinstitute.org/.

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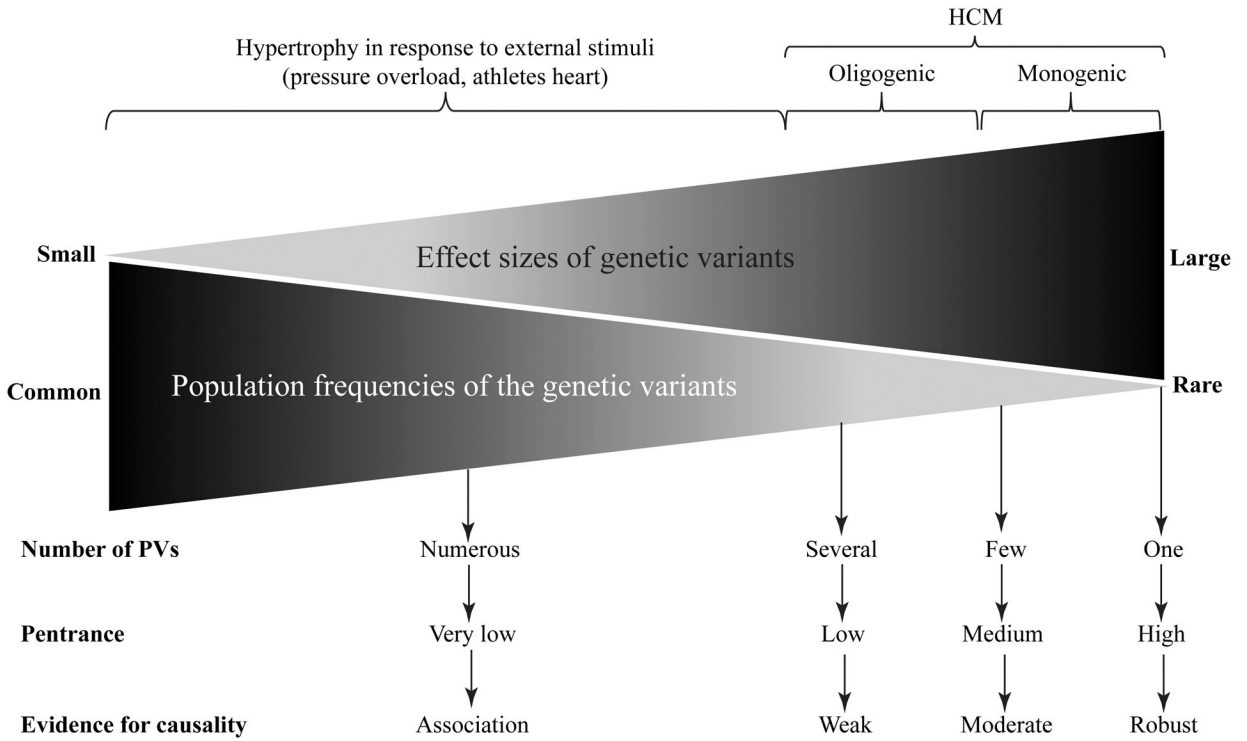**Figure 1. Gradient of effect sizes of the pathogenic variants (PVs) and their population frequencies in the context of familial and sporadic HCM**

A schematic presentation of the effect sizes of the PVs that vary from small to large. The effect size typically inversely correlates with the population frequency of the variant. Rare variants with large effect sizes are more common in familial HCM and those with moderate effect sizes are often found in the sporadic cases and in small families with HCM.
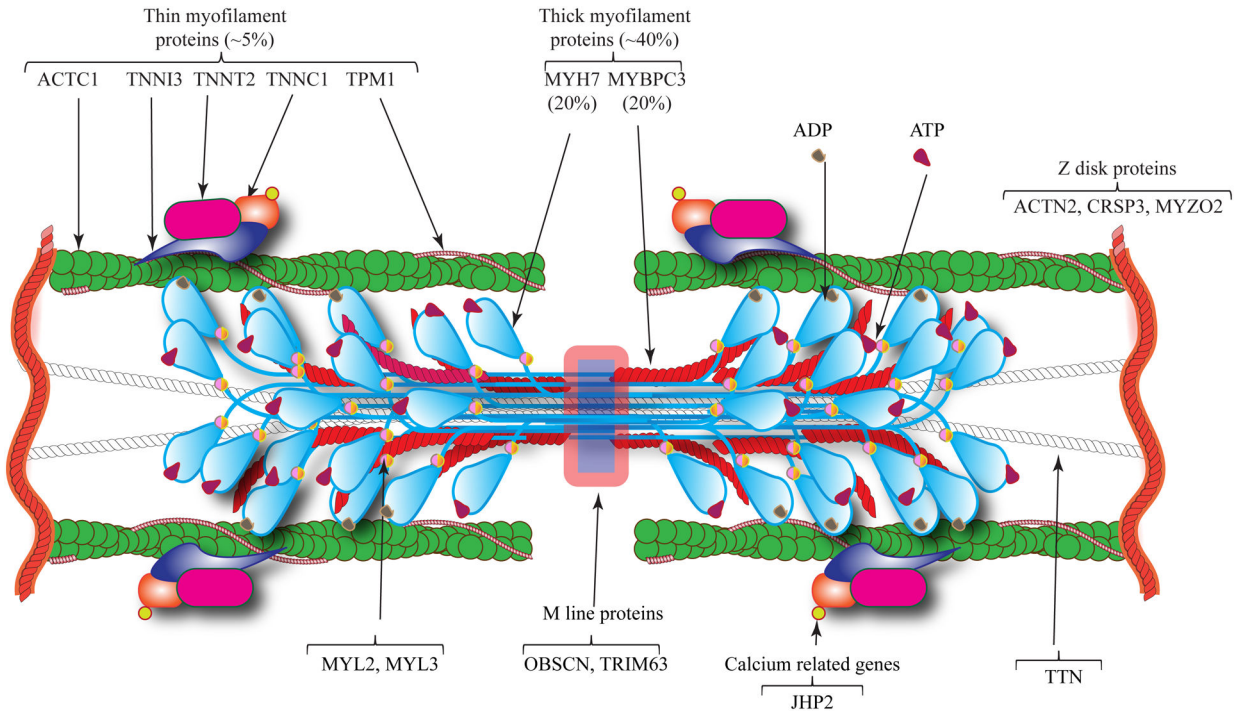
Figure 2. Schematic illustration of sarcomere proteins involved in HCM.

HCM is mainly a disease of sarcomere proteins, which are comprised of thick and thin filaments. Thick filaments are mainly composed of myosin heavy chain 7 (MYH7) and myosin binding protein C3 (MYBPC3), which are the most commonly affected proteins by mutations causing HCM. Thin filaments are comprised of cardiac α actin 1 (ACTC1), troponin complex (TNNT2, TNNI3, and TNNC1) and α tropomyosin (TPM1), which are affected by mutations in about 5% of the HCM cases. Examples of the Z and M line proteins implicated in HCM are also illustrated, which are rare causes of HCM.
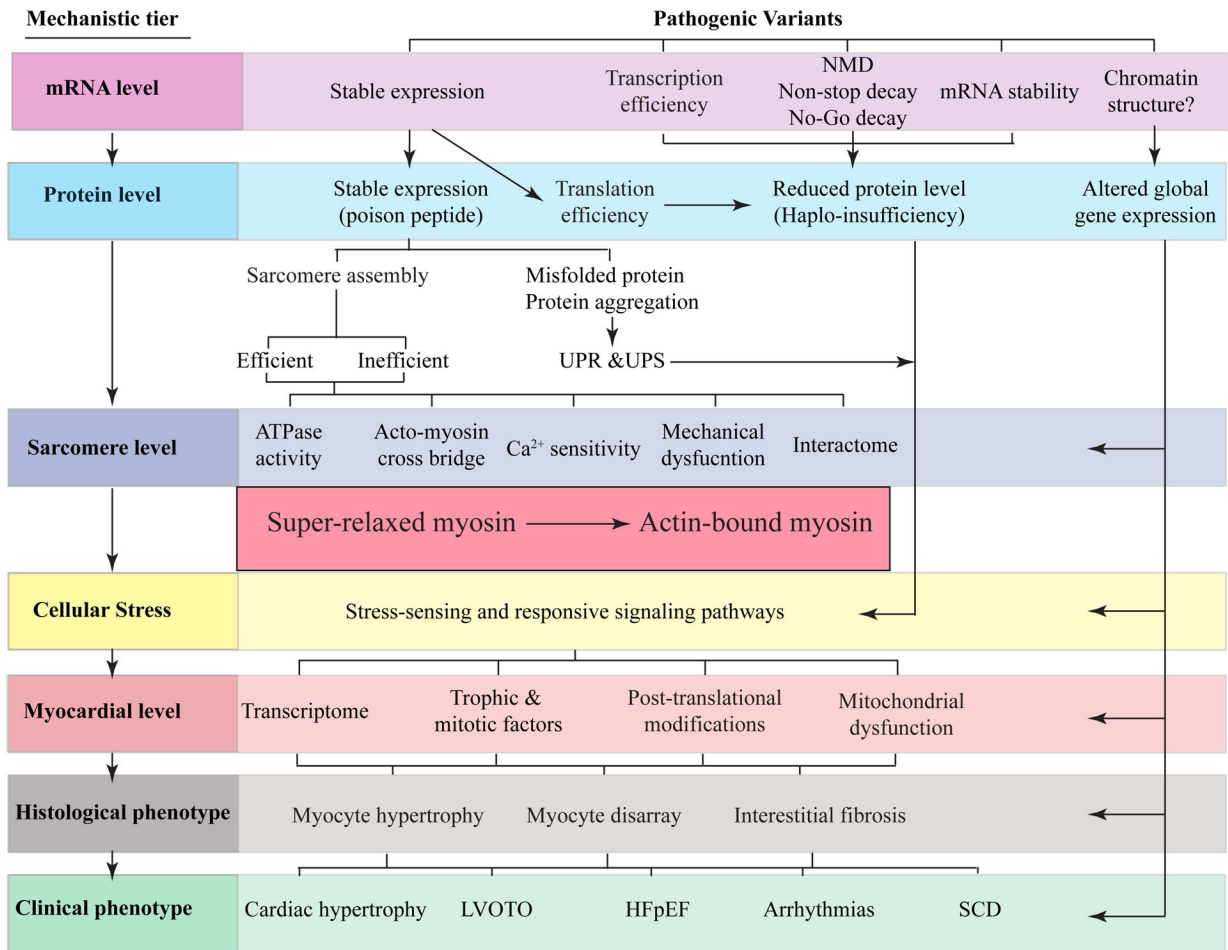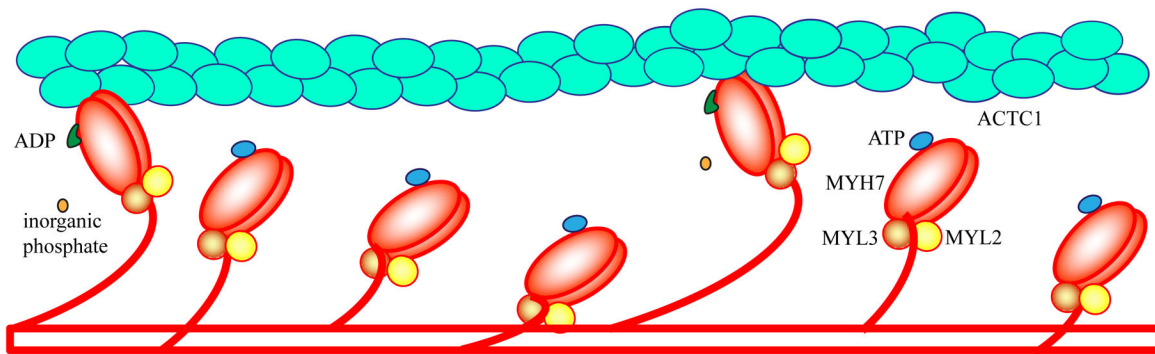
Figure 3. Pathogenesis of HCM.

The primary defect in HCM is the mutation in the sarcomere proteins, composed of thick and thin filaments, M line and the Z disks (or lines). The effects of the mutations on mRNA, protein, sarcomere, myocyte, myocardium (molecular level), histology, and clinical phenotypes are depicted as sequential layers. A change in the amino acid sequence in the sarcomere protein (dominant-negative effect) or the deficiency of a sarcomere protein (haplo-insufficiency) instigates a series of proximal functional defects in cardiac sarcomeres, such as altered calcium sensitivity and ATPase activity. A consequence of these initial defects is a shift in the number of myosin molecules in the super-relaxed state with a low ATPase activity toward the myosin molecules that are in strong bound state with the actin molecule and have a high ATPase activity. The changes activate expression of a stress-sensing (mechanical, biochemical, and energetics) intermediary molecular phenotypes, such as altered transcriptomics and expression of trophic and mitotic factors. The latter set of the molecular changes induce histological and morphological changes in the myocardium, such as myocyte hypertrophy and fibrosis, which collectively lead to the clinical phenotypes, such as cardiac arrhythmias and heart failure.
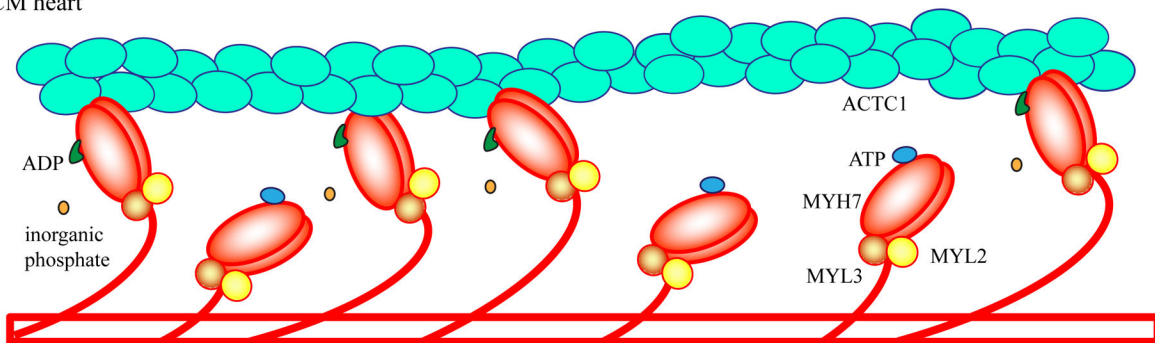
A: Normal heart

B: HCM heart

Figure 4. Impaired acto-myosin interaction in the pathogenesis of HCM
Binding of ATP to the globular head of myosin results in its dissociation from the thin filament actin, whereas hydrolysis of ATP to ADP and inorganic phosphate leads to association of the MYH7 and ACTC1 molecules. Release of the inorganic phosphate induces conformational changes in the MYH7 and flexion of the myosin globular head at lever arm over ACTC1 in the thin filament. The flexion displaces the thin filament by about 10 nm.

In the normal heart, at any given moment during diastole, only a small fraction of the MYH7 molecules (estimated to be about 10%) is bound to the actin (ACTC1) molecule. Approximately 50–60% of the myosin molecules are totally dissociated from the actin molecule and are in the super-relaxed state. The remaining myosin molecules are weakly associated with the actin filaments (Panel A). In HCM caused by mutations in the *MYH7* and *MYBPC3* genes, there is a shift from myosin molecules in the super-relax state to the myosin molecules in the strong bound state with the actin molecule (Panel B). The increased number of acto-myosin complex (strong bound state) is responsible for increased contractility and impaired relaxation.

TABLE 1

Spectrum of Genetic Variants in the Human Genome

Category	Description	Relevance to HCM
Nonsynonymous SNVs (nsSNVs)	• Change amino acid sequence • Missense variants are most common • A small fraction is non-sense • ~ 12,000 in each genome	• Most common type of mutations (PVs) identified in HCM
Insertion/deletion variants (Indels)	• Second most common variants in the genome • ~ 100,000 to 1,000,000 in each genome • Involve 1 to 50 nucleotides • ~ 110 indel in each genome maintain coding frame • ~ 40 indels in each genome result in a frame shift	• Uncommon causes of HCM • Mostly identified in the MYBPC3 gene • In-frame indels are benign but rate cause disease (such as cystic fibrosis)
Splice junction variants	• Could lead to alternative splicing by affecting canonical donor or acceptor site or their vicinity • Could introduce frame shift and lead to haploinsufficiency	• Uncommon causes of HCM • Mostly identified in the *MYBPC3* gene • Responsible for ~ 10% of human diseases
Synonymous SNVs (silent variants) (synSNVs)	• No change in amino acid sequence • Could affect transcriptional and translational efficiency • Could introduce cryptic splice • ~ 12,000 in each genome	• Unclear role in HCM
Regulatory variants	• Variants in the 5' regulatory region could affect transcriptions • Variants in the 3' regulatory region could affect mRNA stability and microRNA binding	• Unclear role in HCM
Intronic and intergenic variants	• The vast majority of the variants in the human genome • Might affect enhances • Might introduce cryptic splice sites	• Unclear role in HCM
Structural variants (SVs)	• Defined as large indels and genomic rearrangements • Involve > 50 nucleotides, typically more than 1,000, and occasionally > million nucleotides • Could affect copy number of the involved genes and hence, their expression levels • Difficult to identify by short-read sequencing	• Unclear role in HCM

Abbreviations:

SNVs: Single nucleotide polymorphism; HCM: Hypertrophic cardiomyopathy; PV: Pathogenic variant s; MYBPC3: Myosin binding protein C3; Indel: insertion/deletion

TABLE2

Categorization of the Genetic Variant for Clinical Applications in HCM

Category	Definition	Characteristics
PVs	Variants for which there is a strong genetic evidence for their involvement in the pathogenesis of HCM	• PVs are predicted to distort structure and function of the encoded proteins, often leading to premature truncation of the protein and haplo-insufficiency • Frameshift variants, such as indels and splice junction variants • Variants leading to gain or loss of a stop codon • Missense variants with a high CADD score • Rare in the general population • Typically involve genes that are intolerant to LoF variants
LPVs	As for PVs, except that genetic evidence for their involvement in the pathogenesis of HCM is less robust	• LPVs have characteristics similar to those of the PVs • Typically they involve genes that are partially intolerant to LoF variants
Functional variants with undefined clinical significance	Variants affect gene expression and/or protein function but are not associated with a clinical phenotype	• Relatively common in the genome • Functional effects at the mRNA and/or protein levels • Involve genes that are tolerant to LoF variants
Variants with unknown significance	Undefined functional or clinical significance	• Common in the genome • Often located in intergenic regions or introns
Likely benign variants	These variants are not expected to cause discernible phenotypic effects. They might have modest effects on gene expression but are not related to HCM	• Common in the general population • Are not predicted to exert functional or clinical effects
Benign variants	Variants with no discernible functional, biological, or clinical effects	• Common in the general population • These variants are expected to be inconsequential at the functional and clinical levels

Abbreviations:

PVs: Pathogenic variants; LPVs: Likely pathogenic variants; LoF: Predicted loss of function; CADD: Combined Annotated Dependent Depletion, which is a score calculated upon integration of more than 60 genomic features of single nucleotide variants and indels.