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REVIEW ARTICLE

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Abstract

Hypertrophic cardiomyopathy (HCM) is one of the most common types of inherited cardiomyopathy with a wide range of clinical manifestations ranging from subtle myocardial hypertrophy to debilitating heart failure, cardiac arrhythmias, and sudden cardiac death. We reviewed the literature on the latest knowledge regarding the pathophysiology and molecular genetic basis of HCM. This will include laboratory studies on animal models and human pluripotent stem-cell derived cardiomyocytes and the theory of proximal mechanisms involving calcium handling and energy expenditure underlying HCM. The current review will also illustrate the pathogenicity of various associated genetic variants, genotype-phenotype correlation and the optimal approach to genetic testing in HCM relevant to current practice. With technological advancements in sequencing technique and increasing availability of genetic testing in cardiology practice, a grasp of foundational knowledge on inherited cardiovascular conditions such as HCM, various sequencing techniques, interpretation of genetic testing results as well as counselling techniques become imminent to modern practice of personalized medicine.

Keywords: Hypertrophic cardiomyopathy, Molecular genetic

Introduction

Hypertrophic cardiomyopathy (HCM) is one of the most common types of inherited heart muscle disease affecting every 1 in 500 individuals [1]. It is characterized by otherwise unexplained left ventricular hypertrophy, defined as wall thickness of ≥15 mm, which is heritable from one generation to another. It is caused by mutations in the genes encoding sarcomeric proteins and in around 40−60% of cases, a definite causative gene mutation can be identified [2]. Clinical manifestations of HCM vary widely between affected individuals, ranging from subtle myocardial hypertrophy to debilitating heart failure and cardiac arrhythmias. According to recent reports, Asian population suffering from HCM were at an annual rate of cardiovascular death of 1−2% as a result of sudden cardiac death (0.5−1%), heart failure (0.5−1%) and thromboembolism (0.1%) [3−5].

Familial occurrence of HCM was first recognized in 1949 by Evans, who named the condition "familial cardiomegaly" [6]. In a case series of 8 young sudden cardiac death patients published in 1957, Teare described autopsy findings of asymmetrical septal hypertrophy and histological features of disorganized arrangement of muscle fibers which later became the hallmark of the disease. In the case series, he also described a familial link in the families of the affected individuals [7]. In 1961, Pare studied a large family of 87 members across 5 generations and illustrated a dominant non-sex-linked mode of transmission of the disease which was later recognized as the predominant mode of inheritance in HCM [8]. In 1990, Christine Seidman and Jonathan Seidman discovered the first genetic locus (14q1)

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associated with familial HCM in this large family by linkage analyses. This was later identified as myosin heavy chain 7 (*MYH7*) and marked the beginning of molecular genetic basis of HCM [9].

This review aimed to summarize the current evidence and knowledge regarding the pathophysiology and molecular genetic basis of HCM. In particular, we reviewed the proximal mechanisms underlying the pathophysiology of HCM from animal models and human pluripotent stem cell-derived cardiomyocyte studies, pathogenicity of various associated genetic variants, genotype-phenotype correlation and the optimal approach to genetic testing in HCM relevant to current practice.

We reviewed the medical literature in the PubMed database and Google Scholar, using the key terms "hypertrophic cardiomyopathy", "genetic basis", "molecular genetics" for studies published up to March 2023. There was no language restriction. Abstracts were reviewed to determine the relevance to our current review. Case reports and articles with unclear or inappropriate research methods were excluded.

Pathophysiology

Animal models and human pluripotent stem cell-derived cardiomyocyte (hPSC-CM)

Before the advent of transgenic mouse model and the recent advancement in developing hPSC-CM, studies on the mechanisms underlying HCM were largely based on surgically excised or explanted heart tissues from the unfortunate end-stage diseased patients, which to certain extent could only reveal the hallmark or the terminal results of the disease process [10,11]. These tissue models were limited in availability and were prone to preparation artefacts. Development of animal models using transgenic techniques revolutionized the study of many cardiovascular conditions. Rodents, in particularly, were extensively used to model HCM in human, allowing easily available cell and tissue samples [12]. However, such models were imperfect in recapitulating human disease mechanisms in various ways due to species differences, in addition to its time-consuming genetic manipulations. In the past decade, the development of hPSC-CM enabled studies of more detailed disease mechanisms in HCM, in particular the mechanical contractile behavior in vitro as well as genotype–phenotype relationships [13]. These advancements fostered the identification of more proximal mechanisms in the disease pathway from the initial change at nucleic acid level to the eventual clinical manifestation in HCM [14].

Proximal mechanisms—altered calcium handling and increased energy expenditure

MYH7, encoding sarcomere protein β-myosin heavy chain, and myosin-binding protein C (MYBPC3) are among the most common causative genes responsible for HCM, elucidating the genetic etiology in approximately half of the patients with familial HCM (Figure 1) [15–17]. In the majority of cases, HCM is caused by missense variants in the implicated genes, which lead to nonsynonymous changes in the amino acid sequence and hence alter the functions of sarcomeric proteins. An exception to this is MYPBC3, in which gain of stop codon or frameshift plays the major role and results in different degradation pathways. This leads to a reduction in synthesis of C protein and hence haploinsufficiency.

Brenner group laid seminal work in demonstrating that allelic imbalance, which was caused by inefficient transcription and translation of the mutant alleles, was compensated in variable degrees by the wild-type allele in different cardiomyocytes. This resulted in functional imbalance within the myocardial network [18]. The unbalanced force generation between adjacent cells led to stress-induced signaling, therefore underlying the development of interstitial fibrosis and hypertrophy in HCM [18–20]. Such variable allelic compensation, and hence a cell-to-cell functional imbalance and heterogeneity in the incorporation of mutant proteins into sarcomere, may in part explain the variability in phenotypic expression of HCM [21].

During normal muscle contraction, myosin binds to actin filaments and acts as a motor to drive the sliding of filaments past one another. Following the expression and incorporation of mutant contractile proteins into sarcomere, the encoded mutant proteins exert various effects on actin-myosin cross-bridge cycling, in particular Ca^{2+} sensitization and increased tension-dependent ATP utilization [22,23]. The resultant hyperdynamic contraction and increased energy consumption are accompanied by an impairment in cardiomyocyte relaxation due to a reduction in cross-bridge dissociation rate [24–27].

In cardiac muscles, the super-relaxed state of myosin (SRX) plays an important role in energy conservation by maintaining a low resting metabolic rate, whereas in the disordered relaxed state (DRX), ATP usage is greatly increased to generate force [28–30]. In contrast to a balanced ratio between SRX and DRX in the normal myosin molecules, a disruption of such physiological balance, for example in HCM, would alter cardiomyocyte contraction, relaxation and metabolism [31].

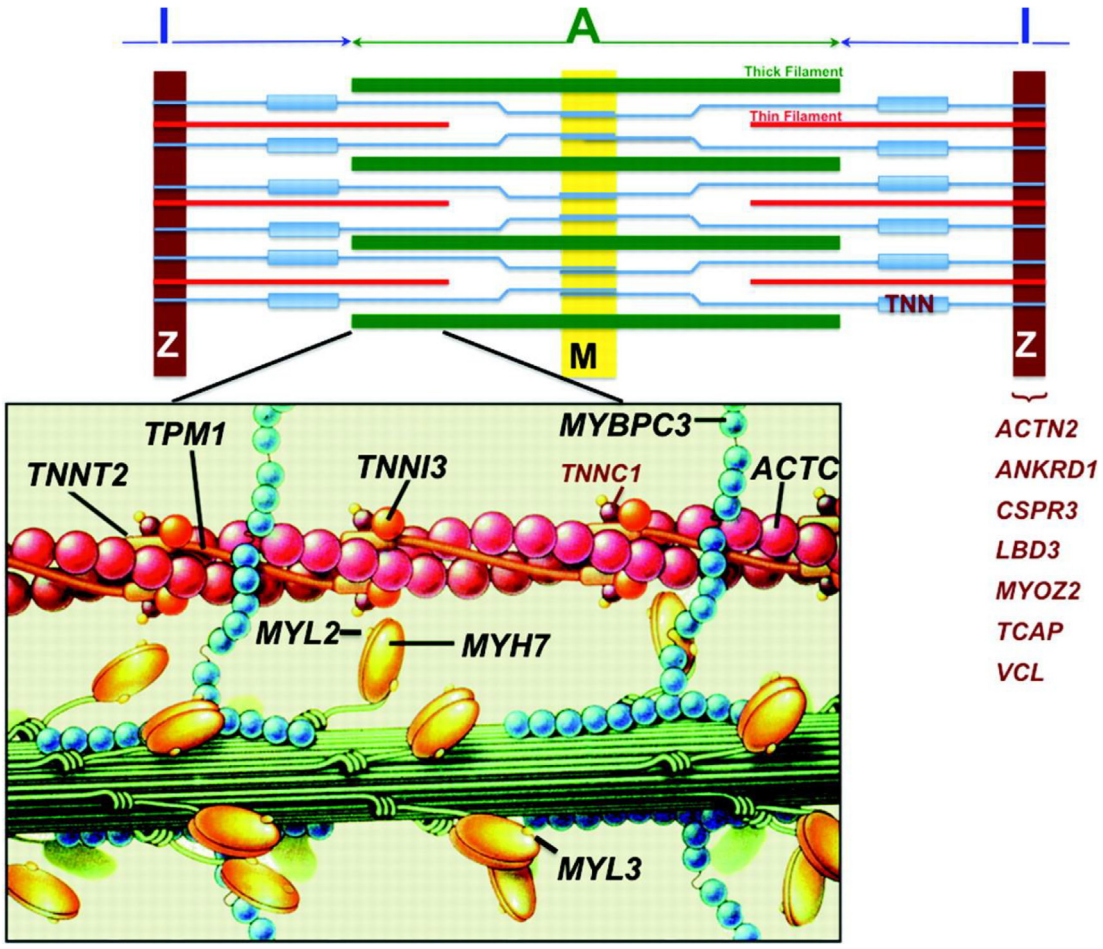

Fig. 1. Adapted from CE. Seidman, JG. Seidman, J. Robbins and H.Watkins, Identifying Sacromere Gene Mutations in Hypertrophic Cardiomyopathy: a personal history. Circ Res. 2011 Mar 18; 108(6):743–50. Accessed 3 August 2023, https://doi.org/10.1161/CIRCRESAHA.110.223834.

MYPBC3 mutation, a gene encoding proteins responsible for stabilization of super-relaxed state of myosin heads, leads to an incomplete arrest of actin-myosin cross-bridge cycling in diastole [29]. These proximal biophysical alterations were shown to precede the development of cardiac hypertrophy in transgenic rabbits, providing an insight in early mutational consequences in HCM [32].

Unlike the thick filament proteins, thin filament proteins uniquely influence the ability of cardiac troponin C to sense and respond to Ca^{2+} [33]. Within the structure of a thin filament, the addition of actin-tropomyosin to the troponin complex downregulates Ca^{2+} sensitivity and accelerates the Ca^{2+} dissociation rate from the regulatory domain of troponin C. Under resting conditions, the myosin-binding sites on the thin filament are blocked by tropomyosin. During contractions, the influx of Ca^{2+} into cytosol binds to troponin C, which triggers a series of signaling pathway and eventually unblocks the myosin-binding sites on actin [34]. Genetic variants affecting genes encoding the thin filaments, such as cardiac troponin T, troponin I and α-tropomyosin, increase myofilament Ca^{2+} sensitivity and alter ATPase activity, consistent with consequences of other HCM mutations [35–39].

These proximal defects in the pathophysiological pathway are fundamental to our understanding in the development of cardiac hypertrophy as well as other morphological and histological phenotypes in HCM via the actions of a series of activated stress-responsive trophic and mitotic factors such as the calcineurin and transforming growth factor β pathways, among others [40–45].

Genetic basis of HCM

Christine Seidman and Jonathan Seidman identified the p. Arg403Glu (pR403Q) mutation in the

MYH7 gene as the first mutation in HCM(9). This pivotal discovery nucleated a genetic repertoire of HCM and identified seven more core sarcomeric genes implicated in the development of HCM with mutations in *MYH7* and *MYBPC3* being the most common [46−54]. Table 1 illustrates the list of core sarcomeric genes, associated proteins and functions, and the predominant mechanisms of mutation causing HCM. Classically, HCM is a predominantly autosomal dominant Mendelian disorder with incomplete and age-related penetrance [55,56]. Variability in penetrance as well as phenotypic expression were observed even within HCM families carrying the same gene, hence speaking for the presence of other control mechanisms such as modifier genes and environmental factors [57]. In fact, following the discovery of the first HCM causal gene in 1990, there was an exponential increase in the number of proposed genes that were reported to be causative in HCM, up to 64 genes by 2016. Many of these genes were reported based on the fact that such variants were absent in the controls without other supporting evidence [58]. This highlighted the significance of a systematic assessment for the pathogenicity of genetic variants.

Pathogenicity of genetic variants

Genetic variants occur as a consequence of DNA replication errors and these errors introduce a certain number of *de novo* variants in every individual genome. Kong studied the genome-wide mutation rates by sequencing entire genomes of 78 Icelandic trios and found an average *de novo* variants of 1.20×10^{-8} per nucleotide per generation [59]. Palamara described a similar finding after studying 498 Dutch trios and found an estimate of 60 *de novo* variants per genome [60]. Common variants, defined as minor allele frequencies (MAF) > 5%, are usually shared across populations whereas rare variants, defined as MAF <1%, are restricted to certain populations [61]. The most common type of variant is single nucleotide polymorphism, followed by small insertion/deletion.

Traditionally, family-based studies were used to assess the pathogenicity or disease causality of Mendelian variants by demonstrating segregation of mutant alleles with clinical phenotypes. The benefit of these studies lies in the similarity of genetic background between studied subjects and hence their ability to control for some genetic confounding. However, there are also disadvantages with family studies and one of them is the difficulty and high cost in identifying, recruiting and enrolling the entire pedigrees for investigation [62]. In silico prediction of variant has provided an alternative instrument in studying pathogenicity by determining the anticipated effects of genetic variants based on computer simulations and model analysis [63]. An example is the effect on polarity, charge and hydropathy of the involved amino acid, and hence the mRNAs and proteins. However, this is still limited to experiments performed in virtual environments, which might not necessarily reflect the in-vivo biology in human.

Population frequency has become a useful criterion in gauging the pathogenicity to genetic variants. Theoretically, a genetic variant with significant deleterious effects on the phenotype would eventually be eliminated via natural selection, hence being less transmissible in the population. Loss-of-function (LoF) variants, such as non-sense, gain of stop codon, splice-site and frameshifts, typically interrupt protein coding function of the involved genes, leading to formation of unstable truncated proteins. These variants are expected to have low occurrence in the general population given the deleterious effects. However, some LoF variants can be tolerated in healthy individuals and hence are commonly found in general population [64−66]. Thus, population frequency, to a certain extent, provides some clues to the pathogenicity of the variants. By analyzing the population databases of exome and genome data, many variants that were previously claimed to be causative in HCM were in fact likely benign in nature given their relatively high population frequencies [67−69]. Clinical Genome Resource (ClinGen) program thus represents an example of consorted efforts among public and private, academic and non-academic institutions in pooling together scientific resources on variant data and annotations [70]. Ingles evaluated and curated 57 frequently tested genes in HCM and found only 8 genes were categorized as definitive (*MYBPC3, MYH7, TNNT2, TNNI3, TPM1, ACTC1, MYL2* and *MYL3*). 3 genes had moderate evidence (*CSRP3, TNNC1* and *JPH2*) and 22 had limited or no evidence. Among the remaining 24 syndromic genes, 12 genes were definitely associated with hypertrophic phenotype [58].

The ACMG/AMP guidelines establish the standard for interpretation of variants, which serve as the basis for evaluation of specific diseases. It recommends standard terminologies by categorizing variants into “pathogenic”, “likely pathogenic”, “uncertain significance”, “likely benign” and “benign”. Conceivably, when being applied by majority of laboratories, interpretation of variants would rely primarily on the availability of adequate variant-specific information for classification. A lack

Table 1. Eight core sarcomeric genes implicated in hypertrophic cardiomyopathy.

Genes Encoding Thick Filament Proteins of the Sarcomere					
Gene	Protein structure	Protein function	Genetic locus	Predominant mutation	Remarks
MYH7	B-myosin heavy chain	Carry ATPase site	Chromosome 14	Missense	Responsible for 30% of HCM cases Can also cause dilated, non-compaction and restrictive cardiomyopathy [71–73]
MYBPC3	Myosin binding protein C3	Assemble and stabilize thick filaments Regulates actin-myosin cross-bridges	Chromosome 11p11.2	Splice-site, indels and nonsense	Responsible for up to 40% of HCM cases Homozygous or compound heterozygous truncating *MYBPC3* mutations cause severe neonatal non-compaction cardiomyopathy and septal defects [74]
MYL2	Myosin regulatory light chain 2	Regulates cardiac myosin cycling kinetics, torsion and function	Chromosome 12	Missense	Responsible for <1% of HCM cases Largely tolerant to missense and LoF variants
MYL3	Myosin essential light chain 3	Modulate myosin crossbridge kinetics	Chromosome 3	Missense	Requires cosegregation, context of population frequency and functional data to assess pathogenicity
Genes Encoding Thin Filament Proteins of the Sarcomere					
TNNT2	Cardiac troponin T	Make up troponin protein complex to regulate muscle contraction	Chromosome 1q	Missense	Responsible for <5% of HCM cases Can also cause dilated cardiomyopathy [75,76]
TNNI3	Cardiac troponin I		Chromosome 19q13.4	Missense	Responsible for <5% of HCM cases Can also cause restrictive cardiomyopathy [73,77] Partially intolerant to missense and LoF variants
TPM1	Alpha tropomyosin	Binds to actin filaments	Chromosome 15	Missense	Responsible for <5% of HCM cases Can also cause dilated cardiomyopathy [78]
ACTC1	Alpha actin cardiac muscle 1	Major constituent of the thin filaments	Chromosome 15q14	Missense	Rare causal gene for HCM Among the least tolerant genes to missense and LoF variants

of such information thus translates into under-calling of pathogenicity and generation of "variants of uncertain significance". This is particularly relevant to HCM as many variants are considered private. On the other hand, missense variants, being the predominant mechanism of mutation in most sarcomeric genes, are intrinsically more difficult to interpret than LoF variants. Hence knowledge sharing between different laboratories and centers through databases such as ClinVar is critical.

Optimal approach to genetic testing in HCM

Targeted gene panel is currently the most adopted approach to genetic testing for HCM in clinical practice. With the advancement in sequencing technologies, whole-exome and whole-genome approaches are anticipated to be increasingly affordable, and perhaps more cost effective in the future. Yet, a recent study, as part of the MedSeq Project, compared the use of multigene panels with whole genome sequencing (WGS) on the detection rate of HCM gene variants. They found that WGS failed to identify all variants detected by targeted panel due to low coverage but were able to identify other gene variants implicated in cardiomyopathy which were not included in the gene panel [79]. Although WGS harbors the potential to increase the yield of identifiable causative genes, with the flexibility of revisiting the achieved genetic data in the future, adequate coverage of focused variants should be optimized. A virtual panel testing approach is a viable option to restrict analyses to genes that can yield interpretable variants, or genes that are actionable, preserving the flexibility for future data reanalysis. Another advantage of WGS over gene panel approach is the study of copy number variants (CNV) and non-coding regions such as introns. This is of relevance to HCM as several studies have implicated these mechanisms in HCM, though in rare occasions [80−82]. However, the literature evidence regarding mechanisms of copy number variants and non-cording regions in causing HCM was scarce and has not been systematically studied. The added benefit of such analysis in the setting of HCM may be low and may not outweigh the increased cost and complexity of incorporating it into routine diagnostic testing.

Genotype-phenotype correlation

Following the discovery of numerous HCM causal genes, there was an enthusiasm in the attempt to correlate genotype information with phenotypic expression and natural history of HCM. Mutations in the genes encoding thin filament proteins, such as *TNNT2* and *TNNI3*, demonstrated relatively milder hypertrophy but a higher incidence of advanced heart failure, compared with the thick filament group, supporting the notion that thin filament HCM is phenotypically distinct from the more common thick filament HCM [83−86]. In thin-filament HCM, the odds of developing new systolic dysfunction were twice the incidence in thick-filament HCM, at approximately 2.5% per year. On the other hand, development of restrictive physiology with preservation of systolic function was also more prevalent in thin-filament HCM, at a rate of 16% after 5 years in a study, leading to a more severe diastolic dysfunction and high incidence of moderate to severe atrial dilatation [84]. This was consistent with the nature and function of cardiac troponin proteins which regulate the dissociation of actin-myosin cross-bridge, aligning with other collateral evidence that troponin I mutations occasionally cause primary restrictive disease [73,77]. These adverse remodeling processes in thin-filament HCM constitute a considerable prevalence of moderate to severe heart failure symptoms in this cohort, with 15% of clinically quiescent patients progressing into NYHA class III or IV symptoms after 5 years, 3 times the prevalence in thick-filament HCM. In contrast to earlier reports of high sudden death risk in thin-filament HCM, recent studies reported a comparatively lower rates of malignant ventricular arrhythmias, sudden cardiac arrest or death, and appropriate ICD interventions, which were not significantly different between thick- and thin-filament HCM [83,84,87]. The observed patterns of hypertrophy were also different in that thin-filament disease usually resulted in hypertrophy in apical or concentric pattern, compared with classical asymmetrical septal hypertrophy often seen with thick-filament disease. As a result, the well-known dynamic left ventricular outflow tract obstruction was less commonly seen in thin-filament HCM due to the morphological differences [84].

Apart from the distinct phenotypic differences between thick- and thin-filament HCM, substantial heterogeneity also existed among patients carrying mutations in the same causal gene [88]. Earlier reports attempted to link individual genotype information precisely with the clinical manifestations and outcomes. For example, a report of a three-generation Chinese familial HCM family suggested that the Arg453Cys mutation in *MYH7* was associated with a malignant clinical course with high risks of sudden death and end-stage heart failure [89]. In another report, Met877Ile mutation in the same

gene was mapped in one family with high degree of penetrance, affecting three of the four members across two generations [90]. This novel mutation was associated with low to severe degree of hypertrophy, mid-ventricular as well as septal hypertrophy, obstructive and non-obstructive left ventricular outflow tract, suggesting considerable phenotypic variation within families carrying the same mutation. Similar heterogeneity was also observed in reports from different ethnic groups and many of these mutations were private [90,91]. The observation that mutations in the same gene, for example *MYH7*, led to a variety of clinical phenotypes such as dilated, non-compaction and restricted cardiomyopathy added to the limited predictability of natural history from individual genotype [71–73]. Mutations in *MYBPC3* also showed similar heterogeneity in terms of age at diagnosis, pattern of hypertrophy and prognosis [92]. Thus far, limited correlation between genotype and phenotype could be found as huge phenotypic variability were seen among patients harboring different causal mutations, as well as family members carrying the same mutation [93]. The presence of other modifier gene variants, gene dosage effects and environmental factors, such as concomitant hypertension, likely contribute to disease pathogenesis.

Sarcomeric-negative HCM

Depending on the source of literature, the positive yield of genetic testing for pathogenic sacromeric gene variants in HCM ranged from 30 to 60%. However, a negative result could not exclude a diagnosis of HCM which essentially is a clinical diagnosis. Furthermore, a negative result could not exclude the nature of a genetic condition. For example, if the causative genetic variant is undiscovered or uncovered in the sequencing panel, one would yield a negative result. Hence, the inclusion of genetic variants leading to HCM phenocopies such as rasopathies, mitochondrial diseases, neuromuscular diseases, metabolic and storage diseases might further increase the positive yield of genetic testing. Moreover, as knowledge in cardiovascular genomics accumulates, growing evidence demonstrated a polygenic nature in many cardiovascular diseases such as obesity and atrial fibrillation. In contrast to monogenic disorders which were commonly known to have causative genes which were rare in the population and highly penetrant with large genetic effect, polygenic disorders were shown to be caused by multiple variants, each of which was common in the population with low penetrance and small genetic effect. As a result, the more variants an individual acquired, the stronger predisposition to these abovementioned conditions was implied [94]. And such polygenic nature in pathogenicity of diseases has also been discussed in HCM [95].

Recently, studies have shown a distinctly different outcome in such cohort compared with sarcomeric HCM. Patients with sarcomeric HCM were shown to be younger at diagnosis, with a higher prevalence of family history of HCM and sudden cardiac death, as well as maximal left ventricular wall thickness and overall cardiovascular death. Similar associations were observed when different sarcomeric genes were being compared [96]. These findings were consistent with analyses from the Sarcomeric Human Cardiomyopathy Registry (SHaRe) [97] which showed a lower incidence of heart failure, atrial fibrillation and stroke in sarcomeric-negative HCM. Given a lower familial recurrence rate in this group, a different approach to family cascade screening should be adopted. On the other hand, given that environmental factors likely play a role in addition to the proposed polygenic mechanism in this cohort, a different management approach has been proposed, for example the control of hypertension [98].

Future directions

With technological advancements in genetic testing alongside a deeper understanding of various inherited cardiovascular conditions, the availability as well as utilization of genetic testing will become a standard of care in modern era of personalized medicine. A grasp of foundational knowledge on basic genetic and genomic disorders, various sequencing techniques, interpretations of genetic testing results as well as delicate counselling techniques become a foreseeable necessity in cardiovascular practice of today. An expanded utilization of genetic testing in patients with hypertrophic cardiomyopathy opens a window for research opportunities of more granular data, for example genotype-phenotype correlations and risk stratifications. Such research effort would be of significance in the local population and instrumental to personalized approach to management of patients with hypertrophic cardiomyopathy.

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Conflicts of interest

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