Genetic therapies in hypertrophic cardiomyopathy

A. Genome editing
B. Gene replacement
C. Allelic-specific silencing
D. Modulation of signaling pathway

Legend:
- Genome editing
- Gene replacement
- Allelic-specific silencing
- Modulation of signaling pathway

Bar chart showing the number of trials from 2010 to 2023.
Gene therapy in cardiology: is a cure for hypertrophic cardiomyopathy on the horizon?

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Short Title: Gene therapy in hypertrophic cardiomyopathy

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ABSTRACT

Hypertrophic cardiomyopathy (HCM) is the commonest genetic cardiomyopathy world-wide, affecting approximately 1 in 500 individuals. Current therapeutic interventions comprise lifestyle optimisation, medications, septal reduction therapies and rarely cardiac transplantation. Advances in our understanding of disease-causing genetic variants in HCM and their associated molecular mechanisms have led to the potential for targeted therapeutics and implementation of precision and personalised medicine. Results from pre-clinical research are promising and raise the question of whether cure of some subtypes of HCM may be possible in the future. This review provides an overview of current genetic therapy platforms including 1) genome editing 2) gene replacement 3) allelic-specific silencing and 4) signalling pathway modulation. The current applicability of each of these platforms within the paradigm of HCM is examined, with an update on current and emerging trials in each domain provided. Barriers and limitations within the current landscape are also highlighted. Despite recent advances, translation of genetic therapy for HCM to clinical practice is still in early development. In realising the promises of genetic HCM therapies, ethical and equitable access to safe gene therapy must be prioritised.

BACKGROUND
Hypertrophic cardiomyopathy (HCM) is the commonest genetic cardiomyopathy world-wide, estimated to affect approximately 1 in 500 individuals (1). It is a primary myocardial disorder in which hypertrophy occurs in the absence of abnormal loading conditions (2).

HCM is a prototypic condition in terms of demonstrating the value of multimodality cardiac imaging and histopathological assessment (Figure 1). Clinically, HCM is very heterogeneous, and may result in no discernible symptoms, left ventricular outflow tract (LVOT) obstruction, arrhythmias, heart failure or sudden death (3). It is also highly genetically heterogeneous. Approximately 30% of HCM is due to mutations in sarcomere genes, eight of which have been defined as pathogenic to date (4). A further approximately 30% of individuals have genetic variants of uncertain significance (VUS) which may or may not be upgraded in the future to being identified as pathogenic (5). In the remainder of individuals, the cause of HCM is unknown and may occur in the absence of any suggestive genetic variants or family history (6), reflecting potentially non-Mendelian causes. Increasingly, HCM is appreciated as a spectrum of disease ranging from a smaller number of patients with monogenic disease-causing variants to a larger number of patients who may have multiple oligogenic variants promoting abnormal hypertrophy (7).

HCM was previously reported to be the most common cause of sudden cardiac death in the young (8). Advances in cascade testing of family members and implantable cardioverter defibrillator (ICD) insertion over recent decades have resulted in HCM becoming a rarer cause of contemporary sudden cardiac death (9, 10). New and emerging advances in gene therapies now offer promise that genetic cardiology may become the ‘new interventional cardiology’, wherein it will be possible to genetically intervene to cure HCM-related symptoms and even the pathology itself. This review describes the current state of therapy for HCM, with a detailed exploration of the mechanisms of genetic therapies, as well as current stages of development and challenges in progress.
1. CURRENT LANDSCAPE OF HCM THERAPIES

HCM therapies currently available can be broadly defined as lifestyle interventions, medical therapies and interventional procedures.

Lifestyle Measures

Adoption of a healthy lifestyle is particularly pertinent in HCM and may result in a more favourable HCM phenotype. In particular, the avoidance of uncontrolled hypertension and obesity are important to minimise additional abnormal loading conditions that may worsen the HCM phenotype. Hypertension may increase septal thickness and worsen diastolic dysfunction (11), while obesity is associated with higher left ventricular outflow tract (LVOT) gradients (12).

People with HCM were previously cautioned against participation in exercise, due to concern regarding worsening of their dynamic LVOT gradient with increased cardiac output. Recent studies have provided reassurance that exercise may play an important role in HCM management, particularly as part of a healthy lifestyle (13, 14). Regular mild and moderate exercise is associated with improved quality of life in people with HCM, while large population studies have demonstrated surprisingly lower cardiovascular mortality in higher bands of self-reported physical activity (15). Even vigorous exercise, once considered contraindicated in HCM, is increasingly accepted. An analysis of 699 patients with HCM undertaking regular vigorous exercise did not demonstrate any increase in rate of death of ventricular arrhythmias compared to controls; a highly reassuring finding (16).

Medical therapy

Rate-reducing agents such as beta-blockers and non-dihydropyridine calcium channel blockers may offer symptomatic benefit by increasing diastolic filling time and decreasing contractility to ameliorate LVOT obstruction in those with obstructive HCM (17). Beta-blockers may also offer prognostic benefit through anti-arrhythmic mechanisms (18). Disopyramide, a sodium-channel antagonist with negative dromotropic effect, is recommended as an add-on therapy for patients with refractory symptoms (2). Diuretics may relieve congestion in the setting of diastolic dysfunction and high filling pressures to provide symptomatic relief of dyspnoea.

Most recently, novel myosin inhibitors such as Mavacamten represent the first generation of HCM-specific therapies, that have been demonstrated to improve both subjective and objective symptoms of dyspnoea in two large trials (19). It is a cardiac-specific molecule that modulates the β-myosin heavy
chain directly to reduce actin-myosin affinity (2, 20). It has not yet been tested in subgroups or in specific genotypes.

Interventional therapies

(i) Prevention of SCD: ICDs are recommended in people with HCM judged to be at high risk of sudden cardiac death. This risk estimation is achieved using a guideline risk calculator (most commonly the European Risk-SCD or American ACC/AHA Risk Calculators (21)) which assign weights high-risk features including septal thickness, presence of non-sustained ventricular tachycardia and family history. The implantation of ICDs in high-risk people with HCM has substantially lowered HCM-related mortality (22).

(ii) Septal reduction therapies:

a. Septal myectomy may offer both symptomatic relief and prognostic benefit with patients with marked septal thickness and LVOT obstruction (23). This is performed as an open cardiac surgery, and typically offered to younger patients. Indications to perform septal myectomy are accepted to be LVOT gradient >50mmHg at rest or with provocation, and heart failure symptoms refractory to medical therapy (24). Extended myectomies involving papillary muscle resection and mitral valve repair may offer superior results (25).

b. Alcohol septal ablation is an alternative septal reduction therapy, performed percutaneously and usually in older patients. Septal perforator arteries are injected with alcohol as a sclerosant, causing controlled infarction, with subsequent regression of the hypertrophic septum (26). It is recommended to be performed when LVOT gradient exceeds 50mmHg at rest or with provocation, and heart failure symptoms refractory to medical therapy are present (25). While alcohol septal ablation is associated with higher rates of arrhythmia, scar and pacemaker dependency compared to septal myectomy, it may have excellent results in high-volume centres (26).

(iii) Cardiac transplantation:

Some patients may rarely be refractory to all existing medical and interventional therapies and require cardiac transplantation. Transplantation is typically performed in HCM for end-stage heart failure but may more rarely be performed for refractory arrhythmias (27). Patients with HCM comprise 1.6% of patients listed for heart transplantation in the United States, with absolute numbers increasing over recent years (28). Survival following cardiac transplantation is generally high, and comparable to post-transplant survival in other cardiomyopathies (27, 28).
Despite this range of existent therapies, ultimately the clinician’s current choice is between symptomatic management or therapies that offer prognostic benefit but not cure. The next frontier in managing HCM may lie in the promise of genetic therapies.

2. GENE THERAPY – THE NEW FRONTIER?

Gene therapy is a promising advanced medical therapy which focuses on the genetic modification of cells with therapeutic intent. It relies on the transfer of nucleic acids (deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) to target the molecular mechanism of a disease.

The journey of gene therapy began over a century ago with the introduction of the term "gene" by the Danish botanist, Wilhelm Johannsen (29). Subsequently, the term “genetic engineering” found its first usage in the 1930s, and it was during the 1960s that the fundamental principles of gene transfer in bacteria came to light (30). Recombinant DNA techniques enabled scientists to incorporate preferred therapeutic genes into engineered vectors. Upon discovering viruses' capability to transfer genetic material, viral vectors arose as an efficient instrument for facilitating gene therapy (31).

The first human gene therapy trial was performed at the National Institutes of Health (NIH), USA, in 1989, providing evidence that human cells could be genetically altered and safely reintroduced to a patient (32). However, initial enthusiasm surrounding the potential of gene therapy was dampened by the death of Jesse Gelsinger in 1999, an 18-year-old who died of multi-organ failure four days after receiving gene replacement therapy with an adenoviral vector encoding a normal copy of ornithine transcarbamylase gene. His death was determined to have been caused by an overwhelming inflammatory response to the viral vector, subduing scientific enthusiasm for a period (33). Subsequent continuous refinement of gene transfer technologies, coupled with greater expertise in orchestrating clinical gene therapy trials and recent breakthroughs in gene therapy trials targeting monogenic immune, retinal, and neurological disorders have revitalized the anticipation surrounding gene therapy’s potential (34).

As of July 2023, a total of 26 gene therapies have received approval worldwide and there are 2,075 gene therapies in various stages of development (35). Gene therapy studies addressing cardiovascular diseases are comparatively limited, with only 55 studies in the overall pipeline and merely 12 studies in the clinical phase (35). HCM is, however, a prototypic candidate cardiovascular disease for trialling the power of gene therapy.
3. GENE THERAPY PLATFORMS

Gene therapies may be deployed through a range of therapeutic approaches, predominantly informed by the molecular basis of the target disease. These platforms encompass genome editing, gene replacement, allelic-specific silencing and the modulation of signalling pathways (Figure 2).

3.1 Genome editing

Genome editing has recently gained more attention due to the emergence of clustered, regularly interspaced, short palindromic repeats (CRISPR)-associated Cas9 (CRISPR/Cas9) nuclease adapted from the bacterial immune system which offer exciting potential for implementation of genetic therapies. Cas9 endonucleases can be programmed to generate DNA double-strand break at a specific sequence by utilizing an engineered guide RNA (gRNA) (36). This DNA break then can be repaired through non-homologous end joining (NHEJ) or homology directed repair process (HDR). In most circumstances, NHEJ is more efficient than HDR because NHEJ remains active throughout approximately 90% of the cell cycle and does not rely on the availability of a nearby homologous donor (37). NHEJ has the capacity to insert or delete genetic sequences randomly at the sites of cleavage. This results in the formation of frameshift mutations or premature stop codons within the open reading frame (ORF) of the target genes (38). In contrast, HDR can achieve accurate modifications in the genome at the target site by employing a homologous DNA repair template (39). Using these mechanisms can allow direct ‘editing’ of the genome to alter disease-causing variants, such as those identified in HCM.

Although CRISPR/Cas9 has been shown to have excellent genome editing capabilities, certain challenges remain to be addressed, including the possible occurrence of off-target effects, the constrained genome-targeting range dictated by protospacer adjacent motif (PAM) sequences, and issues related to efficiency and specificity (40). As a result, many research teams have been trying to optimise this tool by developing adaptive features including dead-Cas9, base editing, Cas9 variant, RNA editing, and prime editing systems (41).
3.2 Gene replacement

Gene replacement therapy is primarily used when a mutation leads to a deficiency or complete absence of the protein, as in cases of haploinsufficiency conditions or recessive loss-of-function. The goal of gene replacement therapy is to introduce a working gene copy that can be translated into a functional protein, replacing a defective counterpart. Gene replacement appears highly promising; of 26 currently-approved gene therapies, seven employ gene replacement strategies (35).

While its applicability is evident in the context of recessively inherited pathologies, particularly in the case of less common causes of infiltrative cardiomyopathy, (42) gene replacement remains pertinent in the context of HCM. Although gene replacement might not directly correct dominant-negative or gain-of-function mechanisms, introducing a wild-type gene copy can still lead to the reduction of the endogenous mutant allele’s activity or, at the very least, enhance the proportion of normal transcripts in autosomal dominant disease. As a result, gene replacement strategy plays a role as a disease-modifying manoeuvre within the clinician’s armamentarium of therapies against HCM.

3.3 Allelic-specific silencing

RNA interference is a naturally occurring molecular phenomenon, whereby microRNAs and certain long noncoding RNAs exert their effects with remarkable precision by forming complementary base pairs with their target RNAs (43). This binding leads to the suppression of the expression of a specific allele. Scientists have harnessed this natural process and integrated it into the realm of gene therapy.

Allelic-specific silencing is frequently employed in instances where one allele of a gene carries a mutation responsible for a genetic disorder, while the other allele retains its functional status. The primary objective of this approach is to selectively address the mutated allele causing the disease, while leaving the healthy allele unaffected. This is accomplished by designing small interfering RNAs (siRNAs), with sequences that exploit the sequence difference between mutant and normal alleles (43). Upon introduction into cells, these molecules bind to the mutated mRNA, prompting its degradation and effectively diminishing the production of the abnormal protein associated with the mutation, while leaving the normal transcript untouched.
3.4 Modulation of signalling pathways

The last contemporary therapeutic approach involves modifying signalling pathways or biological processes that play a crucial role in the pathogenesis of the disease. This strategy resembles the mechanisms underlying many small molecule therapies (44). However, in contrast to small molecules, gene therapy techniques could potentially broaden the range of processes that can be targeted or enable the inhibition of specific cell types, which might be challenging to achieve using small molecule interventions (44). This may enable more personalised care with fewer side-effects. Within the cardiovascular realm, the majority of gene therapies aimed at addressing acquired heart failure utilise genetic signalling pathway-modulation (45).

4. FEASIBILITY OF GENETIC THERAPIES IN HCM

4.1 Genome editing in HCM

For patients with HCM, gene therapy offers hope for ameliorating symptoms and extending lifespan towards normal (Figure 3). For over a decade, scientists have been actively investigating potential molecular targets. The discovery of CRISPR/Cas9 for genome editing led to a paradigm shifting development in HCM gene therapy. The first glimpse of CRISPR/Cas9 genome editing's potential emerged through controversial studies involving human embryos. In these experiments, co-injecting human sperm carrying a 4-base pair pathogenic MYBPC3 deletion with Cas9 genome editing machinery into oocytes possessing a normal MYBPC3 allele yielded 72% of embryos with two normal MYBPC3 alleles (46). While ethical and safety concerns remain unaddressed, this research demonstrated the formidable capacity of CRISPR/Cas9 as a tool for treating hereditary diseases by correcting the genome. Since then, gene editing has also provided a deeper understanding of HCM pathophysiology. Several studies demonstrated how the utilization of CRISPR/Cas9 genome editing enabled the effective creation of HCM disease models and the accurate rectification of genetic anomalies in patient-specific HCM human induced pluripotent stem cells (hiPSCs) (47-49).

Three important preclinical studies were recently published focusing on gene-editing-based therapies to treat HCM (50-52). The first two studies adopted different but complementary strategies involving CRISPR–Cas9 adenine base editing (ABE) techniques. Their aim was to address the missense variant in the MYH7 gene (c.1208G>A; p.Arg403Gln), known for its dominant-negative mechanism, necessitating allele correction or elimination for effective therapeutic effect. In the first study (50), the authors identified an optimal variant ABE and guide RNA (gRNA) combination, ensuring on-target editing of
the variant while minimizing unintended target effects. They employed a humanized mouse model featuring the human MYH7 c.1208G>A variant, enabling direct evaluation of gRNAs designed for human applications. In this model, heterozygous mice developed ventricular hypertrophy and fibrosis by 9 months, while homozygous mice experienced severe cardiomyopathy, often succumbing within a week of birth. Using dual adeno-associated viral vector (AAV9) system with a cardiac troponin T (cTnT) promoter to confer specificity, they delivered ABE and gRNA to cardiomyocytes. High-dose vector injection into homozygous mice on their first day of life achieved approximately 35% transcript-level correction, extending their lifespan to two weeks. In heterozygous mice, which more closely approximate the human situation, the treatment yielded similar correction, rescuing left ventricular hypertrophy, and remodelling for up to 16 weeks.

The second study (51) also utilized the well-established R403Q mouse model of HCM. In contrast to the previous study, the murine variant limited the testing of human-specific gRNAs for gene editing. This mouse model developed cardiomyopathy between 20 and 25 weeks. Treatment entailed the use of a dual AAV9 vector system to deliver ABE and mouse-specific gRNAs under the control of a cardiac specific (Tnnt2) promoter. Vector was administered at 10–13 days of life and resulted in around 68% and 26–39% transcript correction in ventricular and atrial cardiomyocytes, respectively. At 32–34 weeks of life there was rescue of cardiac hypertrophy, improvement in cardiac fibrosis, and normalization of dysregulated gene expression. However, there was significant bystander editing, which increased with sequential AAV injections. This effect may be attributed in part to the choice of a less specific editing machinery (PAM) in the ABE, facilitating engagement with off-target genome sites. An important translational consideration for these approaches is the size of the editing machinery exceeding the AAV packaging capacity. This necessitated splitting the editor gene across two AAVs and relying on cellular trans-splicing to reconstitute the full-length open reading frame. The inefficiency of this process may be limiting when scaling to humans.

In contrast to the previous two studies, Li et al (52) employed the CRISPR–Cas9 HDR technique to repair a premature stop mutation in the MYBPC3 gene. They created a rat model of HCM called "1098hom," (p.W1098x) found in a human HCM pedigree. On the third day after birth, CRISPR/Cas9 system was administered to rat pups using a single dose of AAV9 particles. As HDR is dependent on cell division, vector was administered in the early postnatal period to exploit cardiac mitotic activity. Six months after this injection, CRISPR HDR genome editing successfully corrected a small fraction (3.56%) of the total mutations, restored MYBPC3 protein expression in a minority (6.6%) of cells. Of note, the HCM phenotype was partially corrected despite the low rate of corrected mutations and cellular MYBPC3 expression.
4.2 Gene replacement therapy in HCM

Given that pathogenic variants in MYBPC3 are the most common cause of HCM (53), and a majority of these result in haploinsufficiency, the more straightforward strategy would be to replace the missing protein. Thus far, researchers have explored two potential strategies. The first method employs RNA trans-splicing, wherein the gene therapy product takes the form of a series of wild-type exons which bypass the mutant exon by targeting the pre-mRNA and competing with cis-splicing. This leads to the creation of a mended mRNA molecule that lacks the mutation and yields a fully functional protein. Unlike the gene replacement method using the entire coding sequence, the RNA trans-splicing approach has not gained traction because of its limited ability to produce functional clinical benefits (54, 55).

The second approach involves introducing full-length, wild-type MYBPC3 copy DNA (cDNA). The evidence of the effectiveness of this approach was first demonstrated by lentiviral MYBPC3 gene transfer in mice carrying homozygous loss-of-function MYBPC3 mutations (56). In this study, gene replacement improved myofilament and in vivo cardiac function. Following this, Mearini et al (57) performed MYBPC3 gene replacement using a single systemic delivery of AAV9 in neonatal mice with severe HCM. At the 34-week end point, there was a vector dose-dependent cMYBPC3 protein expression that prevented the HCM phenotype. In line with these findings, AAV-mediated MYBPC3 gene replacement was also associated with restoration of contraction in engineered heart tissue derived from cardiac cells of neonatal MYBPC3 knock-out mice (58, 59). These positive results were successfully extended to MYBPC3-HCM hiPSC-derived cardiomyocytes as well as human embryonic stem cell-derived cardiomyocytes (hESC) carrying a truncating MYBPC3 mutation (55, 60). In both investigations, MYBPC3 gene replacement therapy effectively rectified cMYB-C haploinsufficiency and mitigated hypertrophy.

Currently, two biotechnology companies (in the USA and Switzerland) are actively advancing AAV-mediated MYBPC3 gene replacement therapy for clinical applications. The Switzerland (Dinaqor) gene therapy program is still in the pre-clinical stage, but the United States program (Tenaya Therapeutics) is progressing its MYBPC3 gene therapy, labelled TN-201, into a phase 1b trial. This trial, utilizing an AAV9 vector (34), will commence in a limited group of adult patients with the intention to extend to infants with homozygous mutations, a high-risk population that seldom survives beyond infancy without requiring a heart transplant. (34) This trial will involve two escalating dose groups, enrolling a minimum of 6 and potentially up to 15 patients, all of whom will be administered the active therapeutic. The primary objectives of the study encompass the following: 1) Evaluation of the
frequency and severity of Adverse Events throughout a 5-year period; and 2) Assessment of the number of Serious Adverse Events specifically linked to the study drug. In addition, the trial will investigate changes in markers such as N-terminal pro B-type natriuretic peptide (NTproBNP), high-sensitivity cardiac troponin I (hs-cTnI) levels, symptoms, exercise capacity, and various echocardiographic parameters, as secondary outcomes (61).

An additional gene replacement therapy pertinent to HCM is RP-A501, a product developed by Rocket Pharmaceuticals, designed to address Danon disease (62). Danon disease (DD) is a rare X-linked monogenic condition that affects both the heart and multiple systems, stemming from mutations in the LAMP2 gene. In male Danon disease patients, there is a pronounced development of progressive HCM, left ventricular (LV) dysfunction, and arrhythmias, leading to a median mortality rate below the age of 20 years (62). In the phase 1 trial, patients received IV infusion of RP-A501, an adeno-associated virus with a normal copy of the human LAMP2B gene (AAV9:LAMP2B), given at two different doses: 6.7 x 10^13 genome copies per kilogram (low dose) and 1.1 x 10^14 genome copies per kilogram (high dose). Immunomodulation (IM) therapy consisted of prednisone and rituximab, with additional sirolimus for the most recently treated pediatric patients. From June 2019 to March 2022, the study included 7 male patients with Danon disease, aged between 11.7 and 21.1 years (with a median age of 18.3 years), comprising 5 adults and 2 pediatric patients, consisting of five patients in the low-dose group and two patients in the high-dose group. All patients have remained alive and in stable condition during a follow-up period ranging from 3 to 30 months. One adult patient with initial left ventricular systolic dysfunction required a heart transplant approximately 5 months after receiving RP-A501, likely due to the progression of Danon disease. Any adverse events (AEs) associated with RP-A501 or the immunomodulation therapy were manageable and reversible. Notably, there were no RP-A501-related serious adverse events (SAEs) observed in the pediatric patients (63).

4.3 Allelic-specific silencing in HCM

Another approach to HCM gene repair includes exon skipping. An experimental study has validated the effectiveness of skipping of a mutated exon 6 in the MYBPC3 gene of the HCM mouse model by utilising AAV-vector expressed anti-sense oligonucleotides (64). Treated mice had decreased levels of incorrectly spliced mRNA, restored cardiac dysfunction, and halted development of left ventricular hypertrophy. Despite employing an AAV vector, this approach only conferred a transient effect which may significantly limit its utility.
Similarly, AAV-expressed, small-interfering RNAs (siRNAs) directed at the mutant transcript of the MYH6 gene in R403Q heterozygous mice have been shown to selectively lower the expression of mutant allele while leaving the expression of the wild-type allele unaltered (65). A moderate (29%) reduction in the mutant transcript proved effective in slowing down the advancement of hypertrophy and fibrosis. These studies support the concept that antisense-mediated exon skipping, or allele-specific suppression show potential to be an effective therapeutic strategy.

4.4 Signalling pathway modulation in HCM

The final approach for gene therapy for HCM targets the molecular pathways responsible for controlling contraction and relaxation in the heart. In this context, two promising targets have emerged: sarcoplasmic/endoplasmic reticulum Ca2+ ATPase 2a (SERCA2a) and Myosin Regulatory Light Chain (Myosin RLC). As HCM has been linked to a reduction in SERCA2a levels within cardiomyocytes (66), the effects of augmenting SERCA2a expression in HCM are anticipated to be beneficial (67). In a neonatal mouse model, the introduction of SERCA2a via adenoviral expression led to a reduction in hypertrophy and fibrosis and normalized haemodynamics at 3 months of age. Increasing SERCA2a activity can also be achieved by suppressing its endogenous inhibitor, PLN (phospholamban) (68). However, PLN inhibition needs to be approached with caution as loss of PLN function in humans causes dilated cardiomyopathy (42, 69).

A specific genetic mutation (D166V) within the myosin RLC gene (MYL2) is associated with an aggressive form of HCM linked to a substantial reduction in the natural level of RLC phosphorylation (70). Yadav et al (71) conducted a study to investigate the potential impact of altering cardiac RLC phosphorylation in a humanized D166V mice model. They employed AAV9 to deliver phosphomimetic human RLC variant with serine-to-aspartic acid substitution (S15D) to mimic phosphorylation at this site. Their findings demonstrated that this pseudo-phosphorylation of RLC had the capacity to enhance actomyosin function, improving cardiac function. This study suggested that targeting RLC phosphorylation could be a clinically useful aspect of the clinical management of HCM patients with similar variants.

5. CHALLENGES OF TRANSITIONING HCM GENE THERAPY TO CLINICAL APPLICATIONS
There is much enthusiasm to translate these proof-of-concept studies to clinical applications. There remain, however, multiple significant challenges to be overcome (Figure 4).

While there is significant evidence supporting the safety of the existing AAV-based gene therapy technology, the occurrence of three recent fatalities related to AAV-based gene therapy (72, 73) serves as a reminder of the associated risks and the ongoing necessity to enhance its safety measures. AAVs have emerged as particularly favoured vectors for therapeutic gene delivery due to their rare integrating nature and low immunogenicity (74). However, AAV vectors can still trigger clinically significant innate and adaptive immune responses (75). Some studies have reported instances of innate immune responses elicited by AAVs in various animal models (76). These responses can also sometimes be linked to the transgene. The latest fatality linked to AAV-based gene therapy was suspected to result from an innate immune response, and this incident was connected to AAV9 (73), a widely utilized vector in cardiovascular genomics. The safety of other frequently employed vectors in cardiovascular medicine, such as AAVrh10 and AAVrh74, remains to be ascertained. Furthermore, due to adaptive immune reactions and potential cross-reactivity with naturally occurring AAV in the environment, a significant proportion of patients (40–60%) may possess pre-existing neutralizing antibodies that render them ineligible for treatment with certain therapeutic products (77). The development of adaptive immune responses following initial treatment also hinders the possibility of re-administering the same AAV serotype. This limitation to a single administration raises concerns about achieving the correct dosage required to ensure adequate transduction in the target organ for durable transgene expression. The need for single-dose therapeutic transduction efficacy must be balanced carefully, as administering high AAV doses has been associated with unintended off-target organ adverse effects in humans, including hepatotoxicity, renal failure, and thrombocytopenia (78). These complexities emphasize the importance of prudent decision-making and ongoing need for rigorous safety and efficacy assessment in gene therapy.

It is also important to recognize the variation in AAV’s ability to target specific tissue across different species (79). Although AAV9 has been the preferred serotype for successful cardiac transduction in pre-clinical studies, these results may not directly apply to humans. Therefore, it is imperative to thoroughly evaluate and confirm the suitability of any AAV variants that show promise for human myocardium or employ models that reliably predict their performance in humans. To date, numerous strategies have been developed to enhance AAV tropism for gene therapy, with a particular focus on improving the AAV capsid and vector genome design (80). Engineering AAVs with specific and enhanced cardiac tropism helps mitigate off-target transduction and reduces the required doses for efficacy with consequent reduction in dose-dependent immunogenicity. Furthermore, improved
tropism with a consequent reduction in AAV dose requirements is favourable for manufacturing purposes.

Genome editing using CRISPR-Cas9 presents unique safety concerns including the potential for off-target effects and editing threshold required for therapeutic efficacy. While the off-target events can lead to dangerous mutations in essential genes, such as tumour suppressors and genomic stability (81), uncertainties persist regarding the possible repercussions of incomplete editing and the ensuing functional 'cardiac mosaicism' (82). In addition, in vivo genome editing for a genetically heterogenous disease such as HCM has additional challenges. These include the limitations and constraints associated with adenine and cytosine base editors, the requirement for the development of distinct guide RNAs (gRNAs) for each causal variant, and the restricted availability of appropriately positioned PAM sequences for each variant (82, 83).

Despite significant progress in gene therapy and the growing number of molecular targets and therapeutic strategies, many challenges to the field remain. There is still a pressing need for further research, particularly in the context of developing safe and effective vectors and delivery techniques. These advancements are essential before gene therapy can be established as primary treatment options for patients with HCM. In the immediate future, initiating phase 1 clinical trials involving patients with advanced HCM and limited treatment options can provide valuable insights, as these trials would contribute to a better understanding of efficacy, safety, potential toxicity, immunogenicity, and the ability to rescue specific disease phenotypes.

If successful genetic therapy for HCM can be implemented, additional ethical challenges may arise. Germ-line gene-editing therapy for pathogenic HCM variants may have the potential to prevent further inheritance. Currently, pre-natal genetic counselling and pre-implantation genetic diagnosis are available and subsidised to various degrees internationally (24). It is well-established that a socioeconomic gradient already exists with regards to HCM patients’ access to specialty HCM referral centres and access to relatively common medications and interventions (84). Implementing a process by which gene-positive HCM can be effectively eradicated may create a socioeconomic gradient in HCM prevalence itself, further worsening existing HCM health disparities (85-87).

Ultimately, the greatest challenge limiting the promise of genetic therapies may be that the majority of HCM does not appear to be monogenic in nature. Although a pathogenic genetic variant may be the main driver of HCM in a proportion of cases, the ongoing challenge of gene-elusive HCM highlights gaps in our current understanding and limits the utility of targeted gene therapies. Subgroups such as non-familial HCM appear to have differing genetic yields as well clinical characteristics and prognosis.
when compared to the classical Mendelian disease (6). Additionally, genome-wide association studies have identified significant polygenic inheritance of HCM, contributing to variable expressivity and penetrance (88). Until genetic therapies are able to target the large sarcomere-mutation-negative population, existing medical and interventional therapies will retain clinical relevance in the management of HCM.

**CONCLUSIONS**

Advances in our understanding of disease-causing genetic variants in HCM and their associated molecular mechanisms have led to the potential for targeted therapeutics and improved implementation of precision and personalised medicine. Results from pre-clinical research are promising and raise the question of whether cure of some subtypes of HCM, and extended to other cardiomyopathies such as dilated and arrhythmogenic cardiomyopathies, is possible. Despite recent advances, translation to clinical practice is still in early development and ethical and equitable access to safe gene therapy must be prioritised.
REFERENCES


Figure 1. Hypertrophic cardiomyopathy (HCM) is a clinically and phenotypically heterogeneous disease.
Figure 2. Different genetic therapy platforms used in modern gene therapy.
Figure 3. Applicability of different gene therapy strategies to the management of HCM, categorised according to A) year and B) HCM genotype.
Considerations in the successful implementation of gene therapies in HCM span concerns regarding equity, safety outcomes and efficacy of therapy.
**CLASSIC INVESTIGATION FINDINGS**

- **Electrocardiogram**
  - Left ventricular hypertrophy on voltage criteria
  - Marked T wave inversion (particularly in apical variant)

- **Echocardiography**
  - Asymmetric septal hypertrophy
  - Systolic anterior motion of mitral valve
  - Dynamic left ventricular outflow tract obstruction

- **Magnetic resonance imaging**
  - Asymmetric septal hypertrophy
  - Systolic anterior motion of mitral valve
  - Late gadolinium enhancement

- **Histology**
  - Myocyte hypertrophy, myocardial disarray and/or interstitial fibrosis

*not present in all cases*
Genetic therapy strategies under investigation in HCM

![Graph A: Yearly Distribution of Strategies](image1)

![Graph B: Genotype Distribution of Strategies](image2)

**Legend**
- Red: Genome editing
- Blue: Gene replacement
- Yellow: Allelic-specific silencing
- Green: Modulation of signalling pathway
KEY CONSIDERATIONS IN TRANSLATION OF GENETIC THERAPIES IN HYPERTROPHIC CARDIOMYOPATHY

EQUITY

Introduction of gene therapy for HCM may exacerbate existing socioeconomic inequalities in regards to access to HCM therapy

SAFETY

AAV-associated major inflammatory responses

Off-target unintended effects of CRISPR-Cas9 gene therapy (potential for dangerous mutations in other genes)

EFFICACY

~50% of patients may have innate neutralizing AAV antibodies that render gene therapy ineffective

Single-dose therapy requirement in AAV therapy may limit efficacy

Majority of HCM increasingly recognised to be non-Mendelian in inheritance: lack of applicability of existing gene therapy strategies