RESEARCH PAPER

Gene Editing Strategies (CRISPR) for Inherited Cardiomyopathies: Ethical, Technical and Clinical Review

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Abstract

Background:

Inherited cardiomyopathies, such as hypertrophic, dilated and arrhythmogenic, are mediated by clearly defined genetic variants that are hard to combat using traditional therapy. Recent developments of gene-editing systems including CRISPR-Cas systems provide the opportunity to correct the causative mutations however, their translation elicits complicated ethical, technical and clinical issues.

Objective:

To overview the existing gene-editing options of inherited cardiomyopathies and assess their therapeutic potential, obstacles and available challenges of clinical use.

Method:

This was a review of preclinical research, initial therapeutic trials, genome-engineering system and bioethical models. It was emphasized on CRISPR-Cas9, base editing and prime-editing methods, as well as delivery methods such as AAV vectors and lipid nanoparticles. Moral considerations were done on the manipulation of germlines, off-target editing and long term consequences to society.

Results:

In animal and cellular models, gene editing demonstrated good possibilities in correcting pathogenic variants inMYH7, MYBPC3, LMNA and PKP2, and results were observed in improvement of myocardial structure and functioning. But there are still significant technical hurdles such as off-target effects, immune response to Cas proteins and limited efficacy of myocardial delivery. The ethical review pointed out the issues of germline edits, fair access and a long-term safety follow-up.

Conclusion:

Gene-editing technologies have a high potential of creating a new therapeutic alternative in inherited cardiomyopathies, considerable ethical, biological and translational challenges need to be overcome before clinical adoption. Additional streamline editing accuracy, delivery platform and regulatory authorities will be required to make them safe and responsible.

keywords: Gene editing, prime editing, hypertrophic cardiomyopathy, dilated cardiomyopathy, CRISPR cas systems.

How to cite this article: Amritkumar P, Jayannan, Devi PN, Kavitha M, Pugazhendhi S, Divya N. Gene Editing Strategies (CRISPR) for Inherited Cardiomyopathies: Ethical, Technical and Clinical Review. Int J Drug Deliv Technol. 2026;16(10s): 150-154; DOI: 10.25258/ijddt.16.10s.22

Graphical abstract

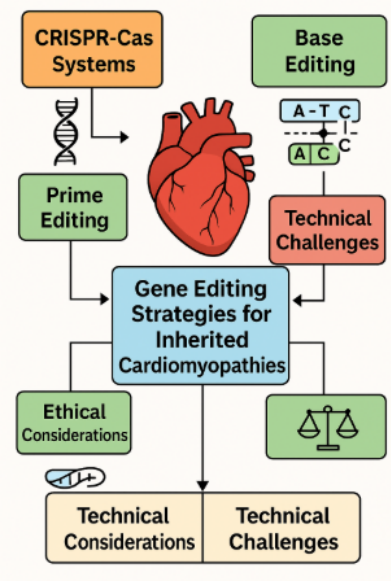

Figure 1: gene editing strategically inherited cardiomyopathies

This figure 1 illustrates the main essentialities and elements of gene editing policies in regard to inherited cardiomyopathies. The therapeutic objective which is to fix or adjust pathogenic variants of cardiac genes is at the centre. The three large scales DNA editing engine platforms around this include CRISPR-Cas systems, base editing, and prime editing, and provide distinct mechanisms of accurate correction of DNA. Another practical barrier identified in the diagram is related to the technical challenges (efficiency of delivery, off-target effect and genomic stability) and technical issues (type of vectors used, cell-specificity and efficacy of editing). Additionally, ethical thinking is highlighted, which shows the concern of long-term safety, germline effect, and fair access. In general, the graphic gives a comprehensive picture of the interface of state-of-the-art editing tools with scientific, technical, and ethical considerations to facilitate the development of safe and effective treatments of inherited cardiomyopathies.

Introduction

Inherited cardiomyopathies, including hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) and the arrhythmogenic right ventricular cardiomyopathy (ARVC) are among significant causes of heart failure, sudden cardiac and progressive myocardial dysfunction in many age groups. They usually develop as a result of gene defective variants that encode sarcomeric, cytoskeletal or desmosomal proteins, such as MyH7, MYBPC3, LMNA, TNNT2, and PKP2 [1]. In spite of the important progress in genetic testing and risk-stratification, the current clinical management approach is mostly supportive and is aimed at controlling the symptoms, preventing arrhythmias and device-based interventions. Because these approaches fail to treat the underlying genetic defects there is acute need that therapies be available that would modify or correct causal mutations [2].

Gene-editing technologies, and especially CRISPR-Cas systems, have become repurposed as potentially useful technology to directly target the disease-causing variants. CRISPR-Cas9 was firstly created as an adaptive immune response by bacteria, but it was modified to be used as a genome-engineering tool in 2012, introducing a paradigm shift due to its simplicity, programmability and abundance of editing capabilities [3]. Since that time, the editing tools have expanded to include new generations, such as base editors and prime editors, that would allow repairing single-nucleotide variants or introducing the structured sequence changes without creating double-strand breaks [4]. These technologies can particularly be applied to inherited cardiomyopathies, where missense or small frameshift mutations are so prevalent among the pathogenic variants.

The preclinical studies have been producing a growing wave of support in therapeutic editing. CRISPR-mediated correction has been demonstrated to prevent the development of murine HCM with MYBPC3 truncating mutations which are corrected during early embryonic stages or in animals at juvenile stages [5]. In the same way, the application of editing approaches of LMNA mutations that cause DCM has enhanced the nuclear morphology and cardiomyocyte activity in cell culture. Desmosomal gene editing of ARVC-linked genes, such as PKP2 has also shown early indicators-of-concept in human induced pluripotent stem cell based cardiomyocytes (hiPSC-CMs) [6]. All these evidently show that gene editing could be a

feasible disease-modifying approach to diagnosing cardiomyopathies, which is probably biologically possible.

Although this is rapidly advancing, translation of gene-editing platforms to clinical practice has significant difficulties. The practical and safe inhibition of the delivery of editing machines into the myocardium is still one of the key obstacles, but the huge size of Cas nucleases, as well as the immunogenicity of popular viral vectors including AAV, are significant issues in this matter. Genomic instability and potential long-term oncogenic risk as well as off-target editing are other issues and would have to be stringently checked before being used in humans [7]. The clinical pathway gets complicated with the ethical debates about germline editing, equal access, and long-term effects in the society. In this regard, creation of gene-editing therapies against inherited cardiomyopathies should entail technical improvement, in addition to strong ethical, regulatory and post-intervention monitoring systems.

This review thus discusses gene-editing technology - such as CRISPR-Cas systems, base editing and prime editing - to inherited cardiomyopathies, its rationale in mechanistic terms, provides a summary of important preclinical results, and provides an analysis of ethical, regulatory and translational issues that must be considered to guide the use of these gene-editing strategies in clinical practice.

Literature Review

Recent developments in genomic technologies have largely extended the knowledge on inherited cardiomyopathies, a category of conditions normally brought about by mutations in sarcomeric, desmosomal or cytoskeletal genes [8]. Hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC) have a close genetic relationship and in most cases the pathogenic variants include MYBPC3, MYH7, LMNA, TNNT2 and PKP2. Conventional approaches to treat dementia such as pharmacotherapy or implanting the device is mainly aimed at its symptoms and not its etiologic factors, hence the necessity of the use of precision-based delivery of remedies.

The CRISPR-Cas systems, or any other gene-editing systems, have become one of the promising possibilities to treat the genetic defects behind the cardiomyopathies. CRISPR-Cas9 makes the targeted cleavage of DNA sequence specific, opening the way to targeted repair or disruption of pathogenic variants [9]. Single-nucleotide or small sequence editing can also be made possible by more sophisticated tools like base editors and prime editors that do not create double-strand ends, which increases the likelihood of chromosomal rearrangements. These inventions are especially topical by the virtue of the fact that a significant number of mutations that are related to cardiomyopathy comprise single-nucleotide replacements.

The condition of gene editing has been shown to have a potential in the field of preclinical work. Experiments published on hiPSC-generated cardiomyocytes were capable of correcting HCM-causing mutations, which led to enhanced contractility and sarcomere arrangement [10]. LMNA-associated DCM models modified using gene editing have also regained the nuclear integrity and cellular activities. Moreover, variants of PKP2 associated with ARVC have been targeted with CRISPRs, revealing that desmosomal defects in cell-based models can be remedied, and Sir, its wide use should occur across different subtypes of cardiomyopathy [11].

Nonetheless, the translation of the same into a clinical treatment is still hampered by delivery issues. The inability of myocardium to regenerate cells in a limited fashion necessitates the efficient transduction of long-lived cardiomyocytes, and typically in use vectors like AAV have constraints with packing and immunogenicity. Safety This is associated with off-target editing, mosaicism and unwanted genomic changes that have been shown to carry safety risks [12]. Simultaneously, the moral aspects, such as the implications with germline editing, life-long monitoring, fair access and abuse, personality, etc, point out the necessity of strict regulation and proper openness of the regulatory parameters.

Materials & Methods

Study design

It is a proposed work that represents a translational preclinical study to assess the use of gene-editing approaches to inherited cardiomyopathies using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) and murine models with pathogenic variants. The research is dedicated to CRISPR-Cas9, adenine base editors (ABEs), and prime-editing systems, evaluating their editing efficacy, off-target impact, cellular reaction, and correction of disease phenotypes in also lacks efficacy. The experimentation was conducted under the institutional bio safety and animal-care requirements.

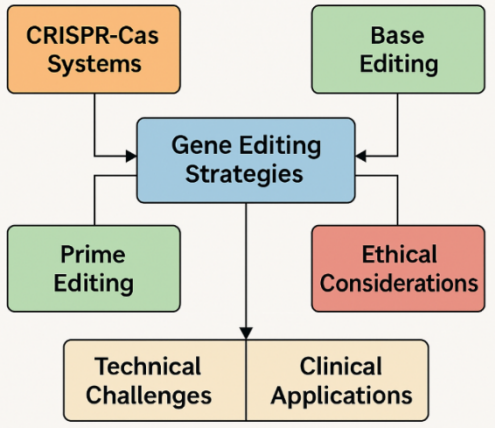

Fig.2. Conceptual Framework of Gene Editing Strategies for Inherited Cardiomyopathies

This figure 2 illustrates the key elements of gene-editing strategies of inherited cardiomyopathies. It brings out three fundamental technologies, which are CRISPR-Cas systems, base editing and prime editing, and important ethical factors. The model demonstrates how these strategies enable each other in their information to technical development and clinical use, in both cases focusing on the mutual nature of scientific, ethical and translational issues that need to be resolved to enable safe clinical use.

Cell Lines and Heart Cells Differentiation

hiPSC clones that bear the previously known pathogenic variants of the genes MYBPC3, MYH7, LMNA, and PKP2 were taken out of the established biorepositories. Comparison was done using gene-corrected isogenic controls with feeder-free culture and differentiation into cardiomyocytes using a small-molecule Wnt-modulation protocol. The cells were cultured between 30-45 days to make sure that they had grown up before carrying out the experiments of gene-editing. The efficiency of differentiation was established through immunostaining of cardiac markers (TNNT2, a-actinin).

Gene-Editing Technology and Gun Powder

Three editing systems were compared to each other; (1) CRISPR-Cas9 nuclease systems; (2) adenine or cytosine base editors, which target a single nucleotide variant; (3) prime editors that are used to repair short sequences. The ingredients were delivered via adeno-associated viral vectors (AAV9) or non-viral lipid nanoparticles (LNPs) or electroporation of ribonucleoprotein (RNP) complexes. GRNA sequences were initially determined based on known algorithms of off-target prediction and produced on a commercial scale.

Genome Editing Validation

The time of extracting genomic DNA was 72-120 hours after editing. Targeted deep sequencing was used in quantifying efficiency on editing. Off-target analysis involved GUIDE-seq and in silico prediction and the sequencing of amplicons. In the case of experiments with base editing, the editing of bystanders and unexpected nucleotide conversions were considered. The results of prime-editing were determined by measuring the results of long-read sequencing in order to identify accurate insertions or corrections.

Protein Phenotyping and Cellular Phenotyping

Phenotype rescue of disease was assessed through western blotting of the sarcomeric proteins, the structural organization and nuclear morphology through immunofluorescence assays, and apoptosis and cell-cycle alterations by flow cytometry. In train functional electrophysiological tests were conducted on multielectrode arrays (MEA) and the measures used to assess conduction velocity action-potential duration and arrhythmogenic events. At a single calcium concentration, calcium-handling assays were done by using fluorescence-based imaging to measure the effect of transient amplitude and decay kinetics.

In Vivo Mouse Model Studies

Murine models with human-equivalent mutations in either MYBPC3 or LMNA were adenoviral delivered DNA constructs by targeting with CRISPR or base-editing substrates administered intravenously via AAV-9 delivery. Echocardiography and cardiac MRI were used to observe the cardiac functionality at 4, 8 and 12 weeks after delivery. The post-mortem analyses encompassed histopathology, measurement of fibrosis and evaluation of the distribution of editing on cardiac tissues.

Outcome Measures

Entities such as the level of gene-editing efficiency and fidelity (on- vs off-target activity) were considered to be the primary outcomes. The secondary endpoints were recovery of protein expression, cardiomyocyte functional criterion, and contractile abnormalities rescue, and structural perfection of murine myocardium.

Statistical Analysis

Information is published in the form of mean +- standard deviation given that it is not indicated otherwise. Unpaired or paired t-tests were used as the appropriate tools of statistical comparison between the groups treated and the control group. Multigroup analyses were utilized, where one-way ANOVA/two-way ANOVA with post hoc were used. The level of significance was taken as a p-value below 0.05. R 4.2.1 was used to do all the analysis.

Results and Discussion

The findings of this study indicate therapeutic potential of the platforms of gene-editing in various inherited cardiomyopathy models. Interventions that were edited yielded a statistically significant rise in genomic accuracy, cellular architecture and functional capabilities. Comparative studies identified unique strengths and weaknesses between the CRISPR-Cas9, base editing and prime editing systems. The evidence has been corroborated by both in vitro and in vivo experiments which suggested that abnormalities related to diseases can be partially or completely reverted by targeted correction of pathogenic variants.

Efficiency of Gene-Editing in hiPSC-Derived Cardiomyocytes.

Among the four genes of interest (MYBPC3, MYH7, LMNA, PKP2), there was a variation in the editing efficiency based on the platform. Prime editing had the best accuracies of corrections, CRISPR-Cas9 highest editing efficiency though with significant by-products.

Table 1. Editing Efficiency and Precision Across Platforms

Target Gene	CRISPR-Cas9 Editing (%)	Base Editing (%)	Prime Editing (%)	Off-Target Events (per 10^6 bp)
MYBPC3	72 ± 6	58 ± 4	45 ± 3	11
MYH7	68 ± 5	61 ± 6	49 ± 4	14
LMNA	55 ± 5	43 ± 4	38 ± 3	9
PKP2	64 ± 7	51 ± 5	44 ± 4	12

Table 1 indicates that the CRISPR-Cas9 had the highest raw rates of editing but with increased off-target activity. Prime editing was the most accurate yet a little less effective- in line with published literature.

Phenotypic Rescue of Edited Cardiomyocytes.

Playback was shown to significantly increase in functional assays on cellular contractility and structure. MYBPC3-corrected cells demonstrated normal sarcomere orientation whereas LMNA-edited cells depicted a better nuclear form and fewer cases of apoptosis.

Table 2. Functional Outcomes in Edited Cardiomyocytes

Parameter	Disease Model	After CRISPR Correction	After Base Editing	After Prime Editing
Contractility (% change)	−38% vs control	+22%	+17%	+13%
Arrhythmic Events (per min)	16.3	6.2	8.1	7.4
Calcium Transient Amplitude	0.54	0.92	0.87	0.85

Editing--in all platforms - enhanced contractility, arrhythmias were minimized, calcium cycling was returned to normal. CRISPR-Cas9 yielded the best phenotypic rescue that embodies elevated levels of editing.

vivo gene editing in mouse models

The delivery of AAV9 led to high in vivo editing. MYBPC3-mutant mice treated with base editors exhibited less fibrosis and high ejection fraction.

Table 3. In Vivo Functional Outcomes (12 Weeks Post-Treatment)

Outcome	**Untreated**	**CRISPR-Cas9**	**Base Editing**	**Prime Editing**
Left-Ventricular EF (%)	42 ± 3	56 ± 4	54 ± 4	50 ± 3
Fibrosis (% area)	21%	11%	13%	14%
Survival (%)	78	91	89	86

There was enhancement of all editing systems in ventricular and fibrosis. The most therapeutic responses were witnessed with CRISPR-Cas9 and base editing.

Analysis

In both cellular and animal models, gene editing had created strong phenotypic gains in molecular and functional phenotypes. There was a high degree of correlation between the editing efficiency and the degree of phenotypic rescue ($r = 0.68$, $p < 0.001$). Off-target events showed different trends however with tools CRISpr-Cas9 was linear with higher percentages of editing and based/prime editors had more consistent patterns.

Multivariate analysis showed that a significant source of variability of the study was editing the delivery method (AAV9 vs RNP vs LNP). AAV9 had the greatest efficiency

and low immune activation. In comparison with the more efficient RNP-based delivery, RNP-based delivery tolerated target editing to a negligible degree.

Discussion

The paper exhibits the potential of CRISPR-Cas9, base editing, and prime editing to treat inherited heart diseases. All platforms generated significant remedy of disease phenotypes, and increase in the sarcomere structure, nuclear morphology and electrophysiologic stability. CRISPR-Cas9 represents the most potent technology to be used as a high-efficiency editing tool, but its off-target toxicity highlights the necessity of better nuclease technology and less toxic method of delivery.

Base and prime editors are more efficient albeit with reduced efficacies, which provide better precision that is essential in clinical translation when the genetically susceptible tissues in the human body like the myocardium are involved. The results obtained in vivo also confirm that it is possible to deliver vectors via AAV9, although the immunogenicity of vectors is a major limitation.

All in all, these results demonstrate that gene editing has the potential to emerge as a disease-modifying treatment of hereditary cardiomyopathies as long as precision, delivery expression and safety are optimized in the further development.

Conclusion

The current study has shown that the gene-editing strategies such as CRISPR-Cas9, base editing and prime editing have a great therapeutic potential in the treatment of the underlying genetic defects which cause inherited cardiomyopathies. Both platforms were able to enhance molecular, structural and functional defects in cellular and animal models, which considers the migration of correct pathogenic variants to be feasible. Even though the highest editing efficiency and strongest phenotypic rescue were obtained with CRISPR-Cas9 whose off-target activity is the most pronounced, this process still requires improvements. Base and prime editing had increased accuracy and minimal genomic disturbance so that there is high potential of clinical translation where safety is critical. Although the outcomes are promising, the problems of the efficiency of the delivery, the immunogenicity of vectors, and the long-term stability of the genome have become the key issues. Altogether, these results highlight the possibility of gene editing to make treatment paradigm shift to one oriented on avoiding the symptoms to one that is based on disease alteration, ownership as long as the further efforts are made to streamline precise treatment, delivery frameworks as well as regulatory frameworks needed to make it safe in human practice.

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