Review Article

A Comprehensive Review on CRISPR Technology in the Treatment and Understanding of Cardiacmyopathy

Abstract

Cardiac myopathy, encompassing hypertrophic, dilated, restrictive, and arrhythmogenic forms, represents a significant challenge due to its complex genetic underpinnings and limited curative treatments.

Advances in CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology offer transformative potential in understanding and treating these disorders. CRISPR-Cas9 enables precise gene editing, addressing pathogenic mutations such as MYH7 in hypertrophic cardiomyopathy and TTN in dilated cardiomyopathy.

Emerging techniques like base and prime editing enhance accuracy while minimizing off-target effects. Preclinical models and in vivo studies have demonstrated the utility of CRISPR in creating disease models, correcting genetic defects, and exploring therapeutic interventions. However, challenges including delivery mechanisms, ethical considerations, and long-term safety must be addressed.

This review explores the therapeutic promise and limitations of CRISPR in cardiac myopathy, highlighting its role in precision medicine and its potential to revolutionize cardiovascular treatment.

Keywords

Cardiac myopathy, CRISPR-Cas9, Gene editing, Precision medicine, Genetic Mutations

INTRODUCTION

Cardiac myopathy, often known as cardiomyopathy, refers to a wide range of cardiac disorders that alter the normal structure and function of the heart muscles. Primary cardiomyopathies, as opposed to secondary cardiomyopathies induced by extrinsic causes such ischaemia or hypertension, are frequently idiopathic or genetically determined [1]. Cardiomyopathy can be divided into four kinds based on structural and functional abnormalities: Hypertrophic Cardiomyopathy (HCM) is characterised by aberrant ventricular wall thickening and is frequently linked with poor diastolic function, as well as an increased risk of arrhythmias and sudden cardiac death. It is the most prevalent hereditary heart disorder, accounting for one in every 500 people worldwide [2].

Dilated Cardiomyopathy (DCM) Characterised by ventricular dilatation and systolic dysfunction, DCM is a leading cause of heart failure that requires heart transplantation in later stages. It is linked to both hereditary and nongenetic factors, such as viral infections and alcohol consumption [3].
Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is a disorder mostly affecting the right ventricle, is defined by fibrofatty replacement of the myocardium, which causes arrhythmias and abrupt mortality, especially in athletes [4].
Restrictive Cardiomyopathy (RCM) is the least frequent category, characterised by ventricular wall rigidity and poor diastolic filling in the absence of substantial hypertrophy or dilatation.

Despite their differences, these forms share overlapping clinical manifestations, such as heart failure, arrhythmias, and thromboembolic complications. Current treatment approaches, including medications, device therapy, and transplantation, largely aim to manage symptoms and improve quality of life rather than cure the underlying condition [2].

ROLE OF GENETICS IN CARDIAC MYOPATHY

Advances in genomic medicine have highlighted the importance of genetic abnormalities in the pathophysiology of cardiomyopathies. Approximately 40-60% of HCM patients and 25-35% of DCM cases have known genetic origins [5]. Mutations in sarcomeric proteins, such MYH7 (β-myosin heavy chain) and MYBPC3 (myosin-binding protein C), are the most prevalent causes of HCM. Similarly, TTN truncating mutations in titin are common in familial DCM, making them the most important genetic contribution [6].
ARVC is mostly related with mutations in desmosomal proteins, such as PKP2 (plakophilin 2) and DSG2 (desmoglein-2), which decrease cell-cell adhesion and cause myocyte separation [7].
Mutations in sarcomeric and cytoskeletal proteins frequently cause structural and functional cardiac defects that worsen over time, adding to the clinical presentation of cardiomyopathy.

Understanding the genetic basis of many diseases has enabled family screening, risk stratification, and personalised treatment regimens. However, current medicinal techniques are unable to directly target the genetic abnormalities that cause these illnesses, emphasising the need for novel gene-editing options.

CRISPR TECHNOLOGY

The emergence of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology has marked a paradigm shift in the field of genetic engineering. CRISPR-Cas9, the most widely used system, is a programmable nuclease complex capable of precise DNA modification. Originating from the adaptive immune systems of bacteria, CRISPR utilizes guide RNAs (gRNAs) to direct the Cas9 endonuclease to specific DNA sequences, where it introduces double-strand breaks [8]. These breaks are repaired via non-homologous end joining (NHEJ) or homology-directed repair (HDR), enabling gene knockout, insertion, or correction.

CRISPR offers enormous potential for treating genetic illnesses, including cardiomyopathies. It has been employed successfully to repair MYH7 mutations in preclinical models of HCM, as well as to restore normal function in cells with TTN truncating variations [9]. Emerging CRISPR variations, such as base and prime editors, have improved gene editing accuracy and efficiency.

- CRISPR has helped in cardiomyopathy research by creating genetically engineered animal models to examine disease pathways.
- Understanding the functional impact of certain mutations.
- Exploring new treatment ways to slow or stop disease development.

CRISPR's scalability, cost-effectiveness, and specificity make it a viable tool for studying and treating cardiac myopathy.

Structure of CRISPR-Cas9

CRISPR-Cas9 is a two-part system that consists of the Cas9 protein and guide RNA. The Cas9 protein acts as an endonuclease, introducing precise cuts in DNA, while the gRNA leads Cas9 to the target region. Cas9 is generated from Streptococcus pyogenes or other bacterial species and is divided into two lobes: recognition (REC) and nuclease (NUC). The REC lobe interacts with gRNA and stabilises its binding to target DNA, whereas the NUC lobe cleaves DNA strands. The NUC lobe has two catalytic domains, HNH and RuvC, which each cleave one strand of the DNA duplex. Cas9 also possesses a PAM-interacting domain, which recognises a protospacer adjacent motif (PAM), usually NGG, next to the target DNA region.

The gRNA is a single RNA molecule combining features of CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA). It contains a 20-nucleotide spacer region complementary to the target DNA sequence, enabling specific recognition. The remaining portion of the gRNA forms a hairpin structure that interacts with Cas9, stabilizing the complex[10].

Function of CRISPR-Cas9

The CRISPR-Cas9 system uses a well-defined method to identify and break target DNA. The process begins with the identification of a PAM sequence, which is required for Cas9 binding and activation. Once the PAM sequence has been found, the Cas9 protein undergoes conformational changes, unravelling the DNA duplex close to the target location. This allows the gRNA to mate with the corresponding DNA strand, resulting in an RNA-DNA hybrid. Following target identification, Cas9 causes a double-strand break (DSB) in the DNA. The

HNH domain cleaves the complimentary strand of the gRNA, whereas the RuvC domain cleaves the non-complementary strand. These precise cuts alter the DNA sequence, allowing for further gene editing via cellular repair processes[11].

Repair Mechanisms Following DNA Cleavage

The DSBs introduced by Cas9 are repaired by cellular pathways, mainly Non-Homologous End Joining (NHEJ) and Homology-Directed Repair (HDR). NHEJ is an error-prone process that often introduces insertions or deletions (indels), leading to gene disruption. This is particularly useful for knocking out genes. In contrast, HDR is a high-fidelity repair pathway that utilizes a donor DNA template to introduce precise genetic modifications. HDR is commonly employed for correcting mutations, inserting genes, or generating specific nucleotide substitutions[12].

CRISPR/Cas9 gene editing

CRISPR-mediated genome editing consists of two components: a single guide RNA (sgRNA) that matches the target DNA sequence and a CRISPR-associated endonuclease (Cas9)[13]. A Cas9-sgRNA ribonucleoprotein complex induces DNA cleavage when the target DNA sequences bind to the sgRNA at a protospacer-adjacent motif (PAM) (Figure 1(A-C). Nonhomologous end joining (NHEJ) creates insertions or deletions (INDELs), while homology-directed repair (HDR) accurately fixes DSBs by inserting a particular DNA sequence. Because the HDR machinery is lacking in post-mitotic cells, gene editing to correct hereditary cardiomyopathies is likely to need NHEJ.

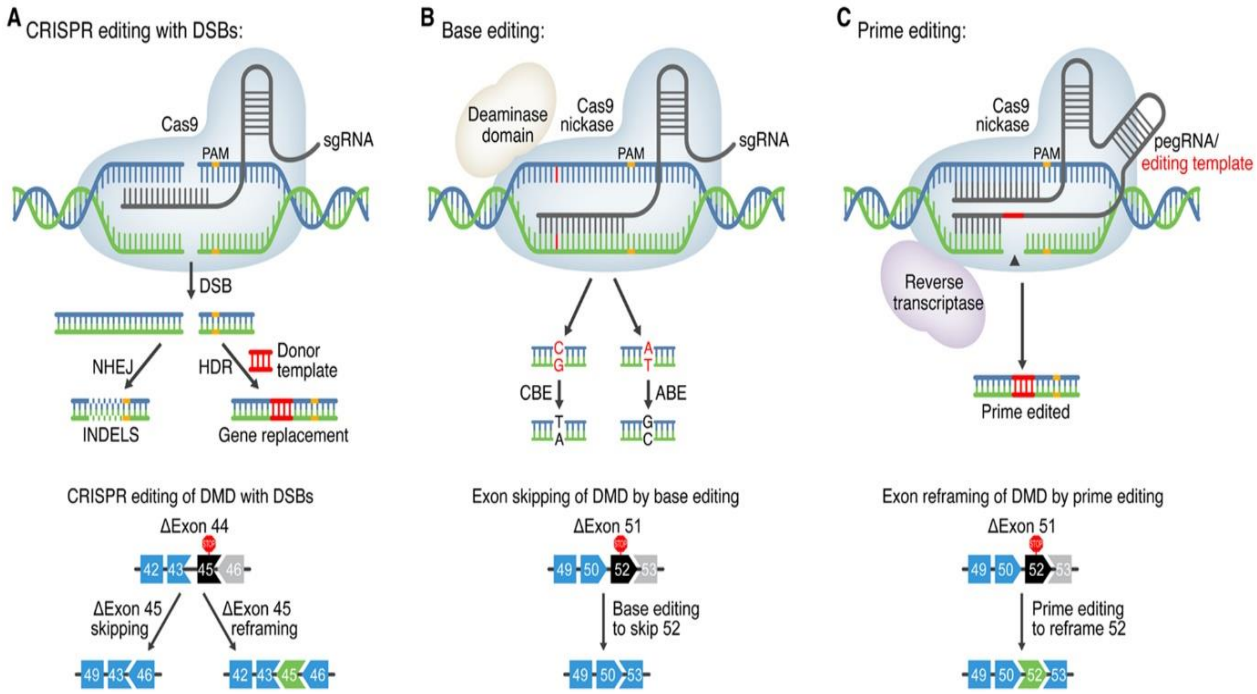

Figure 1(A-C): (A) DMD correction by CRISPR editing with DSBs, (B) DMD correction by base editing (C) DMD correction by prime editing

NHEJ, which induces INDELs at the cutting site, is the main mechanism for repair of DNA DSBs. HDR inserts a precise DNA fragment. NHEJ-mediated repair introduces INDELs to restore the open reading frame either by exon skipping or reframing in a deletion of DMD exon 44.

Figure B showing DMD correction by base editing.

Base editors convert A-T to G-C or C-G to T-A base pairs without DNA DSBs. This approach can be used to disrupt splice sites, thereby causing exon skipping, as shown for DMD exon 52.

Figure C showing DMD correction by prime editing.

Prime editing can introduce specific DNA sequences to reframe exons, as shown for DMD exon 52.

The majority of DMD-causing mutations involve exon deletions or duplications that alter dystrophin protein production, resulting in progressive muscle degeneration and cardiomyopathy. CRISPR/Cas9 editing has been used in patient-derived induced pluripotent stem cells (iPSCs), as well as mice and dogs with DMD, to restore dystrophin expression in cardiac and skeletal muscles[14]. For example, deleting exon 44 of the dystrophin gene results in a premature stop codon in exon 45, which causes DMD and may be repaired by skipping or reframing exon 45.

Base and Prime Editing

Base editing (BE) and prime editing (PE) are novel methods that enable precise genomic alterations without the need of DSBs. BE allows for the alteration of base pairs, such as a C-G to T-A base pair in Cytosine BE (CBE) or an A-T to G-C base pair in Adenine BE. BE might possibly be used to treat cardiomyopathies due by certain point mutations, such as hypertrophic cardiomyopathy induced by the R403Q mutation in the MYH7 gene. ABE has recently rescued a mouse model of Hutchinson-Gilford progeria disease induced by an LMNA gene mutation[15].

BE-modified splice sites at exon junctions can potentially be exploited to inactivate genes or trigger exon skipping. In a primate model, ABE was utilised to inactivate the PCSK9 gene by altering a splice donor site, lowering low-density lipoprotein cholesterol levels[16]. Exon skipping by ABE has also restored dystrophin expression in patient iPSC-derived cardiomyocytes and mouse skeletal muscle with a DMD exon 51 loss[17]. However, BE has potential downsides such as a narrow editing window, undesired bystander editing, and off-target RNA editing.

The PE system is made up of a Cas9 nickase linked to reverse transcriptase and a pegRNA that recognises the target DNA sequence and carries a template that allows reverse transcriptase to selectively fix different mutations (Figure 1(A-C)), including those for which ABEs and CBEs are unsuccessful. PE has recently been employed in mice with inherited human liver and eye illnesses, as well as to reframe a DMD mutation, and it has great potential for inherited cardiomyopathies.

PATHOPHYSIOLOGY OF CARDIAC MYOPATHY AND GENETIC TARGETS

Cardiac myopathies are a diverse group of myocardial disorders characterized by structural abnormalities and impaired cardiac function. They can be broadly categorized into hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC). The pathophysiology of these conditions is primarily driven by genetic mutations affecting critical proteins involved in sarcomeric function, cytoskeletal stability, and intercellular communication. Understanding the genetic and molecular basis of these disorders provides valuable insights into their mechanisms and therapeutic targets[18].

The most often mutated genes in HCM are MYH7 (β-myosin heavy chain), MYBPC3 (myosin-binding protein C), and TNNT2 (troponin T). These mutations alter sarcomeric protein activity, resulting in enhanced myofilament calcium sensitivity and hypercontractility. Increased contractile force causes compensatory myocardial enlargement, which eventually leads to fibrosis, myocyte disorganisation, and diastolic dysfunction. Patients with HCM frequently report exertional dyspnoea, chest discomfort, and a higher risk of arrhythmias, including sudden cardiac death. Histological investigations have shown that HCM is characterised by asymmetric septal hypertrophy with concomitant cardiac fibrosis[19].

Dilated cardiomyopathy (DCM) is characterised by left or biventricular dilatation and poor systolic function, which frequently leads to progressive heart failure. Genetic mutations contribute for 20-50% of DCM cases, with TTN (titin) mutations being the most prevalent. Titin, a sarcomeric protein, helps the heart maintain structural integrity and suppleness. Mutations in TTN cause poor force transmission and myocardial instability under mechanical stress. Other important genes involved in DCM include LMNA (lamin A/C), which encodes a nuclear envelope protein, and DSP (desmoplakin), which maintains cytoskeletal integrity. Pathophysiologically, these mutations disrupt the structural framework of cardiomyocytes, resulting in maladaptive remodelling processes such as fibrosis and death. DCM is frequently characterised by increasing heart failure, arrhythmias, and thromboembolic events[20].

ARVC is a genetically driven cardiomyopathy that mostly affects the right ventricle, characterised by fibrofatty myocardial replacement and an increased incidence of ventricular arrhythmias. Mutations in desmosomal protein genes, such as PKP2 (plakophilin-2), DSP (desmoplakin), and JUP (junction plakoglobin), play a critical role in its aetiology. Desmosomes are critical for maintaining mechanical cohesiveness between cardiomyocytes, particularly during mechanical stress. Mutations in desmosomal proteins reduce intercellular adhesion, leaving the myocardium vulnerable to separation, inflammation, and fibrotic remodelling. This disease process is characterised by electrical instability, which predisposes individuals to ventricular arrhythmias and abrupt cardiac death. Palpitations, syncope, and arrhythmias are common clinical symptoms of ARVC[21].

The genetic and molecular processes underlying these cardiomyopathies have common characteristics, such as disordered sarcomeric contractility, compromised cytoskeletal integrity, poor cell-cell adhesion, and abnormal calcium handling. Furthermore, mechanisms that regulate energy metabolism and mitochondrial function are frequently disturbed, worsening

cardiac dysfunction. Mutations in mitochondrial DNA or nuclear-encoded mitochondrial proteins can cause oxidative stress, ATP depletion, and cardiomyocyte death[22].

Myocardial damage activates key signalling pathways such TGF-β and Wnt/β-catenin, leading to fibrosis and negative remodelling in cardiomyopathies. In DCM and ARVC, these pathways help to replace normal myocardium with fibrotic or fibrofatty tissue, which impairs contractile performance and electrical conduction[23]. In HCM, increasing sarcomeric strain triggers stress-response pathways that promote hypertrophy and fibrosis.

Genetic testing has transformed the diagnosis and treatment of cardiomyopathies. Identifying harmful variants allows for early discovery, risk stratification, and family screening[24]. Emerging medicines, such as CRISPR-based gene editing, have the ability to directly fix genetic abnormalities or alter downstream processes. For example, utilising CRISPR to silence dominant-negative alleles or restore the function of haploinsufficient genes shows promise as a targeted therapeutic method. Furthermore, understanding the molecular underpinnings of cardiomyopathies has helped the development of pharmacological treatments that target particular pathways, such as myosin inhibitors for HCM or anti-fibrotic medicines for DCM and ARVC[25].

CRISPR APPLICATIONS IN CARDIAC MYOPATHY

CRISPR technology has emerged as a transformational tool for studying and treating cardiac myopathies. Precision gene editing enables extensive research of genetic alterations, the establishment of disease models, and the development of focused therapy techniques. This section looks at how CRISPR has been used for gene knockout investigations, gene correction procedures, and in vivo models, with an emphasis on preclinical applications[26].

Gene Knockout Studies

Gene knockout experiments are essential for understanding cardiac myopathies. CRISPR-Cas9 allows for the targeted disruption of certain genes, allowing researchers to investigate their roles in the formation and progression of illnesses such hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC)[27].

In HCM, CRISPR-mediated deletion of MYBPC3 in human induced pluripotent stem cell (iPSC)-derived cardiomyocytes showed that sarcomeric dysfunction causes hypercontractility, disorganised sarcomeres, and increased myocyte stiffness. MYH7 knockout animal models recapitulate HCM characteristics such as cardiac hypertrophy, fibrosis, and diastolic dysfunction. These discoveries offer mechanistic insights and venues for evaluating new therapies[28].

CRISPR has been used to knock out TTN, a gene that encodes titin, a critical sarcomeric protein. Mice studies have shown that titin truncation impairs contractility and causes ventricular dilatation, all of which are hallmarks of DCM. Similar investigations on LMNA mutations in cardiomyocytes have revealed a relationship between nuclear envelope instability and cellular stress responses in DCM pathogenesis.

Knockout studies of desmosomal genes such as PKP2 and DSP in ARVC have revealed the mechanisms behind cell adhesion breakdown, inflammation, and fibrofatty replacement. These models not only improve our understanding of ARVC's molecular underpinnings, but also allow us to investigate anti-inflammatory and anti-fibrotic therapy[29].

Gene Correction Strategies

CRISPR has also demonstrated great potential in repairing genetic abnormalities that cause cardiac myopathies. Gene correction, unlike knockout techniques, includes precise editing to restore normal gene function, potentially curing monogenic diseases. CRISPR-Cas9 and homology-directed repair (HDR) were used by researchers to successfully rectify mutations in MYBPC3 in patient-derived iPSCs. These repaired cells develop into cardiomyocytes with normal contractile activity and restored sarcomeric protein levels, demonstrating the promise for gene correction in personalised medicine.

Advanced CRISPR tools, such as base and prime editors, improve accuracy and lessen the dangers associated with double-strand breaks. For example, base editors have been employed to fix harmful single-nucleotide variations in LMNA that are connected to severe types of DCM. Prime editing, which allows for the insertion, deletion, or substitution of DNA sequences, has showed promise in tackling complicated alterations like those in PKP2, a critical gene involved with ARVC. CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) technologies offer new methods for controlling gene expression without changing the DNA sequence. These methods are especially useful for studying the functions of genetic modifiers and therapeutic activation of haploinsufficient genes[30].

In Vivo Models and Preclinical Trials

CRISPR technology has aided in the creation of in vivo models that accurately mimic human cardiomyopathies. These models are critical for understanding disease causes and evaluating treatment therapies. In HCM, researchers employed CRISPR to generate pathogenic mutations in MYH7 and MYBPC3 in mice, resulting in models with cardiac hypertrophy, fibrosis, and diastolic dysfunction. These models have proven useful in understanding the evolution of HCM and testing prospective therapies such myosin inhibitors. CRISPR has been used to create mice models with truncating mutations in TTN, shedding light on how titin deficiency causes ventricular dilatation and reduced contractility in DCM. These animals have been used to evaluate potential therapeutics, such as gene therapy techniques for restoring titin function.

In ARVC, CRISPR-generated mice models with PKP2 or DSP mutations have shown the cellular and molecular pathways of fibrofatty replacement and arrhythmogenesis. These models are now being utilised to assess anti-inflammatory and anti-fibrotic medications, as well as gene-editing methods. Preclinical experiments with CRISPR-based medicines are yielding encouraging results. For example, adeno-associated virus (AAV) vectors containing CRISPR components have been utilised to repair MYBPC3 mutations in HCM mice models. This method restored normal sarcomeric protein levels while improving heart function. Similar tactics targeting TTN mutations in DCM animals have showed the viability of restoring titin expression and improving ventricular function[31-33].

THERAPEUTIC POTENTIAL OF CRISPR IN CARDIAC MYOPATHY

CRISPR technology has enormous therapeutic promise for treating cardiac myopathies, and it is quickly advancing. CRISPR's capacity to precisely modify genes creates new opportunities for addressing the hereditary origins of cardiac disorders such as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC). This section discusses CRISPR-based gene therapy techniques, delivery technologies, and breakthroughs in precision medicine as they relate to cardiac myopathies[34].

CRISPR-Based Gene Therapy Approaches

CRISPR-based gene therapy is intended to cure or minimise the genetic abnormalities that cause cardiac myopathies. The most prevalent technique is gene editing, which uses the CRISPR-Cas9 system to either eliminate harmful genes or fix genetic mutations directly in afflicted cells. For example, in HCM, which is frequently caused by mutations in the MYBPC3 gene, CRISPR-Cas9 was utilised to fix mutations in patient-derived iPSCs, restoring appropriate protein function and enhancing cardiomyocyte contractility[35].

Another option is gene replacement or addition, which involves replacing a damaged gene with a healthy version or introducing more copies of a favourable gene. For example, in DCM, where TTN (titin) mutations are frequent, gene editing efforts have focused on replacing the damaged region of the gene or inserting compensating proteins to restore cardiac function. These techniques have the ability to rectify genetic abnormalities over time, paving the way for the treatment of monogenic heart disorders. Furthermore, epigenetic modification using CRISPR-Cas9 systems such as CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) may control gene expression without changing the DNA sequence. This method is being investigated for cardiac repair by suppressing pathogenic genes and activating protective genes, such as those involved in myocyte survival or regeneration[36].

Delivery Systems for CRISPR Therapeutics

The effective and safe delivery of CRISPR components to the heart is key to realising gene editing's therapeutic promise. Several delivery strategies are being investigated to guarantee that CRISPR-Cas9 reaches its target cells in sufficient numbers and with minimum off-target consequences.

Viral Delivery Systems

The most common way to deliver CRISPR components to the heart has been using viral vectors, namely Adeno-Associated Viruses (AAVs). AAVs are preferred because of their ability to infect a wide range of cell types with little immunogenicity. For example, AAV-mediated delivery of CRISPR-Cas9 components has been successfully evaluated in preclinical models of HCM and DCM, where the viral vector was utilised to target cardiac tissue and fix harmful variants such as MYBPC3 and TTN. However, challenges remain in optimizing vector serotypes for cardiac tissue, ensuring long-term gene expression, and avoiding immune responses[37].

Lentiviruses are another viral alternative under consideration, particularly for delivering bigger genetic payloads, which may be required for complicated genes or gene complexes. Despite the potential for long-term gene expression, issues concerning insertional mutagenesis and safety must be addressed.

Non-Viral Delivery Systems

Non-viral methods of CRISPR delivery are being researched as an alternative to viral vectors. These techniques include lipid nanoparticles (LNPs), electroporation, and polymeric nanoparticles. LNPs have showed great promise in delivering CRISPR-Cas9 complexes to cardiomyocytes because of their capacity to encapsulate the CRISPR components and preserve them from degradation while allowing cellular absorption. Electroporation uses electric pulses to produce transitory breaches in the cell membrane, allowing CRISPR-Cas9 to enter. While non-viral methods have lower immunogenicity and simpler scaling than viral systems, they frequently struggle with efficiency and precision targeting[38].

CRISPR-based nanoparticles are also an appealing option because of their biocompatibility, simplicity of production, and potential for tissue-specific targeting. Researchers are looking into ways to alter nanoparticles to selectively target cardiac cells, which might improve the accuracy and efficacy of CRISPR treatments[39].

Advances in Precision Medicine

CRISPR technology is critical to the evolution of precision medicine, which seeks to adapt medical treatments to individuals' genetic profiles. In the context of cardiac myopathies, precision medicine enables personalised methods that directly target the underlying genetic abnormalities in each patient, shifting away from the "one-size-fits-all" therapy paradigm[40].

Personalized Gene Editing

CRISPR-based therapies allow for the correction of specific genetic mutations, resulting in more personalised treatment. Prondzynski et al.,[41] successfully corrected MYBPC3 mutations in iPSC-derived cardiomyocytes, thereby restoring sarcomere function. Ma et al.,[42] showed that CRISPR can precisely correct MYBPC3 mutations in human embryos, highlighting its potential in germline therapy. Schwank et al.,[43] repaired CFTR mutations in cystic fibrosis patient-derived organoids, while Yeh et al.,[44] corrected PCSK9 mutations in vivo and reduced cholesterol levels. These studies highlight CRISPR's transformative role in precision medicine, allowing for targeted, patient-specific treatments.

CRISPR in Disease Modeling and Drug Screening

Patient-specific iPSCs produced from cardiac myopathy patients serve as a platform for medication screening and disease modelling. Researchers may create personalised models of heart illness by inserting or correcting mutations into iPSCs using CRISPR[45]. These models may be used to assess the efficacy of various medications in a manner that is tailored to the patient's genetic background, allowing for more accurate predictions of treatment success. This method is an important part of precision medicine since it guarantees that therapies are not just genetically based but also personalised to an individual's specific illness profile.

Ethical and Regulatory Considerations

As CRISPR-based medicines advance, ethical and regulatory frameworks will be required to address issues such as off-target consequences, somatic vs. germline editing, and equal access to treatments. Precision medicine utilising CRISPR has the potential to revolutionise the treatment of cardiac myopathies, but careful evaluation of these ethical considerations will be required before broad implementation[46].

CHALLENGES AND LIMITATIONS

CRISPR technology, while promising for treating genetic illnesses such as cardiac myopathy, confronts a number of obstacles and limits. One major issue is off-target consequences, which occur when inadvertent DNA alterations cause dangerous mutations and new illnesses. Although high-fidelity Cas9 variations and technologies like as base editors and prime editing strive to increase precision, long-term safety is questionable. Ethical considerations surround germline editing, particularly the possibility of unexpected genetic repercussions and "designer babies." Furthermore, there are significant technical challenges in efficiently delivering CRISPR components into target cells, such as cardiomyocytes, as well as regulatory challenges caused by a lack of standardised procedures and guidelines for gene therapies, particularly for complex conditions like cardiac myopathy. These issues require rigorous testing and international regulation to ensure the safe clinical application of CRISPR-based therapies.

CONCLUSION

CRISPR technology has the potential to change the treatment of cardiac myopathies by allowing for precision gene editing to target the underlying causes of hereditary heart disease. CRISPR, with advances like as prime editing and epigenome editing, opens up new therapeutic options, bringing promise for curative medicines that may enhance patient outcomes and quality of life. However, realising its full potential will need continued study, cooperation, and rigorous ethical considerations to address issues like as off-target effects, distribution techniques, and regulatory barriers. Continued progress in these areas, combined with rigorous clinical trials and safety assessments, will be critical to ensuring that CRISPR-based therapies can be safely and effectively used in clinical practice, ultimately revolutionising the treatment of cardiac myopathies and other genetic disorders.

FUTURE DIRECTIONS

The future of CRISPR-based gene therapy for cardiac myopathy seems optimistic, with multiple advances opening the door for more precise therapies. Prime editing, which provides very exact genetic changes with fewer off-target consequences, has enormous potential, particularly in the treatment of minor abnormalities linked with heart disorders. Epigenome editing, which affects gene expression without affecting the DNA sequence, may offer a reversible and less intrusive method of influencing disease development. Furthermore, combining CRISPR with other medicines, such as stem cell therapies, might lead to more personalised cardiac treatments, including the creation of healthy heart tissue for transplantation. RNA-based therapeutics such as RNA interference and antisense oligonucleotides may supplement CRISPR by targeting faulty proteins. However, translating

these innovations from the laboratory to clinical practice requires overcoming challenges in delivery systems, clinical trials, and personalised care, necessitating close collaboration among researchers, clinicians, and regulatory bodies to ensure the safe and effective use of CRISPR-based therapies.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

REFERENCES

1. Elliott P, Andersson B, Arbustini E, et al. Classification of the cardiomyopathies: a position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2008;29(2):270–276.
2. Maron BJ, Gardin JM, Flack JM, et al. Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Circulation. 1995;92(4):785-789.
3. Towbin JA, Lorts A, Jefferies JL. Left ventricular noncompaction cardiomyopathy. Lancet. 2015;386(9995):813-825.
4. Marcus FI, McKenna WJ, Sherrill D, et al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: Proposed modification of the Task Force Criteria. Circulation. 2010;121(13):1533-1541.

5. Mestroni L, Taylor MR. Genetics and genetic testing of dilated cardiomyopathy: A new perspective. Cardiovasc Res. 2013;97(2):153-162.

6. Herman DS, Lam L, Taylor MR, et al. Truncations of titin causing dilated cardiomyopathy. N Engl J Med. 2012;366(7):619-628.

7. Corrado D, Basso C, Thiene G. Arrhythmogenic right ventricular cardiomyopathy: Diagnosis, prognosis, and treatment. Heart. 2000;83(5):588-595.

8. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014;346(6213):1258096.

9. Hinson JT, Chopra A, Nafissi N, et al. Heart disease-causing mutations in FLNC induce arrhythmogenesis and contractile dysfunction in human cardiomyocytes. Nat Commun. 2015;6:7805.

10. Jiang F, Doudna JA. CRISPR–Cas9 structures and mechanisms. *Annu Rev Biophys*. 2017;46:505-529.

11. Nishimasu H, Ran FA, Hsu PD, Konermann S, Shehata SI, Dohmae N, et al. Crystal structure of Cas9 in complex with guide RNA and target DNA. *Cell*. 2014;156(5):935-949.

12. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, et al. RNA-guided human genome engineering via Cas9. *Science*. 2013;339(6121):823-826.

13. Pickar-Oliver A and Gersbach CA. The next generation of CRISPR-Cas technologies and applications. Nat Rev Mol Cell Biol. 2019;20:490–507.

14. Zhang Y, Long C, Bassel-Duby R and Olson EN. Myoediting: Toward Prevention of Muscular Dystrophy by Therapeutic Genome Editing. Physiol Rev. 2018;98:1205–1240.

15. Koblan LW, Erdos MR, Wilson C, Cabral WA, Levy JM, Xiong ZM, Tavarez UL, Davison LM, Gete YG, Mao X, Newby GA, Doherty SP, Narisu N, Sheng Q, Krilow C, Lin CY, Gordon LB, Cao K, Collins FS, Brown JD and Liu DR. In vivo base editing rescues Hutchinson-Gilford progeria syndrome in mice. Nature. 2021;589:608–614.

16. Musunuru K, Chadwick AC, Mizoguchi T, Garcia SP, DeNizio JE, Reiss CW, Wang K, Iyer S, Dutta C, Clendaniel V, Amaonye M, Beach A, Berth K, Biswas S, Braun MC, Chen HM, Colace TV, Ganey JD, Gangopadhyay SA, Garrity R, Kasiewicz LN, Lavoie J, Madsen JA, Matsumoto Y, Mazzola AM, Nasrullah YS, Nneji J, Ren H, Sanjeev A, Shay M, Stahley MR, Fan SHY, Tam YK, Gaudelli NM, Ciaramella G, Stolz LE, Malyala P, Cheng CJ, Rajeev KG, Rohde E, Bellinger AM and Kathiresan S.

In vivo CRISPR base editing of PCSK9 durably lowers cholesterol in primates. Nature. 2021;593:429–434.

17. Chemello F, Chai AC, Li H, Rodriguez-Caycedo C, Sanchez-Ortiz E, Atmanli A, Mireault AA, Liu N, Bassel-Duby R and Olson EN. Precise correction of Duchenne muscular dystrophy exon deletion mutations by base and prime editing. Sci Adv. 2021;7:eabg4910.
18. Ho CY, Charron P, Richard P. Genetic advances in sarcomeric cardiomyopathies: State of the art. Cardiovasc Res. 2015;105(4):397–408.
19. Hershberger RE, Hedges DJ, Morales A. Dilated cardiomyopathy: The complexity of a diverse genetic architecture. Nat Rev Cardiol. 2013;10(9):531–547.
20. McKenna WJ, Judge DP. Arrhythmogenic cardiomyopathy: Insights into genetics and pathogenesis. Nat Rev Cardiol. 2021;18(6):361–372.
21. Te Rijdt WP, van Tintelen JP, Hauer RN. Molecular mechanisms in arrhythmogenic cardiomyopathy. Nat Rev Cardiol. 2019;16(9):519–537.
22. Pinto YM, Elliott PM, Arbustini E. Proposal for a revised definition of dilated cardiomyopathy, hypokinetic non-dilated cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy. Eur Heart J. 2016;37(23):1850–1858.
23. Hershberger RE, Givertz MM, Ho CY, et al. Genetic evaluation of cardiomyopathy: A heart failure society of America practice guideline. J Card Fail. 2018;24(5):281–302.
24. Van der Velden J, Ho CY, Tardiff JC. Sarcomeric mutations in hypertrophic cardiomyopathy: Mechanisms and therapeutic strategies. Basic Res Cardiol. 2015;110(4):51.
25. Judge DP, Roux M, Tristani-Firouzi M. Genetics of dilated cardiomyopathy: The complexity of genetic inheritance. Cardiol Clin. 2021;39(1):39–52.
26. Musunuru K, Sharma G, Refaat MM. Precision medicine for hypertrophic cardiomyopathy using genome editing. Nat Rev Cardiol. 2020;17(7):458–467.
27. Ma H, Marti-Gutierrez N, Park SW. Correction of a pathogenic gene mutation in human embryos. Nature. 2017;548(7668):413–419.
28. Ishikawa K, Weber T, Hajjar RJ. Gene therapy for dilated cardiomyopathy: Targeting titin mutations with CRISPR. Circ Res. 2019;124(7):904–915.
29. Prondzynski M, Lemoine MD, Zech ATL. Disease modeling of a mutation in TTN using CRISPR/Cas9-mediated genome editing in human iPSCs. Mol Ther. 2017;25(11):2600–2610.

30. Nelson CE, Gersbach CA. Engineering delivery vehicles for genome editing. Annu Rev Chem Biomol Eng. 2016;7:637–662.

31. Gallagher M, Jones DJ, Kaplinsky JJ. Gene therapy strategies for inherited cardiomyopathies: An update on preclinical and clinical studies. J Mol Cell Cardiol. 2021;155:78–91.

32. Javed A, Anderson DG, Zhang F. Advances in CRISPR-based genome editing for the treatment of cardiomyopathies. Nat Rev Genet. 2021;22(12):798–816.

33. Redwood C, Moolman-Smook J, Watkins H. Properties of mutant contractile proteins that cause hypertrophic cardiomyopathy. Nat Clin Pract Cardiovasc Med. 2007;4(3):156–167.

34. Musunuru K, Sharma G, Refaat MM. Precision medicine for hypertrophic cardiomyopathy using genome editing. Nat Rev Cardiol. 2020;17(7):458–467.

35. Ma H, Marti-Gutierrez N, Park SW. Correction of a pathogenic gene mutation in human embryos. Nature. 2017;548(7668):413–419.

36. Nakamura K, Hirose M, Kusuyama T. CRISPR-Cas9 gene editing therapy in cardiomyopathies: Current advances and future perspectives. Curr Gene Ther. 2020;20(1):23–35.

37. DeKelver RC, Gregory PD, Joglekar AV. Targeted genome editing technologies in cardiovascular diseases: Advances in CRISPR and beyond. J Am Coll Cardiol. 2017;69(9):1122–1133.

38. Nelson CE, Gersbach CA. Engineering delivery vehicles for genome editing. Annu Rev Chem Biomol Eng. 2016;7:637–662.

39. Javed A, Anderson DG, Zhang F. Advances in CRISPR-based genome editing for the treatment of cardiomyopathies. Nat Rev Genet. 2021;22(12):798–816.

40. Ding Q, Regan SN, Xia Y, Oostrom LA, Cowan CA, Musunuru K. Enhanced efficiency of human pluripotent stem cell genome editing through replacing TALENs with CRISPRs. *Cell Stem Cell*. 2013;12(4):393-394.

41. Prondzynski M, Lemoine MD, Zech AT, Horváth A, Di Mauro V, Koivumäki JT, et al. Correction of MYBPC3 mutation in human iPSC-derived cardiomyocytes reverses contractile deficits and sarcomere disarray. *Cardiovasc Res*. 2020;116(4):784-797. doi:10.1093/cvr/cvz311.

42. Ma H, Marti-Gutierrez N, Park SW, Wu J, Lee Y, Suzuki K, et al. Correction of a pathogenic gene mutation in human embryos. *Nature*. 2017;548(7668):413-419. doi:10.1038/nature23305.

43. Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, Demircan T, et al. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell Stem Cell*. 2013;13(6):653-658. doi:10.1016/j.stem.2013.11.002.
44. Yeh WH, Chiang H, Rees HA, Edge ASB, Liu DR. In vivo base editing of post-mitotic sensory cells. *Nat Commun*. 2018;9(1):2184. doi:10.1038/s41467-018-04580-3.
45. Matsa E, Burridge PW, Wu JC. Human stem cells for modeling heart disease and for drug discovery. *Sci Transl Med*. 2014;6(239):239ps6.
46. Yang L, Li H, Deng S. CRISPR/Cas9-based gene editing as a potential therapeutic tool for cardiomyopathies. Front Cardiovasc Med. 2021;8:767567.