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# Recent Developments in Crispr-Based the Genome Alteration Technology: New Approaches and Uses in Cardiovascular Studies

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**ABSTRACT:**

Significant advances in domains of medicine we have resulted from the quick creation of technologies for genome editing. With this development of the CRISPR - associated protein (Cas) nucleases and new uses in the conjunction with other effectors and genome alteration toolkit based on short palindromic repeats that are clustered regularly interspaced (CRISPR) has been a significantly grown in recent years. Research on the cardiovascular disease (CVD's) has also been transformed by CRISPR-based gene editing technology. Genome editing has demonstrated its worth as potent tool for modelling or perhaps treating a wide range of diseases over the last 25 years. After the development of protein-guided systems like as transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs), which initially made DNA editing possible, RNA-guided techniques have brought about a paradigm change. The CRISPR/Cas9 technique, which is based on the bacterial antiphage system and has produced a versatile and flexible the DNA-editing system that been able to get past a number of drawbacks of previous approaches and quickly emerging as the most widely used tool for both therapeutic research and disease modelling. Prime and Base editing are two newly developed CRISPR/Cas9-derived techniques that have expanded the scope and precision of possible genomic alterations in the recent years. With an emphasis on models for the investigation and management of cardiac disorders, this review attempts to give a broad an overview of the most of recent advancements in field of genome alteration or editing and its uses in the biomedical studies or research.

**Keywords:** CRISPR, cardiovascular diseases, DNA, gene, zinc finger nucleases

## INTRODUCTION:

Cardiovascular diseases (CVDs) are the continue to be one of the world's most serious health issues and a leading cause of death worldwide. Over the past three decades, the prevalence of CVDs has been increased by 93% worldwide, from 271 million in 1990 to 523 million in 2019. Furthermore, the overall number of the fatalities from CVDs has risen by the roughly 54%, accounting for roughly one-third of all the deaths worldwide. It was shown that by 2030, cardiovascular disease (CVD) would account for about 23 million fatalities globally, or almost 30.5% of all deaths [1].

Cardiomyopathy, , hypertensive heart disease, peripheral arterial disease, rheumatic heart disease, cerebrovascular disease, heart failure, and several other cardiac issues are the examples of CVDs. Although lifestyle choices and environmental factors have been identified as risk factors for the development of the CVDs, they only account for a small portion of the occurrences. In order to understand cases that are not clearly linked to the established risk factors for the development of CVDs, it is crucial to investigate the underlying molecular processes. Genetic susceptibility has been shown to be a major factor in the development of CVDs [2].

The molecular foundation of an increasing in number of heart disorders has been discovered thanks to genetic techniques. Apart from the genes that are known to function in the cardiovascular system, investigating additional genes linked to heart conditions could yield innovative treatment approaches for the CVDs. One of the best ways to support medicinal efforts is through genome editing techniques. Genome editing techniques have drawn a lot of attention as potential therapeutic options for preventing CVDs by either deleting particular genes or fixing disease-causing mutations [3].

It has been reported that videlicet, mutations that gain function in the pro-protein convertase subtilisin-like kexin type 9 (PCSK9) gene, a key regulator of LDL-cholesterol concentrations and LDL-receptor levels, raise LDL-C levels, increasing the risk of a high cholesterol level and coronary heart disease (CHD). On the other hand, research on PCSK9 loss-of-function mutations shows that PCSK9 inactivation lowers LDL-C levels and lowers CHD, indicating that PCSK9 inhibition is a legitimate therapeutic approach for the treatment of hypercholesterolaemia and associated conditions [4].

Function of many intracellular defence and regulatory systems is depended upon on complementary base pairing between the nucleic acid species. Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR is related with the proteins (CRISPR-Cas) systems use of the base-pairing of nucleic acid to inhibit or to eliminate the genetic targets, in addition to prokaryotic Argonaute-based systems and eukaryotic RNA interference (RNAi) [5]. These systems are required to maintain and keep flexible the memory of the sequence targets that were acquired from prior bacteriophage exposure. A sequence of brief palindromic repeats (i.e. 25–35 bp each) divide the targeted sequences, which are kept as the spacers of 30–40 base pairs (bp) apiece. The "CRISPR array" is made up of the cas genes themselves, these spacers and repeats, Furthermore, proteins necessary for Cas nuclease activity, the foreign nucleic acid detection and uptake into CRISPR array, and the recognition of invasive mobile genetic elements are required for the activity of the CRISPR-Cas-mediated defences [6,7].

Genome alteration is making changes in DNA in various cells and organisms. This can involve adding or removing out pieces of DNA. The results significantly, like turning off certain genes, fixing harmful mutations in genes or introducing new traits [8-10]. Lately, as life sciences have rapidly progressed, genome editing has become a go- to method for exploring how genes work, uncovering the causes of inherited diseases, finding new targets for gene therapy, and even creating new types of crops, among other exciting uses [11-14].

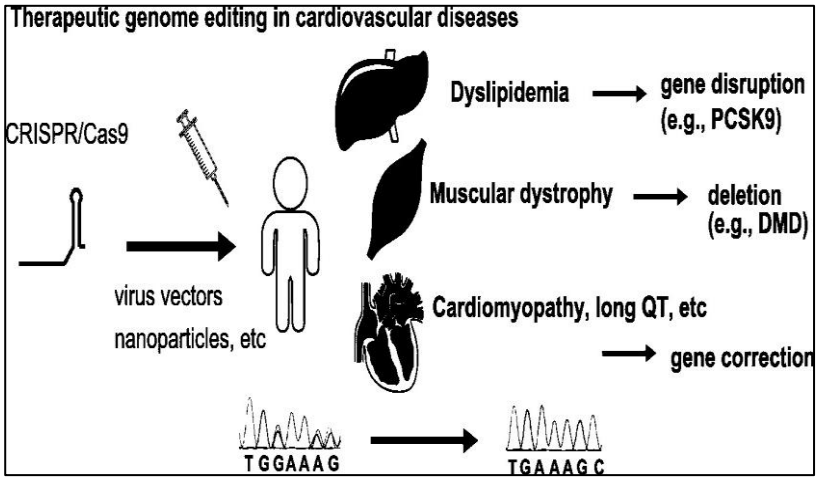


**Fig.1.1** Therapeutic genome editing in cardiovascular diseases

RNA-guided CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated) nucleases systems, transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs) are recently the three widely used genome alteration tools in the world [15,16]. CRISPR-Cas systems are now the most used genome editing method in the molecular biology labs worldwide because of its quick cycle, high efficiency, low cost, strong repeatability, and simple design [17,18]. This review will provide an overview of the CRISPR-Cas systems, covering their advances and applications in gene therapy and human illness research, as well as the potential and problems that will arise in their practical use.

Based on the structure of effector complexes - CRISPR-Cas systems are classified into 2 different classes: Class 1 systems and Class 2 systems [18,19].

Based on variations in Cas protein participation, and CRISPR locus topologies. These classes are further separated into 6 kinds and numerous subtypes:

- Class 1 systems: Cas types I, III, and IV
- Class 2 systems: Cas types II, V, and VI The two separate processes of

(i) Immunisation

(ii) The molecular mechanisms of prokaryotic adaptive immunity based on CRISPR are defence or resistance.

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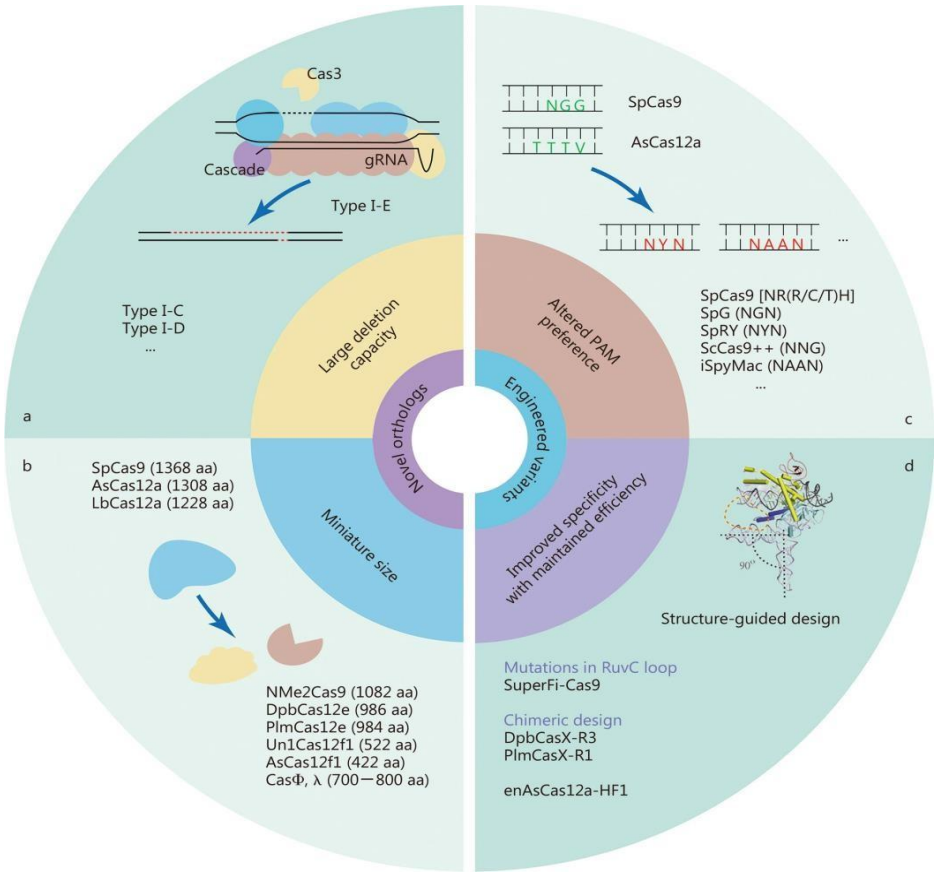


**Fig. 1.2** Aspect of designed and genetically modified system in genetic engineering

Alien genetic components have novel spacer sequence called a protospacer. The protospacer sequence needs to be close to a short motif called the protospacer-adjacent motif (PAM) for a number of CRISPR-Cas system types. The host CRISPR array is dependably shielded from destruction by its own CRISPR-Cas machinery by recognising this pattern. The Cas1 and Cas2 proteins' activity determines the adaption process. During the crRNA biogenesis step and transcription of CRISPR array elements produces precursor pre-crRNA or crRNAs, which are then processed into mature crRNA [20]. Depending on the specific CRISPR-Cas type, either cellular ribonucleases or Cas proteins carry out this maturation. Mature crRNA is incorporated into the Cas protein effector complex. This complex uses the crRNA to examine foreign genetic material for sequencing complementarity. If the requirements for compatibility and a PAM region are satisfied, the Cas protein nuclease is activated, precisely targeting and destroying the foreign genetic code [21,22].

The high specificity, productivity, and adaptability of the CRISPR-Cas system have made it a significant genetic modification method. This technology has improved our ability to control gene expression that produce new drugs to change genomes and diagnose, prevent, to treatment of illnesses. CRISPR-Cas systems used for the management viral infections.

A double-stranded break (DSB) brought on by sgRNA attracting Cas9 endonuclease to a particular location in the genome during genome editing can be repaired by two endogenous self-repair mechanisms: the prone to error non-homologous end joining (NHEJ) route and the homology-oriented repair (HDR) pathway. Since NHEJ is the active for over the 90% of the cell cycle and is independent of the neighbouring homology donor, it is generally more effective than that of HDR. NHEJ has the ability to introduce the random insertions or deletions into the cleavage sites of which can result in premature stop codons or the frameshift alterations inside the target genes open reading frame (ORF), the ultimately rendering them inactive [23-28].

As an alternative, HDR can use as a homologous DNA repair template to the precisely alter the genome at the target spot. Additionally, utilising the several sgRNAs targeting one or more genes could be the result in massive fragment deletions and simultaneous knockout of many of genes [27-28].

## ADVANCES IN CRISPR EFFICIENCY:

### Base Editing:

Irreversible and the direct conversion of one DNA base to another without double strand breaks (DSBs) or a donor DNA template are possible using our base editors, which provide precision. Ideally they are composed of a catalytically impaired Cas protein (mostly dCas9 or a Cas9 nickase) fusion to a DNA modifying enzyme like a cytidine deaminase or an adenosine deaminase. Cytosine base editor (CBE) refers to converting CG to TA while adenine base editors (ABE) convert AT to GC within a defined editing window [29]. Base editors do not create DSBs, thus not forming insertions/deletions typically seen with non-homologous end joining (NHEJ). Therapeutic potential of base editing has been in correcting point mutation of genetic disorders, including familial hypercholesterolemia by editing PCSK9 gene [30].

### Prime Editing:

A more recent technique, specifically called Versatile Search-and-Replace Prime editing, is a relatively new technique that uses both a Cas9 nickase along with a a prime editing guide RNA and reverse transcriptase enzyme and a prime editing guide RNA, or pegRNA. A wide variety of precise edits, including all point mutations, small insertions and deletions, are possible without using DSBs or donor templates [31]. After the Cas9 nickase makes the cut to one DNA strand a reverse transcriptase builds the planned genomic alteration through the pegRNA template. The broad flexibility of prime editing surpasses the targeting limitations of base editors because it allows various mutations to be corrected. Prime editing successfully performed cardiac gene corrections in preclinical models thereby creating excellent prospects for its application in disease therapy [32].

### Challenges and Limitations:

Advancements in CRISPR technologies have not resolved all limitations since they encounter several challenges such as poor edition rates in specific cell types together with off-target consequences and complicated delivery approaches. Engineers have developed Cas9 variants exhibiting high fidelity together with properly designed guide RNAs to minimize off target effects [33]. Mobile CRISPR delivery proves to be a major obstacle for successful deployment in specific tissues such as the heart. Scientists at the moment research lipid nanoparticles along with engineered viral vectors to improve delivery systems and their effectiveness [34].

## CRISPR TECHNOLOGY IN CARDIOVASCULAR RESEARCH:

### Base and Prime Editing Applications:

Base and prime editing provide accurate molecular methods for treating cardiovascular disease-related genetic defects. Studies of gene editing in living organisms reveal that adenine base editing deactivates PCSK9 in liver tissue which produces long-lasting LDL cholesterol decrements [29]. Like prime editing, prime editing also corrects point mutations causing cardiomyopahthies in human induced pluripotent stem cell derived cardiomyocytes, which provides a possibility for personalize gene therapy. [32].

### CRISPRi and CRISPRa for Gene Regulation:

First, CRISPRa and CRISPRi utilize a catalytically dead Cas9 (dCas9) fused to transcriptional repressors or activators, respectively. They are reversible, and do not change the underlying DNA sequence. CRISPRi has been used to knock down arrhythmia genes, and CRISPRa to boost protective genes to protect the heart against cardiac stress in cardiovascular research [31]. Such tools have important value in gene function studies and may represent early therapeutic approaches for temporary gene modulation for conditions that benefit from such modulation.

### In Vivo Delivery Strategies:

The therapeutic achievement depends heavily on proper CRISPR component delivery to cardiac tissue. AAV vectors of serotype 9 represent effective vectors that deliver components to cardiac muscle according to preclinical models [36]. The long-term activation of these delivery agents within the body can initiate immune-related reactions. Other than AAV vectors serotype 9 lipid nanoparticles serve as effective delivery vehicles which recently succeeded in delivering base editors to human livers during clinical trials. Investigators are using two strategies to improve LNPs' heart-specific targeting capabilities or create replication-specific capsids to enhance heart delivery [34].

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**APPLICATIONS OF CRISPR IN CARDIOVASCULAR DISEASE**

Cardiovascular diseases have been a major cause of death and even leading to life time medications for an individual for survival. The factors that are responsible for cardiovascular disease are obesity, hypertension, unhealthy food, heredity, smoking etc. To reduce the deaths caused by the cardiovascular disease and to treat the patient completely and make that person healthy with the help of gene editing technology that is CRISPR. CRISPR is a very advanced technique which can be used for altering the genomic material of a specific individual. There are various genes that are responsible for various diseases in an individual and thereby making alterations in the gene can recover the individual from the disease. It is a very advanced research which is ongoing and can bring a great revolution in various cardiovascular diseases and other diseases. It has a wide range of application in cardiovascular disease:

**Genetic disorders and cardiomyopathies:**

These are one of the major ongoing problems for cardiovascular disease. The most common type of cardiomyopathies is hypertrophic and dilated cardiomyopathy. There are specific genes which can be targeted and altered for modification to cure the disease. There are various studies that have been done on the hypertrophic cardiomyopathy and dilated cardiomyopathy.

1) **Hypertrophic cardiomyopathy: -** It is a disorder which is characterized by thickening of heart muscle which can lead to failure of heart or arrhythmia [37]. Researches were performed on rats who didn't express MYBPC3 protein they developed a very high thickness of ventricular wall and low functions of cardiac. After this using CRISPR genome editing the researchers saw a significant MYBPC3 protein expression and it also gave a conclusion of that CRISPR editing genome tool can be used for treating cardiovascular disease [38].

2) **Dilated cardiomyopathy: -** It is a heart disease characterized which occur due to genetic mutation or due to infection and can cause weakening of the hearts ventricles. A study on rat was performed to showcase the activity dilated cardiomyopathy. By deletion of LMNA gene from rat the researchers saw the reduced life span of the rat and when they inserted the LMNA gene with the help of adeno associated virus they a saw a significant increase in life span of rat [39]. CRISPR editing tool can be used to permanently fix the problem rather than just temporary symptom management.

**Atherosclerosis and lipid disorders:**

Atherosclerosis can narrow the passage of the blood flow by building up bad fat and can increase the chances of heart attack or stroke. Many researches have been performed and from them in one of the research cynomolgus monkey (*Macaca fascicularis*). A specific gene in liver PCSK9 was knockdown by inserting lipid nanoparticles and there was increase in low density lipoprotein which led to death by atherosclerotic cardiovascular disease. The other group of monkey were also treated same way by knocking out PCSK9. After this the genome modification technology was used which included CRISPR-Cas enzymes [40-42] and CRISPR base editors [43,44]. Then by using CRISPR genome editing tool the researchers deactivated PCSK9 which further led to increase in the low-density lipoprotein receptor and cholesterol clearance in blood was increased [45].

**Hypertension and vascular disease:**

By using CRISPR genome editing tool we can treat the high blood pressure in an individual by suppressing the angiotensinogen gene which is a precursor for angiotensin peptide II. Angiotensin II increases blood pressure by constricting the blood vessels which causes high vascular tension. With the help of CRISPR-Cas 9 it can permanently disable the angiotensinogen gene resulting in reduction of blood pressure [46].

**Myocardial fibrosis gene editing:**

CRISPR genome editing tool has a made a great advancement in treating myocardial infractions, myocardial fibrosis. COL1A1 and COL3A1 are major and essential components which are responsible for the fibrotic tissue. By using CRISPR gene editing tool or RNAi and directly targeting COL1A1 and COL3A1 to suppress them and reduce the collagen deposition [47].

**Cardiac regeneration with stem cell therapy:**

After a heart attack there are many damaged heart muscle tissue including cardio myocytes affecting the hearts' ability to contract effectively. To treat it we can use stem cells because of their great efficacy and they can displace the damaged cell. Modifying an individuals genome can significantly enhance survival and potential of the stem cells if a specific gene has been targeted for promoting cell survival [48].

## ETHICAL, SAFETY AND REGULATORY CHALLENGES

CRISPR/Cas9 tool is a powerful weapon contributing wide ranging benefits in areas of clinical therapeutics, transgenic animals and agriculture [49]. The dark side of this technology appears due to ethical, moral and safety concerns [49].

### Off-target effects:

The role of gRNA is to target CRISPR to specific DNA sequence but sometimes the gRNA may malfunction and instead of targeting the CRISPR to desired location of genes, it may target to some other regions of DNA causing off-targeting [50]. The root cause lies in the size of the guide RNA [51]. Larger the size of guide RNA, more the identical sequences of DNA it will be containing leading to more off-targets. To overcome this issue, the size of guide RNA has to be precisely maintained. Guide RNA containing less than 20 nucleotides gives less off-target effects. At the same time, it should not be smaller than 15 base pairs where it loses its binding ability to target site [52]. Off-target mutations may get inherited as such and disturbs the future generation, it occurs via process known as gene drive [53,54].

### Germ-line editing:

Another ethical concern revolves around germ-line editing, as germ cells are involved in the reproduction process, modification in these cells might cause genetic mutation and these genetic defects/mutations get transferred to future generation. It is not possible to predict mutations/genetic defects before experiment as these effects appear after sometime in future generation [54]. Also, some individuals can misuse it for their own benefits, for example, improving health by changing the genetic make-up of sports person or modifying desired phenotypic character [53]. The research of CRISPR is found less in germ-cell due to fear of unpredictable mutations thus scientist focus on research involving somatic cell lines [55].

### Regulatory landscape and clinical trial progress:

The harmful effects of CRISPR-Cas9 technology such as off-targeting and germ-line editing makes the regulatory procedures a complex process [56]. The jurisdiction attempts to minimize the ethical considerations and maximize the scientific potential of CRISPR [56]. In some countries, there are less problems for regulatory acceptance with genome edited crops and thus rising a concern on safety in public [57,58]. Whereas in countries like Europe, there are stringent regulations for genetic modified organisms thus assuring the public safety [59]. The ethical concern of germ-line editing made the World Health Organisation to establish a global governance framework which could help stabilize the standards among nation [60]. In United States, more emphasis is given on establishing regulations that could balance safety and it has also restrictions for funding on germ-line research [61]. China and Japan focus on improving therapeutics by permitting gene editing to a small extent thus leading a concern for public safety [62].

### Clinical trial progress:

The vast advantages of CRISPR technology is replacing the old gene editing methods. It has found its applications in gene modification for neurological, cardiovascular, renal and genetic disorders [63]. It helps in allowing thorough understanding of disorder by development of disease models and making it ready for clinical research [63,64].

## CONCLUSION:

New advancement of the life science and medicine has been aided by the new Cas nucleases and the wide range of uses, which have significantly increased the CRISPR-based genome editing toolbox and have a wide range of uses. Cardiovascular research has been transformed by CRISPR-based genome editing technology, which has sped up the creation of genetically altered models of Cardio Vascular Diseases and their use in treating various forms of CVD. To avoid misuse, this technology should be tightly regulated as it may also pose serious biological risks and proper care and management should be by regulatory authority.

## FUTURE SCOPE, PERSPECTIVES AND CLINICAL TRANSLATION IN RESEARCH

### Next generation CRISPR techniques:

Epigenome editing refers to making selected changes in epigenetic aspects mainly DNA methylation, it does not involve change in DNA sequence [65]. Thus, it broadens the therapeutic power of CRISPR system in comparison to traditional gene editing methods [65].

**CRISPR as a tool for personalized cardiovascular medicine:**

CRISPR-Cas9 technology is a powerful tool in treating cardiovascular diseases due to its ability to detect the gene responsible for disease and modulate it. It also provides a extra edge to design disease models which helps to understand the cause of disease and plan its treatment accordingly [66].

**Potential for CRISPR based gene therapies to enter mainstream clinical practice:**

CRISPR-Cas9 tool has optimised the way in which genetic disorders can be treated by allowing the necessary modification of genes. Although it provides a lot of advantages, its ethical and legal concerns should also be handled with appropriate measures [56]. Being easy to use and efficient in action, it has initiated clinical trials in cancer, cardiovascular disorders, neurological disorders, immunological disorders, allergy, eye related disorders and viral infection [67].

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