Exploring Gene Editing for the Treatment of Heart Diseases

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1. What is CRISPR and CAS9?

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats, and it's a revolutionary technology developed for gene editing. It allows scientists to be able to make precise, crucial changes in one's DNA. CAS9 is a protein that "cuts" DNA at a specific, well chosen location. This technology was first developed as a virus protectant, but then was used for other purposes. In 2020, Jennifer Doudna and Emmanuelle Charpentier were awarded a Nobel Prize for using this technology for gene editing. At the time, this was a big deal as there were two females who significantly changed cardiac treatment for the better.

1.1 How does CRISPR and CAS9 work?

The mechanism of CRISPR-Cas9 is relatively simple. The CRISPR-Cas9 utilizes RNA to direct the Cas9 protein to a "target location". It then cuts the DNA allowing for gene insertion, deletion, or correction of the gene. The benefit of this protein is its accuracy. It is able to target specific points in the DNA sequence without affecting other points, which results in fewer off-target effects (unwanted mutations).

1.2 Heart Mutations

Majority of affecting heart diseases (hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmias, and congenital heart defects, etc.) are caused by genetic mutations. These genetic mutations can be fatal, disrupting the normal function of cardiac cells, impairing the contraction of heart muscles, and compromising the structural integrity.

1.3 How does CRISPR and CAS9 help heart mutations?

CRISPR-Cas9 can help reduce the severity of these heart diseases by changing the DNA sequence. There are three major ways that this protein can help avoid heart diseases. These are correction, addition, and point change.

1.4 Correction

Correction involves fixing a specific mistake (mutation) in the genetic sequence. An example of this is used for the treatment of hypertrophic cardiomyopathy. CRISPR-Cas9 is used to fix the mutated gene that causes heart muscle thickness (leading to hypertrophic cardiomyopathy).

1.5 Addition

Addition is when inserting a nitrogenous base (Adenine, Thymine, Guide, Uracil, Cytosine) manages to fix the mutation. An example of this is shown during the treatment of Duchenne Muscular Dystrophy (genetic disorder affecting cardiac and skeletal muscles). Patients diagnosed with DMD are lacking a properly functioning **dystrophin gene,** necessary for muscular stability.Scientists have been able to successfully use CRISPR-Cas9 to add a function copy of this gene into the sequence. This approach has successfully improved strength of muscles in humans and animals, and holds a promising future for more DMD treatments.

1.6 Point Change

A point change is when one specific, single DNA base is replaced by another. Most diseases are caused by mutations of one DNA base only, so this alone can treat/cure most patients. An example where a point change is used to fight disease is in LMNA Gene Mutations. Mutations in the LMNA genes are extremely dangerous as they cause heart failure. Currently, point changes are being used to alter the mutation, and prevent heart problems.

1.7 Addition

Some more advanced forms of using CRISPR-Cas9 to help de-escalate heart problems are Base Editing (BE) and Prime Editing(PE). BE is when a point change is done without damaging the double-stranded DNA sections. PE is a versatile tool used to precisely allow inversion, deletion, or replacement of a DNA sequence without damage to the rest of the DNA strand.

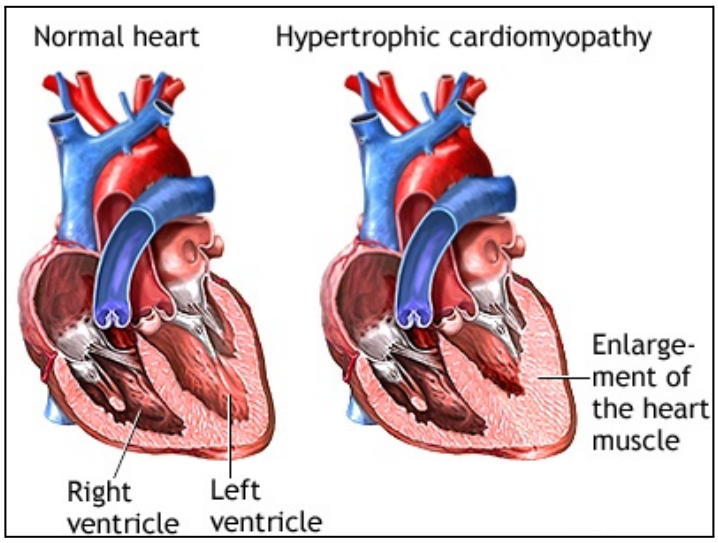
1.8 Risks

Although the use of CRISPR-Cas9 has been developed and almost perfected over recent years, there are still some challenges/risks that come with using this technique of treatment. There are some unwanted effects such as other unintended genetic modifications. It is rare, but sometimes these effects are worse than the disease itself. Another issue that may arise alongside the use of these proteins is the body's natural immunity response. This issue may cause the body to attack the components of CRISPR, resulting in decrease of effectiveness and more problems.

2. Future of CRISPR and CAS9

2.1 Targeted Genes and Common Genetic Heart Disorders

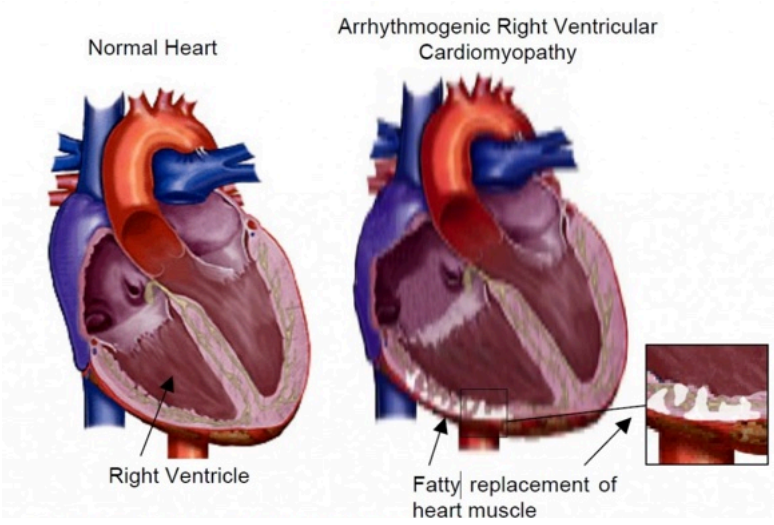
Two of the most common genetic heart disorders are hypertrophic cardiomyopathy (HCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC). These are inherited disorders caused by mutations in specific genes that affect the heart’s structure and function.



Key Genes Involved

- MYH7 – This gene makes a protein called beta-myosin heavy chain, which helps the heart muscle contract.
- MYBPC3 – Another common gene involved, it produces a protein that helps regulate heart muscle contraction. Mutations here can lead to faulty or missing proteins
- TNNT2 and TNNI3 – Changes in these genes can disturb the heart's rhythm and pumping ability.

ARVC is a disease where healthy heart muscle in the right ventricle is gradually replaced by fat and scar tissue. This weakens the heart and increases the risk of life-threatening arrhythmias which are irregular heartbeats.



2.2 Key Genes Involved

- PKP2 (Plakophilin-2) – The most common gene linked to ARVC. It helps cells in the heart stick together. Mutations weaken the connections, allowing cells to die and be replaced by scar tissue.
- DSP (Desmoplakin) and DSG2 (Desmoglein-2) – When these genes are mutated, it leads to structural damage in the heart muscle.

2.3 Future Potential in Gene Editing

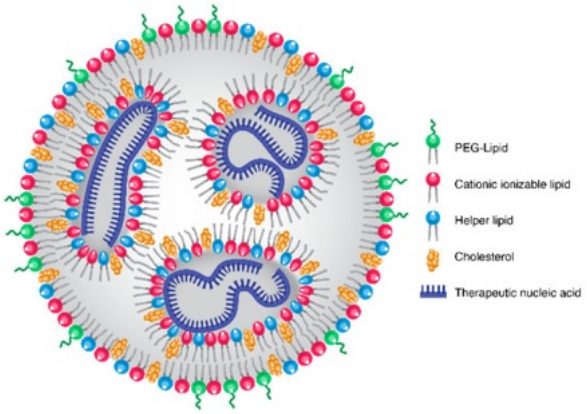
Advancements and developments in gene editing, such as CRISPR/Cas9, are progressing to treat genetic heart disorders. For example, studies have shown that genome editing has successfully been used in animal models such as mice to help prevent hypertrophic cardiomyopathy (HCM) by correcting mutations in genes. This information shows the potential of gene editing to address these kinds of problems such as cardiomyopathies.

However, this can also lead to challenges as scientists need to make sure that gene editing is safe and needs to be thoroughly evaluated. Additionally, another challenge is optimizing delivery methods to ensure efficient and precise editing in the heart cells so that it works efficiently and safely.

Despite these challenges, the development of CRISPR technologies and their applications to cardiovascular diseases show that gene editing may become a powerful tool for preventing or treating genetic heart conditions in the near future.

3. Two Types of Delivery Mechanisms for Cardiac Gene Editing

3.1 Viral Vectors



Corrects Wrong Genes - fixing mutations that cause inherited heart conditions like familial cardiomyopathies

3.2 Delivery of Viral Vectors to Heart Tissue

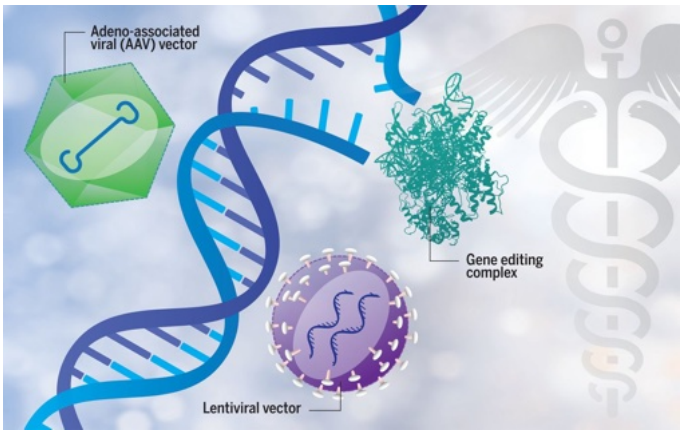
Viral vectors are commonly delivered to heart tissue through intravenous injection or directly into the coronary arteries. These vectors, often based on adeno-associated viruses (AAV), are engineered to carry therapeutic genes. Once in the bloodstream, the viruses target and enter heart cells, delivering the genetic material that can modify or enhance cellular function.

3.3 How This Helps People with Heart Disease

This method helps treat heart disease by repairing damaged tissue or improving heart function at the molecular level. For example, it can boost the production of proteins that support heart muscle strength or blood vessel growth, offering hope for conditions like heart failure or genetic heart disorders. Gene therapy using viral vectors is a promising step toward long-term solutions rather than just symptom management.

3.4 Lipid nanoparticles

Lipid nanoparticles are used for delivering nucleic acids like mRNA, siRNA, and CRISPR components into cells.



3.5 Delivery of Lipid nanoparticles to Heart Tissue

Lipid nanoparticles (LNPs) can be engineered to specifically target heart tissue by modifying their surface with ligands or antibodies that bind to receptors uniquely expressed on cardiac cells. Once administered through intravenous injection, they circulate in the bloodstream and accumulate in the heart.

After reaching the heart, the LNPs fuse with cell membranes and release their genetic payload, such as mRNA or gene-editing tools, into cardiac cells, enabling therapeutic effects like promoting regeneration or correcting genetic defects.

Even though these two methods haven't been fully developed, there are going to be more ways in the future to help those with heart diseases using other delivery mechanisms.

4. Ethical Implications and Concerns

4.1 Informed consent

One of the most significant ethical issues in genome editing is informed consent. Informed consent in experiments is a participant's granted permission to take part in the experiment despite the possible risks involved with the experiment; the participant is aware of any possible consequences and still agrees to join the experiment. Since genome editing can change germline cells, there is an ongoing concern that any alterations made to the genes can affect the embryos and possibly the lives of future generations that haven't given consent to the treatment. In fact, many countries have restricted or banned germline editing in order to prevent these risks.

4.2 Lack of Research and Off-target Mutations

Another issue is the lack of current research. Due to the ambiguity of the potential consequences of genome editing, experts believe that it is unsafe to start using genome editing technologies such as CRISPR-Cas9 and TALENs regularly on patients until further research is conducted. Additionally, because of gene drift in a population, the risk of off-target mutations, an unintended change in the genes that can occur as a result of gene editing, will continue—and possibly increase— in the future.

4.3 Genetic Mosaicism

In some cases, certain cells could contain the edit needed to treat a certain heart condition, while others don't. This risk is called genetic mosaicism, and can lead to some embryos lacking any of the cells that contain the gene edit, potentially causing the disease to occur. Additionally, the effects of mosaicism can cause several other unintended consequences, such as impairing or causing embryos to develop incorrectly, or even creating a new disease entirely.

4.4 Equality

Many advancements in medicine and healthcare have constantly benefited communities with more resources and wealth than developing or disadvantaged populations, creating a constant disparity. Like other recent and advanced technologies, gene editing technologies are very expensive, and are available to those with the wealth and social status needed to access the treatments. This risks intensifying the existing inequality in the healthcare system.

Furthermore, minority groups and communities are constantly underrepresented in gene editing research. Many initiatives have set diversity targets, aiming for about half of all participants in research studies and experiments to be from a minority population. Yet, an analysis conducted in 2016 found that only 4% of participants in genome editing research are of African, Hispanic, or Indigenous descent, and little has been done since to reach the target population.

In conclusion, through gene-editing technologies like CRISPR-CAS9 and other delivery mechanisms such as viral vectors and lipid nanoparticles, there is great potential for treating heart diseases using gene-editing. However, there are several ethical concerns about fairness and consent that come with this technology. With more research into this field, genome editing could be a more powerful tool to treat heart diseases. This would greatly improve the way healthcare treats heart mutations, and has potential to revolutionize the healthcare industry. These technologies are full of potential, and can benefit the lives of people of all ages and health conditions.

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