A review of CRISPR Cas9 for ASCVD: treatment strategies and could target PSCK9 gene using CRISPR cas9 prevent the patient from atherosclerotic vascular disease?

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ABSTRACT

Background: Targeting PCSK9 by maintaining hemodynamic shear stress stability has been shown in several studies to reduce LDL-C, arterial plaque formation, and PCSK9 expression in atherosclerotic cardiovascular disease. Genome editing with CRISPR-associated regularly interspersed short palindromic repeats (CRISPR/Cas9) have therapeutic potential for atherosclerotic cardiovascular disease. This study aims to evaluate the role of CRISPR/Cas9 in targeting PCSK9 as an effective therapeutic and long-term effect on atherosclerotic vascular disease.

Methods: The method used in this study summarizes the article by looking for keywords that have been determined in the title and abstract. The authors used official guidelines from Science Direct, PubMed, the American College of Cardiology, Google Scholar, and PERKI to select full-text articles published within the last decade, prioritizing searches within the last five years.

Results: CRISPR/Cas9 deletion of PCSK9 in mouse models reduces LDL-C, Plaque accumulation in the arteries, and PCSK9 expression. Furthermore, CRISPR/Cas9 deletion in PCSK9 saves the stability of Hemodynamic shear stress to control the PCSK9 expression that causes Atherosclerotic cardiovascular disease.

Conclusion: PCSK9 targeting by CRISPR/Cas9 deletion effectively reduces LDL-C, plaque buildup in the arteries, and PCSK9 expression. However, more research is needed to determine its side effects and safety.

Keywords: ASCVD, Hemodynamic shear stress, CRISPR-Cas9, genome editing, PCSK9.


INTRODUCTION

As the name suggests, atherosclerotic cardiovascular disease (ASCVD) is characterized by high levels of low-density lipoprotein (LDL-C) cholesterol and the development of arterial plaque.1,3 Men and women with a strong family history of atherosclerotic cardiovascular disease are at increased risk due to several factors, including low-density lipoprotein (LDL) cholesterol levels, high blood pressure, diabetes mellitus, and smoking (male relatives younger than 55 years and females who are younger than 65 years). Increased risk of heart disease is associated with inactivity, obesity, a high-fat diet, and specific genetic variants. Low HDL cholesterol levels have long been considered a risk factor for ASCVD, but side effects from drugs used to raise HDL levels have cast doubt on this theory.1-6

It is common knowledge that ischemic heart disease and stroke are the most common causes of death and disability in many middle-income countries.2 A third of all deaths in Indonesia are attributed to ASCVD, the leading cause of morbidity and mortality. Premature death from coronary heart disease (CHD) and cerebrovascular disease (CVD) costs 3,299 and 2,555 years of life, respectively. In Indonesia, high cardiovascular risk is shared among adults over 40, with low rates of preventive treatment.2 ASCVD can generally lead to complications, including myocardial infarction, ischemic stroke, and angina pectoris, which are significant causes of mortality and morbidity worldwide.9-10 Many factors can trigger the occurrence of ASCVD, including smoking, hypertension, diabetes, abdominal obesity, and physical inactivity.9,11

In particular, studies on the genetics of lipid levels confirmed that elevated LDL-C plays an important role in the development of atherosclerosis.10 Mutations in the LDL-C receptor gene that cause it to lose function increase LDL-C levels in the blood. People who inherit the LDL-C receptor mutation from parents (heterozygous) usually have LDL-C levels above 200 mg/dL. People in their third and fourth decades of life are at risk of developing early clinical Cardiovascular Disease (CVD). In
people with two defective copies of the LDL-C receptor gene, LDL-C levels range from 650 to 1000 mg/dL. (Homozygous familial hypercholesterolemia). They may experience CVD events in their teens or early twenties. There was a 28 percent reduction in LDL-C cholesterol levels when the PCSK9 protein lost its ability to convert LDL-C to LDL receptors, lowering circulating LDL particles. PCSK9i, as an adjunct to statin therapy, can reduce LDL-C levels by 50-60% compared to statin therapy alone by preventing the destruction of the LDL receptor.

Studies using stem cells such as induced pluripotent stem cells, mesenchymal stem cells, and human-derived pluripotent stem cells (hiPSCs) have been less successful due to the scarcity of data regarding their efficacy. Transplantation occurs in a completely different environment, requiring much greater attention to detail. Arteriosclerosis has been successfully treated by trial therapy using autologous adipose-derived mesenchymal stem cells (Ad-MSC). Non-pharmacological treatment associated with lifestyle interventions such as maintaining healthy behavior (no smoking, exercise, maintaining a diet by consuming dietary fiber in whole grains, protein food, fruits, and vegetables) and paying attention to health factors (cholesterol, blood pressure, glucose levels) can be used to prevent Atherosclerosis Cardiovascular Diseases.

As a result, there is currently no effective treatment for atherosclerotic cardiovascular disease. Based on those mentioned above, gene therapy could be used as a long-term treatment for patients with ASCVD, according to this literature study.

METHODS

The methodology used is a literature review. The study sources consist of relevant journals from several databases Google Scholar, PubMed, Cochrane, Science Direct, and the American College of Cardiology. The author searches with keywords and synonyms CRISPR Cas9 OR Gene editing OR Genome Editing OR Clustered Regular interspersed Short Palindromic Repeats-CRISPR-related AND ASCVD OR Atherosclerotic Vascular Disease AND PCSK9 gene OR proprotein convertase subtilisin/Kexin type 9 AND Hemodynamic shear stress. The search was limited to publications from 2012 to 2022 to ensure the articles used were up to date. Language restrictions are also applied to limit the search to articles published only in English and Indonesian. All relevant articles were screened and analyzed for inclusion or exclusion from the literature review based on quality and relevance to the review topic, questions and objectives. The title and abstract of each article were tailored to the research question. The full text of the article is checked for availability if the title and abstract match. Finally, the researcher reads the entire article to see if it is relevant and valuable to the subject.

RESULTS

Atherosclerotic cardiovascular disease

Ischemic heart disease, strokes, and peripheral vascular disease are all caused by Atherosclerosis, a chronic inflammatory disease of the large and medium arteries. It’s the leading cause of heart disease and stroke. It has always been a leading cause of death in developed countries, especially in Asia, South America, and Central America. When immune-competent cells in the lesion predominantly produce pro-inflammatory cytokines due to high levels of Low-Density Lipoprotein (LDL) cholesterol, it causes atherosclerosis. Decomposing cells and oxidized forms of LDL cholesterol (oxLDL) are everywhere. Inflammatory phospholipids such as phosphorylcholine are found in oxLDL, which has pro-inflammatory and immune-stimulating properties and causes more cell death (PC). This condition is responsible for myocardial infarctions (MIs), congestive heart failure (CHF), stroke, and claudication.

Intimal plaques, particularly at the vessels’ bifurcations, form in the intima of many medium and large arteries due to Atherosclerosis. Aortic stenosis can affect all arterial systems from the aorta to the coronaries. Some conditions, characteristics, or behaviors have been associated with an increased risk of developing Atherosclerosis, but the exact causes and risk factors are still unknown. All the significant risk factors, including low HDL, high cholesterol, an inactive lifestyle, smoking, high blood pressure, obesity, diabetes, and Atherosclerosis, can be managed or prevented. The proprotein convertase subtilisin/Kexin type 9 inhibitor (PCSK9) has been shown to reduce the risk of Atherosclerosis.

High-risk signs include large plaque volume, poor CT attenuation, napkin ring sign, patchy calcification, and positive remodeling. The first step in the progression of Atherosclerosis, a cardiovascular disease, is the conversion of low-density lipoprotein (LDL) to high-density lipoprotein. The malondialdehyde factor is a marker of lipid peroxidation, a sign of oxidative stress and cardiovascular disease. Atherosclerosis’ clinical effects include artery constriction with symptoms (angina pectoris) and acute coronary syndromes caused by plaque instability.

PCSK9, LDL-C, and ACSVD

Plasma LDL-C is tightly regulated by PCSK9 (proprotein convertase subtilisin/ Kexin type 9), an essential player in lipid metabolism. The liver largely handles protein production and excretion. There is a direct correlation between the amount of LDL-C in blood and the amount of the receptor on the surface of the liver cells capable of binding to the LDLR. PCSK9 gain-of-function mutations cause autosomal dominant hypercholesterolemia. People with PCSK9 deletion mutations had lower LDL-C and a lower risk of coronary artery disease (CAD) than those with PCSK9 function mutations. The PCSK9 locus was also linked to LDL-C and cardiovascular disease risk in a genome-wide association study. As a result, it’s a good idea to block PCSK9 activity. Alirocumab and evolocumab, two PCSK9 monoclonal antibodies approved and shown to reduce LDL-C in various patients, are now available on the market. In a significant outcome study, evolocumab was found to significantly lower the risk of future cardiovascular events.
These findings offered compelling evidence for developing pharmacological ways to limit PCSK9 activity. Aside from reducing LDL-C and limiting atherosclerosis development, PCSK9 has been linked to inflammation, platelet activation, triglyceride-rich lipoprotein metabolism, viral infections, and cancer.

Gene associated with ACSVD
CVD is caused by two proteins, TG and TGR1, which interact. Diabetes with severe dysregulation, alcoholism, and rare cases of homozygotes for mutations in the APOC2, APOA5, LPL, GPHBP1, LMF1, and GPD1 genes results in extremely high triglyceride levels. Mild-to-moderate hypertriglyceridemia is usually multigenic, resulting from a combination of common and rare variants in over 30 genes. Proteins such as angiopeptins 3 and 4 (ANGPTL3 and ANGPTL4) inhibit triglyceride (TG) hydrolysis by regulating lipoprotein lipase (LPL) activity, triglyceride (TG) hydrolysis by regulating lipoprotein lipase (LPL) activity, and 4 (ANGPTL3 and ANGPTL4) inhibit genes.

Experimental gene therapy for Atherosclerosis
Since no mutation occurs in non-coding RNA, it’s not the best candidate for gene-editing treatment for Atherosclerosis. Due to the broad downstream target gene binding sites, direct transfection of non-coding RNA may have unexpected biological effects in gene replacement therapy. Table 1 summarizes the relevant gene therapy studies for Atherosclerosis.

Gene editing: The CRISPR/Cas System as a Tool for Adaptive Bacterial Immunity
The CRISPR/Cas system provides adaptive immunity. A bacterial genome must have unique nucleic acid sequences if it is ever going to adapt to previously encountered attackers, like viruses. CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats and is a shortened way of saying CRISPR. Short exogenous nucleic acids derived from viruses or plasms that integrate into the bacterial genome after the organism is challenged by exogenous genetic material are known as “repeating elements.” Bacterial DNA fragments complementary to the viral or plasmid genetic material that invaded the host are transcribed into RNA. The CRISPR RNA is thought to be supplemented by previously encountered foreign DNA entering bacterial cells. Cas, a nuclease that silences foreign DNA by causing double-strand breaks in it, is the target of the complex.

Genome editing: The Application of the CRISPR/Cas system
The CRISPR/Cas system, developed in 2012 by a previous study, was awarded the 2020 Nobel Prize in Chemistry for his discoveries in genome editing via programmable RNA. When the CRISPR/Cas9 system of pyogenic Streptococcus was discovered, the use of the CRISPR/Cas system for genome editing exploded. Genome editing with CRISPR/Cas9 requires only one Cas9 nuclease and one guide RNA (gRNA). Both will be discussed in more detail in the following paragraphs. One example of the current application is studying genetic diseases in cell and animal models to gain insight into pathophysiology and assess the impact of genetic variation. Adding mutations to treat diseases caused by specific mutations and diseases not primarily caused by genetic alterations are two potential therapeutic options.

To help cut foreign DNA material, the CRISPR/Cas system in bacteria requires two types of RNA called “crRNA” and

<table>
<thead>
<tr>
<th>Gene Editing Tool</th>
<th>Diseases</th>
<th>Animal Model</th>
<th>Efficiency</th>
<th>Security</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRISPR/Cas9 (ADV)</td>
<td>AS PCSK9</td>
<td>C57BL/6 mice</td>
<td>Mutagenesis rate of PCSK9: (&gt;50%) Increased hepatic LDL levels, Decreased plasma cholesterol levels PCSK9 has a high Rate of mutagenesis (more than 40%). Total cholesterol levels were reduced by 40%.</td>
<td>There was no evidence of off-target mutagenesis.</td>
<td>Ding et al.28</td>
</tr>
<tr>
<td>CRISPR/SaCas9 (AAV)</td>
<td>AS PCSK9</td>
<td>ApoB knockout</td>
<td>PCSK9 Mutagenesis rate: 42-47%. Total cholesterol levels were unchanged.</td>
<td>There was no evidence of off-target mutagenesis.</td>
<td>Ran et al.29</td>
</tr>
<tr>
<td>CRISPR/SpCas9 (ADV)</td>
<td>AS PCSK9</td>
<td>FRG KO Mouse with human hepatocytes</td>
<td>The average frequency of base editing is 63%. LDL cholesterol levels have been reduced by 90%. Plasma cholesterol level has been reduced by 60%</td>
<td>At a dosage of 1.0 mg kg/L, one off-target editing site was found (1%).</td>
<td>Wang et al.30</td>
</tr>
<tr>
<td>CRISP Adenine base editor (lipid nanoparticles)</td>
<td>AS PCSK9</td>
<td>Macaca fascicularis</td>
<td></td>
<td></td>
<td>Musunury et al.31</td>
</tr>
</tbody>
</table>

AS: Atherosclerosis; AAV: Adeno-Associated Virus; ADV: Adenovirus; LDL: Low-Density Lipoprotein; LDLR: Low-Density Lipoprotein Receptor; HDL: High-Density Lipoprotein.
Cas9 alternatives have been developed. Nucleases have been discovered, and more the Cas9 nuclease is required. Many Cas9 defective (gRNAs) are programmable RNAs that attach to specific DNA sequences known as “protospacers.”

"tracrRNA." The complexes are directed to specific DNA spots by guide sequences carried by crRNAs. A significant breakthrough occurred for genome editing using the CRISPR/Cas system: developing a synthetic gRNA that can act as a replacement for two bacterial RNA molecules. Watson-Crick bases in the gRNA pair with the first 20 nucleotides of the target DNA sequence, guiding nucleases to specific genomic regions. The corresponding DNA sequence is referred to as a “protospacer.” It must be close to the PAM (Protospacer Adjacent Motif), which interacts with nucleases (for example, the PAM sequence that interacts with S. pyogenes Cas9 is NGG, where “N” is any nucleotide and “G” is guanine). The CRISPR/Cas9 system can repair breaks in target-DNA and endogenous DNA using nuclease double-strand breaks, which has led to repair systems attempting to use specific points in the protospacer DNA.

When using adenine base editors, adenine (A) is deaminated to inosine (I) at the peak edit point (paired with thymine (T) on the other DNA strand). There was yet another snip of hair. Instead of T, Inosine (I) is attached to cytosine (C) through nick repair, and then G (G) is substituted for me. As a result, A-T becomes G-C, eliminating the need for a second break.

Even though the CRISPR-Cas system has a lot of potential for changing genomes, there are a lot of advantages and disadvantages to consider before using it in translational medicine (Table 2). Some researchers have used base editors by attaching Cas9 proteins to cytidine or adenosine deaminase enzymes that are not catalytically active. Gene-regulatory RNAs (gRNAs) are essential for Cas9 and other base editors to function correctly. Instead of causing double-stranded breaks, base editors cause single-nucleotide changes within the protospacer, peaking at specific points in the protospacer DNA.

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Figure 1. The genome-editing mechanism of regularly clustered short palindromic repeats (CRISPR)/CRISPR-associated (Cas) is outlined. Guide RNAs (gRNAs) are programmable RNAs that attach to specific DNA sequences known as “protospacers.”

Jiang et al. found that therapeutic targeting of the PCSK9 gene is possible in CRISPR-Cas9 mice. An additional atherosclerosis study has shown that genome editing of CRISPR-Cas9 can also disrupt the Ldlr gene and increase the PCSK9 gene in adult mice. Tessadori colleagues and friends CRISPR-Cas9 genome editing used in a zebrafish model to correct human congenital cardiovascular disease. Cardiovascular diseases, particularly those linked to inherited cholesterol problems, may be treatable with CRISPR-Cas9, according to these findings.

**ACSVD: PCSK9 Gene editing by CRISPR Cas9**

Adenoviruses were used to express CRISPR-associated 9 and a CRISPR guide RNA specifically targeting PCSK9 in mouse livers genetically engineered to describe both of these factors. Virus injection increased the PCSK9 mutagenesis rate in the liver by 50% within 3 to 4 days. As a result, plasma PCSK9 levels dropped, hepatic LDL receptor levels rose, and blood cholesterol levels decreased (35-40 percent lower). There was no evidence of off-target mutagenesis at ten randomly chosen sites. Genome editing using the CRISPR-CRISPR associated nine systems effectively inactivates the PCSK9 gene and reduces blood cholesterol in mice.

**ACSVD: Effect of Hemodynamic Shear Stress On PCSK9**

The experiments yielded the following results: With increasing shear stress, the level of PCSK9 expression decreases from its peak of 3-6 dynes/cm² to as low as 12-30 dynes/cm². Static and maximum shear stress (30 dynes/cm²) PCSK9 expression was nearly identical (control). LPS treatment significantly increased PCSK9 expression in EC and SMC cells for the second time. Shear stress remained constant at 15 dynes/cm² while the LPS response increased dose-dependently (10-1000 ng/ml). SMCs expressed PCSK9 at all shear stress levels, regardless of the presence or absence of LPS. Under various shear stress conditions, the response of PCSK9 expression to LPS was examined. According to the findings, low shear stress increased the expression of PCSK9 and the production of ROS in vascular ECs and ACEs.
SMCs. Reactive oxygen species appear to influence PCSK9 expression (ROS). The role of PCSK9 and ROS in developing moderately sheared ductus arteriosclerosis has been hypothesized.  

ACSVD: Effect of Shear Stress on ROS Production

Blood flow shear stress determines vascular pathology and modulates native and oxidized low-density lipoprotein (oxLDL) accumulation or uptake. Endothelial and smooth muscle cells (SMC) are affected by shear stress. When shear stress or strain is altered, reactive oxygen species are generated (ROS). The role of ROS as a second messenger has recently been proposed. Under shear flow, superoxide production in ECs was increased, as evidenced by an increase in ROS production. As long as there is shear flow, the superoxide production will remain high. As a result of ROS, endothelial NO synthase, respiratory chain enzymes, and cytochrome P450 monoxygenases contribute to ROS formation. PCSK9 expression is reduced in ECs and SMCs by ROS inhibitors, which reduce ROS production. 

ACSVD: Effect of ROS Inhibition and PCSK9 ekspress

PCSK9 expression is influenced by shear stress. Pseudo-normal shear stress increased PCSK9 expression in vascular smooth muscle and endothelial cells, which decreased. PCSK9 was expressed at a higher level in SMCs than ECs. Regarding ROS production, PCSK9 expression was precisely like ROS generation. PCSK9 expression was reduced by the ROS inhibitors diphenyliodonium chloride and apocynin. PCSK9 expression is induced by low shear stress through ROS production mediated by NADPH oxidase. Low shear stress in vascular ECs and SMCs increased PCSK9 expression and ROS generation. PCSK9 term appears to be regulated by ROS. In the progression of atherosclerosis, the PCSK9-ROS interaction, we hypothesize, may play an essential role in developing low-shear ductus arteriosclerosis. 

Ethical concerns with CRISPR

Consequently, CRISPR/Cas9 can modify embryos, pluripotent stem cells and somatic cells. But deciding which cells to target for genome editing can raise some technical and ethical concerns. A cell’s ability to repair DNA varies depending on its type and state (NHEJ or HDR). Cells actively proliferating and postmitotic are both capable of NHEJ; HDR is more effective in actively increasing cells.

CRISPR-Cas9 gene editing has some drawbacks, such as targeting specific genes. Targeting and binding to particular genome regions is accomplished using Cas9 and 100-nucleotide synthetic guide RNA in the CRISPR-Cas9 system. A common cause of nonspecific targeting in CRISPR is the short sequences (about 20 nucleotides long) used in the technology. These results suggest CRISPR guides bind to lines other than the target sequences but share close homology with up to six mismatches. Mutations can be introduced into other genes and affect their function, leading to cancer if the transformation occurs in an oncogene and Cas9 cleaves these sequences when the repair is defective as expected in NHEJ or external templates are used for HDR. The guide is more likely to bind to mismatches at the other end of the PAM sequence. For the CRISPR seed sequence to be effective, the first 12 bases of the guide sequence must complement the effective target sequence. Mismatches between bases 4 and 7 will also prevent the guide from binding to the directory. Other gene-editing mechanisms, such as meganucleases, which identify sequences of up to 40 nucleotides, use larger spans of nucleotides. However, they are challenging to target since changing the sequence they recognize is difficult. Many other issues arise when attempting to repair the damaged DNA. For example, HDR rarely occurs when the correct fragment is not provided, and about 20% of cells prefer NHEJ.

The delivery of the CRISPR construct is not to blame for low in vivo success but rather the CRISPR itself. As a result, when intravenous injection is used, the success rate is significantly lower, implying that large doses are required to achieve a sufficiently high transformation efficiency to obtain a sizeable therapeutic effect. However, other methods for administering CRISPR therapy may require lower doses but are more invasive or non-translatable in animal models. Adeno-Associated Virus, one of the most prominent vector options for tissue-specific therapeutic effects, has a limited natural tropism, limiting its use to only a few organs. Vectors can be customized to target specific tissues, or tropisms in nature can be used more strongly.

<table>
<thead>
<tr>
<th>Issues</th>
<th>Pros</th>
<th>Cons</th>
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<tr>
<td>gRNA design</td>
<td>The simple RNA: DNA base pairing of a gRNA makes it possible to design a gRNA that is highly specific to its target.</td>
<td>Large genomes frequently contain sequences that are highly homologous to the target site.</td>
</tr>
<tr>
<td>Off-target</td>
<td>Design of gRNAs for controlling the expression of CRISPR system components using powerful online software; availability of new high-fidelity variants.</td>
<td>Due to the lack of cell or tissue specificity, the RNA polymerase I promoter cannot be used for gRNA expression in conventional designs. During the S, G1, and G2 phases, NHEJ is in charge of DNA repair.</td>
</tr>
<tr>
<td>gRNA production</td>
<td>Any promoter can be used for efficient transcription and cleavage thanks to the design of RGR, an artificial ribozyme flanking gRNA (RGR).</td>
<td>At the one-cell stage of embryonic development, CRISPR-Cas9 may or may not cleave DNA</td>
</tr>
<tr>
<td>Multiple on-target mutations</td>
<td>Effective HDR-inducing strategies such as Cas9n, SpCas9-Gem, RS-1, and others.</td>
<td></td>
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<tr>
<td>Biallelic mutations</td>
<td>Biallelic mutations can be induced using inducible CRISPR, inducible knockout (K), and conditional KO.</td>
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</table>

Table 2. The Pros and Cons of CRISPR. 

The reason for using CRISPR as an effective therapy for ACSVD

Genes identified as therapeutic targets, such as CRISPR, can be used to disrupt PCSK9 to treat PCSK9 disease. The PCSK9 gene has LOF mutations introduced by a CRISPR-Cas nuclease system derived from Streptococcus pyogenes or Staphylococcus aureus. LDLR expression at the beginning of animal studies increased by 35-40%, PCSK9 levels decreased by 50%, and plasma cholesterol decreased by 50%; these results indicate liver-directed mutagenesis. It has been 24 weeks since PCSK9 groups began to fall. In particular, there was no mutagenesis outside of the target. CRISPR gene editing has shown promising results, but problems with double-strand damage repair and off-target mutations persist.23

Using the CRISPR or Cas system for genome editing has significant

### Table 3. CRISPR-Cas9 research and its potential application in the treatment of various human diseases.

<table>
<thead>
<tr>
<th>Disease Type</th>
<th>Disease-Related Gene/Protein</th>
<th>Remarks</th>
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<tbody>
<tr>
<td>Duchenne Muscular Dystrophy</td>
<td>Dystrophin (DDN)</td>
<td>Using CRISPR-Cas9, a gene mutation that causes Duchenne muscular dystrophy was corrected.</td>
</tr>
<tr>
<td>Cardiovascular diseases</td>
<td>Proprotein Convertase Subtilisin/Kexin Type 9 (PSCK9)</td>
<td>CRISPR-Cas9 was used to correct the PSCK9 gene in a mouse model of atherosclerosis.</td>
</tr>
<tr>
<td>Huntington disease</td>
<td>Huntingtin (HTT) gene</td>
<td>In a mouse model, CRISPR-Cas9 was used to suppress the mHTI gene selectively.</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>Presenilin 1 (PSEN1) and presenilin 2 (PSEN2) genes</td>
<td>Alzheimer’s disease has been linked to mutations in the PSEN gene that were corrected using CRISPR-Cas9.</td>
</tr>
<tr>
<td>Metabolic liver disease</td>
<td>Pah</td>
<td>CRISPR-Cas9 was used to correct the Pah&lt;sup&gt;−/−&lt;/sup&gt; gene in metabolic liver disease.</td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>17 Fanconi Anemia (FA)</td>
<td>Fanconi anemia was treated with CRISPR-Cas9.</td>
</tr>
<tr>
<td>Hereditary tyrosinemia</td>
<td>Fumarylacetoacetase (Fah)</td>
<td>In a mouse model, CRISPR-Cas9 was used to correct for the Fah mutation.</td>
</tr>
<tr>
<td>Sickle cell anemia</td>
<td>β-globin gene</td>
<td>The CRISPR-Cas9 gene-editing technique treats the blood of sickle cell disease patients.</td>
</tr>
<tr>
<td>β-Thalassemia</td>
<td>Hemoglobin Subunit Beta (HBB) gene</td>
<td>CRISPR-Cas9 was used to correct HBB gene mutations in human iPSCs from thalassemia patients.</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene</td>
<td>Stem cell cultures of cystic fibrosis patients were used to correct the CFTR gene using CRISPR-Cas9 technology.</td>
</tr>
<tr>
<td>Retinitis pigmentosa</td>
<td>RP1, RHO, and RPGR genes</td>
<td>CRISPR-Cas9 was used to interrupt the Rho (S334) mutation.</td>
</tr>
<tr>
<td>Cataract</td>
<td>αA-crystallin gene</td>
<td>The researchers used CRISPR-Cas9 to investigate the association between the αA-crystallin mutation and human congenital cataracts.</td>
</tr>
<tr>
<td>Human Immunodeficiency Virus (HIV)</td>
<td>Long Terminal Repeats (LTRs) in HIV</td>
<td>CRISPR-Cas9 was used as a tool to mutate LTRs of HIV-1 DNA.</td>
</tr>
<tr>
<td>Hepatitis B virus (HBV)</td>
<td>Covalently Closed Circular DNAs (cccDNAs) in HBV</td>
<td>CRISPR-Cas9 was used to target cccDNAs of HBV.</td>
</tr>
<tr>
<td>Cancer</td>
<td>Ephrin Receptor Tyrosine Kinase A2 (EphA2)</td>
<td>A therapeutic strategy was developed using the EphA2 extracellular domain.</td>
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<tr>
<td></td>
<td>Additional Sex Combs Like 1 (ASXL1)</td>
<td>In mouse xenografts, CRISPR-Cas9 was used to reduce leukemic cell growth.</td>
</tr>
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<td></td>
<td>Myeloid Cell Leukemia 1 (MCL-1)</td>
<td>Human BL cells had MCL-1 deleted and apoptosis induced using CRISPR-Cas9.</td>
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<td>Cyclin-Dependent Kinase 11 (CDK11)</td>
<td>Using CRISPR-Cas9, osteosarcoma was silenced by CDK11.</td>
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<td>SHC SH2-binding protein 1 (SHCBP1)</td>
<td>SHCBP1 inhibits the proliferation of breast cancer through CRISPR-Cas9.</td>
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<td></td>
<td>Kelch Domain Containing 4 (KLHDC4)</td>
<td>CRISPR-Cas9 knocked out the KLHDC4 gene in a nasopharyngeal carcinoma cell line.</td>
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<td></td>
<td>Epidermal Growth Factor Receptor (EGFR)</td>
<td>If acquired drug resistance mutations in EGFR could be corrected, CRISPR-Cas9 was used.</td>
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<td>Melanoma Cell Adhesion Molecule (MCAMMUC18)</td>
<td>The cell surface glycoprotein MUC18 was knocked out using CRISPR-Cas9 technology in human primary nasal airway epithelial cells.</td>
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<tr>
<td></td>
<td>Janus kinase 3 (JAK3)</td>
<td>It was found that CRISPR-Cas9 could restore normal T cell development in cells lacking the JAK3 protein.</td>
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</table>
advantages over previous methods. Designing and manufacturing RNAs (CRISPR/Cas systems) is inexpensive and straightforward. In addition, CRISPR/Cas allows multiple gRNAs in the same vector to simultaneously target different genomic regions. According to research, the effectiveness of gene editing in hepatocytes is rather high, which is crucial for the possible future therapy of dyslipidemia.

Furthermore, gene editing differs from other proven pharmaceutical therapy methods in that it seeks to cause permanent genetic modifications. Although germline cell modifications might be handed down to progeny, this is not the case with the techniques detailed here, solely targeting somatic cells. While modifying just one person’s DNA is typically seen as more bearable, a systematic and critical ethical discussion should occur beforehand.

The limitation of this literature was the quantity of data-based studies; there were only three data-based papers, which may have resulted in other similar articles not being included in the search. Furthermore, further research is needed to describe the implementation of reflection learning in health institutions.

CONCLUSION

Genome editing with the CRISPR/Cas9 system could be a promising treatment option for ACSVD patients with PCSK9 gene deletions. This therapy has a lot of promise as a treatment with long-term effects because it is less expensive, quicker, and more efficient. The efficacy of PCSK9 ablation therapy using CRISPR/Cas9 is expected to be studied further. For this reason, the method has been clinically tested to identify potential side effects. As a result, this therapy may one day be used as the primary treatment for ACSVD patients.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ETHICS APPROVAL

Not applicable for the literature study.

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AUTHOR’S CONTRIBUTION

All authors contribute to the study from the conceptual framework, data acquisition, study design, writing and submitting the manuscript, and interpreting the study results through publication.

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REFERENCES


Figure 2. The framework of the deletion PCSK9 gene against LDL-R mutation and Hemodynamic Shear Stress.


