Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/20939
DOI URL: http://dx.doi.org/10.21474/IJAR01/20939

RESEARCH ARTICLE

NEW FRONTIERS IN GENE THERAPY FOR CARDIOVASCULAR DISEASES

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Manuscript Info

Manuscript History
Received: 25 March 2025
Final Accepted: 28 April 2025
Published:May 2025

Keywords:-
Cardiovascular disease, Genetic engineering, Genetic mutations, Gene therapy, and Molecular medicine.

Abstract

Gene therapy is a medical approach that is highly acknowledged and is a modern way to treat diseases or prevent them by making changes or corrections in defective genes. This review paper mainly focuses on an outline of gene therapy and how it is carried out. The main emphasis of this paper is on cardiovascular diseases, which are leading global issues. A variety of important cardiovascular diseases are discussed here, stating which genes are responsible for causing them, along with how the advancement in gene therapy has significantly enhanced the prospect of their effective treatment. Lastly, the review paper highlights the future of gene therapy related to the proper treatment of cardiovascular diseases.

Introduction:-

On a global scale, cardiovascular diseases (CVDs) account for the vast majority of deaths and illnesses.This exerts an immense burden on healthcare in spite of decades of therapeutic progress. Conventional interventions such as various pharmacological agents, surgical processes, and lifestyle changes have shown improvement but they still fall short in the context of addressing the underlying molecular and genetic reasons of these diseases. Gene therapy has emerged as an approach that promises to rectify or modify the disease from its root rather than just controlling the symptoms. It is thus an innovative way to directly manipulate the disease genes that serve as the prime cause of various cardiovascular disorders. Thus, gene therapy holds the potential to transform the way of addressing cardiovascular diseases in the near future, unleashing a new era in the field of biotechnology and biomedical research.

Gene therapy

1.1 Introduction to Gene Therapy

One area of molecular medicine that could have a big impact on human health in the twenty-first century is gene therapy [2]. It is expected to offer novel therapies for numerous hereditary and acquired illnesses [1]. The notion that underpins gene therapy is something that can be understood easily. Insert a genetic fragment into target cells that will either cure the illness or reduce its growth. Technologies that can transfer genes into a wide range of cells, tissues, and organs are necessary for gene therapy to accomplish this goal. The creation of secure and efficient vectors to transfer genetic material into cells is one of the main obstacles to the successful, broad implementation of such genetic therapies.

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1.2 History of Gene Therapy

An overview of the history of the development of gene therapy [8] in chronological order is shown in Table 1.

Table 1: An overview of the history of development of gene therapy in chronological order.

YEAR	DEVELOPMENT
1909	The term "gene" was first used by Wilhelm Johannsen, who set the way for gene therapy to begin[8].
1930s	The phrase “genetic engineering” was coined [3].
1953	The world-famous discovery that DNA has a double-helix structure was achieved by James Watson and Francis Crick [8].
1960s	The fundamentals of bacterial gene transfer were identified [3].
1970s	Researchers were able to incorporate a specific therapeutic gene or genes into manufactured vectors through the use of recombinant DNA techniques [4].
1989	To better monitor infiltrated lymphocytes during immunotherapy for melanoma, a neomycin-resistance marker was introduced onto tumor-infiltrating lymphocytes in 1989 using a retrovirus [5].
1990	Ashanti DeSilva, a little girl diagnosed with severe combined immunodeficiency (SCID), was the subject of the first successful gene therapy clinical study in 1990 when researchers from the University of Pennsylvania began treating her. A retroviral vector was used to introduce a normal copy of adenosine deaminase (ADA) into her T cells, which were then engineered from there [8].
1999	Dead from major coagulation problems and consequent multi-organ failure, Jesse Gelsinger became the first person to die from gene therapy [6].
2003	After receiving permission from the Chinese State Food and Medicine Administration (SFDA) in 2003, “Gendicine” became the first gene therapy drug to be permitted for the treatment of head and neck cancer. To this objective, the first medicine to receive approval was "Gendicine" [7].
2012	“Glybera”, authorized for lipoprotein lipase deficiency in 2012, was the first gene therapy medication approved by the European Medicines Agency (EMA) [8].
2022 and beyond	According to the results of prior clinical trials and the drugs that are now approved, it would appear that this potentially fruitful area of medicine is advancing at a rate that is unprecedented [8].

1.3 Types of gene therapy

Two primary classifications can be distinguished within the field of gene therapy [9]. An example of gene therapy for germ cells is the modification of germ cells through the introduction of functional genes into their genome. This characteristic is inherited through the generations. Concerns over potential hazards, ethicalconsiderations, and technological limitations have kept germ-line gene therapy from being widely used in humans. Generating genes from somatic cells: Instead of using germ cells or undifferentiated stem cells, somatic cell gene therapy can insert a functional gene into any kind of cell. This characteristic is inherited through the generations. When it comes to studying and treating gene-related disorders, cancer, immunization, and wound healing, somatic cell gene therapy is where it's at.

1.4 Strategies in gene therapy

Ex vivo gene therapy and in vivo gene therapy [10] are two distinct approaches that have emerged as a result of scientific advancements [11]. The process of ex vivo gene therapy involves the collection of cells from a patient, which is then followed by the altering of those cells through genetic modification in a laboratory setting. In most

cases, the quantity of genetically changed cells is increased, and then the transduced cells are given back to the patient. The goal of gene therapy in vivo is to modify the genetic repertoire of target cells to accomplish therapeutic goals by applying genetic material, usually DNA, to these cells.

1.5 Techniques of gene therapy

The most popular method of gene therapy [9] involves replacing a dysfunctional gene with the proper gene by inserting it into a nonspecific region of the genome. Following a subsequent cell cycle, the altered gene or transformed cell resumes producing normal proteins and reverts to the healthy phenotype. A few essential components are necessary for gene treatments to work. Gene therapy requires a healthy gene, a diseased gene, and a vector, which is a highly effective delivery mechanism. The vector can be either nonviral or viral (genetically designed virus) [12]. Viral vectors are altered such that they can enter a cell and transfer their gene to the host cell's chromosomes without causing illness.

1.6 Mechanisms Related to Gene Therapy

Different gene treatment strategies exist [14]. Gene addition/augmentation is the process of adding or replacing genes that have a loss-of-function defect [13]. When a gene is augmented, both the damaged gene and the newly transplanted functioning copy of the gene are found in the cell nucleus. When gain-of-function deficiencies are the source of the disease, a different strategy is needed. Importing inhibitory sequences (such as microRNAs or short hairpin RNAs) into target cells can suppress gain of function. Editing the genome: In situ repair of genetic abnormalities is now feasible and all thanks to the development of new instruments that can precisely target and modify DNA. This method, known as genome editing, enables single base-pair accuracy in correcting genetic flaws [13].

CARDIOVASCULAR DISEASES

Worldwide, cardiovascular diseases are responsible for the deaths of 12 million people each year. Despite a general decrease in cardiovascular mortality brought about by advancements in patient care, cardiovascular illness still accounts for more than 50% of deaths in both industrialized and developing nations. During the early phases of cardiovascular disorders, patients frequently do not display any symptoms at all. Furthermore, patients are often unaware that there are lifestyle modifications that can help slow the progression of the disease. These modifications include quitting smoking, increasing the amount of exercise they get, and eating a healthy diet. This is one of the problems that is associated with cardiovascular diseases in general. The unfortunate reality is that treatment for these conditions does not begin until after the manifestation of clinical signs. By then, the condition has established itself and has almost always resulted in significant, permanent damage to the heart and circulatory system. Most of the treatments for cardiovascular disorders that are now on the market just address the symptoms rather than the underlying cause. Additionally, the limited therapeutic windows and severe side effects of many cardioactive medications may restrict their clinical utility. Therefore, it would be extremely beneficial for the patient as well as the nation whose healthcare systems are in charge of caring for a growing number of patients if more targeted and highly successful treatment approaches were developed to treat particular cardiovascular disorders [15].

THE MAIN CARDIOVASCULAR DISEASES MENTIONED IN THIS PAPER:

1.7 High Blood pressure:
- 1.7.1 Hypertension(Hypertensive heart disease)

1.8 Artery Health:
- 1.8.1 Atherosclerosis

1.9 Heart's Blood Supply:
- 1.9.1 Coronary Artery Disease
- 1.9.2 Angina/Angina Pectoris
- 1.9.3 Myocardial Infarction (Heart attack)

1.10 Heart's Pumping Function:
- 1.10.1 Heart Failure (Congestive heart failure)
- 1.10.2 Cardiac arrest.

1.11 Brain's Blood Supply:
- 1.11.1 Stroke

1.12 An inherited cardiovascular disease:
- 1.12.1 Familial Hypercholesterolemia

Note:Atherosclerosis starts first, if atherosclerosis leads to blockage in the coronary artery, then it is coronary artery disease. There is a correlation between coronary artery disease and angina, which ultimately results in myocardial infarction.

Hypertension

Cardiovascular health and healthcare systems in India are significantly impacted by hypertension [17]. The majority of the deaths in India from stroke, as well as coronary heart disease, are directly caused by hypertension. The World Health Organization (WHO) identifies hypertension as one of the top causes of death on a global scale [16].

Types include Essential hypertension (EH) and primary hypertension which are interchangeable terms. Hypertension has been the subject of much study, yet its exact origins remain unknown. A person's age, diet, way of life, and genes are all factors that experts think contribute to hypertensive heart disease. Some lifestyle variables that can contribute to health problems include smoking, excessive alcohol consumption, stress, excess body fat, excessive salt consumption, and insufficient physical activity. It is possible to determine the underlying cause of secondary hypertension and, in some instances, reverse the disease. This is the case in circumstances where the ailment is secondary. The secondary form of hypertension is responsible for only about five to ten percent of all cases. The prevalence of secondary hypertension is over thirty percent among individuals who have hypertension and are between the ages of eighteen and forty [18].

Advancements in gene therapy include the renin-angiotensin-aldosterone system (RAAS), the β -1 adrenergic receptors (β1-Rs), and the endothelial dysfunction that is mediated by a reduction in nitric oxide (NO) [23]. The activation of RAAS is a significant contributor to the development of essential hypertension [23], which is why it is important to target this enzyme. Angiotensin I (AngI) is changed into Angiotensin II (AngII) by the renal and pulmonary endothelium's angiotensin-converting enzyme (ACE). Through the angiotensin I receptor (AT1R) and the angiotensin II receptor (AT2R), AngII has a variety of cellular effects. AngII's association with AT1R causes peripheral vessel walls to constrict, become inflamed, and fibrosis, whereas its association with AT2R vasodilates and encourages anti-inflammatory and anti-fibrotic effects. Therefore, essential hypertension can be treated with gene therapy by targeting the angiotensinogen genes. Therefore, activating AT2R improves vasodilation and decreases inflammation, while downregulating AT1R using various gene therapy techniques can help patients with EH have lower blood pressure (BP) grades. Both AngII and blood pressure were reduced by the antisense synthetic oligodeoxynucleotides (AS-ODNs). Targeting β1-Rs: The heart, kidneys, and cells of adipose tissue are the primary locations for β1-Rs [23]. G protein-coupled receptors are linked to β1-Rs. It was established by Zhang et al. that AS-ODNs can inhibit the expression of β1-R mRNA. According to Liang et al., hypertensive rats that were treated with AS-ODNs against β1-R showed a decrease of BP up to 37 mmHg. According to the data, Sensitive hypertensive rats that undergo AAV vector gene therapy and shRNA downstream of β1-R had lower Systolic blood pressure levels than the control group. Targeting Nitric Oxide Synthetase (NOS): The pathophysiology of EH is partially based on a decrease in NO, leading to endothelial dysfunction. In Table 2 we can see the eNOS gene delivery procedure.

Table 2: The method of gene administration using eNOS for hypertension.

STUDY	METHOD OF GENE ADMINISTRATION	OUTCOME
Chao *et al*.,1998	eNOS (endothelial nitric oxide synthase) gene direct delivery	In rats with salt-responsive hypertension, a drop in blood pressure was noted [19].
Miller *et al*.,2005	eNOS gene transfer using an AAV vector	Hypertensive models with a drop in blood pressure [20].
Gava *et al*.,2008	eNOS gene transfer using an AAV (Adeno-associated virus) vector.	Preventing hypertension of the renovascular arterial system [21].
Zhao *et al*.,2009	eNOS complementary DNA administration	Hyperglycemia causes a drop in blood pressure in rats [22].

Atherosclerosis

Cardiovascular complications continue to be the top cause of death globally, and atherosclerosis is the disease that causes this buildup of lipids, fibrous elements, and calcification inside the large arteries. The process starts with the stimulation of the endothelium and then continues through a sequence of events that restrict the arteries and activate inflammatory pathways, which finally results in the creation of atheroma plaque [24].

The early onset and progression of atherosclerosis are facilitated by lipid accumulation. It has been demonstrated that the most important initiators of atherosclerosis pathogenesis are hyperlipidemia, particularly low-density lipoprotein cholesterol (LDL-C) [25].

Advancements in gene therapy include the prospective targeting of lipid metabolism [25]: Autosomal dominant hypercholesterolemia and atherosclerosis progression are accelerated when PCSK9 (Proprotein Convertase Subtilisin/Kexin type 9) gains function mutations, enhancing the affinity for LDLR (low-density lipoprotein receptor) and speeds up its degradation. CRISPR technology shows promise as a permanent solution to the problem of high human liver PCSK9 levels. To investigate the possibility of in vivo loss function editing of PCSK9 in the lives of mice, the group led by Kiran at Harvard University attempted to use the Adenovirus-CRISPR-Cas9 (ADV-CRISPR-Cas9) technology. A decrease in PCSK9 protein level, an increase in plasma LDLR level, and a reduction of total plasma cholesterol of 35–40% were all results of a loss-of-function mutation that affected more than half of the PCSK9 gene. Cynomolgus monkeys had PCSK9 silenced in a recent study by Musunuru et al. using CRISPR-based editors. Plasma PCSK9 levels were strongly urged to be reduced by 90% and plasma LDL-C levels to be reduced by 60% by the CRISPR base editors. To lower total cholesterol levels, the AAV-SaCas9 system was created by Zhang Feng's lab to disrupt PCSK9 in the liver of mice [25]. ApoC3, also known as apolipoprotein C3, is a protein that is abundantly expressed in the liver and to a lesser amount in the intestine. It is a prospective target for the treatment of atherosclerosis. Using the CRISPR/Cas9 method, an ApoC3 knockout hamster model was generated. The results showed that the quantity of triglycerides was greatly reduced, and there was also a reduction in the number of atherosclerotic lesions [25]. By using an adenoviral vector to introduce Apolipoprotein A-I into an atherosclerosis model in rabbits, Wacker et al. [25] were able to reduce plaque volume and suppress inflammation. An investigation conducted by Hu et al. [25] revealed that the overexpression of PPARγ (Peroxisome proliferator-activated receptor gamma) has the effect of stabilizing atherosclerotic plaques by decreasing the accumulation of lipids, alleviating the infiltration of macrophages, and promoting the proliferation of smooth muscle cells. Taking action to reduce inflammation [23] is another option as shown in Table 3.

Table 3: Taking action to reduce inflammation in atherosclerosis.

STUDY	GENE USED	MODEL	VECTOR	KEY OUTCOMES
Thusen*et al*., 2001	IL-10 (Interleukin-10)	Not specified	AAV	Useful in the treatment of atherosclerosis [26].
Yoshioka *et al*.,2004	IL-10 (Interleukin-10)	Not specified	AAV	Helps reduce levels of MCP-1 (Monocyte Chemoattractant Protein-1) in the blood and keeps the cholesterol-making enzyme HMG-CoA reductase suspended [27].
Liu et al.,2006 & Namiki *et al*.,2004.	IL-10 (Interleukin-10)	Not specified	AAV	Blocking the progression of atherogenesis [28], [29].

Li et al., 2006	TGF-β (Transforming Growth Factor-beta)	LDLR-deficient mice	AAV	A decrease in the amount of reactive oxygen species (ROS) found in the walls of the vessels and a reduction in inflammation [30].
Khan et al., 2010	STAT3 (Signal Transducer and Activator of Transcription 3)	LDLR-deficient rats	AAV8	According to the findings of this investigation, STAT3 may have a potential cardioprotective activity because it was able to stop the progression of atherogenesis in rats that were given gene therapy [31].

Coronary Artery Disease

One cardiovascular condition that has been identified as the primary cause of death in both industrializedand developing nations is coronary artery disease (CAD) [33]. There is a substantial gender, ethnicity, and location-based variation in the prevalence of coronary artery disease [32].

High levels of lipid density level are causally linked to an increased risk of coronary artery disease (CAD), according to studies on familial hypercholesterolemia that involve mutations in the LDLR gene [34].

The incidence of autosomal dominant syndrome is influenced by mutations that alter the expression or function of the gene encoding the low-density lipoprotein receptor (LDLR), according to recent developments in gene therapy. Moderate increases in plasma LDL and early onset of coronary artery disease are symptoms experienced by patients who inherit a single mutant allele [35]. In humanized mice that express human ApoB100 (Apolipoprotein B100) and lack LDLR and APOBEC-1 (Apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1), a low dose of an AAV8-based vector containing the human LDLR complementary DNA can be injected intravenously to lower plasma cholesterol levels comparably [35].

Angina/ Angina Pectoris

Myocardial ischemia episodes, which can result from an imbalance in the heart's oxygen supply demand, can induce angina pectoris [36]. This imbalance can be caused by either an increase in the oxygen demand, which depends on factors like left ventricular volume, wall thickness, heart rate, and myocardial contractility, or a decrease in the supply of oxygen, which can be caused by factors like anemia, reduced coronary blood flow, or other factors that reduce the blood's oxygen-carrying capacity. Atherosclerotic coronary plaque blocking the vessel lumen is a common cause of diminished coronary low reserve, which in turn causes angina [36].

It is possible to have angina in a variety of forms. Most people with angina have chronic stable angina. Exertion is a common trigger for this type of pain, which often only lasts for a short period before going away as the body relaxes [15]. In the early 1970s, “unstable angina” was the word that was initially used to refer to unstable angina [37], which had been alluded to in other previous publications under a variety of different titles on several occasions. There are a few different categories that have been defined for unstable angina. The fresh onset of severe or rapid angina that had been present for less than two months and did not show any signs of resting pain was included in Class I. The rest discomfort that occurred during the previous month was included in Class II, although it did not occur during the last two days. Those who were diagnosed with Class III had angina at rest within the previous two days. Further categorization of unstable angina was based on the clinical conditions that led to its occurrence, as well as the quantity of cardiac medication that was administered. The researchers Prinzmetal et al. [38] handled a total of 32 cases all having "variant" angina pectoris. They settled on the word "variant" to describe these patients' complaints of resting chest discomfort that was not triggered by normal emotional or physical states.

Advancements in gene therapy: The goal of growth factor gene therapy [39] is to encourage angiogenesis, or the development of new blood vessels, in heart disease patients. This is accomplished by supplying the heart with particular growth factors, such as Vascular Endothelial Growth Factor (VEGF) and Fibroblast Growth Factor (FGF). Fibroblast Growth Factor (FGF) Therapy – AGENT Trials: The results of the AGENT-1 experiment, which included 79 patients with acute angina, demonstrated that it was technically feasible to use a catheter to inject

Ad5FGF (adenovirus 5 fibroblast growth factor), which increased myocardial uptake. Better perfusion imaging compared to baseline was the outcome of the 52-patient randomized, placebo-controlled AGENT-2 Trial. The purpose of the AGENT-3 and AGENT-4 studies was to lower angina symptoms and enhance exercise duration in large-scale Phase IIb/III studies in Europe, Latin America, and Canada. Nonetheless, there was no discernible improvement when contrasted with the placebo group [39]. Vascular Endothelial Growth Factor (VEGF) Therapy: Direct intramyocardial injections of VEGF gene therapy [39] were investigated for their potential to enhance blood flow to the heart. In a Phase II trial conducted by Stewart et al., 67 patients showed an improvement in blood flow, an increase in exercisetime, and a decrease in ST-segment depression. Eighty individuals who had percutaneous delivery of intramyocardial-VEGF plasmids were part of another phase II trial called the Euroinject One Trial. Three months after treatment, there was no statistically significant improvement in cardiac function or perfusion imaging compared to placebo. In a study conducted by Beanlands and colleagues, patients undergoing LAD (Left Anterior Descending artery) revascularization were given plasmid injections containing VEGF and L arginine for three months, as opposed to a placebo. The results showed that the combination of the two increased the contractility and motility of the heart walls.

Myocardial Infarction

The condition that affects the blood arteries that supply the heart muscle (the myocardium) is referred to as myocardial infarction (MI), which is more often referred to as a heart attack. The term "myocardial infarction" describes the overall process, whereas "infracted region" describes the specific area of the heart muscle that has been damaged [40]. Fractured regions of the heart muscle have either no flow or flow that is so low that they are unable to maintain cardiac muscle function.

There are two basic types of MI: The term "transmural" refers to its association with atherosclerosis which affects the main coronary artery. Three subcategories can be applied to it: anterior, posterior, and inferior. Typically caused by a complete blockage of blood supply to the area, transmural infarcts are defined by penetrating the entire thickness of the heart muscle [40]. Subendocardial: involves a tiny area in the left ventricle subendocardial wall, the ventricular septum, or the papillary muscles of the affected individual. One possible cause of subendocardial infarcts is a localized decrease in blood flow, which could be caused by a narrowing of the coronary arteries. Because of its isolation from the heart's blood supply, the subendocardial area is a prime location for this kind of pathology [40].

Advancements in Gene Therapy: Advancements in Gene Therapy for Promoting Angiogenesis Post Myocardial Infarction include VEGF Gene Therapy, HGF Gene Therapy, and FGF Gene Therapy. VEGF Gene Therapy: Angiogenesis [41] depends on VEGF (Vascular Endothelial Growth Factor) attaching to certain receptors on the surface of endothelial cells. VEGF-165 gene therapy is thought to be a very successful way to encourage angiogenesis. Through non-viral treatment, the VEGF-165 gene can promote angiogenesis and increase the rate of short-axis contraction in rats or rabbits following MI. Myocardial infarction (MI) models in pigs and dogs show that VEGF-165 can thicken and strengthen the heart wall, improve left ventricular contractility, reduce infarct area, and boost myocardial survival rate. In one trial, the symptoms of angina pectoris and myocardial blood flow significantly improved. HGF Gene Therapy: The effects of HGF (Hepatocyte Growth Factor) gene therapy on animal models of cardiac illnesses were demonstrated to include reduced inflammatory levels, improved cardiac function, increased arteriole density, and angiogenesis [41]. In addition, HGF may inhibit cell death and improve heart contractile performance. A more robust and long-lasting effect of angiogenesis was observed with the combined administration of fibroblast growth factor-2 and HGF, which successfully decreased cardiac remodeling and enhanced left ventricular functioning. Enhancement of cardiac function and gene transfection efficiency in a rat model of myocardial infarction (MI) was achieved by a combination of HGF gene therapy and ultrasound-mediated microbubble-breaking technology. FGF Gene Therapy: The increase of HIF-1α (Hypoxia-inducible factor 1-alpha), stimulation of angiogenesis [41], and suppression of apoptotic effects in cardiac endothelial cells were all stimulated by FGF (Fibroblast Growth Factor). Gene therapy using FGF-4 has been shown to improveperfusion and MI-related dysfunction in a pig model of the disease. Gene therapy with FGF-5 promotes mitosis in myocardial cells, which improves blood flow and local myocardial dysfunction. Advancement in Gene Therapy to Reduce Muscle Reperfusion Injury After MI can be done by Gene Therapy of Endothelial Nitric Oxide Synthetase (eNOS). A signal molecule that serves several protective roles, NO is a signal molecule. After myocardial infarction surgery, adenovirus-mediated human eNOS gene therapy has the potential to improve cardiac remodeling, reduce the area of myocardial infarction, and lower apoptosis, JNK (c-Jun N-terminal kinase) phosphorylation level, and caspase-3 activity. After a myocardial infarction, eNOShas the ability to improve vascular function, as well as minimize the amount of fibrosis that has developed [41], [42].

Heart Failure

The final result of several cardiovascular illnesses is heart failure [43], which is one of the leading causes of morbidity and death on a global scale. Heart failure makes it impossible for the ventricle to adequately fill or empty with blood. This is a symptom of heart failure.

Reduced ejection fraction heart failure (HFrEF) is the medical term for a kind of heart failure in which the left ventricular ejection fraction (LVEF) is less than forty percent. It is more frequently linked to diseases such as myocarditis, cardiomyopathies, or ischemic heart disease and is frequently linked to compromised heart muscle contractile performance. Patients with preserved ejection fraction heart failure (HFpEF) have an LVEF that is much higher than 50%. Increased left ventricular stiffness and poor relaxation are linked to HFpEF, which frequently arises in the setting of diabetes, hypertension, or age [44], [45].

Advancements in gene therapy: It has been demonstrated that the utilization of an adeno-associated virus (AAV) vector in gene therapy leads to an increase in the effectiveness of the SERCA2a (sarcoplasmic reticulum calcium-ATPase 2) enzyme, which in turn leads to an increase in the quantity of calcium that is reabsorbed into the sarcoplasmic reticulum [47]. Several studies have shown that by administering AAV/SERCA2a to pre-clinical animal models of heart failure with preserved ejection fraction (HFpEF), cardiac function was improved. Human gene therapy experiments that targeted SERCA2a have only been carried out in patients with heart failure with reduced ejection fraction (HFrEF), and they all were unsatisfactory experiments.[46]. Among patients with heart failure with reduced ejection fraction (HFrEF), the phase 1/2 CUPID 1 trial demonstrated an acceptable safety profile as well as improvements in three crucial parameters: symptom, function, and biomarker The success of this trial led to the launch of the CUPID 2 experiment, which was both larger and more global in scope. There was no improvement in disease outcomes for HFrEF patients with CUPID 2, which is disappointing because it failed to repeat the beneficial effects of CUPID 1 [46]. In CUPID 1 and 2, the percentage of viral capsids that were empty was twenty-five percent and eighty-five percent, respectively. There was a distinction between the two groups, as demonstrated by this. These empty capsids may act as a decoy and reduce interference from the immune system. Gene therapy can be less effective if the body contains low-level neutralizing antibodies or other substances that interfere with its functioning. In contrast to CUPID 1, patients who participated in CUPID 2 were required to have elevated levels of BNP (B-type natriuretic peptide) and NT pro-BNP (N-terminal pro-B-type natriuretic peptide). This was done because BNP has the potential to influence exogenous SERCA2a. It is possible that this was one of the factors that led to the failure of the trial [46]. The ongoing phase I MUSIC HFpEF trial holds considerable importance as it represents the inaugural gene therapy study evaluating the effects of increased SERCA2a activity [46] in patients with HFpEF. The outcomes of this study are highly anticipated and will reveal the impact, if any, of elevated SERCA2a levels in the myocardium on cardiac function, while also guiding future SERCA2a gene therapy initiatives for HFpEF.

Cardiac Arrest

In the absence of immediate resuscitation efforts, cardiac arrest [48], which is the abrupt cessation of the heart's mechanical function, results in death within minutes. Almost all instances of cardiac arrest may be traced back to structural heart disease as the underlying cause. It is believed that ischemic coronary disease, the primary cause of cardiac arrest, accounts for 70% of all instances [50]. Cardiac tamponade, arrhythmogenic right ventricular dysplasia, congenital coronary artery anomalies, hypertrophic obstructive cardiomyopathy, and congestive heart failure are other anatomical reasons. There are a number of non-structural cardiac causes, including the congenital long QT syndrome, the Brugada syndrome, and the Wolf Parkinson-White syndrome [49].

Advancements in gene therapy: Various gene therapy methods are there for cardiac arrest which are demonstrated in Table 4.

Table 4: **Gene therapy approach for cardiac arrest.**

GENE	ROLE IN CARDIAC ARREST	GENE THERAPY APPROACH
KCNH2 (potassium voltage-gated channel subfamily H member 2)	Long QT Syndrome	A "suppression-and-replacement" (SupRep) KCNH2 gene therapy was produced by cloning a custom-designed KCNH2 short hairpin RNA with approximately 80% knockdown as the suppression component and a "short hairpin RNA-immune" KCNH2 cDNA as the replacement component [51].
KCNQ1(Potassium Voltage-Gated Channel Subfamily Q Member 1)	Long QT Syndrome	A KCNQ1 short hairpin RNA and an immunogenic KCNQ1 cDNA with synonymous variations at the short hairpin RNA target location were cloned together to provide a dual-component suppression-and-replacement (SupRep) KCNQ1 gene therapy [52].
MYBPC3 (Myosin-Binding Protein C3)	Hypertrophic cardiomyopathy	Reducing the size of the AAV genomic cassette while maintaining its ability to express specific genes in the heart is possible through engineering [53].
RYR2 (Ryanodine Receptor 2)	CPVT	Genome editing using AAV9-mediated CRISPR/Cas9 might effectively wipe out the defective Ryr2 allele [54].

Stroke

A neurological condition known as stroke [56] is typified by blood vessel obstruction. When clots form in the brain, they disrupt the flow of blood, which in turn causes arteries to get clogged and blood vessels to break, which ultimately results in bleeding. When the arteries that provide blood to the brain are ruptured, a stroke occurs The sudden death of brain cells as a consequence of a shortage of oxygen is the consequence of this outcome. Stroke survivors may also experience depression and dementia. If we look at the world from a global viewpoint, stroke is the second leading cause of mortality. Over 5.5 million people lose their lives to it annually, and another 13.7 million are affected by it. According to estimates, 10–25% of strokes are secondary (second-time) hemorrhages, while the majority are primary (first-time) hemorrhages [60], [61]. It is seen that the more a person grows, the more the risk of having a stroke increases. As the person grows beyond the age of 55 the chance rises two times. Hypertension, coronary artery disease, and hyperlipidemia are some of the preexisting medical conditions that raise the risk even further. Several critical events play a role in the pathology of stroke, including inflammation, energy failure, disruption of homeostasis, acidosis, elevated intracellular calcium levels, excitotoxicity, toxicity mediated by free radicals, cytokine-induced cytotoxicity, complement activation, blood-brain barrier impairment, glial cell activation, oxidative stress, and leukocyte infiltration [57], [58].

There are two basic categories according to which strokes can be classified. Within the stroke population, ischemic strokes [56] account for about 85% of fatalities. Brain thrombotic and embolic disorders can be caused by ischemic occlusion [55]. Thrombosis occurs when atherosclerosis causes the blood arteries to constrict, reducing blood flow. Plaque buildup narrows blood vessels and causes clots to develop, which in turn causes thrombotic stroke. Embolic strokes occur when there is a sudden drop in blood flow to a specific area of the brain, which in turn generates embolisms thus causing the inability to handle stress and ensuing brain cell death. Hemorrhagic stroke has a high death rate and causes between 10–15% of all strokes [56]. Blood vessels burst in this disease due to internal injury and stress on the brain tissue. Infarction is the result of its harmful effects on the vascular system [59]. Hemorrhages can be classified as either intracerebral (ICH) or subarachnoid. In ICH, blood vessels burst, causing an abnormal

accumulation of blood in the brain. The main reasons for intravascular hemorrhage (ICH) include high blood pressure, dysfunctional vasculature, and excessive use of anticoagulant and thrombolytic drugs.

Advancements in gene therapy: Ischemic stroke [62] gene therapy tries to control inflammatory responses by targeting specific molecular pathways and modifying specific genes; it also promotes tissue repair, neuroregeneration, and angiogenesis [64]. Neurotrophic factor, anti-inflammatory cytokine, growth factor, and stem cell-specific gene therapy are among the gene therapy modalities studied for their possible therapeutic impact in ischemic stroke [64]. Neuronal recovery following an ischemic stroke may be aided by targeting these factors by gene therapy or exogenous delivery. A gene therapy method that shows a great deal of promise is the transplantation of the vascular endothelial growth factor (VEGF) gene into patientswho have suffered an ischemic stroke. A powerful angiogenic factor, VEGF increases blood flow to ischemic brain tissue by stimulating the formation of new blood vessels [65], [66]. Animal models of ischemic stroke have demonstrated that VEGF gene therapy can enhance neurological function, decrease infarct size, and induce neurovascular remodeling [65], [66]. There are various methods for delivering VEGF gene therapy, including viral vectors, non-viral vectors, or cell-based approaches. One of these approaches can sustain the therapeutic gene's expression in the brain [67], [68]. Patients suffering from chronic cerebral ischemia were found to be safe and viable candidates for VEGF gene therapy in a phase I/II clinical study. Gene therapy shows promise as a new way to treat ischemic strokes, according to these findings. Compared to the control group, patients who received VEGF gene therapy had improvements in metabolic activity, cerebral blood flow, and neurological function [69]. By allowing for targeted gene expression, precise genome remodeling, and repair of genetic mutations, gene editing technologies like CRISPR-Cas9 have transformed the area of gene therapy [70]. Novel gene therapy techniques for ischemic stroke, including modifying disease-related signaling pathways, improving endogenous repair mechanisms, and correcting genetic risk factors, are greatly encouraged by these technologies. In the early 1990s, RNA interference was developed which sparked a series of studies that contributed to the development of siRNA gene therapy [63]. These studies helped advance the field. Gene silencing by the use of small interfering RNA (siRNA) is an innovative approach that has the potential to be therapeutic for intracerebral hemorrhage (ICH). Researchers are now able to restrict harmful protein pathways as a means of inducing neuroprotection, restoring motor function, and reducing inflammation following a stroke [71]. This is made possible by recent advancements in gene silencing technologies. Diseases with a long incubation time, such as the secondary damage phase following ICH, may be treatable with the use of siRNA. This is accomplished by silencing detrimental gene components.

4.6.1. Familial Hypercholesterolemia

Despite being one of the most common hereditary conditions, familial hypercholesterolemia (FH) [72] is still mostly underdiagnosed and undertreated globally [73]. Several complications, including vascular deposits, tendon and skin xanthomas, xanthelasma, and extravascular deposits, can develop in FH due to high blood levels of LDL-C (Low-Density Lipoprotein Cholesterol). These complications can lead to coronary heart disease (CHD), early and progressive atherosclerosis, and an increased risk of death and morbidity [74].

Advancements in gene therapy: This can be done using Adeno-associated virus (AAV), exomes, and various other methods. Using AAV: When compared to other viral vectors, AAV is a single-stranded linear DNA-deficient virus that is widely recognized as an excellent gene therapy vector. This is because it hardly ever elicits any immune response, has a low risk of integrating into the host genome, and has the ability to express therapeutic genes in a permanent manner [75]. AAV-mediated therapy has demonstrated encouraging outcomes in pre-clinical trials for the treatment of Duchenne muscular dystrophy (DMD) [76]. In 2020, “phase ½” clinical research was finished to assess the safety and effectiveness of the AAV vectors in nine individuals with HoFH (Homozygous Familial Hypercholesterolemia) [82]. The injection of the thyroxin binding globulin (TBG) promoter through intravenous administration, which was directed to regulate the AAV, increased liver-specific LDLR (Low-Density Lipoprotein Receptor) expression (AAV8.TBG.hLDLR) with a decline in peripheral blood LDL-C levels [77]. The T cell immunological responses to the carrier capsid might have been the cause of the asymptomatic increases of liver transaminases that were observed in the subjects, as indicated by the preliminary data [78]. To produce novel AAV vectors, researchers combined AAV8 with the intervening sequence 2 of the human beta-globin gene (also known as AAV8-IVS2). The researchers administered a single dose of AAV8-IVS2 with LDLR (hLDLR011-T) to 11-week-old double knockout mice that were deficient in the LDLR and APOBEC1 (Apolipoprotein B mRNA editing enzyme catalytic polypeptide 1) genes [79]. According to the data, the blood LDL-C levels decreased by 99% on day three and continued to do so until day 120. Using exomes: The endosome in the nucleus gives rise to exosomes, which are disk-shaped vesicles that range in diameter from 40 to 160 nm and have a density of 1.13 to 1.18 g/mL [80]. Because of their status as "natural nanoparticles," exosomes can withstand the many biofilm barriers and

transport mRNA, miRNA, proteins, and plasmid DNA. An exosome-based LDLR gene therapy for Familial hypercholesterolemia was recently described in a mouse model that has both LDLR genes knocked out [82]. To increase the amount of LDLR in exosomes (ExoLdlr), the vector was introduced into packing cells using transfection. The expression of the LDLR protein in the liver was considerably enhanced and the amount of LDL-C was considerably reduced (from 6.5 mmol/L to 4.5 mmol/L) after the injection of ExoLdlr into the mouse. Compared to the PBS (Phosphate-Buffered Saline) control group, the ExoLdlr mice had a substantially less atherosclerotic area of 3% [81]. In December 2021, a phase I clinical trial (NCT05043181) including 30 patients with HoFH was launched, marking the first human investigation of exosome-based LDL mRNA delivery.

CONCLUSION:

THE FUTURE OF GENE THERAPY FOR CARDIOVASCULAR DISEASE

Gene therapy has enormous promise as a game-changing approach to cardiovascular disease management. This method offers the possibility of disease prevention, long-lasting cures, and the reduction of the need for lifelong pharmacotherapy. In order to bring these promises into clinical reality, it is necessary to overcome substantial difficulties. The precise targeting of therapeutic genes to particular cardiovascular tissues, the reduction of immune responses to delivery vectors, the mitigation of off-target effects caused by genome editing tools, the identification of the patient population that possesses the greatest therapeutic potential, and the establishment of long-term efficacy and safety profiles are some of the key obstacles that need to be overcome. Gene therapy possesses a tremendous potential to transform personalized medicine by tailoring it to the genetic and clinical characteristics of individual patients. This potential can be realized through coordinated efforts across multiple disciplines [83]. The CRISPR-Cas9 technology provides a novel and exciting approach to treating cardiovascular diseases (CVDs) by regulating gene expression and specifically targeting the genetic abnormalities that cause these disorders [84]. Additionally, CRISPR-Cas9 can be used to validate cell-based therapeutics, such as editing iPSC-derived cardiomyocytes for individualized regenerative medicine, and to build disease models to study the pathophysiology of CVDs. Developing strong delivery mechanisms, especially for modifying the mitochondrial genome, should be a primary goal of future research, along with increasing accuracy and decreasing hazards. Genome editing has far-reaching consequences for germline and somatic cells, and new ethical and regulatory frameworks are required to handle these issues. Thus, CRISPR-Cas9 is an innovative tool for the treatment of cardiovascular disorders; it has the ability to greatly enhance patient outcomes and quality of life while also reshaping the field of cardiovascular medicine. If this technology keeps getting better, it could completely change the way genetic illnesses are handled. If we want to improve gene delivery and avoid the immune system, we need to keep working on vector engineering, especially on new capsid architectures [85].More research into the mechanisms behind cardiovascular disease should lead to the identification of new gene therapy targets. By utilizing pharmacogenomics, the most promising individuals can be identified for gene therapies. With this newfound knowledge, practitioners will be equipped to correct signaling pathways that are not working properly, perhaps reversing the progression of cardiovascular disease. Efforts to reduce the immunogenicity and off-target effects of viral carriers will likely persist. More research into the mechanisms behind cardiovascular disease should lead to the identification of new gene therapy targets. By utilizing pharmacogenomics, the most promising individuals can be identified for gene therapies. With this newfound knowledge, practitioners will be equipped to correct signaling pathways that are not working properly, perhaps reversing the progression of cardiovascular disease [86].

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