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REVIEWS

Recent applications of CRISPR-Cas systems in animal models of human cardiovascular disease: Review article

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Cardiovascular disease (CVD) causes more deaths, more years of life lost, and more years lived with disability than any other major category of disease worldwide. Gene editing technologies, including the CRISPR-Cas9 system and its offshoots, are increasingly applied in CVD animal research models to identify genetic drivers of disease processes and to develop and test targeted therapeutics. To explore the potential impact and limitations of using animal models for these applications, this review examines studies in which the creation of a CRISPR-Cas9 animal model has been used to find genetic drivers and develop therapies. Studies were identified using OVID Medline, searching the past 10 years of the primary literature across 4 CVD domains: congenital heart disease, hypertrophic cardiomyopathy, heart failure, and atherosclerosis. The studies illustrate the vast range of gene editing applications and the value of models that parallel human genetic pathophysiology for specific disease processes. Besides furthering anatomical, clinical, and natural historical understanding of CVD pathologies and providing biological substrates in the development of screening and diagnostic tools, animal models support foundational stages in the development of genetic therapeutics, from gene target identification to discovery and development of gene-editing therapy mechanisms and delivery vehicles, through conducting therapeutic trials to assess the risk-benefit ratio.

INTRODUCTION

Since 1990, the Global Burden of Disease Study has collated data on all domains of human disease, quantifying and organizing the contributions that distinct categories of disease make to death and disability worldwide. Its most recent report, GBD 2023, tallies the global burden of cardiovascular disease (CVD): 19.1 million deaths, 31.9% of the global total, and 436.2 million disability-adjusted life-years, 15.6% of the global total—the most of any of the 22 Level 2 disease categories the report encompasses.[1] The cardiovascular system, comprising the heart and vessels that pump and shuttle blood to meet the body's metabolic needs, is subject to both intrinsic disease and insults arising from its connections to other organ systems. Animal models, while controversial in how well they mirror human disease physiology, have provided necessary insight into CVD etiology, pathogenesis, natural history, transmission, and treatment. Mice, the most common species used for animal

models, are inexpensive, have large litters, and have a well-understood genome for editing, making mice suited to initial live-organism research. Other animal species offer distinct advantages in distinct contexts; eg, porcine models offer a closer approximation to human leukocyte proportion in hematologic research. Other large animal models, though more expensive and less readily available, supply better platforms for surgical trials. Recently, transgenic models have benefited from and aided in advances in gene engineering technologies.[2]

These advances arose partly from research into the immunologic defenses of microbes whose genomes include fragmentary genetic sequences from previously encountered pathogens stored as clustered regularly interspaced short palindromic repeats (CRISPR). Transcripts of these sequences act as guide RNAs (gRNAs) for CRISPR-associated (Cas) endonucleases, forming CRISPR-Cas ribonucleoproteins that defend the microbe by inducing targeted breaks in pathogen nucleic acids.[3] As genome editing, transcription modulation, and transcript editing have evolved, modifications of this microbial defense have created several CRISPR-Cas systems, many based on Cas9, an endonuclease that, unmodified, induces double-strand breaks at locations determined by 2 associated RNA transcripts. Modifications include streamlined targeting using single-guide RNAs (sgRNAs) as well as inactivation of one or both of Cas9's catalytic domains; inactivation of one domain yields targeted nickases for single-strand breaks, whereas inactivation of two domains yields "dead" Cas9s that act solely as targeting systems.[4] These tools are already being used in clinical applications: CRISPR-Cas9–based therapies have been used in gene editing approaches for conditions including sickle cell disease and transthyretin amyloidosis, demonstrating the technology's translational potential beyond the laboratory.[5,6]

Animal models and therapeutic applications have evolved—from targeted gene knockout (KO) via Cas9-induced double-strand breaks and nonhomologous end-joining, to improved KO and knock-in editing via Cas9-induced homology-directed repair (HDR), to base-editor mechanisms in which gRNA, modified Cas9, and nucleotide deaminases produce precise single-base genome edits. Persistent challenges for CRISPR-Cas9 genome editing include off-target or inefficient editing, payload size, targeting limitations in delivery (mechanical, viral, and plasmid-based), and immunogenicity.[4]

This review synthesizes how CRISPR-Cas9 genome editing is currently applied in animal models of CVD, examining its use across disease modeling, therapeutic target identification, and gene therapy, to characterize the state of the field and highlight promising directions for translational research.

METHODS

Ovid MEDLINE was used to identify studies included in our review. While specific search terms varied, all searches included the exploded MeSH term "CRISPR-Cas Systems," combined with CVD-related terms. Inclusion criteria consisted of studies in the English language published between 2015 and 2025 and the presence of primary data from single experiments. Studies lacking primary data, not directly addressing the use of CRISPR-Cas9, or in a format other than a research article were excluded from the analysis. Included studies were published between 2016 and 2025. A summary of specific findings from the studies can be found in the Tables section (Tables 1-4).

RESULTS

Using Animal Models and CRISPR/Cas9 to Identify and Analyze Congenital Heart Defects

Congenital heart defects (CHDs) affect up to 1% of live births.[7] These defects are subdivided into cyanotic vs noncyanotic, depending on the location of the defect within the heart and the severity of the defect. These defects present at birth or later in life, depending on the type of defect and its severity.

To study the development, clinical presentation, and natural history of CHD, animal models, specifically mice and zebrafish, can be genetically modified using CRISPR-Cas9. These models, while not perfect imitations of humans, are an effective tool to study congenital defects and their implications for healthy physiology. Ovid MEDLINE yielded 8 studies through the exploded search terms "CRISPR-Cas Systems", "Cardiovascular Disease", "Models, Animals", and "Heart Defects, Congenital." Two studies were excluded as they lacked a focus on CHDs, and two additional studies were excluded as they paralleled studies that were already included. A summary of the studies that were analyzed can be found in Table 1. The goal of this review is to represent the current use of CRISPR-Cas9 in genetically edited animal models to validate theories identifying genes responsible for the development of specific CHDs.

Some genes, such as *LRRC56*, are pleiotropic. When knocked out using CRISPR-Cas9, phenotypic abnormalities occur, including hydrocephalus, situs inversus, male infertility, and bronchiectasis. Mutations in *LRRC56* disrupt the structural integrity of dynein arms in cilia, impairing their motility and, thereby, causing defects in mucociliary clearance and laterality determination.[8] As this gene is involved in the embryological development of many organ systems, mice were used to examine the systemic consequences of the *LRRC56* KO. In comparison to the genetic modifications discussed later in this section, mice without *LRRC56* maintained healthy heart, liver, and kidney function.[8] The *LRRC56* KO resulted in situs inversus, a condition

Table 1. CRISPR-Cas9 Gene Targets, Animal Models, and Cardiac Outcomes in Recent Studies of Congenital Heart Defects*

Study	Gene(s) Targeted	Gene(s) Function	Animal Model	Method of CRISPR-Cas9 Delivery	Outcome
Wu et al, 2025	*LRRC56* (exon 4-5)	Needed for dynein arm assembly	Mice	gRNA	Primary ciliary dyskinesia Hydrocephalus, situs inversus, male infertility, bronchiectasis
Santinha et al, 2023	*Dgcr8* *Dgcr14* *Gnb1l* *Ufd1l*	Component of microprocessor complex for primary miRNA into precursor miRNA Component of C complex spliceosomes Protein of unknown function Needed for degradation of ubiquitinated proteins	Mice	AAV-Perturb-seq gRNA	22q11.2 deletion syndrome
Wang et al, 2018	*Tfap2b* *c.435_438delCCGG*	Transcription factor involved in cell cycle regulation and embryonic development, enriched in neural crest	Mice	mRNA sgRNA c.435_438delCCGG mutant oligos	Homozygous mutant: Char syndrome Heterozygous mutant: PDA
Liu et al, 2017	*Sap 130* *Pcdha9*	Sin3A-associated protein in histone deacetylase complex, coregulator of genes for mitotic cell cycle, mitotic checkpoint control, chromatin-regulatory components Cell-adhesion protein, coregulator of Notch signaling, epithelial-mesenchymal transformation (EMT), and mesenchymal cell differentiation	Mice Zebrafish	T7 promoter sgRNA	Left ventricular hypoplasia Aortic valve abnormalities

*Genes were induced using CRISPR to elicit congenital heart defects.

caused by primary ciliary dyskinesia, where ectodermal cilia are unable to properly conduct currents needed for mesoderm spreading, resulting in a mirrored anatomy without compromising function. Congenital heart defects occur on a spectrum that ranges from no effect on cardiac physiology to lethal. They can occur with other abnormalities due to the mutation of a single gene or, depending on the number of mutated copies, may be the only defect.

Another study investigated transcription factor *TFAP2B*, which is associated with Char syndrome, an autosomal dominant disorder, characterized by patent ductus arteriosus (PDA) as well as facial and finger abnormalities.[9] *TFAP2B* is involved in cell cycle regulation and, in neural crest cells, key regulator of cardiac valve and outflow tract formation.[9] In a prior study analyzing a human family with PDA, the researchers discovered a 4–base–pair deletion within the *TFAP2B* gene, leading to a premature stop codon, which resulted in a truncated protein. This mutation resulted in PDA alone in heterozygous individuals, suggesting that one mutated copy produces PDA, whereas two mutated copies produce the full Char syndrome phenotype.[9] Notably, because Char syndrome follows autosomal dominant inheritance in humans, the absence of hand and facial abnormalities in the heterozygous family members, who instead presented with isolated PDA, is a particularly striking finding. To examine this hypothesis, Wang et al[9] used CRISPR-Cas9 to create mouse models bearing this specific deletion within *TFAP2B*. In both humans and mice, *TFAP2B* is crucial to ductus smooth muscle cells closing the ductus arteriosus after birth, as well as the collecting duct and tubular epithelium.[9] Double mutant mice died shortly after birth due to tubular dilation and polycystic kidney disease, accompanied by PDA and resultant congestive heart failure, whereas single mutant mice developed PDA alone.[9] The mutation produced a decrease in smooth muscle cell distribution and an increase in fibronectin, indicating that abnormal smooth muscle cells impaired closure of the ductus arteriosus.[9] Importantly, unlike human patients carrying the same mutation, these mice were able to achieve ductus arteriosus closure after birth, indicating that the smooth muscle cell signaling mechanisms governing ductus arteriosus closure differ between mice and humans. This is a species difference that limits the animal model's ability to fully replicate the human phenotype regarding the pathogenesis of CHDs like PDA.[9]

In contrast to PDA, another study used CRISPR-Cas9 in mice and zebrafish models to demonstrate that hypoplastic left heart syndrome (HLHS) is multigenic, with specific genes responsible for the individual components.[7] Hypoplastic left heart syndrome is characterized by hypoplasia of the left ventricle, aorta, and mitral valve with an atrial septal defect.[7] Previously, scientists hypothesized that hemodynamic factors were the primary causes of

HLHS. However, this study[7] identified 2 genes that are expressed in cardiac cushions. Mutations in *Pcha9*, which coregulates cellular differentiation of the embryonic germ layers, were shown through animal models to drive aortic valve defects.[7] *Sap130*, a co-regulator of mitotic activity, resulted in left ventricular hypoplasia when mutated, owing to the block of cardiomyocyte proliferation and differentiation.[7] Additionally, researchers noted incomplete penetrance in the gene-edited mice and zebrafish animal models, indicating that epigenetic effects may also contribute to the development of HLHS.[7] This study emphasizes that through CRISPR-Cas9–edited animal models, scientists can validate and identify genetic mutations that interact to produce complex CHD.

CRISPR MODELS OF CHD-ASSOCIATED GENETIC SYNDROMES

In addition to studying complex CHD, genetically edited mouse models can be used to examine the genotype-phenotype relationship of genetic syndromes, in which CHD is a component, such as 22q11.2 deletion syndrome.[10] Three separate genes, *Dgcr8*, *Dgcr14,* and *Gnb1l*, were found to alter gene expression associated with 22q11.2 deletion syndrome.[10] Unlike in the study by Liu et al,[7] where specific genes could be linked to discrete parts of HLHS, the genes within the 22q11.2 locus cannot be identified as primary drivers of the 22q11.2 deletion syndrome phenotype.[10] In the future, CRISPR-Cas9 may be used to examine other genetic syndromes that have CHD as part of their phenotype, to further our understanding of disease biology and potential therapeutic targets.

The shared goal of the studies presented is to identify the genetic causes of CHD, whether a single gene or several, that may inform future therapeutics or genetic screening. Even though species differences can affect how mutations manifest, CRISPR-Cas9–edited animal models remain an effective tool for validating existing hypotheses and characterizing the natural history of CHD.

Hypertrophic Cardiomyopathy

For a focused review of studies on hypertrophic cardiomyopathy (HCM), whose etiologies include inherited single-gene pathogenic variants,[11] an Ovid MEDLine search using MESH terms ("exp Models, Animal/ and exp CRISPR-Cas Systems/ and exp hypertrophic cardiomyopathy/") was performed, yielding 3 articles, 2 in the primary literature, i.e., 2 articles reporting and discussing original study results. Iterated cross-referencing of bibliographies led to a preliminary pool of studies for review, 3 of which were selected for recency and representation of (1) diverse CRISPR-Cas9–based editor tools and (2) connections between the development of novel models and the evaluation of gene editing therapeutics. The summaries of these studies can be found in Table 2.

Table 2. CRISPR-Cas9 Editor Technologies, Delivery Methods, and Outcomes in Recent Animal Model Studies of Hypertrophic Cardiomyopathy

Study	Gene(s) Targeted	Gene Function	CRISPR Editor Technology Used	Models Created	Method of CRISPR-Cas9 Delivery	Outcome
Huang et al, 2020	*Gaa*	Codes for GAA (acid-α galactosidase)	HDR via (spCas9 + dual sgRNA + ssODN template)	Ortholog mouse myoblast, ortholog mouse	Nucleofection (in vitro); pronuclear injection (in vivo)	KI mouse model closely approximating human IOPD
Nie et al, 2023	*MYBPC3*	Codes for sarcomeric linking protein	HDR via (spCas9 + sgRNA + donor template [unspecified type])	Ortholog rat fibroblast, ortholog rat	Dual AAV vectors	KI mouse model approximating human HCM; reversed SNV with Cas9-based HDR therapy
Chai et al, 2023	Mouse *Myh6*, human *MYH7*	Codes for β-myosin heavy chain	BE via (modified spCas9 + sgRNA + ABE)	Human iPSCs, humanized mouse	plasmid nucleofection (in vitro human iPSCs); dual AAV vectors (in vivo mouse model)	KI humanized mouse model approximating human HCM; reversal of SNV in humanized model via ABE; treatment of HCM in humanized model

Abbreviations: ABE, adenine base editor; HCM, hypertrophic cardiomyopathy; HDR, homology directed repair; IOPD, infantile-onset Pompe disease; KI, knock-in; SNV, single nucleotide variant.

Huang et al[12] applied CRISPR-Cas9 to an advanced murine model that was a phenotypic and genotypic approximation of human infantile-onset Pompe disease (IOPD), an inherited metabolic disorder in which a pathogenic variant of *Gaa*, which codes for acid α-glucosidase. Infantile-onset Pompe disease causes enzyme deficiency, lysosomal glycogen accumulation, and, without enzyme replacement, results in severe morbidity, including progressive hypertrophic cardiomyopathy, followed by early death.

After in vitro evaluation of targeting accuracy and efficiency in murine myoblasts to select between candidate sgRNAs and single vs dual sgRNA-directed HDR, the authors knocked an ortholog pathogenic *Gaa* variant into the murine genome via HDR, combining Cas9, 2 overlapping sgRNAs, and a single-stranded oligodeoxynucleotide template. Based on biomolecular and histologic similarity to human disease in the resulting knock-in murine cells, the authors proceeded to in vivo application by pronuclear injection of mouse embryos. Subsequent genetic screening and breeding produced a final homozygous knock-in transgenic model line.

This novel model improved the genotypic approximation of human IOPD by incorporating an orthologous pathogenic *Gaa* variant not seen in KO models, supporting research into gene editing therapies for IOPD. It is less clear whether the model improves in KO alternatives in an approximation of IOPD at levels other than the genomic. Notably, the knock-in mice did not significantly differ from the KO mice in *Gaa* transcript levels or GAA activity; similarly, it is unclear to what degree early-onset vs late-onset hypertrophic cardiomyopathy is a significant pathophenotypic advance, separating this model from KO mice. The net effect of the study is to support dual sgRNA-directed, Cas9-mediated HDR as a tool to produce models of CVD suitable for the study of novel genome-level therapies of human IOPD, and to offer a murine line not inferior to extant KO models in the pathophenotypic approximation of human IOPD.

Nie et al[13] used CRISPR-Cas9 HDR to create and evaluate a novel in vivo, postnatal treatment for HCM owing to pathogenic variants of *MYBPC3*, which codes for myosin-binding protein C3, a sarcomere constituent. Having identified a pathogenic human *MYBPC3* single-nucleotide variant with a premature stop codon in a heterozygous mother-son pair with HCM, the authors created a rat model homozygous for an ortholog of this single-nucleotide variant, then aimed to reverse the single-nucleotide substitution via HDR. Candidate sgRNAs were evaluated in vitro on *MYBPC3* variant-homozygous rat fibroblasts by direct introduction of plasmids coding for sgRNA, spCas9, and donor sequence. The sgRNA demonstrating the highest in vitro editing activity was selected for in vivo testing on 3-day-old rat pups split into homozygous treatment, homozygous control treatment, and wild-type (WT) treatment groups.

For postnatal delivery, the investigators split Cas-system components between two adeno-associated virus (AAV) vectors. Terminal analysis of the control treatment group indicated successful generation of a novel rat model of HCM, with gross cardiac hypertrophy, thickened ventricular walls and hemodynamic impairment, as well as biomolecular and histologic findings approximating human HCM. The treatment group, by contrast, showed significantly less functional, structural, and biomolecular impairment.

The results signal promise for CRISPR-Cas therapeutics for HCM, with important caveats.[13] In the rat model, only homozygotes for the pathogenic variant develop HCM. By contrast, heterozygosity is sufficient for HCM in humans. This divergence limits the study's informativeness for potential human application, especially because the therapy's targeting does not discriminate between WT and pathogenic variant alleles. Therapeutic benefit for animal models with HCM provides evidence that benefits for human homozygotes could greatly outweigh therapeutic risks, but it does not address therapeutic risks for heterozygotes. Most critically, the inability to assess off-target editing effects in humans represents a meaningful translational gap, as

edits to an initially functional WT allele could introduce new pathogenic variants with consequences that remain uncharacterized in the human genomic context.

A third study targeted a single-nucleotide missense variant of *MYH7*, the gene encoding β-myosin heavy chain. Loss of normal *MYH7* function or the presence of this missense variant in human heterozygotes disrupts sarcomere structure and leads to progressive, severe HCM.[14] The authors developed and evaluated an adenine base editor therapy, incorporating catalytically inactive Cas9 as a targeting mechanism to restore the WT *MYH7*. Cas9-editing was also used to generate pathogenic variant human induced pluripotent stem cells and humanized mouse models for therapeutic evaluation. In vitro preliminary application of adenine base editor therapy to human induced pluripotent stem cells was accomplished with direct plasmid nucleofection, whereas in vivo application to humanized mice required a different delivery method, for which a dual AAV system was chosen. Advantages of this study include its use of humanized mice, in which the sequence targeted by the therapy was identical to, rather than orthologous to, the human variant of interest. Moreover, the novel murine model exhibited significant HCM in heterozygotes, as is the case in humans. The therapy showed partial reversal of the pathophenotype in heterozygotes, demonstrating promise for further model testing and translation into human medicine.

Use of Mouse Models and CRISPR-Cas9 in Heart Failure

Heart failure is described as a decrease in the heart's function and physiology, resulting in a reduced cardiac output to the body; animal models effectively allow us to better understand and treat this. The Ovid MEDLINE search for studies outlining heart failure with CRISPR-Cas9 and animal models resulted in 8 primary sources. Two were excluded for having an overlapping focus with another topic of this review, one was excluded for focusing on a specific disorder, one was excluded because of its gene similarity, and one was excluded for using additional gene editing techniques; this resulted in 3 primary sources. See summary in Table 3.

The first study used CRISPR-Cas9 in mice with T287A to delete the autophosphorylation of calcium ion/calmodulin-dependent protein kinase IIδ (CaMKIIδ).[15] This protein is important for the signal transduction and structure of cardiomyocytes, but its autophosphorylation leads to heart failure. In this study, severe transverse aortic constriction surgery was used as a heart failure model in 10-week-old mice. Two weeks after the severe transverse aortic constriction surgery, T287A mice showed increased survival and a higher percentage of fractional shortening, whereas WT mice showed a greater chance of heart failure, because survival and fractional shortening had decreased. The WT mice's left ventricular end-diastolic size, volume, and mass increased, all markers of heart failure, whereas these decreased significantly in T287A mice. This study also found more pronounced hypertrophy and

Table 3. CRISPR-Cas9 Gene Targets, Delivery Methods, and Functional Outcomes in Mouse Models of Heart Failure

Study	Gene Targeted	Gene Function	Animal Model	Method of CRISPR-Cas9 Delivery	Outcome	Results
Lebek et al, 2023	*CaMKIIδ* Autophosphorylation Deletion	Signal transduction and cardiomyocyte physiology	Mice with surgically induced heart failure	T2β87A sgRNA	Reduction of heart failure	T287A mice showed markedly improved survival (1/9 deaths vs 17/26 in wild-type mice), preserved fractional shortening, reduced ventricular dilation and mass, less cardiac hypertrophy, cardiac fibrosis, and cardiomyocyte apoptosis, and a favorable transcriptomic profile with upregulation of cardiac metabolic genes and downregulation of cardiac dysfunction genes.
Carroll et al, 2016	*Myh6* Inactivation	α-Myosin heavy chain promoter	Mice	AAV9 exon 3 and exon 8 sgRNA	Induction of heart failure	*Myh6*-inactivated mice developed cardiac hypertrophy, atrial and ventricular dilation, ventricular wall thinning, reduced fractional shortening, *Myh7* upregulation, elevated natriuretic peptide stress biomarkers, and abnormal cardiomyocyte structure.
Kaneko et al, 2016	*Pln* Inhibition	Inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase 2a and regulator of Ca^{2+} activity in cardiac cells	Mice with surgically induced heart failure	T7 promoter sgRNA	Reduction of heart failure	*Pln*-inactivated mice showed modestly improved survival (mean 55 vs 50 days, *P*=.04), improved diastolic and systolic function, preserved mean blood pressure and heart rate, and reduced pulmonary and atrial mass, in contrast to wild-type mice who showed cardiomyocyte dysfunction, elevated end-diastolic pressure, and increased cardiac chamber mass.

Abbreviation: Ca^{2+}, calcium ion.

cardiac dilation in the WT mice, with significant increases in heart and lung mass that were not present in the T287A mice. The WT mice showed increased cardiac fibrosis, not seen with the T287A mice, and the WT mice had an increased number of apoptotic cardiomyocytes. RNA analysis showed that the WT mice had upregulated genes for cardiac dysfunction in addition to downregulated genes for cardiac metabolism and performance, whereas the T287A mice had upregulated genes for cardiac metabolism and performance along with downregulated genes for cardiac dysfunction.[15] This mouse model demonstrates that CaMKIIδ overactivity leads to heart failure and cardiac disease, and addressing this is a potential treatment for heart failure in humans.

Another study used a unique modality of CRISPR-Cas9 to inactivate *Myh6*, the gene encoding α-myosin heavy chain promoter in cardiomyocytes.[16] An intraperitoneal injection, 10 days after birth, of adeno-associated virus 9 (AAV9) with sgRNA that targeted exon 3, rather than the typical zygote-mediated approach of CRISPR-Cas9, allowed for a postnatal study instead of an in utero study.[16] After 5 weeks, *Myh6*-inactivated mice showed signs of heart failure, with substantial cardiac hypertrophy, dilated atria, and dilated thin-walled ventricles. The edited mice had reduced fractional shortening and switched expression by upregulating *Myh6*, which is also seen in heart failure. Natriuretic peptides A and B and cardiomyocyte stress biomarkers were upregulated in *Myh6*-inactivated mice, in addition to their abnormally structured cardiomyocytes, highlighting cytoskeletal dysfunction within the heart. Researchers also used the intraperitoneal injection of AAV9 sgRNA to target exon 3 and exon 8 to determine the effects of this use of CRISPR-Cas9.[16] Similar outcomes were seen in the second generation of *Myh6*-inactivated mice: they had significantly reduced fractional shortening, natriuretic peptides A and B were upregulated, and they showed cardiac hypertrophy and dilation. *Myh6* is necessary for cardiomyocyte function; in addition, intraperitoneal injection of AAV9 sgRNA in CRISPR-Cas9 gene editing is effective in generating outcomes with this mouse model, successfully displaying possible future human treatment.[16]

The third source used CRISPR-Cas9 to inactivate *phospholamban (Pln)* in mice. *Phospholamban* is a gene encoding a protein that inhibits sarcoplasmic reticulum calcium ion-ATPase 2a and affects calcium ion activity in cardiac cells. Mice used in this study overexpressed calsequestrin, a sarcoplasmic reticulum calcium ion-binding protein whose overexpression saturates the calcium ion buffering capacity of the sarcoplasmic reticulum, producing pathological calcium ion dysregulation that closely mimics the cellular mechanism of human heart failure. This made the mice a well-validated model population in which to study the effects of *Pln* inactivation.[17] Inactivated *Pln* mice had increased survival rates compared with the WT mice. Inactivated *Pln* mice showed improved diastolic and systolic cardiac function, with no change in mean blood pressure or heart rate, whereas

the WT mice showed significant dysfunction of their cardiomyocytes, with lower blood pressure and increased left ventricular end-diastolic pressure. The WT mice showed an increase in pulmonary, atrial, and ventricular mass, whereas inactivated *Pln* mice only showed increased ventricular mass (hypothesized to be from physiological hypertrophy). The study by Kaneko et al[17] hypothesized that *Pln* inactivation improved blood supply and decreased arrhythmias, which are believed to be the cause of death of the WT mice. The mouse model in this study displayed that *Pln* inactivation reduces the characteristics of heart failure. These findings suggest that *Pln* represents a candidate therapeutic target in humans; if *Pln* inactivation similarly enhances sarcoplasmic reticulum calcium ion uptake and reduces pathological calcium ion dysregulation in human cardiomyocytes, it could improve both diastolic and systolic function and reduce the arrhythmic burden that drives mortality rates in heart failure patients. Clinical translation would require establishing delivery mechanisms capable of achieving sufficient *Pln* inactivation in human myocardium, as well as confirming that the survival and functional benefits observed in this calsequestrin-overexpression model extend to the heterogeneous etiologies of human heart failure.

The deletion of the autophosphorylation of CaMKIIδ in the study by Lebek et al[15] and the inactivation of *Pln* in the study by Kaneko et al[17] showed protection against heart failure markers and increased survival, increased cardiomyocyte function, and decreased atrial and pulmonary mass. CaMKIIδ autophosphorylation deletion demonstrated the broadest functional rescue, producing simultaneous reductions in ventricular mass, hypertrophy, fibrosis, and cardiomyocyte apoptosis alongside favorable transcriptomic shifts toward cardiac metabolic genes—a multi-domain benefit profile that makes it a particularly compelling therapeutic candidate. *Pln* inactivation similarly improved survival and biventricular function, though its benefits were concentrated in calcium ion handling and arrhythmia reduction. The most important finding of the study of *Myh6* by Carroll et al[16] is that intraperitoneal injection of the AAV9 sgRNA allows mouse genes to be altered after birth, avoiding embryonic lethality while also limiting off-target gene alterations, which has significant implications for future research into the treatment of heart failure. The targeting of the CaMKIIδ and *Pln* genes and the use of AAV9 sgRNA in CRISPR-Cas9 all provide promising avenues for the treatment of heart failure in humans.

Use of CRISPR-Cas9 in Animal Models for Atherosclerosis

Atherosclerosis is a major risk factor for developing CVD, characterized by atheroma plaques formed by endothelial injury, accompanied by lipid accumulation and inflammatory involvement in arteries.[18] Ultimately, these plaques can obstruct blood flow and result in severe cardiovascular sequelae, such as myocardial infarction. These studies focus on animal models in which

CRISPR-Cas9 was used to target genes involved in cholesterol metabolism and inflammation, with a further goal of advancing our understanding of atherosclerosis risk and identifying potential therapeutic targets.

A targeted Ovid MEDLINE search yielded 10 relevant studies from the following exploded search terms: "Models, Animal," "CRISPR-Cas Systems," "Atherosclerosis," and "Clustered Regularly Interspaced Short Palindromic Repeats." All studies were published between 2017 and 2021. The main focus was on primary research articles, and 2 of these studies were excluded from analysis owing to one being a research letter that lacked primary data and another published as a book chapter. A third study focused on induced pluripotent stem cells and human hepatocytes in addition to mouse models and, thus, was excluded based on misalignment with the remaining studies that focused solely on animal models. Of the remaining 7 studies, 6 used CRISPR-Cas9 to generate animal models with atherosclerosis. Only one study used CRISPR-Cas9 to reduce atherosclerotic development, highlighting a primary trend of using gene editing in animal models for atherosclerosis induction or modeling, rather than as a tool to mitigate it. The studies analyzed are summarized in Table 4.

Two prominent genes have been targeted in a single and double KO manner to create models of atherosclerosis in rabbits and rats. *Low-density lipoprotein (LDL) receptor (LDLR)* encodes a receptor that binds circulating LDLs containing cholesterol and promotes the uptake of cholesterol into peripheral tissues. *Apolipoprotein E (APOE)* is likewise significant for cholesterol transport and uptake. For example, one study[19] utilized two sgRNA guides to target exon 7 of the rabbit *Ldlr* gene, as both the rabbit and human versions of *LDLR* have 18 exons and 17 introns; rabbits also parallel humans in their lipid metabolism. Researchers analyzed specific markers of dyslipidemia (ie, plasma total cholesterol [TC], triglycerides, LDL cholesterol [LDL-c], and high-density lipoprotein cholesterol [HDL-c]), inflammation (ie, interleukin 1β), and atherosclerosis (ie, staining of aortic atherosclerotic lesions), and found that 2 of 7 rabbits presented with spontaneous, severe hypercholesterolemia and atherosclerosis, resembling disease progression in humans.[19] Similar outcomes are seen in an sgRNA guide-mediated deletion of *LDLR* in hamsters, leading to spontaneous atherosclerotic lesions and hyperlipidemia in those fed a high-fat diet. Ezetimibe was most effective in lowering TC levels in these models.[20] Hamsters are particularly advantageous in this context because their lipoprotein metabolism, including cholesterol absorption, transport, and the distribution of lipoproteins across HDL and LDL fractions, more closely mirrors that of humans than do standard mouse models, making hamsters especially informative for studies of diet-induced dyslipidemia and drug response. Ezetimibe, a cholesterol absorption inhibitor that acts at the intestinal brush border, was found to be the most effective agent in lowering TC levels in these hamster models, a finding that is notable because it validates the hamster as a model for testing lipid-lowering therapies

Table 4. CRISPR-Cas9 and Atherosclerosis

Study	Gene(s) Targeted	Gene(s) Function	Animal Model	Method of CRISPR-Cas9 Delivery	Outcome: Induction or Reduction of Disease
Lu et al, 2018	*LDLR* – KO; biallelic modifications	Cholesterol uptake from circulation (LDLs) into peripheral tissue	Rabbit	Two sgRNA sequences targeting exon 7 of rabbit *LDLR* – zygote microinjection	Induction
Guo et al, 2017	*LDLR*	Cholesterol uptake from circulation (LDLs) into peripheral tissue	Hamster	sgRNA sequence targeting *LDLR* – zygote microinjection	Induction
Yuan et al, 2019	*LDLR* (single KO), *APOE* – double KO	Cholesterol uptake from circulation (LDLs) into peripheral tissue	Rabbit	Four sgRNA sequences targeting rabbit *LDLR*; two sgRNA sequences targeting rabbit *APOE* – zygote microinjection	Induction
Zhao et al, 2018	*LDLR, APOE* – single/double KO	Cholesterol uptake from circulation (LDLs) into peripheral tissue	Rat	Two sgRNA sequences targeting exon 4 of rat *LDLR*; two sgRNA sequences targeting exon 4 of rat *APOE* – zygote microinjection	Induction
Jarrett et al, 2019	*LDLR* – somatic deletion	Cholesterol uptake from circulation (LDLs) into peripheral tissue	Adult mouse	AAV-CRISPR – intraperitoneal injection	Induction
Wang et al, 2019	*LDLR, APOE* – single/double KO	Cholesterol uptake from circulation (LDLs) into peripheral tissue	NOD mouse	Three sgRNA sequences targeting each gene: *LDLR*, *APOE* – zygote microinjection	Induction
Murtazina et al, 2021	*TAAR9* – KO	Found in olfactory neurons, with associations to various disease conditions	Rat	sgRNA sequence targeting rat *TAAR9* – zygote microinjection	Reduction

Abbreviations: KO, knockout; LDL, low-density lipoprotein.

and suggests that intestinal cholesterol absorption plays a prominent role in this model's hypercholesterolemia. Another study similarly utilized multiple sgRNAs to target rabbit *Ldlr* and *Apoe*, comparing the development of aortic and coronary atherosclerosis in *Ldlr* single-KO rabbits to *Ldlr/Apoe* double-KO rabbits.[21] With primary outcomes paralleling those from the study by Lu et al,[19] both types of rabbits had higher plasma lipids and

lipoproteins compared with WT rabbits, with *Ldlr* single-KO rabbits having more pronounced hypercholesterolemia and aortic atherosclerotic lesions than double-KO rabbits.

Rat models of *Ldlr* and *Apoe* have also been generated through CRISPR-Cas9. In one study investigating *Apoe*/*Ldlr* single-KO rats and double-KO rats, all three mutants demonstrated accelerated atherosclerotic development as well as dyslipidemia in response to a Western diet compared with WT rats.[22] In particular, *LDLR* KO rats that were fed a Western diet had markedly elevated plasma triglyceride levels, but also had significantly increased HDL-c levels, where all mutants had elevated TC and LDL-c levels. The atherosclerotic acceleration was driven by upregulation of inflammatory genes, including *vascular cell adhesion molecule-1 (VCAM-1)* in all three mutants and *toll-like receptor 4 (TLR4)* in the single-KO rats.[22] These inflammatory mediators represent candidate targets for future CRISPR-Cas9–based interventional studies, as their causal roles in atherosclerotic progression, identified herein, suggest that their suppression could attenuate disease development.

In the studies[19-22] of *Ldlr* in animal models, sgRNAs were used to initiate KO mutations in zygotes, leading to loss of *Ldlr* from birth (ie, a germline mutation). Furthermore, these studies speculate that the mechanism of hypercholesterolemia involves a mutant Ldlr protein having a higher affinity for binding to proprotein convertase subtilisin/kexin type 9 (PCSK9) protein, leading to increased degradation of LDL receptors and, thus, increased circulating cholesterol levels from impaired uptake of cholesterol.[19] Of note, however, another study used an AAV approach to CRISPR-Cas9 to target the mouse *Ldlr* gene.[23] To determine the most effective atherosclerosis model, researchers compared plasma lipid levels and atherosclerotic burden, through assessing aortae for plaque formation, in AAV-targeted *Ldlr* mice with sgRNA-generated germline *Ldlr* KO mice as well as AAV-generated gain-of-function PCSK9 mice. Results indicate that an AAV-CRISPR approach to *Ldlr* KO is comparable to inducing a germline *Ldlr* mutation and more effective than inducing overexpression of PCSK9 in causing more severe hypercholesterolemia and atherosclerosis.[23]

The aforementioned studies[19-23] highlight *Ldlr* and *Apoe* as primary genes targeted to induce atherosclerosis in otherwise healthy animal models. In addition to optimizing CRISPR-Cas9 technology to effectively create models of CVD, there is growing interest in studying atherosclerosis in animal models with a background of metabolic disorders. Another study[24] investigates diabetes as a potential mediator, using nonobese diabetic mice to induce single-KO *Ldlr* or *Apoe*. Results of this study indicate that while a double KO of *Ldlr* and *Apoe* in nonobese diabetic mice leads to severe atherosclerosis, it is accompanied by a form of resistance, as these mice also release significant levels of anti-atherosclerotic CXCL13 and IL-1Ra

cytokines in their inflammatory response to the hyperlipidemia.[24] Among the animal models reviewed, the *Ldlr* KO rabbit models most closely approximate human atherosclerosis pathophysiology, given rabbits' LDL-rich lipoprotein profiles, cholesteryl ester transfer protein (CETP) activity, and ApoB messenger RNA editing patterns that parallel human lipid metabolism more closely than rodent models, as well as the spontaneous development of severe hypercholesterolemia and aortic lesions in *Ldlr* KO rabbits on a normal chow diet, which is a feature not seen in equivalent mouse KOs.[19] Interestingly, one study was salient in reducing atherosclerotic development, rather than inducing it, as seen in the rest of the studies. Researchers used CRISPR-Cas9, specifically an sgRNA, to target *Taar9*, a gene encoding an olfactory receptor, to better understand its role in lipid and cholesterol homeostasis.[25] *Taar9* KO rats exhibited lower levels of plasma TC and LDL-c compared with WT rats; while they did not study atherosclerosis-specific outcomes, namely lesion or plaque development, the TC-to-HDL-c ratio was measured as an atherogenic index and was higher in one *Taar9* KO rat strain compared with WT.[25]

Collectively, these studies highlight the use of CRISPR-Cas9 to manipulate atherosclerosis-associated genes, resulting in animal models that closely mirror the pathophysiology of human atherosclerosis and related dyslipidemias. Further research is necessary to identify and understand genes that can improve disease outcomes.

CONCLUSION

This article reviewed data from studies of CRISPR-Cas9 gene editing tools used in animal models of CVD, focusing on 4 significant contributors to and sequelae of CVD: CHDs, hypertrophic cardiomyopathy, heart failure, and atherosclerosis. In each analysis, CRISPR-Cas9 was used to induce or mitigate the effects of the condition in specific genes and animal models. The gene manipulations and animal models reviewed were found to be effective reproductions of human disease processes.

CRISPR-Cas9 editing in CHD studies focused on inducing defects in mice and zebrafish to study development, clinical presentation, and natural history. These studies identified genes involved in CHD and evidence that CHD may result from the interplay of several mutations or as part of a genetic syndrome. Of the 3 studies reviewed on HCM, one created a mouse model of human IOPD with early-onset HCM, while others produced novel (rat and mouse) models en route to evaluating novel CRISPR-Cas9–based therapies. Tailoring delivery methods to context was a prominent theme: nucleofection and pronuclear injection sufficed in creating organismal models; therapeutic in vivo delivery relied on AAV vectors.

The studies of heart failure pertained to CRISPR-Cas9 and mouse models. The deletion of the autophosphorylation of *CaMKIIδ* (Lebek et al[15]) decreased the prevalence of heart failure, which was also seen in the inactivation of *Pln*[17]; both represent proposed therapeutic targets for heart failure. By contrast, inactivation of Myh6 in the study by Carroll et al[16] worsened heart failure markers, suggesting that preserved Myh6 expression may be important to normal cardiac function, though this does not by itself establish it as a direct treatment target. This study also demonstrated the utility of intraperitoneal injection of AAV9 sgRNA in postnatal CRISPR-Cas9 gene editing, highlighting its potential as a delivery strategy for future heart failure research.

Regarding CRISPR-Cas9 use in affecting atherosclerotic development, studies generated rabbit, rat, mouse, and hamster models of *LDLR* and *APOE* to simulate atherosclerotic development. Primary outcomes, including plasma lipid and cholesterol levels, as well as the extent of atherosclerotic lesions, were measured to ensure effective creation. Most studies utilized sgRNAs to initiate germline mutations, while one focused on a newer application of AAV vectors to create somatic mutations. One study demonstrated promise for CRISPR-Cas9 as a therapeutic tool in reducing atherosclerotic outcomes: Murtazina et al[25] showed that CRISPR-Cas9–mediated KO of *Taar9* in rats produced lower plasma TC and LDL-c levels compared with WT rats, suggesting that targeting this gene may attenuate atherogenic lipid profiles. In terms of future directions, as explored in a study[24] of atherosclerosis in mouse models with a background of diabetes, an established link exists between metabolic risk factors and CVD and is ripe for further investigation.

Evaluation of these studies supports the utility of CRISPR-Cas9 tools for the etiologic and pathophysiologic investigation of CVD through animal models, where applications of these tools create models genetically suitable for evaluating gene editing therapies and facilitate innovation in the therapies themselves. It seems reasonable to expect important therapeutic advances for CVD through gene editing tools as their delivery vehicles continue to improve.

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