

# Gene Therapy to Treat Cardiovascular Disease

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Coronary artery disease, heart failure, and cardiac arrhythmias are major causes of morbidity and mortality in the United States. The overall death rate for all cardiovascular diseases was 236.1 per 100 000 persons, accounting for 1 of every 3 deaths in America.<sup>1</sup> Cardiac diseases can affect every age group, but prevalence is seen in patients 65 or older. For instance, heart failure incidence approaches 1 in 100 older Americans.<sup>2</sup> As of 2009, cardiac arrest accounted for  $\geq 14\%$  of all deaths in the United States.<sup>1</sup> Pharmacologic drugs and device therapies have multiple limitations, and there exists an unmet need for improved clinical outcomes without side effects. Interventional procedures including angioplasty and ablation have improved the prognosis for patients with ischemia and arrhythmias, respectively. However, large subgroups of patients are still left with significant morbidity despite those therapies. This limitation in currently available therapies has prompted extensive investigation into new treatment modalities. Sequencing information from the human genome and the development of gene transfer vectors and delivery systems have given researchers the tools to target specific genes and pathways that play a role in cardiovascular diseases. Early-stage clinical studies have demonstrated promising signs of efficacy in some trials, with few side effects in all trials. Preclinical studies suggest that myocardial gene transfer can improve angiogenesis with vascular endothelial growth factor (VEGF) or fibroblast growth factor (FGF), increase myocardial contractility and reduced arrhythmia vulnerability with sarcoplasmic reticulum  $\text{Ca}^{2+}$  adenosine triphosphatase, induce cardiac repair with stromal-derived factor-1 (SDF-1), control heart rate in atrial fibrillation with an inhibitory G protein  $\alpha$  subunit, and reduce atrial fibrillation and ventricular tachycardia vulnerability with

connexins 40 and 43, the skeletal muscle sodium channel SCN4a, or a dominant-negative mutation of the rapid component of the delayed rectifier potassium channel, KCNH2-G628S.<sup>3–11</sup>

The field of myocardial genetic manipulation is vast because of complex and multifaceted disease mechanisms. Many different gene products can be targeted to ameliorate clinical phenotypes, and there exist several delivery vectors for use in the clinic. This review focuses on 3 main cardiac diseases that are currently being evaluated for therapeutic benefit of gene therapy: coronary artery disease, heart failure, and arrhythmias. Status of preclinical or clinical trial progress will be noted as well as a description of the genes being manipulated.

## Gene Vectors and Delivery

Gene therapy describes the transfer of genes to a target cell or organ to treat or prevent disease. Successful delivery of a gene to the target is paramount to therapeutic efficacy. In some cases, this can be as simple as transduction of a few cells to secrete a hormone or growth factor; in more stringent cases, the requirement may be as extensive as transduction of most or all cells in the target organ. A number of gene delivery methods have been developed using both viral- and non-viral-based vectors. Nonviral methods include using naked DNA alone or complexed with liposomes.<sup>12,13</sup> Naked DNA vectors are simple closed circular DNA plasmids that at a minimum contain a promoter driving a gene of interest and a polyadenylation site.<sup>14</sup> Naked DNA vectors are inefficient, as only a small percentage of target cells express reporter genes after transfection.<sup>13</sup> They are easy to produce and are used extensively for applications that do not require high-density gene transfer. Importantly, the gene of interest does not need to encode a specific protein to be of therapeutic value. Mechanisms that use antisense oligonucleotides or short interfering RNA, which can reduce or eliminate protein function, have been uncovered. In the case of short interfering RNA, the promoter drives production of the effector RNA sequence rather than production of a messenger RNA that is then translated into an effector protein.

Viral vectors are more commonly used for cardiovascular applications because they transfer genes to cardiac myocytes

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*J Am Heart Assoc.* 2013;2:e000119 doi: 10.1161/JAHA.113.000119.

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much more efficiently than any of the nonviral methods. Commonly used viral vectors include retroviruses (including the lentivirus family of which the human immunodeficiency viruses are members), adenoviruses (ADs) and adeno-associated viruses (AAVs). Retroviruses have been used for a number of noncardiac applications, but they do not efficiently transduce cardiomyocytes because they require active cell division for integration and function. Lentiviruses do not require active cell division, so they have been used extensively for cardiac applications. A limitation of lentiviral vectors has been the inability to generate sufficient concentrations of virus for delivery by coronary perfusion. Successful examples of lentiviral gene transfer to the heart predominately use intramyocardial injection.

ADs and AAVs have the advantage of infecting nondividing cells. Both have been shown to transduce the heart with reasonable efficiency. A considerable limitation of AD vectors is that they trigger immune responses that ultimately limit AD-mediated gene expression to a period lasting days to weeks after gene transfer. AAVs have a much more limited immune response, allowing AAV-mediated gene expression to last much longer than that of ADs.<sup>15</sup> Some preclinical studies have shown persistent AAV-mediated gene expression years after vector delivery, and a hemophilia clinical trial docu-

mented persistent expression in skeletal muscle 1 year after AAV injection.<sup>16</sup> Disadvantages of AAVs include limited size of the gene insert (4.5 to 5 kb total) and the requirement of a helper virus or other complex methods for amplification.<sup>17</sup>

One of the limitations for widespread use of gene therapy is efficient delivery of the gene transfer vector to the target. Methods of reported cardiovascular gene delivery include intramyocardial injection, coronary perfusion, and pericardial delivery.<sup>3,4,18–23</sup> All these techniques are moderately successful, but each is hindered by efficacy, tolerability, or access. See Table 1 for a list of positive and negative points regarding each delivery system.

Several trials reviewed here made use of the myocardial injection method. In this simple method, virus is injected from a syringe through a needle embedded in the myocardium. The local area around the injection site has a high density of gene transfer, but even 5 to 10 mm from the needle, gene expression is negligible.<sup>28</sup> With this limitation, a single injection can transduce the majority of a mouse left ventricle, but multiple injection sites are required for adequate coverage in larger mammals. In addition, studies have reported acute inflammation at the injection site because of tissue disruption, which needs to be kept in mind when considering this type of gene delivery.<sup>29</sup>

**Table 1.** Delivery Techniques for Gene Therapy

Gene Delivery Technique	Pro	Con
Myocardial injection <sup>24</sup>	<ul style="list-style-type: none"> <li>● High density of gene transfer limited to cardiac muscle</li> <li>● Simple and safe, able to inject percutaneously</li> </ul>	<ul style="list-style-type: none"> <li>● Small area of gene expression</li> <li>● Multiple injection sites needed</li> <li>● Acute inflammatory response and potential scarring from the injection</li> </ul>
Coronary perfusion-antegrade and retrograde perfusion	<ul style="list-style-type: none"> <li>● Delivers genes globally across the myocardium</li> <li>● Distributes gene vector more homogeneously</li> <li>● Cardiac specific</li> </ul>	<ul style="list-style-type: none"> <li>● Inefficient</li> <li>● Delivered into systemic circulation</li> </ul>
Aortic cross-clamp LV cavity infusion <sup>4</sup>	<ul style="list-style-type: none"> <li>● Increased gene transfer efficiency</li> </ul>	<ul style="list-style-type: none"> <li>● Accessibility, need open chest</li> <li>● High risk of myocardial injury</li> <li>● Not cardiac specific</li> </ul>
Cardiopulmonary bypass perfusion and “closed-loop” system <sup>25</sup>	<ul style="list-style-type: none"> <li>● Specifically enhances coronary perfusion by separating it from systemic circulation</li> <li>● Allows for temperature control of solution, cold increased transfection efficiency</li> <li>● Increased contact time with gene vector through multiple-pass cardiac recirculation</li> </ul>	<ul style="list-style-type: none"> <li>● Significant morbidity risk from cardiopulmonary bypass procedure (CBP)</li> <li>● Intended for patients undergoing CBP</li> </ul>
Epicardial painting <sup>23</sup>	<ul style="list-style-type: none"> <li>● Cardiac specific</li> <li>● Complete transmural penetration (atrial)</li> <li>● High degree of gene transfer</li> </ul>	<ul style="list-style-type: none"> <li>● Accessibility, currently needs open chest</li> </ul>
Ultrasound and microbubbles <sup>26</sup>	<ul style="list-style-type: none"> <li>● Increased permeability of capillary and cell membrane</li> </ul>	<ul style="list-style-type: none"> <li>● Only slightly improved gene transfer efficiency from myocardial injection alone</li> </ul>
Electroporation <sup>27</sup>	<ul style="list-style-type: none"> <li>● Enhanced transfer of naked DNA via myocardial injection and retrograde perfusion</li> <li>● Cardiac specific</li> </ul>	<ul style="list-style-type: none"> <li>● Pulse needs to be delivered in sync or ventricular fibrillation occurs</li> </ul>

LV indicates left ventricle.

Intracoronary perfusion of gene transfer vectors has been reported by percutaneous catheterization, open-chest aortic cross-clamp with left ventricular cavity infusion, and cardiopulmonary bypass with direct coronary arterial perfusion. Gene transfer to the myocardium is considerably less efficient by intracoronary infusion than by intramyocardial injection. The principle limitation is escape from the vasculature to access cardiac myocytes.<sup>30,31</sup> Preclinical studies have shown that treatment with vasodilatory and vascular permeability enhancing agents increases gene transfer. A number of agents have been used: nitroglycerin, nitroprusside, serotonin, bradykinin, histamine, VEGF, and reduced perfusate calcium concentration, among others.<sup>31–34</sup> The best cocktail of permeability and vasodilatory agents to increase uptake of the gene therapy vector is currently unknown. An advantage of intracoronary perfusion is the ability to deliver more globally across the myocardium than is achievable with intramyocardial injection. Gene transfer occurs across the entire perfusion territory of the targeted coronary vessel.

A promising delivery technique for cardiac atrial gene therapy is epicardial painting, in which the viral vector is literally painted on to the epicardial surface suspended in a gelatinous polymer. Preclinical data show complete transmural transmission of reporter genes.<sup>23</sup> The down side to this method is that it currently requires an open chest for access to the epicardium. Additional delivery methods are being developed and tested that will eventually allow this delivery strategy to be completely accessible.

Gene therapy is not without risks. Problems that are currently present, but solvable, include inadequate delivery and expression (discussed above), immune response to the viral vector or transgene, off-target effects, insertional mutagenesis, and limited-duration gene expression. Many of these problems can be solved with improved gene transfer vectors. Some of these problems are inherent to either the transgene or delivery method.

A major limitation to gene therapy is the immune response. Particularly with ADs, severe inflammation leading to toxicity and even, in very rare cases, organ failure has been reported.<sup>35</sup> The proteins on the AD viral capsid have undergone extensive study, and mutations and deletions have improved immune response to ADs.<sup>36,37</sup> In the case of AAVs, humans have endogenous antibodies to AAVs that limit gene transfer.<sup>38</sup> Clinical trials using AAVs have prescreening criteria that disallow patients with AAV antibodies into the study. In preclinical work, investigators were able to improve efficacy of AAV gene transfer by purging the liver of venous blood with saline to reduce contact of AAVs with the antibodies.<sup>38,39</sup>

Nontarget side effects are a concern when the viral vector is perfused into circulation and the gene ends up in a different organ than intended. Even in the target organ, uncontrolled

expression of the transgene may be toxic. Ideas to fine-tune gene expression include the use of situation- or tissue-specific promoters that would only express under certain circumstances (eg, expression during ischemia) or in certain tissues (eg, cardiac-specific promoters).

The possibility of causing a tumor from the viral vector surfaced as a concern for gene therapy with the finding of leukemias after retroviral gene therapy in an immunodeficiency clinical trial. This possibility is likely unique to viruses that insert their DNA in the host genome, with the risk that the DNA could be inserted into a wrong place and become oncogenic.

## Gene Therapy for the Treatment of Coronary Artery Disease

The prevalence of coronary artery disease is on the rise and in 2009 accounted for 1 of every 6 deaths in the United States.<sup>1</sup> Long-term survival of patients with coronary disease has increased because of advances in pharmacologics and revascularization techniques. However, a group of patients who are refractory to conventional therapy has emerged. These patients suffer from severe angina pectoris despite maximal medical therapy and are no longer treatable with percutaneous coronary intervention or coronary artery bypass graft surgery. An alternative treatment option is being developed, termed therapeutic angiogenesis. The therapy involves administration of genes for angiogenic growth factors to augment collateral vessel development.<sup>40</sup> Preclinical successes led to the hope that this strategy would provide a solution to the ongoing clinical problem. A series of lackluster clinical trials has dimmed this hope. A widespread conclusion of these investigations is that angiogenesis is a complex mechanism requiring precise timing of multiple growth factors acting on receptors to stimulate and then sustain new vessel growth.

Preclinical angiogenesis studies investigated a variety of angiogenic growth factors including VEGF, FGF, hepatocyte growth factor, platelet-derived growth factor, and hypoxia-inducible factor, among others.<sup>41–45</sup> Clinical trials have focused predominately on VEGF and FGF. Preclinical work showed improved myocardial function and perfusion by increasing angiogenesis after administration of the gene for either VEGF or FGF to ischemic tissue.<sup>5</sup> See Table 2 for a summary.

VEGF is a mitogen for vascular endothelial cells stimulating migration and proliferation. It has also shown vascular permeability and cytoprotective effects.<sup>5</sup> Early clinical work showed bright promise for therapeutic effect with VEGF gene therapy. Multiple phase 1 trials showed that VEGF treatment alone or combined with CABG reduced symptoms and improved myocardial perfusion.<sup>50–52</sup> Randomized, controlled trials were then initiated to look at long-term safety and

**Table 2.** Gene Therapy Targets for Coronary Heart Disease

Molecular Target	Stage in Development	Findings	Model Assessed	Reference
Vascular endothelial growth factor (VEGF)	Clinical trials, phase 2/3 Continued safety and efficacy	Safe but not consistently efficacious with increasing myocardial perfusion. Success with secondary end points, ie, increased exercise capacity and reduction in ischemic area	Human	Hedman et al, <i>Gene Ther</i> , 2009 <sup>46</sup> Stewart et al, <i>Mol Ther</i> , 2009 <sup>47</sup>
Fibroblast growth factor (FGF)	Clinical trials, phase 2/3 Continued safety and efficacy	Safe but most trials have not increased myocardial perfusion. Some have improved exercise capacity and symptom alleviation	Human	Kukula et al, <i>Am Heart J</i> , 2011 <sup>48</sup>
Hepatocyte growth factor (HGF)	Clinical trial, phase 1 Preclinical	Safe with negligible side effects from ADs; HGF in serum not detected after 35 days Increased capillary density and end-diastolic volume Improved cardiac perfusion and reduced apoptosis	Human Rat Pig	Yang et al, <i>Mol Biol Rep</i> , 2009 <sup>49</sup> Jin et al, <i>Gene Ther</i> , 2012 <sup>41</sup> Yang et al, <i>Mol Biol Rep</i> , 2010 <sup>42</sup>
Platelet-derived growth factor (PDGF)	Preclinical	Increased capillary growth and collateral formation from single naked DNA injection	Rabbit	Li et al, <i>Microvasc Res</i> , 2010 <sup>43</sup>
Hypoxia-inducible factor (HIF1 $\alpha$ )	Clinical trial, phase 1 Preclinical	Preliminary safety of ADs after 1 year Increased myocardial perfusion and improved LV function but no improvement in bioactivity end points	Human Pig	Kilian et al, <i>Circ J</i> , 2010 <sup>44</sup> Heinl-Green et al, <i>Eur Heart J</i> , 2005 <sup>45</sup>

ADs indicates adenoviruses; LV, left ventricle.

efficacy. In general, these trials showed safety, with negligible side effects attributable to the gene therapy, but no consistent signal of efficacy. Most trials did not show any significant difference in the primary end point of myocardial perfusion and symptom alleviation. Some suggestion of an effect from secondary end points was seen, such as increased exercise capacity and reduction in ischemic area. A competing issue within these trials was a strong placebo effect. When subgroups were analyzed or VEGF-treated patients compared with themselves at follow-up, improvements were noticed, but when the active treatment arm was compared with the placebo arm in these trials, no significant differences were generally found.<sup>46,47</sup> FGF is a heparin-binding protein family important for angiogenesis, wound healing, and embryonic growth and development (reviewed in Chen and Forough<sup>53</sup>). Work on FGF has shown similar discrepancies, reaching significance in the primary outcome of increased angiogenesis and myocardial perfusion.<sup>48</sup>

Major questions still remain about the area of therapeutic angiogenesis. It is not clear if the trials failed because of a lack of efficacy of the transgene (some have suggested that multiple angiogenic factors might be the solution) or of a problem of inadequate gene delivery or inappropriate target selection. An additional concern raised by Lee et al<sup>54</sup> and Liu et al<sup>55</sup> is the possibility of a narrow therapeutic window, with overdose of angiogenic factors causing hemangiomas and

arteriosclerosis. All these issues need to be sorted out before angiogenesis gene therapy becomes viable.

## Gene Therapy for the Treatment of Heart Failure

Heart failure (HF) is a leading cause of morbidity and mortality in the United States; in 1 in 9 deaths in 2009 HF was mentioned on the death certificate, and currently 5.1 million people are suffering from HF.<sup>1</sup> Estimated direct and indirect costs to the US healthcare system as of 2009 were \$37.2 billion.<sup>56</sup> Device-based treatments and pharmacotherapies have improved patient survival, but HF eventually still leads to degeneration and death. The 5-year survival for individuals with HF is about 50%, and in end stage the HF 1-year survival is as low as 22% regardless of therapy.<sup>57</sup>

Ongoing or completed clinical trials for heart failure gene therapy have included those testing the sarcoendoplasmic reticulum calcium-ATPase 2a (SERCA2a), SDF-1, and adenylate cyclase-6 (AC6). Strategies to judge efficacy noninvasively include measuring ejection fraction on echocardiograms, measuring HF symptoms—6-minute walk test, New York Heart Association class, and quality-of-life questionnaire. In addition to these targets, numerous other transgenes have reported efficacy for heart failure in various mouse or other small mammalian models. Preclinical studies with multiple literature

reports or with efficacy data in large mammalian models include the S100 calcium-binding protein A1 (S100A1), a c-terminal fragment of the  $\beta$ -adrenergic receptor kinase ( $\beta$ ARKct), and parvalbumin (PVALB). See Table 3 for a summary.

One of the key proteins defective in HF is SERCA2a. Numerous studies have shown that SERCA2a expression and function are decreased in heart failure and that this decrease plays a role in the reduced calcium transient that is characteristic of systolic heart failure. The Calcium Upregulation by Percutaneous administration of gene therapy In cardiac Disease (CUPID) trial looked at the safety and efficacy of SERCA2a gene therapy in HF.<sup>58</sup> In the CUPID phase 2 trial, 39 patients with advanced HF as defined by New York Heart Association class III/IV, ejection fraction  $\leq 30\%$ , and maximal oxygen uptake  $< 16$  mL/kg per minute received intracoronary infusion of either recombinant AAV-1 encoding SERCA2a or placebo. Three doses of AAV-SERCA2a were assessed. At the end of 12 months, patients in the high-dose SERCA2a group showed a decrease in HF symptoms, increased functional status, and reversal of the negative LV remodeling.

A phase 1 clinical trial was just completed showing that SDF-1 gene therapy improved HF symptoms in patients with ischemic cardiomyopathy.<sup>59</sup> The proposed mechanism is that SDF-1 activates endogenous stem cells via the SDF-1: chemokine receptor type 4 pathway. The study took 17 patients with ischemic cardiomyopathy and treated them with SDF-1 DNA plasmid injected endomyocardially. At the 4- and 12-month follow-up, patients exhibited improvement in the

6-minute walk test, quality of life, and New York Heart Association class.

The  $\beta$ -adrenergic ( $\beta$ -AR) system has proven to be a source of multiple targets and mechanisms in the effort to prevent HF and maintain normal cardiac function. Alterations in  $\beta$ -AR signaling is a hallmark of HF, as lower myocardial  $\beta$ -AR density and decreased responsiveness to  $\beta$ -agonists have been found in failing cardiomyocytes.<sup>70</sup> Work in animal models has shown inhibition of  $\beta$ -AR receptor desensitization and increased adenylyl cyclase (AC) activity can improve HF. Overexpression of AC6 led to increased LV function and increased cAMP levels during  $\beta$ -AR stimulation.<sup>71</sup> Data demonstrated that AC6 was the rate-limiting step in  $\beta$ -AR generation of cAMP. In the setting of myocardial expression of G $\alpha_q$ , intracoronary delivery of AC6 in an adenoviral vector improved cardiac function after 2 weeks of gene delivery.<sup>60</sup> Additional studies using this model of AC6 overexpression led to improved LV function, reversal of dysfunctional  $\beta$ -AR signaling, and increased survival.<sup>61,62</sup> In the setting of myocardial infarction, overexpression of AC6 in mice reduced mortality compared with controls.<sup>63</sup> Large animal models have also been investigated. Intracoronary delivery of Ad-AC6 in pacing-induced HF in pigs showed improvements in LV contractility compared with controls, showing promise in multiple preclinical models for AC6 as a gene therapy strategy for HF.<sup>64,72</sup>

Limiting  $\beta$ -AR desensitization through inhibition of GRK2 is another strategy being investigated. GRK2, a ubiquitously

**Table 3.** Gene Therapy Targets for Heart Failure

Molecular Target	Stage in Development	Findings	Model Assessed	Reference
Sarcoendoplasmic Reticulum calcium-ATPase 2a (SERCA2a)	Clinical trials, phase 2	Decreased HF symptoms, increased functional status, and reversal of negative LV remodeling	Human	Jessup et al, <i>Circulation</i> , 2011 <sup>58</sup>
Stromal-derived factor-1 (SDF-1)	Clinical trials, phase 1/2	Safe and improved 6-minute walk test, quality of life, and NYHA class	Human	Penn et al, <i>Circ Res</i> , 2013 <sup>59</sup>
Adenylyl cyclase 6 (ADCY6)	Preclinical	Increased LV function, increased cAMP levels, reversal of dysfunctional $\beta$ -AR signaling, and increased survival Improved LV contractility	Mice Pig	Rebollo et al, <i>Hum Gene Ther</i> , 2006 <sup>60</sup> Roth et al, <i>Circulation</i> , 1999, 2002 <sup>61,62</sup> Takahashi et al, <i>Circulation</i> , 2006 <sup>63</sup> Lai et al, <i>Circulation</i> , 2000 <sup>64</sup>
$\beta$ ARKct-carboxy terminal peptide from GRK2	Preclinical	Heart failure rescue Improved $\beta$ -AR signaling and contractile dysfunction	Rabbit Human cardiomyocytes	Shah et al, <i>Circulation</i> , 2001 <sup>65</sup> Williams et al, <i>Circulation</i> , 2004 <sup>66</sup>
S100A1	Preclinical	Increased reuptake SR Ca <sup>2+</sup> , lowered Ca <sup>2+</sup> leak, enhanced cardiac function, and reversed LV remodeling	Rat cardiomyocytes	Most et al, <i>J Clin Invest</i> , 2004 <sup>67</sup> Pleger et al, <i>Circulation</i> , 2007 <sup>68</sup>
Parvalbumin (PVALB)	Preclinical	Increased rate of Ca <sup>2+</sup> removal and improved relaxation rate	Rat	Szatkowski et al, <i>J Clin Invest</i> , 2001 <sup>69</sup>

HF indicates heart failure; LV, left ventricle; NYHA, New York Heart Association;  $\beta$ ARKct,  $\beta$ -adrenergic receptor kinase;  $\beta$ -AR,  $\beta$ -adrenergic; SR, sarcoplasmic reticulum.



expressed cytosolic protein, is shuttled to the plasma membrane when stimulated by G-protein-coupled receptors. It binds and dissociates membrane-embedded  $\beta\gamma$ -subunits of heterotrimeric G proteins, allowing for phosphorylation of agonist-occupied receptors.<sup>73</sup> GRK2 is upregulated in the failing heart, and inhibition of this protein using a peptide from the carboxy terminal end of GRK2, termed  $\beta$ ARKct, has displayed enhanced in vivo cardiac function and reversal of blunted  $\beta$ -AR responsiveness for both AC activation and LV contractility.<sup>74–76</sup> Multiple animal studies have been performed using  $\beta$ ARKct including HF rescue in post-myocardial infarction rabbits and improved  $\beta$ -AR signaling and contractile dysfunction in failing human cardiomyocytes.<sup>65,66</sup> Additive effects have been seen when adding a  $\beta$ -AR antagonist concomitantly with  $\beta$ ARKct gene therapy.<sup>77</sup>

Multiple other molecular targets that reduce or reverse cardiac deterioration in HF are being investigated. Several of these molecules are related to calcium handling, similar to SERCA2a. For instance, S100A1, an EF-hand  $\text{Ca}^{2+}$ -binding protein, is decreased in HF.<sup>67</sup> S100A1 through its regulator targets, the ryanodine receptor and SERCA2a, increases isometric contraction followed by an increase in  $\text{Ca}^{2+}$  pumped into the sarcoplasmic reticulum.<sup>68,78</sup> Gene transfer of S100A1 to failing rat cardiomyocytes increased reuptake of sarcoplasmic reticulum  $\text{Ca}^{2+}$  during the relaxation phase, lowered ryanodine receptor-mediated  $\text{Ca}^{2+}$  leak, enhanced cardiac function, and reversed LV remodeling.<sup>68,79</sup> These actions of S100A1 are independent of the  $\beta$ -AR system because there are no changes in PKA or cAMP activity.<sup>78</sup> An added benefit is that overexpression of S100A1 in the presence of  $\beta$ -AR stimulation results in maximal contractile performance.<sup>80</sup>

Another calcium-binding molecule being targeted to improve HF is PVALB, which is an EF-hand  $\text{Ca}^{2+}$  sequestering protein that can bind 2  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  ions per molecule.<sup>81</sup> In contrast to SERCA2a, PVALB expression in cardiomyocytes causes energy-independent removal of cytosolic  $\text{Ca}^{2+}$  and potentially can correct the prolonged diastolic  $\text{Ca}^{2+}$  decay in HF without further energy deprivation.<sup>82</sup> Proof-of-concept work showed PVALB expression increased the rate of  $\text{Ca}^{2+}$  removal and improved the rate of relaxation in cardiomyocytes.<sup>69</sup> PVALB is not without its complications. Expression of PVALB is a concern because at higher concentrations, sarcomere shortening is depressed and the force of contraction is compromised.<sup>83</sup> With continuing research into HF, more molecules and mechanisms are being uncovered, leading to additional targets for modification with gene therapy.

## Gene Therapy for the Treatment of Arrhythmias

Cardiac arrhythmias constitute an extensive burden on the American healthcare system, accounting for \$26 billion in

total direct costs.<sup>84</sup> Ventricular tachycardia (VT) and/or ventricular fibrillation (VF) were detected in 43% of sudden death cases analyzed, but the true prevalence and incidence are not known.<sup>57</sup> The prevalence of atrial fibrillation (AF) in the United States was as high as 6.1 million in 2010, and the prevalence of AF is expected to rise to 12 million by 2050.<sup>57,85,86</sup> The major burden of arrhythmias is amplified by the lack of an effective cure for more common arrhythmias, which cause extensive morbidity and mortality. No arrhythmia gene therapy has entered human clinical trials as yet. Preclinical work is under way demonstrating the efficacy and feasibility of gene therapy to treat these arrhythmias. See Table 4 for a summary. To determine efficacy, noninvasive tests include ECG monitoring and electrophysiological studies.

Therapies for ventricular arrhythmias have focused predominantly on disruption of reentrant circuits. Investigators have demonstrated reductions in ventricular arrhythmia susceptibility using transgenes that increase either myocyte refractory properties or myocardial tissue conduction velocity. In a porcine model of healed myocardial infarction and inducible VT, gene transfer of KCNH2-G628S shut down the repolarizing  $I_{Kr}$  current, prolonging the myocyte refractory period and eliminating all ventricular arrhythmia inducibility.<sup>10</sup> In the same model, connexin 43 (Cx43) gene transfer in the infarct scar border improved conduction and reduced arrhythmia susceptibility.<sup>7</sup> Both these studies used a coronary perfusion delivery strategy.

An alternative approach to normalizing slow myocardial conduction was explored by Lau et al<sup>11</sup> They noted that the endogenous cardiac sodium channel (SNC5a) was less active at the depolarized membrane potentials typical of damaged myocytes postinfarction. SCN4A, the skeletal muscle form of the sodium channel, was more active at those membrane potentials, so they tested the ability of SCN4a gene transfer to improve conduction and reduce ventricular arrhythmia inducibility in a peri-infarct canine model.<sup>11</sup> They injected Ad-SCN4a into the epicardial border zone and found that it reduced the incidence of polymorphic VT inducibility, increased  $V_{max}$  of phase 0 of the AP causing rapid conduction, and decreased electrogram fragmentation. Using this same model, the same investigation team tested Cx32 (a connexin that remains open at low pH).<sup>96</sup> Unlike Cx43, Cx32 improved gap junctional conductance but had no antiarrhythmic effect, and peri-infarct expression of Cx32 caused a significantly larger infarct size.

As with HF, much attention has been placed on SERCA2a. In a large animal ischemia-reperfusion model, Ad-SERCA2a was delivered by intracoronary perfusion and assessed 7 days later during a 30-minute occlusion and 24-hour reperfusion.<sup>90</sup> Compared with controls, SERCA2a did not have an effect on VT or VF frequency during ischemia, but reduced VT and VF

**Table 4.** Gene Therapy Targets for Arrhythmia

Molecular Target	Stage in Development	Findings	Model Assessed	Reference
KCNH2-G628S	Preclinical	Prolonged refractory period by shutting down $I_{Kr}$ , eliminating arrhythmia inducibility	Pig Dog	Sasano et al, <i>Nat Med</i> , 2006 <sup>10</sup> Amit et al, <i>Circulation</i> , 2010 <sup>87</sup> Soucek et al, <i>Heart Rhythm</i> , 2012 <sup>88</sup>
Cardiac sodium channel 4a (SCN4a)	Preclinical	Reduced VT inducibility, increased $V_{max}$ causing rapid conduction, and decreased electrogram fragmentation	Dog	Lau et al, <i>Circulation</i> , 2009 <sup>11</sup>
Connexin 32	Preclinical	Improved gap junctional conductance but no antiarrhythmic effect and larger infarct size	Dog	Boink et al, <i>J Am Coll Cardiol</i> , 2013 <sup>3</sup>
Connexin 40	Preclinical	Enhanced atrial conduction and prevented atrial fibrillation	Pig	Igarashi et al, <i>Circulation</i> , 2012 <sup>33</sup>
Connexin 43	Preclinical	Improved conduction and reduced arrhythmia susceptibility	Pig	Greener et al, <i>J Am Coll Cardiol</i> , 2012 <sup>22</sup> Igarashi et al, <i>Circulation</i> , 2012 <sup>33</sup> Bikou et al, <i>Cardiovasc Res</i> , 2011 <sup>89</sup>
Sarcoendoplasmic reticulum calcium-ATPase 2a (SERCA2a)	Preclinical	Reduced VT and VF during reperfusion Reduced premature ventricular contraction and nonsustained VT Decreased APD alternans	Pig Rat Guinea Pig	Prunier et al, <i>Circulation</i> , 2008 <sup>90</sup> Lyon et al, <i>Circ Arrhythm Electrophysiol</i> , 2011 <sup>91</sup> Cutler et al, <i>Circ Arrhythm Electrophysiol</i> , 2009 <sup>92</sup>
Adenylyl cyclase 1 (ADCY1)	Preclinical	Increased beating rate, provided stable pacemaker effects	Dog	Boink et al, <i>J Interv Card Electrophysiol</i> , 2011 <sup>93</sup>
Adenylyl cyclase 6 (ADCY6)	Preclinical	Provided biological pacing during catecholaminergic stimulation	Pig	Ruhparwar et al, <i>Tissue Eng Part A</i> , 2010 <sup>94</sup>
Kir2.1	Preclinical	Increased pacemaking	Guinea Pig	Miake et al, <i>Nature</i> , 2002, <i>J Clin Invest</i> , 2003 <sup>62,95</sup>

VT indicates ventricular tachycardia; VF, ventricular fibrillation; APD, action potential duration.

during reperfusion. The main risk reduction was seen in sustained and nonsustained VT, and there was trend toward an increased number of VF episodes. However, in a rat model of post-myocardial infarction, SERCA2a was delivered via AAV9 or AD and shown to reduce premature ventricular contractions and spontaneous or induced nonsustained VT episodes.<sup>91</sup> Investigators showed that after isoproterenol treatment, SERCA2a-overexpressing animals had fewer sustained VT or VF episodes. Additional analysis demonstrated a reduction in spontaneous sarcoplasmic reticulum calcium-release events, total sarcoplasmic reticulum calcium leak, and triggered arrhythmias. In cardiomyopathy, beat-to-beat alterations of the cardiac action potential duration (APD) are linked with ventricular arrhythmia and sudden death.<sup>97</sup> Guinea pigs receiving Ad-SERCA2a, delivered via the aortic cross-clamp coronary perfusion method, had decreased APD alternans.<sup>92</sup> Ad-SERCA2a-overexpressing myocytes displayed faster  $Ca^{2+}$  reuptake kinetics,  $Ca^{2+}$  transient amplitude, and a lack of APD and  $Ca^{2+}$  transient alternans during rapid pacing compared with controls.

Many studies have identified genetic targets for the treatment of AF. The major themes of the proteins targeted are atrial conduction and repolarization. KCNH2-G628S was

used in the setting of AF where pigs were burst-paced to induce AF and treated with an AD containing KCNH2-G628S.<sup>87</sup> The gene therapy was applied using the epicardial painting method described above. After KCNH2-G628S gene transfer, atrial APD was prolonged, and AF was prevented. A similar study used the canine version of the same mutation, CERG-G627S. Those investigators directly injected the AD into the atria followed by electroporation to enhance gene transfer, and they also showed atrial APD prolongation and disruption of AF.<sup>88</sup>

To enhance atrial conduction, both Cx40 and Cx43 have been used in strategies similar to the VT work described above.<sup>9,89</sup> In controls with AF, Cx43 levels were reduced, expression was lateralized, and conduction velocity was decreased. With gene transfer of either Cx40 or Cx43, conduction effects were reversed, and atrial fibrillation was prevented.

Gene therapy to induce pacing activity is an area under intense investigation. As discussed above, AC6, which can induce cAMP and improve left ventricular function in HF, has been shown to play a role in providing biological pacing during catecholaminergic stimulation.<sup>94</sup> Another AC family member, AC1, which is  $Ca^{2+}$  dependent and expressed in the

sinoatrial node, has been shown to play a role in biological pacing.<sup>98</sup> When AC1 was overexpressed with hyperpolarization-activated cyclic nucleotide-gated channel serotype 2 (HCN2), increased cAMP synthesis and basal beating rate were found in ventricular myocyte cultures.<sup>99</sup> Ad-AC1 was injected into the left bundle branch of dogs with complete heart block, basal heart rate of 60 to 70 beats per minute occurred, increasing to 100 beats per minute during activity.<sup>93</sup> On overexpression of HCN2 concurrently, basal rate of 120 beats per minute and maximal rate of 250 beats per minute were exceeded.<sup>93</sup> Interestingly, this result depends on AC1 interacting with endogenous HCN2 because when AC1 was overexpressed in culture with a cAMP HCN2-insensitive mutant, increased beating rates still occurred.<sup>99</sup> Using the same model, the investigators showed that HCN2 also has positive effects on impulse initiation when coexpressed with SCN4A.<sup>96</sup> Stable in vivo pacemaker activity and a more negative action potential were achieved with no dependence on electronic backup pacing. Additional ion channel regulation work has shown that downregulating the repolarizing inward rectifier current,  $I_{K1}$ , with a dominant-negative construct of Kir2.1 increased pacemaking.<sup>95</sup> A follow-up study showed that downregulation of  $I_{K1}$  resulted in excessive prolonged repolarization that will need to be watched for potential arrhythmogenicity.<sup>100</sup>

Proof-of-concept for gene therapy to treat arrhythmias is exciting and impressive, but many obstacles remain. It has been shown that for treatment of arrhythmia, high gene expression is needed for a therapeutic effect, so delivery and access remain a limitation. Epicardial painting for atrial disease and arrhythmia has the greatest promise but access to the epicardial surface of the atria puts this technique at a disadvantage. Modifications to these approaches are being investigated, and gene therapies for arrhythmias will be a treatment option and will probably soon replace drugs as an adjunct therapy.

## Conclusions

The goal of gene therapy is to modify a gene or genetic pathway to provide therapeutic value and prevent or reduce disease. It is important to develop a method that is safe and effective for the treatment of human disease. Important issues such as tolerance and ease of administration need to be translated to the clinic. For cardiovascular disease, gene therapy has been limited due to vectors and delivery to the target cell. Long-term expression with AAVs is limited, and inflammation as a result of host recognition is a problem. As previously mentioned, AAVs provide longer-term expression and to some extent can evade the host immune system, but existing endogenous antibodies to AAVs have been found to limit AAV-mediated gene transfer in some patients. As was done in the CUPID trial, prescreening for AAV antibodies is important for finding a viable population.<sup>58</sup>

To date, clinical studies have shown limited efficacy but no long-term adverse events with angiogenic gene therapy and suggestions of efficacy and safety with early-stage heart failure gene therapy. Preclinical work is currently ongoing for arrhythmia applications. For gene therapy to be successful, the right gene needs to be targeted in the setting of the correct disease. With multiple disease mechanisms for individual diseases, finding the molecule to target is daunting. Modification of multiple genes may be necessary. Genetic modulation in cardiovascular disease is the forefront of new therapeutic options and, with additional development and rigorous testing, could become the paradigm for first-round treatment options in the clinic.

## Sources of Funding

This work was funded by the National Institutes of Health (AG42701, HL67148, HL93486, EB2846) and the Fondation LeDucq Alliance for Calmodulin Kinase Signaling in Heart Disease.

## Disclosures

None.

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**Key Words:** angiogenesis • arrhythmia • gene therapy • heart failure • myocardium