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REVIEW TOPIC OF THE WEEK

Improving Atrial Fibrillation Therapy

Is There a Gene for That?

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ABSTRACT

Atrial fibrillation (AF) is an all-too-common and often challenging reality of clinical care. AF leads to significant morbidity and mortality; however, currently available treatments for AF have modest efficacy and high recurrence rates. In recent years, genetic therapy approaches have been explored in preclinical models of AF, and offer potential as a treatment modality with targeted delivery, tissue specificity, and therapy tailored to address mechanisms underlying the arrhythmia. However, many challenges remain before gene therapy can advance to a clinically relevant AF treatment. In this review, we summarize the available published data on gene therapy and discuss the challenges, opportunities, and limitations of this approach. (J Am Coll Cardiol 2017;69:2088–95) © 2017 by the American College of Cardiology Foundation.

Atrial fibrillation (AF) is the most common sustained arrhythmia, affecting millions of patients worldwide with increasing prevalence (1). This arrhythmia carries significant morbidity and is well known for its stroke risk; however, the risks of congestive heart failure (CHF), dementia, and death are also significantly increased with AF (1,2). Although several epidemiological factors, such as obesity, diabetes, and CHF influence AF incidence, there is also a genetic predisposition to AF. For instance, having a first-degree relative with AF increases the chance of AF incidence (3). In recent years, genome-wide association studies (GWAS) have identified at least 14 distinct genetic loci associated with the arrhythmia (4), in addition to other loci identified through familial linkage studies. Although it is clear that genetics alone does not explain AF incidence, efforts have focused on genetics as an avenue to understand the molecular underpinnings of AF, as well as to hopefully identify novel treatment paradigms to combat this arrhythmia.

Treatment of AF often starts with the decision of rate versus rhythm control. There has been no proven mortality benefit demonstrated for rhythm control despite several randomized trials; hence, rhythm control is commonly pursued for specific patient populations and patients symptomatic from AF (5). Medical therapy is the most common preliminary strategy for rhythm control, yet antiarrhythmic drug use is limited by modest efficacy and significant possible toxicities, including proarrhythmic effects. Hence, use of these medications is limited to a subset of patient populations and not applied to the majority of AF patients. Nonpharmacological therapy in the form of catheter ablation is quite effective in certain

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patient populations, such as young patients with few comorbidities and those with paroxysmal AF, yet this represents a minority of patients with AF (6). When ablation is applied to patients with persistent AF, for example, efficacy rates are lower (7). Therefore, developing new therapies that could be applied more broadly would certainly be beneficial and is a focus of intense research interest. In this review, we briefly describe some of the mechanisms and remodeling that occur in AF, and how these mechanisms may be targeted through a genetic approach to decrease the burden of AF.

MECHANISMS OF AF

Initiation and maintenance of AF requires both a driver for the arrhythmia and the appropriate substrate to maintain the rhythm. Two principal driving mechanisms are felt to be responsible for AF: focal ectopic firing and re-entry. Both of these mechanisms are supported by the particular expression of ion channels and the tissue architecture of the pulmonary veins, which underlie the reason that pulmonary vein isolation has become the cornerstone of ablation therapy for AF.

Focal ectopic activity often arises from the pulmonary veins due, in large part, to decreased coupling to the surrounding atrial substrate, a relatively depolarized resting membrane potential, and short action potential duration (APD) (8). Similar to the relative electrical insulation surrounding the human sinus node (9), decreased coupling reduces the electrotonic load on pulmonary vein cells, which increases the safety factor of conduction from the pulmonary veins to the surrounding atrial myocardium and supports automaticity (10,11). A higher resting membrane potential in pulmonary vein cells is likely due to decreased inward-rectifier potassium current (I_{K1}) expression (12). Elevation of the resting membrane potential decreases the net inward sodium current (I_{Na}) due to partial inactivation of sodium channels, which will slow conduction, and may contribute to the slow conduction in the proximal pulmonary vein that has been seen experimentally (10,13). However, this loss of depolarizing current through partial I_{Na} inactivation may not affect propagation from the pulmonary veins because decreased coupling has a larger effect on the safety factor of conduction, allowing propagation to occur (10). The APD is shorter in pulmonary vein cells compared with atrial cells, due to higher expression of channels underlying the outward repolarization currents, I_{Kr} and I_{Ks}, which reduces the effective refractory period, facilitates rapid firing, and contributes to heterogeneous repolarization, thereby supporting re-entry (12,13). In addition, altered intracellular ionized calcium (Ca^{2+}) homeostasis can lead to afterdepolarizations, triggering focal ectopic activity as well (14). Finally, the pulmonary vein tissue architecture supports re-entry due to anisotropic, heterogeneous conduction at the junction of the pulmonary veins and the atrium (13,15).

Electrical and structural remodeling of the atrial substrate, autonomic modulation, and Ca^{2+}-handling abnormalities all frequently occur in response to cardiac pathology (14). Electrical remodeling is typically characterized by a decrease in the L-type Ca^{2+} current and an increase in the inward-rectifier current, I_{K1}, both of which act to shorten the atrial APD. However, this may vary, depending on comorbid conditions. For instance, in the setting of CHF with AF, an APD change is less pronounced, whereas remodeling of Ca^{2+}-handling proteins is more apparent and unique from that seen in AF or CHF alone (16–18). In addition, alterations in the level of the constitutively active acetylcholine-induced potassium current are frequently present, which can increase the heterogeneity of the APD in the atrial substrate, favoring the maintenance of AF (19). Structural remodeling, characterized by left atrial enlargement and atrial fibrosis, also contributes to heterogeneous conduction and slower conduction velocity, facilitating the maintenance of AF. Any one of these abnormalities could potentially be the target of gene therapy to reduce the likelihood of AF.

GENE THERAPY FOR AF

The use of targeted genetic alterations to customize treatment for AF is an intriguing approach, particularly in an era of increasing calls for the personalization of medical therapy, yet it has been challenging for these efforts to progress beyond investigational tools. The advantages of gene therapy for AF include tissue specificity with less off-target effects and hopefully increased therapeutic specificity as well. For instance, one could envision tailored genetic therapy for an individual patient on the basis of certain characteristics of their disease, yet many challenges exist. Conceptually, the heterogeneity of the AF substrate may decrease the efficacy of a single genetic alteration. For clinical practice, the inherent safety concern of using gene therapy to modify the myocardium is of paramount importance. Another concern is how the genetic material would be delivered: viral vectors, plasmids, or nanoparticles are all possible. Although

ABBREVIATIONS AND ACRONYMS

AF = atrial fibrillation

APD = action potential duration

CHF = congestive heart failure

DNA = deoxyribonucleic acid

GWAS = genome-wide association study

I_{K1} = inward-rectifier potassium current

I_{Na} = inward sodium current

PITX2 = paired-like homeodomain transcription factor 2

RNA = ribonucleic acid

TGF = transforming growth factor

viral vectors are likely the most practical, legitimate concerns about their safety exist. The establishment of lasting gene expression is also a significant hurdle that must be addressed. Although many challenges remain, effective and lasting therapy for AF has proven elusive and therefore innovative approaches are necessary to increase therapeutic options.

MYOCARDIAL GENE DELIVERY

Gene delivery can be accomplished with either viral or nonviral vectors, with varying degrees of gene incorporation and expression. A nonviral vector consists of a deoxyribonucleic acid (DNA) plasmid containing the gene of interest, with or without other coating agents to improve the uptake of DNA into cells (20). Viral vectors have the advantage of incorporation of genetic material into the genome of the target tissue. Both adenoviral vectors and adeno-associated viral vectors have been used in preclinical models (20–22). Although the adenovirus is able to deliver larger gene sizes, adeno-associated viral vectors may produce longer (if not permanent) gene expression and have a more favorable safety profile (20,23).

Delivery of the vector to the myocardium can take multiple approaches (**Figure 1**). Infusion of the vector into a coronary artery is possible, and has been used in preclinical studies for rate control to deliver vectors to the atrioventricular node (24–26). For rhythm control in paroxysmal AF, however, suppression of ectopic foci in the pulmonary veins would be an ideal target, yet the pulmonary veins do not fit a common coronary distribution. In preclinical models, one typical method is to inject the vector into the myocardium and then electroporate the tissue to improve DNA uptake (27,28). One could envision a similar delivery system via a catheter at locations that are currently treated with ablation lesions. Epicardial gene "painting" has also been validated in preclinical models with transmural gene transfer using an adenoviral vector, a weak protease to improve tissue penetration, and a polymerization agent to allow the vector to adhere to the myocardium (21). Using this technique, nearly 100% of cells examined transmurally had evidence of gene transfer. This technique in its current form could be applied during cardiac surgery, but future possibilities could include a pericardial injection or coated-balloon delivery via an endocardial route to the pulmonary veins, similar to drug-coated balloons currently used in peripheral vascular disease (29).

TARGETS FOR GENE THERAPY

A variety of targets may be appropriate candidates for gene therapy of AF, and have been the subject of 2 recent reviews by Lugenbiel et al. (30) and Donahue (20). The targets are derived from our current molecular understanding of AF, and can be largely divided by their contributions to re-entry: shortened action potential (ion channels, autonomic modulation) or delayed conduction (gap junctions, structural remodeling). Parsing the changes that are responsible for AF, as opposed to those that occur secondary to the arrhythmia, may have a significant impact on the therapeutic strategy. In the future, further targets on the basis of ever-expanding population-based genetic studies will undoubtedly present themselves as well. A review of preclinical studies that have used gene therapy to modify atrial electrophysiology is provided later and summarized in **Table 1**.

ION CHANNELS. Pharmacological manipulation of ion-channel function is the basis for currently available antiarrhythmic drugs (31–34). In particular, prolongation of the atrial action potential with consequent increased refractoriness may reduce or prevent AF. Amit et al. (35) reported a gene therapy-based approach using this tactic in a porcine model of AF. Epicardial delivery by atrial painting of an adenovirus containing a dominant-negative variant of the alpha subunit of KCNH2 (the channel responsible for the I_{Kr} current) resulted in significant APD prolongation, with reduction in AF burden and inducibility. Differential levels of KCNH2 between the treated and untreated groups were observed at 7 days post-treatment, but not 21 days post-treatment. A similar approach was taken by Soucek et al. (36), using a different method of delivery to supply the atrium with a dominant-negative version of KCNH2. Further proof of concept that the atrial action potential can be modified by gene therapy was provided by Perlstein et al. (37). Transfection of a clarithromycin-responsive subunit mutation of the I_{Kr} regulatory subunit, hMiRP-1, allowed prolongation of the APD by administration of clarithromycin 2 weeks later. However, the efficacy of this approach in reducing or preventing atrial arrhythmia was not studied.

AUTONOMIC INNERVATION. The left atrium is richly innervated by the parasympathetic nervous system, activation of which via left cervical vagal stimulation causes shortening of the atrial effective refractory period and increased vulnerability to AF, whereas local pharmacological blockade is protective (38). In contrast, vagal nerve stimulation has also been shown to increase atrial refractoriness, possibly through up-regulation of atrial connexin expression, and reduce the inducibility of AF in both canine and lupine models (39,40). From a gene therapy perspective,

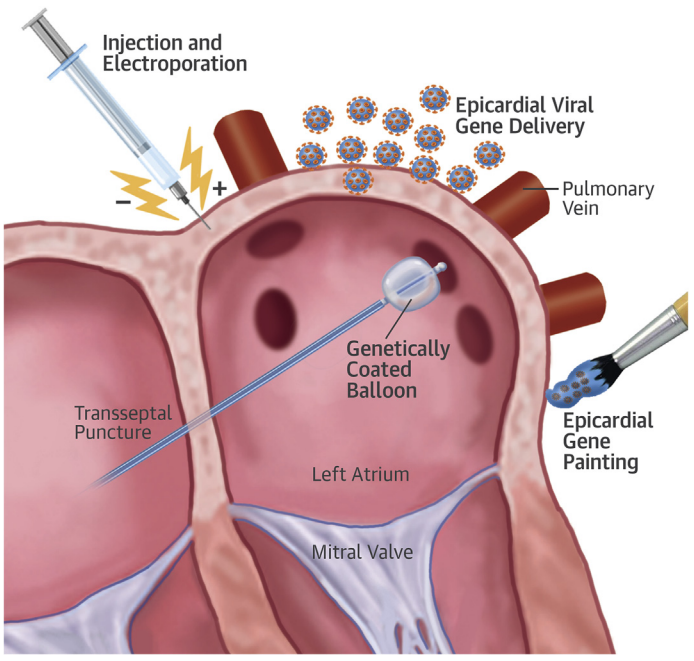
Aistrup et al. (41) endeavored to attenuate vagal signaling in the left atrium by inhibiting the primary effector molecules of the system, $G\alpha_i$ and $G\alpha_o$. Introduction of plasmids containing the complementary DNA for the $G\alpha_i$ and $G\alpha_o$ C-terminus peptides to the left atrium prolonged the action potential and resulted in diminished AF inducibility during vagal stimulation (41).

GAP JUNCTIONS. Reduced expression or abnormal localization of connexins-40 and -43 are associated with impaired electrical conduction in the atrium and an increased risk of developing AF (42,43). Although thought to be a secondary phenomenon, investigators have postulated that restoration of connexin biology to the undiseased state might be beneficial in the management of AF. Accordingly, gene transfer of both of these connexins using an epicardial painting approach significantly improved expression and localization of the proteins, and was associated with improved conduction and a reduction in arrhythmia burden in a porcine atrial-burst pacing model of AF (22). A separate study of connexin 43 alone in the same type of model resulted in similar findings, with a marked reduction in the development of persistent AF in treated animals compared with untreated controls (44).

SUBSTRATE MODIFIERS: APOPTOSIS. AF has been associated with local and systemic inflammation, one facet of which is cardiomyocyte apoptosis (45). In a canine model of AF, apoptosis associated with the arrhythmia was also associated with increased activity of calpain, an intracellular calcium-activated protease, and caspase-3, an apoptotic enzyme (46). With this basis for target selection, genetic knockdown of caspase-3 with an adenovirus-mediated silencing ribonucleic acid (RNA) in a porcine model of AF resulted in prolonged atrial conduction and delayed onset of AF with atrial burst pacing (28).

MicroRNAs are short, noncoding RNAs that are increasingly recognized to play a role in the pathogenesis of AF. Sequencing of microRNAs from the ganglionic plexus of dogs that underwent tachypacing revealed differential expression of distinct microRNAs compared with controls. The most significantly dysregulated was microRNA 206 (miR-206), which targets superoxide dismutase-1, a well-known mediator of apoptosis. Gene therapy of a canine model with a lentivirus containing miR-206 resulted in truncation of the atrial APD beyond what was seen with atrial tachypacing alone. Accordingly, treatment with a lentivirus containing the antagonist anti-miR-206 resulted in atrial effective refractory period prolongation and reduced inducibility of AF (47).

FIGURE 1 Possible Methods of Gene Delivery to the Left Atrium



Preclinical models have demonstrated the feasibility of epicardial injection of a plasmid carrying a gene of interest coupled with electroporation, and epicardial viral gene delivery via epicardial gene painting. Future delivery methods could include balloons coated with either a plasmid or a viral delivery vector delivered to the pulmonary veins with a similar approach to that used currently for pulmonary vein isolation.

SUBSTRATE MODIFIERS: FIBROSIS. Atrial fibrosis is also a well-known factor in the pathogenesis of AF, and may particularly explain the increasing prevalence of the arrhythmia with age. A central feature of age-related fibrosis is up-regulation of transforming growth factor (TGF)-β (48). Kunamalla et al. (27) attempted gene therapy-based modulation of atrial fibrosis by delivering a dominant-negative type II TGF-β receptor to the posterior left atrium in a canine model of AF. The therapy resulted in decreased fibrosis and a reduction in pacing-induced AF in the treated versus control animals (27). Interestingly, this approach also flattened the restitution slope in the treated animals, rendering the tissue more resistant to AF. TGF-β is known to affect the expression of ion channels and the magnitude of currents, such as I_{Na}. The finding of a change in restitution slope in this study suggests that targeting TGF-β affected not only structural remodeling, but also electrical remodeling. In future studies, carefully selecting targets, such as

TABLE 1 Summary of Preclinical Studies Modulating Atrial Electrophysiology Via Gene Therapy

Target Class	First Author, Year (Ref. #)	Model	Genetic Material Delivered	Vector	Delivery Approach	Goal	Outcome
Ion channel	Kikuchi et al., 2005 (21)	Pig	KCNH2: DN mutant (G628S)	Adenovirus	Epicardial painting	↓ I_{Kr}	↑ APD, AERP
	Perlstein et al., 2005 (37)	Pig	KCNE2: sensitizing mutant (Q9E)	Plasmid	Injection	↓ I_{Kr}	↑ APD
	Amit et al., 2010 (35)	Pig: RA tachypacing 7–21 days	KCNH2: DN mutant (G628S)	Adenovirus	Epicardial painting day 0	↓ I_{Kr}	↑ APD → ↓ AF
	Soucek et al., 2012 (36)	Pig: RA tachypacing 14 days	KCNH2: DN mutant (G627S)	Adenovirus	Injection + electroporation day 0	↓ I_{Kr}	↑ APD, AERP → ↓ AF
Autonomic innervation	Donahue et al., 2000 (25)	Pig: acute AF	WT $G\alpha_{i2}$	Adenovirus	Intracoronary infusion to AVN	Rate control via ↑ $G\alpha_{i2}$	Slower AVN conduction, ↑ AVN ERP, ↓ heart rate
	Bauer et al., 2004 (24)	Pig: RA tachypacing, tCMP	$G\alpha_{i2}$: GOF mutant (Q205L)	Adenovirus	Intracoronary infusion to AVN on day 21	Rate control via ↑ $G\alpha_{i2}$	↓ Heart rate, ↑ LVEF w/constitutively active $G\alpha_{i2}$
	Aistrup et al., 2011 (41)	Dog: acute vagal stim, carbachol	$G\alpha_i$ and $G\alpha_o$ C-terminal peptides	Plasmid	Injection + electroporation posterior LA	↓ vagal effect and carbachol on ERP	Attenuated vagal effect on AERP, ↓ carbachol induced AF
	Lugenbiel et al., 2012 (26)	Pig: RA tachypacing	$G\alpha_s$ siRNA	Adenovirus	Intracoronary infusion to AVN on day 0	Rate control via ↓ $G\alpha_s$	↓ Heart rate, preservation of LVEF
Gap junctions	Bikou et al., 2011 (44)	Pig: RA tachypacing	Cx43	Adenovirus	Injection + electroporation	↑ Cx43	↑ Cx43 → ↓ AF
	Igarashi et al., 2012 (22)	Pig: RA tachypacing	Cx40 or Cx43	Adenovirus	Epicardial painting	↑ Cx43 or ↑ Cx40	Homogenized conduction, ↓ AF
Substrate modifier	Trappe et al., 2013 (28)	Pig: RA tachypacing	Caspase 3 siRNA	Adenovirus	Injection + electroporation to RA and LA	↓ Caspase-3 → ↓ cardiomyocyte apoptosis	↓ AF incidence, delayed onset of AF, preserved atrial conduction time
	Zhang et al., 2015 (47)	Dog: RA tachypacing	miR-206 and anti-miR-206	Lentivirus	Injection (left superior fat pad)	Identify miR-206 effect on autonomic remodeling, AERP	miR-206 ↑ autonomic nerve sprouting, ↓ AERP
	Kunamalla et al., 2016 (27)	Dog: RV pacing 3 weeks, tCMP	Type II TGF-β receptor: DN mutant	Plasmid	Injection + electroporation posterior LA	↓ TGF-β signaling	↓ LA fibrosis, homogenized conduction, ↓ restitution slope, ↓ AF duration

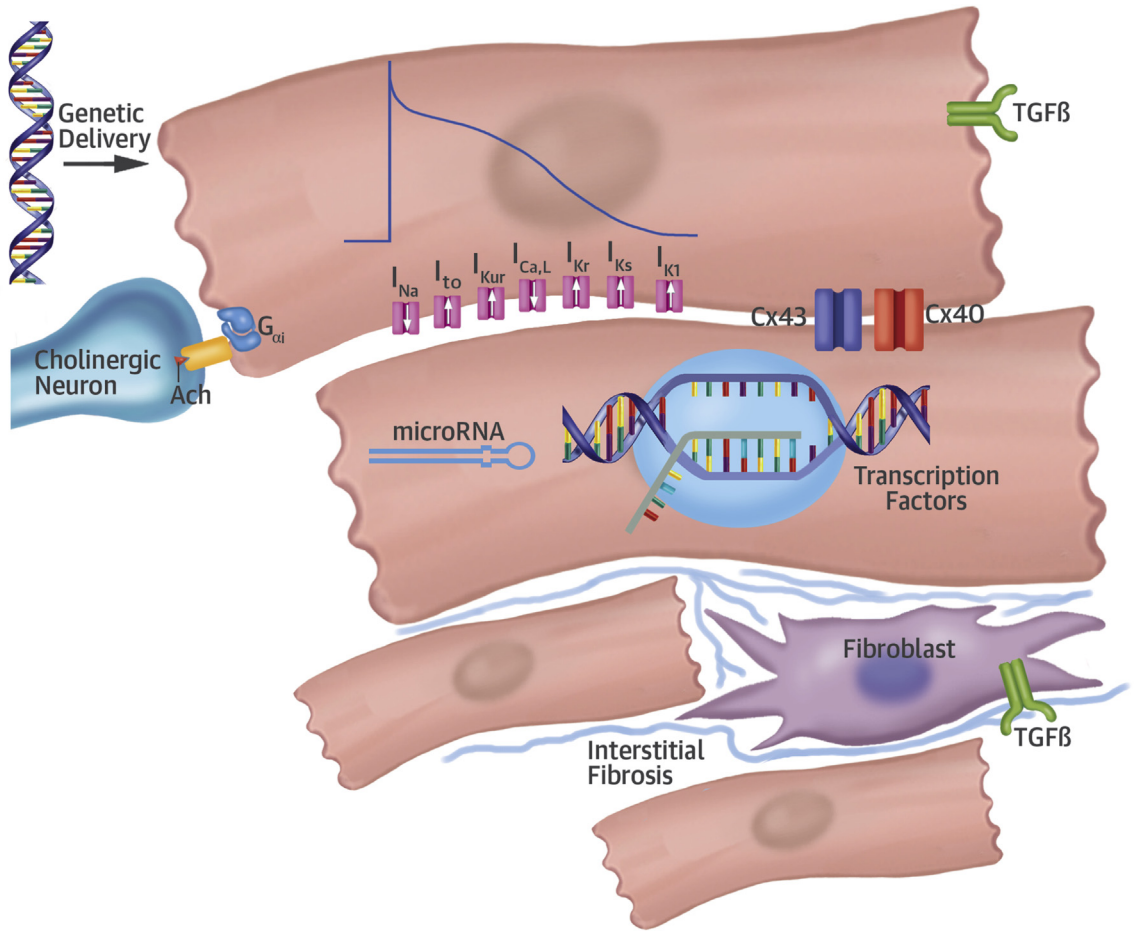
→ = led to; ↑ = increased; ↓ = decreased; AERP = atrial effective refractory period; AF = atrial fibrillation; APD = action potential duration; AVN = atrioventricular node; Cx = connexin; DN = dominant negative; ERP = effective refractory period; GOF = gain of function; I_{kr} = rapidly activating delayed rectifier potassium current; LA = left atrium; LVEF = left ventricular ejection fraction; miR = micro-ribonucleic acid; RA = right atrium; RV = right ventricle; siRNA = small interfering ribonucleic acid; tCMP = tachycardia-induced cardiomyopathy; TGF-β = transforming growth factor beta; WT = wild type.

TGF-β, that affect several aspects of remodeling may prove to be an effective strategy to combat AF. A summary of the targets discussed in this review, and other potential targets, is shown in the **Central Illustration**.

INSIGHT FROM GENETIC STUDIES

As detailed earlier, the targets for gene therapy that have been attempted thus far involve specific genetic targets aimed at addressing the electrical or structural remodeling that occurs in AF. In large GWAS studies of AF patients, the number of loci corresponding to clear electrophysiological phenotypes (ion channels, gap junctions) is relatively few. In contrast, many of the loci that have been identified in GWAS correspond to transcription factors or loci with no currently known function in cardiac physiology, yet their identification through GWAS of thousands of patients with AF strongly suggests a role in the disease. One such locus is near the paired-like homeodomain transcription factor 2 (PITX2) gene, encoding a transcription factor implicated in left atrial development and ion-channel expression, which has the strongest statistical association with AF in GWAS (49). Similar to the idea of targeting TGF-β to manipulate several aspects of remodeling, targeting a transcription factor, such as PITX2, that is upstream to several pathways involved in left atrial development with a gene therapy approach may have far-reaching effects on the atrial substrate beyond that of a single-gene approach. Such a strategy may be very useful in AF, where there is such diversity in remodeling. Intriguingly, a recent study of mice with heterozygous

CENTRAL ILLUSTRATION **Possible Targets for Gene Therapy in AF**



Hucker, W.J. et al. J Am Coll Cardiol. 2017;69(16):2088–95.

Gene therapy delivered to the atrium could target several different pathways involved in atrial remodeling underlying atrial fibrillation (AF). Gene therapy targeting fibroblast proliferation or interstitial fibrosis, differentially expressed ion channels involved in electrical remodeling, gap junctions underlying cardiomyocyte coupling, or autonomic modulation have all been attempted in preclinical models. Future targets of gene therapy may include transcription factors, such as paired-like homeodomain transcription factor 2 (PITX2), affecting several pathways involved in AF susceptibility or microribonucleic acids (microRNAs) to modulate translation of AF targets. Ach = acetylcholine; Cx = connexin; $I_{Ca,L}$ = L type calcium current; I_{K1} = inwardly rectifying potassium current; I_{Kr} = rapidly activating delayed rectifier potassium current; I_{Ks} = slowly activating delayed rectifier potassium current; I_{Kur} = ultra rapid delayed rectifier potassium current; I_{Na} = sodium current; I_{to} = transient outward current; TGF = transforming growth factor.

knockdown of PITX2 were shown to have improved sensitivity to flecainide, suggesting the possibility of using gene therapy to improve the response to antiarrhythmic therapy as well (50).

GENE THERAPY AS AN INVESTIGATIVE TOOL

Rather than using gene therapy as a treatment, an alternative use of gene therapy could be to induce AF in a preclinical model. Inducing AF in animal models is challenging due to the relatively young age of animals used, the size of certain species, and the time required for remodeling to occur. Mouse models have been invaluable in studies of basic mechanisms of AF; however, translating findings in mice to humans is inherently limited for several reasons. First, the resting heart rate of a mouse is approximately an order of magnitude faster than that of a human; therefore, the currents underlying the mouse action potential are fundamentally different, and the APD is much shorter at baseline. This difference influences the susceptibility to AF, as well as the relative impact

of specific electrical remodeling (e.g., down-regulation of L-type Ca^{2+} current or increase of I_{K1}) in mouse versus human. Second, susceptibility to re-entry is influenced by the size of the tissue, and the diminutive size of the mouse atrium influences the probability of initiating a re-entrant circuit, affecting the balance of triggered activity and re-entry in AF induced in a mouse compared with human AF. Third, the mechanisms of and propensity for triggered beats in mice are potentially different than in humans, due to differences in intracellular calcium handling. These inherent differences between mice and humans suggest the need for robust AF models in larger species.

Generating AF models in larger animal species is challenging due to their size, age, and expense. In addition, initiating AF can be difficult and time consuming, often taking weeks of rapid atrial pacing. However genetic alteration of large animal species may offer an alternative method of creating a reproducible model of AF, or perhaps facilitate AF induction and maintenance after rapid atrial pacing. For instance, inducing atrial arrhythmias and AF in a mouse is facilitated by chamber-specific knockout of PITX2 or inactivation of microRNAs regulated by PITX2 (51,52). Applying a similar approach in large animal models may produce a reliable model that could be used to further study mechanisms of AF that are more translatable to human AF, or the efficacy of novel pharmacotherapy and ablation techniques.

The field of gene therapy has many significant challenges that must be overcome before its use will become a clinical reality. Nevertheless, the management of AF remains in urgent need of new treatment paradigms, and gene therapy offers some unique advantages that may be useful in the treatment of AF. In the near term, gene therapy may provide unique opportunities to increase our understanding of the disease, as well as possibly expanding the ability to study AF in a preclinical model. Over a longer time horizon, advances in this field may expand our treatment options for AF, but there is still a long way to go until these approaches will be broadly implemented in clinical practice.

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