Heart, Lung and Circulation (2016) **25,** 808–813
1443-9506/04/$36.00
http://dx.doi.org/10.1016/j.hlc.2016.04.011

REVIEW

# Therapeutic Prospects of Gene Therapy for Atrial Fibrillation☆

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*Received 11 April 2016; accepted 17 April 2016; online published-ahead-of-print 12 May 2016*

Atrial Fibrillation (AF) is one of the most common types of cardiac arrhythmias experienced in clinical practice, increasing the risk of stroke, dementia, myocardial infarction and death. Currently available options for the treatment of AF use either pharmacological agents or catheter-based ablation therapies to restore sinus rhythm or control the ventricular response rate. These current treatment options are suboptimal at best, motivating research into discovering more effective and innovative ways to treat AF. Gene therapy is being explored for its potential to treat various human conditions including cardiac arrhythmias. Gene transfer vectors with increasing transduction efficiency and biosafety have been developed and trialled for cardiovascular disease treatment. With an improved understanding of the molecular mechanisms of AF, several gene therapy targets have been identified and evaluated in an attempt to rate or rhythm control the heart during AF. This review will discuss the gene therapy vectors in use today and methods for delivery of these vectors to the atrium. Further, it will evaluate several gene therapy strategies and approaches for sinus rhythm restoration and ventricular rate control that have the potential to emerge as a therapy for AF.

**Keywords** Arrhythmia • Atrial fibrillation • Gene therapy • Heart • Viral vector

## Introduction

Atrial Fibrillation (AF) is one of the most common types of cardiac arrhythmia disorders experienced in clinical practice, occurring in approximately 2% of the general Australian population. This prevalence increases dramatically with ageing, affecting 5% of people over the age of 65 years and 10% of people over the age of 80 [1–3]. This incidence is projected to double in the next 50 years, as the Australian population continues to age [4]. The impact of AF on the public health system is substantial, with more than 45,000 hospital admissions per year and an estimated AU$1.25 billion per annum for medical and health related costs [5]. The personal impact of AF on patient's health is equally significant. The presence of AF increases the risk of stroke five-fold, increases the risk of dementia two-fold and increases the risk of a myocardial infarction and death by 1.5 to 2-fold each [6–8].

Currently available treatment options for AF aim to either restore sinus rhythm (rhythm control) or control the ventricular response rate during AF (rate control). Both approaches are suboptimal at best. With the rhythm control strategy there are two approaches: pharmacological agents; and catheter-based ablation therapies. Antiarrhythmic drug therapy is limited by a relatively high recurrence rate and side-effects including proarrhythmic effects and non-cardiovascular toxicities [9–11]. Ablative therapies, touted as a potential cure, focus on elimination of electrical triggers located in pulmonary veins and in the left atrium that initiate and perpetuate AF [12]. However, long-term efficacy remains elusive, particularly with chronic AF; ablation affords only modest rates

☆This review follows presentations at the David Leslie Ross Festschrift at Westmead Hospital, held on 12 February, 2016.

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of cure. Procedure-related events such as left atrial perforation, atrio-oesophogeal fistula, pulmonary vein stenosis and thrombo-embolism present high-risk complications. [9,13–15].

The lack of an ideal therapy and the existence of a need for better treatment of AF has motivated research into discovering more effective and innovative ways to treat AF. One exciting avenue, which has the potential to address limitations of current treatment options and expand AF treatments, is cardiovascular gene therapy. With recent improvements in gene transfer vectors, delivery methods, and viable options for clinical translation, several inroads have been developed for gene therapy treatment of cardiovascular diseases. In this review, we discuss the current experimental approaches to gene therapy of AF, and the therapeutic prospects of gene therapy for addressing rhythm and rate control of atrial fibrillation in human subjects.

## General Principles of Myocardial Gene Transfer

In the broadest of terms, gene therapy is the use of nucleic acid sequences to manipulate gene expression in target cells or tissue, for a specific therapeutic outcome. Three major components are required for successful gene therapy. These include the selection of a gene transfer vector, a suitable vector delivery method, and a therapeutic gene target. Based on specific applications, these three components must be designed and employed individually.

There are three main approaches to effective gene therapy. The classic approach is the overexpression of a gene of interest. This involves using a constitutively active or tissue specific promoter to express a gene that is normally down-regulated or whose overexpression is predicted to have a therapeutic effect [16]. The second approach employs regulatory RNA molecules such as siRNAs and miRNAs to target and turn off genes in a sequence dependent manner [17]. The final approach, and the most contemporary, employs molecular techniques to alter the DNA code to repair gene defects [18]. Each of these three approaches has its strengths and limitations.

### Vectors

More often than not, the transfer of nucleic acids for gene therapy entails the use of a viral vector. Viral vectors are exploited for their natural ability to secure a favourable intracellular fate for their genetic material. In gene therapy applications, viral genomes are highly edited so that vectors are non-infectious and replication defective [19,20].

There are several viral vectors that have been exploited to date for gene transfer applications. The three most commonly used vectors are compared to plasmid DNA and are shown in Table 1 [21]. Plasmid DNA is widely used for in vitro transfection experiments as it is easy to produce and has a large packaging capacity. It has been used in cardiac applications. However, its main shortcomings include a very low transfection efficiency in vivo and only transient gene expression, limiting its application [22,23]. The most widely used viral vector in cardiac gene therapy experiments is the adenoviral vector. It is able to transduce the mammalian heart with a very high efficiency and provides a great way to undertake proof-of-principle studies. This vector's main limitation is its ability to induce a rapid inflammatory and immune response resulting in a limited expression window of about two weeks [23]. Lentiviral vectors on the other hand are capable of long-term gene expression by virtue of their ability to integrate into the host cell genome. Lentiviral vectors, however, have a low capacity for cardiac transduction. The most promising vector for cardiac gene therapy is based on recombinant adeno-associated virus (rAAV). This vector is derived from a non-pathogenic parental virus. Depending

**Plasmids and Viral Vectors for Cardiovascular Disease Treatment [20,21]**

| Vector | Plasmid | AAV | Lentivirus | Adenovirus |
|---|---|---|---|---|
| Functional Titre (per mL) | N/A | Up to $10^{13}$ | Up to $10^{9}$ | Up to $10^{13}$ |
| Genome/Size (Kb) | DNA/ N/A | ssDNA/±4.8 | ssRNA/±10 | dsDNA/±36 |
| Insert Capacity | 15kB | 4.8kB | 10kB | 7 to 30kB |
| Integration | No | Yes (Random) | Yes (Random) | No |
| Pattern of Transgene Expression | Up to 2 Months | Long Term | Long Term | Up to 2 Weeks |
| Host/Vector Interactions | Minimally Immunogenic | Minimally Immunogenic | Minimally Immunogenic | Cytotoxic and Immunogenic |
| Cardiac Gene Transfer | Low Cardiac Transduction | Cardiotropic AAV Serotypes | Low Cardiac Transduction | High Cardiac Transduction |
| Disadvantages | Low Transfection Efficiency | Risk of Neutralising Antibodies and T-Cell Responses | Risk of Insertional Mutagenesis | High Antibody and Inflammatory response |
| Clinical Trial Approval | Yes | Yes | Yes | Yes |