Gene Therapy for the Treatment of Catecholaminergic Polymorphic Ventricular Tachycardia

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmic syndrome characterized by exercise- or stress-induced polymorphic ventricular tachyarrhythmias and sudden cardiac death in the context of a structurally normal heart. It is estimated to occur in 1 in 10,000 of the population and has a poor prognosis, with up to 50% mortality by 20 years of age. The pathophysiology has been studied extensively since the first genetic mutation was first identified by Priori and colleagues in 2002. Abnormal calcium storage and release from the sarcoplasmic reticulum (SR) within the cardiomyocytes underpin the disease, with causative mutations in 2 critical proteins in the SR complex: the cardiac SR calcium release channel known as the ryanodine receptor (RyR2) and the calcium buffering and RyR2 regulatory protein calsequestrin (CASQ2). Mutations have been identified in the RyR2 or CASQ2 genes in ≈60% to 70% of individuals with the clinical syndrome.

Diastolic SR calcium release via RyR2 is the molecular basis for delayed afterdepolarizations (DADs), triggered activity, and ventricular ectopy and, if oscillatory, can be sustained ventricular tachyarrhythmias, leading to cardiac arrest and sudden death. Increased sympathetic activity activates the cardiac β-adrenoceptor (βAR) pathways, leading to phosphorylation of RyR2 and increasing the RyR2 channel opening probability. βAR activation also increases sarcoplasmic endoplasmic reticular calcium ATPase2 (SERCA2a) activity via phosphorylation of the inhibitory protein phospholamban, increasing SR calcium loading. Both of these effects are important for the normal increase in cardiac inotropy during exercise or stress as part of the “fight or flight” response.

Individuals with CPVT harbor mutations in RyR2 or CASQ2, lowering the threshold for abnormal SR calcium release during diastole, when the RyR2 channel should be closed. Hence, individuals with CPVT develop complex ventricular tachyarrhythmias secondary to maladaptive handling of SR calcium and increased spontaneous diastolic calcium release. These malignant arrhythmias may develop spontaneously or particularly with increased sympathetic activity during exercise or stress when SR calcium cycling is amplified by βAR activation. In addition to these functional changes in cardiomyocyte SR physiology, recent studies of hearts from CASQ2 knockout mice show dramatic structural changes in the SR volume and morphology, contributing to the functional disturbance in cardiomyocyte calcium regulation.

Current treatments for individuals with CPVT are based on 3 strategies according to the individual and the disease severity: reducing myocardial βAR activation with β-blockers and, in severe cases, surgical cardiac sympathectomy; reducing spontaneous SR calcium and triggered activity with flecainide, which appears to have antiarrhythmic effects via direct RyR2 stabilization and modulation of the gain of reverse excitation-contraction coupling and electric instability; and implanting cardiac defibrillators, which serve as a back-up strategy in high-risk individuals, although there is growing evidence that their efficacy for successful treatment of life-threatening arrhythmia in CPVT patients is unfortunately poor because antiarrhythmic pacing and defibrillation are not as effective for triggered arrhythmia-mediated VT and ventricular fibrillation in individuals with CPVT compared with those arising from other arrhythmia substrates.

Despite the recent impact of the introduction of flecainide on the background of β-blockers as standard maintenance therapy, many individuals with CPVT have ongoing burden of syncope, ICD shocks, and premature mortality. Therefore, new treatment options for individuals with CPVT are required.

Cardiac gene therapy has been evaluated for >20 years, and recently, the application of adeno-associated viral vectors (AAVs) has accelerated the field because AAVs greatly increase the efficiency of prolonged cardiac gene expression after a single delivery compared with previous adenovirus- and plasmid-based gene delivery technologies. Furthermore, the lack of human disease caused by AAV makes them an attractive candidate for clinical cardiac gene therapy. Indeed, the first clinical trials (phases 1 and 2) using AAV to deliver SERCA2a gene therapy to patients with chronic heart failure have been successfully completed and have shown that clinical event rates are significantly lower 3 years after gene transfer in the patients receiving high-dose AAV1.SERCA2a compared with those receiving saline. In addition, these studies have shown that AAV1.SERCA2a gene transfer results in persistence of the SERCA2a gene up to 31 months in cardiac tissues from patients injected with high-dose AAV1.SERCA2a. The early results from these trials, albeit in small patient numbers with follow-up limited to 3 years, have suggested that this approach appears safe, with no suggestion of virus-associated...
adverse events. This raises the possibility of cardiac gene therapy as a strategy for other indications, and inherited cardiac conditions would appear a logical target.

In this issue of *Circulation*, Denegri and colleagues report a novel approach with cardiac gene transfer to treat a transgenic murine model of CPVT. In their study, the investigators used knock-in model of calstabin2 CASQ2/33Q as their disease model. The homologous knock-in CASQ2 R33Q/R33Q mice are characterized by the expression of an endogenous effective CASQ2 protein and by ultrastructural abnormalities, including the widening of the cisternae of junctional SR and a reduction in CASQ2 partners junction and triadin. These mutant mice displayed evidence of DADs and arrhythmias in the presence of βAR stimulation (isoproterenol), recapitulating the CPVT phenotype. Gene transfer with the cardiotropic AAV serotype 9 of wild-type CASQ2 (AAV9.CASQ2) at birth prevented the development of the architectural changes and the arrhythmogenic phenotype in the knock-in mice over a period of 12 months. In a separate series of experiments, AAV9.CASQ2 delivered in adult mice rescued the structural phenotype and arrhythmogenic substrate. These effects were found despite the fact that only ∼40% of cardiomyocytes were infected by AAV9 gene delivery. Bystander effects of gene transfer have been found in the setting of genes that enhance contractile function. The propensity for triggered arrhythmias may worsen with a mosaic of expression across the myocardium, especially when dealing with a protein that alters electric coupling. However, the authors found structural improvements within the cell coupled to reductions of DAD frequency in the presence of βAR stimulation after gene transfer of CASQ2.

The RyR2 receptor forms a supramolecular complex with CASQ2, junctin, and triadin at junctional release sites. Mutations of CASQ2 lead to disruption of this supramolecular complex, which is responsible for regulating Ca2+ sensing at the SR junction. This leads to spontaneous Ca2+ release events, Ca2+ waves, DADs, and triggered arrhythmias. The mechanistic basis for the dysfunctional calcium regulation has been extensively studied and modeled in CPVT. In the presence of βAR stimulation, impaired luminal Ca2+ sensing accelerates the recovery of RyR2, leading to triggered activity. Even though the authors did not examine SR calcium regulation in this study, future studies should probe whether CASQ2 gene transfer would abrogate the accelerated recovery of the RyR2 from inactivation and whether free SR Ca2+ is an important variable in determining the propensity for DADs.

Denegri and colleagues present a rodent study that opens up the exciting prospect of cardiac gene therapy as a potential novel therapeutic option for this malignant inherited arrhythmogenic syndrome. However, a number of challenges remain before this can be effectively studied in humans. The first is the potential requirement to assess the efficacy of AAV-CASQ2 gene transfer across a range of mutant CASQ2-harboring transgenic mouse models to capture the spectrum of genetic abnormalities observed in CPVT patients. This may be necessary to confirm applicability to all CPVT patients harboring a CASQ2 mutation, independently of the specific locus and functional effect. A second is that preclinical models for inherited monogenic cardiac disorders are generally limited to mice, although transgenic rabbits and pigs have been developed. Will the regulatory authorities require efficacy studies in any larger preclinical species, with cardiac anatomy or electrophysiology more closely aligned to the human heart? Or will preclinical studies in larger animals be limited to toxicology and safety studies, which will aid the fast-track development of this approach, given the severity of the human disease and the unmet clinical need? Another challenge is delivering clinical trials in a condition such as CPVT in which patients are rare, patients require genetic testing for confirmation of diagnosis that is not universally available, informed consent can be challenging in the setting of postarrest brain injury, phenotype penetrance for mutation-carrying relatives is variable, and end-point selection for short-term phase 2b efficacy studies can be challenging. A safety phase 2a trial in a small number of patients could potentially be a stepping stone to a definitive efficacy study with mortality or implantable cardioverter-defibrillator shock reduction as the primary end point, given the severity of the disease.

A major hurdle will also be economics. Development of a clinically applicable gene therapy vector requires a large program of preclinical toxicology screening, manufacturing to good manufacturing practices standards, batch review, audit and stability testing, and regulatory submissions and review before embarking on a first-in-human study. The costs for such a development program are exorbitant, measured in tens of millions of US dollars, and if the market is large, for example, patients with coronary artery disease or chronic heart failure, investment may be attractive to the financial and pharmaceutical industries. However, for a relatively small target patient population such as those with CPVT secondary to CASQ2 mutations, even if 1 product could be suitable for all mutation-carrying individuals, it is not clear whether the needed investments can be made. This is not insurmountable, given that the precedent of the only regulatory-approved clinical gene therapy product (Glybera) is for a rare condition, lipoprotein lipase deficiency. However, to accelerate the access of such products to patients at affordable prices, the medical community should work with regulatory agencies to simplify and fast-track the approval process of such biological treatments.

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**Disclosures**

Dr Hajjar is the scientific cofounder of Celladon Co, which is developing AAV1.SERCA2a for the treatment of heart failure. Dr Lyon reports no conflicts.

**References**


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