CRISPR/CAS9 THERAPY ELIMINATES VENTRICULAR TACHYCARDIA IN MOUSE MODEL OF CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA

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Background: Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited, stress-induced arrhythmia syndrome that can lead to sudden cardiac death. CPVT is most commonly caused by mutations in ryanodine receptor 2 (RyR2). RyR2 is a sarcoplasmic reticulum Ca2+ channel responsible for the activation of cardiac muscle contraction. Mutations in RyR2 lead to beta-adrenergic induced Ca2+ leak, leading to pro-arrhythmic conditions, including sudden cardiac death. Initial treatment is inadequate in 50% of patients with limited additional options for those with structural defects. The combination of adeno-associated virus type 9 (AAV9), a virus capable of delivering foreign DNA to the heart, and CRISPR/Cas9, which allows for targeted editing of DNA, is a powerful gene therapy platform. Using this platform, AAV9 can deliver CRISPR/Cas9-guide RNA (gRNA) plasmids to the heart and selectively reduce the expression of the mutant allele in RYR2-R176Q/+ (RQ) mice with CPVT. My hypothesis is that a carefully designed gRNA using AAV9-CRISPR/Cas9 can selectively target the mutant allele of RQ mice, reducing the expression of the mutant allele, and prevent VT in a mouse model of CPVT.

Materials/Methods: A gRNA was designed to target the mutant allele of RQ mice. This gRNA along with SaCas9 was packaged into an AAV9 vector and injected into RQ mice at 5 days. To assess RyR2 mRNA and protein levels, qPCR and western blotting was performed. To assess VT burden, adult mice underwent invasive electrophysiological testing after treatment with caffeine, isoproterenol, and epinephrine. To assess cardiac function, mice underwent echocardiogram assessment.

Results: Our gRNA effectively reduced mRNA and protein levels of RyR2, consistent with successful mutant allele disruption. The incidence of provoked VT was eliminated in our gene-edited mice (0/6) compared to a rate of provoked VT of 6/8 (80%) in our RQ positive controls. Ejection fraction measured by echocardiogram at 3, 6, and 9 months showed no difference between wildtype, RQ, and gene-edited groups.

Conclusions: Our gRNA design was effective in inducing mutant allele-specific disruption, decreasing the levels of mutant protein expressed in RQ mice. This resulted in the elimination of provoked VT in a CPVT mouse model and provides promising results for the future of gene editing as a potential permanent therapy for CPVT.