

pharmacotherapies (2,3). ICDs have frequent device/lead related complications, catheter ablations are subject to recurrence due to the generation of new arrhythmogenic foci, and heart transplantation has a 23% mortality rate 10 years post-procedure (3). These factors highlight the critical need to identify new prophylactic and therapeutic strategies that can target the molecular triggers (cell-cell junction disruption) of ARVC as a means to prevent or halt disease progression.

SUMMARY

[0007] The disclosure describes a recombinant adeno-associated virus (rAAV) vector comprising in 5' to 3' direction: a) a first AAV ITR sequence; b) a promoter sequence; c) a transgene nucleic acid molecule, wherein the transgene nucleic acid molecule comprises a nucleic acid sequence encoding for a plakophilin-2 (PKP2) polypeptide; d) a post-transcriptional regulatory element; e) a polyA sequence; and f) a second AAV ITR sequence.

[0008] The disclosure describes an rAAV vector comprising the nucleic acid sequence set forth in SEQ ID NO: 9, SEQ ID NO: 18, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 29, or SEQ ID NO: 30. In one aspect, the disclosure refers to a recombinant adeno-associated virus (rAAV) viral vector comprising a nucleic acid sequence that is at least 99% identical to SEQ ID NO: 9 and an AAVrh10 capsid protein.

[0009] In some examples, the PKP2 polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 13.

[0010] In some examples, the nucleic acid sequence encoding for a PKP2 polypeptide comprises the nucleic acid sequence set forth in SEQ ID NO: 4 or SEQ ID NO: 14.

[0011] In some examples, the first AAV ITR sequence comprises the nucleic acid sequence set forth in SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 15, SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 20, or SEQ ID NO: 25. In some examples, the second AAV ITR sequence comprises the nucleic acid sequence set forth in SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 15, SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 20, or SEQ ID NO: 25.

[0012] In some examples, the promoter sequence is a cardiac-specific promoter sequence.

[0013] In some examples, the promoter sequence comprises a Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), a cytomegalovirus (CMV) promoter, an SV40 promoter, a dihydrofolate reductase promoter, a beta-actin promoter, a phosphoglycerol kinase (PGK) promoter, a U6 promoter, an H1 promoter, a CAG promoter,
5 a hybrid chicken β -actin promoter, an MeCP2 promoter, an EF1 promoter, a ubiquitous

chicken β -actin hybrid (CBh) promoter, a U1a promoter, a U1b promoter, an MeCP2 promoter, an MeP418 promoter, an MeP426 promoter, a minimal MeCP2 promoter, a VMD2 promoter, an mRho promoter, EF1a promoter, Ubc promoter, human β -actin promoter, TRE promoter, Ac5 promoter, Polyhedrin promoter, CaMKIIa promoter, Gal1
5 promoter, TEF1 promoter, GDS promoter, ADH1 promoter, Ubi promoter, or α -1-antitrypsin (hAAT) promoter. In some examples, the promoter sequence comprises a cardiac troponin T (cTnT) promoter sequence. In some examples, the cTnT promoter sequence comprises the nucleic acid sequence set forth in SEQ ID NO: 2.

[0014] In some examples, the polyA sequence comprises a rabbit beta-globin polyA
10 sequence. In some examples, the rabbit beta-globin polyA sequence comprises the nucleic acid sequence set forth in SEQ ID NO: 6.

[0015] In some examples, the post-transcriptional regulatory element is an oPRE post-transcriptional regulatory element. In some examples, the oPRE post-transcriptional regulatory element comprises the nucleic acid sequence set forth in SEQ ID NO: 5, SEQ ID
15 NO: 27, or SEQ ID NO: 28.

The disclosure describes an rAAV vector of any one of the preceding claims, comprising, in the 5' to 3' direction: a) a first AAV ITR sequence comprising the nucleic acid sequence set forth in SEQ ID NO: 7; b) a promoter sequence comprising the nucleic acid sequence set forth in SEQ ID NO: 2; c) a transgene nucleic acid molecule, wherein the transgene
20 nucleic acid molecule comprises a nucleic acid sequence encoding for a PKP2 polypeptide, wherein the nucleic acid sequence encoding for a PKP2 polypeptide comprises the nucleic acid sequence set forth in SEQ ID NO: 4; d) a post-transcriptional regulatory element comprising the nucleic acid sequence set forth in SEQ ID NO: 5; e) a polyA sequence comprising the nucleic acid sequence set forth in SEQ ID NO: 6; and f) a second AAV ITR
25 sequence comprising the nucleic acid sequence set forth in SEQ ID NO: 8.

[0016] The disclosure describes an rAAV viral vector comprising (i) an AAV capsid protein; and (ii) an rAAV vector of any one of the preceding claims.

[0017] In some examples, the AAV capsid protein is an AAV1 capsid protein, an AAV2 capsid protein, an AAV4 capsid protein, an AAV5 capsid protein, an AAV6 capsid
30 protein, an AAV7 capsid protein, an AAV8 capsid protein, an AAV9 capsid protein, an AAV10 capsid protein, an AAV11 capsid protein, an AAV12 capsid protein, an AAV13 capsid protein, an AAVPHP.B capsid protein, an AAVrh74 capsid protein or an AAVrh10

capsid protein. In some examples, the AAV capsid protein is an AAV9 or AAVrh10 capsid protein.

[0018] The disclosure describes a pharmaceutical composition comprising: a) the rAAV viral vector of any embodiment of the disclosure; and at least one pharmaceutically acceptable excipient and/or additive.

[0019] The disclosure describes a method for treating a subject having a disease and/or disorder involving a *PKP2* gene, the method comprising administering to the subject at least one therapeutically effective amount of the rAAV viral vector of any embodiment of the disclosure or the pharmaceutical composition of any embodiment of the disclosure.

In one aspect, the disclosure refers to use of the rAAV viral vector described herein or the pharmaceutical composition described herein in the manufacture of a medicament for treating arrhythmogenic right ventricular cardiomyopathy (ARVC) in a subject in need thereof, wherein at least one therapeutically effective amount of the rAAV viral vector or the pharmaceutical composition is to be administered to the subject.

[0020] In some examples, the disease and/or disorder involving a *PKP2* gene is a cardiovascular disease characterized by abnormal cardiac cell-cell junction complexes.

[0021] In some examples, the disease and/or disorder involving a *PKP2* gene is arrhythmogenic right ventricular cardiomyopathy (ARVC).

[0022] In some examples, the effective amount improves electrical and structural cardiac integrity in the subject. In some examples, the effective amount rescues and reassembles cell-cell junction proteins in the subject. In some examples, the effective amount improves cardiac function in the subject. In some examples, the effective amount preserves electrical and structural integrity to prevent ARVC in the subject.

[0023] In some examples, the rAAV viral vector or the pharmaceutical composition is administered to the subject at a dose ranging from about 1.0×10^{12} vg/kg to about 2.5×10^{14} vg/kg. In some examples, the rAAV viral vector or the pharmaceutical composition is administered to the subject at a dose ranging from about 1.0×10^{12} vg/kg to about 5.0×10^{13} vg/kg.

[0024] In some examples, the rAAV viral vector or the pharmaceutical composition is administered to the subject intravenously, intrathecally, intracerebrally, intraventricularly, intranasally, intratracheally, intra-aurally, intra-ocularly, or peri-ocularly, orally, rectally, transmucosally, inhalationally, transdermally, parenterally, subcutaneously, intradermally,

intramuscularly, intracisternally, intranervally, intrapleurally, topically, intralymphatically, intracisternally or intranerve.

[0025] In some examples, the rAAV viral vector of any one of the embodiments of the disclosure or the pharmaceutical composition of any embodiments of the disclosure for
5 use in treating a disease and/or disorder involving a *PKP2* gene in a subject in need thereof.

[0026] In some examples, the disease and/or disorder involving a *PKP2* gene is ARVC.

[0027] In some examples, the rAAV viral vector or the pharmaceutical composition is for administration to the subject at a dose ranging from about 1.0×10^{12} vg/kg to about 2.5×10^{14} vg/kg. In some examples, the rAAV viral vector or the pharmaceutical composition is for administration to the subject at a dose ranging from about 1.0×10^{12} vg/kg to about 5.0×10^{13} vg/kg.

[0028] In some examples, the rAAV viral vector or the pharmaceutical composition is for administration to the subject intravenously, intrathecally, intracerebrally, intraventricularly, intranasally, intratracheally, intra-aurally, intra-ocularly, or peri-ocularly, orally, rectally, transmucosally, inhalationally, transdermally, parenterally, subcutaneously, intradermally, intramuscularly, intracisternally, intranervally, intrapleurally, topically, intralymphatically, intracisternally or intranerve. In some examples, the rAAV viral vector or pharmaceutical composition is for administration intravenously.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] FIGS. 1A-1C show Adeno(-associated) virus technology can stably express PKP2 protein. FIG. 1A shows a western blot analysis of mutant (MUT) and wild type (WT) PKP2 protein following adenovirus transduction of neonatal cardiomyocytes at indicated multiplicities of infection (MOI). FLAG antibody recognizes PKP2 and GAPDH serves as the loading control. FIG. 1B shows a schematic for early intraperitoneal injection of AAV9 PKP2 at postnatal day 2 (P2) in wild type (WT) mice and analysis of hearts 4 weeks later. FIG. 1C shows a western blot analysis of PKP2 protein following AAV9 PKP2 injection at postnatal day 2 (P2). PKP2 antibody recognizes endogenous and transduced PKP2, FLAG antibody recognizes only transduced PKP2, and GAPDH serves as the loading control.

[0030] FIG. 2 shows elevating PKP2 protein dose in vitro reassembles the cardiac cell-cell junction. Western blot analysis of desmosomal (PKP2, DSP, DSG2, JUP), fascia-adherens (N-Cad), and gap junction (CX43) proteins following transduction of PKP2 homozygous mutant neonatal cardiomyocytes (Hom CM) with either wild type (WT) or mutant (MUT) PKP2 adenovirus. GAPDH serves as the loading control.

[0031] FIGS. 3A-3C show early AAV9 PKP2 administration restores the cardiac cell-cell junction and improves cardiac morphology. FIG. 3A shows a schematic for early intraperitoneal injection of AAV9 PKP2 at postnatal day 2 (P2) in PKP2 homozygous

We Claim:

1. A recombinant adeno-associated virus (rAAV) viral vector comprising a nucleic acid sequence that is at least 99% identical to SEQ ID NO: 9 and an AAVrh10 capsid protein.
2. A pharmaceutical composition comprising:
 - a) the rAAV viral vector of claim 1; and at least one pharmaceutically acceptable excipient.
3. Use of the rAAV viral vector of claim 1 or the pharmaceutical composition of claim 2 in the manufacture of a medicament for treating arrhythmogenic right ventricular cardiomyopathy (ARVC) in a subject in need thereof, wherein at least one therapeutically effective amount of the rAAV viral vector or the pharmaceutical composition is to be administered to the subject.
4. The use of claim 3, wherein the rAAV viral vector or the pharmaceutical composition is to be administered to the subject at a dose ranging from 1.0×10^{12} vg/kg to 2.5×10^{14} vg/kg.
5. The use of claim 4, wherein the rAAV viral vector or the pharmaceutical composition is to be administered to the subject at a dose of 2.0×10^{13} vg/kg.
6. The use of claim 4, wherein the rAAV viral vector or the pharmaceutical composition is to be administered to the subject at a dose of 6.0×10^{13} vg/kg.
7. The use of any one of claims 3-6, wherein the rAAV viral vector or the pharmaceutical composition is to be administered to the subject intravenously, intrathecally, intracerebrally, intraventricularly, intranasally, intratracheally, intra-aurally, intra-ocularly, or peri-ocularly, orally, rectally, transmucosally, inhalationally, transdermally, parenterally, subcutaneously, intradermally, intramuscularly, intracisternally, intranervally, intrapleurally, topically, intralymphatically, intracisternally or intranerve.

8. The use of any one of claims 3-6, wherein the rAAV viral vector or the pharmaceutical composition is to be administered to the subject intravenously.