PLAKOPHILLIN-2 GENE THERAPY METHODS AND COMPOSITIONS

CROSS-REFERENCE

[0001] This patent application claims the benefit of U.S. Provisional Application No. 63/089,951, filed October 9, 2020, U.S. Provisional Application No. 63/172,053, filed April 7, 2021, U.S. Provisional Application No. 63/216,322, filed June 29, 2021, and U.S. Provisional Application No. 63/227,801, filed July 30, 2021, each of which is incorporated by reference herein in its entirety.

BACKGROUND

[0002] Arrhythmogenic right ventricular cardiomyopathy (ARVC) or arrhythmogenic cardiomyopathy (ACM) is an inherited cardiac disease found in 1/2000 to 1/5000 people. ARVC is characterized by fibrofatty tissue replacement in the myocardium, myocardial atrophy, predominant right ventricular dilation, ventricular arrhythmias, and sudden cardiac death (Wang et al., 2018). The disease is difficult to diagnose by conventional imaging and ECG particularly at its early stage due to its subclinical presentations. At the late stage, the disease progresses to more overt manifestations such as ventricular arrhythmias and morphological abnormalities in the ventricle. Sudden cardiac arrest in the young and athletes is found to be associated with ARVC and exercise-related cardiac wall stress. So far, there is no effective treatment of ARVC (Wang et al., 2018).

SUMMARY

[0003] In one aspect, there are provided methods for treating a heart disease or disorder in an individual in need thereof. In some embodiments, the method comprises administering a composition comprising (a) a gene therapy vector comprising a nucleic acid encoding a plakophilin 2 (PKP2) polypeptide or a fragment thereof operatively linked to a promoter and a 3' element; and (b) a pharmaceutically acceptable carrier or excipient. In some embodiments, the gene therapy vector comprises a viral vector selected from the group consisting of an adeno-associated virus, an adenovirus, a lentivirus, a pox virus, a vaccinia virus, and a herpes virus. In some embodiments, the gene therapy vector is an adeno-associated virus. In some embodiments, the adeno-associated virus is selected from the group consisting of an AAV6, an AAV8, and an AAV9. In some embodiments, the adeno-associated virus is an AAV9 having a nucleic acid sequence with at least 95% identity to SEQ ID NO: 7. In some embodiments, the heart disease or disorder is arrhythmogenic right ventricular cardiomyopathy (ARVC) or arrhythmogenic cardiomyopathy (ACM). In some embodiments, the promoter is a promoter that causes expression in tissues including the heart or a cardiac specific promoter. In some embodiments, the promoter causes expression in the myocardium, the epicardium, or both. In some embodiments, the cardiac specific promoter is a PKP2 promoter, a troponin promoter, or an alpha-myosin heavy chain promoter. In some embodiments, the PKP2 promoter has a nucleic acid sequence having at least 95% identity to SEQ ID NO: 4. In some embodiments, the troponin promoter has a nucleic acid sequence having at least 95% identity to SEQ ID NO: 3. In some embodiments, the 3' element comprises a Woodchuck Hepatitis Virus Posttransciptional Regulatory Element (WPRE), a bovine growth hormone polyadenylation (bGH

polyA) sequence, or a combination thereof. In some embodiments, the gene therapy vector further comprises a cardiac specific enhancer. In some embodiments, the nucleic acid encoding the PKP2 gene has a sequence having at least 95% identity to SEQ ID NO: 1 or SEQ ID NO: 2. In some embodiments, the nucleic acid has a size less than or equal to about 4.7 kb. In some embodiments, the pharmaceutically acceptable carrier or excipient comprises a buffer, a polymer, a salt, or a combination thereof. In some embodiments, the method reverses, reduces, or prevents at least one of fibrofatty tissue replacement; myocardial atrophy; predominant right ventricular dilation; ventricular arrhythmias; sudden cardiac death; exercise-triggered cardiac events; right ventricular cardiomyopathy, dilation, or heart failure; left ventricular cardiomyopathy, dilation, or heart failure; atrial arrhythmias; syncope; palpitations; shortness of breath; or chest pain. In some embodiments, the method reverses, reduces, or prevents fibrofatty tissue replacement in the myocardium, the epicardium, or both. In some embodiments, the method restores desmosome structure and/or function. In some embodiments, the method restores PKP2 mRNA expression and/or PKP2 protein and activity levels. In some embodiments, the method restores expression of one or more genes having a direct or indirect effect on one or more symptoms of the heart disease. In some embodiments, the gene comprises one or more of Ryanodine Receptor 2 (Ryr2), Ankyrin-B (Ank2), Cacna1c (CaV1.2), triadin (Trdn), or calsequestrin-2 (Casq2). In some embodiments, the individual is identified as having at least one variation in a desmosome protein. In some embodiments, the desmosome protein is PKP2. In some embodiments, the variation comprises a deletion, an insertion, a single nucleotide variation, or a copy number variation.

[0004] In one aspect, provided herein are methods for treating a heart disease or disorder in an individual in need thereof. In some embodiments, the method comprises administering a composition comprising a gene therapy vector comprising a nucleic acid encoding a plakophilin 2 (PKP2) polypeptide or a fragment thereof operatively linked to at least one promoter and a pharmaceutically acceptable carrier or excipient. In some embodiments, the gene therapy vector comprises a viral vector. In some embodiments, the viral vector is selected from the group consisting of an adeno-associated virus, an adenovirus, a lentivirus, a pox virus, a vaccinia virus, and a herpes virus. In some embodiments, the gene therapy vector is an adeno-associated virus. In some embodiments, the adeno-associated virus is selected from the group consisting of an AAV6, an AAV8, and an AAV9. In some embodiments, the adeno-associated virus is an AAV9 or a derivative thereof. In some embodiments, the AAV9 has a nucleic acid sequence with at least 95% identity to SEQ ID NO: 7. In some embodiments, the heart disease or disorder is arrhythmogenic right ventricular cardiomyopathy (ARVC) or arrhythmogenic cardiomyopathy (ACM). In some embodiments, the composition is administered intravenously, intracardially, pericardially, or intraarterially. In some embodiments, the promoter is a cardiac specific promoter. In some embodiments, the promoter causes expression in the myocardium, the epicardium, or both. In some embodiments, the cardiac specific promoter is a troponin promoter, or an alpha-myosin heavy chain promoter. In some embodiments, the troponin promoter has a nucleic acid sequence having at least 95% identity to SEQ ID NO: 3. In some embodiments, the promoter is a PKP2 promoter. In some embodiments, the PKP2 promoter has a nucleic acid sequence having at least 95% identity to SEQ

ID NO: 4. In some embodiments, the promoter is a constitutive promoter. In some embodiments, the constitutive promoter is a beta-actin promoter. In some embodiments, the gene therapy vector further comprises a cardiac specific enhancer. In some embodiments, the nucleic acid encoding the PKP2 gene has a sequence having at least 95% identity to SEQ ID NO: 1 or SEQ ID NO: 2. In some embodiments, the pharmaceutically acceptable carrier or excipient comprises a buffer, a polymer, a salt, or a combination thereof. In some embodiments, the method reverses, reduces, or prevents at least one of fibrofatty tissue replacement; myocardial atrophy; predominant right ventricular dilation; ventricular arrhythmias; sudden cardiac death; or exercise-triggered cardiac events; right ventricular cardiomyopathy, dilation, or heart failure; left ventricular cardiomyopathy, dilation, or heart failure; atrial arrhythmias; syncope; palpitations; shortness of breath; or chest pain. In some embodiments, the method reverses, reduces, or prevents fibrofatty tissue replacement in the myocardium, the epicardium, or both. In some embodiments, the method restores desmosome structure and/or function. In some embodiments, the method restores PKP2 mRNA expression and/or PKP2 protein and activity levels. In some embodiments, the method restores PKP2 induced gene expression. In some embodiments, the method restores expression of one or more genes having a direct or indirect effect on one or more symptoms of the heart disease. In some embodiments, the method restores expression of one or more of Ryanodine Receptor 2 (Ryr2), Ankyrin-B (Ank2), Cacna1c (CaV1.2), triadin (Trdn), or calsequestrin-2 (Casq2). In some embodiments, the individual is identified as having at least one variation in a desmosome protein. In some embodiments, the desmosome protein is PKP2. In some embodiments, the variation comprises a deletion, an insertion, a single nucleotide variation, or a copy number variation.

[0005] In another aspect, there are provided gene therapy vectors comprising a plakophilin 2 gene operatively linked to at least one promoter. In some embodiments, the gene therapy vector comprises a viral vector. In some embodiments, the viral vector is selected from the group consisting of an adeno-associated virus, an adenovirus, a lentivirus, a pox virus, a vaccinia virus, and a herpes virus. In some embodiments, the gene therapy vector is an adeno-associated virus. In some embodiments, the adeno-associated virus is selected from the group consisting of an AAV6, an AAV8, and an AAV9. In some embodiments, the adeno-associated virus is an AAV9 or a derivative thereof. In some embodiments, the AAV9 has a nucleic acid sequence with at least 95% identity SEQ ID NO: 7. In some embodiments, the promoter is a cardiac specific promoter. In some embodiments, the promoter causes expression in the myocardium, the epicardium, or both. In some embodiments, the cardiac specific promoter is a troponin promoter or an alpha-myosin heavy chain promoter. In some embodiments, the troponin promoter has a nucleic acid sequence having at least 95% identity to SEQ ID NO: 3. In some embodiments, the promoter is a PKP2 promoter. In some embodiments, the PKP2 promoter has a nucleic acid sequence having at least 95% identity to SEQ ID NO: 4. In some embodiments, the promoter is a constitutive promoter. In some embodiments, the constitutive promoter is an beta-actin promoter. In some embodiments, the gene therapy vector further comprises a cardiac specific enhancer. In some embodiments, the nucleic acid encoding the PKP2 gene has a sequence having at least 95% identity to SEQ ID NO: 1 or SEQ ID NO: 2. In some embodiments, the gene therapy vector is formulated in a

pharmaceutically acceptable carrier or excipient comprising a buffer, a polymer, a salt, or a combination thereof.

INCORPORATION BY REFERENCE

[0006] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0008] An understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0009] **FIG. 1** illustrates how cardiac desmosomes tie cells together.

[0010] **FIG. 2** shows a summary of ARVC disease indications and possible disease mechanisms.

[0011] **FIGS. 3A-3C** show the results of acute silencing of PKP2 in iPSCM at day 8. **FIG. 3A** shows the disappearance of DSP from the cellular membrane. **FIG. 3B** shows a graph illustrating the reduction in sarcomere density. **FIG. 3C** shows the disarray of cell compaction in patterned iPSCM.

[0012] **FIG. 4** shows a quantitative analysis of DSP membrane localization as determined by colocalization with PKG.

[0013] **FIG. 5** shows an immunoblot which illustrates a reduced total amount of DSP protein, detected mainly in the insoluble fraction, in cells where PKP2 is silenced.

[0014] **FIGS. 6A-6B** show results of PKP2 transduction by AAV. **FIG. 6A** shows a vector map of the AAV construct. **FIG. 6B** shows an immunofluorescence image of restoration of DSP membrane localization. **FIG. 6C** shows a quantification of total DSP intensity post PKP2 silencing and AAV-PKP2 transgene rescue.

[0015] **FIGs. 7A-7B** show results of PKP2 transduction by AAV on contraction velocity. **FIG. 7A** shows the experimental timeline. **FIG. 7B** shows two contractility assays which demonstrate functional rescue of reduced velocity post PKP2 silencing.

[0016] **FIG. 8** shows a second generation schematic of an AAV expression cassette of human and mouse PKP2α. The left panel shows all of the elements in the expression cassette. The right panel shows the arrangement of elements in the expression cassettes.

[0017] **FIG. 9A** and **FIG. 9B** show results of the second generation AAV-hPKP2α rescue of contraction velocity post PKP2 silencing in iPSC cardiomyocytes. **FIG. 9A** shows expression in soluble and insoluble fractions in cells transduced in different multiplicities of infection. **FIG. 9B** shows rescue of contraction velocity in cells post PKP2 silencing.

[0018] **FIG. 10** shows expression of the second generation AAV-PKP2α in wildtype mice.

[0019] **FIGS. 11A-11G** show results of pilot expression safety studies of the second generation AAV9 human and mouse PKP2α in wildtype mice. **FIG. 11A** shows body weight before and after AAV9 injection. **FIG. 11B** shows ejection fraction in mice treated with the AAV9 human or mouse PKP2α. **FIG. 11C** and **FIG. 11D** show LV structure measured by internal diameters end diastole and systole. **FIG. 11E, FIG. 11F,** and **FIG. 11G** show electrophysiology activity by QRS (**11E**), QT interval (**11F**) and P/R amplitude (**11G**).

[0020] **FIG. 12** shows a Kaplan-Meier survival curve of *PKP2-cKO* mice.

[0021] **FIGS. 13A-13B** show right ventricle (RV) dilated cardiomyopathy of *PKP2-cKO* mice. **FIG. 13A** (left panel) shows images that illustrate increased RV internal dimension at end-diastole (RVIDd) in *PKP2-cKO* mice. **FIG. 13A** (right panel) shows a graph of RVIDd over time in *PKP2-cKO* mice. **FIG. 13B** (left panel) shows images illustrating the increase in RV area in *PKP2-cKO* mice. **FIG. 13B** (right panel) shows a graph of RV area over time in *PKP2-cKO* mice.

[0022] **FIGS. 14A-14B** show development of left ventricle (LV) dilated cardiomyopathy of *PKP2-cKO* mice compared with control. **FIG. 14A** (left panel) shows images of increased LV internal dimension at end-systole (LVIDs) and end-diastole (LVIDd) in *PKP2-cKO* mice. **FIG. 14A** (right panel) shows a graph which shows the increase in LVIDs and LVIDd in *PKP2-cKO* mice over time. **FIG. 14B** shows a graph of LV performance as measured by percent ejection fraction over time.

[0023] **FIG. 15** shows development of severe electrophysiological phenotypes of *PKP2-cKO* mice compared with control, specifically prolonged QRS interval and increased P/R amplitude ratio in *PKP2-cKO* mice. The top panel shows exemplary electrocardiogram of control and *PKP2-cKO* mice. The bottom panel shows graphs of the increase in QRS interval and increase in P/R amplitude in *PKP2-cKO* mice compared with control.

[0024] **FIGS. 16A-16C** show enhanced expression of fibrosis, tissue remodeling genes, and heart failure markers. **FIG. 16A** shows PKP2 RNA expression in RV and LV (top) and desmosome and Cx43 protein expression (bottom) of *PKP2-cKO* mice compared with control. **FIG. 16B** shows enhanced expression of fibrosis genes: TGFβ1, Col1a1, and Col3a1; and tissue remodeling genes: Timp1 and Mmp2 in *PKP2-cKO* mice compared with control. **FIG. 16C** shows enhanced expression of heart failure markers, NPPA and NPPB, in *PKP2-cKO* mice compared with control mice.

[0025] **FIG. 17** shows the experimental design to evaluate PKP2 efficacy as gene therapy in the *PKP2-cKO* ARVC mouse model.

[0026] **FIG. 18A** shows a schematic of the AAV expression cassettes for human and mouse PKP2α. **FIG. 18B** shows immunoblots of protein expression of mouse and human PKP2α from mice treated with AAV9:PKP2.

[0027] **FIG. 19** shows a Kaplan-Meier survival curve of *PKP2-cKO* mice treated with AAV9:PKP2.

[0028] **FIGS. 20A-20C** show the efficacy of AAV9:PKP2 treatment of *PKP2-cKO* mice in reducing RV and LV dilation and maintaining cardiac function. **FIG. 20A** shows a graph illustrating improvement in ejection fraction in AAV9:PKP2 treated mice. **FIG. 20B** shows a graph illustrating reduction of RV

dilation in AAV9:PKP2 treated mice. **FIG. 20C** shows graphs illustrating improvement in LVIDd (top) and LVIDs (bottom).

[0029] **FIGS. 21A-21B** show improvement in ECG parameters of *PKP2-cKO* mice treated with AAV:PKP2. **FIG. 21A** shows exemplary raw ECG traces of control and *PKP2-cKO* mice treated with AAV9:mPKP2 and buffer. **FIG. 21B** shows graphs illustrating improvement of P/R ratio, QT interval, and QRS interval in *PKP2-cKO* mice treated with AAV9:PKP2 compared with treatment with buffer.

[0030] **FIGS. 22A-22B** show AAV9:PKP2 treatment improvement in arrhythmias in *PKP2-cKO* mice. **FIG. 22A** (top) shows a table grading of severity of arrhythmias. **FIG. 22A** (bottom) shows a graph which summarizes improvement of arrhythmia scores of *PKP2-cKO* mice treated with AAV9:PKP2 compared with control. **FIG. 22B** shows a distribution graph showing improvement in severity of arrhythmias in *PKP2-cKO* mice treated with AAV9:PKP2 compared with control. Each dot represents an animal.

[0031] **FIG. 23** shows the experimental design used to evaluate human PKP2 efficacy as a gene therapy using the *PKP2-cKO* ARVC mouse model.

[0032] **FIGS. 24A-24D** show results of AAV9:hPKP2 gene therapy treatment of *PKP2-cKO* mice. **FIG. 24A** shows results of ejection fraction. **FIG. 24B** show results of right ventricle size. **FIG. 24C** shows LV dilation as measured by LVIDd. **FIG. 24D** shows LV dilation as measured by LVIDs.

[0033] **FIG. 25** shows results of AAV9:hPKP2 gene therapy treatment of *PKP2-cKO* mice for QT interval (top), P/R Ratio (middle), and Arrhythmia Score (bottom).

[0034] **FIGS. 26A-26B** show results of AAV9:hPKP2 treatment of *PKP2-cKO* mice in reducing expression of heart failure markers, fibrosis and tissue remodeling markers in right ventricle (**FIG. 26A**) and left ventricle (**FIG. 26B**).

[0035] **FIGS. 27A-27B** shows results of AAV9:hPKP2 treatment of *PKP2-cKO* mice in reducing fibrosis development. **FIG. 27A** shows histological images of muscle from control and *PKP2-cKO* mice with and without AAV9:hPKP2 treatment. **FIG. 27B** shows a graph of collagen positive tissue from control and *PKP2-cKO* mice with and without AAV9:hPKP2 treatment.

[0036] **FIGS. 28A-28B** show expression of PKP2 and other desmosome proteins in soluble fraction (**FIG. 28A**) and insoluble fraction (**FIG. 28B**).

DETAILED DESCRIPTION

[0037] The most common genetic basis of arrhythmogenic right ventricular cardiomyopathy (ARVC) is mutations in genes encoding desmosomal proteins. Functionally, desmosomes are adhesive intercellular connections that hold intercalated cardiomyocytes together. Plakophillin-2 (PKP2), one of desmosomal genes, is most frequently identified as the causal factor for ARVC. Internal to the membrane-located complex, PKP2 interacts with desmosomal proteins, plakoglobin (PKG) and desmoplakin (DSP). DSP anchors the intermediate filaments, desmin, which form an interwoven network to stabilize the contractile units of cardiac cells, sarcomeres, and other organelles (FIG. 1, Brodehl et al., 2018; Moncayo-Arlandi and Brugada, 2017). It is believed that the loss of desmosome impacts cell-cell adhesion, signal transduction, and electrical coupling of cardiomyocytes (Wang et al., 2018).

Furthermore, the lost signal transduction and electrical coupling are joint defective outcomes by additional collapse of connexin-containing Gap junctions (GJs). GJs are essential in electrically coupling cells and facilitate synchronous beating by allowing flow of small molecules between cells (Green et al., 2019) (FIG. 2 summary on ARVC disease indication and possible mechanisms). In addition, epicardial differentiation can contribute to fibrofatty remodeling that is observed in ARVC or ACM patients (Kohela et al., 2021).

[0038] To delineate the functionality of desmosomes, genetic mouse lines and patient-derived iPSCM models were generated. Cardiac knock-out mouse model of PKP2 (the Delmar mouse model, Cerrone et al., 2017) showed profound early development of biventricular dilation, fibrosis, and a significant reduction of genes regulating Ca^{2+} homeostasis, revealing underling mechanisms for arrhythmias possibly before overt structural changes. Several patient-derived iPSCM lines harboring PKP2 mutations showed reduction of PKP2 expression, Ca^{2+} handling defects, and lipid droplet accumulation induced by culturing in lipogenic induction media (Brodehl et al., 2019).

[0039] Reduction of PKP2 at both mRNA and protein level was reported in ARVC patient heart samples with PKP2 mutations (Akdis et al., 2016; Asimaki et al., 2009). Nonsense-mediated mRNA-decay (NMD) was proposed for some desmosomal gene mutations including PKP2 mutations, suggesting a much less known cellular mechanism in balancing expression of mutated transcripts and proteins (Gerull and Brodehl, 2020; Mura et al., 2003). Those observations suggest a possibility of gene therapy-based intervention of ARVC by restoring expression level of WT PKP2 in heart.

Methods of Treatment

[0040] PKP2 gene therapy vectors provided herein in various aspects are useful for treating an individual with a heart disease or condition. "Treating" or "treatment of a condition or subject in need thereof" refers to (1) taking steps to obtain beneficial or desired results, including clinical results such as the reduction of symptoms; (2) preventing the disease, for example, causing the clinical symptoms of the disease not to develop in a patient that is predisposed to the disease, for example a carrier of a genetic mutation in a desmosome gene such as PKP2, but does not yet experience or display symptoms of the disease; (3) inhibiting the disease, for example, arresting or reducing the development of the disease or its clinical symptoms; (4) relieving the disease, for example, causing regression of the disease or its clinical symptoms; or (5) delaying the disease. In one aspect, provided herein are methods for treating a heart disease or disorder in an individual in need thereof. In some cases, the method comprises administering a composition comprising a gene therapy vector comprising a nucleic acid encoding a plakophilin 2 (PKP2) polypeptide or a fragment thereof operatively linked to at least one promoter and a pharmaceutically acceptable carrier or excipient. In some cases, the heart disease or disorder is arrhythmogenic right ventricular cardiomyopathy (ARVC) or arrhythmogenic cardiomyopathy (ACM). In some cases, methods of treatment herein reduce at least one symptom of a arrhythmogenic cardiomyopathy, including but not limited to the method reverses, reduces, or prevents at least one of fibrofatty tissue replacement; myocardial atrophy; predominant right ventricular dilation; ventricular arrhythmias; sudden cardiac death; or exercise-triggered cardiac events; right ventricular

cardiomyopathy, dilation, or heart failure; left ventricular cardiomyopathy, dilation, or heart failure; atrial arrhythmias; syncope; palpitations; shortness of breath; or chest pain. In some embodiments, the method reverses, reduces, or prevents fibrofatty tissue replacement in the myocardium, the epicardium, or both. In some cases, the method restores desmosome structure and/or function. In some cases, the method restores PKP2 mRNA expression and/or PKP2 protein and activity levels. In some cases, the method restores PKP2 induced gene expression. In some cases, PKP2 induced gene expression comprises expression of genes whose expression are direct or indirect causal factors leading to one or more disease phenotypes. In some embodiments, the method restores expression of one or more genes having a direct or indirect effect on one or more symptoms of the heart disease. In some cases, the method restores expression of one or more of Ryanodine Receptor 2 (Ryr2), Ankyrin-B (Ank2), Cacna1c (CaV1.2), triadin (Trdn), or calsequestrin-2 (Casq2).

[0041] In some embodiments of methods of treatment provided herein, the gene therapy vector comprises a viral vector. Any suitable viral vector is contemplated for use in methods herein including but not limited to a viral vector selected from the group consisting of an adeno-associated virus, an adenovirus, a lentivirus, a pox virus, a vaccinia virus, and a herpes virus. In some cases, the gene therapy vector is an adeno-associated virus. In some cases, the adeno-associated virus is selected from the group consisting of an AAV6, an AAV8, and an AAV9, or a derivative thereof. In some cases, the adeno-associated virus is an AAV9 or a derivative thereof. In some cases, the AAV9 has a nucleic acid sequence with at least 80%, 85%, 90%, 95%, or 99% identity to SEQ ID NO: 7. In some cases, the adeno-associated virus is modified to improve transduction of affected cells in the myocardium or the epicardium, such as cardiomyocytes, for example, in some cases, the adeno-associated virus is a derivative of an AAV6, an AAV8, or an AAV9. In some cases, the derivative is any AAV described in U.S. Patent Application No. 63/012,703, which is hereby incorporated by reference in its entirety.

[0042] In some embodiments or methods of treatment provided herein, the composition comprising a gene therapy vector is administered through any suitable route to reach the affected cells. For example, in some cases, the composition is administered intravenously, intracardially, pericardially, or intraarterially.

[0043] In some embodiments of methods of treatment provided herein, PKP2 is expressed by any promoter suitable for expression in the affected cells and tissues in the myocardium or the epicardium, for example cardiomyocytes. For example, in some cases, the promoter is a cardiac specific promoter. In some cases, the cardiac specific promoter is a troponin promoter or an alpha-myosin heavy chain promoter. In some cases, the promoter is a PKP2 promoter. In some cases, a cardiac specific enhancer is combined with the promoter. In some cases, the troponin promoter has a nucleic acid sequence having at least 80%, 85%, 90%, 95%, or 99% identity to SEQ ID NO: 3. In some cases, the PKP2 promoter has a nucleic acid sequence having at least 80%, 85%, 90%, 95%, or 99% identity to SEQ ID NO: 4. In some cases, the promoter is a constitutive promoter. In some cases, the constitutive promoter is a beta-actin promoter.

[0044] In some embodiments of methods of treatment provided herein the nucleic acid encoding the PKP2 gene has any suitable sequence encoding a PKP2 polypeptide for example, any nucleic acid encoding a polypeptide having a sequence of SEQ ID NO: 8. For example, in some cases, the PKP2 gene has a sequence having at least 80%, 85%, 90%, 95%, or 99% identity to SEQ ID NO: 1. In some cases, the PKP2 gene has a sequence having at least 80%, 85%, 90%, 95%, or 99% identity to SEQ ID NO: 2. In some cases, the nucleic acid sequence encoding the PKP2 gene is codon optimized.

[0045] In some embodiments of methods of treatment provided herein, the gene therapy vector has a gene expression cassette having a size of about 3 kb to about 5 kb. In some embodiments, the gene expression cassette has a size of about 4 kb to about 5 kb. In some embodiments, the gene expression cassette has a size of about 4.2 kb to about 4.8 kb. In some embodiments, the gene expression cassette has a size of about 4.5 kb. In some embodiments, the gene expression cassette has a size no larger than about 5 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.9 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.8 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.7 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.6 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.5 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.4 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.3 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.2 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.1 kb. In some embodiments, the gene expression cassette has a size no larger than about 4 kb. In some embodiments, the gene expression cassette has a size no larger than about 3.9 kb. In some embodiments, the gene expression cassette has a size no larger than about 3.8 kb. In some embodiments, the gene expression cassette has a size no larger than about 3.7 kb. In some embodiments, the gene expression cassette has a size no larger than about 3.6 kb. In some embodiments, the gene expression cassette has a size no larger than about 3.5 kb. In some embodiments, the gene expression cassette has a size of at least about 3.1 kb. In some embodiments, the gene expression cassette has a size of at least about 3.3 kb. In some embodiments, the gene expression cassette has a size of at least about 3.5 kb. In some embodiments, the gene expression cassette has a size of at least about 3.7 kb. In some embodiments, the gene expression cassette has a size of at least about 3.9 kb. In some embodiments, the gene expression cassette has a size of at least about 4.1 kb. In some embodiments, the gene expression cassette has a size of at least about 4.2 kb. In some embodiments, the gene expression cassette has a size of at least about 4.3 kb. In some embodiments, the gene expression cassette has a size of at least about 4.4 kb. In some embodiments, the gene expression cassette has a size of at least about 4.5 kb. In some embodiments, the gene expression cassette has a size of at least about 4.6 kb. In some embodiments, the gene expression cassette has a size of at least about 4.7 kb. In some embodiments, the gene expression cassette has a size of at least about 4.8 kb. In some embodiments, the gene expression cassette has a size of at least about 4.9 kb. In some embodiments, the gene expression cassette has a size of at least about 5 kb.

[0046] In various embodiments of methods herein, the gene therapy vector comprising a PKP2 gene is formulated in a composition comprising a pharmaceutically acceptable carrier or excipient. For example, in some cases, the pharmaceutically acceptable carrier or excipient comprises a buffer, a polymer, a salt, or a combination thereof.

[0047] In some embodiments of methods of treatment provided herein, the individual is identified as having at least one variation in a desmosome protein. In some cases, the desmosome protein is PKP2. In some cases, the variation comprises a deletion, an insertion, a single nucleotide variation, or a copy number variation. In some cases, the individual is identified as having at least one variation in a desmosome protein via DNA sequencing, PCR, qPCR, in situ hybridization, or another other suitable method of identifying a gene variation in an individual.

Gene Therapy Vectors

[0048] In another aspect, there are provided gene therapy vectors comprising a plakophilin 2 gene operatively linked to at least one promoter. In some cases, the gene therapy vector comprises a viral vector. In some cases, the viral vector is any suitable viral vector for treating a heart disease or condition. In some cases, the viral vector is suitable for delivering a gene to cells in the myocardium, the epicardium, or both. In some cases, the viral vector is selected from the group consisting of an adeno-associated virus, an adenovirus, a lentivirus, a pox virus, a vaccinia virus, and a herpes virus. In some cases, the gene therapy vector is an adeno-associated virus. In some cases, the adeno-associated virus is selected from the group consisting of an AAV6, an AAV8, and an AAV9, or a derivative thereof. In some cases, the adeno-associated virus is an AAV9 or a derivative thereof. In some cases, the AAV9 has a nucleic acid sequence with at least 95% identity SEQ ID NO: 7. In some cases, the adeno-associated virus is a derivative of AAV6, AAV8, or AAV9, optimized for transducing cells according to methods of treatment herein. In some cases, the derivative is any AAV described in U.S. Patent Application No. 63/012,703, which is hereby incorporated by reference in its entirety.

[0049] In some embodiments of gene therapy vectors provided herein, PKP2 is expressed by any promoter suitable for expression in the affected cells and tissues, for example cardiomyocytes. In some cases, PKP2 is expressed by a promoter that is active in cells of the myocardium, the epicardium, or both. For example in some cases, the promoter is a cardiac specific promoter. In some cases, the cardiac specific promoter is a troponin promoter or an alpha-myosin heavy chain promoter. In some cases, the promoter is a PKP2 promoter. In some cases, a cardiac specific enhancer is combined with the promoter. In some cases, the troponin promoter has a nucleic acid sequence having at least 80%, 85%, 90%, 95%, or 99% identity to SEQ ID NO: 3. In some cases, the PKP2 promoter has a nucleic acid sequence having at least 80%, 85%, 90%, 95%, or 99% identity to SEQ ID NO: 4. In some cases, the promoter is a constitutive promoter. In some cases, the constitutive promoter is a beta-actin promoter.

[0050] In some embodiments of gene therapy vectors provided herein the nucleic acid encoding the PKP2 gene has any suitable sequence encoding a PKP2 polypeptide for example, any nucleic acid encoding a polypeptide having a sequence of SEQ ID NO: 8. For example, in some cases, the PKP2 gene has a sequence having at least 80%, 85%, 90%, 95%, or 99% identity to SEQ ID NO: 1. In some

cases, the PKP2 gene has a sequence having at least 80%, 85%, 90%, 95%, or 99% identity to SEQ ID NO: 2. In some cases, the nucleic acid sequence encoding the PKP2 gene is codon optimized.

[0051] In some embodiments of gene therapy vectors provided herein, the gene therapy vector comprises a 3' element. In some embodiments, the 3' element stabilizes the transcriptional product of the gene therapy vector (e.g., the PKP2 transcript). In some embodiments, the 3' element comprises a bovine growth hormone (BGH) polyadenylation sequence. In some embodiments, the 3' element comprises a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE).

[0052] In some embodiments of gene therapy vectors provided herein, the gene therapy vector has a gene expression cassette having a size of about 3 kb to about 5 kb. In some embodiments, the gene expression cassette has a size of about 4 kb to about 5 kb. In some embodiments, the gene expression cassette has a size of about 4.2 kb to about 4.8 kb. In some embodiments, the gene expression cassette has a size of about 4.5 kb. In some embodiments, the gene expression cassette has a size no larger than about 5 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.9 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.8 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.7 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.6 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.5 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.4 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.3 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.2 kb. In some embodiments, the gene expression cassette has a size no larger than about 4.1 kb. In some embodiments, the gene expression cassette has a size no larger than about 4 kb. In some embodiments, the gene expression cassette has a size no larger than about 3.9 kb. In some embodiments, the gene expression cassette has a size no larger than about 3.8 kb. In some embodiments, the gene expression cassette has a size no larger than about 3.7 kb. In some embodiments, the gene expression cassette has a size no larger than about 3.6 kb. In some embodiments, the gene expression cassette has a size no larger than about 3.5 kb. In some embodiments, the gene expression cassette has a size of at least about 3.1 kb. In some embodiments, the gene expression cassette has a size of at least about 3.3 kb. In some embodiments, the gene expression cassette has a size of at least about 3.5 kb. In some embodiments, the gene expression cassette has a size of at least about 3.7 kb. In some embodiments, the gene expression cassette has a size of at least about 3.9 kb. In some embodiments, the gene expression cassette has a size of at least about 4.1 kb. In some embodiments, the gene expression cassette has a size of at least about 4.2 kb. In some embodiments, the gene expression cassette has a size of at least about 4.3 kb. In some embodiments, the gene expression cassette has a size of at least about 4.4 kb. In some embodiments, the gene expression cassette has a size of at least about 4.5 kb. In some embodiments, the gene expression cassette has a size of at least about 4.6 kb. In some embodiments, the gene expression cassette has a size of at least about 4.7 kb. In some embodiments, the gene expression cassette has a size of at least about 4.8 kb. In some embodiments, the gene expression cassette has a size of at least about 4.9 kb. In some embodiments, the gene expression cassette has a size of at least about 5 kb.

[0053] In various embodiments of gene therapy vectors provided herein, the gene therapy vector comprising a PKP2 gene is formulated in a composition comprising a pharmaceutically acceptable carrier or excipient. For example, in some cases, the pharmaceutically acceptable carrier or excipient comprises a buffer, a polymer, a salt, or a combination thereof.

[0054] In some embodiments, gene therapy vectors herein comprise nucleic acid sequences provided in Table 1 below.

Table 1: Sequences

Name	Sequence	SEQ ID NO:
Human PKP2	ATGGCAGCCCCCGGCGCCCCAGCTGAGTACGGCTACATCCGGAC CGTCCTGGGCCAGCAGATCCTGGGACAACTGGACAGCTCCAGCC TGGCGCTGCCCTCCGAGGCCAAGCTGAAGCTGGCGGGGAGCAGC GGCCGCGGCGGCCAGACAGTCAAGAGCCTGCGGATCCAGGAGC AGGTGCAGCAGACCCTCGCCCGGAAGGGCCGCAGCTCCGTGGGC AACGGAAATCTTCACCGAACCAGCAGTGTTCCTGAGTATGTCTA CAACCTACACTTGGTTGAAAATGATTTTGTTGGAGGCCGTTCCCC TGTTCCTAAAACCTATGACATGCTAAAGGCTGGCACAACTGCCA CTTATGAAGGTCGCTGGGGAAGAGGAACAGCACAGTACAGCTCC CAGAAGTCCGTGGAAGAAAGGTCCTTGAGGCATCCTCTGAGGAG ACTGGAGATTTCTCCTGACAGCAGCCCGGAGAGGGCTCACTACA CGCACAGCGATTACCAGTACAGCCAGAGAAGCCAGGCTGGGCA CACCCTGCACCACCAAGAAAGCAGGCGGGCCGCCCTCCTAGTGC CACCGAGATATGCTCGTTCCGAGATCGTGGGGGTCAGCCGTGCT GGCACCACAAGCAGGCAGCGCCACTTTGACACATACCACAGACA GTACCAGCATGGCTCTGTTAGCGACACCGTTTTTGACAGCATCCC TGCCAACCCGGCCCTGCTCACGTACCCCAGGCCAGGGACCAGCC GCAGCATGGGCAACCTCTTGGAGAAGGAGAACTACCTGACGGC AGGGCTCACTGTCGGGCAGGTCAGGCCGCTGGTGCCCCTGCAGC CCGTCACTCAGAACAGGGCTTCCAGGTCCTCCTGGCATCAGAGC TCCTTCCACAGCACCCGCACGCTGAGGGAAGCTGGGCCCAGTGT CGCCGTGGATTCCAGCGGGAGGAGAGCGCACTTGACTGTCGGCC AGGCGGCCGCAGGGGGAAGTGGGAATCTGCTCACTGAGAGAAG CACTTTCACTGACTCCCAGCTGGGGAATGCAGACATGGAGATGA CTCTGGAGCGAGCAGTGAGTATGCTCGAGGCAGACCACATGCTG CCATCCAGGATTTCTGCTGCAGCTACTTTCATACAGCACGAGTGC TTCCAGAAATCTGAAGCTCGGAAGAGGGTTAACCAGCTTCGTGG CATCCTCAAGCTTCTGCAGCTCCTAAAAGTTCAGAATGAAGACG TTCAGCGAGCTGTGTGTGGGGCCTTGAGAAACTTAGTATTTGAA GACAATGACAACAAATTGGAGGTGGCTGAACTAAATGGGGTAC CTCGGCTGCTCCAGGTGCTGAAGCAAACCAGAGACTTGGAGACT AAAAAACAAATAACAGGTTTGCTGTGGAATTTGTCATCTAATGA CAAACTCAAGAATCTCATGATAACAGAAGCATTGCTTACGCTGA CGGAGAATATCATCATCCCCTTTTCTGGGTGGCCTGAAGGAGAC TACCCAAAAGCAAATGGTTTGCTCGATTTTGACATATTCTACAAC GTCACTGGATGCCTAAGAAACATGAGTTCTGCTGGCGCTGATGG GAGAAAAGCGATGAGAAGATGTGACGGACTCATTGACTCACTG GTCCATTATGTCAGAGGAACCATTGCAGATTACCAGCCAGATGA CAAGGCCACGGAGAATTGTGTGTGCATTCTTCATAACCTCTCCTA CCAGCTGGAGGCAGAGCTCCCAGAGAAATATTCCCAGAATATCT ATATTCAAAACCGGAATATCCAGACTGACAACAACAAAAGTATT GGATGTTTTGGCAGTCGAAGCAGGAAAGTAAAAGAGCAATACC AGGACGTGCCGATGCCGGAGGAAAAGAGCAACCCCAAGGGCGT GGAGTGGCTGTGGCATTCCATTGTTATAAGGATGTATCTGTCCTT	1

	GATCGCCAAAAGTGTCCGCAACTACACACAAGAAGCATCCTTAG GAGCTCTGCAGAACCTCACGGCCGGAAGTGGACCAATGCCGACA TCAGTGGCTCAGACAGTTGTCCAGAAGGAAAGTGGCCTGCAGCA CACCCGAAAGATGCTGCATGTTGGTGACCCAAGTGTGAAAAAGA CAGCCATCTCGCTGCTGAGGAATCTGTCCCGGAATCTTTCTCTGC AGAATGAAATTGCCAAAGAAACTCTCCCTGATTTGGTTTCCATC ATTCCTGACACAGTCCCGAGTACTGACCTTCTCATTGAAACTACA GCCTCTGCCTGTTACACATTGAACAACATAATCCAAAACAGTTA CCAGAATGCACGCGACCTTCTAAACACCGGGGGCATCCAGAAAA TTATGGCCATTAGTGCAGGCGATGCCTATGCCTCCAACAAAGCA AGTAAAGCTGCTTCCGTCCTTCTGTATTCTCTGTGGGCACACACG GAACTGCATCATGCCTACAAGAAGGCTCAGTTTAAGAAGACAGA TTTTGTCAACAGCCGGACTGCCAAAGCCTACCACTCCCTTAAAG ACTGA	
Human PKP2 (codon optimized)	ATGGCTGCTCCTGGTGCTCCTGCCGAGTACGGCTACATCAGAAC AGTGCTGGGCCAGCAGATCCTGGGACAGCTGGATTCTAGCTCTC TGGCCCTGCCTTCTGAGGCCAAGCTGAAACTGGCCGGCAGTTCT GGAAGAGGCGGCCAGACAGTGAAGTCCCTGCGGATCCAAGAAC AGGTGCAGCAGACCCTGGCCAGAAAGGGCAGATCTTCTGTCGGC AACGGCAACCTGCACAGAACCAGCTCTGTGCCCGAGTACGTGTA CAATCTGCACCTGGTGGAAAACGACTTCGTCGGCGGCAGATCCC CTGTGCCTAAGACCTACGATATGCTGAAGGCCGGCACCACCGCC ACCTATGAAGGCAGATGGGGAAGAGGCACAGCCCAGTACAGCA GCCAGAAAAGCGTGGAAGAGAGAAGCCTGCGGCACCCTCTGCG GAGACTGGAAATCAGCCCTGATAGCAGCCCAGAGAGAGCCCAC TACACCCACAGCGACTACCAGTACTCCCAGAGATCTCAGGCCGG CCACACACTGCACCACCAAGAGTCTAGAAGGGCCGCTCTGCTGG TGCCTCCTAGATACGCCAGATCTGAGATCGTGGGCGTGTCCAGA GCCGGCACAACAAGCAGACAGAGACACTTCGACACCTACCACC GGCAGTATCAGCACGGCAGCGTGTCCGATACCGTGTTCGATAGC ATCCCCGCCAATCCTGCTCTGCTGACATACCCTAGACCTGGCACC TCCAGATCCATGGGCAATCTGCTGGAAAAAGAGAACTACCTGAC CGCCGGACTGACCGTGGGACAAGTTCGACCTCTGGTTCCTCTGC AGCCCGTGACACAGAACAGAGCCAGCAGAAGCAGCTGGCACCA GTCCAGCTTCCACAGCACCAGAACACTGAGAGAAGCTGGCCCTA GCGTGGCCGTGGATTCTTCTGGTAGAAGGGCTCACCTGACAGTT GGCCAAGCAGCTGCAGGCGGAAGCGGAAATCTGCTGACCGAGA GAAGCACCTTCACCGACAGCCAGCTGGGCAACGCCGACATGGA AATGACACTGGAACGGGCCGTGTCCATGCTGGAAGCCGATCACA TGCTGCCCAGCAGAATTAGCGCCGCTGCCACCTTTATCCAGCAC GAGTGCTTCCAGAAGTCTGAGGCCCGGAAGAGAGTGAACCAGCT GAGAGGCATCCTGAAGCTGCTGCAGCTCCTGAAGGTGCAGAACG AGGATGTGCAGAGGGCTGTGTGTGGGGCCCTGAGAAATCTGGTG TTCGAGGACAACGACAACAAGCTGGAAGTGGCCGAGCTGAACG GCGTGCCAAGACTGCTGCAGGTTCTGAAACAGACCCGCGACCTG GAAACAAAGAAGCAGATCACCGGCCTGCTCTGGAACCTGAGCA GCAACGACAAGCTGAAGAACCTGATGATCACAGAGGCCCTGCTG ACCCTGACAGAGAACATCATCATCCCTTTCAGCGGCTGGCCCGA GGGCGATTACCCTAAAGCTAATGGCCTGCTGGACTTCGACATCT TCTACAACGTGACCGGCTGCCTGAGAAACATGTCTAGCGCTGGC GCCGATGGCAGAAAGGCCATGAGAAGATGTGACGGCCTGATCG ACAGCCTGGTGCACTATGTGCGGGGCACAATCGCCGATTACCAG CCTGATGATAAGGCCACCGAGAACTGCGTGTGCATCCTGCACAA CCTGAGCTACCAGCTGGAAGCAGAGCTGCCCGAGAAGTACAGCC AGAACATCTACATCCAGAACCGGAACATCCAGACCGACAACAA CAAGAGCATCGGCTGCTTCGGCAGCCGCAGCCGGAAAGTGAAA GAACAGTACCAGGACGTGCCCATGCCTGAGGAAAAGTCTAACCC	2

	CAAAGGCGTGGAATGGCTGTGGCACAGCATCGTGATCCGGATGT ACCTGAGCCTGATCGCCAAGAGCGTGCGGAATTACACCCAAGAG GCATCTCTGGGCGCCCTGCAGAATCTGACAGCAGGATCTGGCCC TATGCCTACCTCTGTGGCTCAGACCGTGGTGCAGAAAGAGTCTG GCCTGCAGCACACCCGGAAGATGCTGCATGTGGGAGATCCCAGC GTGAAGAAAACCGCCATCAGCCTGCTGAGAAACCTGAGCCGGA ATCTGTCTCTGCAGAATGAGATCGCCAAAGAGACACTGCCCGAC CTGGTGTCTATCATCCCTGACACCGTGCCTAGCACCGACCTGCTG ATTGAGACAACAGCCAGCGCCTGCTACACCCTGAACAACATCAT TCAGAACTCCTACCAGAACGCCCGCGATCTGCTGAACACAGGCG GCATCCAGAAAATCATGGCCATCTCTGCCGGCGACGCCTACGCC TCTAACAAGGCCTCTAAAGCCGCCAGCGTGCTGCTGTATTCTCTG TGGGCCCATACCGAGCTGCACCATGCCTATAAGAAGGCCCAGTT CAAAAAGACCGACTTCGTGAACAGCCGGACCGCCAAGGCCTACC ACTCTCTGAAAGAT	
pcTNT Promoter	GTCATGGAGAAGACCCACCTTGCAGATGTCCTCACTGGGGCTGG CAGAGCCGGCAACCTGCCTAAGGCTGCTCAGTCCATTAGGAGCC AGTAGCCTGGAAGATGTCTTTACCCCCAGCATCAGTTCAAGTGG AGCAGCACATAACTCTTGCCCTCTGCCTTCCAAGATTCTGGTGCT GAGACTTATGGAGTGTCTTGGAGGTTGCCTTCTGCCCCCCAACCC TGCTCCCAGCTGGCCCTCCCAGGCCTGGGTTGCTGGCCTCTGCTT TATCAGGATTCTCAAGAGGGACAGCTGGTTTATGTTGCATGACT GTTCCCTGCATATCTGCTCTGGTTTTAAATAGCTTATCTGAGCAG CTGGAGGACCACATGGGCTTATATGGCGTGGGGTACATGTTCCT GTAGCCTTGTCCCTGGCACCTGCCAAAATAGCAGCCAACACCCC CCACCCCCACCGCCATCCCCCTGCCCCACCCGTCCCCTGTCGCAC ATTCCTCCCTCCGCAGGGCTGGCTCACCAGGCCCCAGCCCACAT GCCTGCTTAAAGCCCTCTCCATCCTCTGCCTCACCCAGTCCCCGC TGAGACTGAGCAGACGCCTCCA	3
PKP2 promoter	CATCTCAGCATCATGGTTGGATGTTTCCACCTGGCTACATAAGCA AGCTTTACACAAGGTGTAATTTGCCTAAATAGTGGTCCATTCTAT TGGGGTGGGAGCAATTGCTTCCAGGACTCACATCCATATGGCTC CCACTTAGCCATGTGGCCTGCTGACAAAGGGTGGCGGAACTGTC ACTACTCTGTTGTCCACGCTTTCAGTCCTTTGGTTTCCTCTTCACT CCCTGGACGCTCATGTAAAAAGGGAGGCCATATACCTGTGCATT GTGTGTCTAAGCATTCAGTGTGTGTCTAAAGGCAGAAGGGTGTG GGTAGGAAAACAAAGACGAGGGAAGCTGCGTTCTCCAAACACT TCAGACTTGAGTAAGTGGGGTTTTGCAGCAATTGAGTGATTTGA GGGAAAGTGAACATACAAACCCAAGCAATCAAAGGGAATATTA TCTTAATACCAGGGATACATGTTTTTCTTTCTGCCTCTTAAGTCC AAAGAGGCAAATCAGGACAAGTGGCTTTGGTTGTAAACTTTAAG GTCAAGGATCCTTTCTGTTGAGCTTAGCTCTCAAGTTCTCAGTAG TCAACTGCGGTGAAACATAATTAATAGCACGATAAATACAAGTT GTGGAAGATTCGATTGAAAGTTGGAGGCCCTCTCCGTGGATCTC TCTACAAAGAGCCTGTAATAAAGAGGACTTAATCAACGTTAGCA GGGCTATTTAAAAAGCATCGTCTATTAAAATTCATTTCTTCTCTA GAGCCTCTTGTTGGAGTTTCTCTGTGTGGGTGTGTTCGTAAGAGA GGAATGGGTTAGCAAGAGTACTGGGTACAATTTGTGTATCCAAG AGAAAACAGAAGCTCTCAATGAGGAAGAACATATGTTTCTGGGA CTGCATCTGTGCAAAAAGTACATAGTCCTGACGTTGTACTAAGA AAAAAAACACTCTCTTTAGAAAGTCTTTTATTTCACACGTTATCT TCTTGGCACATTTCCCTCATATTGCCCTTTCCGCCTGACCAAATA GCCCTTTCTCACCCTCAGGTCCAGGAAAACCAGGAAACGTTTCC AACAGTGCGACAAAGCCTGACTAACCAGACATACTACTCGCTCG GGGATCCCGGAGGCAAGCCTCAGTCCAAGAACAGGAGTGACTCT CGAGGGCTCACCTGCCTGCAGGGCAGCCCCTCCCTGCATCGAGC GGAAATCCATCCTGTCCAGCGCGGGGCGTGGGCAGAGCGGGGC	4

	GCGGCCCCGGCAGGCGGTATCCGCTGGGACTCCGACAACGTGCG CGACCCCAGGCGAACCGCGCCCCTCTCCCCACCTCCCCGCGGGC GGGTACAAGTCTCCAGGTGTCCGCGCGCTCAGCGGGTCCGGCCC GCCCCCGCCCCCGCCCCCGGGCCCGACTGCGCGTGCCCGGCCGG AGCCGCGCCCCCTCCTCAGGGAAGGCCGGGCGTCCGGCCCACGA GGCCGAGCTCCCCCCCCGGCCCGGGCCTCTCACCGGCGCGGGGGG CGGGCCAGGGGCGGGGCCGGACTCGAGCGGGGCGGGGCTCGCG CCAGCGCCCCCAGCTCCGTGGCGGCTTCGCCCGCGAGTCCAGAG GCAGGCGAGCAGCTCGGTCGCCCCCACCGGCCCC	
AAV Human PKP2a Expression Cassette (pcTnT promoter, codon optimized)	ctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgtcgggcgacctttggtcgcccg gcctcagtgagcgagcgagcgcgcagagagggagtggccaactccatcactaggggttccttgtagttaa tgattaacccgccatgctacttatctacgtagccatgctctaggaagatcggaattcGCCCTTAAGT CATGGAGAAGACCCACCTTGCAGATGTCCTCACTGGGGCTGGCA GAGCCGGCAACCTGCCCAAGGCTGCTCAGTCCATTAGGAGCCAG TAGCCTGGAAGATGTCTTTACCCCCAGCATCAGTTCAAGTGGAG CAGCACATAACTCTTGCCCTCTGCCTTCCAAGATTCTGGTGCTGA GACTTATGGAGTGTCTTGGAGGTTGCCTTCTGCCCCCCAACCCTG CTCCCAGCTGGCCCTCCCAGGCCTGGGTTGCTGGCCTCTGCTTTA TCAGGATTCTCAAGAGGGACAGCTGGTTTATGTTGCATGACTGTT CCCTGCATATCTGCTCTGGTTTTAAATAGCTTATCTGAGCAGCTG GAGGACCACATGGGCTTATATGGCGTGGGGTACATGTTCCTGTA GCCTTGTCCCTGGCACCTGCCAAAATAGCAGCCAACACCCCCCA CCCCCACCGCCATCCCCCTGCCCCACCCGTCCCCTGTCGCACATT CCTCCCTCCGCAGGGCTGGCTCACCAGGCCCCAGCCCACATGCC TGCTTAAAGCCCTCTCCATCCTCTGCCTCACCCAGTCCCCGCTGA GACTGAGCAGACGCCTCCAGCCACCATGGCTGCTCCTGGTGCTC CTGCCGAGTACGGCTACATCAGAACAGTGCTGGGCCAGCAGATC CTGGGACAGCTGGATTCTAGCTCTCTGGCCCTGCCTTCTGAGGCC AAGCTGAAACTGGCCGGCAGTTCTGGAAGAGGCGGCCAGACAG TGAAGTCCCTGCGGATCCAAGAACAGGTGCAGCAGACCCTGGCC AGAAAGGGCAGATCTTCTGTCGGCAACGGCAACCTGCACAGAAC CAGCTCTGTGCCCGAGTACGTGTACAATCTGCACCTGGTGGAAA ACGACTTCGTCGGCGGCAGATCCCCTGTGCCTAAGACCTACGAT ATGCTGAAGGCCGGCACCACCGCCACCTATGAAGGCAGATGGG GAAGAGGCACAGCCCAGTACAGCAGCCAGAAAAGCGTGGAAGA GAGAAGCCTGCGGCACCCTCTGCGGAGACTGGAAATCAGCCCTG ATAGCAGCCCAGAGAGAGCCCACTACACCCACAGCGACTACCA GTACTCCCAGAGATCTCAGGCCGGCCACACACTGCACCACCAAG AGTCTAGAAGGGCCGCTCTGCTGGTGCCTCCTAGATACGCCAGA TCTGAGATCGTGGGCGTGTCCAGAGCCGGCACAACAAGCAGACA GAGACACTTCGACACCTACCACCGGCAGTATCAGCACGGCAGCG TGTCCGATACCGTGTTCGATAGCATCCCCGCCAATCCTGCTCTGC TGACATACCCTAGACCTGGCACCTCCAGATCCATGGGCAATCTG CTGGAAAAAGAGAACTACCTGACCGCCGGACTGACCGTGGGAC AAGTTCGACCTCTGGTTCCTCTGCAGCCCGTGACACAGAACAGA GCCAGCAGAAGCAGCTGGCACCAGTCCAGCTTCCACAGCACCAG AACACTGAGAGAAGCTGGCCCTAGCGTGGCCGTGGATTCTTCTG GTAGAAGGGCTCACCTGACAGTTGGCCAAGCAGCTGCAGGCGG AAGCGGAAATCTGCTGACCGAGAGAAGCACCTTCACCGACAGCC AGCTGGGCAACGCCGACATGGAAATGACACTGGAACGGGCCGT GTCCATGCTGGAAGCCGATCACATGCTGCCCAGCAGAATTAGCG CCGCTGCCACCTTTATCCAGCACGAGTGCTTCCAGAAGTCTGAG GCCCGGAAGAGAGTGAACCAGCTGAGAGGCATCCTGAAGCTGC TGCAGCTCCTGAAGGTGCAGAACGAGGATGTGCAGAGGGCTGTG TGTGGGGCCCTGAGAAATCTGGTGTTCGAGGACAACGACAACAA GCTGGAAGTGGCCGAGCTGAACGGCGTGCCAAGACTGCTGCAG GTTCTGAAACAGACCCGCGACCTGGAAACAAAGAAGCAGATCA	5

	CCGGCCTGCTCTGGAACCTGAGCAGCAACGACAAGCTGAAGAAC CTGATGATCACAGAGGCCCTGCTGACCCTGACAGAGAACATCAT CATCCCTTTCAGCGGCTGGCCCGAGGGCGATTACCCTAAAGCTA ATGGCCTGCTGGACTTCGACATCTTCTACAACGTGACCGGCTGCC TGAGAAACATGTCTAGCGCTGGCGCCGATGGCAGAAAGGCCATG AGAAGATGTGACGGCCTGATCGACAGCCTGGTGCACTATGTGCG GGGCACAATCGCCGATTACCAGCCTGATGATAAGGCCACCGAGA ACTGCGTGTGCATCCTGCACAACCTGAGCTACCAGCTGGAAGCA GAGCTGCCCGAGAAGTACAGCCAGAACATCTACATCCAGAACCG GAACATCCAGACCGACAACAACAAGAGCATCGGCTGCTTCGGCA GCCGCAGCCGGAAAGTGAAAGAACAGTACCAGGACGTGCCCAT GCCTGAGGAAAAGTCTAACCCCAAAGGCGTGGAATGGCTGTGGC ACAGCATCGTGATCCGGATGTACCTGAGCCTGATCGCCAAGAGC GTGCGGAATTACACCCAAGAGGCATCTCTGGGCGCCCTGCAGAA TCTGACAGCAGGATCTGGCCCTATGCCTACCTCTGTGGCTCAGAC CGTGGTGCAGAAAGAGTCTGGCCTGCAGCACACCCGGAAGATGC TGCATGTGGGAGATCCCAGCGTGAAGAAAACCGCCATCAGCCTG CTGAGAAACCTGAGCCGGAATCTGTCTCTGCAGAATGAGATCGC CAAAGAGACACTGCCCGACCTGGTGTCTATCATCCCTGACACCG TGCCTAGCACCGACCTGCTGATTGAGACAACAGCCAGCGCCTGC TACACCCTGAACAACATCATTCAGAACTCCTACCAGAACGCCCG CGATCTGCTGAACACAGGCGGCATCCAGAAAATCATGGCCATCT CTGCCGGCGACGCCTACGCCTCTAACAAGGCCTCTAAAGCCGCC AGCGTGCTGCTGTATTCTCTGTGGGCCCATACCGAGCTGCACCAT GCCTATAAGAAGGCCCAGTTCAAAAAGACCGACTTCGTGAACAG CCGGACCGCCAAGGCCTACCACTCTCTGAAAGATTAAtaagcttggatc caatcaacctctggattacaaaatttgtgaaagattgactggtattcttaactatgttgctccttttacgctatgtg gatacgctgctttaatgcctttgtatcatgctattgcttcccgtatggctttcattttctcctccttgtataaatcctg gttgctgtctctttatgaggagttgtggcccgttgtcaggcaacgtggcgtggtgtgcactgtgtttgctgacg caacccccactggttggggcattgccaccacctgtcagctcctttccgggactttcgctttcccctccctatt gccacggcggaactcatcgccgcctgccttgcccgctgctggacagggctcggctgttgggcactgaca attccgtggtgttgtcggggaaATCATcgtcctttccTtggctgctcgcctgtgttgccacctggattctg cgcgggacgtccttctgctacgtcccttcggccctcaatccagcggaccttccttcccgcggcctgctgccg gctctgcggcctcttccgcgtcttcgagatctgcctcgactgtgccttctagttgccagccatctgttgtttgcc cctcccccgtgccttccttgaccctggaaggtgccactcccactgtccttcctaataaaatgaggaaattgca tcgcattgtctgagtaggtgtcattctattctgggggggtggggtggggcaggacagcaagggggaggattg ggaagacaatagcaggcatgctggggaCTGGGGACTCGAGTTAAGGGCgaattcccg ataaggatcttcctagagcatggctacgtagataagtagcatggcgggttaatcattaactacaaggaaccc ctagtgatggagttggccactccctctctgcgcgctcgctcgctcactgaggccgggcgaccaaaggtcgc ccgacgcccgggctttgcccgggcggcctcagtgagcgagcgagcgcgcag	
AAV Human PKP2a Expression Cassette (PKP2 promoter, codon optimized)	ctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgtcgggcgacctttggtcgcccg gcctcagtgagcgagcgagcgcgcagagagggagtggccaactccatcactaggggttccttgtagttaa tgattaacccgccatgctacttatctacgtagccatgctctaggaagatcggaattcGCCCTTAACA TCTCAGCATCATGGTTGGATGTTTCCACCTGGCTACATAAGCAAG CTTTACACAAGGTGTAATTTGCCTAAATAGTGGTCCATTCTATTG GGGTGGGAGCAATTGCTTCCAGGACTCACATCCATATGGCTCCC ACTTAGCCATGTGGCCTGCTGACAAAGGGTGGCGGAACTGTCAC TACTCTGTTGTCCACGCTTTCAGTCCTTTGGTTTCCTCTTCACTCC CTGGACGCTCATGTAAAAAGGGAGGCCATATACCTGTGCATTGT GTGTCTAAGCATTCAGTGTGTGTCTAAAGGCAGAAGGGTGTGGG TAGGAAAACAAAGACGAGGGAAGCTGCGTTCTCCAAACACTTCA GACTTGAGTAAGTGGGGTTTTGCAGCAATTGAGTGATTTGAGGG AAAGTGAACATACAAACCCAAGCAATCAAAGGGAATATTATCTT AATACCAGGGATACATGTTTTTCTTTCTGCCTCTTAAGTCCAAAG AGGCAAATCAGGACAAGTGGCTTTGGTTGTAAACTTTAAGGTCA AGGATCCTTTCTGTTGAGCTTAGCTCTCAAGTTCTCAGTAGTCAA CTGCGGTGAAACATAATTAATAGCACGATAAATACAAGTTGTGG	6

	AAGATTCGATTGAAAGTTGGAGGCCCTCTCCGTGGATCTCTCTAC AAAGAGCCTGTAATAAAGAGGACTTAATCAACGTTAGCAGGGCT ATTTAAAAAGCATCGTCTATTAAAATTCATTTCTTCTCTAGAGCC TCTTGTTGGAGTTTCTCTGTGTGGGTGTGTTCGTAAGAGAGGAAT GGGTTAGCAAGAGTACTGGGTACAATTTGTGTATCCAAGAGAAA ACAGAAGCTCTCAATGAGGAAGAACATATGTTTCTGGGACTGCA TCTGTGCAAAAAGTACATAGTCCTGACGTTGTACTAAGAAAAAA AACACTCTCTTTAGAAAGTCTTTTATTTCACACGTTATCTTCTTGG CACATTTCCCTCATATTGCCCTTTCCGCCTGACCAAATAGCCCTT TCTCACCCTCAGGTCCAGGAAAACCAGGAAACGTTTCCAACAGT GCGACAAAGCCTGACTAACCAGACATACTACTCGCTCGGGGATC CCGGAGGCAAGCCTCAGTCCAAGAACAGGAGTGACTCTCGAGG GCTCACCTGCCTGCAGGGCAGCCCCTCCCTGCATCGAGCGGAAA TCCATCCTGTCCAGCGCGGGGCGTGGGCAGAGCGGGGCGCGGCC CCGGCAGGCGGTATCCGCTGGGACTCCGACAACGTGCGCGACCC CAGGCGAACCGCGCCCCTCTCCCCACCTCCCCGCGGGCGGGTAC AAGTCTCCAGGTGTCCGCGCGCTCAGCGGGTCCGGCCCGCCCCC GCCCCCGCCCCCGGGCCCGACTGCGCGTGCCCGGCCGGAGCCGC GCCCCCTCCTCAGGGAAGGCCGGGCGTCCGGCCCACGAGGCCGA GCTCCCCCCCGGCCCGGGCCTCTCACCGGCGCGGGGGGCGGGCC AGGGGCGGGGCCGGACTCGAGCGGGGCGGGGCTCGCGCCAGCG CCCCCAGCTCCGTGGCGGCTTCGCCCGCGAGTCCAGAGGCAGGC GAGCAGCTCGGTCGCCCCCACCGGCCCCATGGCTGCTCCTGGTG CTCCTGCCGAGTACGGCTACATCAGAACAGTGCTGGGCCAGCAG ATCCTGGGACAGCTGGATTCTAGCTCTCTGGCCCTGCCTTCTGAG GCCAAGCTGAAACTGGCCGGCAGTTCTGGAAGAGGCGGCCAGA CAGTGAAGTCCCTGCGGATCCAAGAACAGGTGCAGCAGACCCTG GCCAGAAAGGGCAGATCTTCTGTCGGCAACGGCAACCTGCACAG AACCAGCTCTGTGCCCGAGTACGTGTACAATCTGCACCTGGTGG AAAACGACTTCGTCGGCGGCAGATCCCCTGTGCCTAAGACCTAC GATATGCTGAAGGCCGGCACCACCGCCACCTATGAAGGCAGATG GGGAAGAGGCACAGCCCAGTACAGCAGCCAGAAAAGCGTGGAA GAGAGAAGCCTGCGGCACCCTCTGCGGAGACTGGAAATCAGCCC TGATAGCAGCCCAGAGAGAGCCCACTACACCCACAGCGACTACC AGTACTCCCAGAGATCTCAGGCCGGCCACACACTGCACCACCAA GAGTCTAGAAGGGCCGCTCTGCTGGTGCCTCCTAGATACGCCAG ATCTGAGATCGTGGGCGTGTCCAGAGCCGGCACAACAAGCAGAC AGAGACACTTCGACACCTACCACCGGCAGTATCAGCACGGCAGC GTGTCCGATACCGTGTTCGATAGCATCCCCGCCAATCCTGCTCTG CTGACATACCCTAGACCTGGCACCTCCAGATCCATGGGCAATCT GCTGGAAAAAGAGAACTACCTGACCGCCGGACTGACCGTGGGA CAAGTTCGACCTCTGGTTCCTCTGCAGCCCGTGACACAGAACAG AGCCAGCAGAAGCAGCTGGCACCAGTCCAGCTTCCACAGCACCA GAACACTGAGAGAAGCTGGCCCTAGCGTGGCCGTGGATTCTTCT GGTAGAAGGGCTCACCTGACAGTTGGCCAAGCAGCTGCAGGCG GAAGCGGAAATCTGCTGACCGAGAGAAGCACCTTCACCGACAG CCAGCTGGGCAACGCCGACATGGAAATGACACTGGAACGGGCC GTGTCCATGCTGGAAGCCGATCACATGCTGCCCAGCAGAATTAG CGCCGCTGCCACCTTTATCCAGCACGAGTGCTTCCAGAAGTCTG AGGCCCGGAAGAGAGTGAACCAGCTGAGAGGCATCCTGAAGCT GCTGCAGCTCCTGAAGGTGCAGAACGAGGATGTGCAGAGGGCT GTGTGTGGGGCCCTGAGAAATCTGGTGTTCGAGGACAACGACAA CAAGCTGGAAGTGGCCGAGCTGAACGGCGTGCCAAGACTGCTGC AGGTTCTGAAACAGACCCGCGACCTGGAAACAAAGAAGCAGAT CACCGGCCTGCTCTGGAACCTGAGCAGCAACGACAAGCTGAAGA ACCTGATGATCACAGAGGCCCTGCTGACCCTGACAGAGAACATC ATCATCCCTTTCAGCGGCTGGCCCGAGGGCGATTACCCTAAAGC	

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PKP2 Protein	MAAPGAPAEYGYIRTVLGQQILGQLDSSSLALPSEAKLKLAGSSGR GGQTVKSLRIQEQVQQTLARKGRSSVGNGNLHRTSSVPEYVYNLH LVENDFVGGRSPVPKTYDMLKAGTTATYEGRWGRGTAQYSSQKS VEERSLRHPLRRLEISPDSSPERAHYTHSDYQYSQRSQAGHTLHHQE SRRAALLVPPRYARSEIVGVSRAGTTSRQRHFDTYHRQYQHGSVSD TVFDSIPANPALLTYPRPGTSRSMGNLLEKENYLTAGLTVGQVRPL VPLQPVTQNRASRSSWHQSSFHSTRTLREAGPSVAVDSSGRRAHLT VGQAAAGGSGNLLTERSTFTDSQLGNADMEMTLERAVSMLEADH MLPSRISAAATFIQHECFQKSEARKRVNQLRGILKLLQLLKVQNEDV QRAVCGALRNLVFEDNDNKLEVAELNGVPRLLQVLKQTRDLETKK QITGLLWNLSSNDKLKNLMITEALLTLTENIIIPFSGWPEGDYPKAN GLLDFDIFYNVTGCLRNMSSAGADGRKAMRRCDGLIDSLVHYVRG TIADYQPDDKATENCVCILHNLSYQLEAELPEKYSQNIYIQNRNIQT DNNKSIGCFGSRSRKVKEQYQDVPMPEEKSNPKGVEWLWHSIVIR MYLSLIAKSVRNYTQEASLGALQNLTAGSGPMPTSVAQTVVQKES GLQHTRKMLHVGDPSVKKTAISLLRNLSRNLSLQNEIAKETLPDLVS IIPDTVPSTDLLIETTASACYTLNNIIQNSYQNARDLLNTGGIQKIMAI SAGDAYASNKASKAASVLLYSLWAHTELHHAYKKAQFKKTDFVN SRTAKAYHSLKD	8
WPRE	TCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCT TAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAAT GCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCC TCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGG CCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGAC GCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTT TCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTC ATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTT GGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCT TTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGA CGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTC CTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTC G	9
hGH polyA signal	CCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCT CCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCC TTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGG TGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGG GGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGACTGGGGA	10
WPRE – hGH polyA signal cassette	TCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCT TAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAAT GCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCC TCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGG CCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGAC GCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTT TCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTC ATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTT GGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCT TTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGA CGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTC CTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTC	11

	GAGATCTGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGT TTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCC CACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCT GAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACA GCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGA CTGGGGACTCGAGTTAAGGGCGAATTCCCGATAAGGATCTTCCT AGAGCATGGCTACGTAGATAAGTAGCATGGCGGGTTAATCATTA ACTACA	
AAV9 capsid amino acid sequence	MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNAR GLVLPGYKYLGPGNGLDKGEPVNAADAAALEHDKAYDQQLKAGD NPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKKRLLEPLGLV EEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGD TESVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSS SGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSN DNAYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNF KLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAHE GCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRT GNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGS GQNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNS EFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGK QGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQ AQTGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPL MGGFGMKHPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSV EIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGVYSEPRPI GTRYLTRNL	12

Viral Vectors

[0055] Suitable viral vectors for methods and gene therapy vectors provided herein include, but are not limited to, viral vectors (e.g. viral vectors based on vaccinia virus; poliovirus; adenovirus (e.g., Li et al. (1994) Invest Opthalmol Vis Sci 35:2543-2549; Borras et al. (1999) Gene Ther 6:515-524; Li and Davidson, (1995) Proc. Natl. Acad. Sci. 92:7700-7704; Sakamoto et al. (1999) Hum Gene Ther 5: 1088-1097; WO 94/12649; WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (e.g., Ali et al. (1998) Hum Gene Ther 9(l):81-86, 1998, Flannery et al. (1997) Proc. Natl. Acad. Sci. 94:6916-6921; Bennett et al. (1997) Invest Opthalmol Vis Sci 38:2857-2863; Jomary et al. (1997) Gene Ther 4:683-690; Rolling et al. (1999), Hum Gene Ther 10:641-648; Ali et al. (1996) Hum Mol Genet. 5:591-594; WO 93/09239, Samulski et al. (1989) J. Vir. 63 :3822-3828; Mendelson et al. (1988) Virol. 166: 154-165; and Flotte et al. (1993) Proc. Natl. Acad. Sci. 90: 10613-10617; SV40; herpes simplex virus; human immunodeficiency virus (e.g., Miyoshi et al. (1997) Proc. Natl. Acad. Sci. 94: 10319-10323; Takahashi et al. (1999) J Virol 73 :7812-7816); a retroviral vector (e.g., Murine-Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, a lentivirus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like. Numerous suitable expression vectors are known to those of skill in the art, and many are commercially available. The following vectors are provided by way of example; for eukaryotic cells: pXTl, pSG5 (Stratagene), pSVK3, pBPV, pMSG, pSVLSV40 (Pharmacia), and pAd (Life Technologies).

However, any other vector is contemplated for use so long as it is compatible with the methods of the present disclosure.

[0056] The ability of certain viruses to infect cells or enter cells via receptor-mediated endocytosis, and express viral genes stably and efficiently have made them attractive candidates for the transfer of foreign nucleic acids into cells (e.g., mammalian cells). Viral vectors are contemplated to include control sequences such as promoters for expression of the polypeptide of interest. Although many viral vectors integrate into the host cell genome, if desired, the segments that allow such integration can be removed or altered to prevent such integration. Moreover, in some embodiments, the vectors do not contain a mammalian origin of replication. Non-limiting examples of virus vectors are described below that are contemplated for use in delivering nucleic acids encoding PKP2 into a selected cell. In some embodiments, the viral vector is derived from a replication-deficient virus.

[0057] In general, other useful viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the polypeptide of interest. Non-cytopathic viruses include certain retroviruses, the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. In general, the retroviruses are replication-deficient (e.g., capable of directing synthesis of the desired transcripts, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high-efficiency transduction of polynucleotide in vivo.

[0058] In some embodiments, a polynucleotide encoding PKP2 is housed within an infective virus that has been engineered to express a specific binding ligand. The virus particle will thus bind with specificity to the cognate receptors of the target cell and deliver the contents to the cell. In some embodiments, the virus is modified to impart particular viral tropism, e.g., the virus preferentially infects fibroblasts, heart cells, or more particularly cardiac fibroblasts (CFs). For AAV, in some cases, capsid proteins are mutated to alter the tropism of the viral vector. For example, lentivirus tropism is often modified by using different envelope proteins; this is known as “pseudotyping.”

[0059] In some embodiments, the viral vector is a retroviral vector. Retroviruses often integrate their genes into the host genome, transfer a large amount of foreign genetic material, infect a broad spectrum of species and cell types, and are often packaged in special cell-lines (Miller et al., Am. J. Clin. Oncol., 15(3):216-221, 1992). In some embodiments, a retroviral vector is altered so that it does not integrate into the host cell genome.

[0060] In some embodiments, the recombinant retrovirus comprises a viral polypeptide (e.g., retroviral env) to aid entry into the target cell. Such viral polypeptides are well-established in the art, for example, U.S. Pat. No. 5,449,614. In some embodiments, the viral polypeptide is an amphotropic viral polypeptide, for example, amphotropic env, which aids entry into cells derived from multiple species, including cells outside of the original host species. In some embodiments, the viral polypeptide is a xenotropic viral polypeptide that aids entry into cells outside of the original host species. In some embodiments, the viral polypeptide is an ecotropic viral polypeptide, for example, ecotropic env, which aids entry into cells of the original host species.

[0061] Examples of viral polypeptides capable of aiding entry of retroviruses into cells include, but are not limited to: MMLV amphotropic env, MMLV ecotropic env, MMLV xenotropic env, vesicular stomatitis virus-g protein (VSV-g), HIV-1 env, Gibbon Ape Leukemia Virus (GALV) env, RD114, FeLV-C, FeLV-B, MLV 10A1 env gene, and variants thereof, including chimeras. Yee et al. (1994) Methods Cell Biol, Pt A:99-l 12 (VSV-G); U.S. Pat. No. 5,449,614. In some cases, the viral polypeptide is genetically modified to promote expression or enhanced binding to a receptor.

[0062] In embodiments, the retroviral construct is derived from a range of retroviruses, e.g., MMLV, HIV-1, SIV, FIV, or other retrovirus described herein. In some embodiments, the retroviral construct encodes all viral polypeptides necessary for more than one cycle of replication of a specific virus. In some cases, the efficiency of viral entry is improved by the addition of other factors or other viral polypeptides. In other cases, the viral polypeptides encoded by the retroviral construct do not support more than one cycle of replication, e.g., U.S. Pat. No. 6,872,528. In such circumstances, the addition of other factors or other viral polypeptides often help facilitate viral entry. In an exemplary embodiment, the recombinant retrovirus is HIV-1 virus comprising a VSV-g polypeptide, but not comprising a HIV 1 env polypeptide.

[0063] In some embodiments, the retroviral construct comprises: a promoter, a multi-cloning site, and/or a resistance gene. Examples of promoters include but are not limited to CMV, SV40, EFla, β-actin; retroviral LTR promoters, and inducible promoters. In some embodiments, the retroviral construct comprises a packaging signal (e.g., a packaging signal derived from the MFG vector; a psi packaging signal). Examples of some retroviral constructs known in the art include but are not limited to: pMX, pBabeX or derivatives thereof. Onishi et al. (1996) Experimental Hematology, 24:324-329. In some cases, the retroviral construct is a self-inactivating lentiviral vector (SIN) vector. Miyoshi et al. (1998) J. Virol 72(10):8150- 8157. In some cases, the retroviral construct is LL-CG, LS-CG, CL-CG, CS-CG, CLG or MFG. Miyoshi et al. (1998) J. Virol 72(10):8150-8157; Onishi et al. (1996) Experimental Hematology, 24:324-329; Riviere et al. (1995) Proc. Natl. Acad. Sci., 92:6733-6737.

[0064] In some embodiments, a retroviral vector is constructed by inserting a nucleic acid (e.g., one encoding a polypeptide of interest or an RNA) into the viral genome in the place of some viral sequences to produce a virus that is replication-defective. To produce virions, a packaging cell line containing the gag, pol, and env genes, but without the LTR and packaging components, is constructed (Mann et al., Cell 33:153-159, 1983). When a recombinant plasmid containing a cDNA, together with the retroviral LTR and packaging sequences is introduced into a special cell line (e.g., by calcium phosphate precipitation or lipid transfection), the packaging sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then secreted into the culture media (Nicolas and Rubinstein, In: Vectors: A survey of molecular cloning vectors and their uses, Rodriguez and Denhardt, eds., Stoneham: Butterworth, pp. 494-513, 1988; Temin, In: Gene Transfer, Kucherlapati (ed.), New York: Plenum Press, pp. 149-188, 1986; Mann et al., Cell, 33:153-159, 1983). The media containing the recombinant retroviruses is then collected, optionally concentrated, and used for gene transfer. Retroviral

vectors are able to infect a broad variety of cell types. However, integration and stable expression typically involves the division of host cells (Paskind et al., Virology, 67:242-248, 1975).

[0065] In some embodiments, the viral vector is a lentiviral vector. Lentiviruses are complex retroviruses, which, in addition to the common retroviral genes gag, pol, and env, contain other genes with regulatory or structural function. Information on lentiviral vectors is available, for example, in Naldini et al., Science 272(5259):263-267, 1996; Zufferey et al., Nat Biotechnol 15(9):871-875, 1997; Blomer et al., J Virol. 71(9):6641-6649, 1997; U.S. Patent Nos. 6,013,516 and 5,994,136, each of which is incorporated herein by reference in its entirety. Some examples of lentivirus include the Human Immunodeficiency Viruses: HIV-1, HIV-2 and the Simian Immunodeficiency Virus: SIV. Lentiviral vectors have been generated by attenuating the HIV virulence genes, for example, the genes env, vif, vpr, vpu and nef are deleted to make the vector biologically safe. The lentivirus employed is sometimes replication and/or integration defective.

[0066] Recombinant lentiviral vectors are capable of infecting non-dividing cells and are sometimes used for both in vivo and ex vivo gene transfer and expression of nucleic acid sequences. For example, recombinant lentivirus capable of infecting a non-dividing cell wherein a suitable host cell is transfected with two or more vectors carrying the packaging functions, namely gag, pol and env, as well as rev and tat is described in U.S. Patent No. 5,994,136, which is incorporated herein by reference in its entirety. In some embodiments, the recombinant virus is targeted by linkage of the envelope protein with an antibody or a particular ligand for targeting to a receptor of a particular cell type. For example, a target-specific vector is sometimes generated by inserting a nucleic acid segment (including a regulatory region) of interest into the viral vector, along with another gene that encodes a ligand for a receptor on a specific target cell type.

[0067] Lentiviral vectors are known in the art, see Naldini et al., (1996 and 1998); Zufferey et al., (1997); Dull et al., 1998, U.S. Pat. Nos. 6,013,516; and 5,994,136 all incorporated herein by reference. In general, these vectors are plasmid-based or virus-based, and are configured to carry the essential sequences for incorporating foreign nucleic acid, for selection and for transfer of the nucleic acid into a host cell. In some cases, a lentiviral vector is introduced into a cell concurrently with one or more lentiviral packaging plasmids, which include, without limitation, pMD2.G, pRSV-rev, pMDLG-pRRE, and pRRL-GOI. Introduction of a lentiviral vector alone or in combination with lentiviral packaging plasmids into a cell, in some embodiments causes the lentiviral vector to be packaged into a lentiviral particle. In some embodiments, the lentiviral vector is a non-integrating lentiviral (NIL) vector. Illustrative methods for generating NIL vectors, such as the D64V substitution in the integrase gene, are provided in US 8,119,119.

[0068] In some embodiments, the viral vector is an adenoviral vector. The genetic organization of adenovirus includes an approximate 36 kb, linear, double-stranded DNA virus, which allows substitution of large pieces of adenoviral DNA with foreign sequences up to 7 kb (Grunhaus et al., Seminar in Virology 200(2):535-546, 1992)). In some cases, PKP2 is introduced into the cell using adenovirus assisted transfection. Increased transfection efficiencies have been reported in cell systems using

adenovirus coupled systems (Kelleher and Vos, Biotechniques, 17(6):1110-7, 1994; Cotten et al., Proc Natl Acad Sci USA, 89(13):6094-6098, 1992; Curiel, Nat Immun, 13(2-3):141-64, 1994.).

[0069] In some embodiments, the viral vector is an adeno-associated virus (AAV) vector. AAV is an attractive vector system as it has a low frequency of integration and it can infect non-dividing cells, thus making it useful for delivery of polynucleotides into mammalian cells, for example, in tissue culture (Muzyczka, Curr Top Microbiol Immunol, 158:97-129, 1992) or in vivo. Details concerning the generation and use of rAAV vectors are described in U.S. Patent Nos. 5,139,941 and 4,797,368, each incorporated herein by reference in its entirety.

[0070] AAV is a replication-deficient parvovirus, the single-stranded DNA genome of which is about 4.7 kb in length including two 145 nucleotide inverted terminal repeat (ITRs). There are multiple serotypes of AAV. The nucleotide sequences of the genomes of the AAV serotypes are known. For example, the complete genome of AAV-1 is provided in GenBank Accession No. NC_002077; the complete genome of AAV-2 is provided in GenBank Accession No. NC_001401 and Srivastava et al., J. Virol., 45: 555-564 (1983); the complete genome of AAV-3 is provided in GenBank Accession No. NC_1829; the complete genome of AAV-4 is provided in GenBank Accession No. NC_001829; the AAV-5 genome is provided in GenBank Accession No. AF085716; the complete genome of AAV-6 is provided in GenBank Accession No. NC_00 1862; at least portions of AAV-7 and AAV-8 genomes are provided in GenBank Accession Nos. AX753246 and AX753249, respectively; the AAV-9 genome is provided in Gao et al., J. Virol., 78: 6381-6388 (2004); the AAV-10 genome is provided in Mol. Ther., 13(1): 67-76 (2006); and the AAV-11 genome is provided in Virology, 330(2): 375-383 (2004). The sequence of the AAV rh.74 genome is provided in U.S. Patent 9,434,928, incorporated herein by reference. Cis-acting sequences directing viral DNA replication (rep), encapsidation/packaging and host cell chromosome integration are contained within the AAV ITRs. Three AAV promoters (named p5, pl9, and p40 for their relative map locations) drive the expression of the two AAV internal open reading frames encoding rep and cap genes. The two rep promoters (p5 and pi 9), coupled with the differential splicing of the single AAV intron (at nucleotides 2107 and 2227), result in the production of four rep proteins (rep 78, rep 68, rep 52, and rep 40) from the rep gene. Rep proteins possess multiple enzymatic properties that are ultimately responsible for replicating the viral genome. The cap gene is expressed from the p40 promoter and it encodes the three capsid proteins VP1, VP2, and VP3. Alternative splicing and non-consensus translational start sites are responsible for the production of the three related capsid proteins. A single consensus polyadenylation site is located at map position 95 of the AAV genome. The life cycle and genetics of AAV are reviewed in Muzyczka, Current Topics in Microbiology and Immunology, 158: 97-129 (1992).

[0071] AAV possesses unique features that make it attractive as a vector for delivering foreign DNA to cells, for example, in gene therapy. AAV infection of cells in culture is noncytopathic, and natural infection of humans and other animals is silent and asymptomatic. Moreover, AAV infects many mammalian cells allowing the possibility of targeting many different tissues in vivo. Moreover, AAV transduces slowly dividing and non-dividing cells, and often persists essentially for the lifetime of those

cells as a transcriptionally active nuclear episome (extrachromosomal element). Of particular importance to the present disclosure, AAV, and AAV9 in particular, are capable of infecting cells of the heart, such as myocardium, epicardium, or both (Prasad et al, 2011; Piras et al, 2016; Ambrosi et al., 2019). The AAV proviral genome is inserted as cloned DNA in plasmids, which makes construction of recombinant genomes feasible. Furthermore, because the signals directing AAV replication and genome encapsidation are contained within the ITRs of the AAV genome, in some cases, some or all of the internal approximately 4.3 kb of the genome (encoding replication and structural capsid proteins, rep-cap) is replaced with foreign DNA. To generate AAV vectors, in some cases, the rep and cap proteins are provided in trans. Another significant feature of AAV is that it is an extremely stable and hearty virus. It easily withstands the conditions used to inactivate adenovirus (56° to 65°C for several hours), making cold preservation of AAV less critical. In some cases, AAV is even be lyophilized. Finally, AAV-infected cells are not resistant to superinfection. The AAV vectors of the disclosure include self-complementary, duplexed AAV vectors, synthetic ITRs, and/or AAV vectors with increased packaging compacity. Illustrative methods are provided in US 8,784,799; US 8,999,678; US 9,169,494; US 9,447,433; and US 9,783,824, each of which is incorporated by reference in its entirety.

[0072] AAV DNA in the rAAV genomes is contemplated to be from any AAV serotype for which a recombinant virus can be derived including, but not limited to, AAV serotypes AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV- 10, AAV-11, AAV- 12, AAV-13 and AAV rh74. Production of pseudotyped rAAV is disclosed in, for example, WO 01/83692. Other types of rAAV variants, for example rAAV with capsid mutations, are also contemplated. See, for example, Marsic et al., Mol. Therapy. 22):1900-09 (2014). The nucleotide sequences of the genomes of various AAV serotypes are known in the art. AAV vectors of the present disclosure include AAV vectors of serotypes AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV39, AAV43, AAV.rh74, and AAV.rh8. Illustrative AAV vectors are provided in US 63/012,703; US 7,105,345; US 15/782,980; US 7,259,151; US 6,962,815; US 7,718,424; US 6,984,517; US 7,718,424; US 6,156,303; US 8,524,446; US 7,790,449; US 7,906,111; US 9,737,618; US App 15/433,322; US 7,198,951, each of which is incorporated by reference in its entirety.

[0073] In some embodiments, the AAV expression vector is pseudotyped to enhance targeting. To promote gene transfer and sustain expression in cardiomyocytes, AAV6, AAV8, and AAV9, are contemplated for use. In some cases, the AAV2 genome is packaged into the capsid of producing pseudotyped vectors AAV2/5, AAV2/7, and AAV2/8 respectively, as described in Balaji et al. J Surg Res. 184:691-98 (2013). In some embodiments, an AAV9 is used to target expression in myofibroblast-like lineages, as described in Piras et al. Gene Therapy 23:469–478 (2016). In some embodiments, AAV1, AAV6, or AAV9 is used, and in some embodiments, the AAV is engineered, as described in Asokari et al. Hum Gene Ther. 24:906-13 (2013); Pozsgai et al. Mol Ther. 25:855-69 (2017); Kotterman et al. Nature Reviews Genetics 15:445-51 (2014); and US20160340393A1 to Schaffer et al. In some embodiments, the viral vector is AAV engineered to increase target cell infectivity as described in US20180066285A1.

[0074] In some embodiments, the AAV vectors of the disclosure comprise a modified capsid, in particular as capsid engineered to enhance or promote in vivo or ex vivo transduction of cardiac cells, or more particularly cardiomyocytes; or that evade the subject's immune system; or that have improved biodistribution. Illustrative AAV capsids are provided in US 7,867,484; US 9,233,131; US 10,046,016; WO 2016/133917; WO 2018/222503; and WO 20019/060454, each of which is incorporated by reference in its entirety. In an AAV capsid (or in particular an AAV9 capsid), one or more substitutions are contemplated to increase infectivity towards cells in the myocardium, epicardium, or both. More particularly, in some embodiments, the AAV vectors of the disclosure, optionally AAV9-based vectors, comprise in their capsid proteins one or more substitutions. In some embodiments, the AAV vectors of the disclosure comprise the AAV-A9 capsid and/or serotype. It will be appreciated that these substitutions and insertions are contemplated to be combined together to generate various capsid proteins useful in the present disclosure.

Methods of Producing Viral Vectors

[0075] In general, a viral vector is produced by introducing a viral DNA or RNA construct into a producer cell. In some cases, the producer cell does not express exogenous genes. In other cases, the producer cell is a "packaging cell" comprising one or more exogenous genes, e.g., genes encoding one or more gag, pol, or env polypeptides and/or one or more retroviral gag, pol, or env polypeptides. In some embodiments, the retroviral packaging cell comprises a gene encoding a viral polypeptide, e.g., VSV-g, that aids entry into target cells. In some cases, the packaging cell comprises genes encoding one or more lentiviral proteins, e.g., gag, pol, env, vpr, vpu, vpx, vif, tat, rev, or nef. In some cases, the packaging cell comprises genes encoding adenovirus proteins such as El A or El B or other adenoviral proteins. For example, in some cases, proteins supplied by packaging cells are retrovirus-derived proteins such as gag, pol, and env; lentivirus-derived proteins such as gag, pol, env, vpr, vpu, vpx, vif, tat, rev, and nef; and adenovirus-derived proteins such as El A and E1 B. In many examples, the packaging cells supply proteins derived from a virus that differs from the virus from which the viral vector is derived. Methods of producing recombinant viruses from packaging cells and their uses are well established; see, e.g., U.S. Pat. Nos. 5,834,256; 6,910,434; 5,591,624; 5,817,491; 7,070,994; and 6,995,009.

[0076] Packaging cell lines include but are not limited to any easily-transfectable cell line. Packaging cell lines are often based on 293T cells, NIH3T3, COS or HeLa cell lines. Packaging cells are often used to package virus vector plasmids deficient in at least one gene encoding a protein required for virus packaging. Any cells that supply a protein or polypeptide lacking from the proteins encoded by such viral vectors or plasmids are contemplated for use as packaging cells. Examples of packaging cell lines include, but are not limited to: Platinum-E (Plat-E), Platinum-A (Plat- A), BOSC 23 (ATCC CRL 11554) and Bing (ATCC CRL 11270). Morita et al. (2000) Gene Therapy 7(12): 1063-1066; Onishi et al. (1996) Experimental Hematology, 24:324-329; U.S. Pat. No. 6,995,009. Commercial packaging lines are also useful, e.g., Ampho-Pak 293 cell line, Eco-Pak 2-293 cell line, RetroPack PT67 cell line, and Retro-X Universal Packaging System (all available from Clontech).

[0077] Virus vector plasmids (or constructs), include: pMXs, pMxs-IB, pMXs-puro, pMXs-neo (pMXs-IB is a vector carrying the blasticidin-resistant gene instead of the puromycin-resistant gene of pMXs-puro) Kimatura et al. (2003) Experimental Hematology 31 : 1007-1014; MFG Riviere et al. (1995) Proc. Natl. Acad. Sci., 92:6733-6737; pBabePuro; Morgenstern et al. (1990) Nucleic Acids Research 18:3587-3596; LL-CG, CL-CG, CS-CG, CLG Miyoshi et al. (1998) J. Vir. 72:8150-8157 and the like as the retrovirus system, and pAdexl Kanegae et al. (1995) Nucleic Acids Research 23 :3816-3821 and the like as the adenovirus system. In exemplary embodiments, the retroviral construct comprises blasticidin (e.g., pMXs-IB), puromycin (e.g., pMXs-puro, pBabePuro), or neomycin (e.g., pMXs-neo). Morgenstern et al. (1990) Nucleic Acids Research 18:3587-3596.

Promoters and Enhancers

[0078] In some embodiments, a nucleic acid encoding a PKP2 is operably linked to a promoter and/or enhancer to facilitate expression of PKP2. Depending on the host/vector system utilized, any of a number of suitable transcription and translation control elements, including constitutive, tissue specific, and inducible promoters, transcription enhancer elements, transcription terminators, etc. are suitable for use in the expression vector (e.g., Bitter et al. (1987) Methods in Enzymology, 153 :516-544).

[0079] Non-limiting examples of suitable eukaryotic promoters (promoters functional in a eukaryotic cell) include CMV, CMV immediate early, HSV thymidine kinase, early and late SV40, long terminal repeats (LTRs) from retrovirus, and mouse metallothionein-I. In some embodiments, promoters that are capable of conferring cardiac-specific expression will be used, including but not limited to promoters that confer expression in the myocardium, the epicardium, or both (Prasad et al., 2011). Non-limiting examples of suitable cardiac-specific promoters include alpha-myosin heavy chain (a-MHC), myosin light chain 2 (MLC-2), cardiac troponin T (cTnT), and cardiac troponin C (cTnC). In some embodiments, a PKP2 or a desmin promoter is used. In some cases, a chimeric promoter with cardiac specific expression is used. In some cases, a cardiac specific enhancer is combined with the promoter.

[0080] Examples of suitable promoters for driving expression PKP2 include, but are not limited to, retroviral long terminal repeat (LTR) elements; constitutive promoters such as CMV, HSV1-TK, SV40, EF-la, β-actin, phosphoglycerol kinase (PGK); inducible promoters, such as those containing Tet-operator elements; and cardiac-specific promoters, such as alpha-myosin heavy chain (a-MHC), myosin light chain 2 (MLC-2), cardiac troponin T (cTnT), and cardiac troponin C (cTnC). In some embodiments, a PKP2 or a desmin promoter is used. In some embodiments, a chimeric promoter with cardiac specific expression is used. In some cases, a cardiac specific enhancer is combined with the promoter.

[0081] In some embodiments, a polynucleotide is operably linked to a cell type-specific transcriptional regulator element (TRE), where TREs include promoters and enhancers. Suitable TREs include, but are not limited to, TREs derived from the following genes: myosin light chain-2, α-myosin heavy chain, AE3, cardiac troponin C, and cardiac actin. Franz et al. (1997) Cardiovasc. Res. 35:560-566; Robbins et al. (1995) Ann. N. Y. Acad. Sci. 752:492-505; Linn et al. (1995) Circ. Res. 76:584-591;

Parmacek et al. (1994) Cell. Biol. 14: 1870-1885; Hunter et al. (1993) Hypertension 22:608-617; and Sartorelli et al. (1992) Proc. Natl. Acad. Sci. USA 89:4047-4051.

[0082] Alternatively, certain advantages will be gained by positioning the coding nucleic acid segment under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with a nucleic acid in its natural environment. A recombinant or heterologous enhancer refers also to an enhancer not normally associated with a nucleic acid sequence in its natural environment. Such promoters or enhancers often include promoters or enhancers of other genes, and promoters or enhancers isolated from any other prokaryotic, viral, or eukaryotic cell, and promoters or enhancers not "naturally occurring," i.e., containing different elements of different transcriptional regulatory regions, and/or mutations that alter expression. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, sequences are sometimes produced using recombinant cloning and/or nucleic acid amplification technology, including PCR, in connection with the compositions disclosed herein (see U.S. Pat. No. 4,683,202, U.S. Pat. No. 5,928,906, each incorporated herein by reference).

[0083] In some embodiments, the vectors of the disclosure include one or more polyA signals. Illustrative polyA signals useful in the vectors of the disclosure include the short polyA signal and the bGH polyA signal. In some embodiments, the vectors of the disclosure include one or more 3' elements. Illustrative 3' elements include the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE).

Gene Therapy Vector Compositions

[0084] To prepare the composition, the vectors and/or the cells are generated, and the vectors or cells are purified as necessary or desired. The vectors, and/or other agents are sometimes suspended in a pharmaceutically acceptable carrier. In some embodiments, the composition is lyophilized. These compounds and cells are often adjusted to an appropriate concentration, and optionally combined with other agents. The absolute weight of a given compound and/or other agent included in a unit dose varies widely. The dose and the number of administrations are contemplated to be optimized by those skilled in the art.

[0085] For example, in some embodiments, about 10^2-10^{10} vector genomes (vg) are be administered. In some embodiments, the dose be at least about 10^2 vg, about 10^3 vg, about 10^4 vg, about 10^5 vg, about 10^6 vg, about 10^7 vg, about 10^8 vg, about 10^9 vg, about 10^{10} vg, or more vector genomes. In some embodiments, the dose be about 10^2 vg, about 10^3 vg, about 10^4 vg, about 10^5 vg, about 10^6 vg, about 10^7 vg, about 10^8 vg, about 10^9 vg, about 10^{10} vg, or more vector genomes.

[0086] Daily doses of the compounds vary as well. Such daily doses often range, for example, from at least about 10^2 vg/day, about 10^3 vg/day, about 10^4 vg/day, about 10^5 vg/day, about 10^6 vg/day, about 10^7 vg/day, about 10^8 vg/day, about 10^9 vg/day, about 10^{10} vg/day, or more vector genomes per day.

[0087] In some embodiments, the method of the disclosure comprises administering a vector or vector system of the disclosure (*e.g.* an rAAV vector) by intracardiac injection, intramyocardiac injection, endocardial injection, intracardiac catheterization, or systemic administration. In some embodiments, the

subject (*e.g.*, a human) is treated by administering between about $1x10^8$ and about $1x10^{15}$ GC of a vector (*e.g.*, an AAV vector or lentiviral vector) by intracardiac injection, intramyocardiac injection, endocardial injection, intracardiac catheterization, or systemic administration. In some embodiments, the subject is treated by administering between about $1x10^8$ and about $1x10^{15}$ GC, between about $1x10^8$ and about $1x10^{15}$ GC, between about $1x10^9$ and about $1x10^{14}$ GC, between about $1x10^{10}$ and about $1x10^{13}$ GC, between about $1x10^{11}$ and about $1x10^{12}$ GC, or between about $1x10^{12}$ and about $1x10^{13}$ GC of vector. In some embodiments, the subject is treated by administering between about $1x10^8$ and about $1x10^{10}$ GC, between about $1x10^9$ and about $1x10^{11}$ GC, between about $1x10^{10}$ and about $1x10^{12}$ GC, between about $1x10^{11}$ and about $1x10^{13}$ GC, between about $1x10^{12}$ and about $1x10^{14}$ GC, or between about $1x10^{13}$ and about $1x10^{15}$ GC of vector. In some embodiments, the subject is treated by administering at least $1x10^8$, at least about $1x10^9$, at least about $1x10^{10}$, at least about $1x10^{11}$, at least about $1x10^{12}$, at least about $1x10^{13}$, or at least about $1x10^{15}$ GC of vector. In some embodiments, the subject is treated by administering at most $1x10^8$, at most about $1x10^9$, at most about $1x10^{10}$, at most about $1x10^{11}$, at most about $1x10^{12}$, at most about $1x10^{13}$, or at most about $1x10^{15}$ GC of vector. In some embodiments, the subject (*e.g.*, a human) is treated by administering between about $1x10^8$ and about $1x10^{15}$ GC/kg of a vector (*e.g.*, an AAV vector or lentiviral vector) by intracardiac injection or systemically. In some embodiments, the subject is treated by administering between about $1x10^8$ and about $1x10^{15}$ GC/kg, between about $1x10^8$ and about $1x10^{15}$ GC/kg, between about $1x10^9$ and about $1x10^{14}$ GC/kg, between about $1x10^{10}$ and about $1x10^{13}$ GC/kg, between about $1x10^{11}$ and about $1x10^{12}$ GC/kg, or between about $1x10^{12}$ and about $1x10^{13}$ GC/kg of vector. In some embodiments, the subject is treated by administering between about $1x10^8$ and about $1x10^{10}$ GC/kg, between about $1x10^9$ and about $1x10^{11}$ GC/kg, between about $1x10^{10}$ and about $1x10^{12}$ GC/kg, between about $1x10^{11}$ and about $1x10^{13}$ GC/kg, between about $1x10^{12}$ and about $1x10^{14}$ GC/kg, or between about $1x10^{13}$ and about $1x10^{15}$ GC/kg of vector. In some embodiments, the subject is treated by administering at least $1x10^8$, at least about $1x10^9$, at least about $1x10^{10}$, at least about $1x10^{11}$, at least about $1x10^{12}$, at least about $1x10^{13}$, or at least about $1x10^{15}$ GC/kg of vector. In some embodiments, the subject is treated by administering at most $1x10^8$, at most about $1x10^9$, at most about $1x10^{10}$, at most about $1x10^{11}$, at most about $1x10^{12}$, at most about $1x10^{13}$, or at most about $1x10^{15}$ GC/kg of vector. It will be appreciated that the amount of vectors and for use in treatment will vary not only with the particular carrier selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient. Ultimately, in some embodiments, the attendant health care provider will determine proper dosage. A pharmaceutical composition is contemplated to be formulated with the appropriate ratio of each compound in a single unit dosage form for administration.

[0088] The compositions are sometimes formulated for sustained release (for example, using microencapsulation, see WO 94/07529, and/or U.S. Patent No.4,962,091). The formulations, where appropriate, are conveniently presented in discrete unit dosage forms and, in some embodiments, are prepared by any of the methods well known to the pharmaceutical arts. Such methods often include the step of mixing the therapeutic agent with liquid carriers, solid matrices, semi-solid carriers, finely divided

solid carriers or combinations thereof, and then, if necessary, introducing or shaping the product into the desired delivery system.

[0089] One or more suitable unit dosage forms containing the compounds, in some embodiments, are administered by a variety of routes including parenteral (including subcutaneous, intravenous, intramuscular and intraperitoneal), intracardially, pericardially, oral, rectal, dermal, transdermal, intrathoracic, intrapulmonary, and intranasal (respiratory) routes.

[0090] The gene therapy vectors provided herein are prepared in many forms that include aqueous solutions, suspensions, tablets, hard or soft gelatin capsules, and liposomes and other slow-release formulations, such as shaped polymeric gels. Administration of gene therapy vectors often involves parenteral or local administration in an aqueous solution. Similarly, compositions containing gene therapy vectors are sometimes administered in a device, scaffold, or as a sustained release formulation. Different types of formulating procedures are described in U.S. Patent No. 6,306,434 and in the references contained therein.

[0091] Vectors, in some embodiments, are formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous infusion) and are often presented in unit dosage form in ampoules, prefilled syringes, small volume infusion containers or multi-dose containers with an added preservative. The pharmaceutical compositions often take the form of suspensions, solutions, or emulsions in oily or aqueous vehicles, and sometimes contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Suitable carriers include saline solution, phosphate buffered saline, and other materials commonly used in the art.

[0092] The compositions sometimes also contain other ingredients such as agents useful for treatment of cardiac diseases, conditions and injuries, such as, for example, an anticoagulant (e.g., dalteparin (fragmin), danaparoid (orgaran), enoxaparin (lovenox), heparin, tinzaparin (innohep), and/or warfarin (coumadin)), an antiplatelet agent (e.g., aspirin, ticlopidine, clopidogrel, or dipyridamole), an angiotensin-converting enzyme inhibitor (e.g., Benazepril (Lotensin), Captopril (Capoten), Enalapril (Vasotec), Fosinopril (Monopril), Lisinopril (Prinivil, Zestril), Moexipril (Univasc), Perindopril (Aceon), Quinapril (Accupril), Ramipril (Altace), and/or Trandolapril (Mavik)), angiotensin II receptor blockers (e.g., Candesartan (Atacand), Eprosartan (Teveten), Irbesartan (Avapro), Losartan (Cozaar), Telmisartan (Micardis), and/or Valsartan (Diovan)), a beta blocker (e.g., Acebutolol (Sectral), Atenolol (Tenormin), Betaxolol (Kerlone), Bisoprolol/hydrochlorothiazide (Ziac), Bisoprolol (Zebeta), Carteolol (Cartrol), Metoprolol (Lopressor, Toprol XL), Nadolol (Corgard), Propranolol (Inderal), Sotalol (Betapace), and/or Timolol (Blocadren)), Calcium Channel Blockers (e.g., Amlodipine (Norvasc, Lotrel), Bepridil (Vascor), Diltiazem (Cardizem, Tiazac), Felodipine (Plendil), Nifedipine (Adalat, Procardia), Nimodipine (Nimotop), Nisoldipine (Sular), Verapamil (Calan, Isoptin, Verelan), diuretics (e.g., Amiloride (Midamor), Bumetanide (Bumex), Chlorothiazide (Diuril), Chlorthalidone (Hygroton), Furosemide (Lasix), Hydro-chlorothiazide (Esidrix, Hydrodiuril), Indapamide (Lozol) and/or Spironolactone (Aldactone)), vasodilators (e.g., Isosorbide dinitrate (Isordil), Nesiritide (Natrecor), Hydralazine

(Apresoline), Nitrates and/or Minoxidil), statins, nicotinic acid, gemfibrozil, clofibrate, Digoxin, Digitoxin, Lanoxin, or any combination thereof.

[0093] Additional agents are sometimes included such as antibacterial agents, antimicrobial agents, anti-viral agents, biological response modifiers, growth factors; immune modulators, monoclonal antibodies and/or preservatives. The compositions provided herein are contemplated to also be used in conjunction with other forms of therapy.

[0094] The viral vectors described herein are suitable for administration to a subject to treat a disease or disorder. In some embodiments, such a composition is in a single dose, in multiple doses, in a continuous or intermittent manner, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is in response to traumatic injury or for more sustained therapeutic purposes, and other factors known to skilled practitioners. The administration of the compounds and compositions of provided herein, in some embodiments, are administered continuously over a preselected period of time or alternatively are administered in a series of spaced doses. Both local and systemic administration is contemplated. In some embodiments, localized delivery of a viral or non-viral vector is achieved. In some embodiments, localized delivery of cells and/or vectors is used to generate a population of cells within the heart. In some embodiments, such a localized population operates as "pacemaker cells" for the heart.

Definitions

[0095] As used herein, the term "cardiomyopathy" refers to any disease or dysfunction of the myocardium (heart muscle) in which the heart is abnormally enlarged, thickened and/or stiffened. As a result, the heart muscle's ability to pump blood is usually weakened. The etiology of the disease or disorder is, in some cases, inflammatory, metabolic, toxic, infiltrative, fibroplastic, hematological, genetic, or unknown in origin. There are two general types of cardiomyopathies: ischemic (resulting from a lack of oxygen) and non-ischemic. In some cases, a cardiomyopathy is arrhythmogenic right ventricular cardiomyopathy (ARVC) or arrhythmogenic cardiomyopathy (ACM).

[0096] "Heart failure (HF) is a complex clinical syndrome that often result from any structural or functional cardiovascular disorder causing systemic perfusion inadequate to meet the body's metabolic demands without excessively increasing left ventricular filling pressures. It is characterized by specific symptoms, such as dyspnea and fatigue, and signs, such as fluid retention. As used herein, "chronic heart failure" or "congestive heart failure" or "CHF" refer, interchangeably, to an ongoing or persistent forms of heart failure. Common risk factors for CHF include old age, diabetes, high blood pressure and being overweight. CHF is broadly classified according to the systolic function of the left ventricle as HF with reduced or preserved ejection fraction (HFrEF and HFpEF). The term "heart failure" does not mean that the heart has stopped or is failing completely, but that it is weaker than is normal in a healthy person. In some cases, the condition is mild, causing symptoms that are noticeable when exercising, in others, the condition is more severe, causing symptoms that are, in some cases, life-threatening, even while at rest. The most common symptoms of chronic heart failure include shortness of breath, tiredness, swelling of the legs and ankles, chest pain and a cough. In some embodiments, the methods of the disclosure

decrease, prevent, or ameliorate one or more symptoms of CHF (e.g., HFrEF) in a subject suffering from or at risk for CHF (e.g., HFrEF). In some embodiments, the disclosure provides methods of treating CHF and conditions that sometimes lead to CHF.

[0097] As used herein "acute heart failure" or "decompensated heart failure" refer, interchangeably, to a syndrome of the worsening of signs and symptoms reflecting an inability of the heart to pump blood at a rate commensurate to the needs of the body at normal filling pressure. AHF typically develops gradually over the course of days to weeks and then decompensates requiring urgent or emergent therapy due to the severity of these signs or symptoms. In some cases, AHF is the result of a primary disturbance in the systolic or diastolic function of the heart or of abnormal venous or arterial vasoconstriction, but generally represents an interaction of multiple factors, including volume overload. The majority of patients with AHF have decompensation of chronic heart failure (CHF) and consequently much of the discussion of the pathophysiology, presentation, and diagnosis of CHF is directly relevant to an understanding of AHF. In other cases, AHF results from an insult to the heart or an event that impairs heart function, such as an acute myocardial infarction, severe hypertension, damage to a heart valve, abnormal heart rhythms, inflammation or infection of the heart, toxins and medications. In some embodiments, the methods of the disclosure decrease, prevent, or ameliorate one or more symptoms of AHF in a subject suffering from or at risk for AHF. In some embodiments, the disclosure provides methods of treating AHF and conditions that sometimes lead to AHF. In some cases, AHF is the result of ischemia associated with myocardial infarction.

[0098] As used herein, the terms "subject" or "individual" refers to any animal, such as a domesticated animal, a zoo animal, or a human. In some cases, the "subject" or "individual" is a mammal like a dog, cat, horse, livestock, a zoo animal, or a human. Alternatively or in combination, the subject or individual is a domesticated animal such as a bird, a pet, or a farm animal. Specific examples of "subjects" and "individuals" include, but are not limited to, individuals with a cardiac disease or disorder, and individuals with cardiac disorder-related characteristics or symptoms, such as arrhythmogenic right ventricular cardiomyopathy (ARVC) or arrhythmogenic cardiomyopathy (ACM).

[0099] The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of tissue culture, immunology, molecular biology, cell biology and recombinant DNA, which are within the skill of the art. See, e.g., Sambrook and Russell eds. (2001) Molecular Cloning: A Laboratory Manual, 3rd edition; the series Ausubel et al. eds. (2007) Current Protocols in Molecular Biology; the series Methods in Enzymology (Academic Press, Inc., N.Y.); MacPherson et al. (1991) PCR 1: A Practical Approach (IRL Press at Oxford University Press); MacPherson et al. (1995) PCR 2: A Practical Approach; Harlow and Lane eds. (1999) Antibodies, A Laboratory Manual; Freshney (2005) Culture of Animal Cells: A Manual of Basic Technique, 5thedition; Gait ed. (1984) Oligonucleotide Synthesis; U.S. Pat. No. 4,683,195; Hames and Higgins eds. (1984) Nucleic Acid Hybridization; Anderson (1999) Nucleic Acid Hybridization; Hames and Higgins eds. (1984) Transcription and Translation; IRL Press (1986) Immobilized Cells and Enzymes; Perbal (1984) A Practical Guide to Molecular Cloning; Miller and Calos eds. (1987) Gene Transfer Vectors for Mammalian Cells (Cold

Spring Harbor Laboratory); Makrides ed. (2003) Gene Transfer and Expression in Mammalian Cells; Mayer and Walker eds. (1987) Immunochemical Methods in Cell and Molecular Biology (Academic Press, London); Herzenberg et al. eds (1996) Weir's Handbook of Experimental Immunology; Manipulating the Mouse Embryo: A Laboratory Manual, 3rd edition (2002) Cold Spring Harbor Laboratory Press; Sohail (2004) Gene Silencing by RNA Interference: Technology and Application (CRC Press); Sell (2013) Stem Cells Handbook.

[00100] Unless the context indicates otherwise, it is specifically intended that the various features of the disclosure described herein can be used in any combination. Moreover, the disclosure also contemplates that in some embodiments, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

[00101] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 1.0 or 0.1, as appropriate, or alternatively by a variation of +/- 15 %, or alternatively 10%, or alternatively 5%, or alternatively 2%. It is to be understood, although not always explicitly stated, that all numerical designations are preceded by the term "about". It is to be understood that such range format is used for convenience and brevity and should be understood flexibly to include numerical values explicitly specified as limits of a range, but also to include all individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly specified. For example, a ratio in the range of about 1 to about 200 should be understood to include the explicitly recited limits of about 1 and about 200, but also to include individual ratios such as about 2, about 3, and about 4, and sub-ranges such as about 10 to about 50, about 20 to about 100, and so forth. It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[00102] It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cardiomyocyte" includes a plurality of cardiomyocytes.

[00103] Also as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

[00104] "Administration," "administering" and the like, when used in connection with a gene therapy vector or composition thereof as provided herein refer both to direct administration, which, in some cases includes administration to non-cardiomyocytes in vitro, administration to non-cardiomyocytes in vivo, administration to a subject by a medical professional or by self-administration by the subject and/or to indirect administration, which, in some cases, is the act of prescribing a composition comprising a gene therapy vector provided herein. When used herein in reference to a cell, it refers to introducing a composition to the cell. Typically, an effective amount is administered, which amount is often to be

determined by one of skill in the art. Any suitable method of administration is contemplated to be used. In some cases, a gene therapy vector is administered to the cells by, for example, by addition of the gene therapy vector to the cell culture media or injection in vivo to the site of cardiac injury. In some cases, administration to a subject is achieved by, for example, intravascular injection, intramyocardial delivery, and the like.

[00105] As used herein the term "cardiac cell" refers to any cell present in the heart that provides a cardiac function, such as heart contraction or blood supply, or otherwise serves to maintain the structure of the heart. Cardiac cells as used herein encompass cells that exist in the epicardium, myocardium, or endocardium of the heart. Cardiac cells also include, for example, cardiac muscle cells or cardiomyocytes, and cells of the cardiac vasculatures, such as cells of a coronary artery or vein. Other non-limiting examples of cardiac cells include epithelial cells, endothelial cells, fibroblasts, cardiac stem or progenitor cells, cardiac conducting cells and cardiac pacemaking cells that constitute the cardiac muscle, blood vessels and cardiac cell supporting structure. In some cases, cardiac cells are derived from stem cells, including, for example, embryonic stem cells or induced pluripotent stem cells.

[00106] The term "cardiomyocyte" or "cardiomyocytes" as used herein refers to sarcomere-containing striated muscle cells, naturally found in the mammalian heart, as opposed to skeletal muscle cells. Cardiomyocytes are characterized by the expression of specialized molecules e.g., proteins like myosin heavy chain, myosin light chain, cardiac α-actinin. The term "cardiomyocyte" as used herein is an umbrella term comprising any cardiomyocyte subpopulation or cardiomyocyte subtype, e.g., atrial, ventricular and pacemaker cardiomyocytes.

[00107] The term "culture" or "cell culture" means the maintenance of cells in an artificial, in vitro environment. A "cell culture system" is used herein to refer to culture conditions in which a population of cells are grown as monolayers or in suspension. "Culture medium" is used herein to refer to a nutrient solution for the culturing, growth, or proliferation of cells. Culture medium is characterized, in some cases, by functional properties such as, but not limited to, the ability to maintain cells in a particular state (e.g., a pluripotent state, a quiescent state, etc.), or to mature cells, such as, in some embodiments, to promote the differentiation of progenitor cells into cells of a particular lineage (e.g., a cardiomyocyte).

[00108] As used herein, the term "expression" or "express" refers to the process by which nucleic acids or polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. If the polynucleotide or nucleic acid is derived from genomic DNA, in some cases, expression includes splicing of the mRNA in a eukaryotic cell. In some cases, the expression level of a gene is determined by measuring the amount of mRNA or protein in a cell or tissue sample.

[00109] As used herein, an "expression cassette" is a DNA polynucleotide comprising one or more polynucleotides or nucleic acids encoding protein(s) or nucleic acid(s) that is configured to express the polynucleotide in a host cell. Typically, expression of the polynucleotide(s) is placed under the control of certain regulatory elements, including constitutive or inducible promoters, tissue-specific regulatory

elements, and enhancers. Such polynucleotides are said to be "operably linked to" or "operatively linked to" the regulatory elements (e.g., a promoter).

[00110] The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00111] "Treatment," "treating," and "treat" are defined as acting upon a disease, disorder, or condition with an agent to reduce or ameliorate harmful or any other undesired effects of the disease, disorder, condition and/or their symptoms.

[00112] As used herein, the term "effective amount" and the like refers to an amount that is sufficient to induce a desired physiologic outcome (e.g., treatment of a disease). An effective amount is sometimes administered in one or more administrations, applications or dosages. Such delivery is dependent on a number of variables including the time period which the individual dosage unit is to be used, the bioavailability of the composition, the route of administration, etc. It is understood, however, that specific amounts of the compositions (e.g., gene therapy vectors) for any particular subject depends upon a variety of factors including the activity of the specific agent employed, the age, body weight, general health, sex, and diet of the subject, the time of administration, the rate of excretion, the composition combination, severity of the particular disease being treated and form of administration.

[00113] As used herein, the term "equivalents thereof" in reference to a polypeptide or nucleic acid sequence refers to a polypeptide or nucleic acid that differs from a reference polypeptide or nucleic acid sequence, but retains essential properties (e.g., biological activity). A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant, in some cases, alters the amino acid sequence of a polypeptide encoded by the reference polynucleotide. In some cases, nucleotide changes result in amino acid substitutions, deletions, additions, fusions and truncations in the polypeptide encoded by the reference sequence. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical.

[00114] As used herein, the term "nucleic acid" and "polynucleotide" are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Non-limiting examples of polynucleotides include linear and circular nucleic acids, messenger RNA (mRNA), cDNA, recombinant polynucleotides, vectors, probes, and primers. As used herein, the word "polynucleotide" or "nucleic acid" preceded by a gene name (for example, "PKP2 nucleic acid") refers to a polynucleotide sequence encoding the corresponding protein (for example, a "PKP2 protein").

[00115] The terms "polypeptide," "peptide," and "protein," are used interchangeably herein and refer to a polymeric form of amino acids of any length, which sometimes include genetically coded and non-genetically coded amino acids, chemically or biochemically modified or derivatized amino acids, and

polypeptides having modified peptide backbones. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous leader sequences, with or without N-terminal methionine residues, immunologically tagged proteins, and the like. As used herein, the word "protein" preceded by a gene name (for example, "PKP2 protein") refers to either the native protein or a functional variant thereof. A "native protein" is a protein encoded by a genomic copy of a gene of an organism, preferably the organism for which the vector is intended (e.g., a human, a rodent, a primate, or an animal of veterinary interest), in any of the gene's functional isoforms or functional allelic variations.

[00116] As used herein, a "functional variant" or "variant" of a protein is a variant with any number of amino acid substitutions, insertions, truncations, or internal deletions that retains the functional attributes of the protein, including, e.g., the protein's ability to induce, in combination with other factors, organization of desmosomes. In some cases, functional variants are identified computationally, such as variants having only conservative substitutions, or experimentally using in vitro or in vivo assays.

[00117] As used herein, a "codon variant" of a polynucleotide sequence is polynucleotide sequence that encodes the same protein as a reference polynucleotide sequence having one or more synonymous codon substitutions. Selection of synonymous codons is within the skill of those in the art, the coding as the genetic code being known. In some cases, codon optimization is performed using a variety of computational tools (such the GENSMART™ Codon Optimization tool available at www.genscript.com). Generally codon optimization is used to increase the expression of protein in a heterologous system, for instance when a human coding sequence is expressed in a bacterial system. The term "codon variant" is intended to encompass both sequences that are optimized in this manner and sequences that are optimized for other purposes, such as removal of CpG islands and/or cryptic start sites.

[00118] The term "vector" refers to a macromolecule or complex of molecules comprising a polynucleotide or protein to be delivered to a host cell, either in vitro or in vivo. A vector is sometimes a modified RNA, a lipid nanoparticle (encapsulating either DNA or RNA), a transposon, an adeno-associated virus (AAV) vector, an adenovirus, a retrovirus, an integrating lentiviral vector (LVV), or a non-integrating LVV. Thus, as used herein "vectors" include naked polynucleotides used for transformation (e.g. plasmids) as well as any other composition used to deliver a polynucleotide to a cell, included vectors capable of transducing cells and vectors useful for transfection of cells.

[00119] As used herein, the term "viral vector" refers either to a nucleic acid molecule that includes virus-derived nucleic acid elements that typically facilitate transfer of the nucleic acid molecule or integration into the genome of a cell or to a viral particle that mediates nucleic acid transfer. Viral particles will typically include various viral components and sometimes also cell components in addition to nucleic acid(s).

[00120] The term "genetic modification" refers to a permanent or transient genetic change induced in a cell following introduction of new nucleic acid (i.e., nucleic acid exogenous to the cell). Genetic change is often accomplished by incorporation of the new nucleic acid into the genome of the cardiac cell, or by transient or stable maintenance of the new nucleic acid as an extrachromosomal element. Where the cell

is a eukaryotic cell, a permanent genetic change is often achieved by introduction of the nucleic acid into the genome of the cell. Suitable methods of genetic modification include viral infection, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate precipitation, direct microinjection, and the like.

[00121] FIG 1 shows cardiac desmosomes tie cells together (Brodehl et al., 2018; Moncayo-Arlandi and Brugada, 2017). The red line in the top panel is depicted as desmin, the intermediate filaments, which forms network to stabilize sarcomeres and other organelles. An EM picture of desmosome is shown in the left corner.

[00122] FIG. 2 shows a summary of ARVC disease indications and possible disease mechanisms.

[00123] FIGS. 3A-3C show results of acute silencing of PKP2 in iPSCM at day 8 showed significant cellular phenotypes. In **FIG. 3A** and **FIG. 3C**, PKP2 in green, DSP in red, and nuclei in blue. In **FIG. 3B**, sarcomeric protein Actn2 in green and MyBPC in magenta.

[00124] FIG. 4 shows a quantitative measurement of DSP membrane localization is estimated by its co-localization with PKG, another desmosomal protein, in response to a range of dosage of siPKP2 at day 8 of silencing.

[00125] FIG. 5 illustrates an immunoblot showing that silencing PKP2 leads to a reduced total amount of DSP protein from the desmosomes, detected mainly in the insoluble fraction, in cells where PKP2 is silenced as compared to the silencing control, siNeg.

[00126] FIG. 6A shows a vector map for AAV-PKP2 gene therapy. **FIG. 6B** shows restoration of DSP membrane location by expressing PKP2 transgene using AAV-mediated gene delivery to iPSCM at day 10 of PKP2 silencing and at day 8 of AAV transduction. GFP is co-expressed with the flag-tagged PKP2. Flag tag is in cyan, DSP in red, and nuclei in blue. FIG. 6C shows AAV-PKP2 transgene restoration of expression of total DSP post PKP2 silencing in iPSC cardiomyocytes. A quantification of total DSP intensity in immunofluorescent signal is showed post PKP2 silencing in the absence or the presence of AAV-PKP2 transgene rescue.

[00127] FIGS. 7A-7B show AAV PKP2 transgene expression rescued contraction velocity of iPSCM post PKP2 silencing. **FIG. 7A** shows PKP2 silencing, AAV transduction, and contractility recording schedule. **FIG. 7B** shows AAV-PKP2 transgene partially restores contraction velocity post PKP2 silencing. Contraction velocity, normalized to the nuclear count, is shown post PKP2 silencing in the absence or the presence of AAV-PKP2 transgene rescue.

[00128] FIG. 8 shows a schematic representation of the second generation AAV expression cassette of human and mouse PKP2α. The left table shows all elements in the expression cassette. The right panel shows the mouse and human expression cassettes.

[00129] FIGS. 9A-9B show preliminary results for the second generation AAV-hPKP2α partially rescues contraction velocity post PKP2 silencing in iPSC cardiomyocytes. **FIG. 9A** shows human PKP2α transgene was expressed in iPSC cardiomyocytes in a dose-dependent fashion. **FIG. 9B** shows human PKP2α transgene showed a partial rescue of contraction velocity post PKP2 silencing at 30K MOI.

[00130] FIG. 10 shows expression analysis of second generation AAV9 human and mouse PKP2α in 12 week-old C57BL/6 animals. The upper panel shows expression of endogenous mouse PKP2α in HBSS control mice and expression of both endogenous and transduced mouse PKP2α at two AAV9 injected doses, 1E13 and 5E13, respectively. The lower panel shows corresponding expression analysis of transduced human PKP2α, a slightly larger homolog.

[00131] FIGS. 11A-G show pilot expression safety studies of second generation AAV9 human and mouse PKP2α in 12 week-old C57BL/6 animals. **FIG. 11A** shows body weight before AAV9 injection and body weight at 3 weeks post AAV9 injection. **FIG. 11B** shows heart function is measured by percentage of ejection fraction at 3 weeks post AAV9 injection of either mouse or human PKP2α. **FIG. 11C** and **FIG. 11D** show LV structure measured by both internal diameters end diastole and systole. **FIGs. 11E-11G** show electrophysiology activity measured by QRS (**FIG. 11E**), QT interval (**FIG. 11F**), and P/R amplitude (**FIG. 11G**).

[00132] FIG. 12 shows a Kaplan-Meier survival curve of *PKP2-cKO* mice after tamoxifen induction. The curve shows that *PKP2-cKO* mice begin declining at three weeks post induction with only one mouse (of seven) surviving to six weeks.

[00133] FIGS. 13A-13B show right ventricle (RV) dilated cardiomyopathy of *PKP2-cKO* mice. **FIG. 13A** (left panel) shows images that illustrate increased RV internal dimension at end-diastole (RVIDd) in *PKP2-cKO* mice at three weeks post tamoxifen induction compared with the control mice. **FIG. 13A** (right panel) shows a graph of RVIDd over time in *PKP2-cKO* mice compared with the control mice. **FIG. 13B** (left panel) shows images illustrating the increase in RV area in *PKP2-cKO* mice. The RV area is illustrated by a dotted line which is shown to increase in area starting at one week post induction, at three weeks, and at four weeks post induction. **FIG. 13B** (right panel) shows a graph of RV area over time in *PKP2-cKO* mice compared with control mice.

[00134] FIGS. 14A-14B show development of left ventricle (LV) dilated cardiomyopathy of *PKP2-cKO* mice compared with control. **FIG. 14A** (left panel) shows images of increased LV internal dimension at end-systole (LVIDs) and end-diastole (LVIDd) in *PKP2-cKO* mice compared with control. The LVIDs is shown as the yellow line to the left and the LVIDd is shown as the red line to the right. **FIG. 14A** (right panel) shows a graph which shows the increase in LVIDs and LVIDd in *PKP2-cKO* mice over time compared with control mice. **FIG. 14B** shows a graph of LV performance as measured by percent ejection fraction over time compared with control mice.

[00135] FIG. 15 shows development of severe electrophysiological phenotypes of *PKP2-cKO* mice compared with control, specifically showing prolonged QRS interval and increased P/R amplitude ratio in *PKP2-cKO* mice. The top panel shows exemplary electrocardiogram of control (top) and *PKP2-cKO* mice (bottom). The increased P wave amplitude is shown in the *PKP2-cKO* mice compared with control. The electrocardiogram also shows a decreased R wave amplitude in *PKP2-cKO* mice compared with control mice. In addition, the QRS interval is prolonged in *PKP2-cKO* mice compared with control. The bottom panel shows graphs of the increase in QRS interval and increase in P/R amplitude in *PKP2-cKO* mice compared with control.

[00136] FIGS. 16A-16C show enhanced expression of fibrosis, tissue remodeling genes, and heart failure markers. **FIG. 16A** shows PKP2 RNA expression in RV and LV (top) and desmosome and Cx43 protein expression (bottom) of *PKP2-cKO* mice compared with control. The *PKP2-cKO* mice show about half the expression of PKP2 compared with control in both the LV and the RV. The bottom panel shows an immunoblot showing reduction in LV protein levels of PKP2, DSP, and PKG in desmosome and Cx43 in gap junction. **FIG. 16B** shows enhanced expression of fibrosis genes: TGFβ1, Col1a1, and Col3a1; and tissue remodeling genes: Timp1 and Mmp2 in *PKP2-cKO* mice compared with control. Here, expression of TGFβ1 and Timp1 is increased between control and *PKP2-cKO* mice in both RV and LV. Col1a1 and Col3a1 are also greatly increased in *PKP2-cKO* mice compared with control mice with slightly more elevated expression in LV compared to RV. Mmp2 is shown to be increased in *PKP2-cKO* mice compared with control mice with slightly more elevated expression in RV compared with LV. Mmp9 was not shown to have a difference in expression between control and *PKP2-cKO* mice. **FIG. 16C** shows enhanced expression of heart failure markers, NPPA and NPPB, in *PKP2-cKO* mice compared with control mice. In both NPPA and NPPB expression is slightly more elevated in LV compared with RV in *PKP2-cKO* mice.

[00137] FIG. 17 shows the experimental design to evaluate PKP2 efficacy as gene therapy in the *PKP2-cKO* ARVC mouse model. A total of six individual treatment groups were included in the studies and all groups were tamoxifen treated for three consecutive days. They are: six WT mice with HBSS buffer treated; ten PKP2-cKO ARVC mice with HBSS buffer treated; ten PKP2-cKO ARVC mice with 3E13 vg/kg of AAV9:human PKP2 treated at 3wks before tamoxifen induction; ten PKP2-cKO ARVC mice with 5E13 vg/kg of AAV9:mouse PKP2 treated at 3wks before tamoxifen induction; ten PKP2-cKO ARVC mice with 5E13 vg/kg of AAV9:mouse PKP2 treated right after tamoxifen induction; and ten PKP2-cKO ARVC mice with 5E13 vg/kg of AAV9:mouse PKP2 treated at 1wk after tamoxifen induction. Baseline readings of body weight, echocardiography, and EKG were collected before tamoxifen induction. All readings post tamoxifen induction were recorded weekly including echocardiography of B-mode, M-Mode (RV, LV), and structure (LV internal diameters) and 30-min ECG for quantifying arrythmias and evaluating electrophysiological parameters.

[00138] FIG. 18A shows a schematic of the AAV expression cassettes for human and mouse PKP2α. The AAV-pTnT600-mPKP2-WPRE has 4199 basepairs which include inverted terminal repeats (ITR) on the 5' end and the 3' end, the pcTNT promoter followed by the coding sequence for mouse PKP2α, then the WPRE and bGH at the 3' end before the 3' ITR. The AAV-pTnT600-hPKP2op-WPRE has 4324 basepairs which include inverted terminal repeats (ITR) on the 5' end and the 3' end, the pcTNT promoter followed by a codon optimized coding sequence for human PKP2α, then the WPRE and bGH at the 3' end before the 3' ITR. **FIG. 18B** shows immunoblots of protein expression of mouse and human PKP2α from wildtype mice treated with AAV9:PKP2 (see full blots in FIG. 10). Buffer treated mice are in the left panel. The middle panel shows an immunoblot from mice treated with 1E13 viral genomes per kg with the AAV9:mPKP2 in the top immunoblot and the AAV9:hPKP2 (codon optimized) in the bottom immunoblot. The right panel shows immunoblots from mice treated with 5E13 viral genomes per

kg with the AAV9:mPKP2 in the top immunoblot and the AAV9:hPKP2 (codon optimized) in the bottom immunoblot.

[00139] FIG. 19 shows a Kaplan-Meier survival curve of *PKP2-cKO* mice treated with AAV9:PKP2. Mice treated with buffer are shown in the red line that begins declining at three weeks post tamoxifen induction and near zero probability of survival at six weeks post-tamoxifen induction. All of the treated groups have 90% or greater probability of survival at six weeks post-tamoxifen induction.

[00140] FIGS. 20A-20C show the efficacy of AAV9:PKP2 treatment of *PKP2-cKO* mice in reducing RV and LV dilation and maintaining cardiac function. **FIG. 20A** shows a graph illustrating improvement in ejection fraction in AAV9:PKP2 treated mice. In this graph buffer treated *PKP2-cKO* mice have a 34% reduction in ejection fraction compared to both wildtype and AAV9:PKP2 treated mice. **FIG. 20B** shows a graph illustrating reduction of RV dilation in AAV9:PKP2 treated mice compared with buffer treated mice which are shown in the red line that is elevated in the graph compared to wildtype mice and AAV9:PKP2 treated mice. **FIG. 20C** shows graphs illustrating improvement in LVIDd (top) and LVIDs (bottom). Each treatment group is illustrated by a separate bar graph with wildtype and buffer treated *PKP2-cKO* mice on the left side.

[00141] FIGS. 21A-21B show improvement in ECG parameters of *PKP2-cKO* mice treated with AAV:PKP2. **FIG. 21A** shows exemplary raw ECG traces of control and *PKP2-cKO* mice treated with AAV9:PKP2 and buffer. The ECG traces of buffer treated control and *PKP2-cKO* mice are shown on the top two panels. AAV9:PKP2 treated *PKP2-cKO* mice are shown in the bottom two traces. **FIG. 21B** shows graphs illustrating improvement of P/R ratio (top graph), QT interval (middle graph), and QRS interval (bottom graph) in *PKP2-cKO* mice treated with AAV9:PKP2 compared with treatment with buffer. In each instance, buffer treated *PKP2-cKO* mice are outliers with the treated and wild type mice.

[00142] FIGS. 22A-22B show AAV9:PKP2 treatment improvement in arrhythmias in *PKP2-cKO* mice. **FIG. 22A** (top) shows a table grading of severity of arrhythmias. Grade 5 represents S-VT/VF/cardiac sudden death; 4 represents NSVT; 3 represents >100 PVCs, couplets and triplets; 2 represents >50, <100 PVCs; 1 represents <50 PVCs, PJCs, and AV block; and 0 represents <10 PVCs. **FIG. 22A** (bottom) shows a graph which summarizes improvement of arrhythmia scores of *PKP2-cKO* mice treated with AAV9:PKP2 compared with control. The buffer treated *PKP2-cKO* mice with an increase in ventricular arrhythmia score starting three weeks post-tamoxifen induction. **FIG 22B** shows a distribution graph showing improvement in severity of arrhythmias in *PKP2-cKO* mice treated with AAV9:PKP2 compared with control. Wildtype mice are shown in the left most bar graph followed by buffer treated *PKP2-cKO* mice which showed an average score of 3. Treated mice are represented by the four bar graphs which show, on average, a dramatic decrease in score.

[00143] FIG. 23 shows the experimental design used to evaluate human PKP2 efficacy as a gene therapy using the *PKP2-cKO* ARVC mouse model. *PKP2-cKO* mice were treated with tamoxifen for three consecutive days and then treated with various doses of AAV9:hPKP2 or HBSS.

[00144] FIGS. 24A-24D show results of AAV9:hPKP2 gene therapy treatment of *PKP2-cKO* mice. **FIG. 24A** shows results of ejection fraction where control mice show a high ejection fraction that is

reduced with tamoxifen induction and not affected by HBSS treatment. Treatment with increasing doses of AAV9:hPKP2 significantly increases ejection fraction in treated mice ($P<0.05$). **FIG. 24B** show results of right ventricle size. Control mice have a relatively small RV size that is increased with tamoxifen induction and not affected by HBSS treatment. AAV9:hPKP2 treatment shows a dose related reduction in RV size. (Lower two doses $P<0.05$, highest dose $P<0.01$). **FIG. 24C** shows LV dilation as measured by LVIDd. **FIG. 24D** shows LV dilation as measured by LVIDs. AAV9:hPKP2 treatment significantly reduced the increase in left ventricle dilation as measured by LVIDd ($P<0.05$) and LVIDs ($P<0.05$, for the 1e14 dose only).

[00145] FIG. 25 shows results AAV9:hPKP2 gene therapy treatment of *PKP2-cKO* mice for QT interval (top), P/R Ratio (middle), and Arrhythmia Score (bottom). AAV9:hPKP2 treatment showed a significant reduction at 4wks post tamoxifen induction in QT interval ($P<0.05$), a trending reduction in P/R ratio, and a trending reduction in arrhythmias in PKP2-cKO mice as compared to PKP2-cKO mice treated with HBSS shown in red bars.

[00146] FIGS. 26A-26B show results of AAV9:hPKP2 treatment of *PKP2-cKO* mice in reducing expression of heart failure markers, fibrosis and tissue remodeling markers in right ventricle (**FIG. 26A**) and left ventricle (**FIG. 26B**). AAV9:hPKP2 treatment of PKP2-cKO mice showed efficacy in reducing expression of heart failure markers, fibrosis and tissue remodeling genes in both right ventricle and left ventricle at 4wks post tamoxifen induction. Endogenous and transgene mRNA levels of PKP2 were estimated in WT control, *PKP2-cKO*, and AAV9:hPKP2 transduced hearts, respectively. Heart failure markers are NPPA and NPPB. Fibrosis genes are Col1a1 and Col3a1. Tissue remodeling gene is Timp1.

[00147] FIGS. 27A-27B shows results of AAV9:hPKP2 treatment of *PKP2-cKO* mice in reducing fibrosis development in both right ventricle and left ventricle at four weeks post tamoxifen induction. **FIG. 27A** shows muscle staining in red color and trichrome staining of fibrosis in blue color. Yellow arrows highlight areas with significant fibrosis in *PKP2-cKO* mouse heart. **FIG. 27B** shows a graph quantifying the collagen positive tissue in control and *PKP2-cKO* mice with and without treatment with AAV9:hPKP2. Untreated *PKP2-cKO* mice have the greatest amount of collagen positive tissue, whereas mice treated with 1e13 have reduced levels by about half. Mice treated with 3e13 or 1e14 have collagen levels close to the control mice.

[00148] FIGS. 28A-28B show expression of PKP2 and other desmosome proteins in soluble fraction (**FIG. 28A**) and insoluble fraction (**FIG 28B**). No DSP is detected in the soluble fractions. Note: * This animal was found dead before echocardiography. **PKP2 protein intensity is normalized to tubulin intensity on the same Western blot. For simplicity, only one of the two tubulin blots is shown here. PKP2 is shown as two bands, the endogenous mouse PKP2 and the larger human PKP2.

EXAMPLES

[00149] The following examples are given for the purpose of illustrating various embodiments of the disclosure and are not meant to limit the present disclosure in any fashion. The present examples, along

with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the claims. Changes therein and other uses which are encompassed within the spirit of the disclosure as defined by the scope of the claims will occur to those skilled in the art.

Example 1: Cellular Model of PKP2 Depletion

[00150] As an initial proof of concept, a cellular model of depletion of PKP2 was created using siRNA. PKP2 was depleted in in induced pluripotent stem cell-derived cardiomyocytes (iPSCM). Acute silencing of PKP2 by siRNAs was performed using siRNAs purchased from Invitrogen including both siPKP2 and negative control siRNA (4390843 Silencer Select Negative Control No. 1 siRNA; 4392420 Assay Id s531202 Silencer Select Pre-Designed siRNA #1; 4392420 Assay Id s531203 Silencer Select Pre-Designed siRNA #2; 4392420 Assay Id s531204 Silencer Select Pre-Designed siRNA #3; and 4392420 Assay Id s10585 Silencer Select Pre-Designed siRNA #4). This silencing led to disappearance of DSP from the cellular membrane at day 8 as shown by immunofluorescence at FIG. 3A. The DSP membrane localization was quantitatively measured (FIG. 4) which illustrated a significant reduction in DSP-PKG co-localization. A reduction of sarcomere density was also observed by immunofluorescence (FIG. 3B). In addition, a disarray of cell compaction in patterned iPSCM was seen by immunofluorescence (FIG. 3C).

[00151] An immunoblot of siPKP2 iPSCM lysate was performed showing that a reduced total amount of DSP protein from the desmosomes is detected mainly in the insoluble fraction of cells were PKP2 is silenced (FIG. 5).

Example 2: AAV9-PKP2 Rescues PKP2 Depletion Phenotype

[00152] By delivering AAV9 variant CR9-01 flag-tagged PKP2 expression driven by 600 nt cardiac troponin (TnT) promoter with GFP to identify transduced cells (FIG. 6A), re-localization of DSP back to the membrane in PKP2 silenced iPSCM was observed (FIG. 6B), thereby restoring desmosome structure. PKP2 transgene was codon optimized to resist siRNA-mediated silencing. Due to a technical difficulty, it was not possible accurately quantify how much DSP was specifically localized to the membrane where cellular junction occurs and desmosomes exist. Therefore, the total cellular DSP intensity, instead of an amount of DSP localized to membrane, was quantified.

[00153] To demonstrate that PKP2 transgene could functionally restore the contractility of cardiomyocytes, bright field-based contraction of iPSCM was recorded by SONY imaging and videos were analyzed by DANA Solutions Pulse analysis software. An experimental timeline is shown in FIG. 7A. In this experiment, siRNA was used to deplete endogenous PKP2 expression in iPSCM cells on day 1. Two siRNA concentrations, 5 and 1.25 nM, were used for either siRNA negative control or siPKP2. Two siPKP2 #3 and #4 were combined to silence the transcript. On day 3, an AAV PKP2 was used to transduce depleted cells resulting in a rescue of contraction velocity was observed in iPSCM in response to PKP2 transgene expression (FIG. 7B). Contractility was recorded at days 3, 4, 5, 6, 7, and 8 post AAV transduction. Contraction velocity was averaged from three 96-well plates and from cells transduced with either AAV 300K MOI or 100K MOI, respectively, at both 5 and 1.25 nM siRNAs. The

velocity value was further normalized to the average nuclear count corresponding to 300K or 100K MOI, respectively.

Example 3: Treatment with Second Generation PKP2α AAV9

[00154] A second generation AAV expression cassette was developed for expressing human or mouse PKP2α. The second generation cassette included a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE) and a bovine growth hormone polyadenylation signal (bGH poly(A)). The second generation vector is illustrated in FIG. 8.

[00155] Transgene rescue studies were conducted in PKP2 silenced iPSC cardiomyocytes. Preliminary results suggested that the second generation AAV-hPKP2α partially rescued contraction velocity post PKP2 silencing in iPSC cardiomyocytes. FIG. 9A shows results where human PKP2α transgene was expressed in iPSC cardiomyocytes in a dose-dependent fashion by different MOI (multiplicity of infection, the average number of virus particles infecting each cells). PKP2 and DSP (desmoplakin) expression were evaluated in both soluble and insoluble fractions of cells at 3 days post AAV transduction. FIG. 9B shows that human PKP2α transgene showed a partial rescue of contraction velocity post PKP2 silencing at 30K MOI as indicated by Student t test with a p value of 0.0103 in contrast to a p value of <0.0001 without AAV PKP2 transgene.

[00156] An experiment was conducted to study expression analysis of the second generation AAV9 human and mouse PKP2α in 12 week-old C57BL/6 animals. The results of this experiment are shown in FIG. 10. Animals were retro-orbital intravenously administrated with AAV9-PKP2α at doses of 1E13 and 5E13 vg/kg, respectively. Heart LV tissues were harvested at 3.5 weeks post injection. Soluble fraction of LV tissues were analyzed here with Western blot. The upper panel shows expression of endogenous mouse PKP2α in HBSS control mice and expression of both endogenous and transduced mouse PKP2α at two AAV9 injected doses, 1E13 and 5E13, respectively. The lower panel shows corresponding expression analysis of transduced human PKP2α, a slightly larger homolog. This human homolog is codon optimized. There were no adverse cardiac event observed at 3.5 weeks post AAV injection by echocardiogram.

[00157] FIGS. 11A-11G show pilot expression safety studies of second generation AAV9 human and mouse PKP2α in 12 week-old C57BL/6 animals did not show any adverse cardiac event at 3 weeks post AAV injection by echocardiogram. Animals were retro-orbital intravenously administrated with AAV9-PKP2α at doses of 1E13 and 5E13 vg/kg, respectively. FIG. 11A shows body weight before AAV9 injection and body weight at 3 weeks post AAV9 injection. FIG. 11B shows heart function measured by percentage of ejection fraction at 3 weeks post AAV9 injection of either mouse or human PKP2α. FIG. 11C and 11D show LV structure measured by both internal diameters end diastole and systole. FIG. 11E-11F show electrophysiology activity measured by QRS, QT interval, and P/R amplitude.

Example 4: *PKP2-cKO* ARVC Mouse Model Characterization

[00158] Four wild-type and seven *PKP2-cKO* ARVC mice, *αMYHC-Cre-ER(T2), PKP2$^{fl/fl}$*, at approximately 3 months of age were intraperitoneally injected for four consecutive days with tamoxifen (20 mg/ml in corn oil 100 µl/mice (approximately 75 mg/kg)). Baseline readings of body weight,

echocardiography, and EKG were collected before tamoxifen induction. All readings post-tamoxifen induction were recorded weekly including echocardiography of B-mode, M-mode (RV, LV), and structure (LV internal diameters) and 30-minute ECG for quantifying arrythmias and evaluating electrophysiological parameters. Terminal tissues, including heart and lung, were collected at the end of the study.

[00159] A survival analysis was performed on the mice (FIG. 12). The Kaplan-Meier survival curve showed a sharp decline of survival of *PKP2-cKO* mice three weeks post-tamoxifen induction, with only one animal reaching six weeks post-tamoxifen induction. Animals showed severe clinical symptoms including sudden death, edema, reduced activity, less tolerance to isoflurane three weeks post-induction.

[00160] *PKP2-cKO* mice developed RV dilated cardiomyopathy at as early as one week post-tamoxifen induction. FIG. 13A, in the left panel, shows that at three weeks post-tamoxifen induction, *PKP2-cKO* mice developed an increased RV internal dimension at end-diastole (RVIDd). The right panel of FIG. 13A summarizes the continuous increases in RVIDd normalized to body weight during four weeks of tamoxifen induction. FIG. 13B, in the left panel, shows images of weekly increases in RV area suggesting RV dilation. The right panel of FIG. 13B summarizes the RV area increases normalized to body weight. P value: Student's t-test. Error bar: s.e.m. *P<0.05, **P<0.01 Vs. Control.

[00161] *PKP2-cKO* mice developed LV dilated cardiomyopathy post-tamoxifen induction. FIG. 14A, left panel, shows that at three weeks post-tamoxifen induction, *PKP2-cKO* developed an increased LV internal dimension at end-systole (LVIDs) and end diastole (LVIDd). The right panel of FIG. 14A summarizes the continuous increases in LVIDs and LVIDd normalized to body weight during four weeks of tamoxifen induction. FIG. 14B shows LV performance as measured by % ejection fraction sharply declined after two weeks post-tamoxifen induction. P value: Student's t-test. Error bar: s.e.m. *P<0.05, **P<0.01, ***P<0.001 Vs. Control.

[00162] *PKP2-cKO* mice developed prolonged QRS interval and increased P/R amplitude ratio suggesting ventricular conduction disturbance and intraventricular block. FIG. 15 top panel shows that at three weeks post-tamoxifen induction, *PKP2-cKO* mice developed an increased P wave amplitude and decreased R wave amplitude. The bottom left graph in FIG. 15 shows the continuous increases in QRS interval and the lower right graph shows the increase in P/R amplitude ratio during four weeks of tamoxifen induction. P value: Student's t-test. Error bar: s.e.m. *P<0.05, **P<0.01, ***P<0.001 Vs. Control.

[00163] *PKP2-cKO* mice developed spontaneous premature ventricular contractions (PVCs). Table 2 shows data obtained during 30 minutes of continuous recording, PVCs were nearly absent at one week, whereas occasional extra systoles were detected in all the *PKP2-cKO* animals at two weeks. The occurrence of PVCs increased further at later times with a majority of animals showing over 100 PVCs. Starting from three weeks, sudden cardiac death was observed in *PKP2-cKO* animals.

Table 2: *PKP2-cKO* ARVC Mouse Model PVC

Animal ID	Week Post Tamoxifen induction			
	Week-1	Week-2	Week-3	Week-4

121	0	5	>100	Died
125	0	12	12	Died
130	0	2	Died	Died
137	0	66	>100	N/A
138	0	20	>100	Died
150	0	1	11	>100
152	4	5	>100	>100

[00164] *PKP2-cKO* mice showed enhanced expression of fibrosis, tissue remodeling genes, and heart failure markers. FIG. 16A, top panel, shows PKP2 mRNA expression in both RV and LV of wild type and *PKP2-cKO* mice. Red and blue dots represent each individual mouse. The bottom panel of FIG. 16A shows representative immunoblots of reduction in LV protein levels of PKP2, DSP, and PKG in desmosome and Cx43 in gap junction. FIG. 16B shows that *PKP2-cKO* mice showed enhanced expression of fibrosis genes, TGFβ1, Col1a1, and Col3a1, and tissue remodeling genes, Timp1 and Mmp2. FIG. 16C shows *PKP2-cKO* mice showed enhanced expression of heart failure markers, NPPA and NPPB.

Example 5: PKP2 Gene Therapy Efficacy in *PKP2-cKO* ARVC Mouse Model

[00165] FIG. 17 shows the experimental design used to evaluate PKP2 efficacy as a gene therapy target using the *PKP2-cKO* ARVC mouse model. A total of six individual treatment groups were included in the studies and all groups were tamoxifen treated for three consecutive days. The treatment groups are as follows: six wildtype mice treated with HBSS buffer; ten *PKP2-cKO* ARVC mice treated with HBSS buffer; ten *PKP2-cKO* ARVC mice treated with 3E13 vg/kg of AAV9:hman PKP2 at three weeks before tamoxifen induction; ten *PKP2-cKO* ARVC mice treated with 5E13 vg/kg of AAV9:mouse PKP2 at three weeks before tamoxifen induction; ten *PKP2-cKO* ARVC mice treated with 5E13 vg/kg of AAV9:mouse PKP2 right after tamoxifen induction; and ten *PKP2-cKO* ARVC mice with 5E13 vg/kg of AAV9:mouse PKP2 at one week after tamoxifen induction.

[00166] Baseline recordings of body weight, echocardiography, and EKG were collected before tamoxifen induction. All readings post tamoxifen induction were recorded weekly including echocardiography of B-mode, M-mode (RV, LV) and structure (LV internal diameters), and 30-minute ECG for quantifying arrythmias and evaluating electrophysiological parameters. Terminal tissues (heart and lung) will be collected at the end of the study.

[00167] AAV9:PKP2 protein expression was detected in wildtype mouse LV heart tissue. FIG. 18A shows a schematic representation of the second generation AAV expression cassette of human and mouse PKP2α. FIG. 18B shows representative immunoblots conducted to show expression of mouse and human PKP2 at three weeks post retro-orbital injection of AAV9:PKP2 (full blots are shown in FIG. 10). A total of five C57BL6 wildtype mice at eight weeks of age were injected for each treatment: HBSS, 1E13 vg/kg, or 5E13 vg/kg.

[00168] A Kaplan-Meier survival curve showed that AAV9:PKP2 extended life span of *PKP2-cKO* mice after 6 weeks post-tamoxifen induction in all AAV9:PKP2 treated groups. Both human and mouse PKP2 demonstrated efficacy in extending life span of treated *PKP2-cKO* mice. In FIG. 19, the red line is *PKP2-cKO* mice treated with HBSS buffer showing a sharp decline after three weeks post-tamoxifen induction. In contrast, all AAV9-PKP2 treated mice survived until 20 weeks post-tamoxifen induction.

[00169] AAV9:PKP2 treatment of *PKP2-cKO* mice showed efficacy in reducing RV and LV dilation and maintaining cardiac function. FIG. 20A shows AAV9:PKP2 treatment prevented a decline in percent ejection fraction compared to HBSS-treated mice (shown in the red line). FIG. 20B shows AAV9:PKP2 treatment showed a reduction of RV dilation at weekly bases as estimated by RV area normalized to body weight. FIG. 20C shows at four weeks post-tamoxifen induction, AAV9:PKP2 treatment significantly reduced LV dilation of *PKP2-cKO* mice as measured by both LV internal dimension at end-diastole (LVIDd) (top graph) and LV internal dimension at end-systole (LVIDs) (bottom graph), both normalized by body weight. Error bar: s.e.m. *P<0.05, **P<0.01, ***P<0.001 Vs. Control.

[00170] AAV9:PKP2 treatment also significantly improved ECG parameters of *PKP2-cKO* mice. FIG. 21A shows examples of raw ECG traces which showed a significant improvement of electrophysiological behaviors of AAV9:PKP2 treated *PKP2-cKO* mouse hearts. FIG. 21B shows AAV9:PKP2 treatment showed significant improvement of P/R ratio (top graph), QT interval (middle graph), and QRS interval (bottom graph) as compared to *PKP2-cKO* mice treated with HBSS shown in red lines.

[00171] AAV9:PKP2 treatment also significantly reduced arrhythmias in *PKP2-cKO* mice. FIG. 22A (top) shows a table with a grading chart to categorize severity of spontaneous arrhythmias during 30 minutes of recording in anesthetized *PKP2-cKO* mice. Premature ventricular contractions (IPVCs), premature junctional complexes (PJCs), AV block (atrioventricular block), non-sustained ventricular tachycardia (NSVT), supraventricular tachycardia (S-VT), and ventricular fibrillation. FIG. 22A (bottom) summarizes averaged scores based on the grading chart showing amelioration of arrhythmias in AAV9:PKP2 treated *PKP2-cKO* mice. FIG. 22B shows a distribution of individual mice in each treatment group at four weeks post-tamoxifen induction. AAV9:PKP2 treatment showed a reduction of both arrhythmia event frequency and severity as indicated by improved arrhythmia scores when compared to *PKP2-cKO* mice treated with HBSS buffer shown in the red bar.

Example 6: PKP2 Gene Therapy Efficacy Studies using AAV9:hPKP2 in *PKP2-cKO* ARVC Mouse Model

[00172] Efficacy of human PKP2 as a gene therapy target was determined using *PKP2-cKO* ARVC mouse model. A total of five individual treatment groups were included in the study. All groups were treated with tamoxifen for three consecutive days. The groups included: four wildtype mice treated with HBSS buffer; four *PKP2-cKO* ARVC mice treated with HBSS buffer; four *PKP2-cKO* ARVC mice treated with 1E13 vg/kg of AAV9:human PKP2 treated at one week after tamoxifen induction; three *PKP2-cKO* ARVC mice treated with 3E13 vg/kg of AAV9:human PKP2 treated at one week after tamoxifen induction; and three *PKP2-cKO* ARVC mice treated with 1E14 vg/kg of AAV9:human PKP2

treated one week after tamoxifen induction. Baseline recordings of body weight, echocardiography, and EKG were collected before tamoxifen induction. All readings post tamoxifen induction were recorded weekly including echocardiography of B-mode, M-mode (RV, LV), and structure (LV internal diameters) and 30-min ECG for quantifying arrythmias and evaluating electrophysiological parameters. Terminal heart tissues were collected at four weeks post tamoxifen induction. FIG. 23 illustrates the experimental design.

[00173] AAV9:hPKP2 treatment of *PKP2-cKO* mice showed efficacy in reducing right ventricle (RV) and left ventricle (LV) dilation and maintaining cardiac function at four weeks post tamoxifen induction. FIGS. 24A-24D show results for this assay. AAV9:hPKP2 treatment prevented decline of percent ejection fraction compared to HBSS-treated mice (FIG. 24A). AAV9:hPKP2 treatment showed reduction of RV dilation as estimated by RV area normalized to body weight (FIG. 24B). AAV9:hPKP2 treatment significantly reduced LV dilation of *PKP2-cKO* mice as measured by both LV internal dimension at end-diastole (LVIDd) (FIG. 24C) and LV internal dimension at end-systole (LVIDs) (FIG. 24D) both normalized to body weight. P value was determined by Student's t-test, error bar: s.e.m. *P<0.05, **P<0.01 compared with control.

[00174] AAV9:hPKP2 treatment showed a significant reduction at four weeks tamoxifen induction in QT interval (FIG. 25 top panel), a trending reduction in P/R ratio (FIG. 25 middle panel), and a trending reduction in arrhythmias (FIG. 25 bottom panel) in *PKP2-cKO* mice as compared to *PKP2-cKO* mice treated with HBSS. P value was determined by Student's t-test, error bar is s.e.m. *P<0.05 compared with control. Due to a small number of animals included in this study, P/R ratio and arrhythmia score did not reach statistical significance. In addition, one animal in the HBSS treated group reached humane endpoint at about 4 weeks post-tamoxifen induction after ECG recording. One animal in the 1e13 dosage group was found dead before echocardiography. Overall, the results of this study suggest that the optimal efficacious dose is 3e13 vg/kg.

[00175] AAV9:hPKP2 treatment of *PKP2-cKO* mice showed efficacy in reducing expression of heart failure markers, fibrosis and tissue remodeling genes in both right ventricle (FIG. 26A) and left ventricle (FIG. 26B) at four weeks post tamoxifen induction. Endogenous and transgene mRNA levels of PKP2 were estimated in wildtype control, *PKP2-cKO*, and AAV9:hPKP2 transduced hearts respectively. Heart failure markers are NPAA and NPPB. Fibrosis genes are Col1a1 and Col3a1. The tissue remodeling gene is Timp1.

[00176] AAV9:hPKP2 treatment of *PKP2-cKO* mice showed efficacy in reducing fibrosis development in both right ventricle and left ventricle at four weeks post tamoxifen induction (FIG. 27A). Muscle staining is shown in red color and trichrome staining of fibrosis is in blue color. Arrows highlight areas with significant fibrosis in *PKP2-cKO* mouse heart. Collagen was quantified in FIG. 27B, showing that treatment with the AAV9:hPKP2 reduced collagen nearly to control levels.

[00177] AAV9:hPKP2 treatment of *PKP2-cKO* mice showed a dose-dependent expression of human PKP2 transgene. The total expression level of PKP2 and other desmosome proteins, DSP and PKG, were estimated it both soluble (FIG. 28A) and insoluble (FIG. 28B) fractions of LV tissue at four weeks post

tamoxifen induction. No DSP is detected in the soluble fractions. Note *This animal was found dead before echocardiography. **PKP2 protein intensity is normalized to tubulin intensity on the same Western blot. For simplicity, only one of the two tubulin blots is shown in FIGS. 28A-B. PKP2 is shown as two bands, the endogenous mouse PKP2 and the larger human PKP2.

[00178] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments described herein may be employed. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for treating a heart disease or disorder in an individual in need thereof, the method comprising administering a composition comprising a gene therapy vector comprising a nucleic acid encoding a plakophilin 2 (PKP2) polypeptide or a fragment thereof operatively linked to at least one promoter and a pharmaceutically acceptable carrier or excipient.

2. The method of claim 1, wherein the gene therapy vector comprises a viral vector.

3. The method of claim 2, wherein the viral vector is selected from the group consisting of an adeno-associated virus, an adenovirus, a lentivirus, a pox virus, a vaccinia virus, and a herpes virus.

4. The method of claim 2 or claim 3, wherein the gene therapy vector is an adeno-associated virus.

5. The method of claim 4, wherein the adeno-associated virus is selected from the group consisting of an AAV6, an AAV8, and an AAV9.

6. The method of claim 5, wherein the adeno-associated virus is an AAV9 or a derivative thereof.

7. The method of claim 6, wherein the AAV9 has a nucleic acid sequence with at least 95% identity to SEQ ID NO: 7.

8. The method of any one of claims 1 to 7, wherein the gene therapy vector targets cells in the myocardium, the epicardium, or both.

9. The method of any one of claims 1 to 8, wherein the heart disease or disorder is arrhythmogenic right ventricular cardiomyopathy (ARVC) or arrhythmogenic cardiomyopathy (ACM).

10. The method of any one of claims 1 to 9, wherein the composition is administered intravenously, intracardially, pericardially, or intraarterially.

11. The method of any one of claims 1 to 10, wherein the promoter is a cardiac specific promoter.

12. The method of claim 11, wherein the cardiac specific promoter directs gene expression in the myocardium, the epicardium, or both.

13. The method of claim 11 or claim 12, wherein the cardiac specific promoter is a troponin promoter, or an alpha-myosin heavy chain promoter.

14. The method of claim 13, wherein the troponin promoter has a nucleic acid sequence having at least 95% identity to SEQ ID NO: 3.

15. The method of any one of claims 1 to 12, wherein the promoter is a PKP2 promoter.

16. The method of claim 15, wherein the PKP2 promoter has a nucleic acid sequence having at least 95% identity to SEQ ID NO: 4.

17. The method of any one of claims 1 to 10, wherein the promoter is a constitutive promoter.

18. The method of claim 17, wherein the constitutive promoter is a beta-actin promoter.

19. The method of any one of claims 1 to 18, wherein the gene therapy vector further comprises a cardiac specific enhancer.

20. The method of any one of claims 1 to 19, wherein the gene therapy vector further comprises a 3' element.

21. The method of claim 20, wherein the 3' element comprises a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), a bovine growth hormone polyadenylation (bGH polyA) sequence, or a combination thereof.

22. The method of any one of claims 1 to 21, wherein the nucleic acid encoding the PKP2 gene has a sequence having at least 95% identity to SEQ ID NO: 1 or SEQ ID NO: 2.

23. The method of any one of claims 1 to 22, wherein the gene therapy vector comprises a nucleic acid sequence having at least 95% identity to SEQ ID NO: 5.

24. The method of any one of claims 1 to 23, wherein the pharmaceutically acceptable carrier or excipient comprises a buffer, a polymer, a salt, or a combination thereof.

25. The method of any one of claims 1 to 24, wherein the method reverses, reduces, or prevents at least one of fibrofatty tissue replacement, myocardial atrophy, predominant right ventricular dilation, ventricular arrhythmias, sudden cardiac death, or exercise-triggered cardiac events.

26. The method of claim 25, wherein the method reverses, reduces, or prevents fibrofatty tissue replacement in myocardium, epicardium, or both.

27. The method of any one of claims 1 to 26, wherein the method restores desmosome structure and/or function.

28. The method of any one of claims 1 to 27, wherein the method restores PKP2 protein and activity levels.

29. The method of any one of claims 1 to 28, wherein the method restores PKP2 induced gene expression.

30. The method of any one of claims 1 to 29, wherein the method restores expression of one or more of Ryanodine Receptor 2 (Ryr2), Ankyrin-B (Ank2), Cacna1c (CaV1.2), triadin (Trdn), or calsequestrin-2 (Casq2).

31. The method of any one of claims 1 to 30, wherein the individual is identified as having at least one variation in a desmosome protein.

32. The method of claim 31, wherein the desmosome protein is PKP2.

33. The method of claim 32, wherein the variation comprises a deletion, an insertion, a single nucleotide variation, or a copy number variation.

34. A gene therapy vector comprising a plakophilin 2 gene operatively linked to at least one promoter.

35. The gene therapy vector of claim 34, wherein the gene therapy vector comprises a viral vector.

36. The gene therapy vector of claim 35, wherein the viral vector is selected from the group consisting of an adeno-associated virus, an adenovirus, a lentivirus, a pox virus, a vaccinia virus, and a herpes virus.

37. The gene therapy vector of claim 35 or claim 36, wherein the gene therapy vector is an adeno-associated virus.

38. The gene therapy vector of claim 37, wherein the adeno-associated virus is selected from the group consisting of an AAV6, an AAV8, and an AAV9.

39. The gene therapy vector of claim 38, wherein the adeno-associated virus is an AAV9 or a derivative thereof.

40. The gene therapy vector of claim 39, wherein the AAV9 has a nucleic acid sequence with at least 95% identity SEQ ID NO: 7.

41. The gene therapy vector of any one of claims 34 to 40, wherein the gene therapy vector targets cells in the myocardium, the epicardium, or both.

42. The gene therapy vector of any one of claims 34 to 41, wherein the promoter is a cardiac specific promoter.

43. The gene therapy vector of claim 42, wherein the cardiac specific promoter directs gene expression in the myocardium, the epicardium, or both.

44. The gene therapy vector of claim 42 or claim 43, wherein the cardiac specific promoter is a troponin promoter or an alpha-myosin heavy chain promoter.

45. The gene therapy vector of claim 43, wherein the troponin promoter has a nucleic acid sequence having at least 95% identity to SEQ ID NO: 3.

46. The gene therapy vector of any one of claims 34 to 45, wherein the promoter is a PKP2 promoter.

47. The gene therapy vector of claim 46, wherein the PKP2 promoter has a nucleic acid sequence having at least 95% identity to SEQ ID NO: 4.

48. The gene therapy vector of any one of claims 34 to 41, wherein the promoter is a constitutive promoter.

49. The gene therapy vector of claim 48, wherein the constitutive promoter is a beta-actin promoter.

50. The gene therapy vector of any one of claims 34 to 49, further comprising a cardiac specific enhancer.

51. The gene therapy vector of any one of claims 34 to 50, wherein the gene therapy vector further comprises a 3' element.

52. The gene therapy vector of claim 51, wherein the 3' element comprises a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), a bovine growth hormone polyadenylation (bGH polyA) sequence, or a combination thereof.

53. The gene therapy vector of any one of claims 34 to 52, wherein the nucleic acid encoding the PKP2 gene has a sequence having at least 95% identity to SEQ ID NO: 1 or SEQ ID NO: 2.

54. The gene therapy vector of any one of claims 34 to 53, wherein the gene therapy vector comprises a nucleic acid sequence having at least 95% identity to SEQ ID NO: 5.

55. The gene therapy vector of any one of claims 34 to 54, wherein the gene therapy vector is formulated in a pharmaceutically acceptable carrier or excipient comprising a buffer, a polymer, a salt, or a combination thereof.

56. A method for treating a heart disease or disorder in an individual in need thereof, the method comprising administering a composition comprising (a) a gene therapy vector comprising a nucleic acid encoding a plakophilin 2 (PKP2) polypeptide or a fragment thereof operatively linked to a promoter and a 3' element; and (b) a pharmaceutically acceptable carrier or excipient.

57. The method of claim 56, wherein the gene therapy vector comprises a viral vector selected from the group consisting of an adeno-associated virus, an adenovirus, a lentivirus, a pox virus, a vaccinia virus, and a herpes virus.

58. The method of claim 56, wherein the gene therapy vector is an adeno-associated virus.

59. The method of claim 58, wherein the adeno-associated virus is selected from the group consisting of an AAV6, an AAV8, and an AAV9.

60. The method of claim 59, wherein the adeno-associated virus targets cells in the myocardium, the epicardium, or both.

61. The method of claim 59, wherein the adeno-associated virus is an AAV9 having a nucleic acid sequence with at least 95% identity to SEQ ID NO: 7.

62. The method of claim 56, wherein the heart disease or disorder is arrhythmogenic right ventricular cardiomyopathy (ARVC) or arrhythmogenic cardiomyopathy (ACM).

63. The method of claim 56, wherein the promoter is a promoter that causes expression in tissues including the heart or a cardiac specific promoter.

64. The method of claim 63, wherein the cardiac specific promoter directs gene expression in the myocardium, the epicardium, or both.

65. The method of claim 63, wherein the cardiac specific promoter is a PKP2 promoter, a troponin promoter, or an alpha-myosin heavy chain promoter.

66. The method of claim 65, wherein the PKP2 promoter has a nucleic acid sequence having at least 95% identity to SEQ ID NO: 4.

67. The method of claim 65, wherein the troponin promoter has a nucleic acid sequence having at least 95% identity to SEQ ID NO: 3.

68. The method of claim 56, wherein the 3' element comprises a Woodchuck Hepatitis Virus Posttransciptional Regulatory Element (WPRE), a bovine growth hormone polyadenylation (bGH polyA) sequence, or a combination thereof.

69. The method of claim 56, wherein the gene therapy vector further comprises a cardiac specific enhancer.

70. The method of claim 56, wherein the nucleic acid encoding the PKP2 gene has a sequence having at least 95% identity to SEQ ID NO: 1 or SEQ ID NO: 2.

71. The method of claim 56, wherein the gene therapy vector comprises a nucleic acid sequence having at least 95% identity to SEQ ID NO: 5.

72. The method of claim 56, wherein the nucleic acid has a size less than or equal to about 4.7 kb.

73. The method of claim 56, wherein the pharmaceutically acceptable carrier or excipient comprises a buffer, a polymer, a salt, or a combination thereof.

74. The method of claim 56, wherein the method reverses, reduces, or prevents at least one of fibrofatty tissue replacement; myocardial atrophy; predominant right ventricular dilation; ventricular arrhythmias; sudden cardiac death; exercise-triggered cardiac events; right ventricular cardiomyopathy, dilation, or heart failure; left ventricular cardiomyopathy, dilation, or heart failure; atrial arrhythmias; syncope; palpitations; shortness of breath; or chest pain.

75. The method of claim 74, wherein the method reverses, reduces, or prevents fibrofatty tissue replacement in myocardium, epicardium, or both.

76. The method of claim 56, wherein the method restores desmosome structure and/or function.

77. The method of claim 56, wherein the method restores PKP2 mRNA expression and/or PKP2 protein and activity levels.

78. The method of claim 56, wherein the method restores expression of one or more genes having a direct or indirect effect on one or more symptoms of the heart disease.

79. The method of claim 78, wherein the gene comprises one or more of Ryanodine Receptor 2 (Ryr2), Ankyrin-B (Ank2), Cacna1c (CaV1.2), triadin (Trdn), or calsequestrin-2 (Casq2).

80. The method of claim 56, wherein the individual is identified as having at least one variation in a desmosome protein.

81. The method of claim 80, wherein the desmosome protein is PKP2.

82. The method of claim 81, wherein the variation comprises a deletion, an insertion, a single nucleotide variation, or a copy number variation.