

# 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

[0002a] In one aspect, provided herein is a use of a composition comprising (a) a viral vector comprising a nucleic acid sequence that is at least 90% identical to SEQ ID NO: 2 encoding a plakophilin 2 (PKP2) polypeptide operatively linked to a cardiac specific promoter and a 3' element; and (b) a pharmaceutically acceptable carrier or excipient in the manufacture of a medicament for treating arrhythmogenic right ventricular cardiomyopathy (ARVC) or arrhythmogenic cardiomyopathy (ACM) in a mammal with ARVC or ACM.

[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 Posttranscriptional 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