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MINIREVIEWS

Disease modeling of desmosome-related cardiomyopathy using induced pluripotent stem cell-derived cardiomyocytes

Shuichiro Higo

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Abstract

Cardiomyopathy is a pathological condition characterized by cardiac pump failure due to myocardial dysfunction and the major cause of advanced heart failure requiring heart transplantation. Although optimized medical therapies have been developed for heart failure during the last few decades, some patients with cardiomyopathy exhibit advanced heart failure and are refractory to medical therapies. Desmosome, which is a dynamic cell-to-cell junctional component, maintains the structural integrity of heart tissues. Genetic mutations in desmosomal genes cause arrhythmogenic cardiomyopathy (AC), a rare inheritable disease, and predispose patients to sudden cardiac death and heart failure. Recent advances in sequencing technologies have elucidated the genetic basis of cardiomyopathies and revealed that desmosome-related cardiomyopathy is concealed in broad cardiomyopathies. Among desmosomal genes, mutations in *PKP2* (which encodes PKP2) are most frequently identified in patients with AC. *PKP2* deficiency causes various pathological cardiac phenotypes. Human cardiomyocytes differentiated from patient-derived induced pluripotent stem cells (iPSCs) in combination with genome editing, which allows the precise arrangement of the targeted genome, are powerful experimental tools for studying disease. This review summarizes the current issues associated with practical medicine for advanced heart failure and the recent advances in disease modeling using iPSC-derived cardiomyocytes targeting desmosome-related cardiomyopathy caused by *PKP2* deficiency.

Key Words: Cardiomyopathy; Advanced heart failure; Induced pluripotent stem cell-derived cardiomyocytes; Desmosome; Genome editing; Gene therapy

Core Tip: Prevention of advanced heart failure caused by cardiomyopathy is an urgent unmet need in the field of cardiovascular medicine. Desmosome, a cell-to-cell junctional component, maintains the structural integrity of heart tissues. Genetic mutations in desmosomal genes cause desmosome-related cardiomyopathy, an intractable disease refractory to standard medical therapies. This review introduces the recent advances in disease modeling of desmosome-related cardiomyopathy caused by *PKP2* mutations using induced pluripotent stem cell-derived cardiomyocytes.

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INTRODUCTION

Heart failure is a clinical syndrome characterized by dyspnea, malaise, swelling, and/or decreased exercise capacity owing to impaired cardiac pumping function[1]. The established optimal medical therapies for heart failure have increased the survival rates of patients in the last few decades[2-4]. However, some patients are refractory to medical therapies and develop symptoms that are diagnosed as advanced heart failure. Currently, the therapeutic strategies available for these patients are heart transplantation and implantation of the ventricular assisting device[1,5]. Cardiomyopathy is a disease of cardiac pump failure due to myocardial dysfunction and is the major cause of advanced heart failure requiring heart transplantation[6-11]. Cardiomyopathies are differentially diagnosed mainly by using imaging modalities, including echocardiography, scintigraphy, computed tomography, magnetic resonance imaging, and cardiac catheterization. Based on the findings of these modalities, cardiomyopathies are classified into dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM), or other rare cardiomyopathies, such as arrhythmogenic right ventricular cardiomyopathy (ARVC)[12]. Among 36,883 heart transplantation recipients registered in the International Society for Heart and Lung Transplantation Thoracic Organ Transplant Registry between 2010 and 2018, the major primary diagnoses were non-ischemic DCM (50.8%), ischemic cardiomyopathy (ICM) (32.4%) with coronary artery disease, RCM (3.5%), and HCM (3.4%)[13]. In Japan, cardiomyopathies [DCM (64%), end-stage HCM with left ventricular systolic dysfunction (12%), and ICM (9%)] account for more than three-quarters of underlying diseases among heart transplant recipients[14]. ARVC, a rare inherited disease, is characterized by the risk of life-threatening arrhythmias, myocardial dysfunction, and fibrofatty replacement of myocardial tissue, predisposing the patients to sudden cardiac death and heart failure[9,11]. The prevalence of ARVC among the registrants for heart transplantation is rare (0.3% and 1%-2% in the United Network for Organ Sharing registry[15] and Japan Organ Transplant Network[14], respectively).

DESMOSOME-RELATED CARDIOMYOPATHY IS CONCEALED IN ADVANCED HEART FAILURE

Recent clinical studies utilizing high-throughput sequencing technologies have elucidated the genetic basis of cardiomyopathies, identified various causative genetic variants, and revealed the correlation between genetic factors and clinical phenotypes or cardiac morphologies in patients with cardiomyopathies[16-20]. ARVC is an inherited disease caused by mutations in desmosomal genes (*PKP2*, *JUP*, *DSC2*, *DSG2*, and *DSP*) (Figure 1)[11,21,22]. These genes encode the structural components of the desmosome, a dynamic junction between cells that maintain the structural integrity of heart tissues[23,24]. The original disease phenotypes of ARVC are characterized by predominant right ventricular enlargement and contractile dysfunction. However, recent studies have reported left ventricular or biventricular involvement in patients with ARVC, resulting in the use of a broad phrase [arrhythmogenic cardiomyopathy (AC)][9,11]. Although the prevalence of AC in patients with advanced heart failure is rare, recent genetic analyses in large cohorts have demonstrated an increased incidence of desmosomal gene mutations in patients with DCM[18,25,26], which is the most frequent basal disease among heart transplantation registrants. Furthermore, homozygosity and compound or digenic heterozygosity of desmosomal genes are not rare, and patients with combined mutations exhibit a severe phenotype[27-30]. Recently, we identified *DSG2*-deficient cardiomyopathy caused by a rare homozygous stop-gain mutation in a patient initially diagnosed with idiopathic sporadic DCM[30]. *Dsg2* deficiency is associated with embryonic lethality in mice. Additionally, *Dsg2*-depleted embryonic stem cells do not proliferate[31]. However, a human male patient with a complete lack of *DSG2*

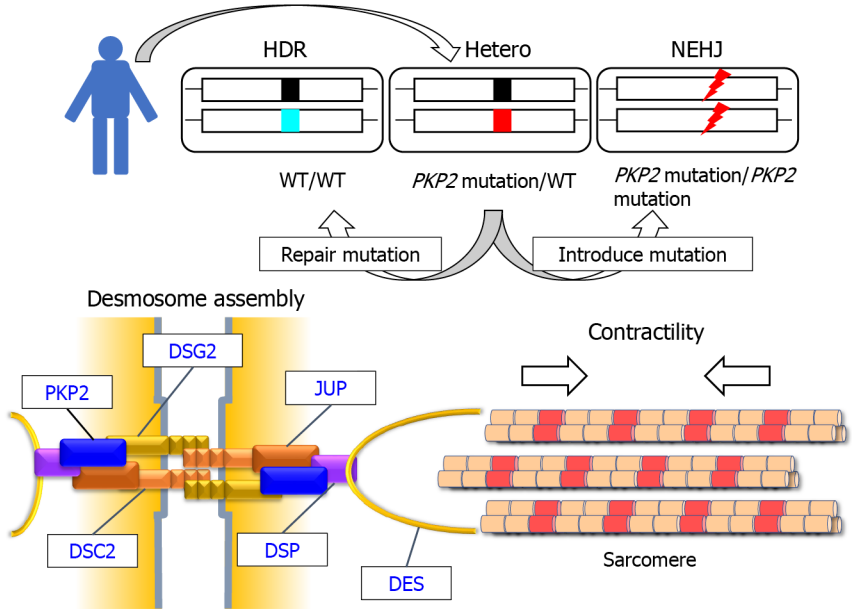

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Figure 1 Modeling impaired desmosome assembly and reduced contractility using isogenic induced pluripotent stem cell-derived cardiomyocytes with the precisely adjusted dose of *PKP2*. Heterozygous frameshift mutation in patient-derived induced pluripotent stem cells (iPSCs) was repaired through homology-directed repair. Homozygous frameshift mutations were introduced in *PKP2* through non-homologous end joining in patient-derived iPSCs. The generated isogenic iPSC-derived cardiomyocytes with the precisely adjusted expression of *PKP2* recapitulated impaired desmosome assembly and reduced contractility caused by *PKP2* deficiency. Desmosomal cadherin proteins (DSG2 and DSC2) form homo-dimers and hetero-dimers. PKP2 is a scaffold protein for desmosomal cadherins, JUP, and DSP. Desmosomes are linked to sarcomere structure *via* the intermediate filament protein DES that targets both desmosome and Z disc structure. HDR: Homology-directed repair; NEHJ: Non-homologous end joining; Hetero: Heterozygous mutation.

expression did not exhibit pathological phenotypes at birth but developed advanced heart failure during the teenage years. Immunohistochemical and transmission electron microscopy analyses of left ventricular heart tissues revealed that the loss of *DSG2* leads to aberrant deposition of desmosomal proteins and disruption of intercalated discs in cardiomyocytes. These findings suggest that desmosome-related cardiomyopathy is concealed in patients with advanced heart failure who are diagnosed with idiopathic DCM. As desmosome impairment is the most upstream molecular change in these patients, experimental studies must focus on elucidating the molecular mechanisms underlying the instability of cell-to-cell junctions to overcome advanced heart failure caused by desmosome-related cardiomyopathy. For disease modeling, patient-derived induced pluripotent stem cells (iPSCs) in combination with genome editing, which allows precise genomic modification of the targeted mutations, are powerful experimental tools to recapitulate pathological phenotypes based on the molecular factors of inherited cardiomyopathies[30,32-35].

PHENOTYPIC RECAPITULATION OF CARDIOMYOPATHY CAUSED BY PKP2 DEFICIENCY USING PATIENT-DERIVED IPSC-CMS

PKP2, which is encoded by *PKP2*, is a desmosomal protein localized to the outer dense plaque and functions as a scaffold for the other desmosome proteins DSG2, DSC2, JUP, and DSP[23,36] (Figure 1). Among the desmosomal genes, mutations in *PKP2* are most frequently identified in patients with AC [11,37-39], and have been extensively studied using patient-derived iPSC-CMs compared to other desmosomal genes (*DSG2*[30,40,41], *DSP*[42,43], and *DSC2*[44,45]). Various clinical phenotypes and pathological characteristics observed in patients with AC harboring *PKP2* mutations, downregulated desmosomal protein expression, upregulated lipogenesis, and increased apoptosis in heart tissues have been recapitulated using genetically engineered mouse models[11] and human cardiomyocytes differentiated from iPSCs[46-54] (Table 1). Most known mutations of *PKP2* are heterozygous and are missense, nonsense, and frameshift mutations. Studies on patient-derived iPSCs have identified that *PKP2* variants are heterozygous missense[48], heterozygous frameshift[46,47,49,50,54], homozygous frameshift[47,51], compound heterozygous, and frameshift[52] mutations. Disease-specific iPSCs are generated from fibroblasts[46-48,51], keratinocytes[49], adipose tissue-derived stromal cells[52], and peripheral blood mononuclear cells[54], whereas control iPSCs are generated from healthy subjects[46-49,51,52], human embryonic stem cells[50], or isogenic cells engineered from patient-derived iPSCs using genome editing[54]. Genome editing allows disease modeling by introducing heterozygous and

Table 1 Human disease model of *PKP2* deficiency using induced pluripotent stem cell-derived cardiomyocytes and experimental pathological phenotypes of arrhythmogenic cardiomyopathy

Genetic mutation	Origin of disease-specific iPSC	Experimental control	Desmosome proteins	Lipid accumulation	Apoptosis	Electrophysiology	Ultrastructure of desmosome	Contractility	Phenotypic rescue by gene replacement	Ref.
Heterozygous missense (c.1841T>C, p.L614P)	Dermal fibroblasts from a 30-yr-old male patient with AC	iPSCs from a 32-yr-old healthy male donor	Decreased JUP; No change in DSP, CDH2, and GJA1 (immunofluorescence staining at weeks 4-5)	Increased oil red O staining after exposure to adipogenic differentiation medium for 2 wk (oil red O staining)	NA	Ventricular-like action potential profile (single-cell patch-clamp recording (without control))	Increased cell width (TEM at weeks 4-5)	NA	NA	Ma *et al*[48]
Heterozygous frameshift (c.971_972ins, pA324fs335X); Heterozygous frameshift (c.148_151delACAG, p.T50SfsX110)	Dermal fibroblasts from a 30-yr-old male patient with AC	iPSCs from a healthy control	Decreased JUP and GJA1 (immunofluorescence staining)	Lipid droplet accumulation (TEM on day 40)	Increased apoptosis after serum starvation (TUNEL)	Prolonged field potential rise time (multielectrode array)	Widened and distorted desmosomes (TEM on day 40)	NA	NA	Caspi *et al* [46]
Homozygous frameshift (c.2484C>T leading to cryptic splicing); Heterozygous frameshift (c.2013delC, p.Lys672ArgfsX12)	Fibroblasts from a female patient with AC; Fibroblasts from a patient with AC	H9 human embryonic stem cell; iPSCs from cardiac fibroblasts of aborted fetus without a family history of AC	Nuclear translocation of JUP (immunofluorescence staining)	Increased lipogenesis after adipogenic stimulation for 4-5 wk (Nile red staining)	Increased apoptosis after adipogenic stimulation for 4-5 wk (TUNEL)	Slow intracellular calcium relaxation; Prolonged relaxation time (calcium imaging using Fura-2 acetoxymethyl on day 60)	NA	NA	NA	Kim *et al* [47]
Heterozygous frameshift (c.1760delT, p.V587Afsx655)	Dermal keratinocytes from a male patient with AC	iPSCs from dermal keratinocytes of a healthy control	Interrupted expression of DSP (immunofluorescence staining)	Lipid droplet accumulation after adipogenic stimulation for 4 wk (oil red O staining at months 3-4)	Genes associated with apoptosis remained unchanged (quantitative real-time PCR)	NA	NA	NA	NA	Dorn *et al* [49]
Homozygous frameshift (c.2484C>T leading to cryptic splicing)	Fibroblasts from a female patient with AC	iPSCs from a healthy control	Reduced JUP (immunofluorescence staining)	NA	NA	NA (decreased co-localization of NaV1.5 with PKP2)	NA	NA (increased pro-fibrotic gene expression after stretch)	NA	Martewicz *et al*[51]
Heterozygous frameshift (c.971_972InsT, p.A324fs335X)	A patient with AC	H9 human embryonic stem cells	Decreased membrane-localized JUP (immunofluorescence staining on day 34)	Increased lipid content (Nile red staining on day 34)	NA	Short action potential and slow spontaneous beat rate in engineered heart slices [optical mapping (relative to monolayer cardiomyocytes)]	NA	NA	NA	Blazeski *et al*[50]
Compound hetero-	Adipose tissue-	Gender-matched	Increased cytoplasmic and	No presence of	Not increased	Reduced sodium current	NA	NA	Restored	Khudiakov

zygous frameshift and missense (c.354delT, p.Y119MfsX23 and p.K859R)	derived mesenchymal multipotent stromal cells from a 14-yr-old female patient with AC	healthy donor	nuclear JUP levels (immunofluorescence staining on days 24-30)	lipid droplets (oil red O staining on day 24)	(PI staining at day 24-30)	density; Decreased action potential upstroke velocity (whole-cell patch-clamp and microelectrodes on days 24-30)			sodium current after lentiviral transduction of *PKP2*	*et al*[52]
Heterozygous and homozygous frameshift mutation (p.D109AfsX10, introduced mutation *via* genome editing)	Wild-type iPSC lines from two different donors with introduced heterozygous and homozygous frameshift mutations	Isogenic wild-type iPSCs	Decreased junctional localization of DSP and GJA1 (immunofluorescence staining); Impaired stability of junctional CDH2 (fluorescence recovery after photobleaching)	NA	NA	Prolonged action potential duration (optical voltage recording on day 30)	NA	Decreased systolic force (three-dimensional cardiac microtissues on day 40)	NA	Zhang *et al* [53]
Heterozygous frameshift mutation (c.1228dupG, p.D410fsX425)	Peripheral blood mononuclear cells from a female patient with AC	Isogenic iPSCs with corrected mutation (wild-type) and introduced homozygous frameshift mutations	Decreased area of desmosomes (DSG2, DSC2, and DSP) (immunofluorescence staining on day 14)	Lipid droplet accumulation in iPSC-CMs with homozygous frameshift mutations (TEM on day 28)	Increased apoptosis in iPSC-CMs with homozygous frameshift mutations (cleaved CASP3 expression on day 28)	Decreased propagation speed in iPSC-CMs with homozygous frameshift mutations (motion vector analysis on day 28)	Increased desmosome gap width (TEM on day 28)	Decreased contractility (contraction velocity and deformation distance evaluated using motion vector analysis on days 14 and 28)	Recovered contractility and desmosome assembly *via* AAV-mediated *PKP2* delivery	Inoue *et al* [54]

Gender of the patient or control donor is indicated if specified. Analytical methods along with time post-cardiomyocyte differentiation (if specified) are indicated. AAV: Adeno-associated virus; iPSC: Induced pluripotent stem cell; iPSC-CMs: Induced pluripotent stem cells-derived cardiomyocytes; PI: Propidium iodide; TEM: Transmission electron microscopy; NA: Not applicable; AC: Arrhythmogenic cardiomyopathy.

homozygous frameshift mutations in wild-type iPSC lines[53]. Decreased expression of desmosomal proteins, aberrant lipogenesis, and apoptosis of cardiomyocytes are observed in the heart tissues of patients with AC[9,55,56]. These pathological phenotypes are recapitulated in iPSC-CMs with *PKP2* mutations as determined using immunostaining[46-54], lipid staining[47-50], electron microscopy[46, 54], terminal transferase dUTP nick end labeling staining[46,47], and cleaved-CASP3 expression analysis [54]. Lethal arrhythmia is a hallmark of patients with AC. Arrhythmia phenotypes are recapitulated using iPSC-CMs with *PKP2* mutations as evidenced by the results of patch-clamp[48,52], multielectrode array[46], calcium imaging[47], and optical voltage recording[53]. In clinical settings, global or regional ventricular contractile dysfunction is defined as a major criterion for the diagnosis of ARVC in modified Task Force criteria[21] and Padua criteria[57]. However, the functional consequence in cardiomyocyte contractility caused by *PKP2* mutations has not been fully studied in human iPSC-CMs.

PKP2 DEFICIENCY AND CONTRACTILE DYSFUNCTION

We established iPSCs from a patient with AC harboring a heterozygous frameshift *PKP2* mutation

(c.1228dupG, p.D410fsX425) and generated an isogenic set of iPSC clones harboring three genotypes [heterozygous mutation (Hetero), homozygously corrected with homology-directed repair (HDR), and homozygously introduced frameshift mutations *via* non-homologous end joining (NHEJ)] using genome editing[54] (Figure 1). These isogenic sets of iPSCs comprise patient-derived Hetero-iPSCs, HDR-iPSCs with two-fold higher *PKP2* expression relative to Hetero-iPSCs, and NHEJ-iPSCs, which do not express *PKP2*, recapitulating both haploinsufficiency and complete loss of *PKP2*. After cardiomyocyte differentiation using the monolayer protocol with chemically defined medium[58], NHEJ-iPSC-CMs lacking *PKP2* expression exhibit lipid droplet accumulation, increased apoptosis, and decreased propagation rate (Table 1). However, patient-derived Hetero-iPSC-CMs with half-dose *PKP2* expression do not exhibit these pathological phenotypes, suggesting that the haploinsufficiency of *PKP2* is not sufficient to induce the above pathological phenotypes within 28 days after differentiation. In contrast, haploinsufficiency of *PKP2* decreased contractility, which was evaluated using motion vector analysis, within 14 days of differentiation. As the monolayer protocol confers strong contraction to iPSC-CMs on culture plates immediately after differentiation[58,59], continuous tensile overload may facilitate the contractile phenotype among isogenic iPSC-CMs. A recent study used isogenic iPSC-CMs in which heterozygous or homozygous frameshift mutation was introduced into wild-type iPSC-CMs[53]. The authors reported that *PKP2* deficiency decreased systolic force in three-dimensional cardiac microtissues. This further supported the functional relationship between *PKP2* deficiency and contractile dysfunction. An experimental study using cardiac tissue-specific *Pkp2* knockout mice demonstrated that the loss of *Pkp2* increased the distance between the cell periphery and DES, an intermediate filament protein in cardiomyocytes[60]. As DES connects Z-discs of sarcomeres to sarcolemmal costameres, desmosomes, and nuclear envelope[11,61], further experimental studies focusing on these cellular networks are required to elucidate the pathogenesis of desmosome-related cardiomyopathy.

DESMOSOME IMAGING USING THE ISOGENIC IPSC-CMS AND AAV-MEDIATED GENE REPLACEMENT

In the isogenic background, the haploinsufficiency of *PKP2* did not affect the localization or expression levels of desmosomal proteins in iPSC-CMs as evidenced by the results of immunostaining or western blotting analyses. However, the desmosome area represented by dot distribution on the cell periphery in Hetero-iPSC-CMs was significantly lower than that in HDR-iPSC-CMs[54], suggesting that desmosome assembly is impaired by *PKP2* haploinsufficiency. The impaired assembly of desmosomal proteins in human iPSC-CMs is supported by another study using isogenic iPSC-CMs. Fluorescence recovery after photobleaching experiments combined with lentivirus-mediated expression of fluorescent protein-tagged N-cadherin provided evidence that molecular stability of junctional N-cadherin is impaired by *PKP2* deficiency[53]. To trace the molecular behavior of endogenous proteins in cardiomyocytes, fluorescent tagging of the structural proteins through genome editing is a powerful tool[62,63]. However, fluorescent tagging of endogenous desmosomal genes might affect desmosome structures or cell-to-cell integrity in iPSCs or iPSC-CMs. We previously identified a patient with *DSG2*-deficient cardiomyopathy due to a rare homozygous stop-gain mutation and demonstrated that complete loss of *DSG2* in human iPSCs does not affect the differentiation or cellular morphology in iPSC-CMs[30]. These findings prompted us to use *DSG2* as the target of endogenous tagging by fluorescent protein to trace desmosome dynamics in live human iPSC-CMs. Genome editing targeting *DSG2* alleles was performed to establish the isogenic iPSC-CMs harboring identical two *DSG2* alleles comprising intact and knocked-in tdTomato alleles under the adjusted *PKP2* expression levels (Figure 2). The desmosome area (represented by desmoglein-2-tdTomato fusion protein) was significantly downregulated due to *PKP2* haploinsufficiency. Adeno-associated virus (AAV), a small, nonenveloped virus with a linear, single-stranded DNA, is widely used for gene therapy targeting human diseases, including heart failure[64,65]. AAV-mediated gene replacement of *PKP2* significantly restored the decreased contractility in Hetero-iPSC-CMs and NEHJ-iPSC-CMs, demonstrating the proof-of-concept for *PKP2* gene therapy in human cells. Furthermore, time-lapse imaging using NHEJ-iPSC-CMs captured the recovery of desmosomes, which gradually assembled at the cell periphery after AAV-mediated *PKP2* replacement (Figure 2). The established isogenic iPSCs harboring knocked-in tdTomato alleles allowed desmosome-imaging in living cells and provided distinct readouts for therapeutic development.

GENE REPLACEMENT THERAPY TARGETING HEART FAILURE

Several clinical trials using AAV-mediated gene replacement have been designed targeting cardiovascular disease[65,66]. A large-scale clinical trial was conducted as a randomized, multinational, double-blind, placebo-controlled phase 2 study targeting up to 250 patients with moderate-to-severe heart failure and reduced contractile function (CUPID2 trial)[67]. The study aimed to deliver

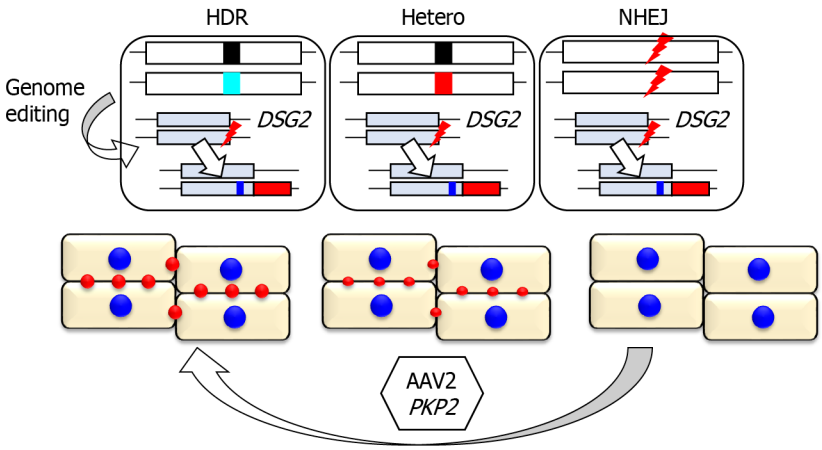

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Figure 2 Allele-specific fluorescent labeling of *DSG2* captures desmosome dynamics in isogenic induced pluripotent stem cell-derived cardiomyocytes. To establish a model for desmosome imaging, the tdTomato fluorescent reporter was knocked-in at the 3'-terminus of *DSG2* in the three established isogenic induced pluripotent stem cells (iPSCs) using genome editing. These isogenic iPSCs carried identical *DSG2* alleles comprising intact and knocked-in alleles distinguished by a synonymous single nucleotide variant (indicated as blue line). These iPSC-derived cardiomyocytes enable desmosome imaging and capturing desmosome recovery after adeno-associated virus-mediated replacement of *PKP2*. HDR: Homology-directed repair; NEHJ: Non-homologous end joining; AAV: Adeno-associated virus; Hetero: Heterozygous mutation.

sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) into heart tissues *via* intracoronary injection. SERCA2a regulates cardiomyocyte contraction and relaxation by transporting Ca^{2+} from the cytosol into the sarcoplasmic reticulum during diastole[68]. The deficiency of SERCA2a is associated with heart failure progression[69,70]. Although promising results were achieved in preceding preclinical and clinical studies[71-73], gene replacement of SERCA2a did not improve the clinical course of patients with heart failure[74]. The two clinical trials of gene therapy targeting patients with heart failure conducted in the same period (AGENT-HF[75] and SERCA-LVAD[76]) were terminated due to the neutral result of the CUPID2 trial and the lack of functional benefit. The amount of vector DNA in heart tissues obtained from patients who received gene therapy and subsequently underwent heart transplantation or mechanical circulatory support device implantation was low, suggesting that only a small proportion of cardiomyocytes expressed AAV-delivered SERCA2a in the myocardium. Although these clinical trials demonstrate the difficulty of gene delivery targeting human heart tissues, they provide the evidence for the safety of cardiac gene therapy and a basis for the design of future gene therapy trials. Recent genetic analysis clarified a large number of genetic mutations that cause cardiomyopathies with advanced heart failure in a loss-of-function manner and can be targeted by specific gene replacement therapy[77,78]. In desmosome-related cardiomyopathy, most of the identified mutations in *PKP2* are heterozygous[22,37,79,80]. However, in extremely rare cases, homozygous mutations of *PKP2* cause lethal infantile heart failure with left ventricular non-compaction or hypoplastic left heart syndrome[81-83]. No effective therapies are available for these patients who require a novel therapeutic approach for desmosome-related cardiomyopathy. Proof-of-concept studies for structural and functional recovery using both human iPSC-CM models and *in vivo* models are required for future clinical application.

CONCLUSION

Although human iPSC-CMs are immature and do not fully recapitulate *in vivo* heart tissues[59], tissue engineering approaches[84,85] will promote the maturation of iPSC-CMs and provide a useful tool in combination with genome editing. The isogenic iPSC-CMs that we established represent a human disease model that recapitulates reduced contractility and impaired desmosome assembly and provides a convenient cellular platform for therapeutic screening to examine upstream molecular targets of desmosome-related cardiomyopathy.

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REFERENCES

1 **Tsutsui H**, Ide T, Ito H, Kihara Y, Kinugawa K, Kinugawa S, Makaya M, Murohara T, Node K, Saito Y, Sakata Y, Shimizu W, Yamamoto K, Bando Y, Iwasaki YK, Kinugasa Y, Mizote I, Nakagawa H, Oishi S, Okada A, Tanaka A, Akasaka T, Ono M, Kimura T, Kosaka S, Kosuge M, Momomura SI. JCS/JHFS 2021 Guideline Focused Update on Diagnosis and Treatment of Acute and Chronic Heart Failure. *J Card Fail* 2021; **27**: 1404-1444 [PMID: 34600838 DOI: 10.1016/j.cardfail.2021.04.023]

2 **Levy D**, Kenchaiah S, Larson MG, Benjamin EJ, Kupka MJ, Ho KK, Murabito JM, Vasan RS. Long-term trends in the incidence of and survival with heart failure. *N Engl J Med* 2002; **347**: 1397-1402 [PMID: 12409541 DOI: 10.1056/NEJMoa020265]

3 **Roger VL**, Weston SA, Redfield MM, Hellermann-Homan JP, Killian J, Yawn BP, Jacobsen SJ. Trends in heart failure incidence and survival in a community-based population. *JAMA* 2004; **292**: 344-350 [PMID: 15265849 DOI: 10.1001/jama.292.3.344]

4 **Jones NR**, Roalfe AK, Adoki I, Hobbs FDR, Taylor CJ. Survival of patients with chronic heart failure in the community: a systematic review and meta-analysis. *Eur J Heart Fail* 2019; **21**: 1306-1325 [PMID: 31523902 DOI: 10.1002/ejhf.1594]

5 **Ono M**, Yamaguchi O, Ohtani T, Kinugawa K, Saiki Y, Sawa Y, Shiose A, Tsutsui H, Fukushima N, Matsumiya G, Yanase M, Yamazaki K, Yamamoto K, Akiyama M, Imamura T, Iwasaki K, Endo M, Ohnishi Y, Okumura T, Kashiwa K, Kinoshita O, Kubota K, Seguchi O, Toda K, Nishioka H, Nishinaka T, Nishimura T, Hashimoto T, Hatano M, Higashi H, Higo T, Fujino T, Hori Y, Miyoshi T, Yamanaka M, Ohno T, Kimura T, Kyo S, Sakata Y, Nakatani T; JCS/JSCVS/JATS/JSVS Joint Working Group. JCS/JSCVS/JATS/JSVS 2021 Guideline on Implantable Left Ventricular Assist Device for Patients With Advanced Heart Failure. *Circ J* 2022; **86**: 1024-1058 [PMID: 35387921 DOI: 10.1253/circj.CJ-21-0880]

6 **Rosenbaum AN**, Agre KE, Pereira NL. Genetics of dilated cardiomyopathy: practical implications for heart failure management. *Nat Rev Cardiol* 2020; **17**: 286-297 [PMID: 31605094 DOI: 10.1038/s41569-019-0284-0]

7 **Marian AJ**, Braunwald E. Hypertrophic Cardiomyopathy: Genetics, Pathogenesis, Clinical Manifestations, Diagnosis, and Therapy. *Circ Res* 2017; **121**: 749-770 [PMID: 28912181 DOI: 10.1161/CIRCRESAHA.117.311059]

8 **McNally EM**, Mestroni L. Dilated Cardiomyopathy: Genetic Determinants and Mechanisms. *Circ Res* 2017; **121**: 731-748 [PMID: 28912180 DOI: 10.1161/CIRCRESAHA.116.309396]

9 **Corrado D**, Basso C, Judge DP. Arrhythmogenic Cardiomyopathy. *Circ Res* 2017; **121**: 784-802 [PMID: 28912183 DOI: 10.1161/CIRCRESAHA.117.309345]

10 **Watkins H**, Ashrafian H, Redwood C. Inherited cardiomyopathies. *N Engl J Med* 2011; **364**: 1643-1656 [PMID: 21524215 DOI: 10.1056/NEJMra0902923]

11 **Austin KM**, Trembley MA, Chandler SF, Sanders SP, Saffitz JE, Abrams DJ, Pu WT. Molecular mechanisms of arrhythmogenic cardiomyopathy. *Nat Rev Cardiol* 2019; **16**: 519-537 [PMID: 31028357 DOI: 10.1038/s41569-019-0200-7]

12 **Kitaoka H**, Tsutsui H, Kubo T, Ide T, Chikamori T, Fukuda K, Fujino N, Higo T, Isobe M, Kamiya C, Kato S, Kihara Y, Kinugawa K, Kinugawa S, Kogaki S, Komuro I, Hagiwara N, Ono M, Maekawa Y, Makita S, Matsui Y, Matsushima S, Sakata Y, Sawa Y, Shimizu W, Teraoka K, Tsuchihashi-Makaya M, Ishibashi-Ueda H, Watanabe M, Yoshimura M, Fukusima A, Hida S, Hikoso S, Imamura T, Ishida H, Kawai M, Kitagawa T, Kohno T, Kurisu S, Nagata Y, Nakamura M, Morita H, Takano H, Shiga T, Takei Y, Yuasa S, Yamamoto T, Watanabe T, Akasaka T, Doi Y, Kimura T, Kitakaze M, Kosuge M, Takayama M, Tomoike H; Japanese Circulation Society Joint Working Group. JCS/JHFS 2018 Guideline on the Diagnosis and Treatment of Cardiomyopathies. *Circ J* 2021; **85**: 1590-1689 [PMID: 34305070 DOI:

10.1253/circj.CJ-20-0910]
13 **Khush KK**, Cherikh WS, Chambers DC, Harhay MO, Hayes D Jr, Hsich E, Meiser B, Potena L, Robinson A, Rossano JW, Sadavarte A, Singh TP, Zuckermann A, Stehlik J; International Society for Heart and Lung Transplantation. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-sixth adult heart transplantation report - 2019; focus theme: Donor and recipient size match. *J Heart Lung Transplant* 2019; **38**: 1056-1066 [PMID: 31548031 DOI: 10.1016/j.healun.2019.08.004]
14 **Nakatani T**, Fukushima N, Ono M, Saiki Y, Matsuda H, Nunoda S, Sawa Y, Isobe M. The Registry Report of Heart Transplantation in Japan (1999-2014). *Circ J* 2016; **80**: 44-50 [PMID: 26638870 DOI: 10.1253/circj.CJ-15-0975]
15 **Giuliano K**, Scheel P 3rd, Etchill E, Fraser CD 3rd, Suarez-Pierre A, Hsu S, Wittstein IS, Kasper EK, Florido R, Tandri H, Calkins H, Choi CW, Sharma K, Kilic A, Gilotra NA. Heart transplantation outcomes in arrhythmogenic right ventricular cardiomyopathy: a contemporary national analysis. *ESC Heart Fail* 2022; **9**: 988-997 [PMID: 35132806 DOI: 10.1002/ehf2.13687]
16 **Coppini R**, Ho CY, Ashley E, Day S, Ferrantini C, Girolami F, Tomberli B, Bardi S, Torricelli F, Cecchi F, Mugelli A, Poggesi C, Tardiff J, Olivotto I. Clinical phenotype and outcome of hypertrophic cardiomyopathy associated with thin-filament gene mutations. *J Am Coll Cardiol* 2014; **64**: 2589-2600 [PMID: 25524337 DOI: 10.1016/j.jacc.2014.09.059]
17 **Marstrand P**, Han L, Day SM, Olivotto I, Ashley EA, Michels M, Pereira AC, Wittekind SG, Helms A, Saberi S, Jacoby D, Ware JS, Colan SD, Semsarian C, Ingles J, Lakdawala NK, Ho CY; SHaRe Investigators. Hypertrophic Cardiomyopathy With Left Ventricular Systolic Dysfunction: Insights From the SHaRe Registry. *Circulation* 2020; **141**: 1371-1383 [PMID: 32228044 DOI: 10.1161/CIRCULATIONAHA.119.044366]
18 **Haas J**, Frese KS, Peil B, Kloos W, Keller A, Nietsch R, Feng Z, Müller S, Kayvanpour E, Vogel B, Sedaghat-Hamedani F, Lim WK, Zhao X, Fradkin D, Köhler D, Fischer S, Franke J, Marquart S, Barb I, Li DT, Amr A, Ehlermann P, Mereles D, Weis T, Hassel S, Kremer A, King V, Wirsz E, Isnard R, Komajda M, Serio A, Grasso M, Syrris P, Wicks E, Plagnol V, Lopes L, Gadgaard T, Eiskjær H, Jørgensen M, Garcia-Giustiniani D, Ortiz-Genga M, Crespo-Leiro MG, Deprez RH, Christiaans I, van Rijsingen IA, Wilde AA, Waldenstrom A, Bolognesi M, Bellazzi R, Mörner S, Bermejo JL, Monserrat L, Villard E, Mogensen J, Pinto YM, Charron P, Elliott P, Arbustini E, Katus HA, Meder B. Atlas of the clinical genetics of human dilated cardiomyopathy. *Eur Heart J* 2015; **36**: 1123-135a [PMID: 25163546 DOI: 10.1093/eurheartj/ehu301]
19 **Suwa Y**, Higo S, Nakamoto K, Sera F, Kunimatsu S, Masumura Y, Kanzaki M, Mizote I, Mizuno H, Fujio Y, Hikoso S, Sakata Y. Old-Age Onset Progressive Cardiac Contractile Dysfunction in a Patient with Polycystic Kidney Disease Harboring a PKD1 Frameshift Mutation. *Int Heart J* 2019; **60**: 220-225 [PMID: 30464138 DOI: 10.1536/ihj.18-184]
20 **Tobita T**, Nomura S, Fujita T, Morita H, Asano Y, Onoue K, Ito M, Imai Y, Suzuki A, Ko T, Satoh M, Fujita K, Naito AT, Furutani Y, Toko H, Harada M, Amiya E, Hatano M, Takimoto E, Shiga T, Nakanishi T, Sakata Y, Ono M, Saito Y, Takashima S, Hagiwara N, Aburatani H, Komuro I. Genetic basis of cardiomyopathy and the genotypes involved in prognosis and left ventricular reverse remodeling. *Sci Rep* 2018; **8**: 1998 [PMID: 29386531 DOI: 10.1038/s41598-018-20114-9]
21 **Towbin JA**, McKenna WJ, Abrams DJ, Ackerman MJ, Calkins H, Darrieux FCC, Daubert JP, de Chillou C, DePasquale EC, Desai MY, Estes NAM 3rd, Hua W, Indik JH, Ingles J, James CA, John RM, Judge DP, Keegan R, Krahn AD, Link MS, Marcus FI, McLeod CJ, Mestroni L, Priori SG, Saffitz JE, Sanatani S, Shimizu W, van Tintelen JP, Wilde AAM, Zareba W. 2019 HRS expert consensus statement on evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy. *Heart Rhythm* 2019; **16**: e301-e372 [PMID: 31078652 DOI: 10.1016/j.hrthm.2019.05.007]
22 **Awad MM**, Calkins H, Judge DP. Mechanisms of disease: molecular genetics of arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Nat Clin Pract Cardiovasc Med* 2008; **5**: 258-267 [PMID: 18382419 DOI: 10.1038/ncpcardio1182]
23 **Al-Jassar C**, Bikker H, Overduin M, Chidgey M. Mechanistic basis of desmosome-targeted diseases. *J Mol Biol* 2013; **425**: 4006-4022 [PMID: 23911551 DOI: 10.1016/j.jmb.2013.07.035]
24 **Delmar M**, McKenna WJ. The cardiac desmosome and arrhythmogenic cardiomyopathies: from gene to disease. *Circ Res* 2010; **107**: 700-714 [PMID: 20847325 DOI: 10.1161/CIRCRESAHA.110.223412]
25 **Pugh TJ**, Kelly MA, Gowrisankar S, Hynes E, Seidman MA, Baxter SM, Bowser M, Harrison B, Aaron D, Mahanta LM, Lakdawala NK, McDermott G, White ET, Rehm HL, Lebo M, Funke BH. The landscape of genetic variation in dilated cardiomyopathy as surveyed by clinical DNA sequencing. *Genet Med* 2014; **16**: 601-608 [PMID: 24503780 DOI: 10.1038/gim.2013.204]
26 **Garcia-Pavia P**, Syrris P, Salas C, Evans A, Mirelis JG, Cobo-Marcos M, Vilches C, Bornstein B, Segovia J, Alonso-Pulpon L, Elliott PM. Desmosomal protein gene mutations in patients with idiopathic dilated cardiomyopathy undergoing cardiac transplantation: a clinicopathological study. *Heart* 2011; **97**: 1744-1752 [PMID: 21859740 DOI: 10.1136/hrt.2011.227967]
27 **Rigato I**, Bauce B, Rampazzo A, Zorzi A, Pilichou K, Mazzotti E, Migliore F, Marra MP, Lorenzon A, De Bortoli M, Calore M, Nava A, Daliento L, Gregori D, Iliceto S, Thiene G, Basso C, Corrado D. Compound and digenic heterozygosity predicts lifetime arrhythmic outcome and sudden cardiac death in desmosomal gene-related arrhythmogenic right ventricular cardiomyopathy. *Circ Cardiovasc Genet* 2013; **6**: 533-542 [PMID: 24070718 DOI: 10.1161/CIRCGENETICS.113.000288]
28 **Gandjbakhch E**, Redheuil A, Pousset F, Charron P, Frank R. Clinical Diagnosis, Imaging, and Genetics of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia: JACC State-of-the-Art Review. *J Am Coll Cardiol* 2018; **72**: 784-804 [PMID: 30092956 DOI: 10.1016/j.jacc.2018.05.065]
29 **Chen K**, Rao M, Guo G, Duru F, Chen L, Chen X, Song J, Hu S. Recessive variants in plakophilin-2 contributes to early-onset arrhythmogenic cardiomyopathy with severe heart failure. *Europace* 2019; **21**: 970-977 [PMID: 30830208 DOI: 10.1093/europace/euz026]
30 **Shiba M**, Higo S, Kondo T, Li J, Liu L, Ikeda Y, Kohama Y, Kameda S, Tabata T, Inoue H, Nakamura S, Takeda M, Ito E, Takashima S, Miyagawa S, Sawa Y, Hikoso S, Sakata Y. Phenotypic recapitulation and correction of desmoglein-2-deficient cardiomyopathy using human-induced pluripotent stem cell-derived cardiomyocytes. *Hum Mol Genet* 2021; **30**: 1384-1397 [PMID: 33949662 DOI: 10.1093/hmg/ddab127]

31 **Eshkind L**, Tian Q, Schmidt A, Franke WW, Windoffer R, Leube RE. Loss of desmoglein 2 suggests essential functions for early embryonic development and proliferation of embryonal stem cells. *Eur J Cell Biol* 2002; **81**: 592-598 [PMID: 12494996 DOI: 10.1078/0171-9335-00278]

32 **Sayed N**, Liu C, Wu JC. Translation of Human-Induced Pluripotent Stem Cells: From Clinical Trial in a Dish to Precision Medicine. *J Am Coll Cardiol* 2016; **67**: 2161-2176 [PMID: 27151349 DOI: 10.1016/j.jacc.2016.01.083]

33 **Kondo T**, Higo S, Shiba M, Kohama Y, Kameda S, Tabata T, Inoue H, Okuno S, Ogawa S, Nakamura S, Takeda M, Ito E, Li J, Liu L, Kuramoto Y, Lee JK, Takashima S, Miyagawa S, Sawa Y, Hikoso S, Sakata Y. Human-Induced Pluripotent Stem Cell-Derived Cardiomyocyte Model for TNNT2 Δ160E-Induced Cardiomyopathy. *Circ Genom Precis Med* 2022; **15**: e003522 [PMID: 35861968 DOI: 10.1161/CIRCGEN.121.003522]

34 **Tabata T**, Masumura Y, Higo S, Kunimatsu S, Kameda S, Inoue H, Okuno S, Ogawa S, Takashima S, Watanabe M, Miyagawa S, Hikoso S, Sakata Y. Multiplexed measurement of cell type-specific calcium kinetics using high-content image analysis combined with targeted gene disruption. *Biochem Biophys Res Commun* 2022; **637**: 40-49 [PMID: 36375249 DOI: 10.1016/j.bbrc.2022.10.088]

35 **Higo S**, Hikoso S, Miyagawa S, Sakata Y. Genome Editing in Human Induced Pluripotent Stem Cells (hiPSCs). *Methods Mol Biol* 2021; **2320**: 235-245 [PMID: 34302662 DOI: 10.1007/978-1-0716-1484-6_21]

36 **Padrón-Barthe L**, Domínguez F, Garcia-Pavia P, Lara-Pezzi E. Animal models of arrhythmogenic right ventricular cardiomyopathy: what have we learned and where do we go? *Basic Res Cardiol* 2017; **112**: 50 [PMID: 28688053 DOI: 10.1007/s00395-017-0640-3]

37 **Ohno S**, Nagaoka I, Fukuyama M, Kimura H, Itoh H, Makiyama T, Shimizu A, Horie M. Age-dependent clinical and genetic characteristics in Japanese patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Circ J* 2013; **77**: 1534-1542 [PMID: 23514727 DOI: 10.1253/circj.cj-12-1446]

38 **Groeneweg JA**, Bhonsale A, James CA, te Riele AS, Dooijes D, Tichnell C, Murray B, Wiesfeld AC, Sawant AC, Kassamali B, Atsma DE, Volders PG, de Groot NM, de Boer K, Zimmerman SL, Kamel IR, van der Heijden JF, Russell SD, Jan Cramer M, Tedford RJ, Doevendans PA, van Veen TA, Tandri H, Wilde AA, Judge DP, van Tintelen JP, Hauer RN, Calkins H. Clinical Presentation, Long-Term Follow-Up, and Outcomes of 1001 Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy Patients and Family Members. *Circ Cardiovasc Genet* 2015; **8**: 437-446 [PMID: 25820315 DOI: 10.1161/CIRCGENETICS.114.001003]

39 **Novelli V**, Malkani K, Cerrone M. Pleiotropic Phenotypes Associated With PKP2 Variants. *Front Cardiovasc Med* 2018; **5**: 184 [PMID: 30619891 DOI: 10.3389/fcvm.2018.00184]

40 **El-Battrawy I**, Zhao Z, Lan H, Cyganek L, Tombers C, Li X, Buljubasic F, Lang S, Tiburcy M, Zimmermann WH, Utikal J, Wieland T, Borggrefe M, Zhou XB, Akin I. Electrical dysfunctions in human-induced pluripotent stem cell-derived cardiomyocytes from a patient with an arrhythmogenic right ventricular cardiomyopathy. *Europace* 2018; **20**: f46-f56 [PMID: 29566126 DOI: 10.1093/europace/euy042]

41 **Buljubasic F**, El-Battrawy I, Lan H, Lomada SK, Chatterjee A, Zhao Z, Li X, Zhong R, Xu Q, Huang M, Liao Z, Lang S, Cyganek L, Zhou X, Wieland T, Borggrefe M, Akin I. Nucleoside Diphosphate Kinase B Contributes to Arrhythmogenesis in Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes from a Patient with Arrhythmogenic Right Ventricular Cardiomyopathy. *J Clin Med* 2020; **9** [PMID: 32050722 DOI: 10.3390/jcm9020486]

42 **Ng R**, Manring H, Papoutsidakis N, Albertelli T, Tsai N, See CJ, Li X, Park J, Stevens TL, Bobbili PJ, Riaz M, Ren Y, Stoddard CE, Janssen PM, Bunch TJ, Hall SP, Lo YC, Jacoby DL, Qyang Y, Wright N, Ackermann MA, Campbell SG. Patient mutations linked to arrhythmogenic cardiomyopathy enhance calpain-mediated desmoplakin degradation. *JCI Insight* 2019; **5** [PMID: 31194698 DOI: 10.1172/jci.insight.128643]

43 **Xia S**, Wang X, Yue P, Li Y, Zhang D. Establishment of induced pluripotent stem cell lines from a family of an ARVC patient receiving heart transplantation in infant age carrying compound heterozygous mutations in DSP gene. *Stem Cell Res* 2020; **48**: 101977 [PMID: 32942234 DOI: 10.1016/j.scr.2020.101977]

44 **Moreau A**, Reisqs JB, Delanoe-Ayari H, Pierre M, Janin A, Deliniere A, Bessière F, Meli AC, Charrabi A, Lafont E, Valla C, Bauer D, Morel E, Gache V, Millat G, Nissan X, Faucherre A, Jopling C, Richard S, Mejat A, Chevalier P. Deciphering DSC2 arrhythmogenic cardiomyopathy electrical instability: From ion channels to ECG and tailored drug therapy. *Clin Transl Med* 2021; **11**: e319 [PMID: 33784018 DOI: 10.1002/ctm2.319]

45 **Reisqs JB**, Moreau A, Charrabi A, Sleiman Y, Meli AC, Millat G, Briand V, Beauverger P, Richard S, Chevalier P. The PPARγ pathway determines electrophysiological remodelling and arrhythmia risks in DSC2 arrhythmogenic cardiomyopathy. *Clin Transl Med* 2022; **12**: e748 [PMID: 35297182 DOI: 10.1002/ctm2.748]

46 **Caspi O**, Huber I, Gepstein A, Arbel G, Maizels L, Boulos M, Gepstein L. Modeling of arrhythmogenic right ventricular cardiomyopathy with human induced pluripotent stem cells. *Circ Cardiovasc Genet* 2013; **6**: 557-568 [PMID: 24200905 DOI: 10.1161/CIRCGENETICS.113.000188]

47 **Kim C**, Wong J, Wen J, Wang S, Wang C, Spiering S, Kan NG, Forcales S, Puri PL, Leone TC, Marine JE, Calkins H, Kelly DP, Judge DP, Chen HS. Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. *Nature* 2013; **494**: 105-110 [PMID: 23354045 DOI: 10.1038/nature11799]

48 **Ma D**, Wei H, Lu J, Ho S, Zhang G, Sun X, Oh Y, Tan SH, Ng ML, Shim W, Wong P, Liew R. Generation of patient-specific induced pluripotent stem cell-derived cardiomyocytes as a cellular model of arrhythmogenic right ventricular cardiomyopathy. *Eur Heart J* 2013; **34**: 1122-1133 [PMID: 22798562 DOI: 10.1093/eurheartj/ehs226]

49 **Dorn T**, Kornherr J, Parrotta EI, Zawada D, Ayetey H, Santamaria G, Iop L, Mastantuono E, Sinnecker D, Goedel A, Dirschinger RJ, My I, Laue S, Bozoglu T, Baarlink C, Ziegler T, Graf E, Hinkel R, Cuda G, Kääb S, Grace AA, Grosse R, Kupatt C, Meitinger T, Smith AG, Laugwitz KL, Moretti A. Interplay of cell-cell contacts and RhoA/MRTF-A signaling regulates cardiomyocyte identity. *EMBO J* 2018; **37** [PMID: 29764980 DOI: 10.15252/embj.201798133]

50 **Blazeski A**, Lowenthal J, Wang Y, Teuben R, Zhu R, Gerecht S, Tomaselli G, Tung L. Engineered Heart Slice Model of Arrhythmogenic Cardiomyopathy Using Plakophilin-2 Mutant Myocytes. *Tissue Eng Part A* 2019; **25**: 725-735 [PMID: 30520705 DOI: 10.1089/ten.TEA.2018.0272]

51 **Martewicz S**, Luni C, Serena E, Pavan P, Chen HV, Rampazzo A, Elvassore N. Transcriptomic Characterization of a

Human In Vitro Model of Arrhythmogenic Cardiomyopathy Under Topological and Mechanical Stimuli. *Ann Biomed Eng* 2019; **47**: 852-865 [PMID: 30569242 DOI: 10.1007/s10439-018-02134-8]

52 **Khudiakov A**, Zaytseva A, Perepelina K, Smolina N, Pervunina T, Vasichkina E, Karpushev A, Tomilin A, Malashicheva A, Kostareva A. Sodium current abnormalities and deregulation of Wnt/β-catenin signaling in iPSC-derived cardiomyocytes generated from patient with arrhythmogenic cardiomyopathy harboring compound genetic variants in plakophilin 2 gene. *Biochim Biophys Acta Mol Basis Dis* 2020; **1866**: 165915 [PMID: 32768677 DOI: 10.1016/j.bbadis.2020.165915]

53 **Zhang K**, Cloonan PE, Sundaram S, Liu F, Das SL, Ewoldt JK, Bays JL, Tomp S, Toepfer CN, Marsiglia JDC, Gorham J, Reichart D, Eyckmans J, Seidman JG, Seidman CE, Chen CS. Plakophilin-2 truncating variants impair cardiac contractility by disrupting sarcomere stability and organization. *Sci Adv* 2021; **7**: eabh3995 [PMID: 34652945 DOI: 10.1126/sciadv.abh3995]

54 **Inoue H**, Nakamura S, Higo S, Shiba M, Kohama Y, Kondo T, Kameda S, Tabata T, Okuno S, Ikeda Y, Li J, Liu L, Yamazaki S, Takeda M, Ito E, Takashima S, Miyagawa S, Sawa Y, Hikoso S, Sakata Y. Modeling reduced contractility and impaired desmosome assembly due to plakophilin-2 deficiency using isogenic iPS cell-derived cardiomyocytes. *Stem Cell Reports* 2022; **17**: 337-351 [PMID: 35063130 DOI: 10.1016/j.stemcr.2021.12.016]

55 **Kirchner F**, Schuetz A, Boldt LH, Martens K, Dittmar G, Haverkamp W, Thierfelder L, Heinemann U, Gerull B. Molecular insights into arrhythmogenic right ventricular cardiomyopathy caused by plakophilin-2 missense mutations. *Circ Cardiovasc Genet* 2012; **5**: 400-411 [PMID: 22781308 DOI: 10.1161/CIRCGENETICS.111.961854]

56 **Rasmussen TB**, Nissen PH, Palmfeldt J, Gehmlich K, Dalager S, Jensen UB, Kim WY, Heickendorff L, Mølgaard H, Jensen HK, Baandrup UT, Bross P, Mogensen J. Truncating plakophilin-2 mutations in arrhythmogenic cardiomyopathy are associated with protein haploinsufficiency in both myocardium and epidermis. *Circ Cardiovasc Genet* 2014; **7**: 230-240 [PMID: 24704780 DOI: 10.1161/CIRCGENETICS.113.000338]

57 **Corrado D**, Perazzolo Marra M, Zorzi A, Beffagna G, Cipriani A, Lazzari M, Migliore F, Pilichou K, Rampazzo A, Rigato I, Rizzo S, Thiene G, Anastasakis A, Asimaki A, Bucciarelli-Ducci C, Haugaa KH, Marchlinski FE, Mazzanti A, McKenna WJ, Pantazis A, Pelliccia A, Schmied C, Sharma S, Wichter T, Bauce B, Basso C. Diagnosis of arrhythmogenic cardiomyopathy: The Padua criteria. *Int J Cardiol* 2020; **319**: 106-114 [PMID: 32561223 DOI: 10.1016/j.ijcard.2020.06.005]

58 **Burridge PW**, Matsa E, Shukla P, Lin ZC, Churko JM, Ebert AD, Lan F, Diecke S, Huber B, Mordwinkin NM, Plews JR, Abilez OJ, Cui B, Gold JD, Wu JC. Chemically defined generation of human cardiomyocytes. *Nat Methods* 2014; **11**: 855-860 [PMID: 24930130 DOI: 10.1038/nmeth.2999]

59 **Gintant G**, Burridge P, Gepstein L, Harding S, Herron T, Hong C, Jalife J, Wu JC. Use of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes in Preclinical Cancer Drug Cardiotoxicity Testing: A Scientific Statement From the American Heart Association. *Circ Res* 2019; **125**: e75-e92 [PMID: 31533542 DOI: 10.1161/RES.0000000000000291]

60 **Pérez-Hernández M**, van Opbergen CJM, Bagwan N, Vissing CR, Marrón-Liñares GM, Zhang M, Torres Vega E, Sorrentino A, Drici L, Sulek K, Zhai R, Hansen FB, Christensen AH, Boesgaard S, Gustafsson F, Rossing K, Small EM, Davies MJ, Rothenberg E, Sato PY, Cerrone M, Jensen THL, Qvortrup K, Bundgaard H, Delmar M, Lundby A. Loss of Nuclear Envelope Integrity and Increased Oxidant Production Cause DNA Damage in Adult Hearts Deficient in PKP2: A Molecular Substrate of ARVC. *Circulation* 2022; **146**: 851-867 [PMID: 35959657 DOI: 10.1161/CIRCULATIONAHA.122.060454]

61 **Stroud MJ**. Linker of nucleoskeleton and cytoskeleton complex proteins in cardiomyopathy. *Biophys Rev* 2018; **10**: 1033-1051 [PMID: 29869195 DOI: 10.1007/s12551-018-0431-6]

62 **Ishizu T**, Higo S, Masumura Y, Kohama Y, Shiba M, Higo T, Shibamoto M, Nakagawa A, Morimoto S, Takashima S, Hikoso S, Sakata Y. Targeted Genome Replacement *via* Homology-directed Repair in Non-dividing Cardiomyocytes. *Sci Rep* 2017; **7**: 9363 [PMID: 28839205 DOI: 10.1038/s41598-017-09716-x]

63 **Kohama Y**, Higo S, Masumura Y, Shiba M, Kondo T, Ishizu T, Higo T, Nakamura S, Kameda S, Tabata T, Inoue H, Motooka D, Okuzaki D, Takashima S, Miyagawa S, Sawa Y, Hikoso S, Sakata Y. Adeno-associated virus-mediated gene delivery promotes S-phase entry-independent precise targeted integration in cardiomyocytes. *Sci Rep* 2020; **10**: 15348 [PMID: 32948788 DOI: 10.1038/s41598-020-72216-y]

64 **Tilemann L**, Ishikawa K, Weber T, Hajjar RJ. Gene therapy for heart failure. *Circ Res* 2012; **110**: 777-793 [PMID: 22383712 DOI: 10.1161/CIRCRESAHA.111.252981]

65 **Ishikawa K**, Weber T, Hajjar RJ. Human Cardiac Gene Therapy. *Circ Res* 2018; **123**: 601-613 [PMID: 30355138 DOI: 10.1161/CIRCRESAHA.118.311587]

66 **Chamberlain K**, Riyad JM, Weber T. Cardiac gene therapy with adeno-associated virus-based vectors. *Curr Opin Cardiol* 2017; **32**: 275-282 [PMID: 28169951 DOI: 10.1097/HCO.0000000000000386]

67 **Greenberg B**, Yaroshinsky A, Zsebo KM, Butler J, Felker GM, Voors AA, Rudy JJ, Wagner K, Hajjar RJ. Design of a phase 2b trial of intracoronary administration of AAV1/SERCA2a in patients with advanced heart failure: the CUPID 2 trial (calcium up-regulation by percutaneous administration of gene therapy in cardiac disease phase 2b). *JACC Heart Fail* 2014; **2**: 84-92 [PMID: 24622121 DOI: 10.1016/j.jchf.2013.09.008]

68 **Hasenfuss G**, Pieske B. Calcium cycling in congestive heart failure. *J Mol Cell Cardiol* 2002; **34**: 951-969 [PMID: 12234765 DOI: 10.1006/jmcc.2002.2037]

69 **Kho C**, Lee A, Hajjar RJ. Altered sarcoplasmic reticulum calcium cycling--targets for heart failure therapy. *Nat Rev Cardiol* 2012; **9**: 717-733 [PMID: 23090087 DOI: 10.1038/nrcardio.2012.145]

70 **Eisner D**, Caldwell J, Trafford A. Sarcoplasmic reticulum Ca-ATPase and heart failure 20 years later. *Circ Res* 2013; **113**: 958-961 [PMID: 24071456 DOI: 10.1161/CIRCRESAHA.113.302187]

71 **del Monte F**, Williams E, Lebeche D, Schmidt U, Rosenzweig A, Gwathmey JK, Lewandowski ED, Hajjar RJ. Improvement in survival and cardiac metabolism after gene transfer of sarcoplasmic reticulum Ca(2+)-ATPase in a rat model of heart failure. *Circulation* 2001; **104**: 1424-1429 [PMID: 11560860 DOI: 10.1161/hc3601.095574]

72 **Jessup M**, Greenberg B, Mancini D, Cappola T, Pauly DF, Jaski B, Yaroshinsky A, Zsebo KM, Dittrich H, Hajjar RJ; Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) Investigators.

Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID): a phase 2 trial of intracoronary gene therapy of sarcoplasmic reticulum Ca2+-ATPase in patients with advanced heart failure. *Circulation* 2011; **124**: 304-313 [PMID: 21709064 DOI: 10.1161/CIRCULATIONAHA.111.022889]

73 **Cutler MJ**, Wan X, Plummer BN, Liu H, Deschenes I, Laurita KR, Hajjar RJ, Rosenbaum DS. Targeted sarcoplasmic reticulum Ca2+ ATPase 2a gene delivery to restore electrical stability in the failing heart. *Circulation* 2012; **126**: 2095-2104 [PMID: 23019291 DOI: 10.1161/CIRCULATIONAHA.111.071480]

74 **Greenberg B**, Butler J, Felker GM, Ponikowski P, Voors AA, Desai AS, Barnard D, Bouchard A, Jaski B, Lyon AR, Pogoda JM, Rudy JJ, Zsebo KM. Calcium upregulation by percutaneous administration of gene therapy in patients with cardiac disease (CUPID 2): a randomised, multinational, double-blind, placebo-controlled, phase 2b trial. *Lancet* 2016; **387**: 1178-1186 [PMID: 26803443 DOI: 10.1016/S0140-6736(16)00082-9]

75 **Hulot JS**, Salem JE, Redheuil A, Collet JP, Varnous S, Jourdain P, Logeart D, Gandjbakhch E, Bernard C, Hatem SN, Isnard R, Cluzel P, Le Feuvre C, Leprince P, Hammoudi N, Lemoine FM, Klatzmann D, Vicaut E, Komajda M, Montalescot G, Lompré AM, Hajjar RJ; AGENT-HF Investigators. Effect of intracoronary administration of AAV1/SERCA2a on ventricular remodelling in patients with advanced systolic heart failure: results from the AGENT-HF randomized phase 2 trial. *Eur J Heart Fail* 2017; **19**: 1534-1541 [PMID: 28393439 DOI: 10.1002/ejhf.826]

76 **Lyon AR**, Babalis D, Morley-Smith AC, Hedger M, Suarez Barrientos A, Foldes G, Couch LS, Chowdhury RA, Tzortzis KN, Peters NS, Rog-Zielinska EA, Yang HY, Welch S, Bowles CT, Rahman Haley S, Bell AR, Rice A, Sasikaran T, Johnson NA, Falaschetti E, Parameshwar J, Lewis C, Tsui S, Simon A, Pepper J, Rudy JJ, Zsebo KM, Macleod KT, Terracciano CM, Hajjar RJ, Banner N, Harding SE. Investigation of the safety and feasibility of AAV1/SERCA2a gene transfer in patients with chronic heart failure supported with a left ventricular assist device - the SERCA-LVAD TRIAL. *Gene Ther* 2020; **27**: 579-590 [PMID: 32669717 DOI: 10.1038/s41434-020-0171-7]

77 **Repetti GG**, Toepfer CN, Seidman JG, Seidman CE. Novel Therapies for Prevention and Early Treatment of Cardiomyopathies. *Circ Res* 2019; **124**: 1536-1550 [PMID: 31120825 DOI: 10.1161/CIRCRESAHA.119.313569]

78 **Hakui H**, Kioka H, Miyashita Y, Nishimura S, Matsuoka K, Kato H, Tsukamoto O, Kuramoto Y, Takuwa A, Takahashi Y, Saito S, Ohta K, Asanuma H, Fu HY, Shinomiya H, Yamada N, Ohtani T, Sawa Y, Kitakaze M, Takashima S, Sakata Y, Asano Y. Loss-of-function mutations in the co-chaperone protein BAG5 cause dilated cardiomyopathy requiring heart transplantation. *Sci Transl Med* 2022; **14**: eabf3274 [PMID: 35044787 DOI: 10.1126/scitranslmed.abf3274]

79 **Calkins H**, Corrado D, Marcus F. Risk Stratification in Arrhythmogenic Right Ventricular Cardiomyopathy. *Circulation* 2017; **136**: 2068-2082 [PMID: 29158215 DOI: 10.1161/CIRCULATIONAHA.117.030792]

80 **van Tintelen JP**, Entius MM, Bhuiyan ZA, Jongbloed R, Wiesfeld AC, Wilde AA, van der Smagt J, Boven LG, Mannens MM, van Langen IM, Hofstra RM, Otterspoor LC, Doevendans PA, Rodriguez LM, van Gelder IC, Hauer RN. Plakophilin-2 mutations are the major determinant of familial arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circulation* 2006; **113**: 1650-1658 [PMID: 16567567 DOI: 10.1161/CIRCULATIONAHA.105.609719]

81 **Ramond F**, Janin A, Di Filippo S, Chanavat V, Chalabreysse L, Roux-Buisson N, Sanlaville D, Touraine R, Millat G. Homozygous PKP2 deletion associated with neonatal left ventricle noncompaction. *Clin Genet* 2017; **91**: 126-130 [PMID: 27030002 DOI: 10.1111/cge.12780]

82 **Katanyuwong P**, Khongkraparn A, Wattanasirichaigoon D. A Novel Homozygous PKP2 Variant in Severe Neonatal Non-compaction and Concomitant Ventricular Septal Defect: A Case Report. *Front Pediatr* 2021; **9**: 801491 [PMID: 35059364 DOI: 10.3389/fped.2021.801491]

83 **Verhagen JMA**, van den Born M, Kurul S, Asimaki A, van de Laar IMBH, Frohn-Mulder IME, Kammeraad JAE, Yap SC, Bartelings MM, van Slegtenhorst MA, von der Thüsen JH, Wessels MW. Homozygous Truncating Variant in PKP2 Causes Hypoplastic Left Heart Syndrome. *Circ Genom Precis Med* 2018; **11**: e002397 [PMID: 30562116 DOI: 10.1161/CIRCGEN.118.002397]

84 **Li J**, Zhang L, Yu L, Minami I, Miyagawa S, Hörning M, Dong J, Qiao J, Qu X, Hua Y, Fujimoto N, Shiba Y, Zhao Y, Tang F, Chen Y, Sawa Y, Tang C, Liu L. Circulating re-entrant waves promote maturation of hiPSC-derived cardiomyocytes in self-organized tissue ring. *Commun Biol* 2020; **3**: 122 [PMID: 32170165 DOI: 10.1038/s42003-020-0853-0]

85 **Li J**, Minami I, Shiozaki M, Yu L, Yajima S, Miyagawa S, Shiba Y, Morone N, Fukushima S, Yoshioka M, Li S, Qiao J, Li X, Wang L, Kotera H, Nakatsuji N, Sawa Y, Chen Y, Liu L. Human Pluripotent Stem Cell-Derived Cardiac Tissue-like Constructs for Repairing the Infarcted Myocardium. *Stem Cell Reports* 2017; **9**: 1546-1559 [PMID: 29107590 DOI: 10.1016/j.stemcr.2017.09.007]

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