PKP2 Mutations in Sudden Death From Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) and Sudden Unexpected Death With Negative Autopsy (SUDNA)

Mingchang Zhang; Fabio Tavora; Joao Bosco Oliveira; Ling Li;
Marcello Franco; David Fowler; Ziqin Zhao; Allen Burke

Background: Plakophilin2 (PKP2) is a desmosome-related protein with numerous armadillo repeats and has been linked to arrhythmogenic right ventricular cardiomyopathy (ARVC). Fatal arrhythmias resulting in sudden death also occur in the absence of morphologic cardiac abnormalities at autopsy, and have been linked to ion channel mutations in a subset of cases, but so far not to PKP2.

Methods and Results: We sequenced all 14 exons of *PKP2* in DNA extracted from postmortem heart tissues of 25 patients dying from ARVC and 25 from sudden unexpected death with negative autopsy (SUDNA). The primers were designed using the Primer Express 3.0 software. Direct sequencing for both sense and antisense strands was performed with a BigDye Terminator DNA sequencing kit on a 3130XL Genetic Analyzer. Mutation damage prediction was made using Mutation Taster, Polyphen and SIFT software. In 6 of the 25 ARVC samples, 6 *PKP2* mutations were identified, 4 of which were likely significant, and 3 of which were novel (p.N641del, p.L64PfsX22, p.G269R). In 6 of the 25 cases of SUDNA samples, 6 *PKP2* mutations were identified, 3 of which were likely significant, and 4 of which were not previously described (p.P665S, p.Y217TfsX45, p.E540, p.S615T).

Conclusions: *PKP2* mutations are not specific for ARVC and may result in SUDNA. The link between ARVC and desmosomal mutations may not be causal but related to an association between defective desmosomal proteins and arrhythmias. (*Circ J* 2012; **76**: 189–194)

Key Words: Arrhythmia; Arrhythmogenic right ventricular cardiomyopathy; Desmosome; Negative autopsy; Sudden death

Armadillo family members are multifunctional proteins that play diverse roles in cell–cell adhesion and signaling. Plakophilins (PKPs) comprise a subgroup of the armadillo protein family, constitutive components of the submembrane plaque in intercellular junctions, including desmosomes and the cardiac area composita.^{1,2} The desmosome is a specialized adhesive junction that interacts with the cytoskeleton and participates in crosstalk with gap and adherens junctions.

Editorial p51

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVC) is an inherited disease characterized by ventricular arrhythmias, and is one of several entities that may cause sud-

den death and ventricular dysrhythmias in children and young adults.^{3–5} It is a leading cause of sudden death among young patients and athletes.⁶ A number of mutations in various genes encoding cardiac desmosomal proteins have been implicated in the pathogenesis of ARVC,^{7–15} and it is estimated that as many as 70% of the mutations linked to familial ARVC are in the gene coding for plakophilin-2 (PKP2),¹⁴ a 98-kDa desmosomal protein. PKP2 interacts with plakoglobin, desmoplakin, and the desmosomal cadherins via its amino terminal (“head”) domain. Mutations in *PKP2* may destabilize the desmosome and result in arrhythmias, by mechanisms that are currently obscure.

Cases of sudden death in children and adults may demonstrate morphologically normal myocardium at autopsy. In adults, this phenomenon has been termed sudden adult death syndrome,¹⁶ sudden unexpected death with negative autopsy

Received July 13, 2011; revised manuscript received August 26, 2011; accepted September 9, 2011; released online October 22, 2011 Time for primary review: 27 days

Department of Forensic Medicine, Shanghai Medical College, Fudan University, Shanghai (M.Z., L.L., D.F., Z.Z., A.B.), China; The Department of Laboratory Medicine, National Institutes of Health, Bethesda, MD (M.Z., J.B.O.), USA; Messejana Heart and Lung Hospital, Fortaleza (F.T.), Brazil; Division of Forensic Medicine, Key Laboratory of Evidence Sciences, China University of Political Science and Law, Beijing (L.L., D.F.), China; University of Maryland Medical Center, Baltimore, MD (M.Z., L.L., D.F., A.B.), USA; and Escola Paulista de Medicina, Sao Paulo (F.T., M.F.), Brazil

Mailing address: Allen Burke, MD, UMMS, 21 S. Greene St, Baltimore, MD 21201, USA. E-mail: aburke@umm.edu

ISSN-1346-9843 doi:10.1253/circj.CJ-11-0747

All rights are reserved to the Japanese Circulation Society. For permissions, please e-mail: cj@j-circ.or.jp

Table 1. Case Characteristics			
	ARVC (n=25)	SUDNA (n=25)	P value
Age, years±SD	34±3	24±3	0.03
Sex, M/F	6/19	8/17	0.5
Race, black/white/other	12/13/0	7/10/1	0.4
Prior syncope, n	4	6	0.8
Family history, n	3*	4**	0.7
Witnessed, n†	20	19	0.9
Exertional, n‡	16	1‡	0.001

*The family history in these cases included long QT syndrome (2), premature sudden death from unknown causes in father (1), premature sudden death for generations with unknown cause (1).
**The family history consisted of premature sudden death in mother (1), brother (1), and father (1), all of unknown cause.
†Collapse seen by others at home (28), workplace (8), in vehicle (1), other (2).
‡Basketball (9), running (5), soccer (1), swimming (1).
‡Weightlifting.

Table 2. Pathologic Characteristics of Arrhythmogenic Right Ventricular Cardiomyopathy	
Heart weight, g	428±88
Biventricular fibrofatty change	13 (52%)
Predominantly LV fibrofatty change	9 (36%)
Predominantly RV fibrofatty change	3 (12%)
Inflammatory infiltrates	12 (48%)
Gross left ventricular scars	17 (68%)
Gross right ventricular involvement	16 (64%)

LV, left ventricle; RV, right ventricle.

(SUDNA), and sudden unexplained death.^{17,18} In this study, we use the term “SUDNA” while recognizing that it is a heterogeneous group of disorders and does not define a specific syndrome. SUDNA accounts for up to 30% of sudden death in adults, and is especially common in children, adolescents and younger adults. SUDNA has been linked to ion channel mutations in up to 25% of cases, indicating that some deaths from SUDNA are the result of channelopathies.^{17,18}

Figure 1. Mutations in arrhythmogenic right ventricular cardiomyopathy. (A) 49-year-old female showing sense primer sequence: c.805 G>A. (B) 15-year-old male showing the antisense primer sequence: c.187_188 insC. (C) 29-year-old male showing sense primer sequence: c.2062 T>C. (D) 52-year-old male showing sense primer sequence (Upper) and antisense primer sequence (Lower): c.1925_1927delACA. (E) 58-year-old male and 46-year-old male showing exon1 sense primer sequence: c.76G>A. (F) 46-year-old male showing exon 9 sense primer sequence: c.1951C>T.

Table 3. PKP2 Mutations in Arrhythmogenic Right Ventricular Cardiomyopathy

	Case no.					
	1	2	3	4	5	6
Age, years	15	38	46	29	49	52
Sex	M	M	M	M	F	M
Exon	1	1	9	1	10	3
cDNA	187_188insC	76G>A	1951C>T	76G>A	2062T>C	805G>A 1925–1927 ACA del
Amino acid	L 64 P fsX22	D26 N	R 651 X	D26 N	S 688 P	G 269 R N 641 del
Novel	Yes	No	No	No	No	Yes
Polyphen	—*	PD	—	PD	D	B
SIFT	—*	D	—	D	D	B
Mutations taster	D	B	D	B	D	B
Family history	None known	None known	None known	None known	None known	None known
History syncope	Absent	Absent	Absent	Present	Absent	Absent
Other history	None	None	None	None	Alport syndrome	None
Activity at death	Witnessed	Exertional	Found dead	Exertional	Found dead	Exertional
Ventricular distribution	Biventricular	Left ventricular	Biventricular	Right ventricular	Biventricular	Left ventricular
Inflammation	Focus	None	None	Focus	None	None

*Indels and nonsense mutations not analyzed by PolyPhen and SIFT.

PD, possibly damaging; D, probably damaging; B, benign; SIFT, Sorting Intolerant from Tolerant.

We speculated that mutations in *PKP2* could also be associated with arrhythmias in the absence of the fibrofatty change of ARVC, and may occur in cases of SUDNA. In fact, we observed severe *PKP2* mutations with the same frequency in ARVC and SUDNA cases.

Methods

Study Subjects

We studied 25 cases of sudden cardiac death with the diagnosis of ARVC and 25 cases of SUDNA from a single medical examiner's office and consulted by a cardiovascular pathologist. The study was approved by the institutional review board. All cases were seen in consultation by the cardiovascular pathologist and forensic pathologist and examined in a similar fashion.

Genotyping and Sequence Alignment

Genomic DNA was extracted from postmortem heart tissue using Qiagen® kit.^{19–21} All coding exons and flanking intronic sequences of *PKP2* variant 2b (NM_004572.3) were amplified by polymerase chain reaction (PCR). Primers and PCR conditions are available on request. The primers were designed using the Primer Express 3.0 software. Direct sequencing for both sense and antisense strands was performed with a BigDye Terminator DNA sequencing kit on a 3130XL Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA). Data was analyzed with Lasergene software for identification of mutations (DNASTAR, Madison, WI, USA); 96 control DNA samples were studied for mutations found in the test samples. Mutation damage prediction was made using Mutation Taster software (<http://www.mutationtaster.org>), PolyPhen (<http://genetics.bwh.harvard.edu/pph2>) and Sorting Intolerant from Tolerant (SIFT; <http://sift.jcvi.org>). Mutations were classified into 2 categories: (1) benign mutation, or missense mutation that was predicted to be benign or possibly damaging by one or more of the 3 bioinformatics analyses; and (2) pathogenic mutations, or nonsense, indels or missense mutations predicted to be probably damaging by all 3 bioinformatics analyses.

Statistical Analysis

The χ^2 test was used to compare the frequency of mutations between the ARVC and SUDNA groups. A P value <0.05 was considered statistically significant.

Results

Clinicopathologic Characteristics

The age, sex, and pathologic findings related to the subjects are presented in **Tables 1** and **2**.

Mutation Analysis of *PKP2* in ARVC Samples

We identified 6 unique genetic variants in *PKP2* in 6 of the 26 ARVC cases. In 1 case there were 2 distinct *PKP2* mutations and in 2 non-related cases there was the same D26N change that was considered a benign polymorphism in previous studies.^{22,23} The 6 different mutations comprised 3 missense substitutions, 1 nonsense substitution, 1 deletion and 1 insertion (**Figure 1**). Details regarding the identified mutations are summarized in **Table 3**. The missense substitutions were 805G>A (G269R), 76G>A (D26N) and 2062T>C (S688P). S688P has been described previously and was predicted to be damaging by all 3 bioinformatics analyses (PolyPhen, SIFT and Mutation Taster). G269R was predicted to be benign by PolyPhen, SIFT and Mutation Taster. The deleterious nonsense substitution was c.1951C>T (R651X), and has already been described.²² The inframe deletion c.1925–1927ACA del (N641del), and frameshift c.187_188insC (L64P fsX22) insertion were novel. In summary, of the 6 mutations found, 4 are pathogenic, and 2 are probably benign polymorphisms. None of the mutations was identified in 96 normal controls.

The mean age of patients with pathogenic mutations was 36 years in the ARVC group, and 28 in the SUDNA group (P=0.3).

Mutation Analysis of *PKP2* in SUDNA Samples

In the 25 SUDNA cases, we identified 6 different heterozygous mutations in 6 cases (24%). None of these mutations was present in the 96 normal controls; 4 were novel and 5 were missense mutations: 1016T>C (F339S), 1592T>G (I531S), 1618G>A (E540K), 1843T>A (S615T) and 1993C>T (P665S). I531S was considered a benign polymorphism in previous

Table 4. PKP2 Mutations in Sudden Unexpected Death With Negative Autopsy							
Age (years)/sex	Exon	cDNA	Protein	Novel	Polyphen	SIFT	Mutations taster
45/M	Exon10	1993C>T	P665 S	Yes	PD	B	B
27/F	Exon7	1592T>G	I 531 S	No	PD	D	B
37/M	Exon3	1016T>C	F 339 S	No	PD	B	B
16/M	Exon3	648_651delATAC	Y217T fsX45	Yes	—*	—*	D
22/M	Exon7	1618G>A	E 540 K	Yes	D	D	D
20/M	Exon9	1843T>A	S 615 T	Yes	D	D	D

*Indels and nonsense mutations not analyzed by PolyPhen and SIFT. Abbreviations see in Table 3.

Table 5. Comparison of Mutations in ARVC and SUDNA			
Diagnosis	Negative	Benign	Pathogenic
ARVC	19 (76%)	2 (8%)	4 (16%)
SUDNA	19 (76%)	3 (12%)	3 (12%)

ARVC, arrhythmogenic right ventricular cardiomyopathy; SUDNA, sudden unexpected death with negative autopsy.

reports, given its presence in normal controls.^{24,25} All three bioinformatics analyses predicted E540K to be probably damaging, as well as S615T. A mutation in the same amino acid (S615F) has been described previously and was also considered pathogenic.^{8,12} F339S and P665S were considered to be possibly damaging by PolyPhen, and to be benign by SIFT and Mutation Taster. We also detected a deletion with frameshift, 648_651delATAC (Y217T fsX45) (Figure 2), which was novel. Details regarding the identified mutations are summarized in Table 4.

Correlation of Bioinformatics Analysis

Each group was classified into 3 categories: (1) negative (no mutation); (2) positive for benign mutation; or (3) positive for pathogenic mutation. No statistically significant differences in mutation frequencies between the ARVC and SUDNA cases were detected ($P=0.842$) (Table 5).

Discussion

Cellular adhesion plays a crucial role in the development and maintenance of multicellular tissues and organs of higher eukaryotes. The desmosome (macula adherens) is the morphologically most conspicuous cellular adhesion structure, and is particularly abundant in tissues such as epidermis and myocardium that are continually assailed by mechanical forces. Desmosomes contain members of at least 3 protein families. Desmosomal cadherins form the intercellular adhesive interface, whereas armadillo and plakin family proteins build up the plaques. It is believed that the cytoplasmic tail of desmogleins (Dsgs) and desmocollins (Dscs) interact with plakoglobin, which in turn binds to desmoplakin. Desmoplakin finally is anchored to the intermediate filament cytoskeleton.²⁶ These interactions seem to be stabilized laterally by plakophilin.²⁷

Recent evidence indicates that mutations in several desmosomal components play a major role in the pathogenesis of ARVC.^{7,8,10–14} Among the genes in which mutations associated with ARVC have been identified, plakophilin-2 accounts for a large proportion. Plakophilins are a family of armadillo-repeat-containing proteins that share a high degree of sequence similarity with the adherens junction component, p120. However, these armadillo-repeat proteins localize to the desmosomal plaque and the nucleus in many epithelial tissues.²⁷ Three members (PKP 1–3, and occasionally p0071, or plakophilin 4) have been characterized and constitute an individual subfamily of armadillo-repeat proteins.²⁸ Although plakophilins are widely expressed in many epithelial tissues and likely perform redundant roles in these tissues, PKP-2 is the sole member of this family expressed in cardiomyocytes.²⁹ PKP2 encodes 2 isoforms, the longer (PKP2b) includes the alternatively spliced exon 6, while the shorter (PKP2a) does not include the sequence encoded by exon 6. Recently, Gandjbakhch et al. found PKP2a was shown to be the major isoform expressed in human heart tissue and PKP2b protein was undetectable.³⁰

PKP 2 interacts with desmoplakin, plakoglobin, Dsg 1 and 2, and Dsc 1a and 2a, with the head domain apparently being critical for the assembly and maintenance of desmosomes.³¹ In addition, PKP 2 is capable of associating with β -catenin, thereby regulating its signaling activity. Grossmann et al²⁹ studied the functional aspects of PKP 2 in a mouse gene knockout model. Homozygous ablation of *PKP2* led to embryonic lethality at midgestation (E10.5–E11) because of aberrations in the architecture and function of the heart, as seen by reduced trabeculation, rupture of cardiac walls, and blood leakage into the pericardial cavity. In contrast, other tissues, in particular epithelia, seemed to be unaffected. Hence, PKP 2 is considered to be an essential morphogenetic and architectural component of the heart. Hall et al analyzed desmosome assembly and stability in epithelial cell lines expressing mutants of plakophilin-2 found in ARVC patients, and found that mutant plakophilin-2 is unable to initiate desmosome assembly.³²

The aforementioned studies have emphasized the structural instability of the cardiomyocyte junction that precedes the fibrous changes in ARVC and that results from mutations. Recently, Oxford et al used RNA silencing technology to decrease the expression of PKP2 in cardiac cells obtained

from neonatal rat hearts, and found loss of PKP2 expression, a decrease in the total Cx43 content, and a significant redistribution of Cx43 to the intracellular space.³³ A significant reduction in Cx43 content is necessary to modify conduction velocity and may therefore directly contribute to arrhythmogenesis.³⁴ Furthermore, Sato et al demonstrated that PKP2 associates with voltage-gated sodium channel Nav1.5, and that knock-down of PKP2 expression alters the properties of the sodium current, and the velocity of action potential propagation in cultured cardiomyocytes.³⁵ These findings suggest that alterations in PKP2 and desmosome structure may be directly associated with arrhythmogenic potential.

The current study demonstrated that mutations in *PKP2* may occur in cases of SUDNA, specifically in patients without evidence of fibrofatty change. SUDNA accounts for up to 30% of sudden death in adults,^{16–18} and has been linked to ion channel mutations in a subset of cases, but so far not to desmosomal mutations. The current study demonstrated that *PKP2* mutations are equally frequent in SUDNA as in ARVC, suggesting that they are not specific for ARVC, and that there may be a general effect on arrhythmogenicity as opposed to the fibrofatty changes characteristic of ARVC. The current study also highlights the rate of exertional deaths among patients with ARVC, which has been described previously.^{6,36} Although the current study does not suggest that *PKP2* mutations predispose to exertional deaths, the sample size is too small to predict if PKP2-related deaths in the absence of ARVC are more or less likely to occur during exercise.

In the current study, the mean of the ARVC patients was significantly greater than that of the SUDNA group, and the mean age of the ARVC patients with potentially lethal mutations was older, although not significantly, than that of patients with SUDNA with mutations. There are several limitations to the current study. First, the small sample size precluded an accurate assessment of the overall rate of *PKP2* mutations in ARVC or SUDNA cases. Furthermore, there is the possibility that the SUDNA patients with *PKP2* mutations may subsequently develop histologic manifestations of ARVC, or that the SUDNA patients in the current study would have developed ARVC if they had not succumbed from their arrhythmia. This is unlikely because inflammatory and fibrous lesions are believed to be arrhythmogenic in themselves, regardless of etiology, and reactive fibro-inflammatory change secondary to desmosomal mutations has not been definitively established. Even if the SUDNA patients with *PKP2* mutations were pre-clinical ARVC, it would suggest that the PKP2 protein defect in itself is arrhythmogenic, and not the fibrofatty change that pathologically defines ARVC. The apparent disconnect between the fibrofatty foci in ARVC and the mutations in desmosomal proteins needs to be further studied, as well as the incidence of desmosomal mutations in sudden death related to other morphologic findings, such as coronary artery disease and hypertrophic cardiomyopathy for example.

In conclusion, our results demonstrate that *PKP2* mutations are similar in incidence and significance in ARVC as well as SUDNA. These findings suggest a link between the desmosome and arrhythmias in the absence of the fibrofatty change characteristic of ARVC. Hence, desmosomal mutations may not in themselves cause fibrofatty change and the syndrome of ARVC. It seems unlikely that the SUDNA cases in the current study represent an early stage of ARVC, as the precursor of fibrofatty change in ARVC has been postulated to be linked to inflammation and previous episodes of myocarditis,^{37,38} which were not evident in these cases. More studies of *PKP2* genotype are necessary in populations other than

ARVC in order to definitively establish a link between ARVC and the desmosome.

Disclosures

Conflict of interest disclosure: None.

References

1. Franke WW, Borrmann CM, Grund C, Pieperhoff S. The area composita of adhering junctions connecting heart muscle cells of vertebrates. I: Molecular definition in intercalated disks of cardiomyocytes by immunoelectron microscopy of desmosomal proteins. *Eur J Cell Biol* 2006; **85**: 69–82.
2. Godsel LM, Getsios S, Huen AC, Green KJ. The molecular composition and function of desmosomes. *Handb Exp Pharmacol* 2004; **(165)**: 137–193.
3. Miyazaki S, Shah AJ, Haissaguerre M. Early repolarization syndrome: A new electrical disorder associated with sudden cardiac death. *Circ J* 2010; **74**: 2039–2044.
4. Song MK, Baek JS, Kwon BS, Kim GB, Bae EJ, Noh CI, et al. Clinical spectrum and prognostic factors of pediatric ventricular tachycardia. *Circ J* 2010; **74**: 1951–1958.
5. Unuma K, Shintani-Ishida K, Tsushima K, Kim GB, Bae EJ, Noh CI, et al. Connexin-43 redistribution and gap junction activation during forced restraint protects against sudden arrhythmic death in rats. *Circ J* 2010; **74**: 1087–1095.
6. Thiene G, Nava A, Corrado D, Rossi L, Pennelli N. Right ventricular cardiomyopathy and sudden death in young people. *N Engl J Med* 1988; **318**: 129–133.
7. Dalal D, Molin LH, Piccini J, Tichnell C, James C, Bomma C, et al. Clinical features of arrhythmogenic right ventricular dysplasia/cardiomyopathy associated with mutations in plakophilin-2. *Circulation* 2006; **113**: 1641–1649.
8. Gerull B, Heuser A, Wichter T, Paul M, Basson CT, McDermott DA, et al. Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. *Nat Genet* 2004; **36**: 1162–1164.
9. Heuser A, Plovie ER, Ellinor PT, Grossman KS, Shin JT, Wichter T, et al. Mutant desmocollin-2 causes arrhythmogenic right ventricular cardiomyopathy. *Am J Hum Genet* 2006; **79**: 1081–1088.
10. Pilichou K, Nava A, Basso C, Beffagna G, Baucé B, Lorenzon A, et al. Mutations in desmoglein-2 gene are associated with arrhythmogenic right ventricular cardiomyopathy. *Circulation* 2006; **113**: 1171–1179.
11. Syrris P, Ward D, Asimaki A, Evans A, Sen-Chowdhry S, Hughes SE, et al. Desmoglein-2 mutations in arrhythmogenic right ventricular cardiomyopathy: A genotype-phenotype characterization of familial disease. *Eur Heart J* 2007; **28**: 581–588.
12. Syrris P, Ward D, Asimaki A, Sen-Chowdhry S, Ebrahim HY, Evans A, et al. Clinical expression of plakophilin-2 mutations in familial arrhythmogenic right ventricular cardiomyopathy. *Circulation* 2006; **113**: 356–364.
13. Syrris P, Ward D, Evans A, Asimaki A, Gandjbakhch E, Sen-Chowdhry S, et al. Arrhythmogenic right ventricular dysplasia/cardiomyopathy associated with mutations in the desmosomal gene desmocollin-2. *Am J Hum Genet* 2006; **79**: 978–984.
14. van Tintelen JP, Entius MM, Bhuiyan ZA, Jongbloed R, Wiesfeld AC, Wilde AA, et al. Plakophilin-2 mutations are the major determinant of familial arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circulation* 2006; **113**: 1650–1658.
15. Yang Z, Bowles NE, Scherer SE, Taylor MD, Kearney DL, Ge S, et al. Desmosomal dysfunction due to mutations in desmoplakin causes arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circ Res* 2006; **99**: 646–655.
16. Fabre A, Sheppard MN. Sudden adult death syndrome and other non-ischaemic causes of sudden cardiac death. *Heart* 2006; **92**: 316–320.
17. Ackerman MJ. State of postmortem genetic testing known as the cardiac channel molecular autopsy in the forensic evaluation of unexplained sudden cardiac death in the young. *Pacing Clin Electrophysiol* 2009; **32**(Suppl 2): S86–S89.
18. Tester DJ, Ackerman MJ. The role of molecular autopsy in unexplained sudden cardiac death. *Curr Opin Cardiol* 2006; **21**: 166–172.
19. Lasota J, Wasag B, Steigen SE, Limon J, Miettinen M. Improved detection of KIT exon 11 duplications in formalin-fixed, paraffin-embedded gastrointestinal stromal tumors. *J Mol Diagn* 2007; **9**: 89–94.
20. Chan PK, Chan DP, To KF, Yu MY, Cheung JL, Cheng AF. Evaluation of extraction methods from paraffin wax embedded tissues for PCR amplification of human and viral DNA. *J Clin Pathol* 2001; **54**: 401–403.
21. Wickham CL, Boyce M, Joyner MV, Sarsfield P, Wilkins BS, Jones DB, et al. Amplification of PCR products in excess of 600 base pairs using DNA extracted from decalcified, paraffin wax embedded bone marrow trephine biopsies. *Mol Pathol* 2000; **53**: 19–23.
22. Fressart V, Duthoit G, Donal E, Probst V, Deharo JC, Chevalier P, et al. Desmosomal gene analysis in arrhythmogenic right ventricular dysplasia/cardiomyopathy: Spectrum of mutations and clinical impact in practice. *Europace* 2010; **12**: 861–868.
23. Klauke B, Kossmann S, Gaertner A, Brand K, Stork I, Brodehl A, et al. De novo desmin-mutation N116S is associated with arrhythmogenic right ventricular cardiomyopathy. *Hum Mol Genet* 2010; **19**: 4595–4607.
24. Lahtinen AM, Lehtonen A, Kaartinen M, Toivonen L, Swan H, Widén E, et al. Plakophilin-2 missense mutations in arrhythmogenic right ventricular cardiomyopathy. *Int J Cardiol* 2008; **126**: 92–100.
25. den Haan AD, Tan BY, Zikusoka MN, Zikusoka MN, Lladó LI, Jain R, et al. Comprehensive desmosome mutation analysis in north americans with arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circ Cardiovasc Genet* 2009; **2**: 428–435.
26. Green KJ, Simpson CL. Desmosomes: New perspectives on a classic. *J Invest Dermatol* 2007; **127**: 2499–2515.
27. Hatzfeld M. Plakophilins: Multifunctional proteins or just regulators of desmosomal adhesion? *Biochim Biophys Acta* 2007; **1773**: 69–77.
28. Izawa I, Nishizawa M, Tomono Y, Ohtakara K, Takahashi T, Inagaki M. ERBIN associates with p0071, an armadillo protein, at cell-cell junctions of epithelial cells. *Genes Cells* 2002; **7**: 475–485.
29. Grossmann KS, Grund C, Huelsken J, Behrend M, Erdmann B, Franke WW, et al. Requirement of plakophilin 2 for heart morphogenesis and cardiac junction formation. *J Cell Biol* 2004; **167**: 149–160.
30. Gandjbakhch E, Charron P, Fressart V, Lorin de la Grandmaison G, Simon F, Gary F, et al. Plakophilin 2A is the dominant isoform in human heart tissue: Consequences for the genetic screening of arrhythmogenic right ventricular cardiomyopathy. *Heart* 2011; **97**: 844–849.
31. Bonne S, van Hengel J, Nollé F, Kools P, van Roy F. Plakophilin-3, a novel armadillo-like protein present in nuclei and desmosomes of epithelial cells. *J Cell Sci* 1999; **112**: 2265–2276.
32. Hall C, Li S, Li H, Creason V, Wahl JK 3rd. Arrhythmogenic right ventricular cardiomyopathy plakophilin-2 mutations disrupt desmosome assembly and stability. *Cell Commun Adhes* 2009; **16**: 15–27.
33. Oxford EM, Musa H, Maass K, Coombs W, Taffet SM, Delmar M. Connexin43 remodeling caused by inhibition of plakophilin-2 expression in cardiac cells. *Circ Res* 2007; **101**: 703–711.
34. Danik SB, Liu F, Zhang J, Suk HJ, Morley GE, Fishman GI, et al. Modulation of cardiac gap junction expression and arrhythmic susceptibility. *Circ Res* 2004; **95**: 1035–1041.
35. Sato PY, Musa H, Coombs W, Guerrero-Sema G, Patino GA, Taffet SM, et al. Loss of plakophilin-2 expression leads to decreased sodium current and slower conduction velocity in cultured cardiac myocytes. *Circ Res* 2009; **105**: 523–526.
36. Burke AP, Farb A, Tashko G, Virmani R. Arrhythmogenic right ventricular cardiomyopathy and fatty replacement of the right ventricular myocardium: Are they different diseases? *Circulation* 1998; **97**: 1571–1580.
37. Basso C, Thiene G, Corrado D, Angelini A, Nava A, Valente M. Arrhythmogenic right ventricular cardiomyopathy: Dysplasia, dystrophy, or myocarditis? *Circulation* 1996; **94**: 983–991.
38. Suzuki H, Sumiyoshi M, Kawai S, Takagi A, Wada A, Nakazato Y, et al. Arrhythmogenic right ventricular cardiomyopathy with an initial manifestation of severe left ventricular impairment and normal contraction of the right ventricle. *Jpn Circ J* 2000; **64**: 209–213.