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Wide Spectrum of Desmosomal Mutations in Danish Arrhythmogenic Right Ventricular Cardiomyopathy Patients

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Abstract

Background: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a lethal condition characterized by ventricular tachyarrhythmias, right- and/or left ventricular involvement and fibrofatty infiltrations in the myocardium. The disease has been associated with mutations in genes encoding desmosomal proteins.

Objective: To thoroughly evaluating an ARVC cohort for desmosomal mutations and large genomic rearrangements and characterized the phenotype associated with double mutation carrier status.

Methods: Sixty-five unrelated patients (55 fulfilling current criteria and 10 borderline cases) were screened for mutations in all known desmosome genes (*DSC2*, *DSG2*, *DSP*, *JUP*, *PKP2*) and *TGFb3*. Presence of genomic rearrangements was assessed by multiplex ligation-dependent probe amplification.

Results: The screening identified 19 different mutations: two mutations in *DSG2*, four in *DSC2*, two in *DSP* (one heterozygous; one homozygous), four in *JUP* (one patient compound heterozygous) and seven in *PKP2*. No genomic rearrangements or mutations in *TGFb3* were identified. Ten of the mutations were novel. Seven families carried more than one mutation. Clinical evaluation of these families showed a variable phenotype associated with the double mutation carrier status. The homozygous desmoplakin mutation (*DSP* p.G2056R+p.G2056R) carrier came from a consanguineous Danish family and had left ventricular involvement, palmar keratoderma and curly hair consistent with a Carvajal-like syndrome.

Conclusions: Thirty-three percent of patients in this Danish ARVC cohort carried desmosomal mutations with a surprisingly wide mutation spectrum. A substantial proportion of patients carried more than one mutation. Our study supports comprehensive desmosomal mutation screening beyond the first encountered mutation, while routine screening for genomic rearrangements does not seem indicated.

Introduction

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC; MIM# 107970) is an inherited cardiomyopathy characterized by ventricular arrhythmias, sudden cardiac death, right and/or left ventricular involvement, and fibrofatty infiltrations in the myocardium. A clinical diagnosis is made according to published Task Force criteria.^{1 2} The original criteria¹ were published in 1994, followed by modified, more sensitive, criteria for first degree relatives in 2002.³ Recently, revised Task Force criteria were published² and these include a number of changes: quantification of histology and right ventricular size, novel electrocardiographic features, genetic testing, and several other parameters are now part of the diagnostic criteria.

Seven genes have so far been associated with the disease. Five of these genes encode desmosomal proteins: desmocollin-2 (*DSC2*; MIM# 610476)^{4 5}, desmoglein-2 (*DSG2*; MIM# 610193)⁶⁻⁹, desmoplakin (*DSP*; MIM# 607450)^{10 11}, plakoglobin (*JUP*; MIM# 611528)^{12 13}, and plakophilin-2 (*PKP2*; MIM# 609040)¹⁴⁻¹⁶. The non-desmosomal gene, transforming growth factor beta-3 (*TGFb3*; MIM# 107970)¹⁷, has also been suggested to be involved in the pathogenesis of ARVC. Recently, a gene of unknown function, *TMEM43* (MIM# 604400), was found causative in ARVC5.¹⁸ The most common form of inheritance is autosomal dominant with variable penetrance and phenotypic expression. In general, the dominantly inherited forms have an isolated cardiac phenotype while the recessive forms also have variable degrees of hair and skin abnormalities. Homozygous mutations in *DSP*¹⁹⁻²¹ and *JUP*¹² have been reported in two cardiocutaneous syndromes named Carvajal Syndrome (MIM# 605676) and Naxos disease (MIM# 601214), respectively.

This study is an extension of our previous work investigating the prevalence of *PKP2* mutations in Denmark.¹⁶ First, we aimed systematically at screening all known desmosome genes without excluding known mutation carriers from further analysis. Secondly, we aimed at clinically evaluating family members in families carrying more than one mutation. And finally, we wished to evaluate the contribution of genomic rearrangements to the pathogenesis of ARVC.

Methods

Study population

The study population comprised a total of 65 unrelated, white individuals. Fifty-five (32 men) patients fulfilled ARVC 1994 Task Force criteria and ten patients (six men) had some ARVC characteristics but did not fulfil the criteria. All patients were identified at a tertiary referral center, Rigshospitalet, Copenhagen University Hospital. The patients were diagnosed in the period 1993-2008. The clinical characteristics of all index patients are summarized in table 1. Initial clinical evaluation of family members routinely consisted of physical examination, 12-lead ECG, signal-averaged ECG, transthoracic echocardiography, 48-hour Holter monitoring, and MRI.

The study was approved by the local ethics committee (KF01-152/04), followed the Helsinki Declaration II, and all participants gave written informed consent prior to inclusion.

Genetic analyses

For all living patients genomic DNA was extracted from whole blood using standard techniques. For deceased patients DNA was extracted from paraffin-embedded tissue (individual III:1, Family G and the proband, Family J) or Guthrie card (individual III:4, Family A). Polymerase chain reaction (PCR) amplification of all exons and flanking introns of the *DSC2*, *DSG2*, *DSP*, *JUP*, *PKP2*, and *TGFb3* genes was performed using standard protocols and PCR fragments were bidirectionally sequenced on an ABI Prism 3730 Analyzer (Applied Biosystems, USA). PCR primers are listed in supplementary table 1. Some fragments were pre-screened using Denaturing High Performance Liquid Chromatography (dHPLC) (Transgenomic, USA) or a LightScanner System (Idaho Technology, USA). Samples pre-screened with dHPLC were mixed with wild type PCR product to enable detection of homozygous variants. DNA extracted from tissue or Guthrie card was amplified and sequenced without any pre-screening. The following reference sequences

were used: *DSC2* (NM_024422/NM_004949), *DSG2* (NM_001943), *DSP* (NM_004415), *JUP* (NM_002230), *PKP2* (NM_004572) and *TGFb3* (NM_003229).

Presence of large genomic insertions and deletions was evaluated with a Multiplex Ligation-dependent Probe Amplification (MLPA) Salsa P168 ARVC kit (MRC-Holland, the Netherlands). The kit has probes for *DSP*, *JUP*, *PKP2*, *RYR2* and *TGFb3*. The analyses were performed in accordance with the supplier's protocol, which consisted of four steps: 1) denaturation of DNA and annealing of MLPA probes, 2) ligation, 3) multiplex PCR reaction with universal primers and 4) fragment analysis using capillary electrophoresis.

For all identified mutations at least 650 self-reported healthy, ethnically matched control individuals were screened using Taqman assays (Applied Biosystems, USA) or a LightScanner System.

A mutation was defined as a sequence variant absent in at least 1300 control chromosomes, and affecting one or more conserved nucleotides/residues, or introducing a change in amino acid polarity in a functionally important protein domain. All other sequence changes were referred to as variants. For clarity, only patients who carried at least one mutation were included in diagnostic yield percentages.

Statistical analysis

For comparisons of frequencies of relevant mutations in the patient and control population Fisher's exact two-sided probability tests were used. A p-value <0.05 was considered significant.

Results

Genetic findings

Eighteen different heterozygous and one homozygous mutation were identified. The mutations occurred in 18 patients fulfilling Task Force criteria. No mutations were found in the borderline group. The mutations identified (table 2) consisted of two in *DSG2* (p.T335A, p.E1020AfsX18), four in *DSC2* (p.E2K, p.G150A, p.S824L, c.2508+5G>A), two in *DSP* (c.939+1G>A, p.G2056R+p.G2056R), four in *JUP* (p.S225L, p.V407I, p.T427M, p.V603L) and seven in *PKP2* (previously reported¹⁶). No genomic rearrangements or mutations in *TGFB3* were identified. Ten of the mutations were novel. All were absent in a minimum of 650 controls. Six rare variants (*DSC2* p.A897KfsX4, *DSG2* p.V56M, *DSG2* p.V920G, *DSP* p.C81Y, *PKP2* p.D26N, *PKP2* p.S140F) occurred in both the patient and control populations. As these variants may contribute to the pathogenesis of ARVC they are mentioned where appropriate. Sequence alignments, *in silico* analysis, and complete listing of all identified non-synonymous variants are provided in supplementary table 2 and 3. Six families (Families A-F) were digenic mutation carriers, one family compound heterozygous for two plakoglobin mutations (Family G), one family had a homozygous desmoplakin mutation (Family H), and nine families (Families I-L and five families only carrying *PKP2* mutations not presented) had a single mutation.

Desmocollin-2

Four mutations were identified in *DSC2*. The *DSC2* p.E2K mutation affects the second residue in the signal peptide which is cleaved off before insertion into the plasma membrane. The patient carrying the *DSC2* p.E2K mutation also carried the *PKP2* p.S140F variant (Family D). Two other identified mutations, *DSC2* p.G150A and *DSC2* p.S824L, both affect highly conserved residues in functionally important parts of the protein. The *DSC2* p.G150A mutation co-occurred with the *JUP* p.S225L (Family A) mutation and the *DSC2* p.S824L mutation carrier (Family E) also had the

PKP2 p.D26N variant. The *DSC2* c.2508+5G>A splice site mutation (Family I) affects a highly conserved nucleotide in the splice donor site of intron 15. One proband and one borderline patient carried the reported *DSC2* p.A897KfsX4 (c.2687_2688insGA) variant. However, this variant was also present in the control population (two out of 65 patients versus 22 out of 650 controls, p=1.0).

Desmoglein-2

Two mutations were identified in *DSG2*. One missense mutation (*DSG2* p.T335A, Family J) affecting a highly conserved residue and one out-of-frame deletion (*DSG2* p.E1020AfsX18, Family K). One proband and one borderline patient carried the reported *DSG2* p.V56M (c.166G>A) and *DSG2* p.V920G (c.2759T>G) variants, respectively. These variants were also present in the control population (1/65 vs. 4/650, p=0.38; 1/65 vs. 3/650, p=0.32).

Desmoplakin

Two mutations were identified in *DSP*. The *DSP* c.939+1G>A mutation (Family L) disrupts the conserved splice donor site of intron 7 and the homozygous *DSP* p.G2056R mutation (Family H) affects the highly conserved domain A of the C-terminal part of the protein. One patient (Family C) carried the *DSP* p.C81Y (c.242G>A) variant. This patient also carried the *PKP2* c.2146-2A>T splice site mutation. The *DSP* p.C81Y variant was present in two out of the 1400 controls screened for this variant (1/65 vs. 2/1400, p=0.13)

Plakoglobin

Five mutations in *JUP* were identified in four patients. All mutations affect highly conserved residues in the armadillo repeat domains. The *JUP* p.S225L mutation occurred in two unrelated patients, of which one came from a family (Family A) with two other mutations resulting in two different digenic carrier statuses within the same family (*JUP* p.S225L+*PKP2* p.S329EfsX24 and *JUP* p.S225L+*DSC2* p.G150A, respectively). The other *JUP* p.S225L mutation occurred in a family (Family B) also carrying the *PKP2* p.R79X mutation. The mother of a sudden cardiac death

victim carried two plakoglobin mutations (*JUP* p.T427M and *JUP* p.V603L, Family G). The *JUP* p.V407I mutation carrier (Family F) also had the *PKP2* p.D26N variant.

Transforming growth factor beta-3

No mutations were found in the coding sequence, flanking introns, 5' untranslated region or 500 base pairs upstream of the *TGFb3* gene. Two novel polymorphisms (c.*739C>T and c.*831G>A) were found in the 3' untranslated region.

Large genomic rearrangements

All patients had normal MLPA profiles excluding large genomic insertions and deletions in the *DSP*, *JUP*, *PKP2*, *RYR2* and *TGFb3* genes.

Clinical findings

The clinical features of the mutation carriers are summarized in table 3.

In family A (figure 1A), the proband was diagnosed with ARVC at age 18 years prior to her sudden death. The patient's first-degree relatives were evaluated without abnormal findings. Seven years later her 16 year old sister unfortunately died and autopsy showed fibrofatty infiltrations in the myocardium. All family members were re-evaluated and the mother and father, an uncle and two aunts fulfilled criteria for ARVC. Genetic screening revealed a truncating *PKP2* mutation (*PKP2* p.S329EfsX24) on the maternal side and two different missense mutations on the paternal side (*JUP* p.S225L and *DSC2* p.G150A). All mutations individually segregated with disease. Individual III:4 carried both the fathers and mothers' mutations. Individual II:8, who had a different digenic carrier status (*DSC2* p.G150A and *JUP* p.S225L), had significantly left ventricular involvement (ejection fraction of 30%). Her son (individual III:6) carried the same two mutations, but had a positive signal-averaged ECG as the only abnormal diagnostic finding at the age of 33 years. An ECG, microscopy photo and an electropherogram from the family are shown in figure 2A-C.

In Family B (figure 1B), the proband had symptom onset at the age of 24 years and was diagnosed with ARVC. His father and brother also had features of ARVC. All family members had normal left ventricular function and there was no history of sudden cardiac death. At the time of diagnosis the proband had documented ventricular tachyarrhythmias, but for the past 11 years of follow-up he has not received ICD-therapy. His father (individual I:1), currently aged 75 years, has not had any ARVC-related symptoms for 20 years. Genetic screening identified two mutations (*PKP2* p.R79X and *JUP* p.S225L). The proband and his father were digenic carriers and the brother only carried the *PKP2* p.R79X mutation.

In Family C (figure 1C) the proband had syncope and ventricular tachycardia and was diagnosed with ARVC. Family evaluation showed that his younger brother (individual II:3) fulfilled Task Force Criteria. His older brother (individual II:4) also had some ARVC characteristics. Both the proband's daughters (individual III:1 and III:2) were phenotypically normal. Genetic screening identified one splice site mutation (*PKP2* c.2146-2A>T) and one rare variant (*DSP* p.C81Y) in the proband, but only the *PKP2* mutation in the clinically affected younger brother. One of the daughters carried the *DSP* p.C81Y variant.

In Families D, E and F (figures 1D-F) all evaluated family members were phenotypically normal and carried none of the proband's mutations.

In Family G (figure 1G) the proband died suddenly aged 31 years and autopsy revealed fibrofatty infiltrations in the myocardium. Clinical evaluation of the mildly symptomatic mother showed premature ventricular contractions, but otherwise normal clinical findings. Her brother and son (individuals II:3 and III:2) were asymptomatic but had some diagnostic features of ARVC. Genetic screening showed that the proband was *JUP* p.T427M mutation carrier, the mother was compound plakoglobin mutation carrier (*JUP* p.T427M and *JUP* p.V603L) and the mother's brother and her sons were *JUP* p.V603L mutation carriers. The two mutations segregated with disease individually.

In Family H (figure 3A-D) the proband had symptom onset at the age of 35 years and was diagnosed with ARVC with left ventricular involvement. In addition, palmar keratoderma was noted (figure 3C). No significant plantar skin manifestations or woolly hair were seen. His mother died driving a moped aged 35 years and his father died of cancer aged 78 years. The mother and father were cousins. None of the proband's three children had any cardiac symptoms. The proband carried a homozygous *DSP* p.G2056R mutation and his parents and children were obligate heterozygous mutation carriers. His brother and sister declined clinical and genetic evaluation.

Discussion

On the basis of our present results combined with our previous *PKP2* screening we conclude that 33% of patients in this Danish ARVC cohort had mutations in desmosomal genes. Our cohort had a surprisingly wide mutation spectrum with a high frequency of *JUP* and *DSC2* mutations. Previous studies have shown 10-43% *PKP2* mutations^{14-16 22}, 0-12% *DSG2* mutations^{6-9 23 24}, 1-16 % *DSP* mutations^{6 11 24} and 0-5 % *DSC2* mutations^{4 8 23 24}. Only three dominant mutations in *JUP* have previously been described.^{13 24 25} A single report has associated mutations in *TGFb3* with ARVC1¹⁷, but mutations have not been described in other populations. However, most previous studies have excluded patients after the first identified mutation.

In six families (11%) digenic mutation carriers were identified where at least one of the mutations were absent in the control population. In Families A, B and G the individual mutations segregated with the disease and were individually regarded as pathogenic. In family A the digenic mutation carriers had a severe phenotype with sudden cardiac death and left ventricular involvement. In contrast, the digenic carriers in family B and G had a mild phenotype with minimal arrhythmia, no left ventricular involvement or sudden cardiac death. Two recent papers suggested a gene dosage effect with patients carrying more than one mutation having a more severe phenotype.^{8 26} Our data partially support these findings although the phenotypic variation is large. In Family C the disease segregated with one of the mutations (*PKP2* c.2146-2A>T) suggesting that the rare variant (*DSP* p.C81Y) is not necessary for the development of the ARVC phenotype. Whether the identified rare variants also present in our control population are benign or have a disease-modifying effect is unknown. In other inherited cardiac arrhythmia syndromes polymorphisms can modify the phenotype.²⁷

One patient from a consanguineous Danish family (Family H) was a homozygous *DSP* mutation carrier. Homozygous mutations have been reported for all five desmosomal genes. Except for the homozygous *DSG2* and *PKP2* mutation carriers all have had variable degrees of hair and

skin abnormalities. A single report has shown a homozygous *DSP* missense mutation (p.G2375R+p.G2375R), located in the B domain of the C-terminal part of the protein, as cause of Carvajal Syndrome.¹⁹ The reported case had significant hair- and skin involvement with woolly hair and a pemphigous-like skin disorder. Interestingly, our patient with a similar glycine to arginine substitution (p.G2056R+p.G2056R) in a homolog region located in the A domain, only showed mild cutaneous abnormalities and no woolly hair. The C-terminal part of desmoplakin consists of three plakin repeat domains each composed of four and a half copy of a unique 38 amino acid repeat motif. Functional evidence suggests that all three domains have similar binding properties to the intermediate filaments.²⁸ The variable phenotype between the two homozygous missense mutation carriers is therefore not likely to be caused by a weaker binding of the intermediate filaments to the A domain. Our patient had significant left ventricular involvement consistent with two out of three reports on homozygous *DSP* mutation carriers.¹⁹⁻²¹ In addition to homozygous mutations, compound heterozygous and dominant *DSP* mutations with variable degrees of cardiac and cutaneous involvement have been reported.²⁹

The present study furthermore aimed at investigating large genomic rearrangements. Only a single report from the Netherlands has implicated large genomic deletions in the pathogenesis of ARVC with a diagnostic yield below one percent.³⁰ However, only 26 patients out of the 120 patients screened in this study fulfilled Task Force criteria.³⁰ Our results are in keeping with the Dutch study, as no rearrangements were identified in our cohort. Although a substantial part of the reported desmosomal mutations are truncating with haploinsufficiency as the proposed molecular mechanism, large genomic deletions does not seem to be a common cause of ARVC. In contrast to this, MLPA analysis of a selected group of long QT syndrome patients showed that 11.5% of patients had disease-causing large genomic rearrangements.³¹

None of the 10 borderline patients had potentially disease-causing mutations as only two variants of unknown significance (*DSC2* p.A897KfsX4 and *DSG2* p.V920G) were found. This

finding may be coincidental and reflect the small sample size. The average period of follow-up was long in these patients (data not shown) suggesting that time-dependent reduced expressivity is not the case, and that these patients may represent phenocopies.

The following limitations apply to this study: cohort size, referral bias to tertiary center, a limited number of family members available for evaluation, issues related to mutation analysis on DNA extracted from paraffin-embedded tissue, and the lack of functional tests available for verification of the disease causing effect of identified mutations. Furthermore, the MLPA kit had a limited number of probes and the sensitivity of the kit may not be 100%. Due to few patients carrying more than one mutation and unclear pathogenicity of a number of the identified mutations no meaningful statistical genotype-phenotype comparisons between single and double mutation carriers were possible. Lastly, due to the anonymous nature of our control population no systematic evaluation for ARVC was possible and it cannot be definitively ruled out that some controls had silent disease.

In conclusion, 33% of the patients in this Danish ARVC cohort had desmosomal mutations with a wide mutation spectrum and a substantial proportion of patients carried more than one mutation. The phenotype associated with the double carrier status was variable. Our data clearly support comprehensive mutation screening beyond the first encountered mutation, while routine screening for large genomic rearrangements does not seem indicated.

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Competing interests

None.

Patient consent

Obtained.

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Figure legends

Figure 1. Pedigrees of families with digenic or compound inheritance. Women are illustrated by circles and men by squares. Filling in the circles/squares indicates fulfilment of ARVC criteria (black), modified criteria (grey), unaffected (white), or unknown (question mark). Probands are marked with an arrowhead. Deceased family members are illustrated with diagonal lines. Below the gender symbol the molecular genetic finding is listed: Mutation (gene name and position), wild type (wt) or untested (question mark). An asterix indicates obligate carrier status. Bottom numbers are the generation and individual numbers.

Figure 2. A) ECG from individual II:8 (Family A), who had significant left ventricular involvement, showing left axis deviation and negative T-waves in V2-V6, I-III and AVF. B) Myocardial tissue from the autopsy of individual III:4 (Family A) showing fibrofatty replacements. C) Electropherogram from individual II:6 (Family A).

Figure 3. A) Pedigree of the family (Family H) carrying the homozygous *DSP* p.G2056R mutation. B) Picture of the face and hair of the proband (individual IV:1) showing curly, but not woolly, hair. C) Picture of the hand of the proband showing palmar keratoderma. D) Electropherogram from the proband. Written informed consent was given for the publication of these images.

Table 1. Clinical characteristics of the cohort according to the 1994 Task Force criteria.

		ARVC group	Borderline group
Age at symptom onset, years (range)		35 (2–62)	36 (12-52)
Gender, men		32/55 (58%)	6/10 (60%)
Reduced left ventricular function		10/55 (18%)	2/10 (20%)
Family history	Major	4/55 (7%)	0/10 (0%)
	Minor	8/55 (15%)	1/10 (10%)
Abnormal depolarization	Major	18/55 (33%)	1/10 (10%)
	Minor	17/55 (31%)	4/10 (40%)
Abnormal repolarization	Minor	25/55 (46%)	1/10 (10%)
Arrhythmia	Minor	49/55 (89%)	10/10 (100%)
Structural or functional abnormality of the right ventricle	Major	11/55 (20%)	1/10 (10%)
	Minor	29/55 (53%)	3/10 (30%)
Tissue characteristics	Major	31/55 (56%)	3/10 (30%)

Table 2. Overview of the mutations.

Family	Gene	Nucleotide change	Amino acid or RNA change	Change in polarity	Evolutionary conservation	Novel	Protein domain
A, III:4	<i>PKP2</i>	c.983_984insGG	p.S329EfsX24*			-	N-terminal
	<i>JUP</i>	c.674C>T	p.S225L	+	+	+	Arm3
A, III:6	<i>DSC2</i>	c.449G>C	p.G150A	-	+	+	EC1
	<i>JUP</i>	c.674C>T	p.S225L	+	+	+	Arm3
B	<i>JUP</i>	c.674C>T	p.S225L	+	+	+	Arm3
	<i>PKP2</i>	c.235C>T	p.R79X			-	N-terminal
C	<i>PKP2</i>	c.2146-2A>T	r.spl			-	Arm8
	<i>DSP</i>	c.242G>A	p.C81Y	-	+	+	N-terminal
D	<i>DSC2</i>	c.4G>A	p.E2K	-	-	+	Signal peptide
	<i>PKP2</i>	c.419C>T	p.S140F	+	-	-	N-terminal
E	<i>DSC2</i>	c.2471C>T	p.S824L	+	+	+	Cytoplasmic
	<i>PKP2</i>	c.76G>A	p.D26N	-	+	-	N-terminal
F	<i>JUP</i>	c.1219G>A	p.V407I	-	+	+	Arm7
	<i>PKP2</i>	c.76G>A	p.D26N	-	+	-	N-terminal
G	<i>JUP</i>	c.1280C>T	p.T427M	+	+	+	Arm8
	<i>JUP</i>	c.1807G>T	p.V603L	-	+	+	Arm11
H	<i>DSP</i>	c.6166G>C+c.6166G>C	p.G2056R+p.G2056R	+	+	+	DACT
I	<i>DSC2</i>	c.2508+5G>A	r.spl?			+	Cytoplasmic
J	<i>DSG2</i>	c.1003A>G	p.T335A	+	+	-	EC3
K	<i>DSG2</i>	c.3059_3062delAGAG	p.E1020AfsX18			+	Cytoplasmic
L	<i>DSP</i>	c.939+1G>A	r.spl			-	N-terminal

Arm: Armadillo repeat; DACT: Domain A, C-terminal; EC: Extracellular repeat. Variants present in control population are in shaded grey. *Reported as *PKP2* E329fsX352 due to alternative nomenclature.

Table 3. Clinical characteristics of mutation carriers.

Individual	Age at symptom onset	Symptoms	Family history	Depolarization abnormalities	Repolarisation abnormalities	Arrhythmias	Tissue characteristics	Structural/functional alterations	LVEF	Criteria (major/minor)
Family A										
III:3	18	Palpitations, SCD	-	-	+	+	++	++	normal	2/2
II:2	-	None	+	-	-	-	NA	-	normal	0/1
II:5	50	Chest pain	++	-	-	- (400 PVC/24h)	++	+	normal	2/1
III:4	16	SCD	++	NA	NA	+	++	NA	NA	2/1
III:5	-	None	++	-	-	-	-	+	normal	1/1
II:6	-	None	++	+	-	-	-	++	50%	2/1
II:7	45	Syncope	+	+	+	-	NA	+	normal	0/4
II:8	55	Chest pain	+	-	+	+	NA	+	30%	0/4
II:10	30	Palpitations	+	++	-	+	++	-	normal	2/2
III:6	-	None	+	+	-	-	NA	-	normal	0/2
Family B										
II:1	24	Palpitations	-	++	+	+	NA	-	normal	1/2
I:1	40	Palpitations	+	++	-	+	NA	+	normal	1/3
II:3	-	None	+	++	-	-	NA	-	normal	1/1
Family C										
II:1	36	Palpitations, syncope	-	-	+	+	NA	++	normal	1/2
II:3	40	Syncope	+	+	-	+	NA	+	normal	0/4
III:2	-	None	+	-	-	-	NA	-	normal	0/1
Family D										
II:1	15	Palpitations	-	-	+	+	++	+	50%	1/3
Family E										
II:1	60	Dizziness	-	+	+	+	++	-	normal	1/3
Family F										
II:2	55	Dizziness	-	-	-	+	++	++	normal	2/1
Family G										
III:1	31	SCD	-	-	-	+	++	-	-	1/1
II:2	30	Palpitations	++	-	-	+	NA	-	normal	1/1
I:1	-	None	-	-	-	-	NA	-	normal	0/0
II:3	-	None	-	-	-	-	NA	+	normal	0/1
II:6	-	None	-	+	-	-	NA	-	normal	0/1
III:2	-	None	++	+	-	-	NA	-	normal	1/1
III:3	-	None	++	-	-	-	NA	-	normal	1/0
Family H										
IV:1	35	Palpitations, dyspnea	-	++	+	+	++	++	35%	3/2
V:1	-	None	+	-	-	-	NA	NA	NA	0/1
V:2	-	None	+	-	-	-	NA	NA	NA	0/1
V:3	-	None	+	-	-	-	NA	NA	NA	0/1
Family I										
Proband	41	Palpitations	-	+	-	+	NA	++	normal	1/2
Family J										
Proband	49	SCD	+	NA	NA	+	++	NA	NA	1/2
Family K										
Proband	54	Palpitations, syncope	-	++	+	+	-	++	normal	2/2
Family L										
Proband	22	Palpitations, syncope	-	++	+	+	-	+	50%	1/3

+: minor criterion; ++: major criterion, LVEF: left ventricular ejection fraction; NA: not available; PVC: premature ventricular contractions; SCD: sudden cardiac death.

A 10 mm/mV 25 mm/s filter:35 Hz

C

JUP normal

JUP 674C>T

A

B

C

D

Normal

Homozygous
DSP 6166G>C