



Arrhythmogenic right ventricular cardiomyopathy caused by deletions in plakophilin-2 and plakoglobin (Naxos disease) in families from Greece and Cyprus: genotype–phenotype relations, diagnostic features and prognosis

Loizos Antoniades¹, Adalena Tsatsopoulou², Aris Anastasakis³, Petros Syrris⁴, Angeliki Asimaki⁴, Demosthenes Panagiotakos³, Costas Zambartas¹, Christodoulos Stefanadis³, William J. McKenna⁴, and Nikos Protonotarios^{2*}

¹Department of Cardiology, Nicosia General Hospital, Nicosia, Cyprus; ²Department of Cardiology, Yannis Protonotarios Medical Centre, Naxos, Cyclades, Greece; ³1st Department of Cardiology, University of Athens, Athens, Greece; and ⁴Department of Medicine, University College London and University College London Hospital Trust, London, UK

Received 1 May 2006; revised 6 July 2006; accepted 20 July 2006; online publish-ahead-of-print 7 August 2006

KEYWORDS

Cardiomyopathy;
Cell adhesion molecules;
Death;
Sudden;
Electrocardiography;
Echocardiography

Aims To evaluate clinical disease expression, non-invasive diagnosis, and prognosis in families with dominant vs. recessive arrhythmogenic right ventricular cardiomyopathy (ARVC) due to mutations in related desmosomal proteins plakophilin-2 (PKP2) and plakoglobin (JUP), respectively.

Methods and results One hundred and eighty-seven individuals belonging to ARVC families, four with dominant PKP2 mutations and 12 with recessive JUP mutation underwent serial non-invasive cardiac assessment. Survival and arrhythmic events were evaluated prospectively up to 21 years (median 8.5 years). Sixteen of 22 PKP2 carriers and all 26 homozygous JUP carriers fulfilled the diagnostic criteria for ARVC, the youngest by the age of 13 years. Clinical disease expression did not differ significantly between PKP2 and JUP carriers. T-wave inversion in leads V1–V3, right ventricular wall motion abnormalities, and frequent ventricular extrasystoles were the most sensitive/specific markers for identification of mutation carriers. QRS dispersion ≥ 40 ms was an independent predictor of syncope but not of sudden death.

Conclusion Mutations in PKP2 and JUP express similar cardiac phenotype. Non-invasive family screening may largely be based on T-wave inversion, right ventricular wall motion abnormalities, and frequent ventricular extrasystoles to identify mutation carriers.

Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetically determined heart muscle disorder presenting clinically with potentially lethal ventricular arrhythmias underlied by progressive loss of cardiac myocytes and fibrofatty replacement.^{1,2} Following the identification of plakoglobin (JUP) mutation as the causative gene for recessive ARVC, mutations in desmoplakin, plakophilin-2 (PKP2), and desmoglein-2 have been identified to cause dominant form.^{3–7} These proteins are important components of desmosomal plaque securing structural and functional integrity of adjacent myocardial cells.⁸ JUP and PKP2 are armadillo

proteins with similar properties functioning at the outer dense plaque of desmosomes. A 2 bp deletion in JUP causes recessive ARVC always associated with palmoplantar keratoderma and woolly hair (Naxos disease),^{9,10} whereas mutations in PKP2 have been identified as a common cause of dominant non-syndromic ARVC.^{5,7} In recent studies from northern Europe, PKP2 mutations have been identified as a cause of dominant ARVC in nearly half of index patients and in the vast majority of familial cases.¹¹

The established diagnostic criteria for ARVC¹² facilitate diagnosis; however, the most specific ones lack sensitivity, particularly in detecting disease at early stages. In the clinical screening of ARVC families, the identification of asymptomatic carriers potentially at increased risk for arrhythmic events and sudden death is challenging. In families with recessive ARVC, cutaneous phenotype

* Corresponding author. Tel: +30 22850 23234; fax: +30 22850 26175.
E-mail address: nprotnaxos@altecnet.gr

apparent from infancy reveals homozygous carriers. In families with dominant ARVC where diagnosis is hampered by low penetrance and variable phenotypic expression, there is need of sensitive non-invasive markers for early identification of mutation carriers. Risk stratification evaluating non-invasive markers and arrhythmic events has identified a profile of arrhythmic risk in selected ARVC populations.¹³⁻¹⁷ Such a profile has not yet been determined in the context of asymptomatic familial disease.

Systematic evaluation and close follow-up with standard non-invasive protocol of 16 families with dominant and recessive ARVC due to mutations in related desmosomal proteins PKP2 and JUP, respectively, from Greece and Cyprus, permitted a genotype-phenotype assessment with respect to diagnosis and clinical disease expressivity. We tried to define non-invasive markers able to identify mutation carriers and to predict the risk for arrhythmic events.

Methods

Study population

Eleven consecutive families with dominant ARVC and 12 families with recessive ARVC (Naxos disease) were screened for mutations in the known ARVC genes. In four of 11 families with dominant ARVC mutations in PKP2 were identified. In all 12 families with recessive ARVC (Naxos disease), the known JUP mutation was reconfirmed. Genotype-phenotype assessment in these 12 families with Naxos disease has been previously reported.¹⁰

All living family members underwent cardiac and molecular genetic investigation. The study complies with the Declaration of Helsinki, the locally appointed Ethics Committee has approved the research protocol, and informed consent has been obtained from all individuals. Initial cardiac assessment included a detailed history of cardiac events, physical examination, resting 12-lead electrocardiogram (ECG), 24 h ambulatory ECG, and two-dimensional echocardiography. Additionally, signal-averaged ECG ($n = 49$), electrophysiological studies ($n = 12$), angiography ($n = 16$), and cardiac biopsies ($n = 12$) were performed. The diagnosis of ARVC was based on criteria of ESC/ISFC.¹⁵

Follow-up

All individuals underwent serial non-invasive testing every 12 months or more frequent in those with clinical events in a prospective evaluation of up to 21 years (median 2 years) and all clinical events were recorded. Detailed historical data were available from medical records or from the patient at the time of the clinical investigation. Major clinical events including episodes of sustained ventricular tachycardia, syncope (with or without documented ventricular tachycardia), and sudden death were recorded by age. Syncope was defined as transient loss of consciousness associated with loss of postural tone.¹⁰ Aborted sudden death as a non-self-terminated event was estimated together with sudden death.

Genetic study

Genomic DNA from all family members and 100 healthy Greek and Cypriot volunteers unrelated to the families (control group) was extracted from whole blood with the use of Qiagen QIAamp DNA blood mini kits. Seventy-eight members of Naxos disease families had been previously genotyped for the JUP mutation.¹⁰ All exons of JUP and PKP2 were amplified and screened for mutations by direct sequencing as described before.^{3,6}

Electrocardiography and echocardiography

Standard 12-lead ECG was recorded at rest (10 mm/mV with speed 25 mm/s and 20 mm/mV with speed 50 mm/s). QRS complex duration was measured by a calliper at usual ECG format. Epsilon waves (low amplitude waves at the end of QRS complex) were included in these measurements. QRS dispersion was estimated as the difference between the widest QRS complexes from leads V1, V2, or V3 when compared with V6. Inversion of T-waves (including flattened T) in the precordial leads was noted. Signal-averaged ECG was performed using time-domain analysis with a bandpass filter of 40 Hz according to previously described protocols.¹⁸ A 24 h ambulatory ECG was recorded on an outpatient basis. Ventricular extrasystoles and episodes of ventricular tachycardia (three or more consecutive ventricular complexes at a rate of ≥ 120 bpm) were noted. Ventricular tachycardia was defined as sustained when lasting more than 30 s. On 24 h ambulatory ECG, ventricular extrasystoles were characterized as frequent when they were more than 1000/24 h for the probands and 200/24 h for the family members.¹⁹

Echocardiography was performed with a 2.2 MHz transducer. Measurements of the right ventricular dimensions in two-dimensional echocardiographic recordings were selected from the outflow tract on parasternal long-axis view and from the inflow tract on apical four-chamber view according to the protocol by Foale *et al.*²⁰ Dilatation of the right ventricle was classified as mild (2-3 SD from normal values) and severe (more than 3 SD from normal values). Wall motion abnormalities of the right ventricle (hypokinesia, akinesia, dyskinesia, diastolic bulging, and aneurysm) were documented.²¹ Left ventricular involvement was detected by evaluating wall motion abnormalities of the left ventricle.²¹

All electrocardiographic and echocardiographic measurements were performed by an expert cardiologist with a long experience on ARVC. Normal limits of these measurements were defined by assessment of 100 healthy Greek and Cypriot volunteers (50 men and 50 women), age 35 ± 19 years (range: 12-88 years).

Statistical analysis

Continuous variables were expressed as mean values \pm SD. Categorical variables were expressed as frequency. Sensitivity was calculated as the true positive rate, whereas specificity as the true negative rate. Ninety five per cent confidence intervals of sensitivity and specificity were estimated. Relationships between categorical variables were tested by contingency tables and χ^2 test using the Fisher criterion for small numbers of subjects. Associations between normally distributed continuous and categorical variables were evaluated using Student's *t*-test. Normality was assessed using the Kolmogorov-Smirnov test. The Mann-Whitney criterion was applied to test associations between skewed and categorical variables. Logistic regression analysis adjusted for age and gender of subjects was used to associate ECG and echocardiographic parameters to different genotypes. Event-free survival rates for the different genotypes were estimated by the Kaplan-Meier method and compared by log-rank test. However, to evaluate the association of genotype, gender, and ECG/echocardiographic variables with arrhythmic events and sudden death, multivariable logistic regression analysis was applied. A conventional survival analysis (i.e. Cox proportional hazards models) was not used because of the lack of accurate data regarding the initiation of the patients' characteristics. Because of potential inter-dependencies between family members, a latent variable was created and coded all family members. This variable was used as a potential confounding factor in all multivariable analyses. Reported *P*-values were based on two-sided hypotheses and compared to a significant level of 5%. Statistical calculations were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Mutation screening

One hundred and eighty-seven individuals (91 men and 96 women) age 39 ± 18 years (range: 12–88 years) belonging to four families with dominant and 12 families with recessive ARVC (12 ± 9 members per family, range: 3–29) were screened for mutations in the PKP2 and JUP genes. We identified two novel and one known PKP2 mutations in 22 members of dominant ARVC families and one known JUP mutation in 72 members of recessive ARVC (Naxos disease) families. One hundred healthy volunteers from Greece and Cyprus screened for the identified mutations were proven carriers for the normal alleles.

In particular, in Family A, five individuals carried a novel deletion of 10 bases in exon 3 of the PKP2 gene (971_980del10). This mutation is predicted to cause a frameshift and introduce a premature termination codon in the N-terminus of the PKP2 protein (G324fsX348) (Figure 1A). Eighteen family members were homozygotes for the normal allele.

In Family B, 11 individuals were heterozygous for a novel deletion of 50 bases in exon 13 of the PKP2 gene (2569del50) (Figure 1B). Similarly, this mutation would result in a frameshift and a premature termination on the PKP2 protein (R857fsX858). Fifteen family members had the normal allele.

The same PKP2 mutation (2509delA) was detected in Families C and D (Figure 1C). In total, there were six mutation carriers and three family members homozygous for the wild-type allele. Deletion 2509delA has been previously seen in ARVC patients⁵ and would lead to the introduction of a number of additional amino acid residues to the C-terminus of PKP2 (V837fsX930).

Finally, the known 2157del2 mutation in JUP was found in 12 families with Naxos disease (Figure 1D).³ Of 129 family

members, we identified 26 homozygotes and 46 heterozygotes for this mutation. Fifty-seven individuals had a normal genotype. All subjects who were homozygous for the 2157del2 mutation presented with palmoplantar keratoderma and woolly hair, whereas none of the PKP2 mutation carriers had any clinical cutaneous abnormality.

Diagnosis of ARVC

Non-invasive cardiac assessment demonstrated abnormalities, fulfilling the established diagnostic criteria for ARVC in 42 subjects (affected). In particular, 12 subjects fulfilled one major plus at least two minor criteria, whereas the other 30 fulfilled at least two major criteria. Sixteen affected (10 men, six women) were heterozygous carriers of PKP2 mutation and 26 affected (13 men, 13 women) were homozygous carriers for JUP mutation. The age at diagnosis of ARVC was 38 ± 17 years (range: 13–64 years) in affected PKP2 mutation carriers and 37 ± 17 years (range: 13–74 years) in affected JUP homozygous carriers. Diagnosis was supported histologically in 12 patients: from endomyocardial biopsy in six, from surgical biopsy in four (Figure 2), and from autopsy in two. Coronary arteries were normal in all 16 subjects who underwent coronary arteriography.

Six PKP2 mutation carriers age 48 ± 22 years (range: 13–71 years) did not fulfil the criteria for ARVC at last follow-up. No cardiac abnormality was detected in any of them except of mild hypokinesia of the right ventricular apex in a 40-year-old man. Also none of the heterozygous JUP mutation carriers aged 51 ± 19 (range: 15–87 years) fulfilled the criteria for ARVC. However, 11 of them showed minor ECG/echocardiographic abnormalities: eight had T-wave inversion in leads V1 and V2, a 36-year-old woman had T-wave inversion in leads V1 through V3, two

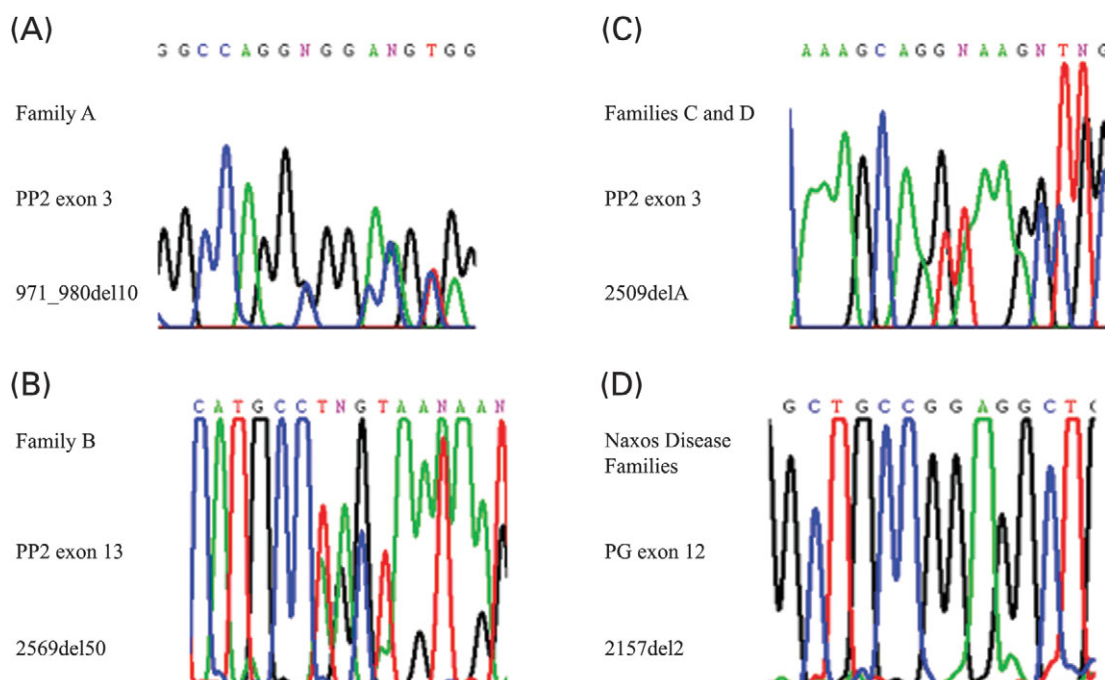


Figure 1 Sequence electropherograms of three plakophilin-2 (PP2) heterozygous mutations (971_980del10, 2569del50, and 2509delA) and one plakoglobin (PG) homozygous mutation (2157del2).

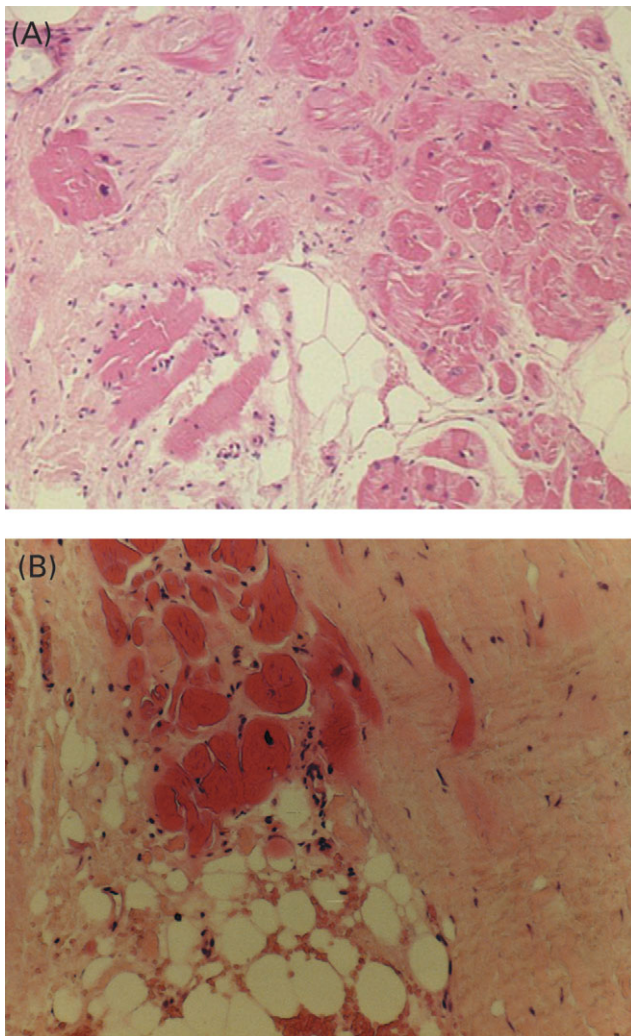


Figure 2 Surgical biopsy samples from right ventricular free wall of two affected carriers with PKP2 (A) and JUP (B) mutation. Surviving myofibres surrounded by fibrous and fatty tissue (Haematoxylin-Eosin-stained sections; magnification $\times 100$).

had diastolic bulging of the right ventricular inflow tract, and a 74-year-old man presented frequent ventricular extrasystoles of left bundle branch block configuration. None of the 93 subjects who were homozygous for the normal alleles aged 39 ± 19 (range: 14–92 years) fulfilled the diagnostic criteria for ARVC. However, 26 of them showed minor ECG or echocardiographic alterations (Table 1): 17 had T-wave inversion in leads V1 and V2 extending to V3 in three 15-year-old adolescents and in a 71-year-old woman, two presented frequent ventricular extrasystoles of left bundle branch block configuration, three showed minor right ventricular wall motion abnormalities and four mild right ventricular dilatation. Five of the 16 homozygous for the normal alleles who were submitted to signal-averaged ECG fulfilled the criteria for late potentials.

Application of diagnostic criteria revealed that T-wave inversion in leads V1 through V3, right ventricular wall motion abnormalities, and frequent ventricular extrasystoles of left bundle branch block configuration showed more than 76% sensitivity and more than 95% specificity in identifying homozygous JUP mutation carriers (Table 1).

The above minor criteria had also the highest sensitivity (64–76%) in identifying PKP2 mutation carriers. T-wave inversion in leads V1 and V2 reached the highest sensitivity of 85% with 82% specificity. Major criteria such as epsilon waves, QRS complex prolongation in leads V1 to V3, right ventricular aneurysms, and severe right ventricular dilatation though of 100% specificity showed low sensitivity.

Electrocardiographic and structural characteristics of affected carriers

Summarized in Table 2 are the electrocardiographic and echocardiographic findings of 42 carriers of PKP2 and JUP mutation who were diagnosed with ARVC. Resting ECG was abnormal in 94% of affected PKP2 carriers and in 92% of JUP homozygous carriers (Figure 3). T-wave inversion in leads V1–V3 was the principal ECG finding in both groups, tending to appear more commonly in affected PKP2 carriers. Depolarization abnormalities were mostly detected in JUP homozygous carriers. The only significant differences between the two groups were QRS prolongation in leads V1–V3 ($P = 0.002$) and QRS dispersion ≥ 40 ms ($P = 0.02$). Structural/functional abnormalities were similarly detected in both groups (Figure 4); right ventricle was involved in all affected, whereas left ventricular involvement was observed in less than 25% in both groups.

Clinical events

All affected carriers underwent serial cardiac assessment in a prospective evaluation of up to 21 years (median 8.5 years) and all clinical events were recorded. Detailed historical data were available from medical records. The age at the end of follow-up was 42 ± 17 years in affected PKP2 carriers and 48 ± 20 years in JUP homozygous carriers ($P = 0.32$). Major clinical events appeared in seven affected PKP2 carriers and 18 JUP homozygous carriers ($P = 0.11$) (Table 2). The age at first event was 27 ± 13 years (range: 14–49 years) in PKP2 carriers and 32 ± 18 years (range: 12–68 years) in JUP homozygous carriers ($P = 0.34$). Sustained ventricular tachycardia and syncope presented without significant differences in both groups (Table 2). Oral antiarrhythmic treatment was applied in 22 patients, surgical ablation was performed in three, and an antitachycardia pacemaker defibrillator was implanted in nine. Seven JUP homozygotes developed heart failure; symptoms of heart failure were associated with severe right ventricular deterioration in two individuals and biventricular deterioration in five. Sudden death occurred in three PKP2 carriers and six JUP homozygous carriers ($P = 0.75$) at the age of 28 ± 14 years (range: 15–53 years). A 15-year-old affected PKP2 carrier suffered an episode of aborted sudden death. He had a history of syncope but on electrophysiological investigation sustained ventricular tachycardia was not induced.

Kaplan–Meier curves for event-free survival did not differ significantly between affected PKP2 carriers and JUP homozygous carriers (Figure 5). By the age of 40 years, event-free survival was 61 ± 13 and $53 \pm 10\%$ respectively.

Risk stratification

During follow-up in 25 out of 42 affected carriers major clinical events were recorded: sustained ventricular tachycardia

Table 1 Sensitivity and specificity of International Task Force Criteria in 16 families with ARVC

12-lead ECG, echocardiography, and arrhythmias	Plakoglobin mutation carriers				Plakophilin-2 mutation carriers		Homozygous normals	
	Homozygous		Heterozygous		(n = 22)	SENS (%) (95% CI)	(n = 93)	SPEC (%) (95% CI)
	(n = 26)	SENS (%) (95% CI)	(n = 46)	SENS (%) (95% CI)				
Demographics								
Age (years)	37 ± 17		43 ± 18		40 ± 18		37 ± 19	
Male:female	13:13		21:25		11:11		46:47	
Repolarization abnormalities								
T-wave inversion in leads V1 and V2	22	85 (71–99)	8	17 (6–28)	15	68 (49–87)	17	82 (74–90)
T-wave inversion in leads V1–V3 (minor)	20	77 (61–93)	1	2 (0–6)	15	68 (49–87)	4	96 (92–100)
Depolarization abnormalities								
QRS width >110 ms in leads V1–V3 (major)	19	73 (56–90)	0	0 (–)	4	18 (2–34)	0	100 (–)
Epsilon waves (major)	12	46 (27–65)	0	0 (–)	4	18 (2–34)	0	100 (–)
RV abnormalities								
Regional WMA (minor)	26	100 (–)	2	4 (0–10)	16/21	76 (58–94)	3	97 (94–100)
Localized aneurysm (major)	16	62 (43–81)	0	0 (–)	10/21	48 (27–69)	0	100 (–)
Mild segmental or global dilatation (minor)	3	12 (0–24)	0	0 (–)	3/21	14 (0–28)	4	96 (92–100)
Severe segmental or global dilatation (major)	12	46 (27–65)	0	0 (–)	4/21	19 (3–35)	0	100 (–)
Arrhythmias of RV origin								
Frequent ventricular extrasystoles (minor)	25	96 (88–100)	1	2 (0–6)	14	64 (44–84)	2	98 (96–100)
Ventricular tachycardia (minor)	10	38 (19–57)	0	0 (–)	7	32 (13–51)	0	100 (–)

CI, confidence interval; RV, right ventricular; SENS, sensitivity; SPEC, specificity; WMA, wall motion abnormalities.

Table 2 Clinical features of affected carriers fulfilling ARVC criteria

Characteristic	Plakophilin-2 mutation carriers (n = 16)	Plakoglobin mutation carriers (n = 26)	P-value
Electrocardiographic abnormalities			
T-wave inversion in leads V1–V3	15 (94%)	20 (77%)	0.15
T-wave inversion beyond V3	12 (75%)	15 (58%)	0.26
QRS width >110 ms in leads V1–V3	4 (25%)	19 (73%)	0.002
QRS dispersion ≥40 ms	4 (25%)	16 (62%)	0.02
Epsilon waves	4 (25%)	12 (46%)	0.17
RV dilatation			
Outflow tract diameter >33 mm ^a	7/15 (47%)	15 (58%)	0.48
Inflow tract diameter >40 mm ^a	6/15 (40%)	11 (42%)	0.88
Severe RV dilatation			
Outflow tract diameter >36 mm ^b	3/15 (20%)	11 (42%)	0.14
Inflow tract diameter >45 mm ^b	2/15 (13%)	7 (27%)	0.28
Functional alterations			
RV wall motion abnormalities	15/15 (100%)	26 (100%)	1
RV aneurysms	10/15 (67%)	16 (62%)	0.74
LV wall motion abnormalities	3/15 (20%)	6 (23%)	0.82
Major clinical events			
Sustained ventricular tachycardia	3 (19%)	9 (35%)	0.28
Syncope	6 (38%)	15 (58%)	0.21
Sudden death	3 (19%)	6 (23%)	0.75

LV, left ventricular; RV, right ventricular.
^aMean normal + 2 SD.
^bMean normal + 3 SD.

in 12, syncope (with or without documented ventricular tachycardia) in 21, and aborted or non-aborted sudden death in nine (Table 2). The independent correlation of ECG/echocardiographic variables at initial assessment (T-wave inversion, QRS dispersion, right ventricular dilatation/dysfunction, and left ventricular involvement) with major clinical events was assessed by multivariable logistic regression analysis. QRS dispersion (≥ 40 ms) was an independent predictor of syncope (odds ratio 5.32, 95% CI 1.02–27.61; $P = 0.047$) but not of sudden death ($P = 0.577$). QRS dispersion tended to predict sustained ventricular tachycardia (odds ratio 5.79, 95% CI 0.80–42.03; $P = 0.082$), whereas left ventricular involvement tended to predict sudden death (odds ratio 5.67, 95% CI 0.80–40.09; $P = 0.082$). No significant interdependencies between family members were observed.

Discussion

Molecular basis of ARVC

Mutations in genes encoding proteins involved in myocardial cell–cell adhesion are increasingly recognized as a primary molecular defect in ARVC pathogenesis.^{3–7} Desmosomes are intercellular junctions abundant in myocardium and epidermis, tissues that experience constant mechanical stress.⁸ JUP and plakophilin are armadillo proteins located at the outer dense plaque of desmosome, found also in the nucleus and involved in signalling mechanisms.²² PKP2 isoform of plakophilin is found in desmosomes of myocardial cells.⁵

Mutation in PKP2 was identified as the causative gene in four out of 11 (36%) families with dominant ARVC. Two novel PKP2 mutations were identified in two families from Cyprus and an already reported one in two families from

Greece. Recent studies compared the clinical expression of patients with PKP2 mutation to a genetically non-homogeneous group of ARVC patients.^{7,23} This is the first study relating the disease phenotype in genetically homogeneous populations with familial ARVC due to mutations in desmosomal proteins with similar properties.

Diagnosis and disease expressivity

In four families with dominant ARVC due to PKP2 mutation, 16 out of 22 mutation carriers fulfilled the established diagnostic criteria, the youngest by the age of 13 years. Penetrance of PKP2 mutation was estimated from the larger family up to 55%. Incomplete penetrance has been reported in dominant ARVC, particularly in PKP2 families the highest reported penetrance reached 66% by adolescence.⁶ Cutaneous abnormalities did not exist in any of the PKP2 mutation carriers. In recessive ARVC, due to JUP mutation, cardiac phenotype showed 100% penetrance by adolescence whereas cutaneous abnormalities were fully expressed in all homozygotes from infancy.¹⁰ Minor ECG/echocardiographic findings identified in a few JUP heterozygous carriers did not differ from those identified in homozygous normals.

The clinical expression of heart disease between PKP2 and JUP-affected carriers did not differ significantly except for depolarization abnormalities which were detected more commonly in homozygous JUP mutation carriers. T-wave inversion in leads V1–V3 was the main ECG finding in both groups, appearing more commonly in affected PKP2 carriers, a finding confirmed by other studies too.¹¹ Therefore, it might be assumed that the mutations in PKP2 and JUP express quite similar cardiac phenotype. Given that the structural profile of the disease appears similar in both groups, depolarization abnormalities more commonly



Figure 3 Recordings from baseline 12-lead ECG (25 mm/s, 10 mm/mV) of two affected carriers with PKP2 (A) and JUP (B) mutation presented with episodes of sustained ventricular tachycardia. Low voltage in all leads, T-wave inversion, and depolarization abnormalities (QRS complex prolongation and epsilon waves) in leads V1–V4 are observed in both. Depolarization abnormalities are more prominent in (B).

detected in JUP mutation might reflect a more extensive defect in the electrical coupling that might be attributed to gap junction remodelling.²⁴ Nevertheless, gap junctions in PKP2 mutation carriers remain to be examined.

Non-invasive markers for identification of mutation carriers

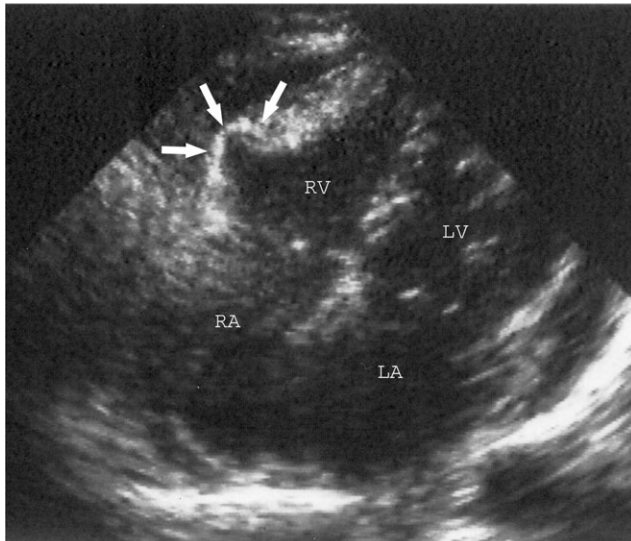
The use of sensitive non-invasive markers for early detection of gene carriers might identify individuals at risk and guide the genetic evaluation within affected families. Diagnostic criteria from ECG and two-dimensional echocardiography, which have been used to screen family members, were validated in this study as markers for identification of mutation carriers. Recessive ARVC presenting full penetrance and homogeneous expression permitted testing of non-invasive markers for diagnostic sensitivity. T-wave inversion in leads V1 to V2 or V3, right ventricular wall motion abnormalities, and frequent ventricular extrasystoles of left bundle branch block configuration showed high sensitivity and specificity in identifying homozygous JUP mutation carriers as well as

PKP2 mutation carriers. Major ECG/echocardiographic criteria though of 100% specificity showed low sensitivity.

Risk stratification in mutation carriers

The growing number of identified disease-causing genes in ARVC probands increases the incidence of asymptomatic carriers within families that are potentially at risk for arrhythmic events and sudden death being occasionally the first disease manifestation. Non-invasive markers able to predict the risk of life-threatening ventricular arrhythmias are considered important in clinical management. Risk stratification in selected genetically non-homogeneous populations of ARVC patients, predominantly those with an arrhythmia presentation, revealed severe right ventricular dilatation/dysfunction as a predictor of arrhythmic events, whereas QRS dispersion (≥ 40 ms) and left ventricular involvement were predictors of sudden death.^{13–17} This study is the first one seeking to explore arrhythmic risk within genotyped ARVC families. Multivariable logistic regression analysis revealed that QRS dispersion (≥ 40 ms) was an

(A)



(B)

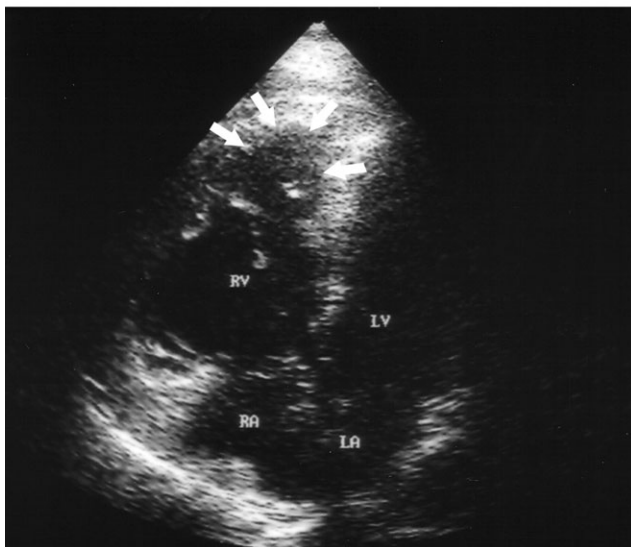


Figure 4 Two-dimensional echocardiographic images recorded from two affected carriers with PKP2 (A) and JUP (B) mutation. Right ventricular aneurysms (white arrows) are exhibited on inflow tract (A) and apex (B) by a modified apical four-chamber view.

independent predictor of syncope but not of sudden death. Left ventricular involvement tended to predict sudden death.

Conclusions

Alterations in desmosomal proteins are increasingly identified to underlie ARVC. Mutations in genes encoding closely related proteins PKP2 and JUP functioning at the outer dense plaque of desmosomes result in similar cardiac phenotype with right ventricular preponderance. They both express ARVC by adolescence with penetrance reaching 55% in PKP2 carriers and 100% in JUP homozygous carriers. Non-invasive family screening for identification of gene carriers may largely be based on precordial T-wave inversion, right ventricular wall motion abnormalities, and frequent

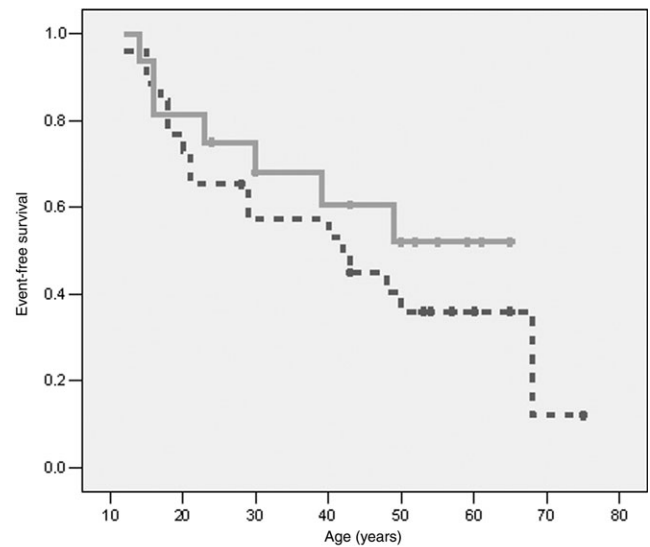


Figure 5 The Kaplan-Meier survival curves for event-free survival in PKP2 (continuous line) and JUP (dotted line) affected carriers.

ventricular extrasystoles of left bundle branch block configuration. QRS dispersion (≥ 40 ms) was an independent predictor of syncope but not of sudden death.

Acknowledgements

This work was supported by the European Commission 5th Framework Program (ARVC/D Project QLGI-CT-2000-01091) and the British Heart Foundation. We thank the patients, the family members, and all who volunteered to take part in this study. We are grateful to Professor Cristina Basso, Padua, for her contribution in the pathological investigation of the most of our patients.

Conflict of interest: none declared.

References

- Marcus FI, Fontaine GH, Guiraudon G, Frank R, Laurenceau JL, Malergue C, Grosgeat Y. Right ventricular dysplasia: a report of 24 adult cases. *Circulation* 1982;65:384-398.
- 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.
- McKoy G, Protonotarios N, Crosby A, Tsatsopoulou A, Anastakis A, Coonar A, Norman M, Baboonian C, Jeffery S, McKenna WJ. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). *Lancet* 2000;355:2119-2124.
- Bauce B, Basso C, Rampazzo A, Beffagna G, Daliento L, Frigo G, Malacrida S, Settimo L, Danieli GA, Thiene G, Nava A. Clinical profile of four families with arrhythmogenic right ventricular cardiomyopathy caused by dominant desmoplakin mutations. *Eur Heart J* 2005;16: 1666-1675.
- Gerull B, Heuser A, Wichter T, Paul M, Basson CT, McDermott DA, Lerman BB, Markowitz SM, Ellinor PT, MacRae CA, Peters S, Grossmann KS, Michely B, Sasse-Klaassen S, Birchmeier W, Dietz R, Breidhardt G, Schulze-Bahr E, Thierfelder L. Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. *Nat Genet* 2004;36:1162-1164.
- Syrris P, Ward D, Asimaki A, Sen-Chowdry S, Ebrahim HY, Evans A, Hitomi N, Norman M, Pantazis A, Shaw AL, Elliot PM, McKenna WJ. Clinical expression of plakophilin-2 mutations in familial arrhythmogenic right ventricular cardiomyopathy. *Circulation* 2006;113:356-364.

7. Pilichou K, Nava A, Basso C, Beffagna G, Bauce B, Lorenzon A, Frigo G, Vettori A, Valente M, Towbin J, Thiene G, Danieli GA, Rampazzo A. Mutations in desmoglein-2 gene are associated with arrhythmogenic right ventricular cardiomyopathy. *Circulation* 2006;**113**:1171–1179.
8. Green KJ, Gaudry CA. Are desmosomes more than tethers for intermediate filaments? *Nat Rev Mol Cell Biol* 2000;**1**:208–216.
9. Protonotarios N, Tsatsopoulou A, Patsourakos P, Alexopoulos D, Gezerlis P, Simitis S, Scampardonis G. Cardiac abnormalities in familial palmoplantar keratosis. *Br Heart J* 1986;**56**:321–326.
10. Protonotarios N, Tsatsopoulou A, Anastasakis A, Sevdalis E, McKoy G, Stratos K, Gatzoulis K, Tentolouris K, Spiliopoulou C, Panagiotakos D, McKenna W, Toutouzas P. Genotype–phenotype assessment in autosomal recessive arrhythmogenic right ventricular cardiomyopathy (Naxos disease) caused by a deletion in plakoglobin. *J Am Coll Cardiol* 2001;**38**:1477–1484.
11. Van Tintelen JP, Entius MM, Bhuiyan ZA, Jongbloed R, Wiesfeld ACP, Wilde AAM, van der Smagt J, Boven LG, Mannens MMAM, van Lagen IM, Hofsta RMW, Otterspoor HLC, Doevendans PAFM, Rodriguez LM, van Gelder IC, Hauer RNW. Plakophilin-2 mutations are the major determinant of familial arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circulation* 2006;**113**:1650–1658.
12. McKenna WJ, Thiene G, Nava A, Fontaliran F, Blomstrom-Lundqvist C, Fontaine G, Camerini F. Diagnosis of arrhythmogenic right ventricular dysplasia/cardiomyopathy. Task Force of the Working Group Myocardial and Pericardial Disease of the European Society of Cardiology and of the Scientific Council on Cardiomyopathies of the International Society and Federation of Cardiology. *Br Heart J* 1994;**71**:215–218.
13. Turrini P, Corrado D, Basso C, Nava A, Bauce B, Thiene G. Dispersion of ventricular depolarization-repolarization: A non-invasive marker for risk stratification in arrhythmogenic right ventricular cardiomyopathy. *Circulation* 2001;**103**:3075–3080.
14. Corrado D, Leoni L, Link MS, Della Bella P, Gaita F, Curnis A, Salerno JU, Igdibashian D, Raviolo A, Disertori M, Zanotto G, Verlato R, Vergara G, Delise P, Turrini P, Basso C, Naccarella F, Maddalena F, Estes NA III, Buja G, Thiene G. Implantable cardioverter-defibrillator therapy for prevention of sudden death in patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Circulation* 2003;**108**:3084–3091.
15. Wichter T, Paul M, Wollmann C, Acil T, Gerdes P, Ashraf O, Tjan TDT, Soeparwata R, Block M, Borggreffe M, Scheld HH, Breithardt G, Bocker D. Implantable cardioverter/defibrillator therapy in arrhythmogenic right ventricular cardiomyopathy: Single-center experience of long-term follow-up and complications in 60 patients. *Circulation* 2004;**109**:1503–1508.
16. Roguin A, Bomma CS, Nasir K, Tandri H, Tichnell C, James C, Rutberg J, Crosson J, Spevak PJ, Berger RD, Halperin HR, Calkins H. Implantable cardioverter-defibrillators in patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy. *J Am Coll Cardiol* 2004;**43**:1843–1852.
17. Hulot JS, Jouven X, Empana JP, Frank R, Fontaine G. Natural history and risk stratification of arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circulation* 2004;**110**:1879–1884.
18. Oselladore L, Nava A, Buja G, Turrini P, Daliento L, Livolsi B, Thiene G. Signal-averaged electrocardiography in familial form of arrhythmogenic right ventricular cardiomyopathy. *Am J Cardiol* 1995;**75**: 1038–1041.
19. Hamid MS, Norman M, Quraishi A, Firoozi S, Thaman R, Gimeno JR, Sachdev B, Rowland E, Elliott PM, McKenna WJ. Prospective evaluation of relatives for familial arrhythmogenic right ventricular cardiomyopathy/ dysplasia reveals a need to broaden diagnostic criteria. *J Am Coll Cardiol* 2002;**40**:1445–1450.
20. Foale R, Nihoyannopoulos P, McKenna W, Klienebenne A, Nadazdin A, Rowland E, Smith G. Echocardiographic measurements of the normal adult right ventricle. *Br Heart J* 1986;**56**:33–44.
21. Blomstrom-Lundqvist C, Beckman-Suurkula M, Wallentin I, Jonsson R, Olsson SB. Ventricular dimensions and wall motion assessed by echocardiography in patients with arrhythmogenic right ventricular dysplasia. *Eur Heart J* 1988;**9**:1291–1302.
22. Zhurinsky J, Shtutman M, Ben-Ze'ev A. Plakoglobin and β -catenin: protein interactions, regulation and biological role. *J Cell Sci* 2000;**113**:3127–3139.
23. Dalal D, Molin LH, Piccini J, Tichnell C, James C, Bomma C, Prakasa K, Towbin JA, Marcus FI, Spevak PJ, Bluemke DA, Abraham T, Russell SD, Calkins H, Judge DP. Clinical features of arrhythmogenic right ventricular dysplasia/cardiomyopathy associated with mutations in plakophilin-2. *Circulation* 2006;**113**:1641–1649.
24. Kaplan SR, Gard JJ, Protonotarios N, Tsatsopoulou A, Spiliopoulou C, Anastasakis A, Prost Squarcioni C, McKenna WJ, Thiene G, Basso C, Brousse N, Fontaine G, Saffitz JE. Remodeling of myocyte gap junctions in arrhythmogenic right ventricular cardiomyopathy due to a deletion in plakoglobin (Naxos disease). *Heart Rhythm* 2004;**1**:3–11.