Clinical Expression of Plakophilin-2 Mutations in Familial Arrhythmogenic Right Ventricular Cardiomyopathy

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Background—Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiac disorder characterized by loss of cardiomyocytes and their replacement by adipose and fibrous tissue. It is considered a disease of cell adhesion because mutations in desmosomal genes, desmoplakin and plakoglobin, have been implicated in the pathogenesis of ARVC. In a recent report, mutations in plakophilin-2, a gene highly expressed in cardiac desmosomes, have been shown to cause ARVC.

Methods and Results—We investigated 100 white patients with ARVC for mutations in plakophilin-2. Nine different mutations were identified by direct sequencing in 11 cases. Five of these mutations are novel (A733fsX740, L586fsX658, V570fsX576, R413X, and P533fsX561) and predicted to cause a premature truncation of the plakophilin-2 protein. Family studies showed incomplete disease expression in mutation carriers and identified a number of individuals who would be misdiagnosed with the existing International Task Force and modified diagnostic criteria for ARVC.

Conclusions—In this study, we provide new evidence that mutations in the desmosomal plakophilin-2 gene can cause ARVC. A systematic clinical evaluation of mutation carriers within families demonstrated variable phenotypic expression, even among individuals with the same mutation, and highlighted the need for a more accurate set of diagnostic criteria for ARVC. **(*Circulation*. 2006;113:356-364.)**

Key Words: arrhythmia ■ cardiomyopathy ■ death, sudden ■ diagnosis ■ genetics

Arrhythmogenic right ventricular (RV) cardiomyopathy (ARVC) is an inherited cardiac disorder characterized by progressive replacement of myocytes by adipose and fibrous tissue. It affects primarily the RV, but left ventricular manifestations also have been documented.[1] ARVC often presents with ventricular arrhythmias, heart failure, and sudden death.[2,3] ARVC is a major cause of sudden cardiac death during adolescence and early adulthood.[1,4,5] Treatment includes use of antiarrhythmic medications and implantation of a cardioverter defibrillator (ICD). The latter is the treatment of choice for individuals at high risk of sudden death.[6]

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Clinical diagnosis of ARVC is based on diagnostic criteria proposed by the International Task Force of the European Society of Cardiology and International Society and Federation of Cardiology that encompass morphological changes, histology, electrical abnormalities, rhythm disturbance, and family history.[7] In light of the possibility of variable penetrance within a family, we reevaluated these diagnostic criteria and proposed that, in the context of proven ARVC in a family, any first-degree relative with a positive signal-averaged ECG or an otherwise unexplained ECG, Holter, or echocardiographic abnormality should be considered to have familial disease. Furthermore, it was proposed that the threshold for significant frequency of ventricular ectopy in this context should be reduced from 1000 to 200 extrasystoles in a 24-hour period.[8]

ARVC is thought to be familial in up to 50% of all cases.[8] It is usually inherited in an autosomal dominant manner with variable penetrance, but autosomal recessive forms have been reported. Several chromosomal loci associated with dominant ARVC have been ascertained by linkage analysis (14q23–q24, 1q42–q43, 14q12–q22, 2q32.1–q32.3, 3p25, 10p12–p14, 10q22, and 6p24).[9] A causative gene encoding for plakoglobin (JUP), has been reported in Naxos disease, a recessive form of ARVC, palmoplantar keratoderma, and woolly hair.[10] Dominant and recessive mutations in desmo-

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TABLE 1. Summary of PKP2 Mutations

Exon	Nucleotide Change	Coding Effect	Position	Family	Comments
1	144_148delCAGA	S50fsX110	N-terminal	I	Previously described[18]
3	C419T	S140F	N-terminal	G	Previously described[18]
5	C1237T	R413X	Arm repeat 2	F	Novel
7	1597_1600delATCC	P533fsX561	Arm repeat 4	K	Novel
8	1755_1756insTTGACTCA	L586fsX658	Arm repeat 5	D	Novel
8	1709delC	V570fsX576	Arm repeat 5	E	Novel
9	C1844T	S615F	Arm repeat 6	H	Previously described[18]
11 (Splice acceptor site)	2146–1G>C	Abnormal splicing	Arm repeat 8	J	Previously described[18]
11	2197_2202delCACACCinsG	A733fsX740	Arm repeat 8	A, B, C	Novel

plakin (DSP) have been shown to cause ARVC in either a purely cardiac phenotype or as part of a syndrome also involving skin or hair abnormalities.[11–13] Familial ARVC type 2, associated with a distinct phenotype of catecholaminergic polymorphic ventricular tachycardia (VT), is caused by mutations in the cardiac ryanodine receptor gene.[14,15] A more recent study has implicated mutations in the noncoding region of transforming growth factor-β3 gene in familial ARVD type 1.[16]

Desmosomes are protein structures situated in cell membranes that maintain adhesion between neighboring cells and serve as anchoring sites for the intermediate filaments. They are found in tissues that experience mechanical stress, including epidermis and myocardium. In addition to cell adhesion, they are involved in cell communication, tissue morphogenesis, and differentiation.[17] Desmosomes consist of 3 major protein families: cadherins (desmocollins and desmogleins), Armadillo (Arm) repeat proteins (plakoglobin and plakophilins), and plakins (desmoplakin, plectin, etc).

The discovery of mutations in DSP and JUP in ARVC has led to the hypothesis that the disorder may be a disease of the desmosome. This hypothesis is supported by a recent study that has implicated a third desmosomal gene, plakophilin-2 (PKP2), in the pathogenesis of ARVC. A large number of PKP2 mutations were found in a cohort of 120 affected individuals, suggesting that mutated forms of PKP2 are a major cause of this condition.[18] However, that study lacked a detailed clinical evaluation of probands, and data on family members were limited.

We have screened 100 consecutive white patients with ARVC (in whom mutations in desmoplakin and plakoglobin had been excluded) for mutations in PKP2 and have identified 9 disease-causing changes. Furthermore, we investigated, both clinically and genetically, the families of those patients with PKP2 mutations to provide a detailed analysis of the genotype-phenotype relation in individuals carrying a mutation.

Methods

Clinical Evaluation

Informed consent was obtained from all participating patients and family members, and the study was approved by the University College London Hospitals Trust ethics committee. Probands and affected family members were clinically evaluated according to the International Task Force of the European Society of Cardiology and International Society and Federation of Cardiology diagnostic criteria[7] and the proposed modified diagnostic criteria.[8] It included clinical history, 12-lead ECG, signal-averaged ECG with a 40-Hz filter, transthoracic echocardiography, contrast echocardiography when considered appropriate, maximal bicycle exercise testing, 24-hour ambulatory ECG, and MRI in some cases.

Mutation Screening

Genomic DNA from patients and family members was extracted from whole blood with QIAamp DNA Blood mini kits (Qiagen). All patients were previously found free of mutations in known ARVC genes, desmoplakin, and plakoglobin. On the basis of the published sequence of the long form of the PKP2 gene (PKP2b, Ensembl gene no ENSG00000057294), primer pairs for all PKP2b exons were designed from flanking intronic sequences. PCR amplification was carried out using standard protocols (AmpliTaq Gold, Applied Biosystems) for all fragments except exon 1, which, because of its high GC content, was amplified with the GC RICH PCR system (Roche). Primer sequences and PCR conditions are available on request. After amplification, PCR fragments were sequenced in both directions on an ABI PRISM 3100 DNA analyzer using BigDye Terminator chemistry (version 3.1) and analyzed by Seqscape version 2.0 software (Applied Biosystems).

DNA samples from 200 healthy volunteers of the same ethnic origin were used as controls.

Results

Mutations in PKP2

One hundred affected individuals with ARVC were examined for mutations in PKP2. We identified 5 novel and 4 known heterozygous PKP2 mutations in 11 patients: 1 deletion/insertion, 1 insertion, 3 deletions, 1 nonsense, 2 missense, and 1 splice site mutation (Table 1).

A deletion/insertion (2197_2202delCACACCinsG) was detected in families A, B, and C (Figure 1A). This mutation causes a frameshift that leads to a premature termination codon (A733fsX740) within the Arm repeat 8 of PKP2.

An insertion of 8 bases (1755_1756insTTGACTCA) was found in family D (Figure 1B), whereas 2 deletions, 1709delC and 1597_1600delATCC, were detected in family E (Figure 1C) and family K, respectively. These 3 mutations are located in the conserved Arm repeat domain and result in frameshifts and premature termination of translation several amino acid residues downstream (L586fsX658, V570fsX576, and P533fsX561, respectively).

A C→T transition (C1237T) was found in exon 5 in family F (Figure 1D). This change is predicted to replace an arginine with a termination codon at position 413 (R413X).

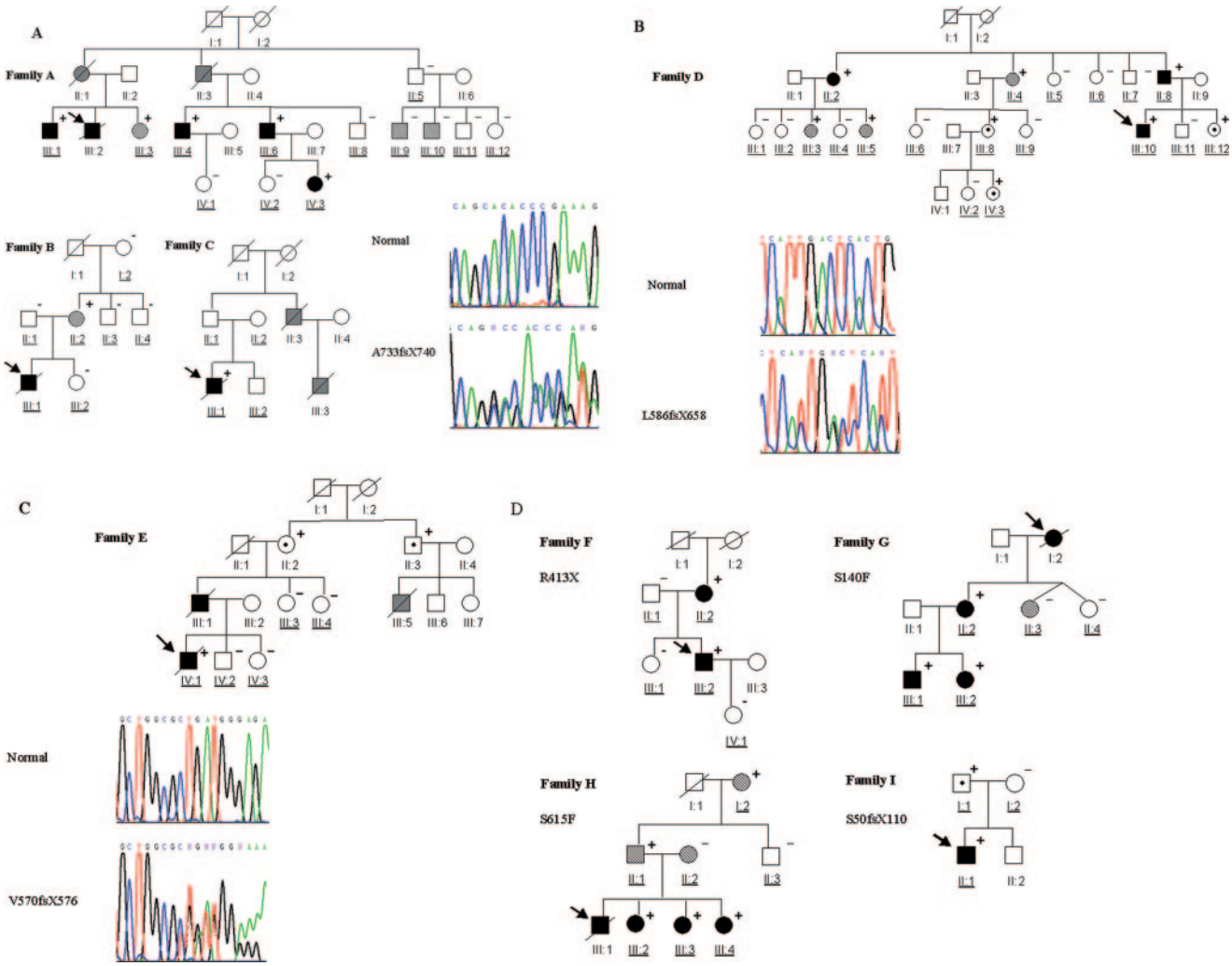

Figure 1. A, B, C, Pedigrees of ARVC families A through E and sequence electropherograms of PKP2 showing mutant sequences vs normal control. D, Pedigrees of ARVC families F through I. Squares denote males; circles, females; solid symbols, individuals fulfilling International Task Force diagnostic criteria for ARVC[7] and/or those confirmed as affected at postmortem; shaded symbols, individuals fulfilling the modified diagnostic criteria only[8]; dotted symbols, mutation carriers not fulfilling ARVC diagnostic criteria (individuals II:2 and II:3 in family E refused clinical evaluation); open symbols, unaffected individuals; gray symbols, deceased individuals who did not undergo clinical evaluation and are considered probably affected because of unexplained sudden death (<40 years of age) or by their position in the pedigree as obligate gene carriers; underlined numerical designations, individuals who underwent clinical evaluation; slanted bars, deceased individuals; and + and −, the presence and absence of a PKP2 mutation, respectively. The index patient in each family is marked with an arrow. Pedigrees shown reflect those nuclear families available for evaluation or for whom available information was verifiable.

Finally, 4 patients carried mutations that have been reported before.[18] These included mutations in exons 1 and 11 (145_148delCAGA and 2146-1G→C in families I and J) and 2 missense mutations (S140F and S615F) detected in families G and H, respectively (Figure 1D).

All mutations were absent in 400 control chromosomes.

Clinical Findings

In family A, a heterozygous A733fsX740 mutation was detected in 5 individuals (Figure 1A). It shows autosomal dominant mode of inheritance through 3 generations, with 6 individuals clinically affected with ARVC. The proband (III:2) died suddenly at 39 years of age with no known previous cardiac history. Postmortem findings confirmed the diagnosis of ARVC. The proband's brother (III:1) had presented with recurrent VT 13 years earlier, but the underlying cause was not identified until the proband's death. Thirteen family members consented to clinical and genetic screening. Three (III:4, III:6, and IV:3) fulfilled clinical diagnostic criteria and were subsequently confirmed to carry the mutation. Individuals III:1, III:6, and IV:3 now have ICDs. Three family members did not fulfill diagnostic criteria but raised a suspicion of incomplete disease expression, 1 on the basis of precordial T-wave inversion (III:3) and 2 brothers, 1 of whom had mild RV enlargement on echocardiography (III:9) and 1 with >1000 multifocal (biventricular) ectopic beats on 24-hour Holter monitoring (III:10). All 3 individuals (III:3, III:9, and III:10) fulfilled modified diagnostic criteria. However, only III:3 was subsequently confirmed to be positive for the A733fsX470 mutation; III:9, III:10, and their father (II:5) did not carry the mutation.

The same deletion was detected in 2 other families, B and C (Figure 1A). In family B, the proband (III:1) presented with 2 syncopal episodes and was found to have precordial T-wave inversion on ECG, positive signal-averaged ECG, RV enlargement with minor regional wall motion abnormality, and ventricular tachycardia of RV origin. He was managed medically, offered an ICD, which he refused, and died suddenly at 21 years of age. Postmortem findings confirmed the diagnosis of ARVC. The immediate family consisted of 11 subjects over 3 generations, 6 of whom consented to screening and are shown in Figure 1A. Only his mother (II:2), who carries the A733fsX740 mutation, had any abnormalities clinically, and although she did not meet task force diagnostic criteria, she satisfied the modified criteria on the basis of characteristic T-wave inversion in V_1 through V_3 on ECG.[8]

In family C, the proband (III:1) died suddenly at 15 years of age (Figure 1A). He had presented with syncope at 7 years of age, and a clinical diagnosis of ARVC was made when he was 8 years of age. He had right precordial T-wave inversion and marked RV enlargement with poor systolic function. A first-degree relative, III:3, had previously died suddenly (at 26 years of age), and fibroelastosis was found on postmortem. Of the 10 available family members spanning 3 generations, only 3 (II:1, II:2, and III:2) were available and consented to evaluation. None had evidence of disease expression. DNA was available only from the proband. He was found to be heterozygous for the A733fsX740 mutation.

In family D, mutation L586fsX658 was found in the proband (III:10) and 8 of his relatives (Figure 1B). Individual III:10 presented with palpitation and presyncope and was found to have recurrent VT. ECG was typical of ARVC, and the RV was enlarged with impaired systolic function on echocardiography. An ICD was implanted. The family included 43 members over 3 generations. Nineteen family members consented to screening (Figure 1B). Clinical evaluation revealed 3 family members who met task force (II:2, II:8) or modified diagnostic (II:4) criteria and were subsequently found to be carriers of mutation L586fsX658. Individual II:8 had symptomatic VT and had an ICD implanted. Another 3 family members in the third generation (III:3, III:5, and III:8) had minor echocardiographic abnormalities and were heterozygous for this mutation.

In family E, mutation V570fsX576 was detected in the proband (IV:1), who died suddenly at 15 years of age (Figure 1C). His postmortem examination showed extensive fibrosis of an area of thin pale myocardium in the RV free wall. The RV was mildly dilated. He had been subjected to periodic cardiac review all his life after surgery for coarctation of the aorta at 10 weeks of age. When he was 5 years of age, his father (III:1) died suddenly at 37 years of age. The postmortem examination at the time cited "floppy mitral valve" as the cause of death, although the RV was mildly enlarged, and subsequent review of histology revealed areas of fibrosis in both ventricles. ARVC was suspected as the cause of death of another relative (III:5) who died suddenly during physical exercise. His father (II:3) was found to be a carrier of this mutation but refused clinical evaluation. Screening of the immediate family members over 3 generations found no other individuals carrying the mutation except the 89-year-old paternal grandmother (II:2). She was found to have an abnormal ECG (right bundle-branch block), but it could have been due to age-related conduction disease. Echocardiography showed no abnormalities, and she refused further clinical evaluation. All 4 gene-negative family members were clinically normal.

Mutation R413X was found in 2 affected individuals (II:2 and III:2) in family F (Figure 1D). The proband (III:2) arrested playing field hockey at 40 years of age and was successfully resuscitated. He had precordial T-wave inversion, an enlarged, hypokinetic RV, and a positive signal-averaged ECG. The immediate family for screening was small, encompassing only 4 additional members over 3 generations who consented to screening (II:1, II:2, III:1, and IV:1). They were asymptomatic. The proband's mother (II:2) who carried the R413X mutation had a normal ECG and no evidence of arrhythmia but had an aneurysmal segment in the RV outflow tract on echocardiography.

Affected individuals in family G have mutation S140F (Figure 1D). The proband (I:2) died suddenly at 36 years of age with postmortem confirmation of ARVC; her 43-year-old sister had died suddenly 6 years previously with no cause identified (not shown in pedigree). Surviving family members available for screening included 17 members over 3 generations, 5 of whom have so far consented to participate in the screening program and are shown in Figure 1D. At initial evaluation 4 years ago, only 1 member (II:2) fulfilled diagnostic criteria. Over the following 4 years, both her children (III:1 and III:2), now 19 and 17 years of age, have advanced to fulfill criteria by virtue of progressive ECG changes, newly positive signal-averaged ECGs, increasing ventricular ectopy, and regional wall motion abnormality on echocardiography. All 3 individuals carry the S140F mutation and now have ICDs. Another member (II:3) was thought to have regional wall motion abnormalities on echocardiography and MRI and fulfilled modified diagnostic criteria. However, she does not carry this mutation.

In family H, a S615F mutation was detected (Figure 1D). The proband (III:1) had 2 syncopal episodes in the months preceding his sudden death at 15 years of age. Family evaluation encompassed both parents' immediate families, a total of 41 members over 4 generations, 19 of whom consented to evaluation (not all shown in pedigree). We initially suspected that the proband's mother (II:2) was the mutation carrier on the basis of symptoms and progressive and dynamic ECG changes. At initial evaluation, only the proband's 3 sisters met diagnostic criteria (III:2, III:3, and III:4). They were found to have characteristic T-wave inversion in right precordial leads on ECG (Figure 2A), whereas cardiovascular magnetic resonance showed RV regional wall motion abnormalities and localized wall thinning (Figure 2B). The proband's father (II:1) has now been found to have minor regional wall motion abnormalities on echocardiography and MRI, and his 97-year-old mother (I:2) has characteristic T-wave inversion on her ECG. Both II:1 and I:2 fulfill modified diagnostic criteria. All of these, and none on the maternal side, are heterozygous for the S615F mutation.

In family I, the proband (II:1) was found to carry a known deletion in exon 1 of PKP2. He presented with recurrent

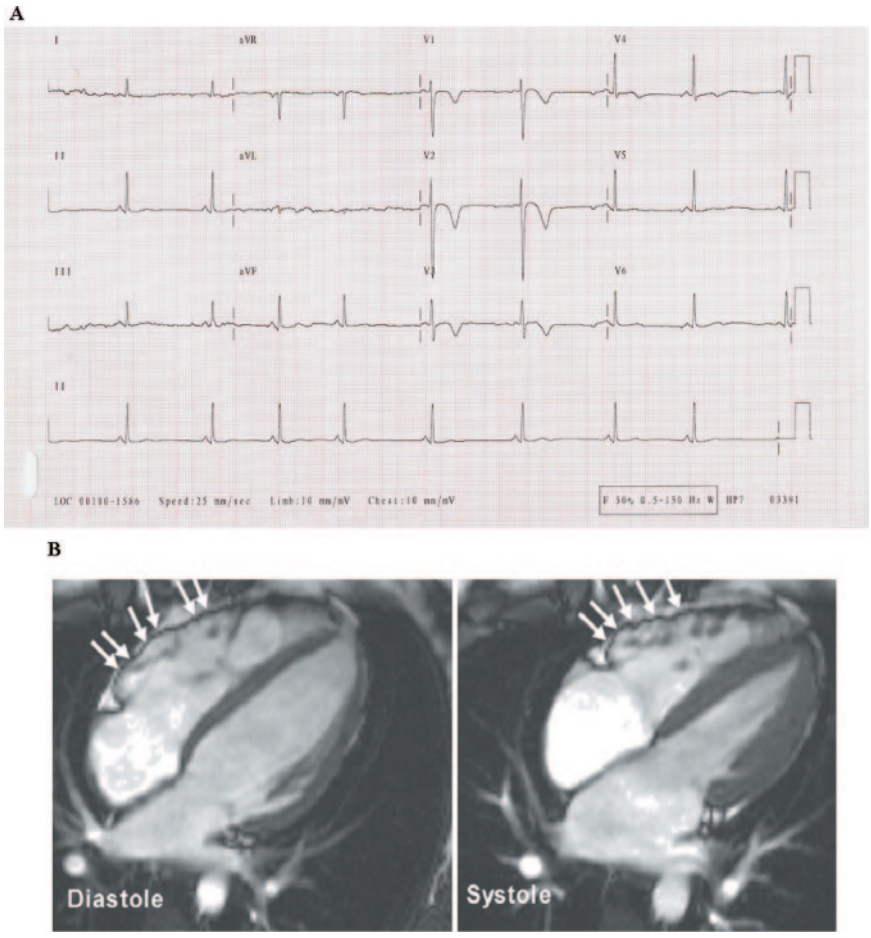

Figure 2. A, ECG from individual III:3 (family H) showing sinus rhythm with prominent T-wave inversion in right precordial leads. B, Cardiovascular magnetic resonance (TrueFISP) images in 4-chamber view from individual III:2 (family H). Volumetric analysis confirmed global RV dilation and systolic impairment. White arrows indicate localized wall thinning, diastolic bulging, and hypokinesia in the RV.

syncope and was found to have VT of RV origin. An ICD was implanted. He has precordial T-wave inversion, >1000 premature ventricular complexes (PVCs) of RV origin on 24-hour Holter monitoring and RV regional wall motion abnormality on MRI. Of 16 family members over 4 generations, to date only his parents (I:1 and I:2) have been available for evaluation. His father (I:1) has palpitations but does not meet task force or modified diagnostic criteria. He is also a carrier of this mutation (Figure 1D).

Finally, 2 mutations were detected in probands in families J and K. Unfortunately, for various reasons, no additional family members were available for clinical or genetic screening. In family J, the proband has a known mutation in exon 11. He presented at 14 years of age with a syncopal episode and was found to have precordial T-wave inversion, RV enlargement with mildly impaired systolic function, and frequent ventricular ectopy of RV origin. There is no known family history of premature cardiac death. In the absence of family history, he does not fulfill diagnostic criteria. In family K, the proband carries the novel deletion P533fsX561. She presented at 49 years of age with palpitations. She had frequent PVCs of RV origin on exercise testing and >9000 PVCs on 24-hour Holter monitoring. There was a family history of premature sudden death in 4 first-degree relatives, which may have been secondary to ARVC but is confounded by angiographically confirmed premature coronary disease on the maternal side. Of 9 first-degree relatives over 3 generations, only 3 survive. Further investigations were declined.

Clinical details of all individuals carrying a PKP2 mutation are summarized in Table 2. Resting ECG was normal in all gene-negative family members, whereas arrhythmia on Holter monitoring (>200 extrasystoles in 24 hours) was detected in 17% and wall motion abnormalities on echocardiography or MRI in 8%.

The age at diagnosis was 8 to 45 years (median, 33 years) in the probands with PKP2 mutations and 8 to 68 years (median, 43 years) in probands in whom no gene was identified. Clinical investigations revealed similar findings in individuals carrying a PKP2 mutation and those probands without a mutation (Table 3). Only the incidence of nonsustained VT, which was higher in the heterogeneous group with no mutation identified, reached statistical significance (Table 3).

Discussion

Here, we described 9 PKP2 mutations in 11 familial cases of arrhythmogenic RV cardiomyopathy. These mutations are predicted to disrupt functionally important domains of the PKP2 protein (Table 1).

PKPs are members of the Armadillo protein family and consist of an N-terminal head domain, followed by a sequence of 10 imperfect amino-acid repeats (Arm domain) and a small C-terminal tail.[17] They are localized in both desmo-

TABLE 2. Diagnostic Features in ARVC Families

Individual	Symptoms	Family History (Major)	Family History (Minor)	Repolarization Abnormalities (Minor)	Depolarization/ Conduction Abnormalities (Major)	Depolarization/ Conduction Abnormalities (Minor)	RV Structural Abnormalities on Imaging (Major)	RV Structural Abnormalities on Imaging (Minor)	>1000 PVCs on 24-h Holter	NSVT/VT/VF	Diagnostic Criteria, Major/Minor
Family A											
III:1 (52 y)	Palpitations, no syncope	+	−	+	−	+	+	−	−	VT	2/3†
III:3 (51 y)	−	+	−	+	−	−	−	−	−	−	1/1‡
III:4 (57 y)	Occasional palpitations	+	−	+	−	+	−	+	−	−	1/3†
III:6 (55 y)	Exertional SOB, presyncope	+	−	+	−	−	−	+	−	NSVT	1/3†
IV:3 (27 y)	Palpitations associated with presyncope	+	−	+	−	−	−	+	−	−	1/2†
Family B											
II:2 (52 y)	Palpitations, exertional SOB	+	−	+	−	−	−	−	−	−	1/1‡
III:1 (21 y)	Syncopal episodes	−	−	+	−	+	−	+	−	VT	0/3
Family C											
III:1 (15 y)	Syncope	−	+	+	−	−	+	−	−	VT	1/3†
Family D											
II:2 (78 y)	−	−	+	+	−	−	+	−	−	−	1/2†
II:4 (73 y)	Exertional SOB, palpitations	−	+	−	−	−	−	+	−*	−	0/2‡
II:8 (76 y)	Palpitations	−	+	+	−	+	−	+	+	VT	0/5†
III:3 (48 y)	Palpitations, presyncope	−	+	−	−	−	−	+	−	−	0/2‡
III:5 (40 y)	Palpitations	−	+	−	−	−	−	+	−	−	0/2‡
III:8 (37 y)	Syncope	−	+	−	−	−	−	−	−	−	0/1
III:10 (46 y)	Palpitations, presyncope	−	−	+	−	+	−	+	+	VT	0/4†
III:12 (44 y)		−	+	−	−	−	−	−	−	−	0/1
IV:3 (15 y)	SOB	−	+	−	−	−	−	−	−	−	0/1
Family E											
II:2 (89 y)	−	+	−	−	−	−	N/A	N/A	N/A	N/A	Clinical investigations declined
II:3 (71 y)	−	+	−	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
IV:1 (15 y)	Exertional SOB and sweating, occasional dizzy spells	+	−	−	−	−	−	−	−	−	1/0
Family F											
II:2 (69 y)	−	−	+	+	+	−	+	−	−	−	2/2†
III:2 (43 y)	Cardiac arrest	−	−	+	−	+	+	+	−	VF	1/3†
Family G											
II:2 (37 y)	Palpitations	+	−	+	−	−	+	−	−	−	2/1†
III:1 (19 y)	Palpitations	+	−	−	+	−	−	+	−*	−	2/1†
III:2 (17 y)	Palpitations	+	−	+	−	+	−	+	−	−	1/3†
Family H											
I:2 (97 y)	−	+	−	+	−	−	−	−	−	−	1/1‡
II:1 (56 y)	−	+	−	−	−	−	−	+	−	−	1/1‡
III:2 (27 y)	Atypical dizzy episodes, atypical chest pain	+	−	+	−	+	−	+	−*	−	1/3†
III:3 (20 y)	Syncope	+	−	+	−	−	−	+	−	−	1/2†
III:4 (28 y)	−	+	−	+	+	+	−	−	−	−	2/2†
Family I											
I:1 (74 y)	Palpitations	−	+	−	−	−	−	−	−	−	0/1
II:1 (28 y)	Recurrent syncope	−	−	+	−	−	+	−	+	NSVT	1/3†
Family J											
Proband (14 y)	Palpitations	−	−	+	−	−	+	−	−	−	1/1
Family K											
Proband (52 y)	Palpitations	−	+	−	−	−	−	−	+	−	0/2‡

SOB indicates shortness of breath; NSVT, nonsustained VT, usually detected on ambulatory monitoring or exercise testing (3 beats, 30-second duration at ≥120 bpm); and VF, ventricular fibrillation.

*Those who have >200 PVCs in a 24-hour period, as per proposed modified diagnostic criteria.[8]

†Those who satisfy International Task Force diagnostic criteria.[7]

‡Those who satisfy proposed modified diagnostic criteria.[8]

TABLE 3. Comparison of Clinical Characteristics in PKP2-Positive Individuals Versus Probands in Whom No Mutation Was Identified

Clinical Characteristics	Individuals With PKP2 Mutation (n=33), %	Probands With No Mutation (n=89), %	*P*
ECG T-wave inversion	45	37	NS
ECG QRS dispersion	6	21	NS
ECG ϵ wave	3	10	NS
ECG other (incomplete RBBB or RBBB)	12	8	NS
SAECG-positive late potentials	36	48	NS
Holter >200 PVCs in 24 h	36	8	NS
Holter >1000 PVCs in 24 h	28	37	NS
NSVT	18	55	0.001
VT/VF	18	25	NS
Echo minor	55	71	NS
Echo major	6	19	NS
Abnormal cardiac MRI	60	51	NS
Male	42	52	NS
Family history premature sudden death at <40 y	45	55	NS
Diagnosed on family screening	18	49	NS

RBBB indicates right bundle-branch block; SAECG, signal-averaged ECG; NSVT, nonsustained VT; and VF, ventricular fibrillation. Definitions for ECG findings, SAECG, and rhythm disturbance are as previously defined. Echo minor criteria refer to task force criteria[7] (findings of mild global or regional RV enlargement, mild wall motion abnormalities, or mild impairment of systolic function). Echo major criteria, as per task force definition, include severe global or regional dilation of the RV, severe systolic impairment, or aneurysm formation.[7] Any morphological abnormalities on MRI imaging, including wall motion abnormalities and late enhancement postgadolinium injection, are recorded. For statistical analysis, χ^2 and Student *t* tests were used to compare variables between the 2 groups ($P<0.05$).

somes and nuclei and play an essential role in desmosome formation and cell signaling.[17,19] PKP1 is believed to be important in establishing cell contact and desmosomal plaque size and organization. PKP2 can bind to a large number of desmosomal components, including desmoplakin, plakoglobin, desmogleins 1 and 2, and desmocollins 1a and 2a.[19]

None of the patients investigated in this study had cutaneous involvement. This is consistent with the findings by Gerull et al[18] in which probands carrying PKP2 mutations were apparently free of skin abnormalities. In myocardial cells, PKP2 is the only PKP isoform found in desmosomes, whereas PKP1a seems to be confined exclusively to the nucleus.[20] In contrast, in suprabasal cell layers of epidermis, PKP1a is detected in desmosomal plaques, and PKP2 is located in the nucleus.[21] Therefore, it seems possible that a compensatory mechanism involving Pkp1 may explain the absence of a skin phenotype in individuals carrying a PKP2 mutation. Conversely, mutations in PKP1 result in ectodermal dysplasia/skin fragility syndrome characterized by skin, hair, and nail abnormalities but no cardiac phenotype.[22–24]

Mutations in PKP2 were first described by Gerull et al[18] in a study that evaluated only 2 of a possible 32 kindreds with ARVC. Our study is the first to provide comprehensive clinical evaluation of ARVC families harboring mutations in PKP2. The data show that clinical expression of PKP2 mutations is heterogeneous even among first-degree relatives ranging from a complete lack of symptoms and/or clinical manifestations to a severe disease phenotype.

Retrospective evaluation of genotyped family members exposes the limitations of the currently available diagnostic criteria. Strict adherence to the International Task Force diagnostic criteria would have led us to omit the probands from families J and K from our cohort, although both have been found to carry previously described disease-causing PKP2 mutations. The same applies to many gene-positive relatives with incomplete disease expression who also do not satisfy International Task Force diagnostic criteria. This could potentially lead to a less vigilant follow-up than may be desirable.

Modifications of the International Task Force diagnostic criteria have been proposed to evaluate family members with incomplete disease expression.[8] Use of these modified criteria in our study does expand the diagnostic yield but also leads to false-positives because several family members assumed to be clinically affected were subsequently found to be gene negative. In family G, the twin daughters of the proband I:2 (II:3 and II:4) were asymptomatic, yet II:3 was thought to have RV regional wall motion abnormalities on imaging. The area of abnormal motion, however, was at the point of insertion of a sizeable moderator band, leading to a false-positive diagnosis, which by extension included her monozygotic twin. Both were subsequently confirmed not to carry the disease-causing mutation.

In family H, interpreting the ECG changes on the proband's mother (II:2) led us initially to focus screening to her side of the pedigree. These changes were subsequently explained by the occurrence of a septal infarct secondary to embolization from an atheromatous ectatic left anterior descending artery. Evaluation of the father's (II:1) side of the pedigree identified incompletely penetrant disease.

In family A, subjects III:9 and III:10 were thought to be affected on the basis of family history and RV enlargement (III:9) and frequent PVCs (III:10). The individual diagnostic criteria are sufficiently nonspecific that using only 1 criterion and a positive family history leads to inclusion of phenocopies of ARVC within families. Pulmonary disease, tethering of localized areas of RV as a result of structural variations, other cardiomyopathies, and drug misuse may mimic features of ARVC. In this study, misdiagnoses did not relate to abnormalities on resting ECG but were largely accounted for by ventricular ectopy detected on Holter monitoring and by overinterpretation of wall motion abnormalities on imaging. These data suggest a need to review, yet again, the diagnostic criteria for ARVC and allow the possibility of including genetic information. These cases highlight the benefits of genetic testing of ARVC families for mutations in PKP2 and more broadly in desmosomal genes.

Genotype-phenotype correlations are an attractive prospect in theory but in practice generally disappoint. With increasing awareness that in some families ARVC may affect the left ventricle to a greater or lesser degree, it has been postulated that perhaps the mutated gene, or even the location of the mutation within the gene, would dictate to what degree the

left ventricle is affected. A review of the affected families with PKP2 mutations shows that the majority have evidence of right-sided involvement only. In 4 families, 5 of the 37 affected individuals also have evidence of left ventricular enlargement with mild impairment of systolic function (III:1, family B; III:1, family C; II:2 and III:1, family G; and proband, family J). However, even in these individuals, right-sided changes predominate (although in at least 1 individual, II:2 in family G, ECG changes suggestive of left ventricular disease were present before RV changes developed). It can be suggested therefore that PKP2 mutations are likely to result in predominantly RV disease but that exceptions will occur.

The prevalence of sudden death or documented ventricular tachycardia in gene-positive individuals and those with confirmed ARVC at postmortem was ≈34%. This is obviously difficult to interpret because it may be subject to bias in either direction: Either family members with symptoms and hence a higher likelihood of arrhythmia were more likely to come forward for screening, artificially elevating the proportion, or, equally possible, there may have been substantially more ARVC-related deaths in these families than postmortems confirmed. Of note, there does not appear to be a correlation between left ventricle involvement and a propensity to ventricular arrhythmia because only 2 individuals (III:1, family B, and III:1, family C) presented with both.

The comparisons between the mutation positive individuals and the 89 probands in whom no mutation in desmosomal genes was identified are of limited value because the latter group is likely to comprise patients with a variety of causes for the ARVC phenotype. Interestingly, no statistically significant difference between clinical characteristics in the 2 groups was observed, except for nonsustained VT. However, the increased incidence of nonsustained VT observed in patients with no mutation is likely to reflect symptomatic presentation in probands versus identification on family and genetic screening in the PKP2-positive individuals.

Although the phenotypic expression of a mutation is likely to be multifactorial, evidence from the larger families reported here would suggest a high degree of penetrance, with the lowest rate being 66% for complete penetrance but approaching 100% if subjects with incomplete penetrance are included. The age at which penetrance is defined as complete or incomplete is important but somewhat arbitrary, and the age of disease expression appears to vary quite widely in these families. One proband was diagnosed clinically by 8 years of age (family C), and 2 additional probands died of their disease at 15 years of age with pathologically advanced disease (families E and H), yet 2 of the younger subjects who were clinically unaffected at 13 and 14 years of age now meet diagnostic criteria 4 years later (family G). Serial follow-up of gene-positive young individuals will better define the age and mode of disease expression.

Conclusions

This study provides further evidence that PKP2 is involved in the pathogenesis of ARVC and strengthens the view that ARVC is a desmosomal disorder. The data highlight the need for a more accurate set of diagnostic criteria for ARVC and demonstrate that genetic screening for mutations in PKP2 can be valuable in identifying asymptomatic mutation carriers at risk of developing the disease.

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Disclosures

None.

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CLINICAL PERSPECTIVE

ARVC is an inherited heart muscle disease that classically presents with palpitation and/or syncope in young adults. However, sudden cardiac death is the first manifestation of the disease in 50% of probands, underscoring the importance of evaluating asymptomatic family members. Clinical abnormalities in ARVC include arrhythmia of RV origin, inverted T waves in V_1 through V_3, and structural and functional abnormalities of the RV on imaging. The task force criteria facilitate diagnosis but lack sensitivity for early disease, when clinical findings are often subtle. This limitation has prompted proposal of modified diagnostic criteria for familial ARVC. The underlying rationale is that a single disease feature may be sufficient for diagnosis in first-degree relatives, within the context of autosomal dominant inheritance. Disease-causing mutations have been identified in the cell adhesion proteins plakoglobin, desmoplakin, and most recently, PKP2. This study presents, for the first time, a systematic analysis of genotype-phenotype correlation in families with defects in PKP2. Heterogeneous phenotypic expression was observed among individuals with the same mutation. Strict adherence to the task force criteria would have precluded clinical diagnosis in 2 probands and several relatives with incomplete disease expression. The modified criteria increased the diagnostic yield at the cost of reduced specificity. False-positives arose from inclusion of relatives with frequent ventricular extrasystoles and overinterpretation of normal variants in RV wall motion. The findings therefore highlight the limitations of existing diagnostic guidelines and suggest a key role for genetic analysis in identifying individuals with early disease while simultaneously excluding phenocopies.

Clinical Expression of Plakophilin-2 Mutations in Familial Arrhythmogenic Right Ventricular Cardiomyopathy

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