Chapter 8

Plakophilin-2 Mutations Are the Major Determinant of Familial Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy

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Abstract

Background Mutations in the plakophilin-2 gene (*PKP2*) have been found in patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVC). Hence, genetic screening can potentially be a valuable tool in the diagnostic workup of patients with ARVC.

Methods and Results To establish the prevalence and character of *PKP2* mutations and to study potential differences in the associated phenotype, we evaluated 96 index patients, including 56 who fulfilled the published task force criteria. In addition, 114 family members from 34 out of these 56 ARVC index patients were phenotyped. In 24 of these 56 ARVC patients (43%), 14 different (11 novel) *PKP2* mutations were identified. Four different mutations were found more than once; haplotype analyses revealed identical haplotypes in the different mutation-carriers, suggesting founder mutations.

No specific genotype-phenotype correlations could be identified, except that negative T-waves in V_2 and V_3 occurred more often in *PKP2* mutation carriers ($P<0.05$).

Of the 34 index patients of whom family members were phenotyped, 23 familial cases were identified. *PKP2* mutations were identified in 16 out of these 23 ARVC index patients (70%) with familial ARVC. On the other hand, no *PKP2* mutations at all were found in 11 probands without additional affected family members ($P<0.001$).

Conclusions *PKP2* mutations can be identified in nearly half of the Dutch patients fulfilling the ARVC criteria. In familial ARVC, even the vast majority (70%) is caused by *PKP2* mutations. However, nonfamilial ARVC is not related to *PKP2*. The high yield of mutational analysis in familial ARVC is unique in inherited cardiomyopathies.

INTRODUCTION

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVC) is a disease with primarily right ventricular involvement that is characterized by fibrofatty infiltration of the myocardium and inflammatory infiltrates.[1-7] The clinical presentation is highly variable but characterized mainly by ventricular arrhythmias, syncope and sudden cardiac death (SCD) during various activities.[1,5,8-16] Diagnosis may be difficult in individual cases but is facilitated by criteria proposed by a generally accepted consensus report (task force).[17-20] These criteria are based on morphological, functional, and ECG features in addition to family history.[17] At least 50% of patients have affected relatives, suggesting a genetic basis for ARVC. The mode of inheritance is autosomal

dominant with reduced and age-related penetrance.[20-25]

Until recently, 5 loci and 4 potentially causative genes encoding plakoglobin (*JUP*), desmoplakin (*DSP*), transforming growth factor-β3 (*TGFβ3*), and the cardiac ryanodine receptor (*RYR2*) had been identified. However, only a small subset of ARVC patients showed mutations in these genes.[26-29]

The recent discovery of plakophilin-2 (*PKP2*) mutations in 32 of 120 probands (27%) with ARVC of western European descent suggests an important role of this gene in the pathogenesis of this disorder.[30] Plakophilin-2, an armadillo-related protein, forms with other proteins including plakoglobin and desmoplakin, an integral part of cardiac desmosomes. These major cell adhesion complexes link desmosomal cadherins with desmoplakin and the intermediate filament system, providing structural and functional integrity to adjacent cells.[31] These structures are considered important for rigidity of cells and cell signaling.[32-34] The exact pathogenesis of *PKP2* mutations in ARVC is speculative, but cell-cell contact is believed to be impaired leading to disruption of cardiomyocytes in response to mechanical stretch or stress, particularly in the so-called triangle of dysplasia (right ventricular outflow tract, inferobasal area and apex).[7,30]

If the *PKP2* mutations indeed occur as frequently as recently suggested, genetic screening would constitute an important tool in diagnosing persons at risk for this potentially life-threatening disorder. To evaluate this tool, screening of the *PKP2* gene in a large cohort of Dutch ARVC patients referred to 4 tertiary referral centers throughout the Netherlands was performed. The goals of this study were to establish the prevalence and character of *PKP2* mutations and to study phenotypic differences.

METHODS

Clinical evaluation and diagnostic criteria

Ninety-six white unrelated index patients were evaluated in 4 university hospitals in the Netherlands. A history was taken from all patients, and they were evaluated by physical examination, 12-lead ECG, 24-hour Holter monitoring, exercise testing, and 2-dimensional transthoracic echocardiography. In addition, 60 patients underwent MRI, 46 had nucleotide scintigraphy, 38 had a signal-averaged ECG, 72 underwent left and right ventricular cine-angiography, 62 had an electrophysiologic study, and 27 had a right ventricular endomyocardial biopsy.

The diagnosis of ARVC in index patients was established in accordance with criteria proposed by a task force.[17] To verify the diagnosis, all patients were discussed by experienced cardiologists from the different centers in a

consensus meeting. The onset of ARVC manifestations was defined as the age at which initial symptoms most likely related to ARVC emerged, including paroxysmal tachycardia, prolonged syncope and successful resuscitation.

The local institutional review committees approved of the study. Informed consent was obtained from all participating patients.

The population consisted of 56 index patients (mean age at presentation 35.2 yrs; 14 women) fulfilling the ARVC criteria: ≥ 2 major criteria (n=20), 1 major and 2 minor criteria (n=34), or 4 minor criteria (n=2). In 34 of these index patients, additional family members had been clinically investigated to address the task force criteria. ARVC was considered proven familial when ≥1 additional family members were found to fulfill these criteria. Suspected familial ARVC was defined as having either 1 major and 1 minor or 3 minor criteria in another family member.

In addition, we tested 40 index patients with some ARVC features who did not fulfill the task force criteria.

Mutational analysis

DNA for *PKP2* sequence analysis was isolated from peripheral blood samples according to standard protocols. Most patients were pre-screened using denaturing gradient gel electrophoresis (DGGE) or denaturing high-performance liquid chromatography (DHPLC). Polymerase chain reaction products showing aberrant patterns by DGGE or DHPLC were reamplified and sequenced. DNA from a subset of patients was sequenced completely. The screening included all coding sequences but also 60-100 bp of flanking intronic sequences. Primers and conditions for DGGE and DHPLC are available upon request; primers used for direct sequencing were obtained from Gerull et al.[30] Direct sequencing for both sense and antisense strands was performed by using a BigDye Terminator DNA sequencing kit (version 2.0) on a 3100 Genetic Analyzer (Applied Biosystems, Foster City, Calif) with SeqScape software (version 2.1.1, Applied Biosystems). If a novel mutation was identified, at least 150 ethnically matched control individuals were screened to recognize common polymorphisms. In 9 families (U2, U3, U4, U6, A3, M6, G1, G2 and G6) we were able to study the segregation of 6 different mutations because both clinical data and DNA from family members were available.

Haplotype analysis

To determine whether the identical mutations found are recurrent or have

a common founder, we performed haplotype analyses using 5 repeat markers within a region of 300 000 bp, including the entire genomic region of the *PKP2* gene. For the positioning of the markers related to the human sequence, the August 2004 human reference sequence (NT_009714 region 25602762-25908664 bp), based on NCBI Build 35 version 1, was used. Primers used to amplify these markers are available on request. For each mutation that was found more than once, there was at least 1 index-patient from which additional family members were available for haplotype analysis. This enabled the reconstruction of haplotypes and the verification of the phase. Subsequently, these haplotypes were investigated in other index patients carrying the identical mutation.

Statistical analysis

Clinical characteristics in ARVC patients with and without a *PKP2* mutation were compared by χ^2 test. Values of $P<0.05$ were considered significant. All data were analyzed with the Statistical Package for Social Sciences (SPSS version 12.0; SPSS, Inc., Chicago, Ill).

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

RESULTS

Mutational analyses

Unique sequence variants were identified in 24 of 56 unrelated index patients fulfilling the ARVC criteria (43%) (Table 1 and Figure 1). One patient (U1) had 2 variants. Mutations were identified in 7 of 20 patients (35%) with ≥ 2 major criteria, 16 of 34 (47%) patients with 1 major and 2 minor criteria, and 1 of 2 patients with 4 minor criteria.

In the additional group of 40 patients not fulfilling the criteria, 2 mutations (c.397C>T and c.2386T>C) were identified (5%).

Four different mutations were found more than once: c.235C>T (5 index patients), c.397C>T (2 index patients), c.2386T>C (5 index patients) and c.2489+1G>A (3 patients). Of the 14 different mutations, 4 were missense, 7 were nonsense, 2 were insertion/deletion-frameshift and 1 was a splice site mutation. Eleven of these were novel (Table 1 and Figure 1).

Pathogeneity of the mutations identified

Twelve of 14 sequence variants identified were considered to be disease

Table 1. Overview of *PKP2* mutations in patients fulfilling the ARVC criteria

Index Patient	Mutation	Exon	Amino Acid Change	Novel	Type Mutation
A6	76G>A	1	Asp26Asn	+	Missense: UV
M12	148_151delACAG	1	Thr50_ Val51SerfsX60	+	Deletion; frameshift
A1	**235C>T**	2	Arg79X	-	Nonsense
G1	**235C>T**				
U2	**235C>T**				
A4	**235C>T**				
G6	**235C>T**				
G10	258T>G	2	Tyr86X	+	Nonsense
A3	**397C>T**	3	Gln133X	+	Nonsense
U3	**397C>T**				
U4	1211-1212insT	5	Val 406SerfsX3	+	Insertion; frameshift
G2	1848C>A	9	Tyr616X	+	Nonsense
G19	2028G>A	10	Trp676X	+	Nonsense
A8	2062T>C	10	Ser688Pro	+	Missense
U5	**2386T>C**	12	Cys796Arg	-	Missense
G3	**2386T>C**				
G4	**2386T>C**				
A5	**2386T>C**				
A7	**2386T>C**				
A2	2421C>A	12	Tyr807X	+	Nonsense
M6	**2489+1G>A**	12		-	Splice site
M3	**2489+1G>A**				
U1	**2489+1G>A**				
	184C>A	1	Glu62Lys	+	Missense: UV
U6	2544G>A	13	Trp848X	+	Nonsense

UV indicates unclassified variant. Identical mutations are represented in bold.

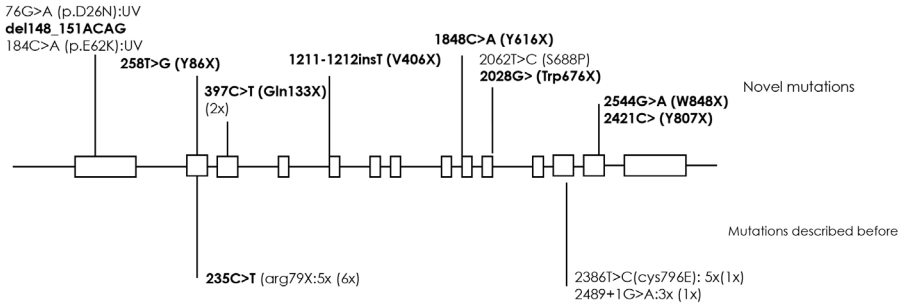

Figure 1. *PKP2* gene structure and (position of the) mutations identified in this study in patients fulfilling the ARVC criteria. Mutations indicated in bold represent truncating mutations, mutations above the schematic gene are novel mutations, mutations below the gene have been described previously. The number after the mutation indicates the number of times that mutation occurred in this study. The numbers in brackets indicate the numbers of times the mutation was found by Gerull et al.[30] UV indicates unclassified variant

causing because of change of charges or predicted major rearrangements of the protein. Moreover, they all affect highly conserved residues. Of the missense mutation c.76G>A (p.Asp26Asn) and the second mutation identified in patient U1, c.184C>A (p.Glu62Lys), the pathogeneity cannot be established with certainty because they were not located in highly conserved regions or amino acids, were located outside functional domains, or did not change the polarity of the amino acid involved. Until functional assays are available, these mutations have to be considered "unclassified variants". None of the 14 variants identified were found in 300 control alleles, thus excluding the possibility of being a common polymorphism. In 9 patients, we studied the segregation of 6 different mutations in family members (c.235 C>T in U2, G1 and G6, c.397C>T in A3 and U3, c.1211-1212insT in U4, c.1848C>A in G2, c.2544G>A in U6, and c.2489+1G>A in M6). No disconcordances were found; ie, affected family members also carried the identical mutation. Furthermore, we identified 2 missense mutations (c.209G>T [5 times] and c.2615C>T [2 times]), that were considered to be polymorphisms. The c.209G>T missense mutation was identified in 4 of 300 control alleles and did not segregate with the disease in relatives of patient U2 whereas the truncating mutation (Arg79X) in that family did. The c.2615C>T mutation is not located in a highly conserved region, nor does it lead to a substantial change in biochemical properties of the amino acid involved. Moreover, this mutation was identified in unrelated patients A3 and U3, both of whom were carriers of a cosegregating truncating mutation (Gln133X).

Haplotype analyses

Haplotype analyses revealed allele sharing among the patients carrying an identical mutation (including patients G7 [c.397C>T] and A10 [c.2386T>C] that did not meet the criteria). The shared alleles were identical in patients having the same mutation, but were (largely) different between the different mutations. The most likely associated haplotypes in all index patients with the different identical mutations are represented in Tables 2 through 5. These data suggest that the frequent mutations are from common founders rather than being recurrent.

Clinical data and comparison between mutation and non-mutation carriers

Index patients with established ARVC were referred because of ventricular tachycardia episodes (n=52), ventricular fibrillation (n=3) and SCD in a sibling with proven ARVC at autopsy (n=1). The age at initial presentation, diagnostic

Table 2: Haplotype (in bold) associated with the c.235C>T mutation

	Index patient									
	U2		A1		A4		G1		G6	
Position										
32.700K	294	**296**	296	**296**	288	**296**	290	**296**	296	**296**
32.830K exon14	207	**215**	207	**215**	217	**215**	207	**215**	205	**215**
32.940K exon 1	259	**261**	259	**261**	259	**261**	261	**261**	263	**261**
32.970K	354	**356**	360	**356**	360	**356**	354	**356**	362	**356**
33.001K	357	**348**	353	**348**	361	**348**	353	**348**	348	**348**

Table 3: Haplotype (in bold) associated with the c.397C>T mutation

	Index patient					
	U3		A3		G7 (criteria -)	
Position						
32.700K	294	**294**	294	**294**	294	**294**
32.830K exon14	215	**207**	217	**207**	209	**207**
32.940K exon 1	261	**261**	259	**261**	259	**261**
32.970K	358	**358**	360	**358**	360	**358**
33.001K	353	**355**	359	**355**	346	**355**

Table 4: Haplotype (in bold) associated with the c.2386T>C mutation

	Index patient											
	U5		A5		A7		A10		G3		G4	
Position							(criteria-)					
32.700K	296	**294**	294	**294**	288	**294**	294	**294**	296	**294**	290	**294**
32.830K exon14	207	**215**	217	**215**	215	**215**	213	**215**	206	**215**	215	**215**
32.940K exon 1	261	**259**	259	**259**	259	**259**	261	**259**	263	**259**	259	**259**
32.970K	362	**360**	358	**360**	364	**360**	366	**360**	358	**360**	364	**360**
33.001K	361	**346**	359	**346**	361	**346**	353	**346**	349	**346**	359	**346**

Table 5: Haplotype (in bold) associated with the c. 2489+1G>A mutation

	Index patient					
	M6		M3		U1	
Position						
32.700K	288	**288**	290	**288**	297	**288**
32.830K exon14	219	**215**	215	**215**	207	**215**
32.940K exon 1	259	**261**	263	**261**	259	**261**
32.970K	360	**356**	356	**356**	358	**356**
33.001K	361	**348**	359	**348**	359	**348**

criteria and the follow-up of individual patients fulfilling the ARVC criteria are presented in Tables 6 and 7 for *PKP2* mutation carriers and patients without a *PKP2* mutation. Comparing *PKP2* mutation carrier and noncarrier ARVC patients showed no significant differences in terms of the average age at initial presentation, occurrence of familial sudden death, and characteristics according to the task force criteria, with the exception of negative T waves in V_2 and V_3 (in 23 of 24 mutation carriers versus 22 of 32 in noncarriers; $P<0.05$). In addition, no significant differences were noted in follow-up duration and number of implantable cardioverter-defibrillators (Table 8). In all patients with implantable cardioverter-defibrillator therapy, additional anti-arrhythmic drug treatment was used. All other patients were treated only with drugs.

Finally, the occurrence of end-point events (documented sustained ventricular tachycardia episodes, ventricular fibrillation, appropriate implantable cardioverter-defibrillator therapy, successful resuscitation and SCD) did not reach significance between the groups.

Figure 2 shows a flowchart of investigation of family members in relation to the yield of molecular analyses. Briefly, in 34 of the 56 index patients fulfilling the ARVC criteria, cardiologic evaluation of family members had been carried out previously. Twenty-three (68%) had familial ARVC (per index patient, average 3.1; range 1-12 family members analyzed). In 11 index patients, no

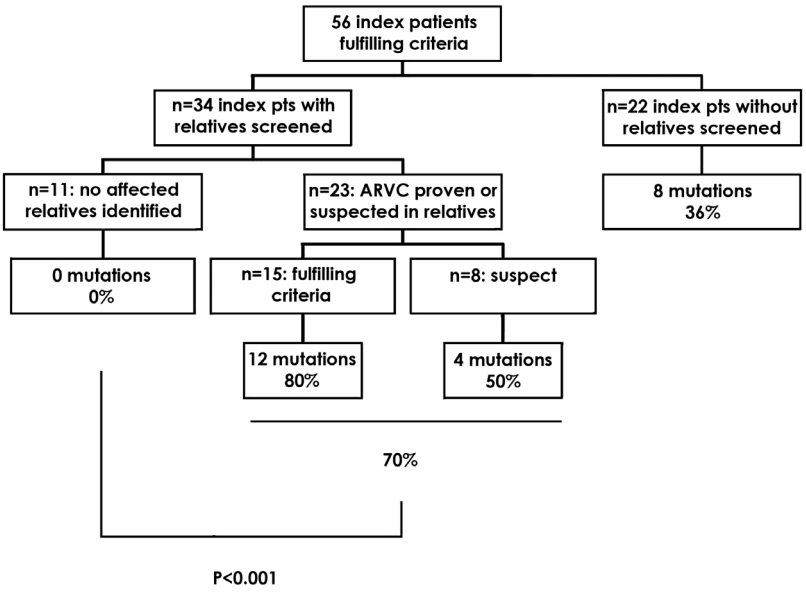

Figure 2. Yield of mutational analyses in relation to the outcome of family screening.

Table 6: Clinical characteristics of ARVC probands with PKP2 mutations.

Index patient	Sex	Mutation	Age at onset, y	Structural and functional changes	Tissue characterization	Abnormal repolarization (negative T-wave V_2-V_3)	Abnormal depolarization	Arrhythmias	Family history	Age of family members with SCD (degree related), y	additional family members evaluated (ARVC+, ARVC±), n	Follow-up , y	End points , y
G1	m	235C>T	27	na	++	+	++	+			5(1;1)	0	SCD 27
G2	f	1848C>A	31		++	+	+	+		36 (1st)	5(0;2)	1	
G3	m	2386T>C	33	++	++	+	+	+	+		3(0;1)	5	↯37
G4	m	2386T>C	30	+	++	+		+			4(0;2)	10	SusVT, ↯39
G6	m	235C>T	43	+	na	+	++	+	+	15 (1st)	1(1;0)	1	
G10	m	258T>G	41	++	na	+	++	+			0	14	susVT
U1	m	2489+1G>A;184C>A	20	+	na	+	++	+			1(1;0)	24	susVT
U2	m	235C>T	22	++	na	+		+	+	47(1st)	9(2;1)	33	susVT
U3	m	397C>T	28	++	na	+		+	++	17(3rd)	12(5;1)	35	susVT
U4	m	1211-12insT	35	++	na	+		+	++	17(1st)	4(3;1)	9	susVT
U5	f	2386T>C	40	++	na	+	++	+			5(1;1)	11	susVT
U6	m	2544G>A	35	++	na	+		+	+		1(1;0)	4	susVT
A1	f	235C>T	40	++	na	+		+			0	6	susVT
A2	f	2421c>A	30	+	na	+		+	++	18(1st)	2(1;1)	4	
A3	f	397C>T	50	++	na			+	+		1(1;0)	1	
A4	m	235C>T	29	++	na	+		+			0	5	
A5	f	2386T>C	37	+	++	+		+	+		1(0;1)	27	susVT,VF 55
A6	m	76G>A	39	++	na	+		+			0	11	susVT
A7	m	2386T>C	21	+	na	+		+	++	39(1st) 17(2nd)	0	1	susVT
A8	f	2062T>C	42		na	+	+	+	+		2(1;0)	5	↯ 43; ↯47
G19	m	2028G>A	38			+	++	+			0	8	susVT
M3	m	2489+1G>A	17	++	na	+	+	+			0	3	↯19
M6	m	2489+1G>A	17	++	na	+		+	++	16(1st) 16(2nd)	1(1;0)	7	susVT, ↯↯ 24
M12	m	c.148_151delACAG	32	++	na	+		+				13	↯35,37

++ indicates major criterion; +, minor criterion; ↯, appropriate implantable cardioverter-defibrillator discharge; ARVC+, patients fulfilling ARVC criteria; ARVC±, suspected ARVC; na, not available; SCD, sudden cardiac death; susVT, sustained ventricular tachycardia; and VF, ventricular fibrillation. Only the presence of a feature is indicated.

Table 7: Clinical characteristics of ARVC probands *without* PKP2 mutations.

Index patient	sex	Age at onset, y	Structural and functional changes	Tissue characterization	Abnormal repolarization (negative T-wave V_2-V_3)	Abnormal depolarization	Arrhythmias	Family history	Age of family members with SCD (degree related), y	additional family members evaluated (ARVC+; ARVC±), n	Follow-up, y	End points, y
U10	m	34	++	na	+	++	+			2(1;0)		susVT
U11	m	51	++	na	+	++	+	+		2(0;0)	6	susVT
U16	m	16	++	++			+			0	12	Died VF (28)
U9	m	40	++	na		++	+			4(0;0)	11	susVT
U14	m	33	++	na	+	++	+	++	35 (1st)	1(1;0)	11	susVT
U7	m	69	++	na		++	+			3(0;0)	8	
U18	m	43	++	na	+		+			9(0;0)	10	
G7	f	39	++	++	+		+	+		1(0;1)	1	↗39
G8	m	17	++	++	+		+			3(0;1)	8	susVT
G9	m	30	++	na	+	++	+	+	34 (1st)	0	11	
A9	m	28	++	na		++	+			0	11	susVT
U17	f	17	++	na	+		+			2(0;0)	13	susVT
U15	m	12	++	na	+		+	+	9 (1st)	2(0;0)	22	susVT
U19	f	41	++	na	+		+			9(0;0)	11	
G11	f	22		++	+	+	+			5(0;2)	6	susVT, ↗23
G12	m	46		++	+	+		+	43 (1st)	1(0;0)	1	
									32 (3rd)			
G13	m	58	+	++	+	+	+			6(0;0)	4	susVT
G14	m	38		++			+	+	33 (1st)	0	6	
G15	m	58	+	++		+	+			2(0;0)	3	
G16	m	22		++	+		+			0	9	susVT
G17	f	44	++	na	+		+			0	6	
A11	f	41		na	+	++	+			0	4	
A12	m	36	++	na	+		+	+		1(0;1)	1	
A13	m	29	++	na	+		+			0	5	↗32
A14	m	43	++	na	+		+			0	5	
G18	m	48	+		+	+	+			0	5	
A15	m	47	+	na		++	+			3(0;0)	2	
A16	m	48	++	na		++	+			0	6	
A17	f	58	+	na		++	+				1	susVT
M1	m	24	++	na	+	++	+			0	9	susVT
M4	m	21	++	na				++	39,20,21 (all 1st)		12	
M7	m	46	++	na	+	++	+				9	susVT

Abbreviations as in Table 6. Only the presence of a feature is indicated.

Table 8: Comparison of ARVC patients with and without PKP2 mutations

	PKP+ (n=24)	PKP- (n=32)	P
Sex (M/F)	17/7	25/7	NS
Mean ±SD age of initial symptoms, y	32.3±8.8	37.3±14	NS
negative T-waves in V_2 and V_3, n (%)	23 (96%)	22 (69%)	<0.05
Index patients with family members screened, n (%)	16 (67%)	18 (56%)	NS
Average family members investigated per index patient, n	3.1	3.9	
familial ARVC, n (%)	16/16 (100%)	7/18 (39%)	
Index patients with SCD ≤35 y in family history, n (%)	6 (25%)	6 (19%)	NS
Mean follow-up (range), y	9.9 (0-35)	7.8 (1-22)	NS
ICDs implanted, n (%)	17 (71%)	19 (59%)	NS
Appropriate ICD therapy, sustained VT, VF, or SCD, n (%)	19 (79%)	17 (53%)	NS

ICD indicated implantable cardioverter-defibrillator; VT, ventricular tachycardia; and VF, ventricular fibrillation.

affected family members could be identified (average, 3.9; range 1-9 family members per index patient analyzed).

In the absence of familial disease, no *PKP2* mutations were identified, whereas in familial forms of ARVC, a *PKP2* mutation was found in 16 of 23 index patients (70%) (P<0.001).

DISCUSSION

Prevalence and spectrum of PKP2 mutations in ARVC patients

In the population studied, 14 different mutations were identified in 24 of 56 patients fulfilling the ARVC criteria (43%). Gerull et al.[30] identified 25 different mutations in 32 of 120 probands (27%) fulfilling the task force criteria. In both studies, the majority of mutations results in a truncated or aberrant protein as a result of insertion-deletion, nonsense or splice site mutations. These results highlight the importance of the *PKP2* gene in the pathogenesis of ARVC in the Dutch population.

The fact that 10 of 14 *PKP2* mutations are predicted to result in a truncated protein product suggests a loss of function, resulting in haploinsufficiency. On the other hand, missense mutations giving amino acid substitutions, most likely result in protein variants with defects in protein function(s) and/or instable protein products. Moreover, these nonfunctional variants might interfere with the function of the normal allele product. Because *PKP2* has been shown to form an essential component of desmosomes, the functional consequences

of mutations can be expected at the level of desmosome formation and consequential effects in cell-cell adhesion and signalling.[31,33,34]

Recurrent or founder mutations?

Three mutations from the initial study by Gerull et al.[30] were supposed to be recurrent (c.235C>T, c.2146-1G>C and c.2203C>T; identified 6, 2 and 2 times respectively) because no shared alleles were identified. Interestingly, one of their recurrent mutations (c.235C>T) was found 5 times in this study. In contrast to their results, our haplotype analyses did show allele sharing in all 5 index patients carrying this mutation, suggesting a common founder. It should be noted that in this study parental genotypes were only partially available as well. The occurrence of founder mutations in the Dutch population, also in inherited cardiological disorders, is clearly recognized.[35-36] The population reported by Gerull et al.[30] is likely to be more heterogeneous as they indicate it is from western European descent. In addition, for the c.397C>T, c.2386C>T and c.2489+1C>A mutations that we identified 3, 6 and 3 times, respectively, our results suggest a founder rather than a recurrent mutation.

Penetrance and variable phenotypes

Hamid et al.[24] already recognized that 11% of relatives from ARVC index patients were found to have isolated minor cardiac abnormalities, most often T-wave inversion in the right precordial leads. These persons, however, did not fulfill the task force criteria. Given the mode of inheritance, Hamid et al. suggested that the ECG abnormalities are likely to represent early disease. Nava et al.[37] also ascertained less severe clinical forms in family members with a more favorable outcome than previously thought. They also considered subtle ECG and echocardiographic abnormalities diagnostic for ARVC. Given the clinical variability and reduced and age-dependent penetrance of ARVC, the clinical criteria lack sensitivity in family members.[24,37]

This variable expression of the *PKP2* gene was also confirmed in our additional patient group that did not fulfill the ARVC criteria because 2 ARVC-related mutations were identified in these 40 patients. Although the clinical course of the disease can not be predicted from DNA analysis, it helps in identifying those persons at risk for developing disease-related symptoms. In those individuals, regular follow-up is advisable.

Genotype-phenotype relationships

No specific genotype-phenotype correlation could be detected in the group of patients carrying a *PKP2* mutation. This may be due to small sample

size; however, given the intrafamilial variability major genotype-phenotype correlations were not expected.[24,37] Moreover, the presence or absence of a *PKP2* mutation could not be related to age at onset, events during follow-up, or any specific clinical manifestation, except for negative T-waves in V_2 and V_3 that were more frequently encountered in mutation carriers.

Regular screening of family members of the index patients was strongly advised and started already years before the DNA analyses were initiated. Nevertheless, family members from 22 index patients fulfilling the ARVC criteria have not been evaluated for various reasons. In the other 34 patients, 114 additional family members underwent cardiological screening to evaluate manifestations of ARVC. In 70% of these index patients with affected family members, a *PKP2* mutation was identified, whereas no mutations were found in 11 index patients in whom investigation of family members revealed no signs of ARVC ($P<0.001$). This high yield of mutations in familial ARVC underscores the importance of this gene in the pathogenesis of inherited forms of this disease. On the other hand, the absence of *PKP2* mutations in nonfamilial ARVC cases suggests the possibility of a nongenetic origin, eg, myocarditis or alternatively a spontaneous mutation in another gene. [38,39]

Finally, all 5 index patients with familial ARVC and sudden death in family members <35 years of age appeared to be *PKP2* mutation carriers.

Study limitations

Patients referred to tertiary referral centers probably reflect the more severe end of the disease spectrum; therefore, the population studied cannot be regarded as representative of the variable expression of the disease. The familial character of ARVC could be established only in family members who voluntarily agreed to cardiological examination after genetic counseling. Different reasons, eg, psychological or socioeconomic, might underlie the choice to refrain from participation in screening, leading to a bias in family evaluation.

The evaluation and interpretation of data obtained after cardiological investigation and interpretation of criteria may vary between different centers. To prevent this, consensus meetings were organized, with all centers participating.

In 15 ARVC patients (5 *PKP2* mutation carriers and 10 non-carriers) the *RYR2* gene was excluded as the causative gene. Because patients not carrying a *PKP2* mutation and *PKP2* mutation carriers might have mutations in other ARVC-related genes that have not been screened, this is a potential

limitation. In DNA analysis, large rearrangements (duplications/deletions) of a gene can be missed by DGGE, DHPLC, or sequencing analysis. Besides, mutations in the promotor region of the gene can not be excluded. Given the size of the control group, it can not be completely excluded that a certain mutation represents a low-frequency polymorphism.

Conclusions

PKP2 mutations can be identified in nearly half of Dutch patients fulfilling the ARVC task force criteria. However, in familial ARVC, mutation detection increases up to 70% of patients, and even up to all cases of familial ARVC combined with a positive family history of premature SCD. This remarkably high yield of *PKP2* mutations demonstrates its predominance in the genetic origin of ARVC and is unique in inherited cardiomyopathies. However, in nonfamilial ARVC, *PKP2* mutations were absent, suggesting a different origin.

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