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Penetrance of Mutations in Plakophilin-2 Among Families With Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy

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OBJECTIVES The purpose of our study was to characterize the penetrance of *PKP2* mutations among family members of people with arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) and to examine clinical features and predictors of disease among *PKP2* mutation carriers.

BACKGROUND Arrhythmogenic right ventricular dysplasia/cardiomyopathy is an inherited cardiomyopathy characterized by fatty-fibrous myocardial replacement of the right ventricle, ventricular arrhythmias, and right ventricular dysfunction. Mutations in *PKP2*, the gene encoding plakophilin-2, are found in 11% to 43% of ARVD/C probands.

METHODS The study population was composed of 64 individuals in 9 families with an ARVD/C proband previously shown to carry a pathogenic *PKP2* mutation. The diagnosis of ARVD/C was established based on task force criteria (TFC) set by the European Society of Cardiology.

RESULTS In addition to the probands, *PKP2* mutations were present in 52% of relatives screened. Forty-nine percent of *PKP2* mutation carriers met TFC. Among mutation carriers who did not meet full TFC, 50% met at least some TFC criteria besides family history. Pedigrees showed wide intra-familial variability, ranging from severe disease with early death to individuals who were completely asymptomatic late in life. Male *PKP2* mutation carriers were more likely to have structural and conduction abnormalities as determined by imaging studies, signal-averaged electrocardiography, and 24-h ambulatory electrocardiography ($p < 0.05$).

CONCLUSIONS *PKP2* mutations in a group of North American families with ARVD/C have both reduced penetrance and variable expressivity. Gender may have an influence on penetrance of *PKP2* mutations, with male mutation carriers more likely to develop specific phenotypic manifestations of this disease. (J Am Coll Cardiol 2006;48:1416–24) © 2006 by the American College of Cardiology Foundation

Arrhythmogenic right ventricular (RV) dysplasia/cardiomyopathy (ARVD/C) is an inherited cardiomyopathy characterized histologically by fibro-fatty myocardial replacement of the RV and clinically by ventricular arrhythmias and RV dysfunction (1,2). Patients with ARVD/C typically present in their mid-teens to mid-forties with symptomatic ventricular tachycardia (VT) of a left bundle branch block morphology (3). Sudden cardiac death may be the first manifestation of the disease (3–5). Clinical diagnosis 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 take into account arrhythmic, electrocardiographic, structural, and histopathologic abnormalities, as well as family history (6).

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Arrhythmogenic RV dysplasia/cardiomyopathy is a genetic disorder transmitted with reduced penetrance and variable expressivity. To date, 6 genes have been identified with mutations causing ARVD/C. Both dominant and recessive forms of ARVD/C are associated with mutations in *DSP*, encoding a desmosomal protein, desmoplakin (7,8). Atypical forms of ARVD/C are caused by mutations in plakoglobin (9) and the cardiac ryanodine receptor (10). Altered expression of TGF-beta-3 has been identified in 2 families with dominant ARVD/C (11). Mutations in *PKP2*, encoding plakophilin-2, result in a dominant form of ARVD/C (12). The role of mutations in *PKP2* has been well established in three different cohorts of ARVD/C probands across the world (12–14). The proportion of *PKP2* mutations among apparently unrelated individuals with ARVD/C ranges between 11% and 43%. On the basis of the hypothesis that ARVD/C is caused by mutations in genes encoding components of the cardiac desmosome, 2 groups have recently identified mutations in ARVD/C patients in a fourth desmosome gene, *DSG2*, encoding desmoglein-2 (15,16).

One family-based investigation has reported the penetrance of the disease among *PKP2* mutation carriers to be

Abbreviations and Acronyms

ARVD/C = arrhythmogenic right ventricular dysplasia/cardiomyopathy
DNA = deoxyribonucleic acid
ECG = electrocardiogram
ICD = implantable cardioverter-defibrillator
RV = right ventricle/ventricular
SAECG = signal-averaged electrocardiogram
TFC = task force criteria (for diagnosis of ARVD/C)
VT = ventricular tachycardia

47%, although the influence of age and gender on clinical expression was not reported (14). The purpose of our study is to further characterize the penetrance and clinical features of *PKP2* mutations in a North American cohort of families, and to examine the influence of age and gender on the clinical manifestation of the disease among family members of ARVD/C patients with *PKP2* mutations.

METHODS

Study population. The study population was composed of 64 individuals in 9 ARVD/C families previously shown to carry a pathogenic *PKP2* mutation (13). The proband in each family met criteria set by the Task Force of the Working Group of Myocardial and Pericardial Disease of the European Society of Cardiology (6). Through an iterative process, clinical evaluation and/or review of medical records and mutation screening was performed on all available first-degree relatives of individuals carrying a *PKP2* mutation. All subjects gave written informed consent to participate in this study, which was approved by a Johns Hopkins School of Medicine Institutional Review Board.

Patient evaluation and clinical testing. Subjects were interviewed to determine family structure and family history of ARVD/C. The medical history of each subject was obtained by review of medical records, clinical evaluation, and patient interview. Pedigrees for each family were constructed on the basis of this information.

The clinical protocol for screening relatives included 12-lead electrocardiogram (ECG), signal-averaged electrocardiogram (SAECG), 24-h Holter monitoring, 2-dimensional echocardiography, exercise tolerance testing, and cardiac magnetic resonance imaging. Cardiac biopsy was recommended for relatives whose non-invasive testing was suggestive but not diagnostic of ARVD/C. Information regarding symptoms, sustained VT, diagnosis of ARVD/C, sudden cardiac death, and other causes of death was recorded by age.

The results of noninvasive testing were obtained in those diagnosed while living and in family members. QRS duration and the duration of the S-wave upstroke on a 12-lead ECG were measured with the image analysis software SigmaScan Pro (Version 5.0) (17). The presence of epsilon waves and the distribution of T-wave inversions on the ECG were also determined (17). Signal-averaged ECGs using time-domain analysis with a bandpass filter of 40 Hz were evaluated in subjects who did not have a pre-existing complete or incomplete right bundle branch block pattern. The SAECG was considered positive for late potentials if any 2 of the following were present: filtered QRS duration >114 ms, low-amplitude signal duration >38 ms, or RMS <20 μV (18). The results of Holter monitoring, baseline ECG, and exercise stress test were used to determine the presence of sustained or non-sustained VT as well as the morphology of ventricular ectopy and/or VT. The severity and extent of RV dysfunction was determined by imaging studies.

Diagnosis of ARVD/C. The diagnosis of ARVD/C was established based on the criteria set by the Task Force of the Working Group of 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 (6) , as listed in Table 1. The diagnosis is established by the presence of 2 major criteria, 1 major criterion plus 2 minor criteria; or 4 minor criteria. The patients were classified as having ARVD/C when they met the full criteria. Family members who satisfied an incomplete set of criteria were noted, but not considered to have been diagnosed with ARVD/C. Autopsy diagnosis was established in subjects whose first clinical presentation was death. Those with the recommended gross as well as histopathologic evidence of ARVD/C were considered to be diagnostic of ARVD/C (5).

Mutation screening. *PKP2* mutation identification of index cases in each pedigree has been described (13). For each available family member, genomic deoxyribonucleic acid (DNA) was extracted from leukocytes present in whole blood using QIAmp DNA blood maxi kits (Qiagen, Inc., Valencia, California). Intronic *PKP2* primers flanking each exon were used as previously described (12). For mutations that result in gain or loss of a restriction enzyme digest site, all family members were compared with the mutation carrier for presence or absence of the mutant allele by restriction enzyme digest of polymerase chain reaction amplicons using intronic primers flanking the exon with the mutation. All other mutations were assessed by bidirectional sequence analysis using an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, California) and chromatograms were analyzed with Sequencher 4.1 software.

Statistical analysis. Qualitative data obtained as recommended by the task force criteria (TFC), were expressed as frequency (%). Continuous variables were expressed as mean $\pm$ SD. A two-tailed chi-square or Fisher exact test was used for categorical variables and a two-tailed t test was used for continuous variables to test the null hypothesis of no difference between subgroups of patients.

Kaplan-Meier analysis was used to examine the freedom from the following events since birth (i.e., by age): 1) any symptom consistent with ARVD/C, 2) meeting TFC for ARVD/C, 3) symptomatic VT, and 4) death. This analysis was performed on individuals from all pedigrees and repeated after exclusion of the probands. All statistical anal-

Table 1. Task Force Criteria for the Diagnosis of ARVD/C

I. Global and/or regional dysfunction and structural alterations*
  MAJOR
    — Severe dilation and reduction of right ventricular ejection fraction with no (or only mild) left ventricular impairment
    — Localized right ventricular aneurysms (akinetic or dyskinetic areas with diastolic bulging)
    — Severe segmental dilatation of the right ventricle
  MINOR
    — Mild global right ventricular dilatation and/or ejection fraction reduction with normal left ventricle
    — Mild segmental dilatation of the right ventricle
    — Regional right ventricular hypokinesis
II. Repolarization abnormalities
  MINOR
    — Inverted T-waves in the right precordial leads (V_2 and V_3) of a 12-lead electrocardiogram (in individuals aged more than 12 years of age; in absence of right bundle branch block)
III. Depolarization/conduction abnormalities
  MAJOR
    — Epsilon waves or localized prolongation (>110 ms) of the QRS complex in right precordial leads (V_1–V_3) of a 12-lead electrocardiogram
  MINOR
    — Late potentials on a signal averaged electrocardiogram
IV. Arrhythmias
  MINOR
    — Left bundle branch block type ventricular tachycardia (sustained or non-sustained) on ECG, Holter, or exercise tolerance testing
    — Frequent ventricular extrasystoles (more than 1,000/24 h) on a Holter monitor
V. Tissue characterization of walls
  MAJOR
    — Fibrofatty replacement of myocardium on endomyocardial biopsy
VI. Family history
  MAJOR
    — Familial disease confirmed at necropsy or surgery
  MINOR
    — Familial history of premature sudden death (<35 yrs) due to suspected right ventricular dysplasia/cardiomyopathy
    — Familial history (clinical diagnosis in a family member based on present criteria)

*Detected by echocardiography, angiography, magnetic resonance imaging, or radionuclide scintigraphy.
ARVD/C = arrhythmogenic right ventricular dysplasia/cardiomyopathy; ECG = electrocardiogram.

yses were performed using the STATA statistical software (version 8.2, STATA Corp., College Station, Texas). A p value of <0.05 was considered statistically significant.

RESULTS

Prevalence of ARVD/C in *PKP2* mutation carriers. Eight different *PKP2* mutations (1 recurrent) were present in the 9 unrelated families screened. Family pedigrees indicating mutation status and significant cardiac events in subjects are shown in Figure 1. In addition to the 9 probands, *PKP2* mutations were present in 27 of the 52 genetically screened relatives (52%). Families A, E, and I each included 1 obligate mutation carrier. The obligate carrier in family A (A:I:2) was the brother of a *PKP2* mutation carrier and father of 4 *PKP2* mutation carriers; his spouse did not carry the *PKP2* mutation. He experienced sudden cardiac death during exercise at age 36. Families E and I included deceased identical twins who were obligate carriers (E:III:1 and I:II:3). In total, 39 of the 64 ascertained individuals (61%) were determined to have a *PKP2* mutation. Nucleotide and amino acid changes associated with these mutations are presented in Table 2.

Clinical and genetic data on each of the 39 *PKP2* mutation carriers are presented in detail in Table 2. Four family members (A:II:1, A:I:1, B:II:2, I:II:1) declined to undergo comprehensive clinical screening. Of the remaining 35 *PKP2* mutation carriers, 17 (49%) were diagnosed with ARVD/C on the basis of either the TFC or gross and histopathologic evidence on autopsy. With exclusion of probands, 8 of the 26 mutation carriers (31%) were diagnosed with ARVD/C. The obligate carrier in family A (A:I:2) died suddenly at age 36, but his autopsy, performed almost 40 years ago, was inconclusive for ARVD/C.

Clinical features of *PKP2* mutation carriers. As shown in Table 2, 19 of the 39 *PKP2* mutation carriers (49%) experienced symptoms associated with ARVD/C. The most common symptoms included palpitations, presyncope, and syncope. Among the 17 patients diagnosed with ARVD/C, 13 (76%) had symptoms related to ARVD/C. These included 8 of the 9 probands (89%) and 5 of the 8 family members (63%). Among the remaining 22 *PKP2* mutation carriers, 6 experienced symptoms (27%), including palpitations (n = 4), syncope and palpitations (n = 1), and sudden death (n = 1).

Among those 18 *PKP2* mutation carriers with comprehensive screening who were not diagnosed with ARVD/C, 9 (50%) had evidence of incomplete penetrance or variable expressivity of the *PKP2* mutation by meeting at least some TFC other than family history. Three individuals met a single minor criterion, 4 met two minor criteria, and 2 *PKP2* mutation carriers met one major criterion for ARVD/C other than criteria for family history. Among the 9 *PKP2* muta-

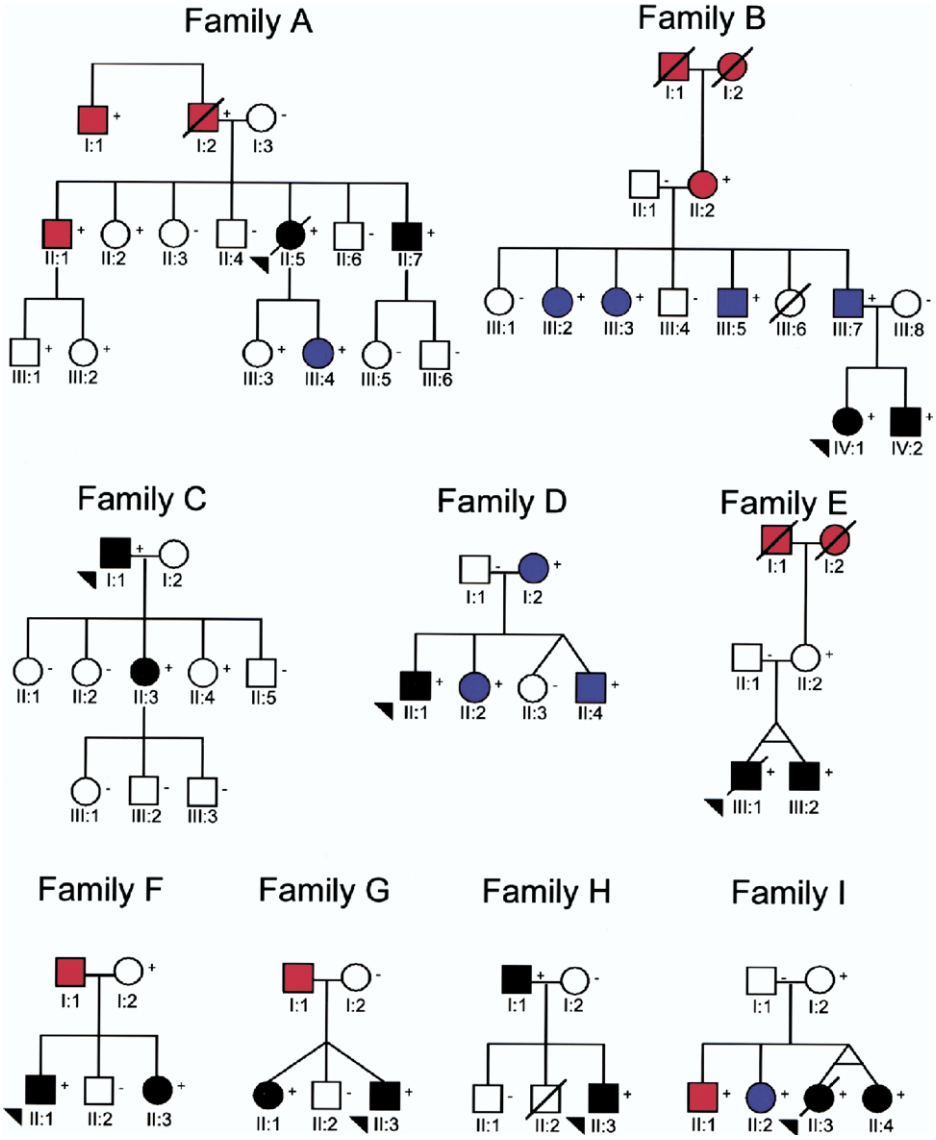

Figure 1. Pedigrees of families with a *PKP2* mutation. **Circles** and **squares** indicate women and men, respectively. **Filling in the circles/squares** indicates the phenotypes of individuals: **white** = unaffected; **black** = affected; **blue** = fulfill an incomplete set of task force criteria; and **red** = inadequate clinical information available. + indicates the presence of a *PKP2* mutation and − indicates the absence thereof. **Arrowhead** indicates the proband of the family. **Slanting bar** indicates a deceased individual.

tion carriers that did not meet any criteria for ARVD/C other than family history, 4 of these individuals were age 20 years or younger.

Among the 39 *PKP2* mutation carriers, 35 were living at most recent follow-up. Four had died from ARVD/C (3 of sudden cardiac death, 1 of biventricular heart failure). Two subjects had received a heart transplant, 1 (C:I:1) at age of 67 because of incessant VT and the other (F:II:1) at age 33 from heart failure and incessant VT. Except for 1 case of sudden death, each of these outcomes occurred in a family's proband.

Penetrance of *PKP2* mutations is age-dependent. Figure 2 presents Kaplan-Meier curves documenting the age dependence of the clinical course of *PKP2* mutation carriers. Figure 2A shows freedom from: 1) symptoms, 2) diagnosis (meeting TFC), 3) ventricular tachycardia, and 4) death in all *PKP2* mutation carriers. The line indicating diagnosis by TFC excludes subjects with incomplete clinical testing. Figure 2B excludes all probands.

For probands and family members, development of symptoms, diagnosis, symptomatic VT, and death all appear to be age-dependent phenomena with incomplete penetrance even at an advanced age.

Influence of gender on penetrance of *PKP2* mutations. Although 10 of 15 (67%) of male *PKP2* mutation carriers who were comprehensively evaluated were diagnosed with ARVD/C, only 7 of 20 (35%) of female mutation carriers met TFC ($p = 0.06$). This trend of gender dependence bordering on statistical significance led us to investigate different measures of severity of the disease between the 2 genders. Table 3 shows the results of cardiac testing for ARVD/C among male and female *PKP2* mutation carriers

Table 2. Clinical and Genetic Characteristics of Subjects With *PKP2* Mutations

Case	Nucleotide Change	Amino-Acid Change	Gender	Presenting Symptom	Age	Fam FH	Depol EW	Depol QRS	Depol LP	Depol DSW	Repol TWI	Arrhyth LBBB	Arrhyth PVC	Struct G.Dil	Struct Aneu	Struct WMA	HP
A, I, 1	1642delG	V548fsX562	M	Asymptomatic	70	HP						−		None	−	−	
A, I, 2	1642delG	V548fsX562	M	SCD	36	−											
A, II, 1	1642delG	V548fsX562	M	Asymptomatic	44	HP						−		None	−	−	
A, II, 2	1642delG	V548fsX562	F	Palpitations	50	HP	−	−	−	−	V_1	−		None	−	−	
A, II, 5	1642delG	V548fsX562	F	Syncope	36	SCD	−	−		+	V_1–V_4	+	+	Mod	−	+	+
A, II, 7	1642delG	V548fsX562	M	Palpitations	37	HP	−	+	−	+	V_1–V_3	−	+	Mild	−	−	
A, III, 1	1642delG	V548fsX562	M	Asymptomatic	20	HP			−		V_1	−		None	−	−	
A, III, 2	1642delG	V548fsX562	F	Palpitations	17	HP	−	−	−	−	V_1	−		None	−	−	
A, III, 3	1642delG	V548fsX562	F	Asymptomatic	16	HP	−	−	−	−	V_1	−	−	None	−	−	
A, III, 4	1642delG	V548fsX562	F	Asymptomatic	13	HP	−	−	−	−	V_1–V_2	−	−	None	−	+	
B, II, 2	2011delC	P671fsX683	F	Asymptomatic	83	TFC											
B, III, 2	2011delC	P671fsX683	F	Asymptomatic	54	TFC	−	+	+	+	—	−					
B, III, 3	2011delC	P671fsX683	F	Syncope, palpitations	53	TFC	−	−		+	V_1–V_4	−		None	−	−	
B, III, 5	2011delC	P671fsX683	M	Asymptomatic	43	TFC	−	+	+	+	—	−		None	−	−	
B, III, 7	2011delC	P671fsX683	M	Palpitations	51	TFC	−	−	+	−	V_1	−	−	Mild	−	−	
B, IV, 1	2011delC	P671fsX683	F	Palpitations, nausea	21	−	−	−	+	+	V_1–V_4	+	+	Mild	+	−	−
B, IV, 2	2011delC	P671fsX683	M	Asymptomatic	23	TFC	+	+	+	+	V_1–V_4	−	+	Mild	−	+	−
C, I, 1	145-148delCAGA	S50fsX110	M	Palpitations, near syncope, nausea	67	−	−	−	+	−	V_2–V_4	+	+	Sev	+	+	+
C, II, 3	145-148delCAGA	S50fsX110	F	Near syncope	42	HP	+	−	+	+	V_1–V_3	+	+	Mild	−	−	
C, II, 4	145-148delCAGA	S50fsX110	F	Asymptomatic	37	HP	−	−	−	+	V_1–V_2	−	−	None	−	−	
D, I, 2	2509delA	V837fsX740	F	Asymptomatic	48	TFC			−		V_1–V_3	−		None	−	−	
D, II, 1	2509delA	V837fsX740	M	Palpitations, VT	25	−	−	−	+	−	V_1–V_6	+	+	Mod	−	−	−
D, II, 2	2509delA	V837fsX740	F	Palpitations	21	TFC	−	−	−	+	V_1–V_3	−	−	Mild	−	−	−
D, II, 4	2509delA	V837fsX740	M	Asymptomatic	15	TFC	−	−	−	+	V_1–V_4	−	−	Mild	−	+	−
E, II, 2	2146-1G→C	abn splice product	F	Asymptomatic	56	HP			−		V_1–V_2	−	−	None	−	−	
E, III, 1	2146-1G→C	abn splice product	M	Presyncope	19	−								Mod			+
E, III, 2	2146-1G→C	abn splice product	M	Presyncope	28	HP	−	+	+	+	V_1–V_4	+	+	Mild	−	+	
F, I, 2	2146-1G→C	abn splice product	F	Asymptomatic	62	HP	−	−	−	+	—	−	−	None	−	−	
F, II, 1	2146-1G→C	abn splice product	M	VT	33	−	+	+	+	+	V_1–V_5	+	+	Sev	−	+	+
F, II, 2	2146-1G→C	abn splice product	F	Palpitations	36	HP	−	+	−	−	V_1–V_2	−	−	None	−	−	
G, II, 1	1613G→A	W538X	F	Asymptomatic	31	TFC	−	+	−	+	V_1–V_3	−	−	Mild	−	−	−
G, II, 3	1613G→A	W538X	M	VT	29	−	+	+	+	+	V_1–V_5	+	+	Mod	−	+	−
H, I, 1	2489+1G→A	abn splice product	M	Asymptomatic	55	HP	−	−	+	−	V_1	−	−	None	−	+	
H, II, 3	2489+1G→A	abn splice product	M	Asymptomatic	18	HP	−	−	−	+	V_1–V_3	+	+	Mild	−	+	
I, I, 2	1271T→C	F424S	F	Asymptomatic	60	TFC	−	−	−	−	—	−	−	None	−	−	
I, II, 1	1271T→C	F424S	M	Asymptomatic	37	TFC						−					
I, II, 2	1271T→C	F424S	F	Asymptomatic	34	TFC	−	−	−		V_1–V_3	−	−	None	−	+	
I, II, 3	1271T→C	F424S	F	VT	27	−	−	+	+	+	V_1–V_5	+	+	Sev	−	+	
I, II, 4	1271T→C	F424S	F	Palpitations	33	TFC	−	−	+	+	V_1–V_4	+	+	None	−	+	−

Aneu = localized aneurysms (akinetic or dyskinetic areas with diastolic bulging); Arrhyth = arrhythmias; Depol = depolarization abnormalities; DSW = delayed S-wave upstroke; EW = presence of epsilon wave in precordial leads on a 12-lead electrocardiogram; F = female; Fam = family history suggestive of ARVD/C; FH = family history; G. dil. = global right ventricular dilation with no left ventricular impairment; HP = indicates diagnosis of a family member with ARVD/C based on histopathologic evidence; HP = histopathologic abnormalities; LBBB = left bundle branch block-type ventricular tachycardia on electrocardiogram, exercise tolerance test or 24-h Holter monitor; LP = late potentials on signal-averaged electrocardiogram; M = male; PVC = more than 1,000 premature ventricular contractions on a 24-h Holter monitor; QRS = localized QRS prolongation in precordial leads on a 12-lead electrocardiogram; Repol = repolarization abnormalities; SCD = sudden cardiac death; SCD (under FH) = indicates sudden cardiac death in a family member at an age <35; Struc = right ventricular structural abnormalities; TFC = indicates diagnosis of ARVD/C in a family member based on International Task Force criteria; TWI = T-wave inversions in leads V_1 through V_3 and beyond on a 12-lead electrocardiogram; WMA = wall motion abnormalities of right ventricle (regional hypokinesia); + = indicates presence of a certain characteristic; − = indicates absence of a certain characteristic.

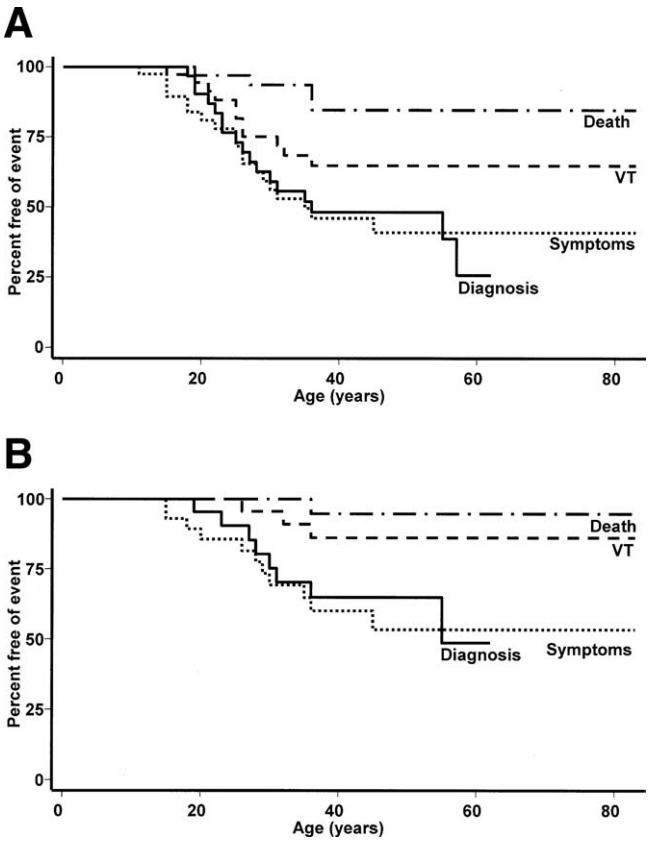

Figure 2. Kaplan-Meier curves demonstrating the proportion of individuals free from: 1) symptoms, 2) diagnosis, 3) symptomatic ventricular arrhythmia, and 4) cardiac death among **(A)** all *PKP2* mutation carriers and **(B)** all family members with a *PKP2* mutation after exclusion of the probands. VT = ventricular tachycardia.

summarizing the frequency of each of the TFC. Men were significantly more likely ($p < 0.05$) to have late potentials on a SAECG, frequent ventricular extrasystoles on a 24-h Holter monitor, and evidence of RV dilation on imaging studies ($p < 0.05$). There was no significant difference in the age of diagnosis of ARVD/C between men (mean 29 ± 14 years) and women (mean 31 ± 11 years). There was no significant difference in age of surviving male and female *PKP2* mutation carriers at most recent follow-up.

Clinical discordance in identical twins. The pedigrees included 2 sets of monozygotic twins (E:III:1 + E:III:2 and I:II:3 + I:II:4) with *PKP2* mutations who were concordant for ARVD/C but discordant for disease severity. In family E, one twin died suddenly during exercise at age 19 with ARVD/C diagnosed on autopsy. He had experienced several presyncopal episodes before his death. His brother was screened at that time and found to meet criteria for ARVD/C, and an implantable cardioverter-defibrillator (ICD) was implanted for primary prevention shortly thereafter. The surviving brother, now age 28, continued to do well. His ICD never discharged to treat VT. He had no symptoms of heart failure, and he had only mild RV dilation and mild RV wall motion abnormalities. The twins' mother also had a *PKP2* mutation; she had an incomplete right bundle branch block pattern on ECG but showed no signs of ARVD/C at age 56.

In family I, the proband initially presented with VT during exercise at age 15. She was eventually diagnosed with ARVD/C in her early '20s and died of biventricular heart failure at age 27. Her twin sister was diagnosed with ARVD/C after being screened at age 28. An ICD was implanted at age 31 after a loop recorder showed several episodes of non-sustained VT. She was doing well at age 33 with one episode of ICD firing for sustained VT, no evidence of heart failure symptoms, and only mild RV dilation.

DISCUSSION

Our results show that *PKP2* mutations in a group of North American families with ARVD/C have both reduced penetrance and variable expressivity. Despite increasing incidence of clinical disease with increased age, some mutation carriers remain without evidence of ARVD/C throughout their lifetime. Despite a similar age at onset of clinical disease, male *PKP2* mutation carriers are more likely to have late potentials on a SAECG, frequent ventricular extrasystoles on a 24-h Holter monitor, and RV dilation on imaging studies.

Importance of understanding penetrance in a genetic disease. Currently, we and others recommend that all first-degree relatives of a proband with ARVD/C undergo comprehensive screening every 1 to 3 years. As sudden cardiac death may be the initial symptom, early use of ICDs in those at highest risk of life-threatening cardiac arrhythmia seems warranted. However, ICD placement is not without risk, including procedural complications, inappropriate shocks, and psychological burden.

As genetic testing for heritable cardiovascular diseases proceeds to clinical use, understanding the implications of a positive genetic test is critical. By itself, inheritance of a *PKP2* mutation that was previously identified in a proband should lead to closer clinical screening for that family member. Asymptomatic *PKP2* mutation carriers may be reassured by the relatively low penetrance associated with such mutations and not automatically assumed to be destined to the clinical course of the proband in that family.

Influence of age and gender. Arrhythmogenic RV dysplasia/cardiomyopathy is an age-dependent disorder, with a median age at presentation in the U.S. of 26 years and a range of onset in symptoms between 2 and 70 years (3). Thus, estimates of penetrance must be tied to the age of the population studied, acknowledging that younger individuals without phenotypic features may later develop this condition. Accurate analysis of penetrance on the basis of age also allows proper counseling for mutation carriers at young ages to estimate their lifetime risk. Our pedigrees include individuals age 70 years or greater who remain free of all discernible manifestations of ARVD/C (A:I:1, B:II:2), suggesting true non-penetrance of *PKP2* mutations. Additionally, there

Table 3. Patient Characteristics in *PKP2* Mutation Carriers

Clinical Characteristics	Men (n = 18)	Women (n = 21)	Total (n = 39)
Presenting symptoms			
Palpitations	4 (22)	7 (33)	11 (28)
Syncope	0 (0)	2 (10)	2 (5)
Near syncope	1 (6)	1 (5)	2 (5)
Asymptomatic	9 (50)	11 (52)	20 (51)
Death			
Sudden	2 (11)	1 (5)	3 (8)
Heart failure	0 (0)	1 (5)	1 (3)
Global and/or regional dysfunction and structural abnormalities			
Dilation and reduction of right ventricular EF with no LV impairment[a]			
Moderate to severe*	5/16 (31)	2/19 (11)	7/35 (20)
Mild†	6/16 (38)	4/19 (21)	10/35 (29)
Localized ventricular aneurysms*	1/15 (7)	1/19 (5)	2/34 (6)
Regional right ventricular hypokinesis†	8/15 (53)	5/19 (26)	13/24 (38)
Depolarization abnormalities			
Prolongation of QRS in leads V_1–V_3*	6/12 (50)	4/19 (21)	10/31 (32)
Presence of epsilon waves†	3/12 (25)	1/19 (5)	4/31 (13)
Late potentials on SAECG[a]†	9/13 (69)	5/18 (28)	14/31 (45)
S-wave upstroke ≥55 ms in leads V_1–V_3	8/12 (67)	11/18 (61)	19/30 (63)
Repolarization abnormalities			
Inverted T-waves in leads V_1 through V_3 or beyond†	9/13 (69)	10/21 (48)	19/34 (56)
Arrhythmias			
Left bundle branch block-type VT documented†	6/16 (38)	5/20 (25)	11/36 (31)
Frequent ventricular extrasystoles[a]†	8/11 (73)	5/15 (33)	13/26 (50)
Family history			
Family history confirmed by biopsy or autopsy*	7 (39)	9 (43)	16 (41)
Sudden cardiac death in family at <35 yrs of age†	1 (0.5)	0 (0)	1 (0.3)
Family members diagnosed by using the present criteria†	4 (22)	10 (48)	14 (36)
Tissue characterization of walls			
Infiltration of RV by fat with presence of surviving strands or cardiomyocytes*	3/7 (43)	1/5 (20)	4/12 (33)

*Major criteria; †minor criteria. [a]Presence of dilation of the right ventricle (RV), >1,000 PVCs on a 24-h Holter monitor, and late potentials on a signal-averaged electrocardiogram (SAECG) were significantly different between men and women ($p < 0.05$).
EF = ejection fraction; LV = left ventricle; SAECG = signal averaged electrocardiogram; PVC = premature ventricular complex; VT = ventricular tachycardia.

are several individuals age 50 years or greater who show few if any signs of ARVD/C (that is, A:II:2, F:I:2, E:II:2).

The influence of male gender on some phenotypic manifestations of ARVD/C achieves statistical significance in our population, suggesting that men may be at greater risk of this condition. Two reports describing the clinical characteristics of family members of ungenotyped ARVD/C cases show a male preponderance of cases. In a study of 37 Italian families with ARVD/C, 62% of individuals with ARVD/C were men (19). Similarly, Hamid et al. (20) found that among first-and second-degree relatives of ARVD/C cases in Western Europe, 10% met TFC for ARVD/C, 72% of them men.

Lower penetrance among women may be the result of lesser exposure to environmental factors hypothesized to trigger ARVD/C in susceptible individuals, such as sustained vigorous athletic activity or exposure to viral agents causing inflammation. Alternately, the reduced penetrance may result from biological differences. For instance, it is thought that myocyte apoptosis plays a role in the pathogenesis of ARVD/C because apoptotic myocytes have been found in endomyocardial biopsies and autopsy tissue from ARVD/C patients (21,22). The inhibitory effect of estrogens on myocardial cell apoptosis has previously been reported (23).

Influence of environmental factors. Our presentation of 2 sets of identical twins with ARVD/C and discordant severity of disease strongly implicates environmental factors in the pathogenesis of this condition. Identical twins not only share the same *PKP2* mutation, but also any other possible genetic modifiers of this condition. Thus, factors such as exposure to viruses and aerobic activity may be responsible for differences in rates of ventricular arrhythmia and severity of structural RV dysfunction.

The mechanism by which male gender results in greater risk of some manifestations of ARVD/C is not known. At physiologic concentration, 17-beta-estradiol prevents programmed cell death in myocardial cells (24). This may be protective in ARVD/C, where increased apoptosis of ventricular myocytes has been invoked in the pathogenesis (21). Although protective hormonal or genetic factors that are gender-linked may be the primary reason, men may also be at higher risk of environmental factors that appear to influence disease outcomes.

Family history in the diagnosis of ARVD/C. Currently, the influence of family history in establishing a diagnosis of ARVD/C is variable, with some cases resulting in a major criterion and others a minor criterion. If a family member has ARVD/C confirmed at autopsy or surgery (such as

cardiac transplantation), all first-degree relatives meet a major criterion for ARVD/C. In contrast, if ARVD/C is clinically and independently diagnosed in a proband, then all first-degree relatives achieve a minor criterion for this condition. Sudden death at age <35 years from suspected ARVD/C also provides family members with a minor criterion. Biopsy, an invasive procedure, is difficult to obtain in all subjects being evaluated for ARVD/C. Moreover, even among those who undergo it, the results lack sensitivity for demonstrating fibrofatty replacement (3,25). As a result, some family members may not receive a major criterion (and sometimes a diagnosis) on the basis of the current recommendations of the task force, in spite of the proband having an overt clinical disorder. If the presence of a *PKP2* mutation is included as a major criterion under family history, the diagnosis of ARVD/C would be made in 50% of family members who carry this mutation. In contrast, under the current criteria, only 30% of mutation carrier family members currently satisfy TFC. As the clinical consequences of mutations in ARVD/C-associated genes become better recognized, the current TFC should be reviewed to evaluate the possibility of substituting genetic mutations for family history as either a minor or major criterion.

Study limitations. Complete cardiac evaluation was not performed in all first-degree relatives in all families, either because some family members were unwilling or unable to undergo comprehensive testing or declined inclusion of such testing in our analysis. This situation may predispose to underreporting of penetrance of *PKP2* mutations in ARVD/C families. Second, all probands were recruited through our research registry. This method of ascertainment may have led to an overestimate of penetrance, as families with multiple affected members may have been particularly likely to enroll in our registry. Third, self-reports of onset of symptoms of ARVD/C are vulnerable to recall bias, as family members are asked to recall age of onset of cardiac symptoms that were sometimes not documented in the medical record. Similarly, age of diagnosis (meeting TFC) is dependent upon the age at which a proband or family member first sought evaluation for ARVD/C. In the case of family members, this often followed the diagnosis of an affected person in the family. As some family members were diagnosed as meeting TFC at their first evaluation, the Kaplan-Meier curves demonstrating freedom from diagnosis almost certainly lag the true age meeting diagnostic criteria. Finally, this study describes penetrance in only 9 families. Although it adds significantly to data from the other families described previously, there remain limited longitudinal data on the course of the disease in family members.

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