Familial Evaluation in Arrhythmogenic Right Ventricular Cardiomyopathy: Impact of Genetics and Revised Task Force Criteria

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Familial Evaluation in Arrhythmogenic Right Ventricular Cardiomyopathy

Impact of Genetics and Revised Task Force Criteria

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Background—With recognition of disease-causing genes in arrhythmogenic right ventricular cardiomyopathy, mutation analysis is being applied.

Methods and Results—The role of genotyping in familial assessment for arrhythmogenic right ventricular cardiomyopathy was investigated, including the prevalence of mutations in known causal genes, the penetrance and expressivity in genotyped families, and the utility of the 2010 Task Force criteria in clinical diagnosis. Clinical and molecular genetic evaluation was performed in 210 first-degree and 45 second-degree relatives from 100 families. In 51 families, the proband was deceased. The living probands had a high prevalence of ECG abnormalities (89%) and ventricular arrhythmia (78%) and evidence of more severe disease than relatives. Definite or probable causal mutations were found in 58% of families and 73% of living probands, of whom 28% had an additional desmosomal variant (ie, mutation or polymorphism). Ninety-three relatives had a causal mutation; 33% fulfilled the 2010 criteria, whereas only 19% satisfied the 1994 version ($P=0.03$). An additional desmosomal gene variant was found in 10% and was associated with a 5-fold increased risk of developing penetrant disease (odds ratio, 4.7; 95% confidence interval, 1.1 to 20.4; $P=0.04$).

Conclusions—Arrhythmogenic right ventricular cardiomyopathy is a genetically complex disease characterized by marked intrafamilial phenotype diversity. Penetrance is definition dependent and is greater with the 2010 criteria compared with the 1994 criteria. Relatives harboring >1 genetic variant had significantly increased risk of developing clinical disease, potentially an important determinant of the phenotypic heterogeneity seen within families with arrhythmogenic right ventricular cardiomyopathy. **(*Circulation*. 2011;123:2701-2709.)**

Key Words: arrhythmogenic right ventricular cardiomyopathy ■ desmosomes ■ genes ■ family

The clinical profile of arrhythmogenic right ventricular cardiomyopathy (ARVC) is based largely on findings in probands with symptomatic ventricular arrhythmia. Only a handful of studies have focused on family assessment,[1,2] most of which preceded the advent of genotyping and used the 1994 Task Force criteria for diagnosis.[3] Estimates of penetrance have been confined to single-gene studies. With the identification of disease-causing genes, the familial basis of ARVC is increasingly recognized, and the clinical identification of the relatives at risk has become central to management. This study evaluated a large cohort of families with ARVC to investigate the prevalence of mutations in known causal genes, the penetrance and disease expression in both probands and relatives, and the utility of 2010 diagnostic criteria in familial diagnosis.

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Methods

Study Sample

The Heart Hospital, University College London Hospitals (London, UK) is a national referral center for the diagnosis and management of inherited cardiac diseases. Specialist weekly clinics operate for each of inherited cardiomyopathies (hypertrophic, dilated, arrhythmogenic right ventricular). The families were drawn from referrals to the ARVC, inherited arrhythmia, and victims of sudden death clinics. The study sample included 100 families with ARVC evaluated in the Inherited Cardiac Disease clinic from July 2003 to June 2009. The proband was defined as "the individual through whom the family was ascertained."[4] Deceased probands were sudden death victims in

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whom an expert cardiac pathologist confirmed the diagnosis postmortem and/or familial evaluation identified a first-degree relative with ARVC. Living probands fulfilled diagnostic criteria (definite/borderline) of ARVC as defined by the 2010 Task Force criteria.[5] Diagnosis was considered definite when 2 major, or 1 major and 2 minor criteria, or 4 minor criteria from different categories, were fulfilled. Diagnosis was considered borderline when 1 major and 1 minor or 3 minor criteria from different categories were fulfilled.[5] Evidence of disease expression in relatives was defined by the presence of a borderline (incomplete disease expression) or definite diagnosis. All first-degree relatives and second-degree relatives from families in whom a disease-causing mutation was found were invited for prospective evaluation.

Clinical Evaluation

Clinical evaluation included 12-lead ECG, signal-averaged ECG, transthoracic echocardiography, symptom-limited exercise test, and 24-hour ambulatory ECG monitoring, according to the 2010 ARVC criteria.[5] Cardiovascular magnetic resonance and electrophysiological studies were performed in selected patients. Left ventricular (LV) systolic dysfunction was defined by the presence of LV ejection fraction ≤50%.

Genetic Analysis

Genetic analysis in living and deceased probands and relatives was performed in accordance with the guidelines of the local ethics committee. All patients or family members of deceased probands gave written consent for genetic analysis. This study conforms to the ethics principles of the Declaration of Helsinki on human research. Blood samples from probands and relatives were screened by direct sequencing for mutations in the 5 desmosomal genes associated with the disorder, as previously reported.[6] Systematic cascade screening was not part of the original study design. To avoid selection bias, penetrance was estimated from families satisfying the following criteria: identification of disease-causing desmosomal mutation and genotyping of all evaluated first-degree relatives regardless of their clinical status.

Mutations and sequence variants were defined according to the 2010 ARVC Task Force criteria.[5] A definite pathogenic mutation was defined as a DNA alteration previously associated with ARVC[7] that was unobserved or rare in a large non-ARVC, ethnically matched control population and was predicted to alter the structure or the function of the encoded protein or cosegregated with disease phenotype in the family. A sequence variant was considered a probable disease-causing mutation when it fulfilled some but not all of these criteria (ie, not reported in previous studies or when it was not possible to show cosegregation). Sequence variants that were also present in control subjects were classified as polymorphisms. Variants previously reported as mutations were considered disease causing unless we could show otherwise. A total of 300 unrelated healthy, ethnically matched volunteers served as control subjects.

Statistical Analysis

Continuous variables are reported as mean±SD; categorical variables are summarized as percentages. Continuous variables were compared by use of the Student *t* test. Categorical variables were compared by use of the χ^2 or Fisher exact test in case of rare events. The 1994 and 2010 ARVC criteria sensitivity in relatives was calculated as the proportion of gene-positive subjects who fulfilled a definite diagnosis of the disease; specificity was calculated as the complementary of the proportion of the gene negative subjects who fulfilled a definite diagnosis of the disease. Binary logistic regression analysis was performed to calculate the odds ratio of disease expression. Cardiovascular mortality was calculated by dividing the number of patients who had a cardiovascular event during the follow-up by the total number of person-years. Follow-up was the interval from initial evaluation to the cardiovascular event or most recent evaluation. The following were considered cardiovascular events: sudden cardiac death, death resulting from heart failure or stroke, and heart transplantation. We calculated the 95% confidence interval for cardiovascular mortality using the assumption of an underlying Poisson distribution of rare events. Cardiovascular mortality was compared by use of the Fisher test. SPSS (version 15.0; SPSS Inc, Chicago, IL) and STATA (version 11.0; Stata Corp LP, College Station, TX) statistical software were used for the statistical analyses.

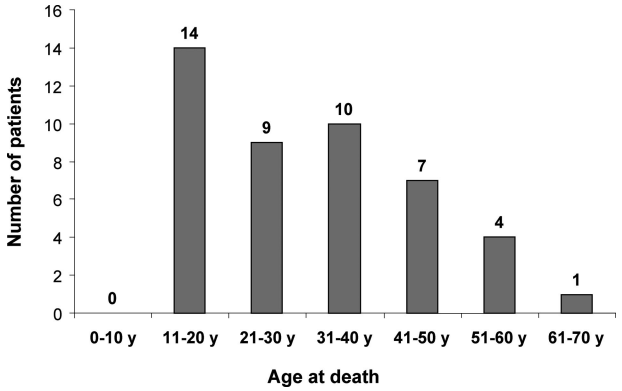

Figure 1. Age at sudden cardiac death of the 45 patients with pathologically proven arrhythmogenic right ventricular cardiomyopathy.

Results

Family Evaluation

In the 100 families, the proband was alive in 49 and deceased in 51; 210 of 463 (45.4%) living first-degree relatives were evaluated. Of the remainder, 9 were children (age <8 years) and 244 were not available (declined evaluation, were reviewed elsewhere, lived abroad, or could not be contacted).

Clinical Findings in the Probands

In 51 families, the reason for evaluation was the sudden cardiac death of the proband. In 45 (88%), the postmortem was consistent with ARVC; in 3 (6%), no cause was identified; in 2 (4%), the death was attributed to other causes (hypertension, hypertrophic cardiomyopathy); and in 1 (2%), no histology was available for review. These 6 families were included because there was at least 1 relative who fulfilled the diagnostic criteria for ARVC. Sudden death was most common in the young; 14 (31%) of pathologically proven deceased ARVC probands died between 14 and 20 years of age (Figure 1).

Of the 49 living probands, 46 were evaluated at The Heart Hospital (Table 1). In 3 probands, it was not possible to confirm the diagnosis that had been made in another hospital. Clinical presentation was cardiac symptoms in 21 (45.7%), sustained ventricular tachycardia in 17 (37.0%), and ventricular fibrillation in 5 (10.9%), whereas the diagnosis was incidental in 3 (6.5%). The majority of patients had at least 1 ECG abnormality (41 of 46, 89.1%) and ventricular arrhythmia (36 of 46, 78.3%), as detailed in Table 1.

Clinical Findings in the First-Degree Relatives

Eighty-eight (41.9%) of the 210 first-degree relatives had evidence of disease expression: 40 (19.0%) fulfilled a definite

Table 1. Clinical Characteristics of the 46 Living Probands

General	
Male, n (%)	27 (58.7)
Age at evaluation at The Heart Hospital, mean±SD, y	42.5±13.2
Age at diagnosis, mean±SD, y	38.7±13.1
Clinical presentation, n (%)	
Cardiovascular symptoms	21 (45.7)
VT	17 (37.0)
Ventricular fibrillation	5 (10.9)
Incidental	3 (6.5)
Family history, n (%)	
Major	
ARVC confirmed in a first-degree relative who meets current Task Force criteria	15/55* (27.3)
ARVC confirmed pathologically at autopsy or surgery in a first-degree relative	…
Identification of a disease-causing or probable disease-causing gene mutation	32 (69.6)
Minor	
History of ARVC in a first-degree relative in whom it is not possible or practical to determine whether the family member meets current Task Force criteria	…
Premature sudden death (<35 y) due to suspected ARVC in a first-degree relative	1 (2.2)
Global and/or regional dysfunction and structural alterations, n (%)	
Major criterion	9 (19.6)
Minor criterion	3 (6.5)
Left ventricular dysfunction, n (%)	13 (28.3)
Tissue characterization of the wall, n (%)	
Major criterion	2/5† (40)
ECG depolarization/conduction abnormalities, n (%)	
Major	
Epsilon waves	1 (2.2)
Minor	
Late potential on signal-averaged ECG	20 (43.5)
Terminal activation duration of QRS ≥55 ms	15 (32.6)
ECG repolarization abnormalities, n (%)	
Major	
Inverted T waves in right precordial leads (V_1–V_3) or beyond in individuals >14 y of age (in the absence of complete RBBB QRS ≥120 ms)	28 (60.9)
Minor	
Inverted T waves in leads V_1 and V_2 in individuals >14 y of age (in the absence of complete RBBB) or in V_4, V_5, or V_6	12 (26.1)
Inverted T waves in leads V_1, V_2, V_3, and V_4 in individuals >14 y of age in the presence of complete RBBB	1 (2.2)
Arrhythmias, n (%)	
Minor	
Nonsustained VT	10 (21.7)
Sustained VT	19 (41.3)
Frequent ventricular extrasystoles (>500/24 h by Holter)	16 (34.8)

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; VT, ventricular tachycardia; RBBB, right bundle-branch block; and LBBB, left bundle-branch block.

*Fifteen of 55 first-degree relatives.

†Two of the 5 in whom myocardial tissue was available.

Table 2. Clinical Characteristics of the 88 First-Degree Relatives With Definite (n=40) or Borderline (n=48) Diagnosis

General	
Male, n (%)	33 (37.5)
Age at The Heart Hospital evaluation, mean±SD, y	42.8±17.3
Family history, n (%)	
Major	
ARVC confirmed in a first-degree relative who meets current Task Force criteria	42 (47.7)
ARVC confirmed pathologically at autopsy or surgery in a first-degree relative	62 (70.5)
Identification of a disease-causing or probable disease-causing gene mutation	36 (40.9)
Minor	
History of ARVC in a first-degree relative in whom it is not possible or practical to determine whether the family member meets current Task Force criteria	…
Premature sudden death (<35 y) due to suspected ARVC in a first-degree relative	4 (4.5)
Global and/or regional dysfunction and structural alterations, n (%)	
Major criterion	3 (3.4)
Minor criterion	5 (5.7)
Left ventricular dysfunction	9 (10.2)
Tissue characterization of the wall	
Major criterion	1/1
ECG depolarization/conduction abnormalities, n (%)	
Major	
Epsilon waves	2 (2.3)
Minor	
Late potential on signal-averaged ECG	34 (38.6)
Terminal activation duration of QRS ≥55 ms	16 (18.2)
ECG repolarization abnormalities, n (%)	
Major	
Inverted T-waves in right precordial leads (V_1–V_3) or beyond in individuals >14 y of age (in the absence of complete RBBB QRS ≥120 ms)	20 (22.7)
Minor	
Inverted T waves in leads V_1 and V_2 in individuals >14 y of age (in the absence of complete RBBB) or in V_4, V_5, or V_6	12 (13.7)
Inverted T waves in leads V_1, V_2, V_3, and V_4 in individuals >14 y of age in the presence of complete RBBB	…
Arrhythmias, n (%)	
Minor	
Nonsustained VT	24 (27.3)
Sustained VT	3 (3.4)
Frequent ventricular extrasystoles (>500/24 h by Holter)	27 (30.7)

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; RBBB, right bundle-branch block; VT, ventricular tachycardia; and LBBB, left bundle-branch block.

diagnosis and 48 (22.9%) a borderline diagnosis according to the 2010 Task Force criteria.[5] Table 2 shows the clinical characteristics of these 88 first-degree relatives. More than half (48 of 88, 54.5%) had at least 1 ECG abnormality, and 41 (46.6%) had ventricular arrhythmia, as detailed in Table 2. Clinical features in the first-degree relatives of living and deceased probands were compared (Table I in the online-only Data Supplement). All 9 first-degree relatives with LV dysfunction and all 3 with sustained ventricular tachycardia came from families with a deceased proband.

Comparison of Clinical Findings in Probands and First-Degree Relatives

Compared with affected first-degree relatives, probands were more commonly men (58.7% versus 37.5%; $P=0.02$) and had a higher prevalence of major right ventricular abnormalities (19.6% versus 3.4%; $P=0.002$), LV dysfunction (28.3% versus 10.2%; $P=0.007$), T-wave inversion in right precordial leads (60.9% versus 22.7%; $P<0.001$), and sustained ventricular tachycardia (41.3% versus 3.4%; $P<0.001$; Tables 1 and 2).

Follow-Up of Probands and Relatives

One hundred twenty-five patients (44 probands, 81 first-degree relatives) were followed up for a mean of 3.4±1.6 years. Two probands underwent cardiac transplantation at 23 and 27 years of age; the latter died 4 months after the heart transplantation as a result of a brain hemorrhage. Two probands died of heart failure at 15 and 54 years of age. One relative died suddenly at 43 years of age while being investigated for palpitation and presyncope. One relative died of cancer. Cardiovascular mortality was 11.6/1000 person-years (95% confidence interval, 4.8 to 27.9/1000 person-years) and was higher in probands compared with relatives (30.2/1000 person-years [95% confidence interval, 11.3 to 80.5/1000 person-years] versus 3.4/1000 person-years [95% confidence interval, 0.5 to 23.9/1000 person-years]; $P=0.04$).

Mutation Analysis in Probands and First-Degree Relatives

Sequencing of the known causal desmosomal genes revealed at least 1 mutation in 56 families (definite in 44, probable in 12); in 3, no family member was available for testing. A total of 67 causal mutations were found with the following distribution: desmoplakin, n=16 (23.9%); plakophilin-2, n=24 (35.8%); desmocollin-2, n=9 (13.4%); and desmoglein-2, n=18 (26.9%) (Table 3). In 48 families, only 1 causal mutation was found. Six families were digenic (4 with compound heterozygous mutations, 2 with double heterozygous mutations); 1 patient had 3 mutations in 3 different genes, and another had 4 mutations in 2 genes (1 in *PKP2* and 3 in *DSG2*).

Mutation analysis was performed in 44 of the 46 living probands, revealing 39 mutations in 32 (73%) of the living probands. Mutations were identified in the following: desmoplakin, n=10 (25.6%); plakophilin-2, n=13 (33.3%); desmoglein-2, n=13 (33.3%); and desmocollin-2, n=3 (7.7%).

DNA extraction from tissue was successful in 8 deceased probands. Genetic analysis for desmosomal gene mutations was performed. Eight mutations and 1 polymorphism were found in 6 (75.0%). One patient had compound heterozygous mutations in desmoglein-2, and another had double heterozygous mutations in plakophilin-2 and desmocollin-2. The frequency of the mutations in each gene was as follows: desmoplakin, n=2; plakophilin-2, n=2; desmoglein-2, n=3; and desmocollin-2, n=1.

Probands with a mutation in one of the known causal genes (group 1) were compared with probands in whom mutation screening was negative (group 2). The prevalence of affected first-degree relatives was similar in both groups (28.9% versus 20.0%, respectively; $P=0.57$). Probands of known genotype had phenotypic features similar to those in whom the mutation remained unknown, with the exception of T-wave inversion in right precordial leads, which was more common in gene-negative probands (91.7% versus 50.0%; $P=0.01$; Table II in the online-only Data Supplement).

Disease Penetrance in First-Degree Relatives

Of 44 first-degree relatives who were mutation carriers, 27 (61.4%) satisfied the 2010 Task Force criteria (definite: n=15, 34.1%; borderline: n=12, 27.3%). The cumulative proportion by age of penetrant disease among proven gene carriers is shown in Figure 2. Disease penetrance was similar in the 30 relatives with a frameshift (insertion or deletion) or a stop codon or splice donor mutation compared with the 14 with a missense mutation (66.7% versus 50.0%, respectively; $P=0.29$).

Comparison of the 1994 and 2010 Arrhythmogenic Right Ventricular Cardiomyopathy Criteria in Genotyped Relatives

The 1994 and 2010 ARVC criteria were compared in 93 gene-positive relatives (first and second degree). Thirty-one (33.3%) fulfilled the definite diagnosis according to the new ARVC criteria compared with only 18 (19.4%) according to the 1994 ARVC criteria ($P=0.03$). None of the 23 gene-negative relatives had a definite diagnosis of ARVC according to the 2010 criteria (specificity, 100%), whereas 2 gene-negative relatives fulfilled the 1994 criteria (specificity, 91%); they had family history of ARVC (major criterion) and minor right ventricular abnormalities on imaging, in addition to nonsustained ventricular tachycardia on 24-hour tape in 1 patient and positive signal-averaged ECG in the other.

Disease Expression in Relation to Multiple Variants

Multiple desmosomal variants were more common in probands than in relatives (9 of 32 [28.1%] versus 9 of 93 [9.7%]; $P=0.01$).

The influence of multiple mutations/variants on disease expression was assessed in relatives. Nine relatives (9.7%) had at least 1 additional desmosomal variant (4 had another

Table 3. Desmosomal Gene Variants in the Arrhythmogenic Right Ventricular Cardiomyopathy Cohort

Gene	Disease-Causing Mutation, DNA Change–Protein Change	Possible Disease-Causing Mutation, DNA Change–Protein Change	Polymorphism, DNA Change–Protein Change
DSP	c.3337C>T-R1113X	c.2723G>A-R908H (2)*	c.8300C>A-T2767N
	c.1755_1756insA-T586fsX594	c.3532C>G-L1178V	c.5498A>T-E1833V
	c.818_819insA-E274fsX288	c.1224C>G-N408K	c.4578C>A-N1526K
	c.946_947insATACGCA-N316fsX324	c.4604T>C-L1535P	
	c.3045delG-S1015fsX1017	c.8275C>A-R2759S	
	c.2131-1G>A-abnormal splicing		
	c.2821C>T-R941X		
	c.1873C>T-Q625X		
	c.1325C>T-S442F		
	c.1520C>T-S507F		
PKP2	c.1844C>T-S615F	c.2456T>A-I819N	c.1592T>G-I531S (3)
	c.2197_2202delinsG-A733fsX740 (3)	c.2258T>A-L753Q	c.1114G>C-A372P
	c.2028G>A-W676X		c.1577C>T-T526M (2)
	c.419C>T-S140F (2)†		c.209G>T-S70I (5)
	c.2146-1G>C-abnormal splicing (6)		
	c.1237C>T-R413X (2)		
	c.145_148delCAGA-S50fsX110		
	c.2489+1G>A-abnormal splicing		
	c.1799delA-V600fsX655		
	c.1613G>A-W538X		
	c.775G>T- E259X		
	c.2062T>C-S688P		
	c.1759G>A-V587I†		
DSC2	c.304G>A-E102K (3)	c.2686_2687dupGA-E896fsX900	
	c.1430delC-M477fsX480	(A897fsX900) (3)‡	
		c.1721G>A-S574N	
		c.2194T>G-L732V	
DSG2	c.3G>C-M1I	c.998T>C-I333T	c.473T>G-V158G (2)
	c.829_840del12-L277_M280del	c.1051A>G-S351G (2)	c.2759T>G-V920G (2)
	c.1773_1774delTG-C591X	c.792T>A-D264E	
	c.1174G>A-V392I	c.716T>C-V239A	
	c.1003A>G-T335A (2)	c.1478A>G-N493S	
	c.1038_1040delGAA-K346del	c.3167C>T-T1056I	
	c.137G>A-R46Q	c.166G>A-V56M§	
	c.991G>A-E331K		
	c.462C>A-D154E		
JUP	…	…	…

DSP indicates desmoplakin gene; *PKP2*, plakophilin-2 gene; *DSC2*, desmocollin-2 gene; *DSG2*, desmoglein-2 gene; and *JUP*, plakoglobin gene. NCBI reference sequences: DSP-NM_004415.2, PKP2-NM_004572.3, DSC2-NM_024422.3, DSG2-NM_001943.3, JUP-NM_021991.2.

*The number of families with the same gene variant is reported in parentheses; this is omitted when the gene variant is sporadic.

†These variants have been found in control subjects, and their pathogenic role is controversial.[11]

‡This variant has been found in controls and its pathogenic role is controversial.[10,12]

§This variant has been reported in control subjects and has a higher prevalence in patients with dilated cardiomyopathy.[8]

gene mutation, 4 had an additional polymorphism, and 1 had 2 additional polymorphisms). Six (66.7%) of these 9 relatives fulfilled a definite diagnosis compared with 25 (29.8%) of the 84 with only 1 identified mutation ($P=0.03$). The presence of >1 variant in a desmosomal gene was associated with a 5-fold increased odds of having penetrant disease, as diagnosed by the 2010 Task Force criteria (odds ratio, 4.7; 95% confidence interval, 1.1 to 20.4; $P=0.04$).

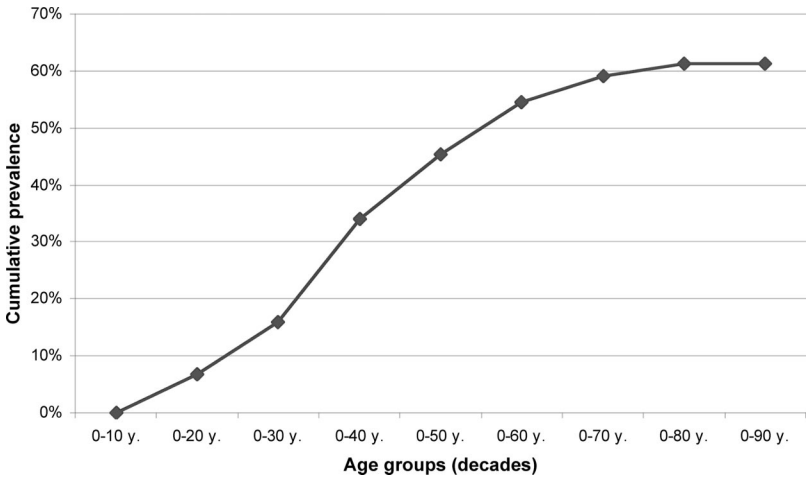

Figure 2. Cumulative proportion by age of penetrant disease among proven gene carrier first-degree relatives.

Problems in the Interpretation of Genetic Analysis

In some families, the interpretation of the genotype-phenotype data was ambiguous (Figure 3). In family A, the proband (II 3) died suddenly with postmortem evidence of ARVC. Subsequent family evaluation showed evidence of disease expression in his sisters. Two *PKP2* gene variants have been found in this family. One variant, S615F, which cosegregated with the disease in the sisters, was previously reported as disease causing.[6,9] The proband's father (I 1), who carried this gene variant, did not show evidence of the disease. Another *PKP2* variant, A372P, reported as a polymorphism and found in control subjects,[10] was carried by the proband's mother (I 2), who had nonsustained ventricular tachycardia on 24-hour Holter.

In family B, the proband (I 1) died of ARVC. A *PKP2* mutation (S140F) was found in the deceased, an affected daughter (II 1), an affected grandson (III 1), and an affected granddaughter (III 2). This mutation has been considered to be disease causing in ARVC, but has also been reported in control subjects.[6,9,11] However, another 36-year-old daughter (II 3) of the proband who did not carry the gene mutation had nonsustained ventricular tachycardia and frequent ventricular ectopics on 24-hour ECG.

The proband of family C presented with a syncopal episode and carried a novel DSP gene variant (R908H), which is located in a conserved position, was not present in 300 control subjects, and was predicted to affect protein function by a predictive program for sequence alterations.[7] His mother

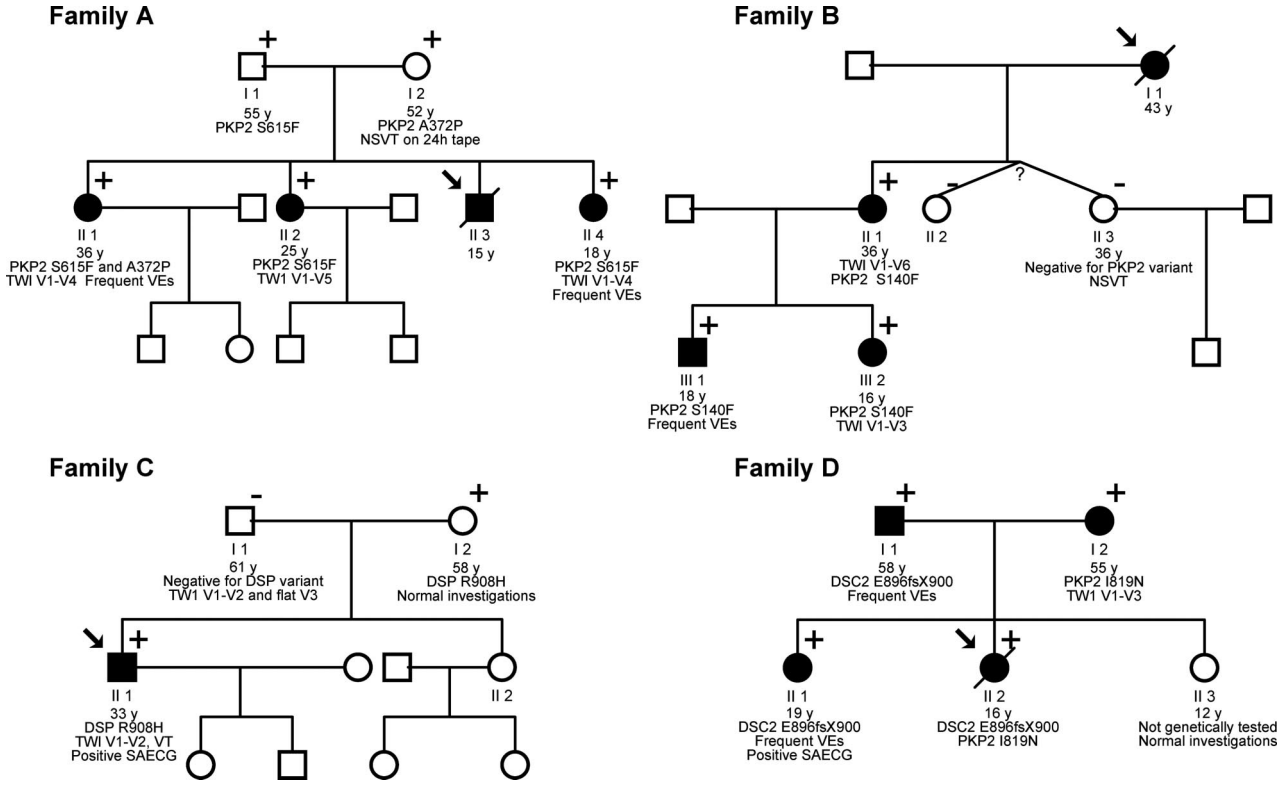

Figure 3. Families with problematic genotype-phenotype correlation (see text for details). NSVT indicates nonsustained ventricular tachycardia; SAECG, signal-averaged ECG; TWI, T-wave inversion; VE, ventricular ectopics; and VT, ventricular tachycardia.

carried the same gene variant, but had no evidence of disease expression. His father, who did not carry the variant, was asymptomatic and had no known cardiovascular disease; his ECG, however, demonstrated T-wave inversion in V_1 to V_2 with flattening in V_3.

The proband from family D died suddenly of ARVC. Tissue was available for DNA extraction, and 2 variants in the *PKP2* and *DSC2* gene were found. The *PKP2* gene variant I819N is a novel variant (not present in 300 control subjects) and was found in the proband's mother, who had T-wave inversion in V_1 to V_3 but no documented arrhythmias. The *DSC2* gene variant E896fsX900 is a frameshift variant causing a premature stop coding and has been described as a polymorphism[10,12] because it has been found in control subjects. In this family, the sister and father of the proband carried the *DSC2* gene variant. The 19-year-old sister had late potentials on signal-averaged ECG and frequent ventricular ectopics (>900 in 24 hours) that required antiarrhythmic therapy. The 58-year-old father, with no known cardiovascular disease, had frequent ventricular ectopics (>700 in 24 hours). Although the E896fsX900 frameshift variant has been reported in control subjects,[10,12] it was associated with the disease in this family, and preliminary and unpublished data from our laboratory showed functional abnormalities of this variant, and, therefore, we considered it as a possible cause of disease.

Discussion

This study examines the impact of genotyping and the 2010 Task Force criteria on familial evaluation in ARVC. Consistent with previous reports, marked intrafamilial phenotype diversity was a key finding, of which the most prominent manifestation was the difference in disease severity between probands and relatives. In more than half of the families evaluated, the proband presented with sudden cardiac death; most of the remainder had an arrhythmic presentation, and in only 3 living probands was the diagnosis incidental. Thirty-one percent of probands died suddenly between 14 and 20 years of age, confirming that adolescence is a particularly vulnerable period for fatal arrhythmic events in patients with ARVC. In contrast to probands, first-degree relatives had milder disease expression; major structural and functional abnormalities of the right ventricle were less common, as was LV dysfunction, T-wave inversion in the right precordial leads, and ventricular tachycardia. Consistent with previous studies,[1] the prognosis in relatives was better. The majority of probands were men, whereas women predominated among affected first-degree relatives, suggesting that men may develop a more severe phenotype that makes the individual more likely to come to medical attention. Disease expression in gene-positive relatives was age related and developed in the fifth decade of life and beyond in ≈50% of affected individuals (Figure 2). This experience of relatively late-onset disease in relatives compared with probands highlights the need for serial diagnostic evaluation and appropriate genetic counseling beyond the conventional second and third decades. It is also raises questions as to what distinguishes a proband that dies suddenly as a teenager from a relative who develops minor disease expression in the fourth or fifth decade. Mutation analysis was not feasible in most of the deceased probands. Of interest, however, was the finding in relatives of more severe disease expression in those who had multiple genetic variants. At least 1 genetic variant considered to be a definite or probable cause of disease was found in 56 families, of whom 8 had ≥2 disease-causing variants. We speculate that those probands who died suddenly as teenagers or young adults may have been carriers of multiple mutations. This was the case in 2 of the 8 deceased probands in whom mutation analysis was possible.

More than 70% of living probands had a causal or possibly causal mutation in one of the known desmosomal genes, a higher percentage compared with recent studies.[10,13–15] The majority of mutations were found in *PKP2* and *DSG*2, followed by *DSP* and *DSC*2. This proportion is similar to that reported recently in 82 ARVC probands from North America by Den Haan et al,[10] although, interestingly, the latter series had only 1% of mutations in the *DSP* gene. This may reflect differences in patient selection because the 1994 Task Force criteria excluded patients with significant LV disease, whereas this study reflects the new 2010 criteria, which recognize LV involvement as an important part of the clinical spectrum of the disease.[16] We speculated that probands in whom no mutation was identified might have sporadic (nonfamilial) disease; however, the prevalence of familial involvement was similar for probands with and without a mutation in one of the implicated desmosomal genes, suggesting that key disease-causing genes remain to be identified.

On the basis of clinical evaluation alone, 41% of first-degree relatives had evidence of disease expression (incomplete or complete), which is consistent with the autosomal dominant pattern of inheritance and comparable to the proportion reported by Nava et al (41.3%).[1] Among relatives who were proven gene carriers, only one third fulfilled the 2010 Task Force criteria for definite diagnosis, whereas an additional 27% had a borderline diagnosis, consistent with incomplete disease expression.[5] The 2010 Task Force criteria were more sensitive than the 1994 version in diagnosing familial disease, emphasizing the dependence of penetrance on clinical definition. The type of mutation (frameshift/stop codon/splice donor versus missense) was not associated with a difference in penetrance.

Recent work[17,18] has explored the prevalence and clinical significance of digenicity in ARVC. Xu et al[17] observed that a single plakophilin-2 variant was frequently insufficient to cause overt clinical disease, with 42% of affected carriers harboring a second hit in the same or a second desmosome-encoding gene. Bauce et al[18] reported patients with >1 rare genetic variant who had severe disease with early-onset heart failure; in general, multiple mutation carriers showed a higher prevalence of LV involvement than monogenic subjects. In our study, probands were significantly more likely than first-degree relatives to harbor >1 rare genetic variant. Within families, diallelic individuals may be more likely to develop symptomatic disease expression and to become the first family member to receive a diagnosis. Among relatives, the presence of >1 rare genetic variant was

associated with a significant (5-fold) increase in risk of disease expression, providing further evidence of gene-gene interactions and gene-dose effects. Taken together, accumulating evidence points to genetic complexity as an important determinant of intrafamilial phenotype diversity in arrhythmogenic cardiomyopathy.

Besides reporting examples of genotype-phenotype discordance, these findings have important implications for genetic counseling and predictive testing. An identified variant cannot be presumed necessary or sufficient for diagnosis. Caution is warranted before relatives with symptoms and/or borderline clinical abnormalities are discharged on the basis of a negative gene test or diagnosis confirmed on the basis of what may be a sequence variant. Further work will shed light on the overall contribution of rare sequence variants to clinical diversity in arrhythmogenic cardiomyopathy, the mechanisms underlying allelic interactions, the utility of genetic markers for diagnosis and prognostication, and the interplay of genetics with environmental factors.

Conclusions

Arrhythmogenic cardiomyopathy is characterized by marked intrafamilial phenotype diversity, which manifested most prominently by an arrhythmic presentation in probands; more than 50% of probands in our series died suddenly, while an arrhythmic presentation predominated among the remainder. Penetrance was definition dependent, with the 2010 criteria showing increased sensitivity for familial diagnosis. Expression was age related and, though uncommon, was observed in the fifth decade of life and beyond. Affected relatives frequently demonstrated limited phenotypes. The mechanisms underlying variable penetrance and expressivity in arrhythmogenic cardiomyopathy have hitherto been elusive, but emerging evidence implicates genetic complexity as a key contributor.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Arrhythmogenic right ventricular cardiomyopathy is a genetically determined autosomal dominant cardiac disorder caused by mutations in desmosomal genes. Clinical presentation is typically with arrhythmia and/or sudden death during the second and third decades. This study confirms this perspective; 51 of 100 probands presented with sudden death, 14 between the ages of 14 and 20 years. The study also examined family members in whom the molecular genetic status was known. Disease expression in relatives was milder, developed later in life, and was associated with a low incidence of arrhythmia/sudden death. Relatives with multiple genetic variants, however, had more severe disease expression with sudden death. It is unclear whether multiple variants explain the early presentation with sudden death seen in >30% of probands, or whether later-onset disease is progressive with clinical arrhythmia complications developing in later decades. The findings of this study underscore the role of mutation analysis with cascade screening of families to enable appropriate genetic counseling and targeted serial cardiac evaluation.

SUPPLEMENTAL MATERIAL

Supplemental Tables

Supplemental Table 1. Comparison of the clinical characteristics the first degree relatives with definite or borderline diagnosis, according to the status of the proband (deceased/alive)

	Family members with deceased proband N=67	Family members with living proband N=21	p-value
BASELINE CHARACTERISTICS			
General			
Male, no. (%)	27 (40.3%)	6 (28.6%)	0.33
Age at first evaluation (years), mean (±SD)	43.0±17.6	42.4±16.7	0.89
Family history, no. (%)			
Major			
ARVC confirmed in a first-degree relative who meets current task force criteria	-	-	-
ARVC confirmed pathologically at autopsy or surgery in a first degree relative	-	-	-
Identification of a pathogenic mutation categorized as associated or probably associated with ARVC	25 (37.3%)	11 (52.4%)	0.22
Minor			
History of ARVC in a first degree relative in whom it is not possible or practical to determine if the family member meets current task force criteria	-	-	-
Premature sudden death (<35 years) due to suspected ARVC in a first degree relative	-	-	-
Global and/or regional dysfunction and structural alterations, no. (%)			
Major criterion	2 (3.0%)	1 (4.8%)	0.56
Minor criterion	5 (7.5%)	0 (0%)	0.20
Left ventricular dysfunction	9 (13.4%)	0 (0%)	0.10
Tissue characterization of the wall			
Major criterion	-	-	-
ECG depolarization/conduction abnormalities, no. (%)			
Major			
Epsilon waves	-	-	-
Minor			
Late potential on signal-averaged ECG	28 (41.8%)	6 (28.6%)	0.28
Terminal activation duration of QRS ≥ 55 msec	10 (14.9%)	6 (28.6%)	0.16
ECG repolarization abnormalities, no. (%)			
Major			
Inverted T-waves in right precordial leads (V_1-V_3) or beyond in individuals > 14 years of age (in the absence of complete RBBB QRS ≥ 120 msecs)	14 (20.9%)	6 (28.6%)	0.46
Minor			
Inverted T waves in leads V1 and V2 in individuals > 14 years of age (in the absence of complete RBBB), or in V4, V5, or V6	8 (11.9%)	4 (19.0%)	0.41
Inverted T waves in leads V1, V2, V3 and V4 in individuals > 14 years of age in the presence of complete RBBB	-	-	-
Arrhythmias, no. (%)			
Non sustained VT*	20 (29.9%)	4 (19.0%)	0.19
Sustained VT*	3 (4.5%)	0 (0.0%)	
Frequent ventricular extrasystoles (more than 500/24 h by Holter)	21 (31.3%)	6 (28.6%)	0.81

Abbreviations: ARVC = arrhythmogenic right ventricular cardiomyopathy; RBBB = right bundle brunch block; VT = ventricular tachycardia; LBBB = left bundle brunch block.
Notes: *of right ventricular outflow configuration, LBBB morphology with inferior axis (positive QRS in II, III, AVF and negative in AVL) or of unknown axis.

Supplemental Table 2. Comparison of the clinical characteristics of the living probands, according to the genetic status.

	Gene negative N=12	Gene positive N=32	p-value
BASELINE CHARACTERISTICS			
General			
Male, no. (%)	9 (75.0%)	18 (56.3%)	0.26
Age at first evaluation (years), mean (±SD)	46.2 ± 12.3	40.8 ± 13.0	0.22
Age at diagnosis (years), mean (±SD)	42.6 ± 11.1	36.9 ± 13.2	0.19
Clinical presentation no. (%)			
Cardiovascular symptoms	4 (33.3%)	16 (50.0%)	0.77
Ventricular tachycardia	5 (41.7%)	11 (34.4%)	
Ventricular fibrillation	2 (16.7%)	3 (9.4%)	
Incidental	1 (8.3%)	2 (6.3%)	
Family history, no. (%)			
Major			
ARVC confirmed in a first-degree relative who meets current task force criteria	2/10 (20.0%)	13/45 (28.9%)	0.57
ARVC confirmed pathologically at autopsy or surgery in a first degree relative	-	-	-
Identification of a pathogenic mutation categorized as associated or probably associated with ARVC	-	-	-
Minor			
History of ARVC in a first degree relative in whom it is not possible or practical to determine if the family member meets current task force criteria	-	-	-
Premature sudden death (<35 years) due to suspected ARVC in a first degree relative	-	-	-
Global and/or regional dysfunction and structural alterations, no. (%)			
Major criterion	3 (25.0%)	6 (18.8%)	0.65
Minor criterion	2 (16.7%)	1 (3.1%)	0.18
Left ventricular dysfunction	3 (25.0%)	8 (25.0%)	1.00
Tissue characterization of the wall	-	-	-
Major criterion			
ECG depolarization/conduction abnormalities, no. (%)			
Major			
Epsilon waves	-	-	-
Minor			
Late potential on signal-averaged ECG	5 (41.7%)	14 (43.8%)	0.90
Terminal activation duration of QRS ≥ 55 msec	4 (33.3%)	10 (31.3%)	0.90
ECG repolarization abnormalities, no. (%)			
Major			
Inverted T-waves in right precordial leads (V_1-V_3) or beyond in individuals > 14 years of age (in the absence of complete RBBB QRS ≥ 120 msecs)	11 (91.7%)	16 (50.0%)	0.01
Minor			
Inverted T waves in leads V1 and V2 in individuals > 14 years of age (in the absence of complete RBBB), or in V4, V5, or V6	1 (8.3%)	10 (31.3%)	0.12
Inverted T waves in leads V1, V2, V3 and V4 in individuals > 14 years of age in the presence of complete RBBB	-	-	-
Arrhythmias, no. (%)			
Non sustained*	2 (16.7%)	8 (25.0%)	0.58
Sustained VT*	6 (50.0%)	12 (37.5%)	
Frequent ventricular extrasystoles (more than 500/24 h by Holter)	5 (41.7%)	11 (34.4%)	0.65

Abbreviations: ARVC = arrhythmogenic right ventricular cardiomyopathy; RBBB = right bundle brunch block; VT = ventricular tachycardia; LBBB = left bundle brunch block.
Notes: *of right ventricular outflow configuration, LBBB morphology with inferior axis (positive QRS in II, III, AVF and negative in AVL) or of unknown axis.