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Desmosomal gene analysis in arrhythmogenic right ventricular dysplasia/cardiomyopathy: spectrum of mutations and clinical impact in practice

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Aims Five desmosomal genes have been recently implicated in arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) but the clinical impact of genetics remains poorly understood. We wanted to address the potential impact of genotyping.

Methods and results Direct sequencing of the five genes (*JUP*, *DSP*, *PKP2*, *DSG2*, and *DSC2*) was performed in 135 unrelated patients with ARVD/C. We identified 41 different disease-causing mutations, including 28 novel ones, in 62 patients (46%). In addition, a genetic variant of unknown significance was identified in nine additional patients (7%). Distribution of genes was 31% (*PKP2*), 10% (*DSG2*), 4.5% (*DSP*), 1.5% (*DSC2*), and 0% (*JUP*). The presence of desmosomal mutations was not associated with familial context but was associated with young age, symptoms, electrical substrate, and extensive structural damage. When compared with other genes, *DSG2* mutations were associated with more frequent left ventricular involvement ($P = 0.006$). Finally, complex genetic status with multiple mutations was identified in 4% of patients and was associated with more frequent sudden death ($P = 0.047$).

Conclusion This study supports the use of genetic testing as a new diagnostic tool in ARVC/D and also suggests a prognostic impact, as the severity of the disease appears different according to the underlying gene or the presence of multiple mutations.

Keywords Cardiomyopathy • Gene • Arrhythmogenic right ventricular dysplasia • Diagnosis • Prognosis • Desmosome

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Introduction

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is an inherited myocardial disease that predominantly affects the right ventricle (RV), whose first comprehensive clinical description was made in 1982.[1] The estimated prevalence in the general population ranges from 1:1000 to 1:5000.[2,3] ARVD/C is characterized histopathologically by fibro-fatty myocardial replacement and clinically by ventricular arrhythmias that may lead to sudden death, especially in young people and athletes.[1–5] Clinical diagnosis is often difficult at an early stage and in mild forms due to a broad spectrum of clinical features and a long period of concealed cardiac expression. Diagnostic criteria integrating a clinical score were proposed by the International Task Force of the European Society of Cardiology and International Society and Federation of Cardiology.[4]

ARVD/C is a familial disease in 30–50% of cases[6] and is typically transmitted as an autosomal dominant trait with variable penetrance.[2,7,8] In recent years, the identification of causative mutations in plakoglobin (*JUP*), desmoplakin (*DSP*), plakophilin-2 (*PKP2*), desmoglein-2 (*DSG2*), desmocollin-2 (*DSC2*) has fostered the view that ARVD/C is a disorder of the desmosome[9–16] and provided new insight into its pathogenesis.[2,7,8] A few additional mutations have also been identified in the cardiac ryanodine receptor 2,[17] the transforming growth factor-beta 3,[18] and *TMEM43* genes.[19]

The major recent advance in the molecular genetics of ARVD/C might result in important clinical impact through early and correct diagnosis in patients and relatives.[2,7,8] This key issue first necessitates clarifying the efficiency of mutation screening in practice and the optimal molecular strategy. Genotyping may also have a prognostic impact if the precise genetic status would influence the severity of the disease.

The aim of the present study was to perform a systematic analysis of the five desmosomal genes in a large population of patients with ARVD/C. We also analysed the clinical factors that might influence the yield of the mutation screening. Because all five genes were analysed in all the patients, we were also able to study the frequency of multiple mutations, and to search for a gene-dose effect on the phenotype.

Methods

Clinical evaluation

This multicentric study that complies with the Declaration of Helsinki was approved by the Pitié-Salpêtrière Hospital ethical committee (CPPRB) and written informed consent was obtained from all participating individuals, recruited in France and Switzerland. Clinical evaluation was performed as we described before,[20] including clinical history, family history, 12-lead ECG, signal-averaged ECG, 24-h ambulatory ECG, transthoracic echocardiography, magnetic resonance imaging, and/or radionuclide scintigraphy in all patients, and contrast angiography in most cases. The diagnosis of ARVD/C was made according to the established European Society of Cardiology/International Society and Federation of Cardiology Task Force major and minor criteria.[4]

Mutation analysis

For each patient, genomic DNA was extracted from blood leucocytes present in the whole blood with the classical technique of Phenol Chloroform. Amplification of each exon and intron–exon junctions for all studied genes was performed with primers designed by PRIMER3 software (characteristics of primers available upon request). Analysis of the entire coding sequence of *JUP* (Online Mendelian Inheritance in Man or OMIM 173325, MN_021991.2, transcript 745aa), *DSP* (OMIM 125647, MN_004415.2, transcript 2871aa), *PKP2* (OMIM 602861, MN_004572.3, transcript 881aa), *DSG2* (OMIM 125671, MN_001943.3, transcript 1118aa), and *DSC2* (OMIM 125645, MN_024422.3, transcript 901aa) genes was performed by direct sequencing with the use of the BYG DYE dideoxy-terminator chemistry (Perkin Elmer) on ABI 3830 DNA sequencer (PE Applied Biosystems). Analysis of the chromatograms was performed with SeqScape (PE Applied Biosystems). Analysis of all the five genes was performed in all patients, even when a mutation was identified in a given gene. A control group of 300 healthy and unrelated subjects (600 alleles) with a Caucasian origin was used as controls.

A genetic variant was considered as a causal mutation on the basis of (i) the absence of the variant in the control population (and in SNPs databases), (ii) a drastic effect expected in the protein (nonsense mutation, frame shift mutation), or in the presence of a missense mutation, when (iiia) the mutated residue was highly conserved among species/isoforms and when (iiib) a damaging effect of the mutation was predicted and was concordant by three appropriate softwares (Polyphen, SIFT, Align GVGD) [both conditions (iiia) and (iiib) are required]. A genetic variant was considered as a genetic variant of unknown significance (GVUS) in the presence of a missense variant absent in the control population but without a causal effect predicted by at least one software or with a mutated residue not highly conserved among species/isoforms. The classification of genetic variants was refined through the segregation analysis within the family, when that information was available. A genetic polymorphism was defined as a variant observed in the control population or in polymorphism databases.

Statistical analysis

Comparisons between two groups were performed with two-tailed χ^2, or Fisher exact test, for categorical variables and a two-tailed *t*-test, or Mann–Whitney test, for continuous variables (expressed as mean $\pm$ standard error) when appropriate. Comparisons between multiple groups were performed with Kruskal–Wallis test or with Fisher exact test for continuous and categorical variables, respectively. For all comparisons, a value of $P < 0.05$ was considered significant.

Results

We studied 135 independent patients with ARVD/C. Mean age at diagnosis was 36.8 years $\pm$13.7; males were predominant with a ratio of 103/32 (or 3.2/1); familial history of ARVD/C was present in 23% of cases (determined by family history and clinical evaluation when available); familial history of premature sudden death (<50 years) in 24%; personal history of sudden death (aborted or during follow-up) in 9%; syncope or sudden death in 41%, symptoms in 87%; sustained ventricular tachycardia was present in 54%; RV morphologic involvement was diffuse in 54% (global RV enlargement or systolic dysfunction) or localized in 46%; left ventricular ejection fraction (LV EF) was decreased (<45%) in 14%; ECG T-wave inversion was present in 66%; late potentials were present on signal-averaged ECG in 69% (evaluated in 113); positive electrophysiological testing was present in 54% (evaluated in 91); an internal cardioverter defibrillator was

Table 1 Mutations in plakophilin-2 (*PKP2*) gene

Nucleotide	Coding effect	Number of index patients with each variant	Reported or novel
c.148_151del	p.Thr50SerfsX61	4	Reported[21,29]
c.223+1G>A	IVS1+1G>A	1	Novel
c.253_256del	p.Glu85MetfsX26	1	Novel
c.275T>A	p.Leu92X	2	Novel
c.587dup	p.Ser197PhefsX19	1	Novel
c.713C>T	p.Pro238Leu	1	Novel
c.987del	p.Ser329ArgfsX23	2	Novel
c.1132C>T	p.Gln378X	2	Reported[24]
c.1170+1G>A	IVS4+1G>A	1	Novel
c.1205del	p.Gln402ArgfsX3	1	Novel
c.1231dup	p.Val411GlyfsX15	1	Novel
c.1237C>T	p.Arg413X	2	Reported[22]
c.1378+1G>C	IVS5+1G>C	2	Novel
c.1630_1631insTT	p.Pro544LeufsX20	1	Reported[12]
c.1643del (c1642Del)	p.Gly584ValfsX15	3	Reported[12,23,29]
c.1688+1G>A	IVS7+1G>A	1	Novel
c.1689-1G>C	IVS8-1G>C	1	Novel
c.1951C>T	p.Arg651X	1	Reported[12]
c.2058T>G	p.Tyr686X	1	Novel
c.2146-1G>C	IVS10-1G>C	7	Reported[22–24,29]
c.2197_2202delinsG	p.His733AlafsX8	1	Reported[22–24,29]
c.2431C>A	p.Arg811Ser	1	Novel
c.2489+1G>A	IVS12+1G>A	3	Reported[21,23,29]
c.2509del	p.Ser837ValfsX94	1	Reported[12,23,24]
Total mutations	24 mutations	42 patients	
c.193G>T	p.Ala65Ser	1	GVUS
c.259G>C	p.Val87Leu	1	GVUS
c.302G>A	p.Arg101His	1	GVUS
c.419C>T	p.Ser140Phe	1	GVUS (but reported in Refs 12,22,23,29)
c.791C>T	p.Ala264Val	1	GVUS
c.1468C>T	p.Arg490Trp	2	GVUS
Total GVUS	Six GVUS	Seven patients	

GVUS, genetic variants of unknown significance.

implanted in 48%. Patients were of European/Caucasian origin in 90.5%, Maghreb in 3%, Caribbean in 2%, other in 4.5%.

We identified 41 different disease-causing mutations, including 28 novel ones, in 62 patients (46% of 135). Distribution of genes was 31% (42 of 135) for *PKP2*, 4.5% for *DSP* (6/135), 10% (14/135) for *DSG2*, 1.5% for *DSC2* (2/135), and 0% for *JUP*. Characteristics of the mutations in the different genes are indicated in *Tables 1* and *2* (see Supplementary material online, *Table S1*). A given mutation was identified in a single family or individual (private mutation) in 39% (24 of 62). In addition, a total of 14 different GVUS were identified in 13 patients (a total of 10% of the 135 patients): six patients with an isolated GVUS, three patients exhibited a double GVUS, and four patients carried one mutation and one GVUS (including one patient with a triple variant).

The yield, or identification rate, of desmosomal mutations was significantly associated with younger age at diagnosis ($P = 0.0065$), the presence of symptoms ($P = 0.0075$), ECG T-wave inversion ($P = 0.0037$), late potentials ($P = 0.0055$), positive ventricular stimulation ($P = 0.0018$), diffuse RV involvement ($P = 0.02$), low LV EF ($P = 0.012$), but not with familial context (*Table 3*). Similar results were obtained when GVUS were pooled with mutations and compared with index patients without genetic variants (*Table 3*).

Analysis of the phenotype according to the underlying gene mutations demonstrated significant differences for sex ratio, ECG T-wave inversion, and LV involvement among the three most frequent genes (*Table 4*). *DSP* mutations were associated with female predominance and low frequency of ECG T-wave inversion. *DSG2* mutations were associated with more frequent LV involvement when compared with *PKP2* mutations ($P = 0.002$). Considering all the desmosomal gene mutations, we observed that, compared with mutations resulting in truncated proteins, patients with

Table 2 Mutations and genetic variants of unknown significance in desmoglein (*DSG2*), desmocollin-2 (*DSC2*), desmoplakin (*DSP*), and plakoglobin (*JUP*) genes

Nucleotide	Coding effect	Number of index patients with each variant	Reported or novel
DSG2			
c.136C>T	p.Arg46Trp	1	Novel
c.137G>A	p.Arg46Gln	3	Reported[15,23]
c.146G>A	p.Arg49His	4	Reported[15,23]
c.523+2T>C	IVS5+2T>C	3	Novel
c.690+1G>A	IVS6+1G>A	1	Novel
c.882_883insA	p.Val295SerfsX6	1	Novel
c.977A>T	p.Asn326Val	1	Novel
c.1919_1932del	p.Gly640AspfsX15	1	Novel
Total mutation	8 mutations	14 patients[a]	
c.437G>T	p.Arg146Leu	1	GVUS
c.792T>A	p.Asn264Glu	1	GVUS
c.806T>C	p.Ile269Thr	1	GVUS
Total GVUS	Three GVUS	Three patients	
DSC2			
c.341del	p.Glu114GlyfsX7	1	Novel
c.394C>T	p.Arg132Cys	1	Novel
c.2368_2370del	p.Gly790del	1	Novel
Total mutation	Three mutations	Two patients	
c.2603C>T	p.Ser868Phe	1	GVUS
Total GVUS	One GVUS	One patient	
DSP			
c.2815G>A	p.Gly939Ser	1	Novel
c.3995_3996delinsAATCGA	p.Ala1332GlufsX15	1	Novel
c.5800C>T	p.Arg1934X	1	Reported[30]
c.5999_6000delinsG	p.Ser2000TrpfsX33	1	Novel
c.6850C>T	p.Arg2284X	1	Novel
c.7999C>T	p.Gln2667X	1	Novel
Total mutation	Six mutations	Six patients	
c.1691C>T	p.Thr564Ile	1	GVUS
c.7027G>A	p.Glu2343Lys	1	GVUS
c.7915C>T	p.Arg2639Trp	1	GVUS (but reported as a mutation in ref 31)
Total GVUS	Three GVUS	Three patients	
JUP			
c.1408G>A	p.Glu470Lys	1	GVUS

GVUS, genetic variants of unknown significance.
[a]One patient carried two mutations (total is therefore 15 mutations, including 8 different ones, observed in 14 patients).

missense mutations were associated with a younger age at diagnosis (25.7 vs. 35.0 years, $P = 0.032$) and more frequent LV involvement, especially when GVUS were taken into account (43.7% vs. 13.6%, $P = 0.028$) (see Supplementary material online, *Tables S2 and S3*).

Complex genetic status with multiple mutations was identified in five patients (4% of the total population or 8% of the patients with a mutation) with two double heterozygotes (*PKP2*/*DSG2*), one homozygote (*DSG2*), and two compound heterozygotes (in *DSG2* and *DSC2*) (see Supplementary material online, *Table S4*). When compared with patients with single mutations, patients with multiple mutations were associated with more frequent sudden death (40 vs. 5.3%, Fisher exact test $P = 0.047$) and tended to be associated with more frequent LV systolic dysfunction (60 vs. 19.3%, Fisher exact test $P = 0.07$) (*Table 5*). When GVUS were taken into account and pooled with disease-causing mutations, a total of 11 patients presented a complex genetic status (8% of the total population, 11 of 135, or 15% of the patients with a mutation, 11 of 71, including one triple variant) which was also associated with more frequent sudden death in these patients

Table 3 The global identification rate of desmosomal mutations according to clinical features of the patients

Phenotype	Mutation ($n = 62$)	GVUS ($n = 9$)	Other ($n = 64$)	*P*-value (three groups)	*P*-value (mutation vs. GVUS + other)	*P*-value (Mut + GVUS vs. other)
Age at diagnosis	33.4 ± 13.1	38.9 ± 8.1	39.9 ± 14.3	0.038	0.0065	0.013
Age at inquest	41.1 ± 15.1	43.2 ± 9.4	44.0 ± 14.5	NS	NS	NS
Gender (M/F)	48/14	8/1	47/17	NS	NS	NS
Family history ARVD/C	27.40%	11.10%	20.30%	NS	NS	NS
Family history SD	22.60%	44.40%	23.40%	NS	NS	NS
Symptoms	95.10%	88.90%	78.10%	0.013	0.0075	0.0056
Sudden death[a]	8.00%	11.10%	9.40%	NS	NS	NS
Syncope/SD	47.50%	55.50%	32.80%	NS	NS	NS
Sustained VT	55.70%	50%	53.10%	NS	NS	NS
ECG T-wave inversion	79.70%	75%	53.10%	0.0059	0.0037	0.0016
Late potentials+ ($n = 113$)	82.40%	71.40%	56.30%	0.012	0.0055	0.0046
EP testing+ ($n = 91$)	71.40%	40%	38.60%	0.0056	0.0018	0.0049
Diffuse RV abn	64.50%	75%	40.60%	0.012	0.02	0.0036
Low LV EF (<45%)	22.60%	37.50%	3.20%	0.0005	0.012	0.0006
ICD	50.00%	62.50%	43.70%	NS	NS	NS

M/F, male/female; SD, sudden death.
[a]Aborted or during follow-up; VT, ventricular tachycardia; EP, electrophysiology; RV abn, right ventricular abnormality (global dilatation or systolic dysfunction); EF, ejection fraction; ICD, implantable cardiovertor defibrillator; NS, not significant.

Table 4 Phenotype according to the underlying gene (causal mutations only)

Phenotype	*PKP2* ($n = 40$)	*DSP* ($n = 6$)	*DSG2* ($n = 12$)	*DSC2* ($n = 2$)	*P*-value *PKP2*/*DSG2*/*DSP*	*P*-value *PKP2*/*DSG2*
Age at diagnosis	34.0 ± 13.7	40.8 ± 9.2	30.3 ± 10.9	18–19	NS	NS
Age at inquest	41.9 ± 14.7	44.0 ± 10.1	40.0 ± 16.4	19–20	NS	NS
Gender (M/F)	33/7	0/6	11/1	2/0	0.0001	NS
Family history ARVD/C	30.00%	33.30%	8.30%	1/2	NS	NS
Family history SD	25.00%	33.30%	8.30%	1/2	NS	NS
Symptoms	92.50%	100%	100%	2/2	NS	NS
Sudden death	7.50%	0%	8.30%	1/2	NS	NS
Syncope/SD	45.00%	50.00%	54.50%	2/2	NS	NS
Sustained VT	53.80%	50.00%	75.00%	0	NS	NS
ECG T-wave inversion	87.50%	33.30%	88.90%	0	0.015	NS
Late potentials+ ($n = 49$)	81.20%	66.70%	90.00%	2/2	NS	NS
EP testing+ ($n = 40$)	73.10%	50.00%	87.50%	0	NS	NS
Diffuse RV abn	67.50%	66.70%	58%	1/2	NS	NS
Low LV EF	10%	33.30%	50%	1/2	0.006	0.002
ICD	47.50%	66.70%	66.70%	0	NS	NS

See *Table 3*. *DSP*, desmoplakin; *PKP2*, plakophillin-2; *DSG2*, desmoglein-2; *DSC2*, desmocollin-2. The two patients who are double heterozygotes (with *PKP2* and *DSG2* mutations) were excluded from the analyses.

(Fisher exact test $P = 0.043$) (see Supplementary material online, *Table S5*).

Discussion

This report describes the systematic screening of five desmosomal genes in a population of 135 unrelated index patients with familial or sporadic forms of ARVC/D. This is the most comprehensive study in that setting, considering the size of the population, the systematic analysis of the different genes (even when a first mutation was identified), and the study of clinical factors that might influence the yield of the mutation screening. In addition, we were very cautious and conservative regarding the causative role of the genetic variants.

Table 5 Phenotype and patients with complex genetic status (double mutation carriers vs. single mutation carrier, whatever the desmosomal mutations)

Phenotype	Double ($n = 5$)	Single ($n = 57$)	*P*-value
Age at diagnosis	30.6 ± 13.4	33.6 ± 13.2	NS
Age at inquest	39.4 ± 19.5	41.3 ± 14.9	NS
Gender (M/F)	5/0	43/14	NS
Family history ARVD/C	40.00%	26.30%	NS
Family history SD	20.00%	22.80%	NS
Symptoms	100%	94.70%	NS
Sudden death	40.00%	5.30%	0.047
Syncope/SD	40.00%	48.20%	NS
Sustained VT	60.00%	55.40%	NS
ECG T-wave inversion	75.00%	80.00%	NS
Late potentials+ ($n = 48$)	100%	81.20%	NS
EP testing+ ($n = 42$)	75.00%	71.10%	NS
Diffuse RV abn	80.00%	63.10%	NS
Low LV EF (<45%)	60%%	19.30%	0.07
ICD	20%	52.60%	NS

see *Table 3*. No GVUS is included here.

Previous data about the efficiency of mutation screening in ARVD/C reported variable and often contrasting results. The frequency of *PKP2* mutations ranged between 11 and 45%[12,21–24] and the yield of mutation screening of all desmosomal genes ranged between 29 and 52%.[7,23] The yield of mutation screening was significantly related to the familial context in one study (70% in the presence of a familial context vs. 0% in sporadic cases)[21] but was not suggested in others.[12,22,23] These variable results might be explained, at least in part, by the quite small populations analysed (56–82 index patients) and by unexhaustive analysis of the desomosomal genes in most studies (analysis of 2, 3, or 4 of the five desmosomal genes).[7]

We identified 41 different disease-causing mutations, including 28 novel ones, in 62 patients (46% of 135). No mutation was identified in the *JUP* gene, which appears therefore restricted to syndromic and recessive ARVD/C (Naxos disease)[9] with rare exceptions.[23,25] The *PKP2* gene was the most frequent gene involved in the disease (31% of patients), followed by *DSG2* (10%), *DSP* (4%), and *DSC2* (1%). The spectrum of the mutations was large with various mutation mechanisms (missense, splicing, frameshift, and so on) and various locations within each desmosomal gene (at full length of the coding regions of the genes), with a high frequency of unique and 'private' mutations (39%). In addition, we identified complex genetic status (multiple mutations) in 4% of the total population (or 8% of patients with a mutation). On the basis of these findings, a molecular diagnosis strategy can be proposed for genotyping in clinical practice. (i) The analysis should include at least the three most frequent genes (*PKP2*, *DSG2*, and *DSP*). (ii) *DSC2* gene analysis can be added but the identification rate is very low. (iii) *JUP* analysis is not required. (iv) Not only the already reported mutations but also all the exons and intron–exon boundaries of the genes should be systematically analysed. (v) The analysis should not be stopped after the identification of a first mutation, in case a second mutation is missed.

However, molecular analysis should be rigorous and cautious as the causal link between a genetic variant and the disease might be difficult to establish. At the end of the screening procedure, we were not able to conclude, and therefore classified as GVUS, a total of 14 variants in 13 patients (in 10% of the total population, including 7% of patients as the sole genetic variant and 3% of patients with another causal mutation). Interestingly, the classification of the genetic variants based on the conditions we used in the index patients (as in routine practice in a diagnostic laboratory) was robust as the analysis of the segregation within the family did not significantly modify the classification. Several additional genetic variants were previously reported in the literature as mutations, but our data led us to conclude that they were rare polymorphisms (see details in Supplementary material online).[23,24,26–32]

Interestingly, we were able to analyse the mutation identification rate according to the phenotype of the patients. We observed that the yield was not different in sporadic and familial forms of ARVD/C (17 of 31 or 55% in familial forms vs. 45 of 104 or 43% in sporadic forms). In some cases, we were able to observe that apparently sporadic cases were related to a non-penetrant mutation in the transmitting parent or were related to a *de novo* mutation in the propositus.[27] These findings have implications for the clinician. Sporadic cases of ARVD/C should be considered as a genetic disease too, with specific recommendations about genetic counselling and cardiological examination in relatives. Genetic screening in clinical practice can be proposed in apparently sporadic cases and should not be restricted to familial cases. In other respects, the yield of mutation screening was significantly associated with a younger age at disease onset, the presence of symptoms, electrical substrate, and extensive structural damage (diffuse RV involvement, low LV EF). In symptomatic patients <35 years with ECG T-waves inversion and extensive structural damage (diffuse RV involvement), the probability of mutation identification was 68% (17 of 25). This might be related to inclusion bias (reflecting only a better yield in patients with typical and advanced disease at a young age), but this might also suggest that desmosomal gene mutations are specifically associated with such specific phenotypes. Interestingly, the latter hypothesis could therefore explain the quite frequent LV involvement recently reported in families with desmosomal mutations.[28] In addition, preliminary phenotype–genotype analyses from the present work suggest that LV involvement is significantly associated with *DSG2* mutations (50% of *DSG2* patients, when compared with 10% in patients with *PKP2* mutations), whereas LV involvement was previously associated with mutations in *DSP* gene.[28] We also observed that, compared with mutations resulting in truncated proteins, patients with missense mutations were associated with a more severe disease. A speculative explanation might be that a dominant-negative effect, usually associated with missense mutations, might have more severe consequences than haploinsufficient effect, usually associated with truncated mutations.

The unexpected identification of multiple mutations in at least 4% of patients with ARVD/C (or 8% if GVUS included) raises a possible new understanding of the variable phenotypic expression of the disease. We observed that multiple mutations were

significantly associated with more frequent personal history of sudden cardiac death (aborted or not). This finding suggests a gene-dose effect on the phenotype and might at least in part explain a severe evolution within a family that may contrast with a more simple evolution in relatives. This finding was also observed in recent reports[33,34] and may therefore suggest the use of genetics as a prognostic marker. Interestingly, the fact that the relation was similar when GVUS were taken into account suggests that GVUS might at least be genetic modifiers influencing the risk of death in ARVD/C.

Study limitations

The findings we observed were obtained mainly from a Caucasian population, with a typical and conservative diagnosis of ARVD/C (fulfilling 1994 Task Force Criteria) and possibly do not apply to a different population. Our conservative approach for the classification of causal mutations may have resulted in an underestimation of the exact yield of desmosomal genes mutation screening, as some variants not observed in controls were classified as 'GVUS', in the absence of robust functional experiments available in ARVD/C.

Conclusion

This study reports the most detailed and comprehensive analysis of desmosomal gene screening in ARVD/C. The findings support the use of genetic testing as a new diagnostic tool in clinical practice, with promising perspectives for early diagnosis in relatives of patients with ARVD/C and for borderline index patients.

The results also suggest a prognostic impact of genetic testing, as the severity of the disease was different according to the underlying gene or the presence of multiple mutations.

Supplementary material

Supplementary material is available at *Europace* online.

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