

Arrhythmia/electrophysiology

Impact of genotype on clinical course in arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated mutation carriers

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Aims

We sought to determine the influence of genotype on clinical course and arrhythmic outcome among arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C)-associated mutation carriers.

Methods and results

Pathogenic mutations in desmosomal and non-desmosomal genes were identified in 577 patients (241 families) from USA and Dutch ARVD/C cohorts. Patients with sudden cardiac death (SCD)/ventricular fibrillation (VF) at presentation ($n = 36$) were younger (median 23 vs. 36 years; $P < 0.001$) than those presenting with sustained monomorphic ventricular tachycardia (VT). Among 541 subjects presenting alive, over a mean follow-up of 6 ± 7 years, 12 (2%) patients died, 162 (30%) had sustained VT/VF, 78 (14%) manifested left ventricular dysfunction ($EF < 55\%$), 28 (5%) experienced heart failure (HF), and 10 (2%) required cardiac transplantation. Patients ($n = 22$; 4%) with > 1 mutation had significantly earlier occurrence of sustained VT/VF (mean age 28 ± 12 years), lower VT-/VF-free survival ($P = 0.037$), more frequent left ventricular dysfunction (29%), HF (19%) and cardiac transplantation (9%) when compared with those with only one mutation. *Desmoplakin* mutation carriers experienced more than four-fold occurrence of left ventricular dysfunction (40%) and HF (13%) than *PKP2* carriers. Missense mutation carriers had similar death-/transplant-free survival and VT/VF penetrance ($P = 0.137$) when compared with those with truncating or splice site mutations. Men are more likely to be probands ($P < 0.001$), symptomatic ($P < 0.001$) and have earlier and more severe arrhythmic expression.

Conclusions

Presentation with SCD/VF occurs at a significantly younger age when compared with sustained monomorphic VT. The genotype of ARVD/C mutation carriers impacts clinical course and disease expression. Male sex negatively modifies phenotypic expression.

Keywords

Arrhythmia • Arrhythmogenic right ventricular dysplasia/cardiomyopathy • Genetics • Prognosis

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Introduction

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is an inherited cardiomyopathy characterized by ventricular arrhythmias, predominantly right ventricular dysfunction, and an increased risk of sudden cardiac death (SCD).^{1,2} Over the last decade, pathogenic mutations have been identified in desmosomal genes: plakoglobin (*JUP*),³ plakophilin-2 (*PKP2*),⁴ desmoplakin (*DSP*),⁵ desmoglein-2 (*DSG2*),⁶ and desmocollin-2 (*DSC2*).⁷ Mutations in several non-desmosomal genes including *PLN*-encoding phospholamban⁸ and *TMEM43*-encoding transmembrane protein 43⁹ have also been reported to cause ARVD/C. While evidence suggests that genotype may influence risk of both life-threatening arrhythmia and heart failure,¹⁰ previous genotype–phenotype studies were largely limited by population size. The objective of this study was to (i) define the long-term clinical course among a large cohort of ARVD/C mutation carriers, (ii) investigate the prognostic influence of genotype, including impact of multiple mutations, and (iii) ascertain the association of sex with phenotypic expression.

Methods

Study population

The study population of 577 subjects was derived from the Johns Hopkins ARVD/C registry ($n = 259$) and the Dutch ARVD/C registry (from the Interuniversity Cardiology Institute of the Netherlands; $n = 318$). All patients harboured a pathogenic ARVD/C-associated mutation. The study complies with the Declaration of Helsinki and locally appointed ethics committee approved the research protocol and informed consent was obtained from the subjects (or their guardians).

Phenotypic characterization

The medical history of each subject was obtained by review of medical records, clinical evaluation, and patient interviews. Detailed clinical information regarding demographics, presentation, symptoms, non-invasive, and invasive studies, and arrhythmia occurrence was obtained (see Supplementary material online, Table S1). Patients were prospectively followed and information regarding major clinical events (including syncope, episodes of sustained ventricular tachycardia (VT) or fibrillation (VF), sudden cardiac arrest (SCA), implantable cardioverter-defibrillator (ICD) implantation, appropriate ICD shocks and anti-tachycardia pacing, heart failure, cardiac transplantation, SCD, and other causes of death was obtained (see Supplementary material online, Table S2 for definitions). The primary outcome measure was the occurrence of a sustained arrhythmic event (a composite measure of the occurrence of SCA, spontaneous sustained VT/VF or an appropriate ICD intervention for sustained VT/VF). The secondary outcome was the occurrence of death or cardiac transplantation in this cohort (see Supplementary material online, Methods A).

Genotype

Comprehensive mutation testing of *PKP2*, *DSP*, *DSG2*, *DSC2*, and *JUP* (dideoxy sequencing or next-generation sequencing) for all probands was performed as reported earlier^{11,12} (see Supplementary material online, Methods B). Mutations were identified or confirmed by dideoxy (Sanger) sequencing in all patients. Multiplex ligation-dependent probe amplification analysis was performed to identify large deletions in *PKP2* among the Dutch cohort.^{11,13} One U.S. family had an incidental finding of a large *DSP* deletion on chromosomal microarray.¹⁴ Non-desmosomal

gene analysis included *TMEM43* and *PLN*. Family members were screened only for the pathogenic mutation(s) found in their respective index patient. The identified mutations were characterized by the gene involved and by the nature of the specific mutation (truncating, splice site, and missense). Nonsense, frame shift, splice site mutations, and exon deletions were all considered to be proven pathogenic unless previously identified as polymorphism or non-pathogenic. Missense mutations were reviewed and considered pathogenic for inclusion in the study when these criteria were met: (i) exome sequencing project (ESP) minor allele frequency $\leq 0.05\%$ (the NHLBI 6500 Exome data sets; EVS; <http://evs.gs.washington.edu/EVS/>) and (ii) *in silico* predictive programs (SIFT) and Polymorphism Phenotyping-2 (PolyPhen-2) (see Supplementary material online, reference 1 and 2) predicted the genetic variants to affect protein function by a tolerance index score of <0.02 (SIFT) and a PolyPhen-2 score of >0.900 . In addition, the presence of the missense mutations was assessed in 1000 Dutch control chromosomes (Genome of The Netherlands, GoNL; www.nlgenome.nl). Subjects who had SCD at presentation and either were obligate carriers or had evidence of ARVD/C on autopsy but did not have genotype studies were assumed to have the same mutation as other genetically affected first degree members of their respective family.

Statistical analysis

Continuous variables are summarized as either mean \pm SD or median (interquartile range, IQR) and compared across groups using a *t*-test, Mann–Whitney *U*-test, or Kruskal–Wallis test as appropriate. Categorical variables are reported as frequency (%) and compared between groups by the χ^2 or Fisher's exact test. Correlation of relevant clinical phenotypic characteristics including arrhythmic risk with the genotype was performed. The cumulative freedom since birth (i.e. by age) from the arrhythmic outcome was determined by the Kaplan–Meier method, and differences in survival between groups evaluated with the log-rank test. All *P*-values were corrected for family membership by using robust variance estimates in models clustered by family membership using *logistic*, *stcox*, and *somersd* commands using STATA (version 13; StataCorp, College Station, TX, USA). A *P*-value of ≤ 0.050 was considered significant. SPSS (version 19; SPSS Inc., Chicago, IL, USA) statistical software was used for all other analyses.

Results

The study population consisted of 577 patients from 241 unrelated families and was derived from the Johns Hopkins ARVD/C registry (259 patients: 102 probands, 157 relatives) and the Dutch ARVD/C cohort (318 patients: 128 probands, 190 relatives). The registries were statistically similar in terms of sex, race, and proband/relative ratios. Men constituted 55% of the overall population with the average age at presentation being 35 ± 17 years.

Genotype

The majority of participants (463, 80%) carried a single copy of a *PKP2* mutation (164 probands, 299 relatives) with significantly fewer heterozygous carriers of mutations in other ARVD/C-associated genes (31 *DSG2*, 31 *PLN*, 19 *DSP*, 8 *DSC2*, 2 *JUP*, and 1 *TMEM43*). Twenty-two individuals (4%) carried two or more pathogenic mutations (compound heterozygote, homozygote, or digenic mutation carriers). Among carriers of single mutations, premature truncating, splice site, and missense mutations were identified in 342 (60%), 130 (23%), and 83 (14%) patients, respectively (genotype details in Supplementary material online, Table S3 and S4).

Presentation with sudden cardiac death

Sudden cardiac death, with or without electrocardiographically proven VF, was the presenting symptom in 36 (6%; 29 families) patients. Among patients presenting with SCD, mutations in *DSP* were significantly more represented (11% vs. 3% in alive; $P = 0.019$) (Supplementary material online, Table S5), while missense mutations were less common (3% vs. 15% in alive; $P = 0.071$). The

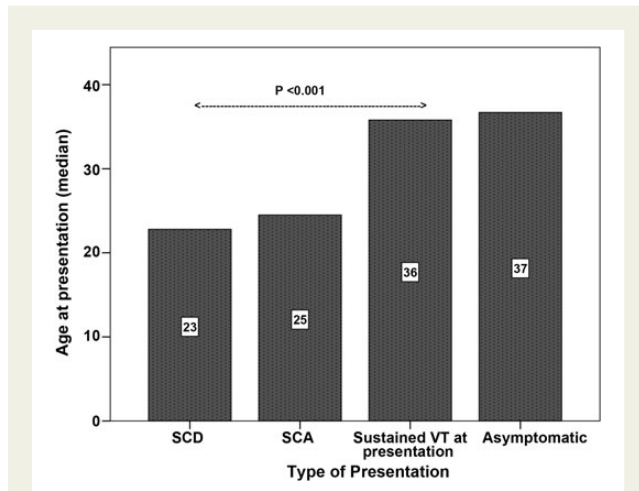


Figure 1 Median age at presentation categorized according to the nature of the presentation. SCD, sudden cardiac death; SCA, sudden cardiac arrest; VT, ventricular tachycardia. * P -values were obtained from non-parametric comparisons of group ranks (Somers's D) adjusting for family clustering.

median age (IQR, range) at SCD was 23 (12; 13–57) years, which was significantly younger than those presenting alive with sustained monomorphic VT with a median age of 36 years (20; 14–78) ($P < 0.001$) (Figure 1).

Alive at presentation

Of the 541 patients presenting alive (220 probands; 321 family members), more than half of the population (53%) was asymptomatic at presentation, whereas one-quarter presented with sustained VT (54% of probands, 4% of family members) (Table 1). The median age at symptom onset for the large *PKP2* group was 30 years (range 10–84), whereas it was significantly ($P = 0.027$) later for those with *PLN* mutations (40 years; range 16–59) (genotype details in Supplementary material online, Table S6). Among probands, no significant difference in sex ($P = 0.175$), occurrence of symptoms ($P = 0.276$), or incidence of composite outcome ($P = 0.719$) was seen between major gene groups (see Supplementary material online, Table S7). The majority of relatives were asymptomatic at presentation (86%) with 24 (8%) experiencing the arrhythmic outcome during follow-up (4 ± 5 years) with no significant differences among gene groups (see Supplementary material online, Table S8). Data for the single *TMEM43* gene mutation carrier are noted in Supplementary material online, Table S9.

Clinical course

Over a mean duration of follow-up of 6 years, 207 (38%) patients experienced the composite sustained arrhythmic outcome (Table 2). The arrhythmic event-free survival for the overall population at age 40 and 60 years was 66 and 42%, respectively (Supplementary material online, Figure S1A). The median age at the first episode of sustained VT/VF was 35 years in patients with *PKP2* mutations, whereas it was

Table 1 Baseline phenotypic characteristics of the study population categorized according to the underlying genotype

Clinical variable	Study cohort (n = 577)	<i>PKP2</i> (n = 463)	<i>JUP</i> (n = 2)	<i>DSG2</i> (n = 31)	<i>DSC2</i> (n = 8)	<i>DSP</i> (n = 19)	>1 mutation (n = 22) ^a	<i>PLN</i> (n = 31)
Male	318 (55)	253 (55)	2 (100)	19 (61)	6 (75)	10 (53)	12 (54)	15 (48)
Proband	230 (40)	164 (35)	2 (100)	16 (52)	5 (62)	9 (47)	15 (68)	18 (58)
Age at presentation; m \pm SD; years	35 \pm 17	35 \pm 18	41 \pm 33	32 \pm 17	41 \pm 19	33 \pm 17	31 \pm 13	42 \pm 12
SCD as presentation	36 (6)	29 (6)	0 (0)	1 (3)	0 (0)	4 (21)	1 (4)	1 (3)
Among those presenting alive								
Type of presentation	(n = 541)	(n = 434)	(n = 2)	(n = 30)	(n = 8)	(n = 15)	(n = 21)	(n = 30)
Asymptomatic	287 (53)	243 (56)	0	15 (50)	3 (37)	8 (53)	7 (33)	11 (37)
SCA	16 (3)	13 (3)	0 (0)	0 (0)	1 (12)	0 (0)	1 (5)	1 (3)
VT at presentation	131 (24)	99 (23)	1 (50)	8 (27)	2 (25)	3 (20)	7 (33)	11 (37)
Cardiac syncope	85 (16)	64 (15)	0 (0)	7 (23)	2 (25)	3 (20)	5 (24)	4 (13)
Age at symptom onset; m \pm SD; years	33 \pm 14	33 \pm 14	41 \pm 33	29 \pm 12	35 \pm 21	33 \pm 15	25 \pm 10	38 \pm 11
Inducibility at EPS ^b	146/200 (73)	107/148 (72)	–	12/14 (86)	2/3 (67)	7/9 (78)	10/11 (91)	7/14 (50)
Holter PVC count; median	910	694	–	518	3883	1723	2562	3169

PKP2, plakophilin-2; *JUP*, plakoglobin; *DSG2*, desmoglein-2; *DSC2*, desmocollin-2; *DSP*, desmoplakin.

^aDigenic, homozygous, and compound heterozygous mutations; *PLN*, phospholamban; EPS, electrophysiological study.

^bamong those that had EP study; ARVD/C, arrhythmogenic right ventricular dysplasia/cardiomyopathy; SCD, sudden cardiac death; SCA, sudden cardiac arrest; VT, ventricular tachycardia; PVC, premature ventricular complex; SD, standard deviation; m, mean.

Table 2 Clinical course including data obtained at presentation and during follow-up in arrhythmogenic right ventricular dysplasia/cardiomyopathy mutation carriers presenting alive categorized according to the underlying genotype

Clinical variable	Study cohort (n = 541)	PKP2 (n = 434)	JUP (n = 2)	DSG2 (n = 30)	DSC2 (n = 8)	DSP (n = 15)	>1 mutation ^a (n = 21)	PLN (n = 30)
Definite ARVD/C	327 (60)	251 (58)	2 (100)	16 (53)	5 (62)	9 (60)	18 (86)	25 (83)
Follow-up; m ± SD; years	6 ± 7	6 ± 7	6 ± 7	5 ± 5	2 ± 3	7 ± 9	10 ± 10	7 ± 5
Composite outcome	207 (38)	153 (35)	2 (100)	13 (43)	4 (50)	6 (40)	12 (57)	16 (53)
Spontaneous Sustained VT	162 (30)	122 (28)	1 (50)	10 (33)	2 (25)	5 (33)	9 (43)	13 (43)
Appropriate ICD intervention (VT/VF)	24 (4)	14 (3)	1 (50)	3 (10)	1 (12)	–	2 (9)	2 (7)
Resuscitated SCA/VF	21 (4)	17 (4)	0 (0)	0 (0)	1 (12)	1 (7)	1 (5)	1 (3)
Age at first sustained VT/VF	35 ± 13	35 ± 13	41 ± 33	32 ± 11	36 ± 23	40 ± 16	28 ± 12	39 ± 11
ICD implantation	241 (44)	179 (41)	2 (100)	15 (50)	4 (50)	8 (53)	14 (67)	18 (60)
Appropriate ICD therapy ^b	117/241	85/179	1/2	7/15	3/4	3/8	9/14	8/18
VT storm	37 (7)	28 (6)	1 (50)	1 (3)	1 (12)	1 (7)	3 (14)	1 (3)
VT ablation	73 (13)	53 (12)	1 (50)	6 (20)	2 (25)	3 (20)	3 (14)	5 (17)
LV dysfunction ^c	78 (14)	39 (9)	0 (0)	4 (13)	3 (37)	6 (40)	6 (28)	20 (67)
Heart failure	28 (5)	15 (3)	0 (0)	0 (0)	0 (0)	2 (13)	4 (19)	7 (23)
Cardiac transplant	10 (2)	6 (1)	0 (0)	0 (0)	0 (0)	0 (0)	2 (9)	2 (7)
Death	12 (2)	7 (2)	0	0	0	0	1 (5)	4 (13)

PKP2, plakophilin-2; JUP, plakoglobin; DSG2, desmoglein-2; DSC2, desmocollin-2; DSP, desmoplakin.

^aDigenic, homozygous, and compound heterozygous mutations; PLN, phospholamban.

^bAmong those that had ICD implanted; NSVT, non-sustained VT; ARVD/C, arrhythmogenic right ventricular dysplasia/cardiomyopathy; SCA, sudden cardiac arrest; SD, standard deviation; LV, left ventricular; m, mean.

^cEF < 55%; VT, ventricular tachycardia; VF, ventricular fibrillation; ICD, implantable cardioverter-defibrillator.

significantly later in PLN mutation carriers (42 years) ($P = 0.031$). Ten patients underwent cardiac transplantation (mean age at transplant: 46 ± 15 years, two primarily for intractable arrhythmias, eight primarily as a result of heart failure) and 12 (2%) died during follow-up. The survival free of death/cardiac transplant at age 40 and 60 years for the overall population was 91 and 85%, respectively (Supplementary material online, Figure S1B). Sudden cardiac death in a proband could not be assessed as risk factor in family members since only one relative died suddenly.

Patients with more than one mutation (compound heterozygote, homozygote, or digenic mutation carriers; 4%) had significantly earlier onset of symptoms [median age 23 years (16–46)] as well as earlier occurrence of sustained ventricular arrhythmias (median age 25 years; $P = 0.041$) when compared those with only one mutation. Overall, these patients had a higher incidence of arrhythmias when compared those with only one mutation (Table 2). The sustained arrhythmia-free survival at age 20 and 40 years was significantly lower (71 and 33%, respectively) among probands with more than one mutation than among single heterozygous mutation carriers (Figure 2).

Left ventricular dysfunction (EF < 55%) was seen in 78 (14%) patients while 28 (5%) experienced congestive heart failure (HF) during follow-up. Plakophilin-2 carriers were least likely to have left ventricular dysfunction (9%), whereas those with DSP mutations had significantly more frequent left ventricular dysfunction (40%) ($P = 0.001$) and HF (13%) ($P = 0.046$). Patients with more than one mutation and PLN mutation carriers had more than three times the amount of left ventricular dysfunction and heart failure. This relationship, stratified among probands and relatives is shown in Figure 3.

Influence of type of mutation on outcomes

Among those presenting alive, premature truncating, splice site, and missense mutations were identified in 319 (59%), 119 (22%), and 82 (15%) patients, respectively (Supplementary material online, Table S6). No significant difference in either the arrhythmic outcome-free survival or the cardiac transplant/death-free survival was seen in the overall population between patients with premature truncating, splice site, or missense mutations (Figure 4A and B).

Sex

Females constituted nearly half (45%) of the overall cohort. However, men were more likely to be probands (68% of probands; $P < 0.001$), be symptomatic at presentation (50% men vs. 37% women; $P < 0.001$) and present with SCD (78% men; $P = 0.004$). In the overall population, the composite arrhythmic outcome occurred significantly more in males (53 vs. 29%; $P < 0.001$) (Figure 5). However, occurrence of HF (6 vs. 4%; $P = 0.246$) and left ventricular dysfunction (16 vs. 12%; $P = 0.151$) was not significantly higher in males.

Discussion

Our study of the largest worldwide cohort of 577 patients (230 index patients, 347 relatives) with ARVD/C associated mutations has three important results. First, our study shows that phenotypic first presentation with SCD and/or VF occurs at a significantly younger age (median 23 years) than presentation with sustained monomorphic VT (median 36 years). Secondly, this large cohort demonstrates the association of genotype with clinical course and disease expression.

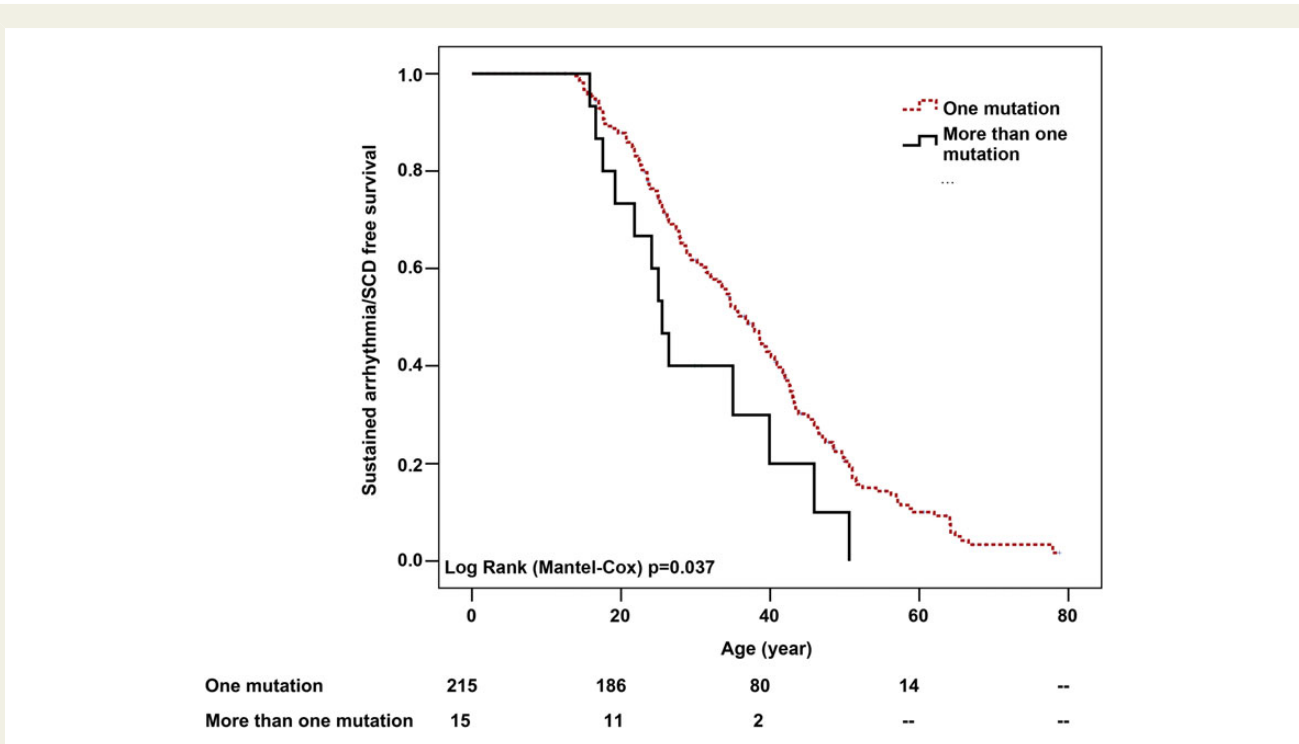


Figure 2 Survival analysis showing significantly lower sustained arrhythmia-free survival in index patients with more than one mutation when compared with those with one mutation only.

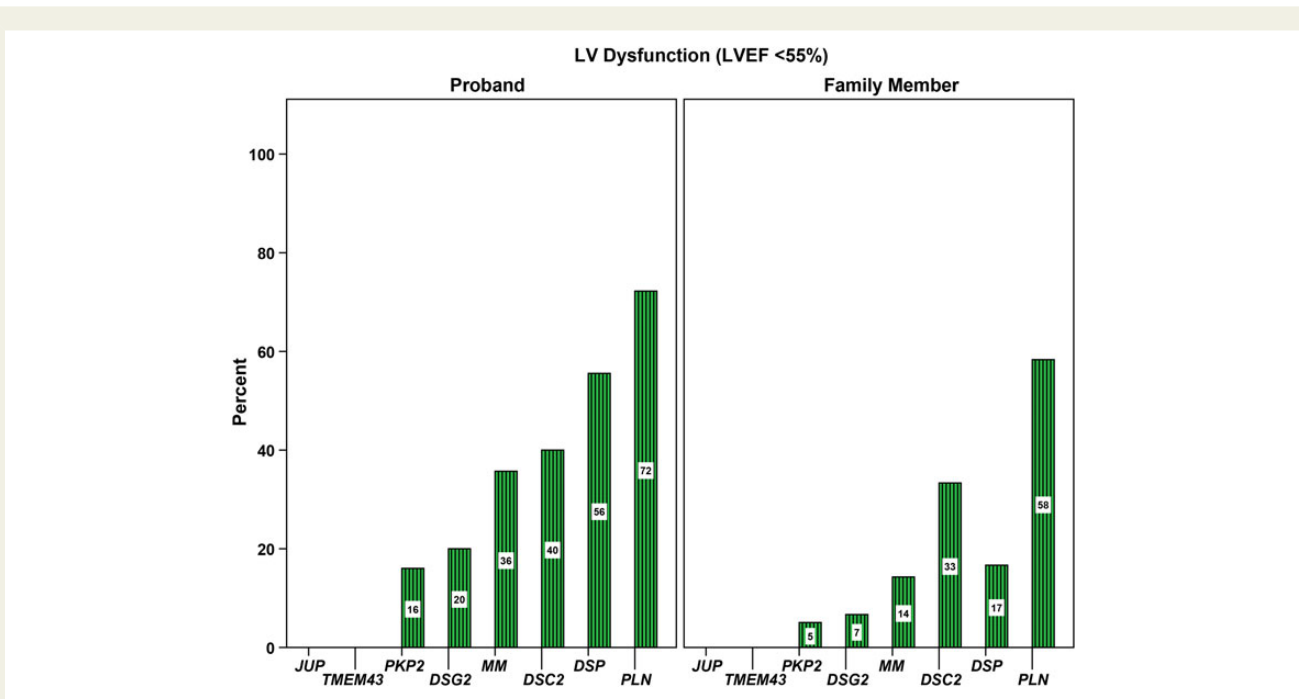


Figure 3 Proportion of patients manifesting left ventricular (LV) dysfunction among probands and family members in each of the major gene groups. PKP2, plakophilin-2; DSP, desmoplakin; DSG2, desmoglein-2; DSC2, desmocollin-2; PLN, phospholamban; JUP, plakoglobin; MM, more than one mutation, including compound heterozygote, homozygote, and digenic patients.

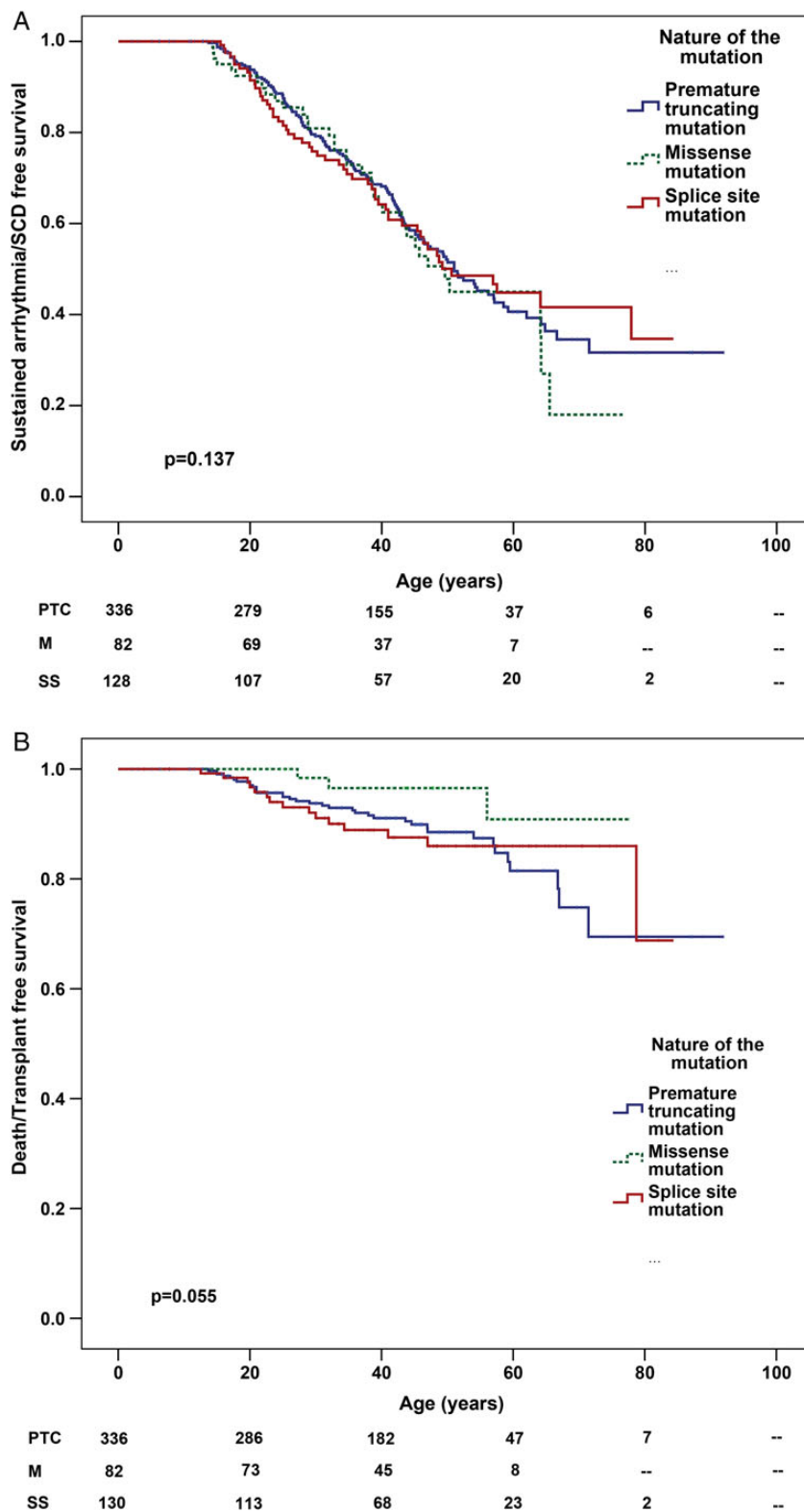


Figure 4 (A) Sustained ventricular arrhythmia-free survival in patients stratified according to the type of underlying mutation. (B) Death or transplant-free survival in patients stratified according to the type of underlying mutation in the study population.

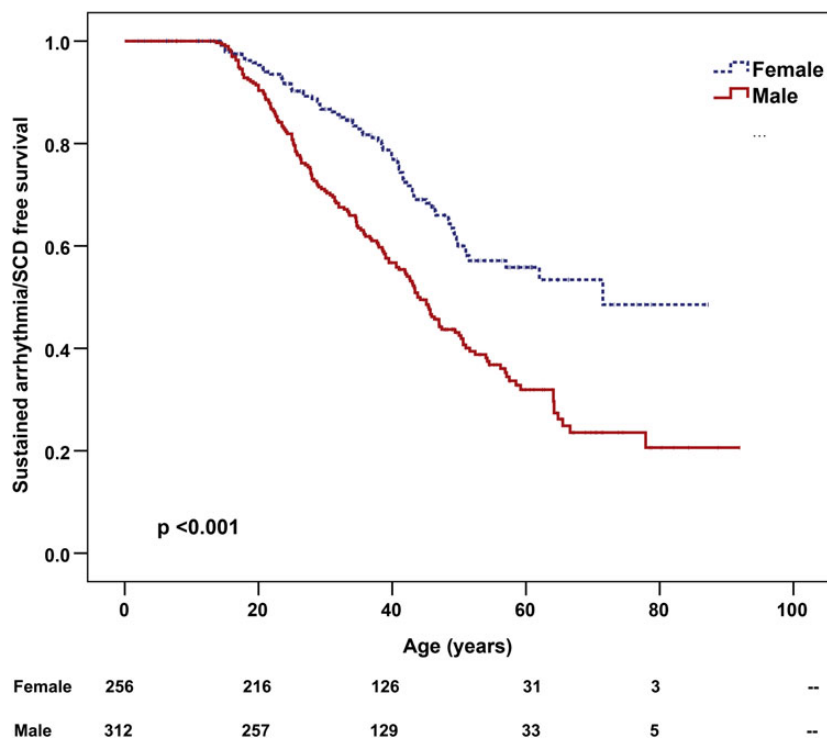


Figure 5 Composite arrhythmic outcome-free survival among study patients stratified by sex.

Thirdly, our study provides evidence that male sex negatively modifies the clinical course.

Sudden death as earliest disease manifestation among arrhythmogenic right ventricular dysplasia/cardiomyopathy mutation carriers

Sudden cardiac death is a well-appreciated risk associated with ARVD/C, with up to half of index cases presenting with SCD.^{10,15} Recent studies suggest that histologic changes may be preceded by gap-junction remodelling mediating electrical coupling and altering the amplitude and kinetics of the sodium current,¹⁶ giving rise to slow conduction. In cellular and mouse models,¹⁷ these changes result in an increased propensity for arrhythmias, also without any evidence of structural heart disease.¹⁷ Our finding that those presenting with SCD/SCA are significantly younger than those presenting with sustained VT supports this mechanism and underscores the importance of molecular, genetic and phenotypic investigation of young family members following the first diagnosis in a family. *Desmoplakin* carriers were significantly more likely to present with SCD than other mutation carriers and accounted for 11% of SCD cases (when compared with 3% of the entire population). This observation confirms a prior report from four Italian families¹⁸ and provides evidence for early identification of young at-risk individuals with *DSP* mutations.

Impact of genotype on heart failure and arrhythmic outcomes

In our study, majority of patients presenting alive as well as those presenting with SCD harboured premature truncating *PKP2* mutations.

Four percent of our population (6% of probands) carried more than one ARVD/C-associated mutation. The lower incidence of digenic, homozygous, or compound heterozygous mutations in our population compared with data in some previous reports^{10,19} reflects the rigorous approach and stringent criteria we used to define pathogenicity of missense mutations. Prior studies have suggested that individuals with more than one mutation may have worse clinical outcomes.^{11,20–24} Our relatively large sample of individuals with more than one mutation alive at presentation shows that these patients have considerably worse clinical course with significantly earlier onset of symptoms and first sustained arrhythmia, a greater chance of developing sustained VT/VF, and a five-fold increase in the risk of developing left ventricular dysfunction and HF than those carrying a single mutation. These results suggest that patients with more than one mutation should be followed carefully for symptoms associated with both arrhythmia and heart failure.

Among carriers of a single mutation presenting alive, our study demonstrates that the risk of left ventricular involvement and development of HF is intrinsically related to the mutated gene. Conversely, carriers of single mutations in all the genes had a high risk of developing a life-threatening arrhythmia, with no significant differences in survival from life-threatening VT/VF among the different genes. *PLN* mutation carriers presented at a significantly older age yet had worse long-term prognosis, with more left ventricular dysfunction and heart failure. Also *DSP* mutation carriers were considerably more likely to develop both HF and signs of LV involvement, confirming the observation from prior smaller cohorts.^{25,26} In contrast to a prior observation,²¹ *DSG2* mutation carriers were not disproportionately likely to develop LV involvement in our study.

Pathogenicity of a missense mutation in ARVD/C is difficult to determine and has been questioned.^{20–22} We demonstrate that ARVD/C-related missense mutations have a similar pathogenic character as non-missense mutations. In contrast to findings of other investigators,²¹ carriers of missense mutations in our population had no difference in survival from death/transplant or from developing a life-threatening arrhythmia when compared with carriers of premature truncating and splice site mutations. This lack of phenotypic difference or outcome suggests that these highly scrutinized missense alleles are indeed pathogenic. This pathogenicity of ARVD/C-associated missense mutations can be correctly predicted by the combined criteria from SIFT, PolyPhen-2, and ESP minor allele frequency.

Sex

Previous reports suggest that the severity of the phenotype is likely influenced by sex.²⁷ However, the impact of sex upon clinical course among ARVD/C mutations carriers has been unclear. Our study shows that male mutation carriers were more likely to present with sudden death, be symptomatic at presentation, develop VT/VF/SCD, and to have less overall survival free from death/cardiac transplant. However, males were not more likely to have LV involvement or heart failure. The mechanism for lower penetrance among women is uncertain although it is likely that this may reflect different levels of participation in competitive athletics among men and women.²⁸ There is some evidence from animal models that strenuous exercise is associated with development of ARVD/C²⁹ and more recently vigorous exercise has been found to be associated with worse clinical outcomes in patients harbouring a desmosomal mutation.²⁸

Limitations

Variations in disease expression between families carrying the same mutation, and among members of the same family, suggest that modifier genes and environmental influences contribute to the overall phenotype in ARVD/C. This study does not evaluate the potential impact of a large number of variants of uncertain significance in modifying the phenotypic expression of a pathogenic mutation. The biophysical function of many missense mutations reported in this study has not been studied in expression systems or models. Also, all *PLN* mutation carriers had the same mutation and results cannot be extrapolated to other mutations in this gene. The presence of founder mutations limits the extent to which the findings may be generalized to other ARVD/C cohorts. Although appropriate ICD therapy constituted a small minority of the composite arrhythmic outcome, overestimation of SCD risk is a possibility. Despite our large cohort, the small number of multiple mutations carriers relative to the overall population demands caution in generalizing these results. Finally, this study focused on patients harbouring pathogenic mutations and comparison with those without identifiable genetic abnormality remains a goal for future studies.

Conclusions

This study of the largest well-defined ARVD/C cohort worldwide demonstrates that SCD and VF occur at much younger age than monomorphic VT. Genotype–phenotype correlation analysis

provides evidence for (i) gene-specific differences in the propensity to life-threatening arrhythmias, left ventricular dysfunction, and heart failure and (ii) substantially worse outcome with presence of more than one pathogenic mutation. The study confirms worse outcome with male sex. We also provide evidence that missense variants, defined as pathogenic by predicting algorithms, are associated with a similar prognosis as premature truncating or splice site mutations.

Supplementary material

Supplementary Material is available at *European Heart Journal* online.

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References

- Marcus FI, Fontaine GH, Guiraudon G, Frank R, Laurenceau JL, Malergue C, Grosgeat Y. Right ventricular dysplasia: a report of 24 adult cases. *Circulation* 1982;**65**:384–398.
- Thiene G, Nava A, Corrado D, Rossi L, Pennelli N. Right ventricular cardiomyopathy and sudden death in young people. *N Engl J Med* 1988;**318**:129–133.
- McKoy G, Protonotarios N, Crosby A, Tsatsopoulou A, Anastakis A, Coonar A, Norman M, Baboonian C, Jeffery S, McKenna WJ. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular dysplasia/cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). *Lancet* 2000;**355**:2119–2124.
- Gerull B, Heuser A, Wichter T, Paul M, Basson CT, McDermott DA, Lerman BB, Markowitz SM, Ellinor PT, MacRae CA, Peters S, Grossmann KS, Drenckhahn J, Michely B, Sasse-Klaassen S, Birchmeier W, Dietz R, Breithardt G, Schulze-Bahr E, Thierfelder L. Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. *Nat Genet* 2004;**36**:1162–1164.
- Rampazzo A, Nava A, Malacrida S, Boffagna G, Baucé B, Rossi V, Zimbello R, Simionati B, Basso C, Thiene G, Towbin JA, Danieli GA. Mutation in human desmoplakin domain binding to plakoglobin causes a dominant form of arrhythmogenic right ventricular cardiomyopathy. *Am J Hum Genet* 2002;**71**:1200–1206.
- Awad MM, Dalal D, Cho E, Amat-Alarcon N, James C, Tichnell C, Tucker A, Russell SD, Bluemke DA, Dietz HC, Calkins H, Judge DP. DSG2 mutations contribute to arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Am J Hum Genet* 2006;**79**:136–142.
- Yarris P, Ward D, Evans A, Asimaki A, Gandjbakhch E, Sen-Chowdhry S, McKenna WJ. Arrhythmogenic right ventricular dysplasia/cardiomyopathy associated with mutations in the desmosomal gene desmocollin-2. *Am J Hum Genet* 2006;**79**:978–984.

8. van der Zwaag PA, van Rijsingen IA, Asimaki A, Jongbloed JD, van Veldhuisen DJ, Wiesfeld AC, Cox MG, van Lochem LT, de Boer RA, Hofstra RM, Christiaans I, van Spaendonck-Zwarts KY, Lekanne dit Deprez RH, Judge DP, Calkins H, Suurmeijer AJ, Hauer RN, Saffitz JE, Wilde AA, van den Berg MP, van Tintelen JP. Phospholamban R14del mutation in patients diagnosed with dilated cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy: evidence supporting the concept of arrhythmogenic cardiomyopathy. *Eur J Heart Fail* 2012;**14**:1199–1207.
9. Merner ND, Hodgkinson KA, Haywood AF, Connors S, French VM, Drenckhahn JD, Kupprion C, Ramadanova K, Thierfelder L, McKenna W, Gallagher B, Morris-Larkin L, Bassett AS, Parfrey PS, Young TL. Arrhythmogenic right ventricular dysplasia/cardiomyopathy type 5 is a fully penetrant, lethal arrhythmic disorder caused by a missense mutation in the TMEM43 gene. *Am J Hum Genet* 2008;**82**:809–821.
10. Quarta G, Muir A, Pantazis A, Syrris P, Gehmlich K, Garcia-Pavia P, Ward D, Sen-Chowdhry S, Elliott PM, McKenna WJ. Familial evaluation in arrhythmogenic right ventricular cardiomyopathy: impact of genetics and revised task force criteria. *Circulation* 2011;**123**:2701–2709.
11. Cox MG, van der Zwaag PA, van der Werf C, van der Smagt JJ, Noorman M, Bhuiyan ZA, Wiesfeld AC, Volders PG, van Langen IM, Atsma DE, Dooijes D, van den Wijngaard A, Houweling AC, Jongbloed JD, Jordaens L, Cramer MJ, Doevendans PA, de Bakker JM, Wilde AA, van Tintelen JP, Hauer RN. Arrhythmogenic right ventricular dysplasia/cardiomyopathy: pathogenic desmosome mutations in index-patients predict outcome of family screening: Dutch arrhythmogenic right ventricular dysplasia/cardiomyopathy genotype-phenotype follow-up study. *Circulation* 2011;**123**:2690–2700.
12. den Haan AD, Tan BY, Zikusoka MN, Llado LI, Jain R, Daly A, Tichnell C, James C, Amat-Alarcon N, Abraham T, Russell SD, Bluemke DA, Calkins H, Dalal D, Judge DP. Comprehensive desmosome mutation analysis in North Americans with arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circ Cardiovasc Genet* 2009;**2**:428–435.
13. Roberts JD, Herkert JC, Rutberg J, Nikkel SM, Wiesfeld AC, Dooijes D, Gow RM, van Tintelen JP, Gollob MH. Detection of genomic deletions of PKP2 in arrhythmogenic right ventricular cardiomyopathy. *Clin Genet* 2013;**83**:452–456.
14. Murray B, Wagle R, Amat-Alarcon N, Wilkens A, Stephens P, Zackai EH, Goldmuntz E, Calkins H, Deardorff MA, Judge DP. A family with a complex clinical presentation characterized by arrhythmogenic right ventricular dysplasia/cardiomyopathy and features of branchio-oculo-facial syndrome. *Am J Med Genet A* 2013;**161A**:371–376.
15. Dalal D, Nasir K, Bomma C, Prakasa K, Tandri H, Piccini J, Roguin A, Tichnell C, James C, Russell SD, Judge DP, Abraham T, Spevak PJ, Bluemke DA, Calkins H. Arrhythmogenic right ventricular dysplasia: a United States experience. *Circulation* 2005;**112**:3823–3832.
16. Cerrone M, Noorman M, Lin X, Chkourko H, Liang FX, van der Nagel R, Hund T, Birchmeier W, Mohler P, van Veen TA, van Rijen HV, Delmar M. Sodium current deficit and arrhythmogenesis in a murine model of plakophilin-2 haploinsufficiency. *Cardiovasc Res* 2012;**95**:460–468.
17. Deo M, Sato PY, Musa H, Lin X, Pandit SV, Delmar M, Berenfeld O. Relative contribution of changes in sodium current versus intercellular coupling on reentry initiation in 2-dimensional preparations of plakophilin-2-deficient cardiac cells. *Heart Rhythm* 2011;**8**:1740–1748.
18. Bauce B, Basso C, Rampazzo A, Boffagna G, Daliento L, Frigo G, Malacrida S, Settimo L, Danieli G, Thiene G, Nava A. Clinical profile of four families with arrhythmogenic right ventricular cardiomyopathy caused by dominant desmoplakin mutations. *Eur Heart J* 2005;**26**:1666–1675.
19. Sen-Chowdhry S, Syrris P, Pantazis A, Quarta G, McKenna WJ, Chambers JC. Mutational heterogeneity, modifier genes, and environmental influences contribute to phenotypic diversity of arrhythmogenic cardiomyopathy. *Circ Cardiovasc Genet* 2010;**3**:323–330.
20. Bauce B, Nava A, Boffagna G, Basso C, Lorenzon A, Smaniotto G, De Bortoli M, Rigato I, Mazzotti E, Steriotis A, Marra MP, Towbin JA, Thiene G, Danieli GA, Rampazzo A. Multiple mutations in desmosomal proteins encoding genes in arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Heart Rhythm* 2010;**7**:22–29.
21. Fressart V, Duthoit G, Donal E, Probst V, Deharo JC, Chevalier P, Klug D, Dubourg O, Delacretaz E, Cosnay P, Scanu P, Extramiana F, Keller D, Hidden-Lucet F, Simon F, Bessirard V, Roux-Buisson N, Hebert JL, Azarine A, Casset-Senon D, Rouzet F, Lecarpentier Y, Fontaine G, Coirault C, Frank R, Hainque B, Charron P. Desmosomal gene analysis in arrhythmogenic right ventricular dysplasia/cardiomyopathy: spectrum of mutations and clinical impact in practice. *Europace* 2010;**12**:861–868.
22. Kapplinger JD, Landstrom AP, Salisbury BA, Callis TE, Pollevick GD, Tester DJ, Cox MG, Bhuiyan Z, Bikker H, Wiesfeld AC, Hauer RN, van Tintelen JP, Jongbloed JD, Calkins H, Judge DP, Wilde AA, Ackerman MJ. Distinguishing arrhythmogenic right ventricular cardiomyopathy/dysplasia-associated mutations from background genetic noise. *J Am Coll Cardiol* 2011;**57**:2317–2327.
23. Bao J, Wang J, Yao Y, Wang Y, Fan X, Sun K, He DS, Marcus FI, Zhang S, Hui R, Song L. Correlation of ventricular arrhythmias with genotype in arrhythmogenic right ventricular cardiomyopathy. *Circ Cardiovasc Genet* 2013;**6**:552–556.
24. Rigato I, Bauce B, Rampazzo A, Zorzi A, Pillichou K, Mazzotti E, Migliore F, Marra MP, Lorenzon A, De Bortoli M, Calore M, Nava A, Daliento L, Gregori D, Illiceto S, Thiene G, Basso C, Corrado D. Compound and digenic heterozygosity predicts lifetime arrhythmic outcome and sudden cardiac death in desmosomal gene-related arrhythmogenic right ventricular cardiomyopathy. *Circ Cardiovasc Genet* 2013;**6**:533–542.
25. Sen-Chowdhry S, Syrris P, Ward D, Asimaki A, Sevdalis E, McKenna WJ. Clinical and genetic characterization of families with arrhythmogenic right ventricular dysplasia/cardiomyopathy provides novel insights into patterns of disease expression. *Circulation* 2007;**115**:1710–1720.
26. Norman M, Simpson M, Mogensen J, Shaw A, Hughes S, Syrris P, Sen-Chowdhry S, Rowland E, Crosby A, McKenna WJ. Novel mutation in desmoplakin causes arrhythmogenic left ventricular cardiomyopathy. *Circulation* 2005;**112**:636–642.
27. Bauce B, Frigo G, Marcus FI, Basso C, Rampazzo A, Maddalena F, Corrado D, Winnicki M, Daliento L, Rigato I, Steriotis A, Mazzotti E, Thiene G, Nava A. Comparison of clinical features of arrhythmogenic right ventricular cardiomyopathy in men versus women. *Am J Cardiol* 2008;**102**:1252–1257.
28. James CA, Bhonsale A, Tichnell C, Murray B, Russel SD, Tandri H, Tedford RJ, Judge DP, Calkins H. Exercise increases age-related penetrance and arrhythmic risk in arrhythmogenic right ventricular dysplasia/cardiomyopathy associated desmosomal mutation carriers. *J Am Coll Cardiol* 2013. doi:10.1016/j.jacc.2013.06.033.
29. Kirchhof P, Fabritz L, Zwiener M, Witt H, Schafers M, Zellerhoff S, Paul M, Athai T, Hiller KH, Baba HA, Breithardt G, Ruiz P, Wichter T, Levkau B. Age- and training-dependent development of arrhythmogenic right ventricular cardiomyopathy in heterozygous plakoglobin-deficient mice. *Circulation* 2006;**114**:1799–1806.