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Shared Desmosome Gene Findings in Early and Late Onset Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy

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Abstract

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is an inherited form of cardiomyopathy with low penetrance and variable expressivity. Dominant mutations and rare polymorphisms in desmosome genes are frequently identified. We reasoned that individuals with earlier onset disease would have more frequent desmosome gene mutations and rare polymorphisms. Three groups were compared: Young with symptoms attributable to ARVD/C or a diagnosis of ARVD/C at age of 21 years or earlier, Middle with first symptoms or diagnosis age of 22–49 years, and Late with first symptoms or diagnosis at age of 50 or more years. deoxyribonucleic acid (DNA) sequence analysis was performed on five cardiac desmosome genes, and the presence of mutations and rare missense polymorphisms was compared among the three groups. In the entire Young cohort, 20 (67%) had one or more cardiac desmosome gene mutations. The prevalence of cardiac desmosome gene mutations was similar in the Middle (48%) and Late (53%) cohorts (P=0.23). Similar numbers of individuals in each cohort had more than one desmosome gene mutation, although the numbers are too small for statistical comparisons. The prevalence of certain rare missense DNA variants was not different among the cohorts (P=0.71), yet these rare missense alleles were more prevalent in the overall study cohort of 112 ARVD/C participants compared to 100 race-matched controls (P=0.027). The presence of these variants did not associate with the age of onset of ARVD/C or ventricular tachycardia. These findings highlight the complex interplay of environmental and genetic factors contributing to this condition.

Keywords

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C); Genetics; Sudden cardiac death; Desmosome

Introduction

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is an inherited form of cardiomyopathy affecting up to 1 in 5,000 individuals in the USA [1, 2]. Although the median age at presentation was 26 years in one study, this included a broad range of ages extending from 2 to 70 years [3]. Factors influencing the age of onset are not well understood.

Over the past several years, it has become clear that many cases of ARVD/C are caused by mutations in genes encoding elements of the cardiac desmosome (*PKP2*, *DSG2*, *JUP*, *DSC2*, and *DSP*) [4–8]. Its transmission is typically autosomal dominant, but recessive variants of ARVD/C have also been described [9, 10]. In rare cases, affected individuals have been reported with three distinct desmosome gene mutations, although the absence of a functional assay for novel desmosome variants leads to difficulty in classification [11].

Determination of the age of onset is complicated by a “latent period” during which overt evidence of disease may be lacking, and sudden cardiac death may be the first symptom [12]. Children under the age of 11 years account for less than 2% of ARVD/C [13]. Very little is known about the genotypic characteristics of this subset of ARVD/C individuals. In

addition, incomplete penetrance and variable expressivity affect the interpretation of family screening for ARVD/C [14, 15]. Similarly, late onset disease is rare but well described [12, 16]. Genetic factors influencing low penetrance and later onset disease are also poorly understood.

The aim of our study is: (1) To investigate the prevalence of desmosome mutations and multiple mutations (compound and digenic heterozygosity) in young people with ARVD/C compared to more typical age of onset and to those with later onset disease, and (2) to determine whether low-frequency amino acid substitutions that are found among individuals with ARVD/C are similarly prevalent among controls.

Methods

The study population consists of 112 unrelated participants with definite and probable ARVD/C from the Johns Hopkins ARVD registry. Three specific subgroups were identified based on the age of onset of symptoms or, if asymptomatic, the age of diagnosis of definite or probable ARVD/C. The Young cohort consisted of 19 definite ARVD/C and 11 probable ARVD/C probands, age≤ 21 years. The Middle cohort consisted of 56 individuals with definite ARVD/C and 11 with probable ARVD/C, age between 22 and 49 years of age. The Late cohort included 15 probands with definite ARVD/C, age≥50 years. The Middle cohort overlaps with a prior report by our group, but the Young and Late cohorts were expanded by ten (Young) and six (Late) to increase the representation of these groups [11]. Only one member of any family is included in this analysis; family members who provided medical records, blood for deoxyribonucleic acid (DNA) testing, and consent for participation were assessed for cosegregation of mutations with ARVD/C, but they are not included in the three cohorts in order to prevent skewed representation of families with high penetrance.

This study was approved by the Johns Hopkins School of Medicine Institution Review Board and written informed consent was obtained from all study subjects. Clinical history and medical records were obtained at enrollment and at yearly intervals. Family history was determined by interviewing participants or family members. The age of onset of symptoms was defined as the age at which those individuals experienced symptoms attributable to ARVD/C. Symptoms include palpitations, syncope, ventricular tachycardia, sudden cardiac death (SCD), chest discomfort, and symptoms related to right heart failure. All participants underwent a thorough physical examination.

Further testing included 12-lead echocardiogram (ECG; *n*=112), exercise testing using standard protocols, signal-averaged electrocardiogram (SAECG; *n*=95), 24-h Holter monitoring (*n*=78), imaging studies such as echocardiography or cardiac magnetic resonance imaging (MRI; *n*=112), and right ventricular endomyocardial biopsy (*n*=39). The presence or absence of ventricular tachycardia or ventricular ectopics and its morphology was determined with the standard 12-lead ECG, the results of 24-h Holter, and exercise testing. SAECG in subjects without preexisting right bundle branch block was considered positive for late potentials if any two of the following were present: (1) filtered QRS duration ≥ 114 ms, (2) low-amplitude signal duration≥38 ms, and (3) RMS20 μV. RV dysfunction and structural abnormalities were determined with imaging studies. Histological evidence of fibrofatty replacement of myocytes was obtained from endomyocardial biopsies of the right ventricular free wall.

The diagnosis of ARVD/C was established based on the criteria set in 1994 by the Task Force of the Working Group of Myocardial and Pericardial Disease of the European Society of Cardiology and the International Society and Federation of Cardiology [17]. The diagnosis of definite ARVD/C is established by the presence of two major criteria, one

major and two minor criteria, or four minor criteria. The diagnosis of probable ARVD/C is established by the presence one major and one minor criterion or three minor criteria. The criteria must come from different categories. (1) Structural alteration of the heart (Major: Severe dilatation and reduction of right ventricular ejection fraction with no significant LV impairment; localized right ventricular aneurysms; severe segmental dilatation of the right ventricle. Minor: Mild global right ventricular dilatation and/or dysfunction with normal left ventricle; mild segmental dilatation of the right ventricle; regional right ventricular hypokinesia). (2) Tissue characterization of walls (Major: Fibrofatty replacement of myocardium on endomyocardial biopsy). (3) Repolarization abnormalities (Minor: Inverted T waves in right precordial leads in people aged >12 years, in the absence of right bundle branch block). (4) Depolarization/conduction abnormalities (Major: epsilon waves or localized prolongation >110 ms of the QRS complex in right precordial leads; Minor: late potentials on signal averaged ECG). (5) Arrhythmias (Minor: left bundle branch block type ventricular tachycardia, sustained or non-sustained on ECG, Holter, or exercise testing; frequent ventricular extrasystoles >1000/24 h) [6]. Family history [Major: familial disease confirmed at necropsy or surgery. Minor: familial history of premature sudden death (<35 years) due to suspected ARVD/C, family members independently diagnosed with ARVD/C] [17].

Mutation Analysis

Each participant had genomic DNA extracted from leukocytes present in whole blood using QIAmp DNA blood maxi kits (Qiagen, Valencia, CA). Intronic *PKP2*, *DSG2*, *DSC2*, *DSP*, and *JUP* primers flanking each exon were used in polymerase chain aeaction (PCR) amplification. The resultant PCR products were purified using the QIAquick PCR Purificatin Kit (Qiagen) followed by bidirectional sequence analysis using the ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) and chromatograms analyzed with Sequencer 4.1 software. Mutations were confirmed by restriction fragment length polymorphisms when possible or by Taqman genotyping assays (Applied Biosystems).

A novel variant in the DNA sequence was defined as a mutation when it does not occur in 400 unrelated, healthy, race-matched, control chromosomes (NIGMS Human Genetic Cell Repository, Coriell Institute for Medical Research), when it alters one or more conserved amino acids, and when it segregates with disease in the family if that information is available. All other novel variants are classified as polymorphisms. Sequence variants that were previously reported as mutations were considered mutations unless we could show otherwise. Novel DNA variants that alter a restriction site were tested for their presence in controls using restriction enzyme digest. If the sequence variants did not alter any restriction sites, the presence among controls was determined by the use of Taqman genotyping assays (Applied Biosystems). Novel missense protein variants were analyzed by ClustalW (MacVector, Cary, NC), Polyphen (http://genetics.bwh.harvard.edu/pph/), and MUpro (http://www.ics.uci.edu/~baldig/mutation.html) for conservation, stability, and likelihood for pathogenic effect.

Low-frequency amino acid substitutions are defined as missense variants that were found in 200 unrelated, healthy, race-matched, control chromosomes (NIGMS Human Genetic Cell Repository, Coriell Institute for Medical Research) at a frequency of less than 5%, or were previously reported as single-nucleotide polymorphisms at a similar low-frequency. The ARVD/C Genetic Variants Database was reviewed for all mutations and low-frequency missense alleles (www.arvcdatabase.info).

Statistical Analysis

Continuous variables were expressed as mean ± SD and compared by unpaired *t* test when comparing two groups and analysis of variance (ANOVA) in cases of multiple groups. Categorical variables were expressed as frequency (%) and compared by chi-squared test in case of large samples and by Fisher's exact test in case of small sample size.

A low-frequency amino acid substitution score was calculated by assigning a score of one for the presence of each missense variant. Participants were then categorized based on this score. Categorical variables were compared based on the score category. For the survival analysis, individuals were categorized into three groups based on the presence or absence of low-frequency amino acid substitution and known desmosome gene mutations. Kaplan–Meier analysis and log–rank test was used to compare arrhythmia-free survival at the age of evaluation as a function of the three groups. Analyses were performed using STATA statistical software (College Station, TX) and a p value< 0.05 was considered statistically significant.

Results

Demographics and Presentation

The demographics and presenting symptoms of individuals in each cohort are summarized in Table 1. The Young cohort consisted of 19 people with definite ARVD/C with a mean age of symptom onset or a diagnosis of ARVD/C at 15.5±4.1 years (min=2, max=20 years). Sixteen were symptomatic before the age of 21 years, and the remaining three were diagnosed due to a family history of ARVD/C prior to symptoms. The 11 individuals with probable ARVD/C in the Young cohort had a mean age of 16.5± 3 years (min=12, max=21). Nine were symptomatic before the age of 21 years, and two were screened and diagnosed due to a family history of ARVD/C prior to symptoms.

The Middle cohort consisted of 56 people with definite ARVD/C with a mean age of symptom onset or ARVD/C diagnosis at 34.3±8.1 years (min=22, max=48). Forty-eight had symptoms prompting their evaluation, and eight were screened due to a family history of ARVD/C prior to symptoms. The 11 individuals with probable ARVD/C in the Middle cohort had a mean age of 36.2±4.7 years (min=29, max=46). Nine had symptoms prompting their phenotypic screening, and two were evaluated for ARVD/C due to a family history of this condition prior to symptoms.

The Late cohort consisted of 15 people with definite ARVD/C with a mean age of symptom onset or a diagnosis of ARVD/C at 56.3±7.0 years (min=50, max=76). Thirteen underwent cardiac testing for symptoms, and two were evaluated due to a family history of ARVD/C prior to symptoms.

Phenotypic Characteristics

The results of phenotypic testing of all 112 participants in this study are shown in Table S1. Analysis was made with comparisons among the three groups, with separate categories for definite and probable ARVD/C. These characteristics varied only based on the presence of epsilon wave, which was most common in the Middle cohort (P=0.03), and abnormal SAECG, which was most common in the Late cohort (P=0.006) among the three groups. There is a decreased likelihood of an abnormal SAECG in the Young cohort (P=0.012), but no phenotypic characteristics were otherwise different in this cohort.

Desmosome Mutations

Thirteen individuals (67%) had one or more mutations identified in the Young cohort with definite ARVD/C (Tables 2 and S1). There were 13 different mutations: four altered a critically conserved nucleotide that forms the intron–exon splice site, one was a nonsense mutation that resulted in a premature termination codon, four were missense mutations disrupting highly conserved residues, and four were insertion–deletion mutations. The distribution of genes in which mutations occurred in each cohort is shown in Fig. 1.

A total of three novel desmosome gene mutations were identified in the Young cohort with definite ARVD/C (*DSP* p.E422K, *DSG2* p.K346del, *DSG2* c.523+2T>C; Fig. S1). The individual with the *DSP* p.E422K (1264G> A) mutation was evaluated for palpitations at age of 15 years. Echocardiogram showed a dilated right ventricle with severe right ventricular dysfunction. There was no family history of ARVD. One participant with recurrent syncopal events since the age of 17 years has two *DSG2* mutations p.K346del and *DSG2* c.523+2T>C (compound heterozygous). She has no family history of ARVD; she has two children, ages 7 and 8 years, each of whom carries one of the *DSG2* mutations. Both are currently asymptomatic and have no phenotypic features of ARVD. *DSG2* c.523+ 2T>C alters a critically conserved nucleotide leading to a mutant splice product. p.K346del (ca. 1038–1040 delGAA) causes in-frame deletion of a lysine residue in the extracellular domain EC3 of desmoglein-2. In the proband, it is possible that nonsense-mediated messenger ribonucleic acid (mRNA) decay of the mutant splice product (c.523+ 2T>C) contributed to the disease mechanism. However, RNA samples from the proband and her two children are not available for analysis.

Seven individuals (64%) had one or more mutations identified in the Young cohort with probable ARVD/C (Tables 2 and S1). There were seven different mutations: one altered a critically conserved nucleotide that forms the intron–exon splice site, three were missense mutations disrupting highly conserved residues, and three were insertion–deletion mutations. A total of three novel desmosome gene mutations were identified in the Young cohort with probable ARVD/C (*DSG2* p.T1047R, *DSC2* p.G220R, *PKP2* p.V587delfsX655).

Twenty-eight individuals (50%) had one or more mutations identified in the Middle cohort with definite ARVD/C (Tables 3, and S1). There were 17 different mutations: two altered a critically conserved nucleotide that forms the intron–exon splice site, four were nonsense mutations that resulted in a premature termination codon, five were missense mutations disrupting highly conserved residues, five were insertion–deletion mutations, and one was a cryptic splice mutation.

Four individuals (36%) had one or more mutations identified in the Middle cohort with probable ARVD/C (Tables 3 and S1). There were four different mutations: one altered a critically conserved nucleotide that forms the intron–exon splice site, one was a missense mutation disrupting highly conserved residue, and two were insertion–deletion mutations.

Eight individuals (53%) had one or more mutations identified in the Late cohort with ARVD/C (Tables 4 and S1). There were ten different mutations: three altered a critically conserved nucleotide that forms the intron–exon splice site, six were missense mutations disrupting highly conserved residues, and one was an insertion–deletion mutation. A total of two novel desmosome mutations (Fig. S1) were identified in the Late cohort with definite ARVD/C (*DSG2* p.D297N, *PKP2* p. V725D). One individual had two desmosome gene variants that have previously been published as mutations (*PKP2* p.S140F and *DSG2* p.V56M), although they also occur rarely in controls and their association with disease has been questioned [18, 19]. However, he also has a clear splice site mutation (*PKP2* ca. 2146-1G>C) and is included as positive for a desmosome gene mutation on that basis.

There were no differences in the prevalence of one or more desmosome mutations among the Young (67%), Middle (48%) and Late (53%; *P*=0.23) cohorts. *PKP2* (Fig. 1) was the most common gene in which mutations were identified. There was no increase in frequency of compound heterozygous or digenic heterozygous mutations in the Young cohort compared to the Middle and Late cohorts (Table 1; *P*=0.072).

Low-Frequency Amino Acid Substitutions

Several low-frequency amino acid substitutions are more common in this cohort of 112 definite and probable ARVD/C participants than among 100 race-matched Coriell controls. A total of 15 missense variants were identified for further analysis (Table 5): six in *PKP2*, two in *DSG2*, one in *JUP*, three in *DSP*, and three in *DSC2*. These missense variants were chosen for further analysis because of their known low frequency in controls (< 5%) and their lack of known association with disease pathogenesis. We reasoned that these variants may be over-represented in ARVD/C compared to controls. Indeed, 21% of the total ARVD/C cohort compared to 13% of the controls had one or more of these variants (*P*=0.027; Fig. 2).

We sought to determine if these rare missense alleles influence the age of onset of ARVD/C by comparing their prevalence among the three cohorts. However, there is no difference in their frequency among the three cohorts (*P*=0.71). The presence of these variants also did not influence the age of onset of ventricular tachycardia (VT). Figure 3 shows the Kaplan–Meier curves documenting freedom from VT for individuals with (1) no desmosome mutations and none of the 15 missense variants, (2) one or more desmosome mutations and none of the 15 variants, and (3) one or more desmosome mutations and at least one of the 15 variants. The median cumulative arrhythmia-free survival was influenced by the presence of desmosome mutations, independent of any of these 15 low-frequency missense variants. The median cumulative arrhythmia-free survival with no desmosome mutation and none of the 15 variants is 45 years, and 32 years when one or more desmosome mutation is present without any of the 15 variants (*P*=0.02). When at least one desmosome mutation is present, the addition of any of the 15 variants did not influence the median cumulative arrhythmia-free survival.

Discussion

Phenotypic heterogeneity in inherited cardiovascular disease is a common occurrence. Affected individuals within a family who harbor the same gene mutation often manifest wide clinical variability. This suggests that other modifying factors, including environmental exposures and other genetic influences, play a role in either exacerbating or attenuating disease manifestation. The exact impact of these factors is poorly understood.

For many inherited cardiovascular diseases, the presence of two or more mutations may result in earlier and more severe disease manifestation. For instance, in hypertrophic cardiomyopathy (HCM), people with homozygous, digenic heterozygous, or compound heterozygous mutations may have a higher incidence of SCD and may have more severe left ventricular hypertrophy [20, 21]. This led to a similar hypothesis in HCM that the presence of sarcomere gene mutations would be different among a cohort with childhood onset. However, Morita et al. [22] demonstrated sarcomere gene mutations in only 46 of 84 individuals (54%) with familial or sporadic HCM diagnosed before the age of 15 years. This did not differ from the overall prevalence of gene mutations (59%) in unrelated probands with HCM.

The genetics of early onset ARVD/C was previously less well characterized than early onset HCM. Recent studies have suggested that for the rare desmosome gene mutations (namely,

DSG2 and *DSC2*), multiple mutations may be required to manifest the ARVD/C phenotype [23]. Multiple desmosome gene mutations have also been shown to have more frequent sudden death [24]. Even common polymorphisms (namely, *PKP2* p.P366L, MAF:17%) may not be completely innocuous but, rather was suggested in one report to confer a more benign phenotype [25]. In our study, at least one desmosome mutation was found in 20 out 30 participants with definite ARVD/C or probable ARVD/C (67%) diagnosed before the age of 21 years. These results did not differ significantly from the Middle or Late cohorts (P=0.23). There was also no increase in prevalence of compound heterozygous or digenic heterozygous mutations in the Young cohort.

The age of onset of ARVD/C is not easily defined. The risk of SCD exists even in the early "concealed" phase of ARVD/C [3]. Progression of disease may not be uniform throughout life and likely involves sporadic periods of greater change as well as quiescent phases. After the age of 40 years, the incidence of SCD due to ARVD/C may decrease [26, 27]. The true incidence of later onset ARVD/C, however, may be severely underestimated due to the presence of other comorbidities such as coronary atherosclerosis and myocardial infarction. Phenotypic testing for ARVD/C after age of 50 years may also be misleading. SAECG for example, may be positive in up to 14% of the elderly with no clinical evidence of heart disease [28]. In this study, 53% of the Late cohort (mean age 56.3±7 years) had one or more desmosome mutation, not different from the Middle and Young cohorts. The reasons for later onset disease in this cohort may pertain to other genetic or environmental factors.

The autosomal dominant monogenic inheritance paradigm, where one gene mutation causes disease, is further challenged by the potential modifier effects of uncommon DNA variants that may influence disease. Crotti et al. [29] reported a family with Long QT syndrome (LQT2) in which the proband had a missense *KCNH2* mutation p.A1116V and a common *KCNH2* polymorphism p.K897T on the non-mutant allele. The proband had ventricular fibrillation and cardiac arrest at the age of 44 years. Family members with the same *KCNH2* mutation p.A1116V without the p.K897T polymorphism, however, only had latent disease. Similar factors may be relevant in ARVD/C.

We attempted to explore the contribution of missense variants in ARVD/C by comparing age of onset as a marker of severity of disease. A total of 15 low-frequency (<5%) amino acid substitutions were analyzed among both cases and controls. The prevalence of these variants was higher (P=0.027) in the entire cohort of 112 probands with definite and probable ARVD/C. When we analyzed the Kaplan–Meier curves of VT-free survival, only the presence of desmosome mutations conferred a worse outcome. The addition of these low-frequency amino acid variants did not influence VT-free survival. The prevalence of these variants is also not significantly higher in the Young cohort. The increased prevalence of these variants in the disease population compared to controls does suggest a contributory role. However, the exact effect of these polymorphisms is difficult to determine without functional assays, and such assays are not currently available for desmosome protein variants.

Xu et al. [30] recently reported desmosome gene analysis in a cohort of 198 individuals with ARVD/C. In this cohort, 26% had a desmosome gene variant or mutation. Furthermore, among the 38 participants with a *PKP2* mutation or variant, 16 also had a second mutation or variant present at low frequency among controls, representing 8% of the total cohort. This also suggests that additional desmosome gene variants may impact disease [30].

For this study, classification of all participants was based on the traditional 1994 Task Force Criteria (TFC) [17]. In a recent analysis of 105 individuals with proven ARVD/C based on the 1994 TFC, only three were reclassified as probable ARVD/C using the new TFC [31].

The impact of the new TFC criteria was predominantly felt in the probable ARVD/C group (64% were reclassified as ARVD/C) and in family members of probands (11% were reclassified as ARVD/C) [31, 32]. Because we included both definite and probable ARVD/C and applied these criteria equally to the three cohorts, the use of the newer criteria would not likely change our results.

Late potentials by SAECG are increasingly recognized as a marker for delay activation in ARVD/C. An abnormal SAECG (by the traditional TFC) could be a nonspecific finding in the elderly. An abnormal SAECG is also less commonly found in the Young cohort, suggesting a decrease in sensitivity in young individuals with ARVD/C. To satisfy a minor criterion, the new TFC only requires one of the three measured parameters in the SAECG to be abnormal [31]. While this increases sensitivity in Young cohort, it comes at a cost of decreasing specificity in the Late cohort.

The addition of a pathogenic mutation as a major criterion in the new TFC could encourage one to stop screening for mutations in other desmosome genes once a mutation is identified. As a result, a second mutation could be missed if comprehensive analysis is not performed. In addition, family members may be mistakenly counseled about their risk of inheritance if the proband is not thoroughly evaluated for desmosome gene mutations.

Study Limitations

Complete evaluation could not be performed in all first degree relatives, which limits our ability to associate familial segregation of many of these DNA variants with disease manifestations. Also, the age of diagnosis is dependent on the age at which a proband first sought evaluation for ARVD/C. Often, this follows the diagnosis of an affected person in the family. In the event that a proband is diagnosed as meeting TFC at the first evaluation, the true age of diagnosis would certainly precede it. On phenotypic evaluation, we relied on qualitative definitions of RV disease on imaging studies as recommended by the 1994 TFC rather than quantitative parameters for imaging studies that are proposed in the new TFC [17, 31]. The numbers of individuals included at early and late age are relatively small, and further multi-center analyses may help to confirm and extend these findings.

Conclusion

The phenotypic heterogeneity and variable penetrance of ARVD/C highlight the complex interplay of environmental and genetic factors contributing to this condition. In this analysis, the prevalence of desmosome mutations and of multiple mutations was similar in the Young, Middle, and Late cohorts. Certain rare missense variants were more common among ARVD/C cases of all ages compared to controls. This suggests that these rare desmosome gene variants may influence the development of ARVD/C but not the age of onset. Additional genetic and environmental modifiers likely impact age of onset of this condition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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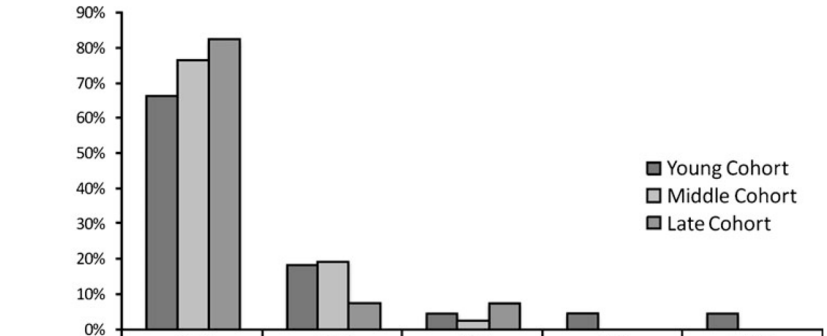

	PKP2	DSG2	DSP	JUP	DSC2
Young Cohort	67%	19%	5%	5%	5%
Middle Cohort	77%	20%	3%	0%	0%
Late Cohort	83%	8%	8%	0%	0%

Fig. 1.

Distribution of desmosome mutations in each cohort. The percentage of mutations in each gene is shown by cohort. Both definite and probable ARVD/C cases were included

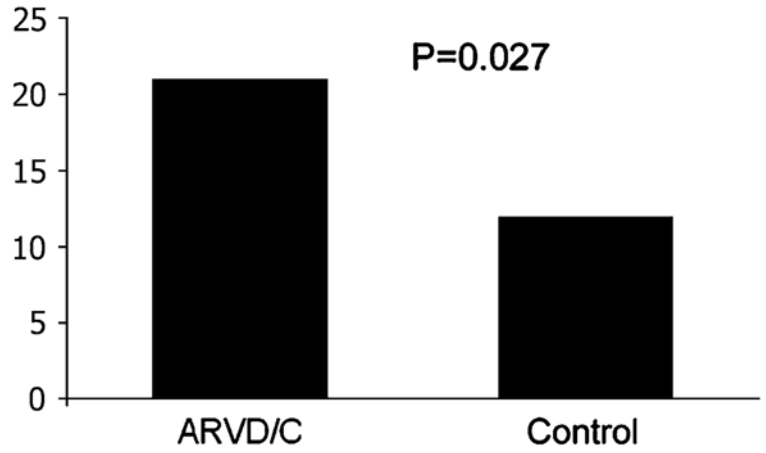

Fig. 2.

Percentage with low-frequency missense alleles. The percentage of participants with one or more of the low-frequency missense polymorphisms is shown. ARVD/C represents the percentage with both definite and probable disease; Control represents 100 race-matched Coriell controls

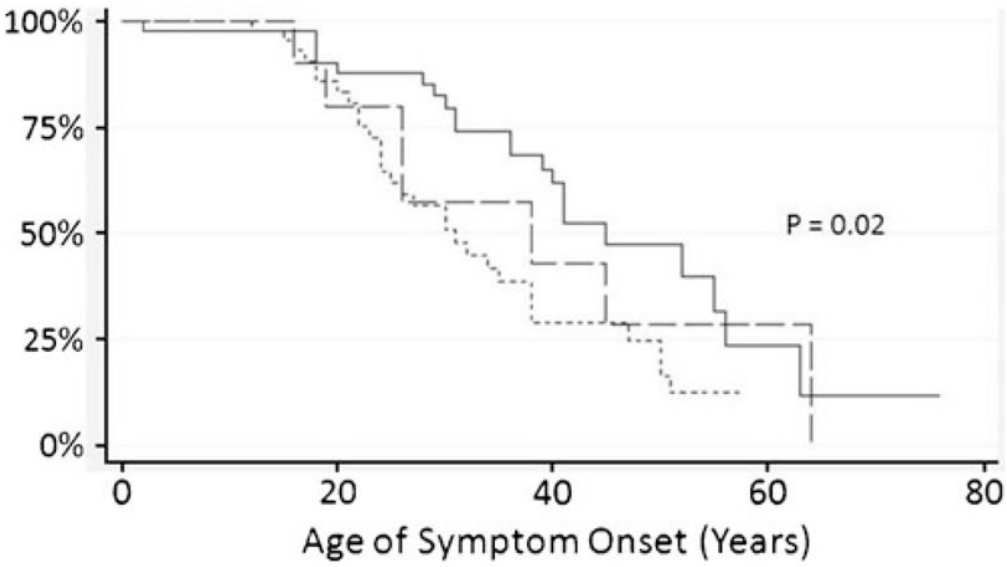

Fig. 3.

Proportion of individuals free from VT. Kaplan Meier curves demonstrate the proportion of individuals free from VT with one or more desmosome mutations and none of the 15 variants (- -), one or more desmosome mutations and at least one of the 15 variants (– –), and no desmosome mutations and none of the 15 missense variants (—). *P*=0.02 represents comparison between those with no desmosome mutation or missense variant and each of the other two groups; comparison between those with desmosome mutation with or without the other variants was not significant (*P*=0.85)

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Table 1

Demographics, presentations, clinical data, and genotypic characteristics

Characteristics	Young cohort with definite ARVD/C (*N*=19)	Young cohort with probable ARVD/C (*N*=11)	Middle cohort with definite ARVD/C (*N*=56)	Middle cohort with probable ARVD/C, (*N*=11)	Late cohort with definite ARVD/C (*N*=15)	*P* values
Male	12	7	29	6	12	0.08
Age at symptom onset	15.5±4.1 (min=2, max=20)	16.5±3.0 (min=12, max 21)	34.3±8.1 (min=22, max 48)	36.2±4.7 (min=29, max=46)	56.3±7.0 (min=50, max=76)	
ARVD/C symptoms: *n* (%)						0.57
Palpitations	8 (42%)	6 (54%)	19 (34%)	5 (45%)	4 (27%)	
Syncope	4 (21%)	1 (9%)	14 (25%)	1 (9%)	3 (20%)	
VT	3 (16%)	1 (9%)	14 (25%)	3 (27%)	3 (20%)	
SCD	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	
Others (asymptomatic, atrial fibrillation, atypical chest pain, shortness of breath)	4 (21%)	3 (27%)	8 (14%)	2 (18%)	5 (33%)	
Family history						0.34
ARVD/C on autopsy	6 (32%)	2 (18%)	13 (23%)	1 (9%)	5 (33%)	
SCD expected to be caused by ARVD/C	5 (26%)	0 (0%)	5 (9%)	0 (0%)	2 (13%)	
ARVD/C based on current criteria	5 (26%)	4 (36%)	13 (23%)	0 (0%)	1 (7%)	
Any family history (major or minor criterion)	9 (47%)	6 (54%)	21 (37%)	2 (18%)	5 (33%)	
Repolarization abnormalities						0.56
T wave inversion V1–V3 [n (%), *N*]	13 (81%), *N*=16	8 (80%), *N*=10	51 (93%), *N*=55	4, (36%), *N*=11	10 (71%), *N*=14	
Depolarization abnormalities: [*n* (%), *N*]						
Epsilon waves in V1–V3	0 (0%), *N*=19	0 (0%), *N*=11	11 (20%), *N*=56	0 (0%), *N*=11	1 (7%), *N*=14	0.03
QRS prolongation in V1–V3	7 (37%), *N*=19	2 (18%), *N*=11	18 (33%), *N*=55	0 (0%), *N*=11	5 (36%), *N*=14	0.65
Late potentials on SAECG	10 (56%), *N*=18	1 (10%), *N*=10	34 (72%), *N*=47	5 (55%), *N*=9	9 (90%), *N*=10	0.006
Arrhythmias [*n* (%), *N*]						
LBBB VT on Holter or ECG	11(58%), *N*=19	5 (45%), *N*=11	32 (58%), *N*=55	7 (63%), *N*=11	8 (53%), *N*=15	0.76
>1000 PVCs on Holter	15 (94%), *N*=16	5 (50%), *N*=10	21 (57%), *N*=37	3 (33%), *N*=9	4 (67%), *N*=6	0.11
Minor dysfunction and structural alterations, [*n* (%), *N*]	9 (47%), *N*=19	9 (82%), *N*=11	32 (57%), *N*=56	6 (55%), *N*=11	7 (47%), *N*=15	0.71

Characteristics	Young cohort with definite ARVD/C (*N*=19)	Young cohort with probable ARVD/C (*N*=11)	Middle cohort with definite ARVD/C (*N*=56)	Middle cohort with probable ARVD/C, (*N*=11)	Late cohort with definite ARVD/C (*N*=15)	*P* values
Major dysfunction and structural alterations, [*n* (%), *N*]	10 (53%), *N*=19	0 (0%), *N*=11	23 (41%), *N*=56	1 (9%), *N*=11	6 (40%), *N*=15	0.92
Fibrofatty replacement on endomyocardial biopsy, [*n* (%), *N*]	5, *N*=8	0, *N*=3	6, *N*=22	0, *N*=1	4, *N*=5	N/A
Genetic characteristics						
1 or more desmosome mutations [*n* (%)]	13 (68%)	7 (64%)	28 (50%)	4 (36%)	8 (53%)	0.23
>1 desmosome mutation [*n* (%)]	1 (5%)	0 (0%)	3 (5%)	0 (0%)	3 (20%)	0.07

Table 2

Summary of mutations in the Young cohort

Gene	Nucleotide change	Amino acid change	Previous report
With definite ARVD/C			
PKP2	1368delA	N456fsX458	Yes
	2146-1G>C	Mutant splice product	Yes
	1171–2A>G	Mutant splice product	Yes
	2489+1G>A	Mutant splice product	Yes
	1613G>A	W538X	Yes
	1271T>C	F424S	Yes
	2011delC	R671fsX683	Yes
	145–148delCAGA	S50fsX110	Yes
DSG2	137G>A	R46Q	Yes
	523+2T>C[a]	Mutant splice product	No
	1038-1040delGAA[a]	K346del	No
DSP	1264G>A	E422K	No
JUP	56C>T	T19I	Yes
With probable ARVD/C			
PKP2	2509delA	V837fs930X	Yes
	2146-1G>C	Mutant splice product	Yes
	1759G>A	V587I	Yes
	2197–2202delCACACC ins G	A733fs X740	Yes
	1759delG	S587fs X 655	No
DSG2	3140C>G	T1047R	No
DSC2	658G>A	G220R	No

[a]Indicates that this mutation was found with another mutation in one or more individuals

Table 3

Summary of mutations in the Middle cohort

Gene	Nucleotide change	Amino acid change	Previous report
With definite ARVD/C			
PKP2	145–148delCAGA[a]	S50fsX110	Yes
	235C>T	R79X	Yes
	1237C>T[a]	R413X	Yes
	1307–15del TAATGGGG1308insATTTAGTT	H436fsX447	Yes
	1613G>A	W538X	Yes
	1642delG	V548fsX562	Yes
	2489+1G>A	Mutant splice product	Yes
	2146-1G>C	Mutant splice product	Yes
	2197–2202insGdel CACACC	A773fsX740	Yes
	2484C>T ([a] homozygous)	Cryptic splicing	Yes
DSG2	1520G>A	C507Y	Yes
	2434G>T	G812C	Yes
	1003A>G[a]	T335A	Yes
	146G>A[a]	R49H	Yes
	918G>A[a]	W306X	Yes
	829-1 delGCTTGAAGGGAT[a]	G277fsX278	Yes
DSP	1331A>G	I445V	Yes
With probable ARVD/C			
PKP2	145–148delCAGA	S50fsX110	Yes
	216insG	Q74fsX85	Yes
	2146-1G>C	Mutant splice product	Yes
DSG2	146G>A	R49H	Yes

[a]Indicates that this mutation was found with another mutation in one or more individuals

Table 4

Summary of mutations in the Late cohort with ARVD/C

Gene	Nucleotide change	Amino acid change	Previous report
PKP2	2489+1G>A	Mutant splice product	Yes
	2197–2202insGdelCACACC	A773fsX740	Yes
	2359C>T	L787F	Yes
	2174T>A	V725D	No
	1759G>A	V587I	Yes
	2145+1G>C	Mutant splice product	Yes
	419C>T[a]	S140F	Yes
	2146-1G>C[a]	Mutant splice product	Yes
DSG2	209G>A[a]	V56M	Yes
	889G>A	D297N	No

[a]Indicates that this mutation was found with another mutation in one or more individuals

Table 5

Summary of low-frequency amino acid substitutions

Gene	cDNA change	Amino acid change	MUpro: prediction	PolyPhen: prediction	Frequency in total study cohort (*N*=115)	Frequency in Coriell controls (*N*=100)
PKP2	76G>A	D26N	Increased stability	Possibly damaging	1/112	2/100
	826C>T	P276S	Increased stability	Benign	1/112	1/100
	1114G>C	A372P	Increased stability	Benign	1/112	0/100
	1577C>T	T526M	Increased stability	Benign	2/112	1/100
	2431C>A	R811S	Increased stability	Possibly damaging	1/112	1/100
	2488G>C	A830P	Increased stability	Benign	1/112	0/100
DSG2	473T>G	V158G	Increased stability	Probably damaging	1/112	0/100
	2648C>T	S883F	Increased stability	Benign	1/112	0/100
JUP	1942G>A	V648I	Increased stability	Benign	4/112	2/100
DSP	4578C>A	N1526K	Increased stability	Benign	1/112	0/100
	4514C>T	A1505V	Increased stability	Benign	1/112	0/100
	4609C>T	R1537C	Increased stability	Probably damaging	4/112	3/100
DSC2	1787C>T	A596V	Increased stability	Possibly damaging	1/112	1/100
	1914G>C	Q638H	Increased stability	Possibly damaging	1/112	0/100
	2687–2688insGA	E896fs X900	N/A	N/A	2/112	2/100

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