Comprehensive Desmosome Mutation Analysis in North Americans With Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy

A. Dénise den Haan, MD; Boon Yew Tan, MBChB; Michelle N. Zikusoka, MD; Laura Ibañez Lladó, MS; Rahul Jain, MD; Amy Daly, MS; Crystal Tichnell, MGC; Cynthia James, PhD; Nuria Amat-Alarcon, MS; Theodore Abraham, MD; Stuart D. Russell, MD; David A. Bluemke, MD, PhD; Hugh Calkins, MD; Darshan Dalal, MD, PhD; Daniel P. Judge, MD

Background—Arrhythmogenic right ventricular dysplasia/cardio-myopathy (ARVD/C) is an inherited disorder typically caused by mutations in components of the cardiac desmosome. The prevalence and significance of desmosome mutations among patients with ARVD/C in North America have not been described previously. We report comprehensive desmosome genetic analysis for 100 North Americans with clinically confirmed or suspected ARVD/C.

Methods and Results—In 82 individuals with ARVD/C and 18 people with suspected ARVD/C, DNA sequence analysis was performed on PKP2, DSG2, DSP, DSC2, and JUP. In those with ARVD/C, 52% harbored a desmosome mutation. A majority of these mutations occurred in PKP2. Notably, 3 of the individuals studied have a mutation in more than 1 gene. Patients with a desmosome mutation were more likely to have experienced ventricular tachycardia (73% versus 44%), and they presented at a younger age (33 versus 41 years) compared with those without a desmosome mutation. Men with ARVD/C were more likely than women to carry a desmosome mutation (63% versus 38%). A mutation was identified in 5 of 18 patients (28%) with suspected ARVD. In this smaller subgroup, there were no significant phenotypic differences identified between individuals with a desmosome mutation compared with those without a mutation.

Conclusions—Our study shows that in 52% of North Americans with ARVD/C a mutation in one of the cardiac desmosome genes can be identified. Compared with those without a desmosome gene mutation, individuals with a desmosome gene mutation had earlier-onset ARVD/C and were more likely to have ventricular tachycardia. (Circ Cardiovasc Genet. 2009;2:428-435.)

Key Words: arrhythmia ■ cardiomyopathy ■ ventricular tachycardia ■ sudden cardiac death ■ genetics ■ desmosome ■ ARVD/C

Arrhythmogenic right ventricular dysplasia/cardiomypathy (ARVD/C) is an inherited form of cardiomyopathy characterized histologically by fibrofatty replacement of the right ventricular myocardium.1,2 Affected individuals typically present with palpitations, syncope, ventricular tachycardia, right heart failure, or sudden cardiac death (SCD).2-4 In 1 reported series of individuals with this condition, 23% experienced SCD as their presenting symptom.4 Currently, the diagnosis is based on clinical characteristics assembled by an expert task force, encompassing a complex series of both major and minor criteria.5

Editorial see page 415
Clinical Perspective on p 435

The incidence and prevalence of ARVD/C is uncertain and may vary regionally. In northeast Italy, ARVD/C was found to be responsible for 22.4% of SCD in young athletes and in 8.2% of SCD in nonathletes.6 A report from Germany indicated a prevalence of 1 per 1000 individuals.7 Others estimate the prevalence in Europe and North America to be 1 per 5000 individuals.8,9

In recent years, research has focused on identifying the genetic basis for this condition. In 2000, McKoy et al identified a homozygous mutation in JUP in patients with Naxos disease.10 This autosomal recessive form of ARVD/C is accompanied by palmoplantar keratodermia and woolly hair. JUP encodes junction plakoglobin, one of the desmosomal proteins. Carvajal syndrome is a similar recessive disorder of left ventricular (LV) cardiomyopathy, with skin and hair abnormalities, caused by homozygous mutations in DSP, encoding desmoplakin.11 These seminal findings led to

Received February 12, 2009; accepted May 27, 2009.
The online-only Data Supplement is available at http://circgenetics.ahajournals.org/cgi/content/full/CIRCGENETICS.109.858217.
Correspondence to Daniel P. Judge, MD, Johns Hopkins University, Division of Cardiology, Ross 1049; 720 Rutland Avenue, Baltimore, MD 21205.
E-mail djudge@jhmi.edu
© 2009 American Heart Association, Inc.

Circ Cardiovasc Genet is available at http://circgenetics.ahajournals.org
DOI: 10.1161/CIRCGENETICS.109.858217

428
the discovery of heterozygous mutations in genes encoding these and other desmosomal proteins in patients with nonsyndromic ARVD/C. In addition to mutations in DSP and JUP, nonsyndromic ARVD/C may be caused by mutations in PKP2, DSG2, or DSC2.12–16 Mutations in genes encoding non-desmosomal proteins may also cause ARVD/C. Alterations of the S' and S' regulatory elements of TGFβ3, encoding transforming growth factor β3, have each been reported.17 Mutations in RYR2, encoding the cardiac ryanodine receptor, result in a form of arrhythmic cardiomyopathy without significant structural abnormalities.18 This phenotype is variably referred to as ARVD/C or catecholaminergic polymorphic ventricular tachycardia.18,19 More recently, a mutation in TMEM43, encoding a transmembrane protein with ties to an adipogenic transcription factor, was reported as the cause for ARVD5, a subtype of ARVD/C with prominent LV involvement.20

An autosomal dominant pattern of inheritance for nonsyndromic ARVD/C is seen among approximately 30% of affected individuals.4 This may reflect both reduced penetrance and variable expressivity in families segregating this condition.21,22 In addition, both compound heterozygosity and autosomal recessive mutations have been reported in nonsyndromic ARVD/C, suggesting that additional genetic factors may influence phenotypic manifestations.15,23,24 To date, a mutation in more than 1 desmosomal gene has not been associated with this condition, suggesting that further genetic analysis may not be necessary if a single mutation is recognized.

Comprehensive desmosomal genetic analysis has been reported by only 1 group in England.22,25 They described analysis of 200 individuals in 69 families, finding a pathogenic mutation in 20 families (29%).25 As clinical use of genetic testing for ARVD/C becomes more widely used, the importance of understanding the likelihood of discovery of a pathogenic mutation becomes more prominent. Just as the rate of PKP2 mutations is different among unique cohorts, variability in other desmosomal genes is also relevant.14,26–29 We report the first comprehensive desmosomal genetic analysis for a large North American cohort of individuals with clinically confirmed or suspected ARVD/C.

**Methods**

**Patient Recruitment and Evaluation**

Eighty-two unrelated ARVD/C probands and 18 unrelated individuals with suspected ARVD/C gave written informed consent to participate in a study approved by the Johns Hopkins University institutional review board. History and medical records were obtained at enrollment and at yearly intervals. The age of onset of ARVD/C symptoms was defined as the age at which a patient experienced symptoms related to ARVD/C. Symptoms include palpitations, syncope, ventricular tachycardia, SCD, edema, and chest pain. Family history was obtained by interviewing patients and family members. Patients were evaluated by physical examination. Additional testing included 12-lead ECG (n = 100), 24-hour Holter monitoring (n = 74), exercise testing, signal-averaged ECG (n = 87), imaging studies, including echocardiography and MRI (n = 100), and right ventricular endomyocardial biopsy (n = 34). The presence and morphology of ventricular ectopy or ventricular tachycardia were identified using the results of standard 12-lead ECGs, Holter monitoring, and stress testing. The signal-averaged ECG was considered positive for late potentials if a patient, not previously known to have a right bundle branch block, showed any 2 of the following abnormalities: (1) a filtered QRS duration ≥ 114 milliseconds; (2) low-amplitude signal duration ≥ 38 milliseconds; or (3) RMS ≤ 20 mV. Biopsies of right ventricular lateral wall were assessed for fibrofatty replacement in the presence of surviving strands of cardiomyocytes.

The diagnosis of ARVD/C was based on the criteria set by the Task Force of the Working Groups of Myocardial and Pericardial Disease of the European Society of Cardiology.5 The diagnosis of suspected ARVD/C was based on meeting at least 2 minor or 1 major and 1 minor criteria from these Task Force Criteria.

**Genetic Analysis**

In this cohort of 100 North American whites, 52 probands had not previously undergone genetic analysis for ARVD/C, and they were added to 48 individuals who had previously been analyzed only for mutations in PKP2 and/or DSG2.19,24,26 All 100 of these patients had novel analysis of 3 genes (DSP, JUP, and DSC2), and 52 patients had initial analysis of all 5 genes, so that the entire cohort had complete sequence investigation of all 5 desmosomal genes in which mutations have been reported to cause ARVD/C.

Genomic DNA was extracted from whole blood using QIAamp DNA blood maxi kits (Qiagen Inc, Valencia, Calif). Polymerase chain reaction products were generated for sequence analysis, using intronic primers (primer sequences available from the authors on request) flanking each exon in PKP2, DSP, DSG2, JUP, and DSC2. Polymerase chain reaction products were purified using the QIAquick PCR Purification Kit (Qiagen Inc). Bidirectional sequence analysis was performed using an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, Calif), and chromatograms were analyzed manually and with Sequencer 4.1 software.

All novel sequence variants were classified as mutation or polymorphism. We defined a mutation as not occurring among 400 unrelated, unaffected, race-matched, control chromosomes (NIHMS Human Genetic Cell Repository, Coriell Institute for Medical Research), altering one or more conserved amino acids, and segregating with disease in the family when that information was available. All other novel variants were classified as polymorphisms. Sequence variants that were reported previously as mutations were considered mutations, unless we could show otherwise. Controls were tested for the presence or absence of a novel restriction enzyme digest site if the DNA variant altered such a site, or by TaqMan genotyping assays (Applied Biosystems) if the sequence variant did not alter any restriction sites. Novel missense protein variants were analyzed by ClustalW (MacVector Inc, 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.

To clarify the sequence of DSG2 cDNA based on discordant reports of sequence for the first exon,30,31 total RNA was extracted from human hearts and reverse transcribed, and 5' rapid amplification of cDNA ends was performed using nested primers in the 3rd and 4th exons. Products were sequenced to determine whether an alternate start site exists in cardiac tissue.

**Statistical Analysis**

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS version 16.0, SPSS Inc, Chicago, Ill). Continuous variables between 2 groups were compared by unpaired t test, when comparing 2 groups, and ANOVA in case of multiple groups. Categorical variables were compared by chi-squared test in case of large samples and by Fisher's exact test in case of small sample size (n ≤ 5 in 2 of the groups). Kaplan–Meier analysis and log-rank test were used when establishing and comparing symptom- and/or ventricular tachycardia (VT)-free survival. Cox proportional hazards model was used to adjust for the effect of gender on VT-free survival. A probability value ≤ 0.05 was considered statistically significant.
Clinical Characteristics and Mutational Analysis

Eighty-two patients (59% men) diagnosed with ARVD/C are included in this study. Clinical characteristics are summarized in Table 1, with more detailed information in supplemental Table I. Mean age at enrollment was 44 ± 14 years. Age of diagnosis varied between 2 and 76 years (mean, 37 ± 14 years), with the first symptoms of ARVD/C arising at age 2 to 76 years (mean, 33 ± 14 years).

Two different sequences have been reported for the first exon of DSG2.30,31 Analysis of DSG2 mRNA from human hearts by 5′ rapid amplification of cDNA ends was performed to determine whether alternative splicing or an alternate first exon was present as reported previously for the DSG2 sequence based on a human cancer cell line.31 This demonstrated only 1 sequence for the DSG2 first exon.30 Transcript sequence analysis was restricted to this first exon, and both cDNA and translated protein numbering were based on NCBI reference sequence NM_001943.

Within the desmosome genes sequenced in this cohort of 100 people, several novel and previously reported DNA sequence variants were identified. Those DNA sequence variants that are predicted not to alter the protein structure or amino acid sequence were not studied further. Some nonsynonymous variants have already been determined to be polymorphisms and were also not studied further. The novel nonsynonymous sequence variants were assessed in a population of 200 race-matched unaffected individuals (400 control chromosomes). Sequence variants that altered a conserved amino acid and were not present among controls were considered to be mutations. Supplemental Table II includes 29 nonsynonymous variants that were excluded as mutations because of the presence among controls or lack of conservation of the encoded amino acid. We observed 31 unique nonsynonymous mutations among the 100 probands; among these, 23 were reported previously, and 8 are novel (Table 2).

Four of the novel mutations result in frameshift and disruption of many conserved amino acids or premature termination, and 4 of the novel mutations are missense alterations of 1 conserved amino acid. Conservation of the mutated amino acid is shown in Figure 1.

In the cohort with ARVD/C, 1 or more desmosome gene mutations were identified in 43 people (52%). Thirty-seven of these (86%) had a single heterozygous desmosome gene mutation, whereas 6 people (14%) had more than 1 mutation (5 patients with 2 mutations and 1 patient with 3 mutations), resulting in a cumulative total of 50 mutations. Forty-five percent of the ARVD/C cohort have one or more mutation in PKP2 (n = 37), 9% in DSG2 (n = 7), 1% in DSP (n = 1), and 1% in JUP (n = 1). We identified no mutations in DSC2. Mutations in more than 1 gene (digenic heterozygosity) were present in 4%. The remaining 48% have no discernible mutation in these desmosome genes.

Among the 55 separate mutations that occurred in the cohort of 100 (Table 2), consequences consisted of disruption of a critically conserved nucleotide at the exon-intron junction in 29%, insertions and/or deletions in 25%, missense substitution of a conserved amino acid in 24%, single-nucleotide substitution resulting in premature termination codon in 18%, and cryptic splicing in 4% (Table 2).

Results

Clinical Characteristics and Mutational Analysis

Within the desmosome genes sequenced in this cohort of 100 people, several novel and previously reported DNA sequence variants were identified. Those DNA sequence variants that are predicted not to alter the protein structure or amino acid sequence were not studied further. Some nonsynonymous variants have already been determined to be polymorphisms and were also not studied further. The novel nonsynonymous sequence variants were assessed in a population of 200 race-matched unaffected individuals (400 control chromosomes). Sequence variants that altered a conserved amino acid and were not present among controls were considered to be mutations. Supplemental Table II includes 29 nonsynonymous variants that were excluded as mutations because of the presence among controls or lack of conservation of the encoded amino acid. We observed 31 unique nonsynonymous mutations among the 100 probands; among these, 23 were reported previously, and 8 are novel (Table 2).

Four of the novel mutations result in frameshift and disruption of many conserved amino acids or premature termination, and 4 of the novel mutations are missense alterations of 1 conserved amino acid. Conservation of the mutated amino acid is shown in Figure 1.

In the cohort with ARVD/C, 1 or more desmosome gene mutations were identified in 43 people (52%). Thirty-seven of these (86%) had a single heterozygous desmosome gene mutation, whereas 6 people (14%) had more than 1 mutation (5 patients with 2 mutations and 1 patient with 3 mutations), resulting in a cumulative total of 50 mutations. Forty-five percent of the ARVD/C cohort have one or more mutation in PKP2 (n = 37), 9% in DSG2 (n = 7), 1% in DSP (n = 1), and 1% in JUP (n = 1). We identified no mutations in DSC2. Mutations in more than 1 gene (digenic heterozygosity) were present in 4%. The remaining 48% have no discernible mutation in these desmosome genes.

Among the 55 separate mutations that occurred in the cohort of 100 (Table 2), consequences consisted of disruption of a critically conserved nucleotide at the exon-intron junction in 29%, insertions and/or deletions in 25%, missense substitution of a conserved amino acid in 24%, single-nucleotide substitution resulting in premature termination codon in 18%, and cryptic splicing in 4% (Table 2).

Genotype-Phenotype Relationship

Differences between patients with and without a desmosome mutation are shown in Table 3. Among men with ARVD/C, the likelihood of a desmosome gene mutation was higher (63%) than among women (38%). Patients with a desmosome mutation were more likely to have experienced VT (73% versus 44%), and experience their first episode of VT at a younger age (33 ± 12 years versus 41 ± 14 years). Figure 2 shows gender-adjusted cumulative VT-free survival in patients with ARVD/C with and without a desmosome mutation, with a median VT-free survival of 32 years in patients with a mutation and 46 years in patients without a mutation. When stratified by gender, this difference in VT-free survival persists among women, with regard to the presence or absence of a desmosome gene mutation (P = 0.001), but not for men (P = 0.072).
affected men and women (Table 4). A mutation was identified in 30 of 48 men (63%) and in 13 of 34 women (38%) \((P<0.05)\). In addition, men show more depolarization abnormalities, and even though there is no significant difference in the prevalence of structural abnormalities, there is a difference in severity of structural abnormalities. Men in this cohort tend to have major structural abnormalities (54%), whereas women tend to have minor structural abnormalities (65%).

**Suspected ARVD/C**

Eighteen patients (39% men) with suspected ARVD/C (2 or more criteria without meeting current Task Force diagnostic criteria) were included in this study. Clinical characteristics are summarized in supplemental Table III. Mean age was 41±8 years. Age of suspected diagnosis varied between 15 and 44 years (mean, 35±8 years), with the first symptoms of ARVD/C arising at age 13 to 40 years (mean, 31±8 years).

A desmosome gene mutation was identified in 5 patients (28%). One of them had a single heterozygous desmosome gene mutation in DSG2, and 4 had an isolated heterozygous mutation in PKP2. The mutations identified in PKP2 consisted of 2 deletions, a disruption of a critically conserved nucleotide at the intron-exon splice site, and an insertion. The isolated DSG2 mutation was a missense substitution of a conserved amino acid.\(^\text{15}\) There were no significant differences between patients suspected of ARVD/C with and without mutations. Although there is a reduced prevalence of desmosome gene mutations in the cohort with suspected ARVD/C compared with those who fulfill current clinical criteria (28% versus 52%), the numbers are too small to achieve statistical significance.

**Multiple Mutations**

One individual in this cohort was found to have 3 different previously reported mutations. This individual (No. 43, supplemental Table I) is a 50-year-old man who underwent evaluation for ARVD/C after an episode of syncope caused by VT. With phenotypic evaluation, he meets 2 major and 3 minor criteria. Desmosome gene sequencing identified 2 previously published mutations in PKP2 (c.2146 to 1G>C and c.419C>T) as well as a previously published mutation in DSG2 (p.V56M).\(^\text{14,30}\) We considered each of these mutations independently; PKP2 c.2146 to 1G>C results in abnormal splicing with loss of exon 11 with frameshift and alteration of many conserved amino acids, strongly supporting its pathogenicity.\(^\text{14}\) The PKP2 missense mutation c.419C>T (p.S140F) alters only a single amino acid, although it has been found repeatedly in association with ARVD/C and was not seen in more than 500 control chromosomes.\(^\text{14,26,28,32}\) However, in the absence of a well-validated functional assay for desmosome protein variants of uncertain significance, one cannot definitively ascribe a pathogenic role to this allele. Finally, the DSG2 V56M variant has also been reported in ARVD/C, and this valine is highly conserved.\(^\text{30}\) Although this allele was recently found among 3 of 617 individuals in mixed German populations (2 groups of cardiac patients without LV cardiomyopathy and 1 cohort of blood donors without phenotypic testing), it was found in 10-fold greater

---

### Table 2. Desmosome Mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide Change</th>
<th>Amino Acid or RNA Change</th>
<th>Previous Report</th>
<th>n</th>
<th>N=55</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKP2</td>
<td>c.145_148delCAGA</td>
<td>p.Thr48SerfsX61</td>
<td>+</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.217_218dup(c.216insG)</td>
<td>p.Asn74AlafsX99</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.235C&gt;T</td>
<td>p.Arg79X</td>
<td>+</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.419C&gt;T</td>
<td>p.Ser140Phe</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.1171-2A&gt;G</td>
<td>r.spl</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.1237C&gt;T</td>
<td>p.Arg413X</td>
<td>+</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.1271T&gt;C</td>
<td>p.Phe424Ser</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.1307_1315del-ins8</td>
<td>p.Leu436HisfsX11</td>
<td>−</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.1368delA</td>
<td>p.Lys456AlafsX3</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.1613G&gt;A</td>
<td>p.Trp538X</td>
<td>+</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.1643delG(c.1642delG)</td>
<td>p.Gly548ValafsX15</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.1759G&gt;A</td>
<td>p.Val587Ile</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.2013delC(c.2011delC)</td>
<td>p.Lys672ArgfsX12</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.2145+1G&gt;C</td>
<td>r.spl</td>
<td>−</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.2146-1G&gt;C</td>
<td>r.spl</td>
<td>+</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.2197_2202delinsG</td>
<td>p.His733AlafsX8</td>
<td>+</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.2359C&gt;T</td>
<td>p.Leu787Phe</td>
<td>−</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.2484C&gt;T</td>
<td>r.0.2483_2489del</td>
<td>+</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.2489+1G&gt;A</td>
<td>r.spl</td>
<td>+</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.2489+1G&gt;T</td>
<td>r.spl</td>
<td>−</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.2509delA</td>
<td>p.Ser837ValafsX94</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DSG2</td>
<td>c.137G&gt;A</td>
<td>p.Arg466Gln</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.146G&gt;A</td>
<td>p.Arg49HIs</td>
<td>+</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.166G&gt;A</td>
<td>p.Val56Met</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.918G&gt;A</td>
<td>p.Trp306X</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.1003A&gt;G</td>
<td>p.Thr335Ala</td>
<td>−</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.1520G&gt;A</td>
<td>p.Cys507Tyr</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.2434G&gt;T</td>
<td>p.Gly812Cys</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.829_835del</td>
<td>r.spl</td>
<td>−</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DSP</td>
<td>c.1331A&gt;G</td>
<td>p.Ile445Val</td>
<td>−</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>JUP</td>
<td>c.506C&gt;T</td>
<td>pThr191le</td>
<td>−</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Each desmosome gene mutation is represented by its nucleotide and amino acid alteration. Those that have previously been reported are indicated with (+) and novel mutations are represented with (−) in column 4. Mutation nomenclature follows the Human Genome Variation Society guidelines (http://www.hgvs.org/mutnomen). For PKP2 mutations that have previously been reported with different cDNA designation, the prior nomenclature is provided in parentheses. Mutations that disrupt a splice site are noted as “r.spl” to indicate abnormally spliced mRNA.

When comparing the consequences of mutations, there was no significant difference found in symptom-free survival or VT survival among those with missense mutations compared with those with truncating gene mutations or splice altering mutations. There was also no significant difference recognized in VT-free survival between patients with mutations in the different desmosomal genes and between patients with 1 versus multiple mutations. In contrast with a previous report, we found no difference with regard to family history of ARVD/C among those with and without a desmosome gene mutation.\(^\text{27}\)

**Gender**

In this study population there is a small male preponderance (59%). There are a few notable differences in comparison of
prevalence among people with nonischemic dilated cardiomyopathy.33 These authors proceeded to show that the presence of the DSG2 V56M allele cosegregates with disease in families with dilated cardiomyopathy, is associated with both abnormal cardiac immunostaining and abnormal electron microscopy, and is putatively functional in protein modeling studies.33 We share these authors conclusion that DSG2 V56M likely plays a contributory role, although it may not be sufficient by itself to result in cardiomyopathy.34

Although compound heterozygosity (2 different mutations in the same gene) has been reported in ARVD/C, digenic heterozygosity (heterozygous mutations in 2 genes) has not been reported previously. Digenic heterozygosity was present in 2 additional individuals with ARVD/C in this cohort, emphasizing the importance of comprehensive genetic analysis even if a single gene mutation is identified.

### Table 3. Comparison Mutation Carriers Versus Nonmutation Carriers

<table>
<thead>
<tr>
<th></th>
<th>With Mutation (N=43)</th>
<th>No Mutation (N=39)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender: male, n (%)</td>
<td>30 (63)</td>
<td>18 (37)</td>
<td>0.03*</td>
</tr>
<tr>
<td>Gender: female, n (%)</td>
<td>13 (38)</td>
<td>21 (62)</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis, y</td>
<td>33±12</td>
<td>40±15</td>
<td>0.04*</td>
</tr>
<tr>
<td>Age at first ARVD/C-related symptoms, y</td>
<td>30±13</td>
<td>37±14</td>
<td>0.015*</td>
</tr>
<tr>
<td>Family history, n (%)</td>
<td>16 (37)</td>
<td>15 (39)</td>
<td>0.91</td>
</tr>
<tr>
<td>Family history proven on autopsy</td>
<td>7 (16)</td>
<td>11 (28)</td>
<td>0.19</td>
</tr>
<tr>
<td>Repolarization abnormalities, n (%)</td>
<td>41 (95)</td>
<td>32 (84)</td>
<td>0.054</td>
</tr>
<tr>
<td>Depolarization abnormalities, n (%)</td>
<td>37 (88)</td>
<td>27 (73)</td>
<td>0.08</td>
</tr>
<tr>
<td>LBBB-VT on ECG, Holter, or stress-ECG</td>
<td>30 (73)</td>
<td>17 (44)</td>
<td>0.007*</td>
</tr>
<tr>
<td>Age of first recorded VT</td>
<td>33±12</td>
<td>41±14</td>
<td>0.007*</td>
</tr>
<tr>
<td>Minor dysfunction and structural alterations, n (%)</td>
<td>20 (47)</td>
<td>22 (56)</td>
<td>0.37</td>
</tr>
<tr>
<td>Major dysfunction and structural alterations, n (%)</td>
<td>21 (49)</td>
<td>15 (39)</td>
<td>0.34</td>
</tr>
<tr>
<td>Fibrofatty replacement on endomyocardial biopsy, n (%)</td>
<td>5 (33)</td>
<td>8 (44)</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Differences between patients with and without a desmosome mutation are shown here. LBBB-VT indicates ventricular tachycardia with left bundle branch block.

*P<0.05.

---

### Figure 1. Conservation of the mutated amino acid in 4 novel missense mutations. Amino acids are represented by their standard abbreviations (I for isoleucine, T for threonine, L for leucine). *Indicates site of mutation. Species are shown by their standard nomenclature.

### Figure 2. Kaplan–Meier survival analysis demonstrating cumulative VT-free survival in the study population stratified by presence of a desmosome gene mutation and adjusted for gender.
Importantly, mutations in more than 1 gene were found in 3 probands (3%). This is also the first identification of 3 previously reported gene mutations in an individual with ARVD/C. The possibility of multiple pathogenic mutations leads to a challenge in genetic counseling. Once a disease causing mutation is identified in an individual with ARVD/C, unaffected family members may choose to be screened for the presence of this mutation to determine their risk of developing ARVD/C. Mutation carriers may modify their lifestyle to decrease the likelihood of SCD. Family members who do not share a single desmosome mutation present in a proband may incorrectly be advised of a low risk of developing ARVD/C if more extensive testing was not performed in the proband. With the possibility of multiple mutations influencing disease expression, comprehensive cardiac desmosome genetic testing should be performed for this condition.

No desmosome gene mutation was identified in nearly half of the patients. The genetic basis for their condition may be the result of mutations in nondesmosome ARVD/C genes, such as RYR2 or TMEM43, although phenotypes of individuals with mutations in these genes are typically different from the probands in our cohort.18,20 Also, traditional sequence analysis may not identify large deletions or gene rearrangements. Additional genes with mutations resulting in ARVD/C are likely to be identified in the future. Finally, it is possible that people with ARVD/C and no discernible desmosome gene mutation may have nongenetic causes for their cardiomyopathy, such as viral infection or autoimmunity.36,37

Study Limitations

There are a few limitations to the study performed. First of all, there is a selection bias. These people came to medical attention because they had symptoms of ARVD/C themselves or because a family member was diagnosed with ARVD/C. The people with the most severe first manifestation of disease, SCD, are not represented in this group. Family members of these individuals are represented in this group, but they may have less severe disease. Another limitation is that there is currently no functional test available to study the consequences of mutations in desmosome genes. Despite rigorous criteria to designate novel sequence alterations as mutations or polymorphisms, misclassification is possible in the absence of functional data.

Conclusions

In a cohort of 100 individuals with clinically confirmed or suspected ARVD/C, desmosome gene mutations are frequently identified. In rare cases, more than 1 gene mutation is present. People with ARVD/C and a desmosome gene mutation present at a younger age and are more likely to have VT. Clinical use of comprehensive cardiac desmosome gene testing for individuals with this condition may identify family members at increased risk of developing ARVD/C.

Acknowledgments

We acknowledge the Johns Hopkins ARVD Program (http://www.arvd.com).
Sources of Funding
This work was supported by funding from the National Institutes of Health (HL088072 to D.P.J.), the France-Merrick Foundation, JHU Friends in Red, and the Jeff Cooper CARE Foundation. Dr. den Haan was supported by the Stichting Dr Hendrik Muller’s Vaderlandsch Fonds, the Van Wijk-Stam-Caspers fund for cardiovascular research, and the Trajectum Scholarship. Dr. Calkins received funding from the Bogle Foundation, the Campanella family, the Wilmending Endowment, the Healing Hearts Foundation, Boston Scientific, Medtronic, and St. Jude Medical to support this project in part.

Disclosures
Dr. Calkins receives honoraria from Boston Scientific, Medtronic, and St. Jude Medical and is a consultant for Medtronic. The authors have no other potential conflicts of interest to disclose.

References
CLINICAL PERSPECTIVE

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is an inherited form of cardiomyopathy characterized by frequent ventricular arrhythmias and predominant involvement of the right ventricle. Mutations have been reported in several genes encoding components of the cardiac desmosome, a cellular adhesion complex that connects cardiac myocytes. To date, comprehensive genetic characterization of a large cohort of North Americans with this condition has not been reported. We performed genetic analysis of 100 individuals for mutations in 5 desmosome genes: PKP2, DSG2, DSP, JUP, and DSC2. Among those who meet current criteria for ARVD/C, 43 of 82 (52%) have a mutation in 1 or more desmosome genes. A cohort of 18 individuals with “suspected” ARVD/C were also tested, and only 5 (28%) had a mutation in 1 of the desmosome genes. Patients with a mutation in a desmosome gene were more likely to have experienced ventricular tachycardia (73% versus 44%), and they presented at a younger age (33 versus 41 years) compared with those without a desmosome gene mutation. These findings have several important implications for clinical practice. First, some people with ARVD/C have more than 1 desmosome gene mutation, emphasizing the importance of testing for mutations in more than a single gene. Second, our data provide a context for interpreting clinical genetic testing for mutations in desmosome genes. DNA variants of uncertain significance remain somewhat common for ARVD/C, and there is not yet a clear assay to determine quantitatively the functional consequence of a desmosome genetic variant. As such, genetic testing for ARVD/C should only be done with genetic counseling in tertiary referral centers.
Comprehensive Desmosome Mutation Analysis in North Americans With Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy

A. Dénise den Haan, Boon Yew Tan, Michelle N. Zikusoka, Laura Ibañez Lladó, Rahul Jain, Amy Daly, Crystal Tichnell, Cynthia James, Nuria Amat-Alarcon, Theodore Abraham, Stuart D. Russell, David A. Bluemke, Hugh Calkins, Darshan Dalal and Daniel P. Judge

_Circ Cardiovasc Genet._ 2009;2:428-435; originally published online June 3, 2009;
doi: 10.1161/CIRCGENETICS.109.858217

_Circulation: Cardiovascular Genetics_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 1942-325X. Online ISSN: 1942-3268

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circgenetics.ahajournals.org/content/2/5/428

Data Supplement (unedited) at:
http://circgenetics.ahajournals.org/content/suppl/2009/06/03/CIRCGENETICS.109.858217.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation: Cardiovascular Genetics_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation: Cardiovascular Genetics_ is online at:
http://circgenetics.ahajournals.org/subscriptions/
SUPPLEMENTAL MATERIAL

Online supplemental materials for “Comprehensive Desmosome Mutation Analysis in North Americans with Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy”


Supplemental Methods: None. All methods are provided in the text.

Supplemental Tables:

Supplemental Table 1 - Clinical and genetic characteristics of patients with ARVD/C
Supplemental Table 2 – Non-conserved desmosome variants excluded as mutations
Supplemental Table 3 - Clinical and genetic characteristics of patients with suspected ARVD/C

Supplemental Figures: None

Supplemental References: None
### Supplemental Table I: Clinical and genetic characteristics of patients with ARVD/C

<table>
<thead>
<tr>
<th>Case</th>
<th>Gene</th>
<th>Nucleotide change</th>
<th>Gender</th>
<th>Age first symptom</th>
<th>Family History</th>
<th>Depol</th>
<th>Repol</th>
<th>Arrhyth</th>
<th>Struct</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PKP2</td>
<td>c.145_148delCAGA</td>
<td>M</td>
<td>28</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>PKP2</td>
<td>c.145_148delCAGA</td>
<td>M</td>
<td>31</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>PKP2</td>
<td>c.235C&gt;T</td>
<td>F</td>
<td>26</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>PKP2</td>
<td>c.235C&gt;T</td>
<td>F</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>PKP2</td>
<td>c.1171-2A&gt;G</td>
<td>M</td>
<td>19</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>PKP2</td>
<td>c.1237C&gt;T</td>
<td>F</td>
<td>33</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>PKP2</td>
<td>c.1271T&gt;C</td>
<td>F</td>
<td>30</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>PKP2</td>
<td>c.1307_1315delins8</td>
<td>M</td>
<td>22</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>PKP2</td>
<td>c.1368delA</td>
<td>F</td>
<td>15</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>PKP2</td>
<td>c.1613G&gt;A</td>
<td>F</td>
<td>18</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>PKP2</td>
<td>c.1613G&gt;A</td>
<td>M</td>
<td>30</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>PKP2</td>
<td>c.1613G&gt;A</td>
<td>M</td>
<td>35</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>PKP2</td>
<td>c.1613G&gt;A</td>
<td>M</td>
<td>26</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>PKP2</td>
<td>c.1613G&gt;A</td>
<td>M</td>
<td>45</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>PKP2</td>
<td>c.1643delG</td>
<td>M</td>
<td>30</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>16</td>
<td>PKP2</td>
<td>c.2013delC</td>
<td>F</td>
<td>11</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>17</td>
<td>PKP2</td>
<td>c.2146_1G&gt;C</td>
<td>M</td>
<td>20</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>18</td>
<td>PKP2</td>
<td>c.2146_1G&gt;C</td>
<td>M</td>
<td>31</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>19</td>
<td>PKP2</td>
<td>c.2146_1G&gt;C</td>
<td>M</td>
<td>26</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>20</td>
<td>PKP2</td>
<td>c.2146_1G&gt;C</td>
<td>F</td>
<td>34</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>21</td>
<td>PKP2</td>
<td>c.2146_1G&gt;C</td>
<td>F</td>
<td>34</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>22</td>
<td>PKP2</td>
<td>c.2146_1G&gt;C</td>
<td>M</td>
<td>47</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>23</td>
<td>PKP2</td>
<td>c.2146_1G&gt;C</td>
<td>M</td>
<td>30</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>24</td>
<td>PKP2</td>
<td>c.2146_1G&gt;C</td>
<td>M</td>
<td>41</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>25</td>
<td>PKP2</td>
<td>c.2197_2202delinsG</td>
<td>F</td>
<td>32</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>26</td>
<td>PKP2</td>
<td>c.2197_2202delinsG</td>
<td>M</td>
<td>25</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>27</td>
<td>PKP2</td>
<td>c.2197_2202delinsG</td>
<td>M</td>
<td>56</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>28</td>
<td>PKP2</td>
<td>c.2359C&gt;T</td>
<td>M</td>
<td>52</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>29</td>
<td>PKP2</td>
<td>c.2489_1G&gt;A</td>
<td>M</td>
<td>22</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>30</td>
<td>PKP2</td>
<td>c.2489_1G&gt;A</td>
<td>F</td>
<td>38</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>31</td>
<td>PKP2</td>
<td>c.2489_1G&gt;A</td>
<td>M</td>
<td>51</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>32</td>
<td>PKP2</td>
<td>c.2489_1G&gt;A</td>
<td>M</td>
<td>24</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>33</td>
<td>DSG2</td>
<td>c.137G&gt;A</td>
<td>M</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>34</td>
<td>DSG2</td>
<td>c.1520 G&gt;A</td>
<td>M</td>
<td>38</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>35</td>
<td>DSG2</td>
<td>c.2434G&gt;T</td>
<td>F</td>
<td>23</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>36</td>
<td>DSP</td>
<td>c.1331 A&gt;G</td>
<td>F</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>37</td>
<td>JUP</td>
<td>c.56C&gt;T</td>
<td>M</td>
<td>17</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>38</td>
<td>PKP2</td>
<td>c.2484C&gt;T</td>
<td>F</td>
<td>23</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>39</td>
<td>PKP2</td>
<td>c.145_148delCAGA</td>
<td>M</td>
<td>27</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>40</td>
<td>PKP2</td>
<td>c.1237C&gt;T</td>
<td>M</td>
<td>22</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>41</td>
<td>PKP2</td>
<td>c.1759G&gt;A</td>
<td>M</td>
<td>64</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>42</td>
<td>DSG2</td>
<td>c.146 G&gt;A</td>
<td>M</td>
<td>24</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>43</td>
<td>PKP2</td>
<td>c.146 G&gt;A</td>
<td>M</td>
<td>50</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>44</td>
<td>F</td>
<td>2</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>45</td>
<td>M</td>
<td>18</td>
<td></td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>46</td>
<td>M</td>
<td>14</td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>47</td>
<td>M</td>
<td>13</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>48</td>
<td>M</td>
<td>18</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>49</td>
<td>F</td>
<td>27</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>50</td>
<td>M</td>
<td>22</td>
<td></td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>51</td>
<td>M</td>
<td>35</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>F</td>
<td>31</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>M</td>
<td>31</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>F</td>
<td>29</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>F</td>
<td>40</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>F</td>
<td>28</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>F</td>
<td>38</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>F</td>
<td>35</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>M</td>
<td>43</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>F</td>
<td>36</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>M</td>
<td>42</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>M</td>
<td>41</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>M</td>
<td>40</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>F</td>
<td>41</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>M</td>
<td>47</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>F</td>
<td>41</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>67</td>
<td>F</td>
<td>48</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>68</td>
<td>F</td>
<td>41</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>F</td>
<td>24</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>F</td>
<td>43</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>71</td>
<td>F</td>
<td>43</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>M</td>
<td>48</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>73</td>
<td>F</td>
<td>53</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>F</td>
<td>42</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>F</td>
<td>41</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>F</td>
<td>45</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>M</td>
<td>46</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>78</td>
<td>F</td>
<td>51</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>M</td>
<td>30</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>M</td>
<td>63</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>81</td>
<td>M</td>
<td>57</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>M</td>
<td>76</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each individual is listed with a unique case number. Gene indicates the gene in which a mutation was identified. The six categories for criteria are shown as Family History, Depolarization abnormalities (Depol), Repolarization abnormalities (Repol), Arrhythmia (Arrhyth), Structural RV disease (Struct), and tissue. For these columns, ‘+’ indicates meeting a minor criterion, ‘++’ indicates meeting a major criterion, and ‘?’ indicates that they were not adequately tested for that criterion.
Supplemental Figure 2

Non-conserved desmosome protein variants excluded due to presence in controls or lack of conservation:

<table>
<thead>
<tr>
<th>PKP2</th>
<th>DSP</th>
<th>DSC2</th>
<th>DSG2</th>
<th>JUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.T526M</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p.I531S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p.A830P*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* refers to a nucleotide alteration which may alter splicing though mRNA was not available to test further
### Supplemental Table 3: Clinical and genetic characteristics of patients with probable ARVD/C

<table>
<thead>
<tr>
<th>Case</th>
<th>Gene</th>
<th>Nucleotide change</th>
<th>Gender</th>
<th>Age first symptom</th>
<th>Family History</th>
<th>Depol</th>
<th>Repol</th>
<th>Arrhyth</th>
<th>Struct</th>
<th>Tissue</th>
<th>Minor criteria</th>
<th>Major criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PKP2</td>
<td>c.145_148delCAGA</td>
<td>F</td>
<td>38</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>PKP2</td>
<td>c.216insG</td>
<td>M</td>
<td>46</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>PKP2</td>
<td>c.2146-1G&gt;C</td>
<td>F</td>
<td>16</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>PKP2</td>
<td>c.2509delA</td>
<td>M</td>
<td>21</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>DSG2</td>
<td>c.146G&gt;A</td>
<td>M</td>
<td>38</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td>M</td>
<td>28</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>F</td>
<td>26</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td>M</td>
<td>36</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td>M</td>
<td>29</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td>F</td>
<td>36</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td>M</td>
<td>33</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td>F</td>
<td>33</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td>F</td>
<td>36</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td>F</td>
<td>37</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td>F</td>
<td>31</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td>F</td>
<td>18</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td>F</td>
<td>40</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td>F</td>
<td>13</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Each individual is listed with a unique case number. Gene indicates the gene in which a mutation was identified. The six categories for criteria are shown as Family History, Depolarization abnormalities (Depol), Repolarization abnormalities (Repol), Arrhythmia (Arrhyth), Structural RV disease (Struct), and tissue. For these columns, ‘+’ indicates meeting a minor criterion, ‘++’ indicates meeting a major criterion, and ‘?’ indicates that they were not adequately tested for that criterion.