Clinical and genetic spectrum of hereditary cardiac arrhythmia syndromes
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Desmoglein-2 and Desmocollin-2 Mutations in Dutch Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy patients: Results from a multicenter study

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Clinical and Genetic Spectrum of Hereditary Cardiac Arrhythmia Syndromes
Abstract

Background

This study aimed to evaluate the prevalence and type of mutations in the major desmosomal genes, Plakophilin-2 (PKP2), Desmoglein-2 (DSG2) and Desmocollin-2 (DSC2), in Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy (ARVD/C). We also aimed to distinguish relevant clinical and ECG parameters.

Methods & Results

Clinical evaluation was performed according to the Task Force Criteria (TFC). We analyzed the genes in: (a) 57 patients who fulfilled the ARVD/C TFC (TFC+), (b) 28 patients with probable ARVD/C (1 major and 1 minor, or 3 minor criteria) and (c) 31 patients with 2 minor or 1 major criteria. In the TFC+ ARVD/C group, 23 patients (40%) had PKP2 mutations, 4 (7%) had DSG2 mutations, 1 patient (2%) carried a mutation in DSC2, while 1 patient (2%) had a mutation in both DSG2 and DSC2. Among the DSG2 and/or DSC2 mutation-positive TFC+ ARVD/C probands, 2 carried compound heterozygous mutations and 1 had digenic mutations. In probable ARVD/C patients and those with 2 minor or 1 major criteria for ARVD/C, mutations were less frequent and they were all heterozygous. Negative T waves in the precordial leads were observed more (p<.002) among mutation carriers than non-carriers, and in particular in PKP2 mutation carriers.

Conclusions

Mutations in DSG2 and DSC2 are together less prevalent (10%) than PKP2 mutations (40%) in Dutch TFC+ ARVD/C patients. Interestingly, bi-allelic or digenic DSC2 and/or DSG2 mutations are frequently identified in TFC+ ARVD/C patients, suggesting that a single mutation is less likely to cause a full blown ARVD/C phenotype. Negative T waves on ECG were prevalent among mutation carriers (p<.002).

Key Words: Arrhythmia • Cardiomyopathy • Desmosomes • Genetics
Clinical and Genetic Spectrum of Hereditary Cardiac Arrhythmia Syndromes

Introduction
Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy (ARVD/C) is a familial disease characterized by progressive fibrofatty replacement of the right ventricular (RV) myocardium.1 The main histological feature is progressive loss of RV myocardium, which is replaced with adipose and fibrous tissue. These changes may be localized and in the early stages are often confined to the so-called “triangle of dysplasia”: the inflow, outflow, and apical regions of the RV.1 Aneurysm formation is typical. Diffuse myocardial involvement leads to global RV dilation. Left ventricular (LV) involvement occurs with disease progression and was present on histology in >75% of cases in a multicenter pathological study.2 ARVD/C is a genetically heterogeneous disease and is most commonly inherited as an autosomal-dominant trait with incomplete penetrance and variable expression. Its clinical picture is mainly characterized by the occurrence of ventricular arrhythmias and related consequences.3-4 Estimates of the prevalence of ARVD/C in the general population range from 1 in 2,000 to 1 in 5,000; it affects men more frequently than women, with an approximate gender ratio of 3:1.3-4 A familial background has been demonstrated in 30-50% of ARVD/C cases 3-5 and the majority of disease-causing mutations have so far been identified in genes encoding proteins of specialized adhesive junctions between cells, also known as desmosomes.5-18 Mutations in the PKP2 gene encoding Plakophilin-2 are the most prevalent and were identified in up to 43% of unrelated ARVD/C index patients.5,7,10 Dominant mutations in genes encoding Desmoglein-2 (DSG2) and Desmocollin-2 (DSC2) have been reported in up to 12% and 5% of ARVD/C patients, respectively.12,16-17,19-20 Rarely, dominant mutations in the Plakoglobin (JUP), Desmoplakin (DSP) and TMEM43 genes have been reported in patients with ARVD/C, with recessive mutations in JUP and DSP being causal for the cardiocutaneous diseases, Naxos disease and Carvajal syndrome, respectively.6,8,21-24 This study is an extension of our previous study in which we elucidated PKP2 mutations in our Dutch ARVD/C cohorts.5 Our goal now was to evaluate the prevalence of mutations in three cardiac desmosomal genes, PKP2, DSG2 and DSC2, and to study their impact on clinical phenotypes in three groups: (a) patients fulfilling the ARVD/C task force criteria (TFC+),25 (b) patients with probable ARVD/C (1 major and 1 minor, or 3 minor criteria), and (c) patients with either 2 minor or 1 major criteria for ARVD/C.25 Genotype-phenotype analysis, of both clinical and ECG features, was also performed to identify potentially distinguishing features.

Methods

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Clinical evaluation and diagnostic criteria
We evaluated 116 white, unrelated, index patients in four university hospitals in the Netherlands. A case history was taken from all patients, and they were physically examined and evaluated by 12-lead ECG, 24-hour Holter monitoring, exercise testing, and 2-dimensional transthoracic echocardiography. Diagnosis of ARVD/C was based on the diagnostic criteria of the Task Force of the European Society of Cardiology/International Society and Federation of Cardiology.25 (Table 1). In cases of doubtful diagnosis, patients were discussed with experienced cardiologists from the four centers in a consensus meeting. The 116 index patients were subdivided into: (a) 57 TFC+ patients, (b) 28 probable ARVD/C (who had 3 minor criteria, or 1 major and 1 minor criteria) and (c) 31 patients with 2 minor or 1 major criteria of ARVD/C (not including family history). 8 TFC+ patients from our previous study were excluded due to absence of DNA.5 Instead, 8 new TFC+ patients were included in this study (Table 2, marked ‘n’). The onset of disease manifestation was defined as the age at which initial symptoms most likely related to ARVD/C emerged, including paroxysmal tachycardia, prolonged syncope, and successful resuscitation.

Genetic studies
Genomic DNA was extracted from whole blood or paraffin-embedded tissues according to the established protocol (Qiagen). Detailed procedures for screening the PKP2 gene were described earlier.5 DSG2 and DSC2 coding regions (exons 1-15 and exons 1-16, respectively) were screened for mutations. Primer sequences and PCR conditions are available on request. Mutational analysis of the amplimers was performed by denaturant high performance liquid chromatography (DHPLC) (Transgenomic Wave) and denaturing gradient gel electrophoresis (DGGE). PCR products with altered DHPLC peak or DGGE variants were purified using QIAquick PCR purification kit (Qiagen) and were sequenced bidirectionally on an ABI 377 sequencer.

In this study, the pathogenic nature of the identified missense mutations were judged on the basis of four criteria (for an overview of criteria potentially useful for classifying variants see Goldgar et al.26): (1) the differences in physico-chemical properties of the amino acids involved in the respective substitutions, (2) the evolutionary conservation of the amino acids across several species, (3) the presence in an evolutionary conserved region, and (4) the localization within a functionally important domain (predicted or unpredicted). Missense mutations were considered pathogenic when the respective amino acid substitutions satisfied two or more of these criteria. Amino acid substitutions were considered unclassified variants (UVs) when these satisfied none or only one of the criteria mentioned above and are not known as single nucleotide polymorphisms. In all cases, the reported mutations/variations were not identified in at least 150 ethnically-matched control persons. Notably, in
some cases the mutations had been reported in previous studies in ARVD/C patients.

**Statistical analysis**
Clinical characteristics in the patients with a PKP2, DSG2 or DSC2 mutation/UV were compared using Fisher’s exact test. Values of $P<0.05$ were considered significant. All data were analyzed with the Statistical Package for Social Sciences (SPSS version 15.0; SPSS, Inc., Chicago, IL).

**Results**

**Clinical evaluation**
Clinical data of the 57 TFC+ and 28 probable ARVD/C patients are shown in Tables 2 and 3, respectively. The average age of first clinical presentation was 33 years in PKP2, 36 years in DSG2, 42 years in DSC2, and 14 years for combined DSC2/DSG2 mutation carriers and 37 years for non-mutation carriers (range 14-68 yrs); these ages did not differ significantly between men and women.

**Genetic studies**
We screened the PKP2, DSG2 and DSC2 genes in 116 patients.

**TFC+ group**

Of 57 TFC+ ARVD/C patients, most of whom were included in our previous study, 23 carried a mutation in PKP2 (Table 2; Figure 1c). Four TFC+ patients had mutations in DSG2 (Figure 1a and 1c and Table 2); two of them (patients-25 and -41) had mutations (and unclassified variants (UVs) in both alleles. Patient-25 carried compound heterozygous, p.Pro205Leu mutation and an UV p.Gly1083Ser in DSG2. Patient-41 was homozygous for p.Asp297Asn in DSG2.

Patients-39 (female) and -52 (male) had an identical mutation p.Arg46Gln in DSG2 (Table 2). In addition they carried p.Val158Gly (DSG2) on the same allele, which has been described as a mutation in a UK study. In our study, we also found this p.Val158Gly in our control population, and similar results were recently reported by Posch et al., thus we should consider p.Val158Gly as a non-synonymous SNP.

Patient-24, a 56-year old male, was the only patient in this group who had a heterozygous mutation p.Ile603Thr only in DSC2 (Table 2 and Figure 1b). Patient-40 carried a heterozygous p.Val392Ile mutation in DSG2 and p.Leu732Val mutation in the DSC2 gene (Figure 1a, Table 2). We could not identify any mutations in any of these three genes in 28 (49%) TFC+ patients (Figure 1c). A schematic representation of the mutation yield from the PKP2, DSG2 and DSC2 screening is shown in Figure 1c.

**TFC- group**

![Figure 1b](image1.png)

*Figure 1b.* Schematic representation of the DSC2 mutations and UVs. UVs are shown in italics.
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<table>
<thead>
<tr>
<th>Major</th>
<th>Minor</th>
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<tbody>
<tr>
<td>Severe RV dilatation and reduction in systolic function with no (or only mild) LV impairment</td>
<td>Mild global RV dilatation and/or reduced EF with normal LV</td>
</tr>
<tr>
<td>Localized RV aneurysms</td>
<td>Regional RV hypokinesia</td>
</tr>
<tr>
<td>Severe segmental RV dilatation</td>
<td>Mild segmental RV dilatation</td>
</tr>
<tr>
<td>Fibrofatty replacement of myocardium on EMB</td>
<td>Negative T-waves (V2 and V3); &gt;12 years of age in the absence of RBBB</td>
</tr>
<tr>
<td></td>
<td>Late potentials on SA-ECG</td>
</tr>
<tr>
<td>Localized RV aneurysms</td>
<td>Global and/or regional dysfunction and structural alterations</td>
</tr>
<tr>
<td>Regional RV hypokinesia</td>
<td>Severe segmental RV dilatation</td>
</tr>
<tr>
<td>Mild global RV dilatation and/or reduced EF with normal LV</td>
<td>Mild segmental RV dilatation</td>
</tr>
<tr>
<td>Negative T-waves (V2 and V3); &gt;12 years of age in the absence of RBBB</td>
<td>Late potentials on SA-ECG</td>
</tr>
<tr>
<td>Sustained and non-sustained VT with LBBB morphology (on ECG, Holter, exercise testing)</td>
<td>Family history of clinical diagnosis based on present criteria</td>
</tr>
<tr>
<td>&gt;1000 ventricular extrasystoles/24 hrs</td>
<td>RV right ventricle, LV left ventricle, EF ejection fraction, EMB endomyocardial biopsy, RBBB right bundle branch block, VT ventricular tachycardia, SCD sudden cardiac death</td>
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Table 1. Task force criteria for the diagnosis of Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy. Either 4 minor criteria, 2 major, or 1 major + 2 minor criteria are sufficient to make a diagnosis of ARVC. EF=ejection fraction, EMB=endomyocardial biopsy, LBB=left bundle branch, LV=left ventricular, RV=right ventricular, SCD=sudden cardiac death, VT=ventricular tachycardia.

Figure 1c: Graphical representation of PKP2, DSG2, DSC2 gene screening in the ARVD/C, probable ARVD/C, and patients with attenuated features of ARVD/C. Filled bars represent TFC+ patients, slashed bars represent probable ARVD/C, and dotted bars represent patients with attenuated features of ARVD/C. The x-axis represents analyzed genes and the y-axis represents percentages of mutations identified in each group. On top of each bar, the number of mutation carriers or non-mutation carriers of the respective genes are represented.

Figure 1d: Graphical representation of PKP2, DSG2, DSC2 gene screening in the ARVD/C, probable ARVD/C, and patients with attenuated features of ARVD/C. Filled bars represent TFC+ patients, slashed bars represent probable ARVD/C, and dotted bars represent patients with attenuated features of ARVD/C. The x-axis represents analyzed genes and the y-axis represents percentages of mutations identified in each group. On top of each bar, the number of mutation carriers or non-mutation carriers of the respective genes are represented.
Table 2. Clinical characteristics of the probands with TFC+ ARVD/C patients.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age initial</th>
<th>Tissue</th>
<th>Repolariz Late</th>
<th>Arg79X</th>
<th>Cys796Arg</th>
<th>Arg79X</th>
<th>Tyr807X</th>
<th>Trp676X</th>
<th>Tyr616X</th>
<th>Arg79X frameshift c.2489+1 G&gt;A</th>
<th>frameshift c.148_151delACAG (homozygous)</th>
<th>Asp297Asn</th>
<th>Arg46Gln</th>
<th>Gly1083Ser</th>
<th>Pro205Leu;</th>
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<tr>
<td>m</td>
<td>34</td>
<td>++</td>
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Probable ARVD/C patients
In the patients with probable ARVD/C (n=28), we detected one heterozygous truncating mutation c.397C>T (p.Gln133X) in PKP2 (patient-63; Table 3). Patient-59 had a heterozygous UV p.Leu15Gln in DSG2 (Figure 1a, and Table 3). Leu15 in DSG2 is not very well conserved across diverse species, but it is located in a functionally important signal sequence domain. Patient-64 carried a heterozygous mutation p.Asp350Tyr and Patient-75 carried an UV p.Pro289Ser, in DSC2 (Figure 1b and Table 3). We were able to study the segregation of the mutation in the family of patient-64: it was also found in a brother and a sister, with a positive signal averaged-ECG as a sole manifestation, at ages 48 and 47 years respectively.

Table 3. Clinical characteristics of the probable ARVD/C patients.
++ indicates a major criterion; + minor criterion; f, female; m, male. Mutations identified in the PKP2, DSG2 and DSC2 are indicated. UVs are shown in italics.
Patients with 1 major or 2 minor criteria for ARVD/C

In this TFC-group of patients (n=31) who had only 1 major or 2 minor criteria of ARVD/C, we detected two UVs in PKP2: c.1592C>T (p.Ile531Ser), which has been reported in the Finnish population as a polymorphism28, and c.1012A>G (p.Thr338Ala). In DSG2 five heterozygous mutations (including one UV) were found: p.Val56Met, p.Ile73Val, p.Val149Phe, p.Val392Ile, p.Asn1067Asp (UV),(Figures 1a, 1b). The patient with p.Val392Ile amino-acid change was a female of 34 years. She had negative T waves at V4-6, a low voltage ECG, was later diagnosed with dilated cardiomyopathy at
the age of 47 years, and experienced an out-of-hospital cardiac arrest due to ventricular fibrillation. She died with RV failure at 56 years and histology showed typical features of ARVD/C in the RV and dilated cardiomyopathy (DCM) with fibrosis in the LV. p.Val392Ile mutation in DSG2 was diagnosed post mortem in paraffin-embedded tissue material. Her sister had died suddenly at the age of 32 years. Her nephew (the dead sister’s son) showed negative T-waves in V4-V6 and was diagnosed with DCM at the age of 49 years after experiencing a near-syncope. He was also found to carry this p.Val392Ile DSG2 mutation. These data suggest a left-dominant arrhythmogenic cardiomyopathy.

**Mutation characteristics**

The DSG2 mutations identified in our study, p.Val392Ile, p.Val56Met, and p.Arg46Gln, were previously reported in studies from the USA, UK and Germany.\(^{12,17,27}\) We identified four novel DSG2 mutations: p.Ile73Val, p.Val149Phe, p.Pro205Leu, and p.Asp297Asn (Figure 1a). In addition, we detected three novel UVs in DSG2 (p.Leu15Gln, p.Asn1067Asp and p.Gly1083Ser) (Figure 1a). In DSC2 we identified three novel mutations, p.Asp350Tyr, p.Asp350Tyr in the DSC2 gene.
p.Ile603Thr, p.Leu732Val, and one UV, p.Pro289Ser (Figure 1b). The mutations in various groups of patients are shown in Figure 1c.

The DSG2 and DSC2 mutations we identified are all missense mutations (Figures 1a, 1b and Tables 2, 3), in contrast to PKP2 mutations, which are mostly truncating/frameshift mutations. In this study, three patients (patients-25 and -41, TFC+ group; and 1 patient with two minor criteria) among the DSG2 mutation carriers were familial cases. The DSC2 mutation carriers were all sporadic, with no family history pertaining to ARVD/C.

**Genotype-phenotype relationships/phenotypic characteristics**

Figures 2a and 2b show examples of a classical ARVD/C ECG recorded from a male symptomatic patient in this study. Figure 3 shows a representative ARVD/C histopathology of myocardial tissue taken from patient-64. We have compared various pertinent phenotypic features (age of onset, cardiac structural alteration, ECG features, VES) among the patients with defects in PKP2 (n=24), DSG2 (n=5), DSC2 (n=3), and both DSG2/DSC2 (n=1). Patients without a mutation in any of these three genes were also compared with the mutation carriers and TFC+ and probable ARVD/C patients were included in this analysis.

The presence of T wave inversions in right precordial leads on 12-lead ECG in individuals with a mutation (PKP2, DSG2 and DSC2) was significantly higher than that of individuals without a mutation (P <.002). Among the 54 patients with a negative T wave, 26 carried mutation/UVs in one of the three genes. In contrast, only 4 patients (of 31) with a normal, positive T wave carried a mutation/UV. This difference in T wave was more evident among the PKP2 mutation carriers. Disease onset was slightly earlier among the small group of DSG2 carrier patients (including the one with an additional DSC2 mutation) compared to those with a PKP2 mutation in the TFC+ group (34 years vs 36 years; not significant. No other significant differences in any other parameters could be established.

**Discussion**

In this multicenter Dutch study we have systematically evaluated a large number of ARVD/C patients, both clinically and genetically. We have screened three cardiac desmosomal genes (PKP2, DSG2 and DSC2) in 116 patients, of whom 57 were TFC+. PKP2 mutations were most prevalent in TFC+ patients, who comprised just under half of the ARVD/C patients (40%). Mutations in DSG2, DSC2 or both were found in 4, 1 and 1 of ARVD/C index patients, respectively (7%, 2%, 2%); this frequency is similar to reports published from other countries. DSG2 mutations ranged between 3.5-17% in our probable ARVD/C patients and in those with 1 major or 2 minor criteria for ARVD/C. The DSC2 mutation frequencies in these groups were 7% and 0%, respectively. In approximately 50% of our TFC+ ARVD/C patients, we identified no mutation in any of the three desmosomal
genes screened, although large deletions or mutations in regulatory regions (promoter/intronic) cannot be excluded. Mutations in other genes related to ARVD/C are believed to be involved far less often and might add only a few more patients to our groups.4 In this study we have identified compound heterozygous or homozygous mutations in DSG2 in two unrelated patients. In addition, a third patient carried mutations in both DSG2 and DSC2. Interestingly, all three patients fall in the full blown ARVD/C group (TFC+). Moreover, in two patients from the TFC+ group, p.Arg46Gln (DSG2) occurred with p.Val158Gly on the same allele. Haplotype analysis confirmed a common founder (data not shown). Previously, p.Val158Gly has been reported in two families as potentially pathogenic,16 however, we found p.Val158Gly in 1% of our control subjects, similar to the frequency reported by Posch et al.27 Thus we cannot conclude at this stage whether the presence of this p.Val158Gly adds to the pathogenicity of the p.Arg46Gln mutation. Though previous studies detected compound heterozygous DSG2 mutations in two TFC+ ARVD/C patients,12,14 the majority of the mutation carriers were heterozygous for a DSG2 mutation, unlike our ARVD/C patients.12,14 But it should be noted that previous studies did not look for digenic mutations in their ARVD/C cohorts.5,7,9,12-16,19,20 This suggests that the heterozygous missense mutations in the genes encoding desmosomal cadherins (DSG2/DSC2) are associated with a less fulminant phenotype. This is corroborated by the fact that we identified mutations in these genes predominantly in the probable ARVD/C patients or group of patients with 1 major or 2 minor criteria. However, this observation in small groups has to be confirmed in a larger series. Further, we have only identified missense mutations in DSG2 and in DSC2 in our Dutch cohort, whereas three published reports reported mainly missense mutations, as well as some truncating mutations, in these two genes in their ARVD/C cohorts.12,14,17 Moreover, except for the family with the p.Val392Ile mutation, we found no significant predilection for left ventricle involvement among DSG2 mutation carriers in our study, as has been reported in a UK population by Syrris et al. 2007.17 Patient-40 (TFC+) and another unrelated patient (not shown in Table) harbored the identical mutation p.Val392Ile in DSG2, but their clinical presentation is quite variable. Patient-40 had full blown features of ARVD/C (TFC+) (Table 2), while the patient from the 2 minor and 1 major criteria group demonstrated a left-dominant arrhythmogenic cardiomyopathy. We think the difference in phenotype could be attributed to the fact that patient-40 (TFC+) harbored a second mutation, p.Leu732Val, in DSC2. During this study, we also observed a frequent polymorphism, p.Glu713Lys (DSG2), equally prevalent among controls and the ARVD/C population (4%), which has also been described by Posch et al. as an innocent SNP.27 However, suspicion about the pathogenicity of p.Val56Met (DSG2) should be verified as we did not detect p.Val56Met in controls in our study, in contrast to Posch et al.27
In DSC2, variant p.E896fsX5 was detected in three different families and in 4% of our control population. We therefore now consider this to be a non-pathogenic common variant in our population, although in a previous study it was considered pathogenic.16

As to the phenotype, we have observed predominance of negative T waves in V1-V3 among the mutation carriers, more specifically among the PKP2 mutation carriers. Turrini et al. (2001)29 while retrospectively studying ECG parameters in various groups of ARVD/C patients, found negative T waves in precordial leads as a predictor of sudden cardiac death. Unfortunately they provided no genotype data for the ARVD/C patients in their study.28

Another study from the Netherlands suggested that repolarization abnormalities in ARVD/C could facilitate the occurrence of ventricular arrhythmias in these patients.29 We observed the significant predominance of negative T waves among individuals with the PKP2 mutation, which could be used to direct genetic screening.5

In conclusion, we found DSG2 and DSC2 mutations in approximately 10% of TFC+ patients in our Dutch ARVD/C cohort. We also identified mutations in these genes in 10% of probable ARVD/C cases. Interestingly, we found compound and digenic mutations exclusively in TFC+ patients, suggesting a dose effect of mutations correlating with disease severity, although larger studies are needed to confirm this observation.

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Disclosures: None

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