Clinical and genetic spectrum of hereditary cardiac arrhythmia syndromes

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Arrhythmogenic Right Ventricular Cardiomyopathy due to a Novel Plakophilin 2 Mutation: Wide Spectrum of Disease in Mutation Carriers Within a Family

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Abstract

**Background:** Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is a familial disease, with male preponderance, characterized by progressive fibrofatty replacement of the right ventricle and ventricular arrhythmias. Mutations in plakophilin-2 (PKP2), a desmosomal protein, have been reported to underlie familial ARVC. We report a novel ARVC PKP2 mutation and present the clinical findings in 3 female mutation carriers.

**Methods and Results:** A female proband presented with resuscitated cardiac arrest, and was diagnosed with ARVC due to right ventricular enlargement and regional hypokinesis, along with repolarization abnormalities and frequent ventricular ectopy. A novel 28 bp insertion in exon 11 of the PKP2 gene was found which causes a frameshift in the coding region. This results in a change in the amino acid sequence of the protein with a premature stop codon at position 740. Four first degree relatives were screened, and the mother and younger sister were identified as mutation carriers. The mother was phenotypically normal, while the younger sister has repolarization abnormalities and frequent ventricular ectopy.

**Conclusions:** We report a novel PKP2 mutation which causes familial ARVC. All mutation carriers in this kindred were women, and the family showed incomplete penetrance and variable expression of ARVC. Premature truncation of the plakophilin-2 protein appears to be the predominant mechanism whereby PKP2 mutations elicit the ARVC phenotype.

**Key Words:** cardiomyopathy, heart arrest, genetics, arrhythmia, women

**Abbreviations**

ARVC = Arrhythmogenic right ventricular cardiomyopathy  
ECG = electrocardiogram  
SAECG = signal-averaged electrocardiogram  
MRI = magnetic resonance imaging  
PCR = polymerase chain reaction  
DHPLC = denaturing high-performance liquid chromatography

**Introduction**

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited disease characterized by progressive fibrofatty replacement of the right ventricular myocardium and
ventricular arrhythmias. It affects males disproportionately, and carries an increased risk for sudden death, particularly during exercise.\textsuperscript{1,2} ARVC is most commonly inherited in an autosomal dominant fashion, with significant genetic heterogeneity and at least 9 different genetic loci mapped.\textsuperscript{3} To date, 5 genes have been implicated in autosomal dominant ARVC, including transforming growth factor-β3 (TGFβ3) in ARVC1,\textsuperscript{4} the cardiac ryanodine receptor (RYR2) in ARVC2,\textsuperscript{5} and 3 desmosomal proteins: desmoplakin (DSP) in ARVC8,\textsuperscript{6} desmoglein-2,\textsuperscript{7} and plakophilin-2 (PKP2) in ARVC9.\textsuperscript{8}

### Table 1: Diagnostic criteria for Arrhythmogenic Right Ventricular Cardiomyopathy

<table>
<thead>
<tr>
<th>Major</th>
<th>Tissue Characterization of Walls</th>
<th>Repolarization Abnormalities</th>
<th>Depolarization Abnormalities</th>
<th>Arrhythmias</th>
<th>Family History</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe dilatation and reduction of RV EF with no (or only mild) LV impairment. Localized RV aneurysms. Severe segmental RV dilatation.</td>
<td>Fibrofatty replacement of myocardium on endomyocardial biopsy</td>
<td>Epsilon waves or localized prolongation (&gt;110ms) of the QRS complex in right precordial leads.</td>
<td>Familial disease confirmed at necropsy or surgery.</td>
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<table>
<thead>
<tr>
<th>Minor</th>
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<th>Family History</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global RV dilatation and reduced EF with normal left ventricle. Regional right ventricular hypokinesis.</td>
<td>Inverted T waves in V2 and V3</td>
<td>Late potentials on SA ECG</td>
<td>LBBB VT on ECG, Holter, exercise testing.</td>
<td>Familial history of premature sudden death due to suspected ARVC. Familial history (clinical diagnosis based on present criteria.)</td>
<td></td>
</tr>
</tbody>
</table>

RV indicates right ventricle; EF, ejection fraction; LV, left ventricle; SAECG, signal-averaged electrocardiogram; LBBB, left bundle branch block; VT, ventricular tachycardia; ECG, electrocardiogram; ARVC, Arrhythmogenic right ventricular cardiomyopathy.
Plakophilins with other proteins assemble to form desmosomes, complex structures which provide structural and functional integrity to adjacent cells. They are abundant in cells that experience mechanical stress and appear to have a primarily structural function.9 Plakophilin-2 interacts with multiple other cell adhesion proteins and is the primary cardiac plakophilin.10 Ablation of mouse Pkp2 results in defects of cardiac morphogenesis and junctional architecture with embryonic lethality.11 It is thought that mutant PKP2 in cardiac desmosomes impairs cell to cell contacts and disrupts adjacent myocytes. Areas of high stress and stretch are thought to be particularly vulnerable, explaining the focal involvement and exercise-related risk of arrhythmias in ARVC.8

Here we report a novel PKP2 mutation in a female proband with ARVC. In addition, 2 female relatives were identified as mutation carriers, and underwent additional clinical screening. We report the spectrum of clinical findings in these 3 female patients with plakophilin-2 mutations.

Methods

Patients and Clinical Variables
All individuals gave written, informed consent for procedures and genetic analysis. Clinical evaluation included 12-lead electrocardiogram (ECG), signal-averaged electrocardiogram (SAECG), 2-dimensional transthoracic echocardiography, cardiac magnetic resonance imaging (MRI), maximal exercise testing according to standard protocols, and 24-hour ambulatory ECG monitoring. Conventional time domain criteria were used to determine an abnormal signal-averaged ECG.12 The proband also underwent intracardiac electrophysiology study with programmed ventricular stimulation, right ventricular angiography, and endomyocardial biopsy. Diagnosis of ARVC was based on the diagnostic criteria of the Task Force of the European Society of Cardiology/International Society and Federation of Cardiology.13 The criteria are given in table 1. Clinical diagnosis requires either: 2 major criteria; 1 major plus 2 minor; or 4 minor, from different categories.

Genetic Studies
Genomic DNA was isolated from peripheral blood lymphocytes (Gentra Systems) in the proband and in her family members. The entire cardiac RYR2 coding region (ref seq. NM_001035, exons 1-105) and PKP2 (ref seq. NM_004572.2, exons 1-14) were screened for mutations. Primer sequences and polymerase chain reaction (PCR) conditions are available on request. Mutational analysis of the amplicons was performed by denaturing high-
performance liquid chromatography (DHPLC, Transgenomic Wave). PCR products with altered DHPLC peak were purified using QIAquick PCR purification kit (Qiagen) and were sequenced bidirectionally on an ABI 377 sequencer.

Figure 1: 12-lead ECG of proband (top) shows low voltage in the limb leads and T-wave inversion in leads V1 to V5. In contrast, T-wave inversion is seen only in leads V1 to V3 in the proband’s sister (bottom).
Results

Clinical Investigation

The proband, a previously healthy 16-year-old girl at the time of presentation, was swimming when she became lightheaded and left the pool. She then lost consciousness and was pulseless. Resuscitation was initiated, and on paramedics arrival she was noted to be in ventricular fibrillation. She was defibrillated successfully. Her electrocardiogram after recovery revealed low voltage in the limb leads, T-wave inversion in leads V1-V5 (Figure 1), and normal QRS duration. Her left ventricle was normal, but her right ventricle was enlarged with free-wall hypokinesis evident by echocardiography, MRI, and angiogram. The right ventricular outflow tract in systole measured 28 mm in the parasternal long-axis view, and 29 mm in the parasternal short-axis view. The RV medial lateral dimension in the apical 4-chamber view in diastole was 45 mm, and in systole 38 mm. Endomyocardial biopsy revealed focal and interstitial fibrosis with myocyte cellular and nuclear enlargement, consistent with, but not diagnostic for ARVC. Subsequent ambulatory ECG monitoring revealed frequent ventricular extrasystoles (>1,000 in 24 hours), and SAECG was positive for late potentials. She thus fulfills criteria for clinical diagnosis of ARVC (4 minor criteria in different diagnostic categories, see Table 2). She underwent electrophysiology study with programmed ventricular stimulation (single, double, and triple extrastimuli at 3 drive cycle lengths from the right ventricular apex and outflow tract). She had multiple nonsustained episodes of polymorphic ventricular tachycardia induced with double and triple extrastimuli, and had a defibrillator implanted. She has remained free of events on beta-blocker therapy, and is now 18 years old. She had no family history of known ARVC or sudden unexplained death.

<table>
<thead>
<tr>
<th></th>
<th>ECG</th>
<th>SAECG</th>
<th>Echo</th>
<th>MRI</th>
<th>Holter</th>
<th>Diagnostic criteria</th>
<th>Modified criteria for relatives of ARVC patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband</td>
<td>TWI</td>
<td>V1-V5</td>
<td>Positive LP</td>
<td>Global RVE</td>
<td>Regional RV HK</td>
<td>1200 PVC's in 20 hours</td>
<td>4 minor (TWI, LP, regional HK, Frequent PVC's)</td>
</tr>
<tr>
<td>Sister</td>
<td>TWI</td>
<td>V1-V3</td>
<td>Negative LP</td>
<td>Normal</td>
<td>Regional RV HK</td>
<td>238 PVC's 24 hours</td>
<td>3 minor (TWI, regional HK, FH)</td>
</tr>
<tr>
<td>Mother</td>
<td>normal</td>
<td>Negative LP</td>
<td>Normal</td>
<td>Normal</td>
<td>10 PVC's 24 hours</td>
<td>1 minor (FH only)</td>
<td>None</td>
</tr>
</tbody>
</table>

ECG indicates electrocardiogram; SAECG, signal-averaged electrocardiogram; MRI, magnetic resonance imaging; ARVC, Arrhythmogenic right ventricular cardiomyopathy; TWI, T-wave inversion; LP, late potentials; RVE, right ventricular enlargement; HK, hypokinesis; PVC’s, premature ventricular contractions; FH, family history

Table 2: Clinical Features of Mutation Carriers
Identification of the PKP2 mutation

Screening of RYR2 (exons 1-105) did not reveal any mutations. Sequencing the PKP2 gene revealed an insertion of 28-nucleotides at cDNA position 2196 in exon 11 which generates a frameshift in the coding region of PKP2. This results in a change in the amino acid sequence after residue 732 in this 881-amino acid protein, with a premature stop codon at position 740 of the mutant protein. After identification of the PKP2 mutation in the proband, her 4 first degree relatives were screened. The same mutation was identified in the mother and sister, but not in the father or brother (Figure 2).

Evaluation of mutation carriers

Clinical evaluation of mutation carriers included 12-lead ECG, SAECG, 2-D echocardiography, cardiac MRI, maximal exercise testing according to standard protocols, and 24-hour
ambulatory ECG monitoring. Invasive tests (intracardiac electrophysiology study and right ventricular biopsy) were deferred. The patient’s mother was 45 years old at the time of clinical investigation. Her 12-lead ECG, SAECG, echocardiogram, and cardiac MRI were normal. She had no arrhythmias on exercise treadmill testing, or on 24-hour ambulatory ECG monitoring. She had only 10 ventricular extrasystoles in 24 hours. Aside from a family history of ARVC based on clinical criteria in her daughter (the proband), she has no diagnostic criteria suggestive of ARVC. At this time, she appears to be a silent mutation carrier. The patient’s younger sister was 16 years old at the time of clinical investigation. She had T wave inversion in leads V1-V3 (Figure 1). Her SAECG and echocardiogram are normal. Cardiac MRI revealed mild segmental hypokinesis of the right ventricular free wall. She had no arrhythmias on exercise testing or on 24-hour ambulatory ECG monitoring, with 238 ventricular extrasystoles in 24 hours. She presently has 3 minor criteria in different categories for ARVC (T wave inversion, regional hypokinesis, and family history, see Table 2). Currently, she is not being treated but has been advised to have prompt evaluation of any cardiac symptoms.

Discussion

We report the clinical findings in 3 female carriers of a novel mutation in plakophilin-2 that underlies ARVC. The proband presented with resuscitated cardiac arrest as her first symptom, but had sufficient clinical features to make the diagnosis of ARVC at the age of 16. Of the 2 other family members with the mutation, 1 appears to be unaffected by ARVC (a silent mutation carrier) at the age of 45. The other has several features of ARVC, and is likely affected but does not currently meet diagnostic criteria. This report highlights the incomplete penetrance and variable expression of ARVC, the importance of screening first degree relatives of patients with genetic arrhythmia syndromes, the need for repeated investigations of young patients with possible ARVC, and the limitations of the current diagnostic criteria.

The initial report of PKP2 mutations in ARVC did not systematically evaluate familial disease in all probands. Two of the 32 kindreds in that report were available for detailed clinical analysis. In both kindreds, several mutation carriers were identified with either no or mild disease phenotypes.8 This is consistent with our finding of incomplete penetrance of ARVC due to plakophilin-2 mutations and recently reported findings in additional families with PKP2 mutations.14-16 Several reasons for incomplete penetrance have been suggested, including modifier genes, environmental triggers, and gender effects. The high proportion of males among individuals affected with ARVC suggests that gender may have a signifi-
cant effect. We observed a wide spectrum of clinical manifestations in 3 female mutation carriers, from no apparent disease at age 45, to ventricular fibrillation as the first symptom at age 16. Similarly variable expression has been previously reported in ARVC due to PKP2 mutations.\textsuperscript{14,16} This suggests that factors other than gender are important in determining penetrance of ARVC due to plakophilin-2 mutations.

It is likely that the newly identified mutation underlies ARVC in this family. The mutation causes a premature stop codon at position 740 in exon 11, and is predicted to result in a truncated protein. Of the 25 initially described PKP2 mutations that underlie ARVC, 18 of them result in premature stop codons.\textsuperscript{8} Two of these previously described mutations (Q726X and R735X) result in truncations in close proximity to our novel mutation. Furthermore, a different deletion/insertion mutation that also results in a premature stop codon at amino acid 740 has recently been reported in 6 families with ARVC in 2 separate publications.\textsuperscript{14,15} It is thought that lack of plakophilin-2 or incorporation of mutant protein into cardiac desmosomes impairs cell to cell contact and disrupts adjacent myocytes. Mutations that result in abnormally truncated proteins appear to be a relatively common mechanism of PKP2 dysfunction in ARVC. Of the now 47 published PKP2 ARVC mutations, 35 result in premature stop codons.\textsuperscript{8,14-16}

The criteria for the clinical diagnosis of ARVD have been widely accepted, and are useful in that they provide a uniform approach to the diagnosis of a disease with a broad spectrum of clinical manifestations. They are, however, imperfect as a true “gold standard” for diagnosis.\textsuperscript{17} The difference between major and minor criteria in the category of structural alterations depends on subjective assessments, prompting the suggestion that the guidelines include quantitative measurements of right ventricular size and function.\textsuperscript{18} Further limitations of the guidelines regarding family history are highlighted by this kindred. The proband meets clinical criteria for diagnosis, and therefore, confers upon her relatives 1 minor criterion. If she were to have her disease “confirmed” by surgery or autopsy, her relatives would then be assigned 1 major criterion. It is important to distinguish between confirmed and suspected disease; however, some patients have unambiguous ARVC, but have not had “proof” of disease by surgery or autopsy. If we consider our proband to have “confirmed” ARVC, she would then confer a major criterion to her sister, in whom clinical diagnosis would then be made (1 major and 2 minor criteria).

Based on prospective evaluation of family members of probands with ARVC, Hamid et al. suggested that the diagnostic criteria be modified for first degree relatives of ARVC patients to require only 1 minor criterion.\textsuperscript{19} Furthermore, this group recommends that the number of
ventricular ectopic beats required to qualify as a minor criterion may be reduced from 1,000 to 200 in a 24 hour period. Using this modified system, the proband’s sister would be considered affected, as she has 3 abnormalities (T wave inversion, > 200 PVC’s in 24 hours and regional hypokinesis) in the context of a relative with ARVC. Although she is affected with ARVC and at some risk for sudden death, her risk is likely lower than patients who meet current Task Force criteria. Also, at age 16 her disease appears to be less severe than her sister who had cardiac arrest at age 16, but who also had more ventricular ectopy, greater degrees of RV structural abnormalities, more diffuse repolarization abnormalities, and an abnormal SAECG. Given her lack of symptoms and mild disease at this time, we have opted not to implant a defibrillator, but to continue close follow-up. A defibrillator will be considered if she has progression of her disease or develops worrisome symptoms.

Although genetic testing for ARVC is not yet in widespread clinical practice, it seems inappropriate to ignore genetic information when determining disease status if available. For example, the more broad criteria for family members of ARVC patients could be applied to the proband’s brother and father as well. If one of these individuals had a minor ECG abnormality, it would be inappropriate to diagnose them with ARVC, as they lack the PKP2 mutation identified in the proband. However, the diagnosis of ARVC cannot be made by genetic information alone, as the proband’s mother appears unaffected, despite carrying the mutation. The incomplete penetrance found in most genetic arrhythmia syndromes highlights the importance of clinical criteria. However, the current Task Force criteria are likely too restrictive to identify patients with ARVC early in the course of the disease, while modified criteria for all family members could result in misdiagnosis of unaffected individuals. Currently, identification of a pathogenic genetic mutation needs to be coupled with clinical diagnostic criteria for ARVC, not only to establish the diagnosis, but also to determine the risk for ventricular arrhythmias and to guide therapeutic decisions.

References


