

Mechanisms of Disease: molecular genetics of arrhythmogenic right ventricular dysplasia/cardiomyopathy

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SUMMARY

Arrhythmogenic right ventricular dysplasia/cardiomyopathy is an inherited cardiomyopathy estimated to affect approximately 1 in 5,000 individuals. Cardinal manifestations include right ventricular enlargement and dysfunction, fibrofatty replacement of myocytes in the right ventricle, characteristic electrocardiographic abnormalities, and ventricular arrhythmia most commonly arising from the right ventricle. The disease is frequently familial and typically involves autosomal dominant transmission with low penetrance and variable expressivity. Approximately 50% of symptomatic individuals harbor a mutation in one of the five major components of the cardiac desmosome. Nevertheless, other genetic modifiers and environmental factors complicate the clinical management of mutation carriers as well as counseling of their relatives. This Review summarizes the known genetic mutations associated with arrhythmogenic right ventricular dysplasia/cardiomyopathy, describes possible origins of recurrent mutations, presents theories on the pathogenesis of disease following a mutation, and discusses the current issues surrounding clinical use of genetic analysis in the assessment of individuals with this condition.

KEYWORDS arrhythmia, arrhythmogenic right ventricular dysplasia, arrhythmogenic right ventricular cardiomyopathy, genetics, sudden cardiac death

REVIEW CRITERIA

This manuscript is based on our experience with ARVD/C and the genetic factors contributing to it, as well as a comprehensive review of published manuscripts on the topic in PubMed. Owing to the many different names for this disorder, we searched using acronyms and full names including “ARVD”, “ARVC”, “ARVD/C”, “ARVC/D”, “Uhl’s anomaly”, and “parchment right ventricle”. Historical documents as early as 1952 were used, although the majority of reviewed references were published between 1982 and the present. Review was restricted to full documents, and references cited within documents were used to expand our search.

CME

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Learning objectives

Upon completion of this activity, participants should be able to:

- 1 Describe the prevalence of arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C).
- 2 Identify clinical features of ARVD/C.
- 3 Describe the histopathology that characterizes ARVD/C.
- 4 Describe differences between Naxos disease and Carvajal syndrome.
- 5 Describe optimal screening intervals for family members of patients with ARVD/C.

Competing interests

The authors, the journal editor H Camm and the CME questions author D Lie declared no competing interests.

INTRODUCTION

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is a heritable condition characterized by replacement of cardiomyocytes, primarily in the right ventricle, by fibrofatty tissue.¹ The resulting disruption of normal myocardial architecture in ARVD/C can result in severe right ventricular (RV) dysfunction, life-threatening arrhythmias and sudden cardiac death. A number of genetic studies have identified mutations in various components of the cardiac desmosome that have important roles in the pathogenesis of ARVD/C (Figure 1). Mutations in ARVD/C-related genes demonstrate incomplete penetrance and variable expressivity, implicating environmental factors and other genetic modifiers in the etiology of this disease. This Review focuses on recent genetic

advances, hypothetical disease mechanisms, and the role of clinical genetic testing in diagnosis and prognosis.

In 1952, Henry Uhl described an 8-month-old girl in whom the parietal surface of the RV had become “paper-thin with no myocardium visible” and the endocardium had become apposed to the epicardium.² In the following decades, most cases of right ventricular myocardial dysfunction were classified as ‘Uhl’s anomaly’.³ By the late 1970s, however, French physicians had begun describing a distinct clinical entity, characterized by patchy fibrofatty infiltration of the RV myocardium and with patients usually presenting later in life.⁴ Over the following decade, this clinical entity was recognized as a familial disease with incomplete penetrance and variable expressivity.^{5,6}

Credit for the original description of ARVD/C is usually granted to a report on 24 cases that focused on the cardiac electrophysiological attributes of the disease.⁷ In addition, the name of this disease state has evolved, from the early eponym of ‘Uhl’s anomaly’ and ‘parchment right ventricle’, to arrhythmogenic right ventricular dysplasia, which referenced the characteristic histopathology of fibrofatty infiltration of the RV myocardium. A more-generalized term is now commonly used—arrhythmogenic right ventricular cardiomyopathy—which does not ascribe a primary role to the fibrofatty infiltration. For this report, we use a hybrid eponym, ARVD/C, in an effort to be inclusive.

MUTATIONS

Plakoglobin

Studies of individuals from the Greek island of Naxos with an autosomal recessive syndrome characterized by the triad of ARVD/C, non-epidermolytic palmoplantar keratoderma and woolly hair (‘Naxos disease’) led to identification of the first causative gene for an ARVD/C-associated disorder. Initial mapping of this disorder pointed to the chromosomal locus 17q21,⁸ and candidate-gene sequencing within this region revealed a homozygous 2 bp deletion (c.2157–2158delGT) in the junction plakoglobin gene (*JUP*) that was present only in affected individuals (Figure 2A).⁹ Subsequent studies showed that among subjects homozygous for this mutation, the disease is completely penetrant by adolescence.¹⁰ A study of a German family recently reported the first dominantly inherited *JUP* mutation (p.Ser39–Lys40insSer) to cause

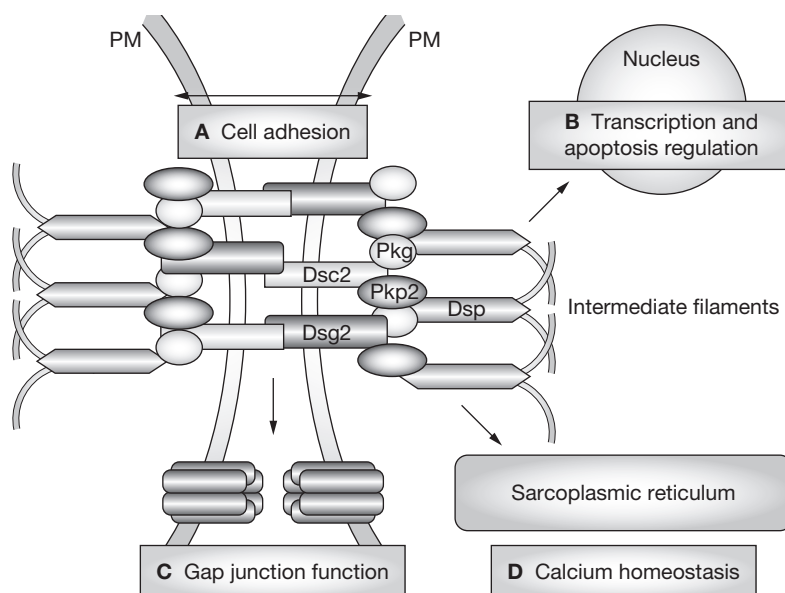


Figure 1 The cardiac desmosome and proposed roles of the desmosome in (A) supporting structural stability through cell–cell adhesion, (B) regulating transcription of genes involved in adipogenesis and apoptosis, and maintaining proper electrical conductivity through regulation of (C) gap junctions and (D) calcium homeostasis. Abbreviations: Dsc2, desmocollin-2; Dsg2, desmoglein-2; Dsp, desmoplakin; Pkg, plakoglobin; Pkp2, plakophilin-2; PM, plasma membrane.

nonsyndromic ARVD/C (i.e. no cutaneous abnormalities).¹¹

Plakoglobin, also known as γ -catenin and a member of the armadillo family of proteins,¹² was the first component of the desmosome to be implicated in the pathogenesis of ARVD/C. Homozygous targeted disruption of the plakoglobin gene in mice (*Jup*^{-/-}) results in embryonic lethality, caused by severe heart defects, beginning at embryonic day 10.5.¹³ In addition to skin blistering, these mice demonstrated ventricular rupture, impaired contractility and an absence of desmosomes in the intercalated discs of the myocardium.^{13,14} Mice with heterozygous plakoglobin mutations (*Jup*^{+/-}) were indistinguishable from their wild-type littermates at birth, but showed RV dilatation and dysfunction, and ventricular arrhythmias by 6 months of age. These phenotypes were exacerbated by exercise (daily swimming for 2 months),¹⁵ supporting the impression that endurance training could accelerate disease progression among individuals with ARVD/C. Despite these findings, histologic analysis of myocardium from *Jup*^{+/-} mice did not show any fibrofatty infiltration, and electron microscopy studies revealed no structural changes

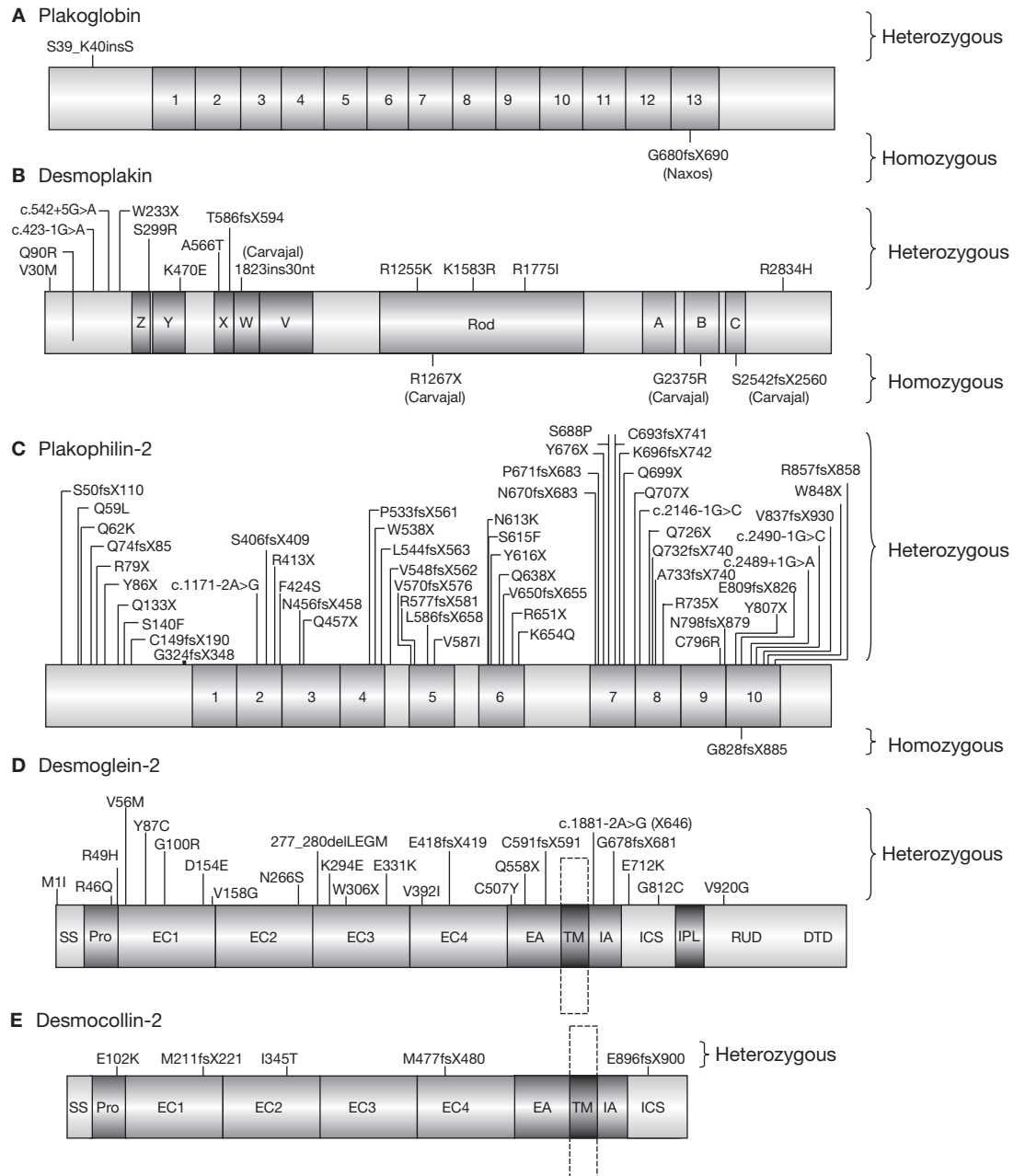


Figure 2 Schematic of the five desmosomal proteins in which ARVD/C mutations have been identified and published. Heterozygous mutations are indicated above each diagram and homozygous mutations are indicated below each diagram. **(A)** Plakoglobin; the 13 armadillo domains are numbered. **(B)** Desmoplakin; the five N-terminal α -helical bundles (Z, Y, X, W, V) are shown, followed by the rod domain required for dimerization and the A, B, and C subdomains of the plakin-repeat domain. **(C)** Plakophilin-2; the 10 armadillo domains are numbered. **(D)** Desmoglein-2 and **(E)** Desmocollin-2. Abbreviations: DTD, desmoglein-specific terminal domain; EA, extracellular anchor; EC1-4, extracellular domains 1-4; IA, intracellular anchor; ICS, intracellular cadherin segment; IPL, intracellular proline-rich linker; Pro, propeptide; RUD, 6 repeated-unit domains; SS, signal peptide sequence; TM, transmembrane domain.

in the desmosomes or adherens junctions. Furthermore, gene-expression profiling of wild-type and heterozygous mice showed no statistical differences in gene expression patterns.¹⁵

Desmoplakin

In the mid-1990s, investigators in India¹⁶ and Ecuador¹⁷ described an autosomal recessive syndrome similar to Naxos disease that was also

characterized by woolly hair and palmoplantar keratoderma, but individuals with this syndrome showed predominantly left-sided dilated cardiomyopathy ('Carvajal syndrome'). In the same year that *JUP* mutations were found in patients with Naxos disease, homozygosity mapping led to the discovery of a desmoplakin gene (*DSP*) mutation (c.7901delG) in three Ecuadorian families with Carvajal syndrome.¹⁸ This report was the second to link desmosome dysfunction with a syndrome that included manifestations of ARVD/C. Since this initial study, a number of novel mutations in *DSP*, both recessive and dominant, have been identified in individuals with a constellation of woolly hair, palmoplantar keratoderma and cardiomyopathy.^{19–21} Interestingly, other missense and nonsense mutations in the desmoplakin gene have been associated with isolated dominant ARVD/C or arrhythmogenic left ventricular cardiomyopathy in which affected patients had no hair or skin abnormalities.^{22–24} Furthermore other *DSP* mutations have been found to cause cutaneous abnormalities without signs of cardiomyopathy.^{25,26} *DSP* mutations are summarized in Figure 2B.

In vivo experiments examining the effects of desmoplakin disruption on heart physiology have provided insight into the pathogenesis of ARVD/C. Desmoplakin, a member of the plakin family, is expressed in all desmosomes, and is a cytoplasmic protein without a transmembrane domain that serves as an intracellular link between desmosomes and intermediate filaments.^{12,27} The desmoplakin mouse knockout (*Dsp*^{-/-}) dies shortly after embryonic implantation at embryonic day 6.5.²⁸ Rescue of desmoplakin expression in the extra-embryonic tissues prolongs the survival of *Dsp*^{-/-} mice to embryonic day 10, but these mice display pronounced defects in heart, epidermis and neuroepithelium development.²⁹ Cardiac-specific deletion of *Dsp* generates a mouse that exhibits several features of human ARVD/C.³⁰ By use of this model, a study found that heterozygous cardiac-tissue-specific *Dsp*^{+/-} mice were normal at birth but displayed a cardiac phenotype at 6 months that included fibrofatty replacement of the right and left ventricular myocardium, thin ventricular walls, impaired left ventricular ejection fraction and ventricular arrhythmias, and premature mortality of 20%.

Another study demonstrated that cardiac-specific overexpression of mutant, but not wild-type, desmoplakin in mice increased myocardial

apoptosis, fibrosis and adipose deposition as well as biventricular dilatation and dysfunction.³¹ Although human ARVD/C caused by aberrant *DSP* overexpression has not been documented, this mouse study indicates that perturbations in the precise ratios of desmosomal components could contribute to disease pathogenesis.

Plakophilin-2

The discovery of mutations in two functionally related genes, plakoglobin and desmoplakin, focused attention on the desmosome in ARVD/C pathogenesis. Plakophilin-2, an armadillo-family member that is expressed in the heart and interacts directly with plakoglobin and desmoplakin,¹² is essential in mice for proper heart morphogenesis and desmoplakin localization.³² Subsequent sequencing of the plakophilin-2 gene (*PKP2*) in 120 unrelated probands with ARVD/C revealed heterozygous mutations in 32 individuals (a prevalence of 27%).³³ Studies in several other cohorts confirmed that *PKP2* mutations in patients with ARVD/C are common, with a prevalence ranging from 11% to 43%.^{34–36} Although the vast majority of known mutations are heterozygous and result in missense, nonsense and frameshift mutations, a recessive mutation has been reported, which is notably not associated with hair or skin abnormalities.³⁷ These mutations are summarized in Figure 2C. The combination of several reported series in which *PKP2* mutation analysis was performed (*n* = 363) resulted in a *PKP2* mutation rate of 26% in unrelated ARVD/C patients.^{33–36,38}

The high prevalence of *PKP2* mutations has enabled statistical analyses of genotype-phenotype correlations in patients with ARVD/C as well as of disease penetrance in their relatives. A North American study showed that, in comparison with ARVD/C probands without a *PKP2* mutation, those with a *PKP2* mutation developed symptoms and arrhythmias at an earlier age, although there was no significant difference in implanted cardioverter-defibrillator firing rates.³⁴ By contrast, a report from The Netherlands showed no significant difference in age at initial presentation or incidence of sudden death among family members with and without a *PKP2* mutation.³⁶ Mutation carriers, however, were more likely than noncarriers to have T-wave inversions in the precordial leads on electrocardiography. Differences in these data could be a result of population variations or the small sample sizes in both studies.

Table 1 Arrhythmogenic right ventricular dysplasia/cardiomyopathy diagnostic criteria. To confirm diagnosis, individuals must fulfill two major criteria, or one major and two minor criteria, or four minor criteria, with each criterion coming from a different group.

Group	Major criteria	Minor criteria
Structural or functional RV abnormality	Severe RV dilation and reduction of RV ejection fraction with little or no LV involvement Localized RV aneurysm Severe segmental dilation of the right ventricle	Mild global RV dilation and/or ejection fraction reduction with normal LV Mild segmental dilation of the right ventricle Regional RV hypokinesia
Tissue characterization	Infiltration of RV myocardium by fibrofatty replacement tissue	No criteria listed
Electrocardiogram depolarization/conduction abnormality	Epsilon waves or localized prolongation (>110ms) of the QRS complex in right precordial leads (V1–V3)	Late potentials on signal-averaged electrocardiogram
Electrocardiogram repolarization abnormality	No criteria listed	Inverted T waves in electrocardiogram leads V1–V3, aged >12 years, without RBBB
Arrhythmias	No criteria listed	LBBB-type ventricular tachycardia (sustained or nonsustained) Frequent premature ventricular contractions (>1,000 per 24 h)
Family history	Family history of ARVD/C confirmed on autopsy or surgery	Family history of ARVD/C clinically and independently diagnosed Familial history of premature sudden death (<35 years) owing to suspected ARVD/C

Abbreviations: ARVD/C, arrhythmogenic right ventricular dysplasia/cardiomyopathy; LBBB, left bundle branch block; LV, left ventricular; RBBB, right bundle branch block; RV, right ventricular. Permission obtained from the BMJ Publishing Group © McKenna W *et al.* (1994) **71**: 215–218.

Among relatives of individuals previously diagnosed with ARVD/C, van Tintelen *et al.* found no *PKP2* mutations in 11 cases of isolated, nonfamilial ARVD/C. Of 23 patients with well-documented familial ARVD/C, however, 70% of these probands harbored *PKP2* mutations, emphasizing the importance of mutation screening, especially in familial ARVD/C.³⁶ Another study by Dalal and colleagues found that among the *PKP2* mutation carrying relatives, 49% met diagnostic Task Force Criteria for ARVD/C (Table 1).³⁹ Of the mutation carriers who did not satisfy diagnostic criteria, 50% met at least one criterion other than family history. Within families, phenotypic variability was high in individuals carrying the same mutation: some family members were completely asymptomatic in later life, whereas some had severe disease and died prematurely. This study also showed that penetrance of *PKP2* mutations was higher with increased age and male sex, with male mutation carriers more likely than female mutation carriers to have both structural and conduction abnormalities.³⁹ These data support previous findings among Italian and Western European populations that ARVD/C incidence is higher in men than women.^{40,41}

The high occurrence of *PKP2* mutations among individuals with ARVD/C probably relates to several factors. Although haplotype studies in Dutch patients with matching *PKP2*

mutations showed inheritance of a common allele, suggesting a founder effect,³⁶ microsatellite analysis in larger, more heterogeneous populations in North America³⁴ and Western Europe³³ confirmed inheritance of identical mutations in unrelated alleles. These recurrent mutations suggest that *PKP2* contains genomic regions that are inherently prone to alteration. Another possible reason for the raised frequency of *PKP2* mutations among different, unrelated families is the presence of the nearby plakophilin-2 pseudogene (*PKP2P1*) located on chromosome 12p13,⁴² which could induce *PKP2* gene conversion. When compared with the *PKP2* coding sequence, the *PKP2P1* sequence contains a 4-bp deletion corresponding to the c.145–148delCAGA mutation described by several independent groups.^{33–36} However, splice site mutations disrupting critically conserved intronic nucleotides are not likely to be caused by gene conversion, as *PKP2P1* is a processed pseudogene and, therefore, devoid of introns. An additional potential mechanism of recurrent mutations is C>T transition at CpG hotspots; indeed, at least four known C>T *PKP2* mutations occur at CpG dinucleotides (c.235C>T, c.1237C>T, c.1951C>T and c.2203C>T).

Desmoglein-2

Desmogleins are desmosomal cadherins, and together with the desmocollins are essential

transmembrane components of the desmosome.⁴³ There are four related members of the desmoglein family, each with a separate gene localized to chromosome 18p. Desmoglein-2, encoded by *DSG2*, is highly expressed in cardiac tissue, making it an attractive ARVD/C candidate gene. Sequence analysis led to recognition of *DSG2* as the fourth desmosomal gene associated with ARVD/C.^{44,45}

To date, the majority of *DSG2* mutations identified (summarized in Figure 2D) are extracellular missense mutations.^{44–46} The prevalence of *DSG2* mutations in published reports varies from 7–26%.^{44,45,47} Importantly, the populations analyzed in these studies also varied; some excluded individuals with recognized *PKP2* or *DSP* mutations,^{44,45} and one study included individuals with left ventricular or biventricular cardiomyopathy.⁴⁷ As such, these figures might not represent the true prevalence of *DSG2* mutations in cohorts of individuals with ARVD/C.

Individuals with *DSG2* mutations—including two probands with compound heterozygous *DSG2* mutations and consequently no normal desmoglein-2—seem to have isolated ARVD/C without skin or hair abnormalities.^{44,45} By contrast, complete loss of desmoglein-2 in mice is lethal.⁴⁸ Although abnormal desmoglein-2 expression has been linked with diffuse-type gastric cancer in humans,^{49,50} there have been no reports of gastric cancer in patients with ARVD/C carrying *DSG2* mutations.

Desmocollin-2

Desmocollin-2—another type I integral membrane cadherin found in desmosomes and similar to desmoglein-2—was the fifth and most recent major component of the cardiac desmosome to be implicated in ARVD/C.^{51,52} Mutations in the desmocollin-2 gene (*DSC2*) seem to be infrequent in ARVD/C; only five *DSC2* mutations have been described to date (summarized in Figure 2E).^{51–53} Although *Dsc2* targeting in the mouse has not yet been reported, zebrafish treated with *dsc2* antisense morpholino oligos demonstrated bradycardia, impaired contractility and chamber dilation.⁵¹

Non-desmosome gene mutations

Catecholaminergic polymorphic ventricular tachycardia resembles ARVD/C, though structural right ventricle disease is not typically seen in this disorder. Mutations in *RYR2*, encoding the cardiac ryanodine receptor, were first reported in

12 individuals with this disorder but no structural RV disease.⁵⁴ Revisiting previous linkage analysis that had shown an association between chromosome 1q42.1–q43 and a phenotype described as ARVD/C then identified mutations in *RYR2* in four families.⁵⁵ This particular subtype of ARVD/C had previously been characterized in extensive detail, with a notable paucity of structural RV disease, but with fibrofatty replacement of right ventricular myocytes on histopathologic examination.⁵⁶ Today, most consider catecholaminergic polymorphic ventricular tachycardia as a distinctly separate disorder from ARVD/C, though interpretation of the clinical criteria used to make these diagnoses can be ambiguous.

An isolated report describes novel variants in the untranslated regions of *TGFB3*, the gene encoding transforming growth factor β 3 in an individual with ARVD/C.⁵⁷ This finding raises interesting questions about the role of the pluripotent transforming growth factor β cytokine family in the pathogenesis of ARVD/C, although a direct causative role has not been proven. Notably, *in vitro* experiments using these noncoding variants demonstrated twofold increased expression of fusion constructs containing the noncoding variants compared with fusion constructs containing wild-type *TGFB3*; however, increased expression of *TGFB3* in patient-derived tissues was not found, downstream effectors of transforming growth factor β signaling were not assessed, and mutational analysis of known desmosomal ARVD/C genes was not reported.

Additional genomic loci

There are several early reports on linkage analysis in families segregating ARVD/C.^{58–61} Despite extensive investigation within or in close proximity to identified loci, related genes with mutations resulting in true ARVD/C have not been reported. One possible reason is that this disorder has both low penetrance and variable expressivity, complicating phenotypic assessment, which is critical for proper assignment of linkage. For example, a family initially reported to show cosegregation of the ARVD/C phenotype with chromosome 2q32.1–q32.3 was subsequently recognized to have a responsible mutation in *PKP2*, which localizes to chromosome 12p11.^{33,60} At least two family members were classified differently with regard to their phenotype in the later report.³³

A genomic locus on the short arm of chromosome 3 was initially reported in 1998 in a large

Newfoundland family segregating ARVD/C.⁵⁸ Subsequently, inclusion of additional individuals and restriction of analysis to only affected individuals refined the locus to 2 cM at chromosome 3p25 with a logarithm of odds score of 9.3.⁶² Though a specific mutation has not been identified, a haplotype involving this locus has been associated with sudden cardiac death, survival and response to treatment with an implantable cardioverter-defibrillator.⁶² Although eventual discovery of the responsible gene mutation will probably improve our understanding of ARVD/C pathogenesis, clinical assessment of the 3p25 haplotype in families is certainly justified.

THEORIES ON MECHANISM OF DISEASE

Several mechanisms have been proposed to explain the association between desmosome gene mutations and RV enlargement and dysfunction, and fibrofatty scar formation (Figure 1). The simplest is a purely structural model that proposes that the loss of myocyte adhesion results in cell death and regional fibrosis. Recent reports, including electron microscopy studies, describe ultrastructural abnormalities of the desmosome associated with desmosome gene mutations, which support this hypothesis.^{11,38} Focal RV scar would then result in the characteristic arrhythmia that typically accompanies ARVD/C. In this structural model, environmental factors such as exercise or inflammation from viral infection could exacerbate impaired adhesion and hasten disease progression. The right ventricle might have greater propensity to disease than the left because of its thinner walls and its normal dilatory response to exercise. The absence of left ventricular disease and the late-onset disease seen in some individuals with clear desmosome gene mutations are not, however, easily explained by this model.

A more complex model invokes the canonical Wnt/ β -catenin signaling pathway. Plakoglobin (γ -catenin), a protein with functional similarities to β -catenin, can localize both to the plasma membrane and the nucleus.⁶³ One recent study demonstrated that disruption of desmoplakin frees plakoglobin from the plasma membrane allowing it to translocate to the nucleus and suppress canonical Wnt/ β -catenin signaling.³⁰ Wnt signaling can inhibit adipogenesis by preventing mesodermal precursors from differentiating into adipocytes.⁶⁴ Suppression of Wnt signaling by plakoglobin nuclear localization could, therefore, promote the differentiation

of adipose tissue in the cardiac myocardium in patients with ARVD/C.³⁰

Apoptosis also seems to have a role in the myocardial cell loss seen in ARVD/C. TdT-mediated dUTP-biotin nick end-labeling (TUNEL) analysis of myocardial tissue from ARVD/C biopsies demonstrated increased DNA fragmentation that is characteristic of programmed cell death.⁶⁵ Other studies have found increased expression of the apoptotic genes *CPP32* (which encodes caspase 3) and *BAX* (which encodes BCL2-associated X protein) in ARVD/C samples but not in age-matched normal controls.^{66,67} Cell-line studies have shown that plakoglobin regulates expression of the antiapoptotic gene *BCL2* (which encodes B-cell CLL/lymphoma 2) and that the Wnt/ β -catenin signaling pathway modulates the apoptotic response in preadipocytes.^{68,69} Although cardiomyocyte apoptosis seems to be a consistent feature of ARVD/C, the contribution of programmed cell death in the progression and pathogenesis of ARVD/C remains unclear.

Elegant functional studies have begun to elucidate the links between mechanical cell junction machinery and electrical gap junction machinery, suggesting a mechanistic link between abnormal desmosomes and arrhythmias.⁷⁰ Interestingly, decreased expression of *PKP2* in cardiac cells disrupts the normal localization and conductivity of the gap junction protein connexin 43.⁷¹ Remodeling of the gap junction in response to an alteration or deficiency in elements of the cardiac desmosome could have a leading role in the genesis of arrhythmias.⁷²

Studies of the first-described dominant *JUP* mutation also shed light on possible mechanisms for ARVD/C pathogenesis.¹¹ This in-frame insertional mutation was shown in a yeast-two-hybrid screen to create a novel interaction between mutant plakoglobin and histidine-rich calcium-binding protein.¹¹ Although this predicted gain-of-function has not yet been confirmed in patient-derived tissues, these experiments indicate that plakoglobin can promote arrhythmias through aberrant calcium homeostasis mediated by histidine-rich calcium-binding protein.¹¹

CURRENT STATUS OF CLINICAL GENETIC ANALYSIS

Comprehensive exonic sequence analysis of the known desmosomal ARVD/C-related genes currently identifies a responsible mutation in approximately 50% of ARVD/C probands. Recognition of additional genes associated with

this condition, improved techniques for identifying large deletions or gene rearrangements, and lower cost sequence analysis should all improve the diagnostic yield of genetic testing in the future.

Patients and their physicians may seek clinical genetic testing for ARVD/C for several reasons. In our experience, the most common reason cited is identification of individuals related to someone with ARVD/C who may be at increased risk of sudden cardiac death or of developing the disorder. In such cases, the affected proband should be tested first and if a mutation is identified, at-risk family members can also seek testing. Genetic diagnosis before implantation is possible, but depends on the policies in the clinical laboratory performing the test. We discourage testing requested simply on the basis of curiosity or to confirm a diagnosis, as imaging and arrhythmia testing have greater diagnostic utility at this point. When the diagnostic criteria for this disorder are revised, inclusion of desmosome gene mutations in establishing this diagnosis might lead to increased ARVD/C genetic testing in the clinic.

Clinical genetic testing for ARVD/C is currently available in at least two laboratories in the US, both of which meet the Clinical Laboratory Improvement Amendments: the Johns Hopkins DNA Diagnostic Laboratory and the Harvard Laboratory for Molecular Medicine. Clinicians and patients who seek such testing should be aware of the possible outcomes, including the distinct possibility of finding a sequence variant of uncertain or unknown significance in one or more of these genes. Accordingly, genetic counseling is essential with such testing. Mutations resulting in insertions, deletions, frameshift, or premature termination are frequently found in this disorder, each of which dramatically alters the predicted protein. Even if they have not been previously reported, mutations may be more easily inferred as pathogenic by a clinical laboratory. Missense substitutions can be harder to interpret without extensive analysis in unaffected populations and functional assessment of these alterations (Figure 3). Clinical genetic testing laboratories are not typically able to perform such analyses.

In our experience, *PKP2* mutations are not typically found among individuals with sub-clinical manifestations of ARVD/C, but are far more common in those who satisfy the current clinical criteria.³⁴ As such, use of such clinical genetic testing in an individual who does not meet the diagnostic criteria for ARVD/C is

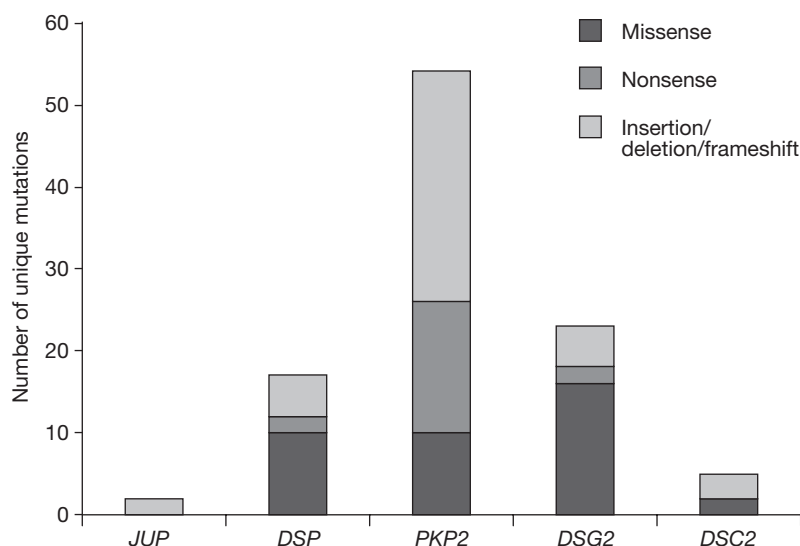


Figure 3 Tallies of the types of unique mutations found in each of the five desmosomal genes mutated in arrhythmogenic right ventricular dysplasia/cardiomyopathy. Shown are missense, nonsense, and insertion/deletion or frameshift mutations. Abbreviations: *DSC2*, desmocollin-2; *DSG2*, desmoglein-2; *DSP*, desmoplakin; *JUP*, junctional plakoglobin; *PKP2*, plakophilin-2.

unlikely to result in a clear diagnosis.³⁴ As the criteria used in the diagnosis of this condition evolve, genetic testing is likely to provide information that is additive to that provided by family history of ARVD/C.

Several reports have demonstrated probands and families with ARVD/C in whom more than one pathogenic mutation has been identified.^{37,44,45} This possibility must also be considered when advising a proband or family member about the likelihood of identifying those in the family who are at highest risk of developing ARVD/C. As previously noted, penetrance of ARVD/C is low and variable expressivity is widely seen.³⁹ Accordingly, identification of a genetic predisposition to ARVD/C should be viewed as only one factor contributing to ARVD/C, and does not independently lead to a diagnosis.

CONCLUSIONS

ARVD/C is a disorder of the cardiac desmosome. Recognition of several genes with mutations contributing to ARVD/C has improved our understanding of the pathogenesis of this condition, but importantly also provides an opportunity to effectively target screening within families. New insights derived from cellular and animal studies of the cardiac desmosome are anticipated to improve both diagnosis of and therapy for this condition in the future.

KEY POINTS

- Mutation in genes encoding any of the five major components of the cardiac desmosome—*PKP2* (encoding plakophilin-2), *DSG2* (encoding desmoglein-2), *DSP* (encoding desmoplakin), *DSC2* (encoding desmocollin-2), and *JUP* (encoding junctional plakoglobin)—can result in arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C)
- Approximately 50% of individuals with ARVD/C who have undergone full sequence analysis of these desmosome genes have a single heterozygous mutation identified, though a few cases of individuals with homozygous or compound heterozygous mutations have also been described
- ARVD/C segregates in families with both incomplete penetrance and variable expressivity; clinical screening of family members is recommended, particularly among those recognized to share a genetic predisposition to ARVD/C
- Owing to the age-dependent onset of ARVD/C, repeat clinical screening is recommended at 2- to 3-year intervals from the age of 12 years in the absence of a known mutation, to help target family members at highest risk; in families with earlier onset disease or sudden cardiac death in children, earlier clinical screening should be performed
- With the recent emergence of clinical genetic testing for ARVD/C, genetic counseling is strongly advised for individuals with ARVD/C and their family members

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Competing interests

The authors declared no competing interests.