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# Arrhythmogenic right ventricular cardiomyopathy: moving toward mechanism

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**Commentary**

Mutations in genes encoding desmosomal proteins have been identified as the major cause of arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVC), in which the right ventricle is “replaced” by fibrofatty tissue, resulting in lethal arrhythmias. In this issue of the *JCI*, Garcia-Gras et al. demonstrate that cardiac-specific loss of the desmosomal protein desmoplakin is sufficient to cause nuclear translocation of plakoglobin, upregulation of adipogenic genes in vitro, and a shift from a cardiomyocyte to an adipocyte cell fate in vivo (see the related article beginning on page 2012). This evidence for potential Wnt/β-catenin signaling defects sets the scene for a comprehensive exploration of the contributions of this pathway to the pathophysiology of ARVC, not only through perturbation of cardiac patterning and development, but also through effects on myocardial differentiation and physiology.



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# Arrhythmogenic right ventricular cardiomyopathy: moving toward mechanism

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**Mutations in genes encoding desmosomal proteins have been identified as the major cause of arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVC), in which the right ventricle is "replaced" by fibrofatty tissue, resulting in lethal arrhythmias. In this issue of the *JCI*, Garcia-Gras et al. demonstrate that cardiac-specific loss of the desmosomal protein desmoplakin is sufficient to cause nuclear translocation of plakoglobin, upregulation of adipogenic genes in vitro, and a shift from a cardiomyocyte to an adipocyte cell fate in vivo (see the related article beginning on page 2012). This evidence for potential Wnt/β-catenin signaling defects sets the scene for a comprehensive exploration of the contributions of this pathway to the pathophysiology of ARVC, not only through perturbation of cardiac patterning and development, but also through effects on myocardial differentiation and physiology.**

## Unique clinical features of ARVC

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVC) is a heart muscle disorder that predominantly affects the right ventricle and is associated with ventricular tachycardia, syncope, and sudden death (1). With the prevention of lethal arrhythmia, contractile failure is emerging as a major source of morbidity and mortality. At autopsy there is loss of myocardial mass with "replacement" by abnormal adipose and fibrous tissue; in essence a myocardial dystrophy. These features patchily involve the right ventricle and preferentially affect 3 areas: the apex, the inflow tract, and the outflow tract. This distribution may reflect a primary defect in morphogenesis. Although right ventricular disease predominates, the left ventricle is involved in about 50% of cases, and in some individuals there may be a global dilated cardiomyopathy (1).

## Human genetics

ARVC is typically inherited as a dominant Mendelian disease, although recessive variants exist and the involvement of family members often can only be detected by directed screening (1). Human genetic studies over the last few years have offered insight into the potential causes of ARVC. Early work demonstrated substantial genetic heterogeneity, and at least 9 independent loci have now been identified. The discovery of cutaneous and hair follicle involvement in recessive forms of ARVC led to the identification of mutations in the desmosomal proteins plakoglobin and desmoplakin (2, 3). These findings also implicated other desmosomal proteins or their partner proteins as candidate causes of the disorder (Table 1). Subsequent work has revealed desmoplakin mutations in a small proportion of dominantly inherited ARVC cases and in arrhythmogenic cardiomyopathy localized to the left ventricle (4–6). The description of mutations in the cardiac ryanodine receptor in families with an exercise-related arrhythmia known as catecholaminergic polymorphic ventricular tachycardia has highlighted phenotypic distinctions from typical ARVC (1).

In the last year, ARVC genetics have taken a significant step forward, led by the observation that mice null for the armadillo protein plakophilin 2 (PKP2), another desmosomal component, die at around E11 with profound cardiac abnormalities (7). These mice fail to form normal cardiac desmosomes, and desmoplakin dissociates from the abnormal junctions accumulating in cytoplasmic aggregates. These findings led in turn to the discovery of dominant mutations in the *PKP2* gene in a large proportion of probands with ARVC and not only established mutant desmosomal proteins as a major cause of the syndrome, but also raised the possibility of genetic testing as a diagnostic tool (8). The initial report in a series of 120 unselected European probands identified PKP2 mutations in almost one-third of these individuals (8). Recent data from more selected cohorts of index patients with evidence of familial involvement have suggested that as many as 70% of such kindreds may have mutations in PKP2 (9). Of note, these investigators also described evidence of founder effects for several PKP2 mutations in remote kindreds, implying less dramatic effects on survival than are seen in other forms of ARVC.

## Possible disease mechanisms

How do these mutant junctional proteins result in a unique, predominantly right ventricular cardiac phenotype? Desmosomal proteins are widely expressed, so the focal nature of apparent pathology in both

**Nonstandard abbreviations used:** ARVC, arrhythmogenic right ventricular dysplasia/cardiomyopathy; PKP2, plakophilin 2.

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**Table 1**
Human cardiac desmosomal diseases

| Disorder | OMIM no. | Genetic locus | Causative gene | Mode of inheritance | Comments | References |
|---|---|---|---|---|---|---|
| ARVC8 | 607450; 125647 | 6p24 | *Desmoplakin 1* | AD | Highly pleiotropic, occasionally involves LV cardiomyopathy | 4, 6 |
| Carvajal syndrome | 605676 | 6p24 | *Desmoplakin 1* | AR | LV involvement, aneurysm, palmoplantar keratoderma, and woolly hair | 3 |
| ARVC9 | 602861 | 12p11 | *PKP2* | AD | Present in 30–70% of cases | 8 |
| Naxos disease | 601214 | 17q21 | *Plakoglobin* | AR | Palmoplantar keratoderma and woolly hair | 2 |
| ARVC | 125671 | 18q12 | *Desmoglein 2* | AD | | 24 |

AD, autosomal dominant; AR, autosomal recessive; OMIM, Online Mendelian Inheritance in Man.

dominant and recessive ARVC led to initial speculation on the role of mechanical stresses (1, 10, 11). Impaired desmosome function under conditions of mechanical stress was proposed to predispose to cardiomyocyte detachment and death, with subsequent inflammatory reaction and fibrofatty replacement. However, consideration of the distribution of skin lesions in recessive variants infers that this mechanism alone is unlikely to be responsible. Several areas of the body subject to substantial physical stresses are not involved, while the hair follicles are uniformly affected. In addition, the prominent adipose replacement suggests not scarring and healing, but rather a more fundamental perturbation of primary tissue architecture.

Three different types of intercellular junction are distinguished at the cardiac intercalated disc: (a) adherens junctions, which anchor actin filaments; (b) desmosomes, which anchor intermediate filaments; and (c) gap junctions, which mediate ion transfer (Figure 1). Cardiac myocytes rely on these specialized structures for both mechanical and electrical

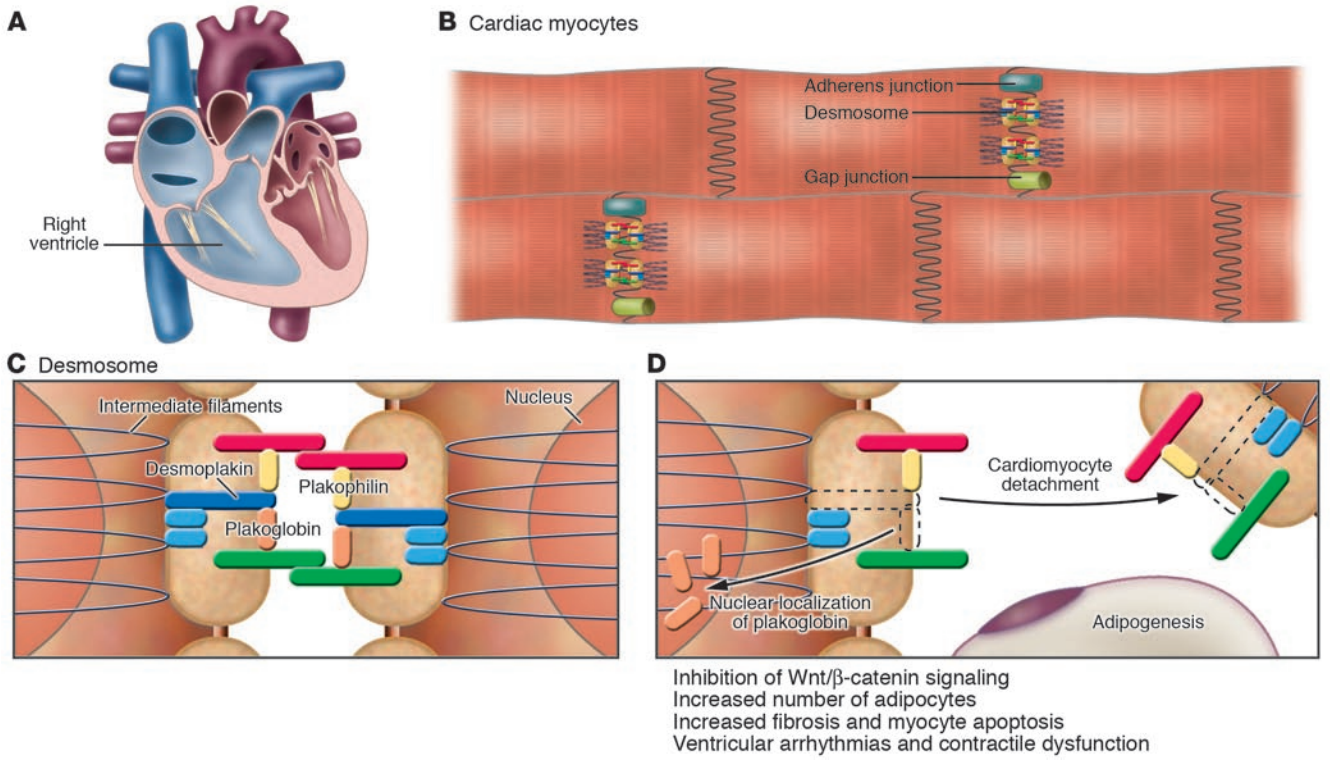


**Figure 1**
Cardiac-specific restriction of the desmosomal protein desmoplakin causes nuclear localization of plakoglobin and reduced Wnt/β-catenin signaling, recapitulating human ARVC. (**A**) ARVC predominantly affects the right ventricle of the heart. (**B**) The intercalated discs of cardiac myocytes are characterized by gap junctions, adherens junctions, and desmosomes. (**C**) Cell-cell adhesion is largely dependent on interaction of intracellular components of the desmosomal plaque such as desmoplakin and plakoglobin. (**D**) In this issue of the *JCI*, Garcia-Gras et al. (16) report that heterozygous cardiac desmoplakin-deficient mice show nuclear localization of plakoglobin and reduced Wnt/β-catenin signaling. This causes increased expression of adipogenic and fibrogenic genes in vitro, abnormal cardiac adipose tissue and fibrosis in vivo, and ventricular arrhythmias similar to human ARVC. Interactions between signaling defects and mechanical stresses are likely to be involved in the genesis of the final phenotype.

coupling of the myocardial syncytium (12). Desmosomes may protect other junctions from mechanical stress, but they also have been implicated in the structural organization of the intercalated disc. Desmosome-dependent orchestration of local membrane and cytoplasmic domains may be critical for many of the physiologic functions of the intercalated disc (12). For example, the destabilization of cell adhesion complexes may perturb the kinetics of gap junction turnover, resulting in heterogeneous conduction, a potential contributor to arrhythmogenesis in ARVC (13, 14).

Desmosomes also participate in intercellular signaling networks, of which the Wnt/β-catenin pathway is the most extensively studied (15). In the archetypal pathway the cytoplasmic concentration of β-catenin is exquisitely regulated by multiple inputs, including secreted ligands of the Wnt family and recruitment of β-catenin to intercellular junctional complexes. Cytoplasmic accumulation of β-catenin leads to its nuclear translocation, association with the T cell factor/lymphoid enhancer factor (Tcf/Lef) family of transcription factors and subsequent changes in gene expression. This evolutionarily conserved pathway plays a central role in many of the most fundamental cellular behaviors and has been directly implicated in the regulation of cell fate, proliferation, and apoptosis. Importantly, the various pathway components are duplicated in higher organisms, and specific isoforms may even be employed serially for discrete functions at different times and at different sites (15). In addition, superimposed on the basic structure of the Wnt/β-catenin signaling network are many subtle feedback loops and points of cross-talk that are only beginning to be uncovered (15).

In this context, in this issue of the *JCI* Garcia-Gras et al. explored the effects of cardiac-restricted desmoplakin deficiency on canonical Wnt/β-catenin signaling (16). Using Cre recombinase driven by an α–myosin heavy chain gene promoter with a floxed desmoplakin allele, desmoplakin expression was eliminated in the heart. Homozygous cardiac deletion of desmoplakin results in substantial embryonic lethality, with evidence of growth arrest at around E11 and cardiac abnormalities reminiscent of the phenotypes seen with germline desmoplakin-null or PKP2-null mice (7, 17). Approximately 5% of cardiac null desmoplakin homozygotes survive to gestation, but these mice die within 6 weeks. Mice heterozygous for the conditional null allele develop age-dependent multichamber cardiac enlargement and dystrophic myocardium with many of the hallmarks of ARVC, including disorganized myocytes and areas of fibrous and adipose tissue. The authors also convincingly demonstrate — using siRNA in HL-1 cells — that inhibition of desmoplakin expression is sufficient to cause nuclear translocation of plakoglobin and upregulation of genes implicated in adipogenesis and generation of new collagen. Increased cardiomyocyte nuclear localization of plakoglobin and ventricular arrhythmias was also observed in heterozygous mice (Figure 1). These results infer that desmoplakin deficiency results in inhibition of canonical Wnt/β-catenin signaling and a consequent shift away from a myocyte fate and toward an adipocyte fate in the heart. These data support previous evidence of competition between plakoglobin and β-catenin and demonstrate that disruption of the Wnt/β-catenin axis is sufficient to push cell fate toward adipogenesis, even in myocardium (15, 18). Clearly more work will be required to reconcile these data with the proposed mechanisms in ARVC families resulting from mutations in plakoglobin itself (13).

## Multiple mechanisms

It remains to be seen how mechanistically faithful this model will prove to be for human ARVC. Human desmoplakin-associated cardiomyopathy results from germline mutations (3–6), and the effects of germline desmoplakin knockout suggest either that the known disease alleles are unlikely to be true nulls or that the mouse is not a perfect model of human disease (17). The relatively late excision of the floxed allele in relation to cardiogenesis and the restriction of the resultant null allele to the heart preclude an assessment of the effects of perturbed Wnt/β-catenin signaling on earlier developmental events that may be central to the pathogenesis of ARVC. Wnt signaling from the neural tube inhibits cardiogenesis in anterior adaxial mesoderm (19), while regional inhibition of Wnt activity is necessary for the normal induction of cardiogenesis in the anterior lateral mesoderm (20, 21). Inhibition of Wnt signaling in the posterior mesoderm is sufficient to induce ectopic cardiac tissue, and conditional knockout of β-catenin in the endoderm results in cardiac duplication in the midline (22). Once the initial heart tube is patterned, restriction of endocardial β-catenin signaling is critical for normal valve formation (23). In addition to these and other possible roles for the Wnt/β-catenin pathway, a direct effect of plakoglobin signaling is also a potential explanation for the observations outlined in the current work (16). The successful dissection of the pathobiology of ARVC will depend on understanding the role of human desmoplakin disease alleles in each of these developmental steps as well as in myocyte proliferation and differentiation and in postnatal physiology.

Garcia-Gras et al. (16) have established a potential role for signaling defects in ARVC, setting the scene for a comprehensive exploration of the interplay of cell adhesion proteins not only as passive players in myocardial architecture, but as key regulators in cardiac patterning and development, in myocyte differentiation, and in the ongoing maintenance of the cellular architecture of the adult heart. Superimposed mechanical stresses may well play a major role in ARVC (14), but the pathogenesis of this disorder is likely to reflect the unique molecular networks responsible for the development and maintenance of the right ventricle. Mouse modeling has taught us that human disease is often the result not of true null alleles, but rather of subtle hypomorphic or gain-of-function effects precisely perturbing complex intersecting pathways. Ultimately, understanding the many roles of the Wnt/β-catenin pathway in the biology of ARVC will require the generation of a series of tissue-specific conditional null alleles and the recapitulation of disease alleles.

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# Neuropeptide signaling and hydrocephalus: SCO with the flow

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**Congenital hydrocephalus affects 0.1–0.3% of live births, with a high mortality rate (~50%) in the absence of surgical intervention. Although the insertion of shunts alleviates the symptoms of the majority of congenital cases, the molecular basis of hydrocephalus and the mechanisms of cerebrospinal fluid (CSF) circulation remain largely unknown. Two important players are the subcommissural organ/Reissner's fiber (SCO/RF) complex and the ventricular ependymal (vel) cells that together facilitate the flow of the CSF through the narrow canals of the ventricular system. In this issue of the *JCI*, Lang et al. demonstrate that overexpression of the pituitary adenylate cyclase–activating polypeptide (PACAP) type I (*PAC1*) receptor gene results in abnormal development of the SCO and vel cells, leading to congenital hydrocephalus (see the related article beginning on page 1924). The ligand for the PAC1 receptor is the neuropeptide PACAP, which uncovers what the authors believe to be a novel role for this signaling cascade in the regulation of CSF circulation.**

Hydrocephalus arises from an accumulation of cerebrospinal fluid (CSF), most frequently due to an impairment of CSF flow within either the ventricular system (noncommunicating hydrocephalus) or the subarachnoid space (communicating hydrocephalus). The CSF is secreted from the choroid plexus, and movement through the ventricular system occurs in a rostrocaudal direction, from the lateral ventricles to the third ventricle via the foramen of Munro, then through the Sylvian aqueduct to the fourth ventricle, and finally into the cisterna magna of the subarachnoid space and the central canal of the spinal cord (Figure 1). Ultimately, CSF fluid is removed through the arachnoid villi into the venous circulation.

Noncommunicating hydrocephalus results from an obstruction of the ventricular system and has many causes, including viral infection, tumors, hemorrhage, and developmental defects (1). Obstruction usually occurs in the narrowed segments of the ventricular system, typically the cerebral aqueduct. Indeed, stenosis of the cerebral aqueduct is considered the primary cause of congenital hydrocephalus (1, 2). Several factors play a role in the maintenance of CSF flow through the narrow canals, including ciliary movement on ependymal cells and a functioning subcommissural organ (SCO), an ependymal gland located in the dorsocaudal region of the third ventricle at the entrance of the Sylvian aqueduct (Figure 2) (3, 4). It is well established that the SCO secretes glycoproteins that aggregate and form a long, threadlike structure known as Reissner's fiber (RF) in most vertebrate species (Figure 2). RF elongates and extends through

**Nonstandard abbreviations used:** CREB, cAMP response element–binding protein; CSF, cerebrospinal fluid; IFT, intraflagellar transport; PAC1, PACAP type I; PACAP, pituitary adenylate cyclase–activating polypeptide; RF, Reissner's fiber; SCO, subcommissural organ; vel, ventricular ependymal.

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