Arrhythmogenic Cardiomyopathy
Transgenic Animal Models Provide Novel Insights Into Disease Pathobiology

Kalliopi Pilichou, PhD; Connie R. Bezzina, PhD; Gaetano Thiene, MD; Cristina Basso, MD, PhD

Arrhythmogenic right ventricular cardiomyopathy (ARVC) (Online Mendelian Inheritance in Man 107970) is a clinically and genetically heterogeneous heart muscle disorder associated with an increased risk of sudden cardiac death, particularly in young persons and athletes.1–3 The pathological hallmark of ARVC consists of progressive (either diffuse or segmental) loss of cardiac myocytes that are replaced by fibrofatty tissue, leading to electric instability with or without impaired mechanical function.4–6 Right ventricular (RV) aneurysms in the so-called “triangle of dysplasia” are considered a pathogenic feature of the disease. With an estimated prevalence of the disease of ≈1:2500 to 1:5000, ARVC can be listed among the rare cardiovascular disorders.6

The first comprehensive clinical description of ARVC was reported in 1982 by Marcus et al7 in adults with ventricular tachyarrhythmias of left bundle branch block (LBBB) morphology. In 1994, an international task force provided standardized criteria to establish the diagnosis of ARVC,8 and these diagnostic criteria have been updated recently to increase sensitivity but with the important requisite of maintaining specificity.9 Onset of clinical symptoms and signs usually occurs in adolescence or young adulthood10 and often is triggered by effort.11 Main clinical features of the disease comprise arrhythmias of RV origin that are commonly associated with syncope or sudden cardiac death, thus underscoring the crucial role of risk stratification to identify patients who require an implantable cardioverter-defibrillator.12

In 1988, a familial background consistent with an autosomal-dominant trait was described in ≈50% of patients with ARVC.13 To date, human genetics studies have identified 12 independent loci and 8 disease genes for this disorder inherited mostly as autosomal-dominant traits with incomplete penetrance and variable expressivity.2,3 Five of the 8 causative genes encode major components of the cardiac desmosomes, namely plakoglobin (JUP), desmoplakin (DSP), plakophilin-2 (PKP2), desmoglein-2 (DSG2), and desmocollin-2 (DSC2) (Table 1). Up to 50% of ARVC probands harbor a mutation in 1 of these genes.2,23 ARVC mutations display wide variability in the severity of phenotype, even among family members sharing the same gene variation, thus suggesting that additional genetic or environmental modifiers can influence the disease phenotype. Moreover, compound heterozygosity has been implicated to explain the wide variability and penetrance in familial ARVC.24,25 Despite the major step forward in human genetics studies, which have led to consideration of ARVC as a disease of the desmosome, early molecular and cellular events underlying the processes of cardiomyocyte injury, fibrous and fatty tissue repair, ventricular remodeling, and arrhythmias remain to be elucidated. The present review focuses on in vivo transgenic animal models of ARVC with the aim of providing an update on disease pathobiology.

Pathogenesis of ARVC
The evolution in disease terminology reflects the major advances with time in the understanding of the disease etiopathogenesis. Several theories have been put forward, including the maldevelopmental (congenital dysplasia, aplasia, or hypoplasia), inflammatory (myocarditis), and degenerative (myocardial dystrophy) ones.4,26

According to the now-abandoned concept of a congenital abnormality, ARVC was viewed as a heart defect present at birth in which the RV myocardium failed to develop during embryonic life. Confusion in the literature about ARVC has been created by the frequent misuse of the term Uhls anomaly (an almost total absence of the myocardium of the RV),4,26 which is characterized differently from ARVC by lack of family history; heart failure and infrequency of arrhythmias as clinical picture; and a significantly earlier age of presentation, usually in childhood.

As far as the inflammatory theory is concerned, it has been debated often whether the inflammatory cells observed in the myocardium of affected patients are a reaction to cardiomyocyte death or, rather, the consequence of infective or immune mechanisms.4 Cardiotropic viruses have been reported in the myocardium of some patients with ARVC, and they have been proposed as possible etiologic agents supporting an infective pathogenesis.27 However, the viral agent might be just an innocent bystander or play a secondary, but still...
Table 1. Desmosomal Genes in Arrhythmogenic Right Ventricular Cardiomyopathy

<table>
<thead>
<tr>
<th>Author (Ref)</th>
<th>Gene</th>
<th>Protein</th>
<th>Chromosome Locus</th>
<th>OMIM</th>
<th>Mode of Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coonar et al,14 McKoy et al15</td>
<td>JUP</td>
<td>Plakoglobin</td>
<td>17q21</td>
<td>#601214</td>
<td>AR*</td>
</tr>
<tr>
<td>Asimaki et al22</td>
<td>DSP</td>
<td>Desmoplakin</td>
<td>6p24</td>
<td>#607450</td>
<td>AD</td>
</tr>
<tr>
<td>Rampazzo et al16</td>
<td>PKP2</td>
<td>Plakophilin-2</td>
<td>12p11</td>
<td>#609040</td>
<td>AD</td>
</tr>
<tr>
<td>Norgett et al17</td>
<td>DSG2</td>
<td>Desmoglein-2</td>
<td>18q12</td>
<td>#601913</td>
<td>AD</td>
</tr>
<tr>
<td>Gerull et al19</td>
<td>DSP</td>
<td>Desmoplakin</td>
<td>6q24</td>
<td>#607450</td>
<td>AD</td>
</tr>
<tr>
<td>Pilichou et al20</td>
<td>DSC2</td>
<td>Desmocollin-2</td>
<td>18q12</td>
<td>#60476</td>
<td>AD</td>
</tr>
<tr>
<td>Syrris et al21</td>
<td>JUP</td>
<td>Plakoglobin</td>
<td>17q21</td>
<td>#601214</td>
<td>AR*</td>
</tr>
</tbody>
</table>

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

*Naxos disease.
†Carvajal syndrome.

important role.28 According to the latter hypothesis, the genetically dystrophic myocardium could favor viral settlement (superimposed myocarditis), leading to progression or the precipitation of the disease phenotype. Even a transdifferentiation theory has been put forward to explain the fibrofatty phenomenon, suggesting that cardiomyocytes transform into fibrocytes, adipocytes, or both, but this theory is questionable because of the limited de-differentiation capabilities of adult cardiomyocytes.29

The most attractive etiopathogenetic theory remains the dystrophic one (termed myocardial dystrophy).4 It stems from the high similarities of the histopathologic features observed in ARVC and in skeletal muscle dystrophies (Duchenne or Becker), which are characterized by progressive and acquired muscular atrophy with replacement by exuberant fatty and fibrous tissue (so-called pseudohypertrophy). Cell death either by apoptosis or necrosis can account for progressive loss of the ventricular myocardium.30 The dystrophic theory was further supported by the heredofamilial background of ARVC, which eventually lead to the identification of the causative mutations in genes encoding for desmosomal proteins. Thus, according to the widely accepted “defective desmosome” hypothesis, genetically determined disruption of desmosomal integrity and function is the key factor leading to cell death and the development of ARVC. These major advances at the bench have evolved into the current perspective of ARVC as a genetically determined myocardial disease, which now is listed among cardiomyopathies.31

Desmosomes mediate cell-cell adhesion through 3 families of proteins, namely the armadillo (eg, JUP, PKPs), cadherins (eg, DSCs, DSGs), and plakins (eg, DSP). These complex networks of proteins primarily are found in tissues subjected to mechanical stress, such as the epidermis and the heart.32 Mutations in desmosomal genes with recessive and dominant patterns of inheritance are associated with cutaneous disease, cardiac disease, or both,15–22 thus demonstrating the important role in epithelial and cardiac structure and function. Moreover, it is becoming increasingly clear that in addition to structural functions, the components of the cell adhesion apparatus possess intracellular signaling capabilities.

The current hypotheses on ARVC pathogenesis are illustrated in Figure 1. However, many questions still remain unanswered. Are signaling pathways involved in disease onset and progression, and if so, which ones? Can age, exercise, or inflammation exacerbate the phenotype? What is the relative role of apoptosis and necrosis in the ongoing cardiomyocyte death? Why does the disease always start from the epicardium with the distinct outer-inner layer “wavefront” progression? What is the mechanistic link among abnormal desmosomes, gap junctions, and arrhythmias? Where does fibrous and fatty tissue repair come from? Although human genetics studies have been successful in identifying disease-causing genes, they are not helpful in answering these questions because of intrinsic limitations such as the various environmental stimuli (ie, exercise, inflammation), the different genetic background, the difficulty in obtaining human cardiac samples, and the small number of individuals carrying the same mutation. Therefore, transgenic animal models have been proposed by the biomedical research community to gain insight into disease pathobiology. Knockin, knockout, and overexpression animal models that mimic the human ARVC phenotype are valuable resources for the understanding of the molecular and cellular mechanisms because of the unique ability to control both the environmental influences and the genetic background.

Desmosome-Related Transgenic Mice

Desmoplakin

DSP is a large coiled-coil protein that has the capacity to associate with itself, with other desmosomal components (JUP, PKP), and with intermediate filaments, forming an ordered array of nontransmembrane proteins that binds the intermediate filaments to the desmosomal cadherins.33 The DSP, exclusive and major desmosomal component, was the first disease gene associated with the autosomal-dominant ARVC phenotype,16 although DSP mutations previously had been described in an autosomal-recessive disease showing cardiocutaneous abnormalities and predominantly left-sided cardiomyopathy (Carvajal syndrome).17,18,34

Up to now, 3 mouse lines have been established for the DSP gene. The first described is a knockout DSP mouse model showing high incidence of mortality shortly after embryonic implantation at E6.5, thus highlighting the important role of this molecule in desmosome assembly, stabilization, and tissue integrity.35 Partial rescue of desmoplakin expression in the extraembryonic tissues promoted survival of the DSP−/− null embryos through gastrulation (E10), but
these mice displayed pronounced defects in heart, epidermis, and neuroepithelium development.36

More recently, Garcia-Gras et al37 engineered cardiac-restricted DSP+/− mice exhibiting a high incidence of early embryonic lethality. The postnatal DSP+/− surviving mice died shortly after birth (2 to 6 weeks of age). Histological examination on DSP+/− embryos showed poorly formed hearts with no chamber specification. In contrast, the cardiac restricted DSP+/− mice developed normally during the embryonic life, exhibiting an age-dependent penetrance of the cardiac phenotype, including a 20% incidence of premature death within 6 months after birth. Echocardiography and gross pathological analysis demonstrated an enlarged RV and left ventricle (LV). Abnormal echocardiographic findings of the LV included wall thinning, increased end-diastolic and end-systolic dimensions, and depressed systolic function with reduced ejection fraction. Of note, histology in DSP+/− and DSP+/− mice showed poorly organized myocytes with large areas of patchy fibrosis, and oil red O staining showed excess accumulation of fat droplets, predominantly at the site of fibrosis.

In addition, the authors showed that JUP, released from the bonds that aggregate this molecule to the desmosomes, translocates to the nucleus in this DSP-deficient model.37 At the nuclear level, JUP acts as a competitor of β-catenin, an effector of the canonical Wnt signaling, with a negative effect on this pathway. They also showed that expression levels of genes targeted by the Wnt/β-catenin pathway (c-myc, cyclinD1) were reduced, whereas the expression of adipogenic genes (PPARγ, adiponectin, C/EBP-α) was increased. Apoptosis, as detected by TUNEL-positive cells, was significantly increased. On the basis of these observations, they suggested that suppression of DSP led to nuclear translocation of JUP and suppression of canonical Wnt/β-catenin signaling pathway. The latter, a key regulator of transcriptional switch from myogenesis to adipogenesis, leads to typical human ARVC features, such as enhanced adipogenesis, fibrogenesis, and myocyte apoptosis. More recently, the same group analyzed both mouse and human hearts and found that adipocytes in ARVC originate from the second heart field progenitor cells, which switch to adipogenesis because of suppressed canonical Wnt/β-catenin signaling due to the JUP nuclear translocation.38 Identification of the second heart field as the cell source of adipocytes in ARVC would offer a plausible explanation for the predominant involvement of the RV in ARVC. However, we should emphasize that the second heart field gives origin to the bulbus cordis and the pulmonary infundibulum, which correspond to the whole RV (ie, inlet, apical, and outlet).39 On the contrary, the subepicardial progenitor cells spread into the RV and LV and are responsible for generating the myocardium and interstitial cells.40 Because ARVC is now considered a biventricular disease both in experimental animal models and in human patients, it is our opinion that subepicardial progenitor cells more than the second heart field play a role in disease pathogenesis and particularly in the process of fibrofatty replacement, which is considered archetypal of this cardiomyopathy.
An alternative approach was used by Yang et al\textsuperscript{31} in the same year. Although multiple attempts to generate cardiac-restricted transgenic mice expressing N-terminal DSP mutants failed because of abnormal cardiac development, they succeeded in developing a transgenic mouse overexpressing a cardiac-restricted C-terminal DSP mutation (R2834H) using the heart-specific α-myosin heavy chain promoter. Reduced cardiac function of both ventricles was evident by 6 months of age. Increased cardiomyocyte apoptosis and cardiac fibrosis with neutral lipid accumulation were visible. The mutant mice displayed aberrant intermediate (desmin) filament localization at intercalated discs. Interruption of DSP-desmin interactions might lead to desmosome instability with reduced resistance to mechanical stress as supported by the ultrastructural evidence of intercalated disc remodeling with widened gaps. This in turn leads to abnormal localization of other cell-cell adhesion molecules and changes in gap junction components. Overall, the authors concluded that DSP is essential for maintaining desmosome stability and cardiac tissue integrity, pointing out that DSP abnormalities can result in ARVC as a result of cardiomyocyte death, changes in lipid metabolism, and defects in cardiac development.

**Plakophilin-2**

PKP2, an armadillo-repeat protein, is a juxtamembranous constituent of desmosomal plaques and an essential stabilizing partner of desmosomal cadherins. Heterozygous mutations of PKP2 gene were first described in 2004 by Gerull et al\textsuperscript{19} in 27% of unrelated patients with ARVC and are now considered the most common in different patient series. Similar to DSP knockout mice, PKP2 knockout mice exhibit lethal alterations in heart morphogenesis and stability at midgestation (E10.5 to E11) characterized by reduced trabeculation, disarrayed cytoskeleton, and ruptured cardiac walls with blood leakage into the pericardial cavity.\textsuperscript{42} In the absence of PKP2, the cytoskeletal linker protein DSP dissociates from the plaques of the adhering junctions that connect the cardiomyocytes and forms granular aggregates in the cytoplasm. By contrast, embryonic epithelia show normal junctions. Thus, PKP2 is important for the assembly of junctional proteins and represents an essential morphogenic factor and architectural component of the heart.\textsuperscript{42}

**Plakoglobin**

JUP, a member of the armadillo family of proteins, is a constitutive component of plaques associated with the 2 major distinct types of intercellular junctions: adherens junctions and desmosomes.\textsuperscript{33} JUP exists in a regulated equilibrium between a diffusible cytosolic form and a plaque-assembled form, which is bound to specific domains of certain desmosomal cadherins (DSCGs and DSCs). The presence of plakoglobin in desmosomes as well as in adherens junctions suggests an important role in regulating cross-talk between these 2 junctions.

In 2002, McKoy et al\textsuperscript{15} identified a homozygous, 2-base pair deletion in the JUP gene in 19 individuals affected by an autosomal-recessive ARVC form associated with palmoplantar keratoderma and woolly hair (Naxos disease). This deletion caused a frameshift and premature termination of the protein. The authors hypothesized that desmosomal abnormalities could trigger structural myocardial and epidermal tissue changes as a consequence of loss of adhesiveness, particularly under mechanical stress. More recently, Asimaki et al\textsuperscript{22} reported a dominant mutation in the JUP-encoding gene in a German family with ARVC and no obvious cutaneous abnormalities. Analysis of a biopsy sample of the RV from the proband showed markedly decreased localization of JUP, DSP, and connexin43 at intercalated discs in cardiac myocytes.

Ruiz and coworkers\textsuperscript{43} in 1996 introduced a targeted null mutation of the JUP gene in mice by using homologous recombination and embryonic stem cell technology. They found JUP to be an essential component of cardiac but not epithelial desmosomes, and its absence led to embryonic death between days 12 and 16 of development because of defects in heart structure, stability, and function. In the hearts of mice lacking JUP, desmosomes were no longer detected, and the remaining junctional structures were drastically altered, resulting in different distribution of DSP and DSG.

At the same time, Bierkamp and coworkers\textsuperscript{44,45} described another JUP null mouse model with some contradictory findings. Although JUP null mutant embryos died at embryonic day 10.5 because of severe heart defects, some mutant embryos developed further and died around birth because of cardiac dysfunction and with skin blistering and subcorneal acantholysis. Ultrastructural analysis revealed that desmosomes were reduced in number and structurally altered both in the skin and in the heart. Both knockout mouse models underline the importance of JUP for cardiac desmosome assembly and function but disagree about its role in the epithelial desmosomes.

In 2006, Kirchhof and coworkers\textsuperscript{46} studied heterozygous JUP-deficient (+/−) mice, showing that depletion of a JUP allele could alter the RV contractility and electrophysiological function without affecting myocardial structure, desmosome integrity, or connexin43 expression. Specifically, by 6 months of age, the JUP+/− mice displayed RV dilatation and dysfunction as well as ventricular arrhythmias. These phenotypes were exacerbated by daily swimming, supporting the knowledge that endurance training could accelerate disease progression among persons with ARVC. However, histological analysis of myocardium from JUP+/− mice did not show any cardiomyocyte abnormality or fibrofatty replacement, and electron microscopy studies revealed no structural changes in the desmosomes or adherens junctions. More recently, the same group demonstrated that a load-reducing therapy (furosemide and nitrates) prevents training-induced (daily swimming) development of ARVC in JUP-deficient (+/−) mice.\textsuperscript{47}

Finally, a conditional cardiac restricted JUP−/− mouse exhibited a phenotype closer to human ARVC,\textsuperscript{48} with altered desmosomal ultrastructure and diminished signal of desmosomal proteins at the intercalated disks. Although this knockout mouse showed many histopathologic features of human ARVC, it did not recapitulate the arrhythmic phenotype. However, the investigators demonstrated that loss of JUP leads to stabilization of β-catenin, with increasing β-catenin/
TCF transcriptional activity, which may contribute to ARVC pathogenesis.

Desmoglein-2

DSG2 is a Ca\(^{2+}\)/H\(^{+}\)-dependent adhesion molecule present in all desmosome-bearing tissues from their earlier appearance onward. This transmembrane glycoprotein interacts with other members of the desmosomal cadherin subfamily on the surface of the same cell in a cis-orientation and between adjacent cells in a trans-orientation, mediating a highly ordered interdigitation along the plane of the membrane in desmosomal junctions.\(^{32}\) Mutations in the DSG2 gene were first associated in 2006 with 10% of unrelated ARVC patients in northern Italy.\(^{20}\)

The first mouse model was described in 2002 by Eshkind and colleagues,\(^{49}\) with germline inactivation of DSG2 by homologous recombination in embryonic stem cells. DSG2\(^{-/-}\) mice and a considerable number of DSG2\(^{+/+}\) mice died at or shortly after implantation (E3.5 to E5.5), showing that DSG2 is necessary for embryonic stem cell viability and proliferation. Heterozygous lethality was independent of whether the mutated allele was inherited from the male or female parent. By immunofluorescence analysis in DSG2-negative blastocysts, they demonstrated markedly decreased expression level of the junctional proteins (DSP and PKP2), whereas ß-catenin and E-cadherin were normally distributed. However, no morphopathological analysis was performed in the DSG2\(^{-/-}\) mouse hearts or skin.

Recently, our group developed a transgenic mouse model overexpressing the mouse homologue of the human mutation N266S identified in an Italian family.\(^{50}\) Overexpression of a dominant-negative form of DSG2 specifically in the heart resulted in a biventricular cardiomyopathy with aneurysms, ventricular arrhythmias, and sudden death, thus recapitulating the human ARVC phenotype (Figure 2). Thirty percent of mice expressing the mutant N271S DSG2 died by 3.6 weeks, and only 20% survived by the age of 20 weeks. Both gross morphology examination and histological analysis of the heart revealed progressive structural abnormalities. In particular, hearts were grossly and histologically normal by 2 weeks of age but then showed pronounced myocardial damage first in the outer subepicardial layers and later transmurally moving toward the endocardium, starting with contraction bands and coagulative necrosis, massive neutrophil infiltrates, and then calcification (granulation tissue repair with fibroblasts and loose connective tissue). Dense fibrosis with collagen bundle deposition leading to thinning, aneurysm formation, and cavity dilatation in both ventricles appeared at the later stages. This age-related phenotype was further supported by echocardiography and electrophysiological abnormalities. Electron microscopy showed ultrastructural features in keeping with cardiomyocyte necrosis (ie, disruption of the sarcolemma, disgregation of myofilaments, and mitochondrial swelling). Mitochondria showed multiple amorphous densities as well as electron-dense calcific deposits and even complete mineralization preceding calcification of cardiomyocytes. Disruption of the sarcolemma was confirmed in vivo by Evans blue dye uptake. Very recently, Krusche et al generated a homozygous DSG2 mutant mouse lacking 2 adhesive extracellular DSG2 domains (DSG2\(^{mut/-}\)).\(^{51}\) Different from our overexpression model,\(^{50}\) this homozygous mutant mouse with extensive myocardial fibrosis and calcification allows, for the first time, the study of the consequences of a mutated endogenous desmosomal protein, highlighting the acquired aspect of the disease.\(^{51}\)

Clinicopathological Findings in ARVC Transgenic Mice

Table 2 summarizes the main clinicopathological findings of the currently available transgenic mice of ARVC, which are either overexpression models carrying dominant-negative
Table 2. Clinicopathological Features of Arrhythmogenic Right Ventricular Cardiomyopathy in Transgenic Mice

<table>
<thead>
<tr>
<th>Mouse Model</th>
<th>Cardiac-Restricted</th>
<th>Cardiac-Restricted</th>
<th>Conditional</th>
<th>Cardiac-Restricted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DSP+/-; Tg-R2834H41</td>
<td>JUP+/-; Tg-R2834H41</td>
<td>JUP+/-; Tg-N271S50</td>
<td>DSG2+/+; Tg-N271S50</td>
</tr>
<tr>
<td>Endurance training</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Premature death</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ventricular arrhythmias</td>
<td>+ (induced)</td>
<td>+ (induced)</td>
<td>+ (induced)</td>
<td>+ (induced)</td>
</tr>
<tr>
<td>QRS prolongation</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Prolonged RV activation times</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Prolonged LV activation times</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>RV dilatation/ reduced EF</td>
<td>NA</td>
<td>+ (trained)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>LV dilatation/ reduced EF</td>
<td>+ (trained)</td>
<td>+ (trained)</td>
<td>+ (trained)</td>
<td>+ (trained)</td>
</tr>
<tr>
<td>Increased heart weight</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>RV aneurysms</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LV aneurysms</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fatty tissue</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lipid droplets</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Necrosis</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Calcification</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Myocyte hypertrophy</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Abnormal desmosomes</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Abnormal CX43</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

CX43 indicates connexin43; EF, ejection fraction; LV, left ventricle; NA, not available; RV, right ventricle; Tg, transgenic.

Desmosome mutations (DSP, DSG2), knockout heterozygous models mimicking haploinsufficiency (DSP, JUP) or homozygous DSG2 mutant mice. With the exception of the heterozygous JUP+/- model showing structurally normal hearts, questioning whether this experimental mouse really should be considered a model of ARVC, all the models are characterized by a biventricular cardiomyopathy with increased heart weight, RV and LV enlargement, and wall thinning. These ARVC transgenic mice recapitulate human ARVC, as recently proven by the comparison of the clinicopathological features between the human carrier who underwent cardiac transplantation and the DSG2 mouse model with the homologous mutation. The concept that ARVC no longer is considered primarily a right-sided disease is not unique to mice because in recent years, both pathology and magnetic resonance investigation has demonstrated a high rate of LV involvement, so the use of the more comprehensive term arrhythmogenic cardiomyopathy has been advanced.

In mice, histology of the myocardium consistently shows myocyte loss with almost exclusive fibrous tissue repair, whereas fatty tissue is not a typical feature of murine ARVC. Only focal neutral lipid accumulation in areas of ongoing myocyte death was evident, suggesting alteration of lipid metabolism. The almost total absence of adipose tissue in ARVC transgenic mice might be due to differences between species, considering that the normal mouse heart does not show physiological epicardial fat compared with the human heart where there is always a certain amount of fatty tissue in the subepicardium. According to Lombardi et al., the identification of the second heart field as the cell source of adipocytes in ARVC would offer a plausible explanation for the predominant involvement of the RV in ARVC. However, we must recognize that their as well as other experimental ARVC animal models always showed a biventricular involvement with prevalent, if not exclusive, fibrous repair. It is our personal opinion that the presence of fatty tissue is either species related (humans versus mice) or ventricle related (right versus left). Moreover, as experimental animal models suggest, it seems that the faster and more extensive the process of myocardial necrosis, the greater the amount of fibrous tissue compared with fatty tissue. As far as molecular events are concerned, the pathways investigated so far include the one involving JUP in intracellular signaling and gene expression regulation in cardiac-restricted DSP+/- and conditional cardiac-restricted knockout JUP mice. Accordingly, suppression of DSP levels results in nuclear translocation of JUP where it competes with β-catenin with suppression of the canonical Wnt/β-catenin signaling pathway, leading to increased expression of genes involved in adipogenesis, fibrogenesis, and apoptosis. The antagonistic role of JUP in the β-catenin-mediated signaling also was investigated in the cardiac-restricted knockout JUP mouse, even though the researchers concentrated on the pathways involved in pathological hypertrophy rather than on the cardiomyocyte death and fibrogenesis. More recently, in the homozygous DSG2 mutant mouse disease progression correlated with increased mRNA expression of c-myc, ANF, BNF,
CTGF and GDF15, which are markers for cardiac stress, remodeling and heart failure.51 From the cellular point of view, apoptosis has been proven to participate in the process of myocyte loss in all ARVC mouse models, although with a variable amount. Contradictory results are found with regard to the structure of intercalated discs.37,41,46,48,50 In fact, only DSP-R2834H overexpressing mice showed disruption of intercalated discs and reduced colocalization of DSP and desmin at the desmosome level, pointing to desmosomal instability as the trigger for cardiomyocyte damage. However, the preliminary findings from these transgenic mouse models, although not homogeneous, highlight that desmosomes not only are adhesive structures among adjacent cells, but also may influence morphogenetic processes due to signaling capabilities in addition to structural and mechanical impairment.

To catch the cellular events involved in the various phases of disease onset and progression, a step-by-step morphopathological investigation recently was undertaken in DSG2 mutation overexpression mice.50 Thus, to our knowledge, the investigation experimentally proved for the first time that myocyte necrosis is the first cellular event initiating progressive myocardial dystrophy in DSG2-related ARVC (Figure 3).

The fact that necrosis is not often reported in both autopsy and endomyocardial biopsy studies of human ARVC likely relates to its focal distribution and possibly episodic nature. Of note, human disease onset and progression sometimes is characterized by the onset of chest pain, with electrocardiographic abnormalities and myocardial enzymes release mimicking acute myocardial infarction, suggesting underlying cardiomyocyte necrosis.2,4,52 Whether necrosis and sarclemmma disintegration are due to cytoskeletal impairment or abnormal intracellular homeostasis remains to be elucidated. Molecular pathways involved in cell necrosis currently are under investigation.

Finally, ARVC mouse models are mostly characterized by ventricular arrhythmias, conduction disturbances, and premature death. In particular, in the DSG2 mouse model, we recently demonstrated that sudden death occurs not only in the advanced stages of the disease, but also at young age when there is yet no morphological evidence of the chronic fibrous repair with aneurysm formation but only of the acute-subacute damage with myocyte necrosis and inflammation.50 This again reproduces accurately the clinical course of human ARVC, where cardiac arrest can manifest at every stage along the natural history. Ventricular tachycardia and fibrillation usually are the mechanisms of sudden death in the hot phase of disease progression likely because of acute myocyte death and reactive inflammation. On the contrary, older patients with a long-lasting ARVC more often experience scar-related ventricular tachycardia.

Recently, it has been hypothesized that intercalated disc remodeling in ARVC can affect gap junctions besides desmosomes.51–58 Reduction in connexin43 protein expression and gap junction remodeling might contribute to conduction slowing, thereby predisposing to electric instability, even well before myocyte damage. However, this hypothesis has not yet been proven, either in humans or in animal models, and will need further assessment.

Figure 3. Stepwise onset and progression of arrhythmogenic right ventricular cardiomyopathy in transgenic mice. Shown are schematic representations of a transverse section of the heart with normal structure (A), early subepicardial myocardial injury (B), transmural myocardial injury (C), and late fibrous repair with wall thinning and aneurysm formation (D). E through H, Trichrome staining on transverse histological sections. I through N, haematoxylin-eosin staining on transverse histological sections (Magnification ×12).
Conclusions
Unraveling the molecular and cellular events underlying the onset and progression of myocardial dystrophy in ARVC is crucial to translate scientific knowledge from bench to bedside, with the final aim of developing target therapy for preventing disease onset and progression. In particular, we need to understand why cardiomyocytes start to spontaneously die. The various disease mechanisms described by different investigators are likely not mutually exclusive but can all contribute to ARVC pathogenesis. Knockin ARVC mouse models, which more closely resemble the human disease, are becoming available for investigation to better understand the cascade of pathobiological events, leading from the gene defects to the morphofunctional disease phenotype.

Sources of Funding
Fondazione Cassa di Risparmio di Padova e Rovigo, Padova, Italy; Telethon, Rome GGP09293; and Veneto Region, Venice, Italy.

Disclosures
None.

References


Arrhythmogenic Cardiomyopathy: Transgenic Animal Models Provide Novel Insights Into Disease Pathobiology
Kalliopi Pilichou, Connie R. Bezzina, Gaetano Thieme and Cristina Basso

Circ Cardiovasc Genet. 2011;4:318-326
doi: 10.1161/CIRCGENETICS.110.959031
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 1942-325X. Online ISSN: 1942-3268

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circgenetics.ahajournals.org/content/4/3/318

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

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

Subscriptions: Information about subscribing to Circulation: Cardiovascular Genetics is online at:
http://circgenetics.ahajournals.org//subscriptions/