



Published in final edited form as:

*Cell Commun Adhes.* 2014 February ; 21(1): 13–23. doi:10.3109/15419061.2013.876016.

## Remodeling of Cell-Cell Junctions in Arrhythmogenic Cardiomyopathy

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### Abstract

Arrhythmogenic cardiomyopathy (AC) is a primary myocardial disorder characterized by a high incidence of ventricular arrhythmias often preceding the onset of ventricular remodeling and dysfunction. Approximately 50% of patients diagnosed with AC have one or more mutations in genes encoding desmosomal proteins, although non-desmosomal genes have also been associated with the disease. Increasing evidence implicates remodeling of intercalated disk proteins reflecting abnormal responses to mechanical load and aberrant cell signaling pathways in the pathogenesis of AC. This review summarizes recent advances in understanding disease mechanisms in AC that have come from studies of human myocardium and experimental models.

### Introduction

Arrhythmogenic cardiomyopathy is a primary myocardial disease characterized by ventricular arrhythmias and sudden cardiac death.<sup>1</sup> Originally described as a right ventricular disease (ARVC), it is now recognized to include left ventricular and biventricular forms which are often misdiagnosed as dilated cardiomyopathy or myocarditis. In light of this broader phenotypic spectrum, the term arrhythmogenic cardiomyopathy (AC) has been adopted.<sup>2</sup>

AC has a prevalence of 1:1000 to 1:5000 in the general population, but it accounts for 11–22% of sudden cardiac deaths among young athletes.<sup>3</sup> It is the major cause of sudden death among athletes in Northern Italy<sup>4</sup> and accounts for 17% of sudden cardiac deaths in young people (< 35 years) in the United States.<sup>5</sup> AC is a familial disease in at least 50% of cases and is usually inherited as an autosomal dominant trait.<sup>6</sup> The overall prevalence may be underestimated because wide phenotypic variation, age-related progression and low genetic penetrance may obscure diagnosis.<sup>6</sup> Currently, the diagnosis of AC rests upon fulfilling a complex set of criteria established by an International Task Force, which although relatively specific are not highly sensitive.<sup>7</sup>

AC is a highly arrhythmogenic disease. Arrhythmias usually arise as the first manifestation of disease, and typically precede structural remodeling of the myocardium.<sup>8</sup> This so-called “concealed” phase is unique among the non-ischemic cardiomyopathies. In hypertrophic

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cardiomyopathy, for example, arrhythmic risk appears to be related at least in part to the underlying substrate of myocyte disarray, hypertrophy, fibrosis and small vessel disease. In dilated cardiomyopathy, arrhythmias occur in the context of significant left ventricular remodeling and contractile dysfunction. By contrast, there is something fundamentally arrhythmogenic about early AC, in which frequent arrhythmias occur in otherwise apparently normal hearts.<sup>2</sup> As the disease progresses, degenerative changes in cardiac myocytes associated with inflammation and accumulation of fibrofatty scar tissue become more prominent. Thus, AC exhibits features of both the inherited arrhythmia syndromes such as long QT and the non-ischemic cardiomyopathies characterized by complex myocardial pathology.<sup>2</sup>

## Genetics of AC

Autosomal dominant inheritance in AC was first described in 1987 in a report on eight Italian families.<sup>9</sup> The first genetic locus linked to AC was identified at 14q23-q24 in 1994 after evaluation of a large Venetian family.<sup>10</sup> It was not until 1998, however, that analysis of patients from the Greek island of Naxos led to identification of the first causative gene mutation in AC.<sup>11</sup> So-called Naxos disease is a highly penetrant recessive syndrome characterized by the clinical triad of ARVC, woolly hair and keratoderma involving pressure areas of the palms and soles. The cutaneous phenotype is expressed from infancy, thereby unequivocally identifying affected individuals and ensuring accurate linkage analysis. The cardiac symptoms characteristically develop from adolescence to early adulthood although arrhythmias have been documented in young children.<sup>11</sup> The disease allele was mapped to 17q21 and shown to involve a homozygous two-base-pair deletion in the gene encoding the desmosomal protein plakoglobin ( $\gamma$ -catenin).<sup>12</sup> This first association of a desmosomal gene mutation with AC paved the way for identification of disease-causing mutations in other desmosomal genes.

A mutation in the desmoplakin gene, *DSP*, resulting in truncation of the C-terminal domain was subsequently implicated in another recessive cardio-cutaneous syndrome described in families from Ecuador.<sup>13</sup> So-called Carvajal syndrome consists of palmoplantar keratoderma, woolly hair and a biventricular cardiomyopathy that exhibits clinical features of dilated cardiomyopathy.<sup>14</sup> Clinical and pathological characterization of Carvajal syndrome is limited, but frequent and complex ventricular arrhythmias have been documented in pre-adolescence.<sup>14</sup> Pathological features include biventricular dilatation with focal aneurysms, and myocyte degeneration and replacement fibrosis (albeit without adipose tissue) preferentially affecting sub-epicardial and mid-myocardial layers while sparing the sub-endocardium.<sup>15</sup> Focal ventricular aneurysms and sub-epicardial/mid-myocardial prominence are typical features of the myocardial pathology of AC.

The first dominantly inherited mutation in *DSP* was identified in 2002 in an ARVC family of Italian descent. This mutation (S299R) was predicted to modify a putative phosphorylation site in the N-terminal domain of desmoplakin and interfere with the protein's binding to plakoglobin.<sup>16</sup> Three additional dominant mutations in *DSP* were reported in 2005 (R1775I, R1255K and c.423-1G>A) in ARVC families characterized by a high occurrence of sudden cardiac death and prominent left ventricular involvement.<sup>17</sup> Soon

after, another mutation was identified (2034insA) in a family showing predominant left ventricular disease, giving rise to the term “arrhythmogenic left ventricular cardiomyopathy”.<sup>18</sup> Yang et al. reported 4 additional variants (V30M, Q90R, W233X, and R2834H) also linked to biventricular forms of the disease.<sup>19</sup>

Once the focus of the gene hunt was directed to the desmosome, mutations in AC families were discovered in genes encoding plakophilin-2 (PKP2), desmoglein-2 (DSG2) and desmocollin-2 (DSC2).<sup>20</sup> *PKP2* appears to be the most commonly mutated gene in ARVC. In the Netherlands, for example, *PKP2* mutations may account for up to 70% of index cases.<sup>21</sup> Prudence, however, must be applied in distinguishing truly pathogenic mutations from background genetic noise, especially in the case of missense mutations. A recent study on a large series of ARVC patients showed that 21% of patients carry missense mutations in desmosomal genes versus a remarkably high 16% of controls.<sup>22</sup> In some cases, a single sequence variant may be insufficient to cause AC by itself but may contribute to disease expression when combined with another sequence variant in the same gene (compound heterozygosity) or in another gene (digenic heterozygosity).<sup>23</sup>

Mutations in non-desmosomal genes have also been associated with AC, the most convincing of which include those encoding transmembrane protein 43 (TMEM43)<sup>24</sup> and phospholamban (PLN).<sup>25</sup> The S358L mutation in *TMEM43*, first identified in a large founder population in Newfoundland, causes a fully penetrant non-classic form of the disease associated with a high incidence of premature death and heart failure in survivors.<sup>24</sup> The *PLN* mutation R14del, first described in Greek families with dilated cardiomyopathy and heart failure,<sup>26</sup> was recently identified in a substantial number of Danish patients clinically diagnosed with ARVC or dilated cardiomyopathy phenotypes, further supporting a broader phenotypic spectrum for AC.<sup>25</sup>

Other extra-desmosomal genes have been associated with AC including those encoding desmin,<sup>27</sup> transforming growth factor beta-3<sup>28</sup> and the cardiac ryanodine receptor,<sup>29</sup> although the latter appears to be more closely linked to catecholaminergic polymorphic ventricular tachycardia.<sup>30</sup> It is currently possible to identify mutations in one or more of the 5 major desmosomal genes in ~60% of probands who fulfill International Task Force criteria for AC. Presumably, other disease alleles yet to be described may be responsible for at least some of the remaining patients with AC.

## More than a disease of abnormal cell-cell adhesion

Since being recognized as a non-ischemic cardiomyopathy by the World Health Organization in 1994,<sup>31</sup> AC has become the focus of active investigations of its molecular pathophysiology and disease mechanisms. While there has been significant progress in identifying AC-causing mutations, much less is known about how the mutant proteins actually cause the disease. One leading hypothesis is that abnormal cell-cell adhesion injures the cardiac myocytes and promotes cell death with subsequent replacement by fibrofatty tissue.<sup>32</sup> Such a mechanism almost certainly plays a role. However, desmosomal proteins may fulfill dual roles as structural proteins in adhesion junctions and as signaling molecules, which can alter signaling pathways and thereby modulate pathological gene expression,

promote cardiac myocyte apoptosis and perhaps mediate expression of a fibrogenic and/or an adipogenic phenotype.<sup>33</sup> Moreover, mononuclear (predominantly T cell) inflammatory infiltrates may be especially prominent in the hearts of AC patients, occurring in nearly 80% in some postmortem series.<sup>34</sup> The sub-endocardium is commonly affected, suggesting that the inflammatory response may reflect extension of the disease to previously unaffected regions of the myocardium. Active disease progression associated with intense inflammation could play a role in the development of “hot phases”, episodic exacerbations following an interval of clinically quiescent disease.<sup>2</sup> However, this remains entirely speculative as no definitive experimental evidence exists to link inflammation in AC to disease pathogenesis and/or progression.

Although desmosomes occur in virtually all solid organs, clinical phenotypes related to desmosomal gene mutations appear to be limited to the heart and skin, tissues that experience the greatest mechanical load.<sup>33</sup> Young competitive athletes with AC have a 5.4-fold relative risk of sudden death compared to non-athletes.<sup>35</sup> Strenuous physical activity has been associated with an increased penetrance and acceleration of the disease phenotype.<sup>36</sup> The right ventricular apex, an area preferentially affected in ARVC, exhibits heterogeneous fiber orientation and elevated incremental strains, potentially creating a mechanically weak spot in the heart.<sup>37</sup> Finally, electron microscopy studies of patient myocardial samples have reported decreased numbers of desmosomes and apparent clefts or widening of various components within the intercalated disks.<sup>38</sup> All of these observations suggest that mechanical stress plays an important role in disease progression and sudden death. However, it should be emphasized that, currently, there is no direct evidence of weakened cell-cell adhesion in the hearts of patients with AC. Thus, despite the interesting potential associations between inflammation and exercise and AC disease pathogenesis, we really have a very limited understanding of the molecular mechanisms responsible for arrhythmias and myocyte injury in AC. Advances in understanding such mechanisms might not only aid in development of preventive measures in AC, but perhaps reveal fundamental new paradigms in sudden death occurring in other, more common forms of heart disease.

### **Disease mechanisms in AC: What have we learned from human studies?**

To gain insights into mechanisms of AC pathogenesis, we have analyzed patient myocardial samples in an effort to discover features of the molecular pathology of this disease spectrum. The great majority of the world-wide human myocardial tissue archive for AC consists of formalin-fixed, paraffin-embedded samples obtained by endomyocardial biopsy or at autopsy. While this limits the repertoire of analytical approaches that may be applied, we have learned a great deal about AC by studying the distribution of intercalated disk proteins using immunohistochemistry.

We first determined whether mutant proteins are expressed in AC and if so whether they are localized at cell-cell junctions or are re-distributed to other (intracellular) compartments. We also asked whether a mutation in a single desmosomal protein could affect the localization of other (non-mutant) desmosomal proteins.<sup>32</sup> Because defects in intercellular mechanical coupling are generally associated with abnormal cell-cell communication via gap junctions,<sup>39</sup> we also determined whether mutations in desmosomal proteins were associated

with changes in the distribution of connexins, proteins that form electrical junctions in the myocardium. Initial studies in myocardial samples from patients with Naxos disease showed that the truncated mutant form of plakoglobin was abundantly expressed in both ventricles but it failed to localize properly at intercalated disks (Fig. 1).<sup>40</sup> A similar analysis of the heart in Carvajal syndrome showed that not only the mutant protein, desmoplakin, failed to localize at cell-cell junctions, but so did its non-mutant binding partner plakoglobin (Fig 1).<sup>15</sup> Thus, a mutation in a single desmosomal protein can affect the localization of other proteins that are not genetically altered.<sup>32</sup> We also observed a marked reduction in the amount of immunoreactive signal for Cx43, the major ventricular gap junction protein, in both Naxos and Carvajal myocardium (Fig 1).<sup>15, 39</sup>

We have subsequently analyzed myocardium from a large number of patients with clinically and/or pathologically documented ARVC related to mutations in one or more of the 5 desmosomal protein genes and involving autosomal dominant inheritance in most cases.<sup>41</sup> We observed that immunoreactive signal for plakoglobin was reduced at cell-cell junctions in the great majority of cases, independent of the underlying pathogenic mutation. It also occurred in cases that fulfilled diagnostic criteria but in which no desmosomal gene mutation could be identified by genetic screening. This consistent finding was associated with decreased amounts of immunoreactive signal for desmoplakin or plakophilin-2 in some cases while in others the signal appeared normal (Fig. 2). Reduced junctional plakoglobin signal occurred diffusely in both ventricles including areas that appeared histologically normal.<sup>41</sup> A similar reduction was not seen in myocardial samples from patients with end-stage heart failure due to hypertrophic, dilated or ischemic cardiomyopathies<sup>41</sup> although subsequent studies have shown that junctional plakoglobin signal is diminished at cell-cell junctions in myocardium from patients with sarcoidosis or giant cell myocarditis, both highly arrhythmogenic forms of granulomatous myocarditis.<sup>42</sup> Taken together, these observations suggest that redistribution of plakoglobin is a consistent feature in AC and raise the possibility of common arrhythmogenic mechanisms in AC and some forms of granulomatous myocarditis.<sup>41,42</sup> Furthermore, as discussed below, redistribution of plakoglobin from junctional to intracellular and/or intranuclear sights may be responsible for modulating Wnt signaling pathways which experimental studies have implicated in AC disease pathogenesis.<sup>43</sup>

An extensive literature exists on the dependence of cell-cell electrical coupling at gap junctions on normal mechanical coupling by intercellular adhesion junctions.<sup>39</sup> Such dependency is probably of particular importance in the myocardium in which extremely large gap junctions, presumably required for safe impulse propagation, are surrounded by extensive points of cell-cell adhesion within intercalated disks.<sup>39</sup> Initial observations of diminished Cx43 immunoreactive signal at cell-cell junctions in Naxos disease<sup>39</sup> and Carvajal syndrome<sup>15</sup> (Fig. 1) have now been confirmed in additional cases of autosomal dominant ARVC associated with mutations in various desmosomal proteins (Fig. 2).<sup>44</sup> Gap junction remodeling in AC occurs diffusely in the myocardium and has been shown in at least one case of Naxos disease to precede development of myocardial degeneration and remodeling.<sup>45</sup> These observations suggest that gap junction remodeling is a consistent feature of AC and raise the possibility that it may contribute to abnormal tissue electrophysiology during the concealed phase of the disease in which arrhythmias occur in

the absence of structural changes.<sup>46</sup> It should be emphasized, however, that thus far no studies have been reported on functional consequences of the apparent gap junction remodeling seen by immunohistochemistry. Thus, the contributions, if any, of gap junction remodeling to arrhythmogenesis in AC remain a matter of speculation.

Very little is known about the molecular pathology of AC caused by mutations in non-desmosomal genes. The TMEM43 protein contains 4 trans-membrane segments and a large hydrophilic domain that is remarkably conserved among species. It is widely expressed, particularly in the placenta, and in most tissues localizes at the inner nuclear membrane and endoplasmic reticulum where it has been shown to interact with emerin and lamins.<sup>47</sup> Immunoreactive signal for plakoglobin was found to be depressed at intercalated in myocardial samples from 3 ARVC patients with *TMEM43* mutations, whereas PKP2, Cx43 and emerin appeared to be normally distributed.<sup>48</sup> The implications of these observations are uncertain but they add weight to the notion that redistribution of plakoglobin from junctional to intracellular and/or intranuclear sites may be an important component of the underlying disease pathway in AC.

Phospholamban regulates the sarcoplasmic reticulum  $\text{Ca}^{+2}$  pump (SERCA2a) in cardiac muscle and is thus important for maintaining  $\text{Ca}^{+2}$  homeostasis.<sup>49</sup> Three different *PLN* mutations have been linked to a dilated cardiomyopathy phenotype associated with malignant ventricular arrhythmias and interstitial fibrosis.<sup>50–52</sup> Given the phenotypic overlap between *PLN* mutation carriers diagnosed with dilated cardiomyopathy and classical ARVC, a large population of patients diagnosed with both disease entities was screened for mutations in this gene.<sup>25</sup> A 3-base pair deletion (R14del) was identified in 39 (15%) dilated cardiomyopathy and 12 (12%) ARVC index patients, none of whom had mutations in desmosomal genes. Interestingly, samples from *PLN* R14del carriers diagnosed with ARVC showed reduced immunoreactive signal for plakoglobin at cardiac IDs in the majority of cases (5 of 7). By contrast, only 1 of 9 R14del carriers diagnosed with dilated cardiomyopathy showed evidence of plakoglobin re-distribution (Fig. 3).<sup>25</sup> These observations suggest that diminished plakoglobin signal at intercalated disks may track with the ARVC phenotype rather than genotype.

As detailed in the next section, new information is emerging from experimental models that expression of mutations linked to AC is associated with reduced  $\text{Na}^{+}$  current density.<sup>53</sup> Although the responsible mechanisms are not known, this observation could have important implications for understanding arrhythmogenesis in AC. Reduced  $I_{\text{Na}}$  has not been documented in patients although reduced signal for Nav1.5 at intercalated disks has been shown by immunohistochemistry in myocardium from patients with AC.<sup>54</sup> Taken together with observations of gap junction remodeling, this raises the possibility that decreased  $I_{\text{Na}}$  combined with reduced electrical coupling could affect impulse propagation and thereby contribute to arrhythmias early in the disease process.<sup>55</sup> This is a promising area of investigation.



## Disease mechanisms in AC: What have we learned from experimental models?

Perhaps the most important insight to emerge from experimental models of AC is the potential role of altered Wnt signaling in disease pathogenesis.<sup>43</sup> Like its homolog  $\beta$ -catenin, plakoglobin ( $\gamma$ -catenin) functions as both an adhesion junction protein and a nuclear signaling molecule modulating Wnt pathways.<sup>56</sup> The N-terminus plays a key role in determining stability and degradation of armadillo proteins. This region appears to be a target for N- and O-glycosylation<sup>57</sup> and is also involved in the ubiquitin-proteasomal degradation pathway.<sup>58</sup> Wnt pathways are involved in fundamental biological processes including cardiac development and differentiation, hypertrophy, cell-cell adhesion, cytoskeletal re-arrangement and calcium homeostasis.<sup>59</sup> Under basal conditions, cytoplasmic plakoglobin undergoes phosphorylation at N-terminal serine residues by glycogen synthase kinase  $3\beta$  (GSK- $3\beta$ ) which targets it for ubiquitylation and proteasomal degradation. Activation of the canonical Wnt signaling pathway inhibits GSK- $3\beta$  which allows plakoglobin to accumulate within the cell and enter the nucleus where it may interact with Tcf/Lef transcription factors and alter gene expression. The precise role of plakoglobin once it enters the nucleus is still incompletely understood but the prevailing view is that nuclear localization of plakoglobin suppresses canonical Wnt signaling and its downstream targets through Tcf/Lef1 transcription factors.<sup>56</sup> In this context, Garcia-Gras et al. showed that siRNA-mediated suppression of desmoplakin expression in an atrial cell line promotes nuclear accumulation of plakoglobin associated with a 2-fold reduction in canonical Wnt signaling.<sup>43</sup> The resulting phenotype also included increased expression of adipogenic and fibrogenic genes and intracellular accumulation of fat droplets.<sup>43</sup> This important study was the first to implicate abnormal Wnt signaling in the pathogenesis of AC. In subsequent studies, these authors provided evidence that suppression of the canonical Wnt pathway in progenitor cells in the heart causes a switch to an adipogenic instead of a myogenic fate which could explain the often prominent accumulation of mature adipocytes in areas of myocyte damage in AC.<sup>60</sup>

Transgenic mice with cardiac-specific expression of the V30M or Q90R N-terminal desmoplakin mutations are embryonic lethal.<sup>19</sup> On the contrary, transgenic mice with cardiac-specific expression of a C-terminal desmoplakin mutation (R2834H) are viable. These animals show increased cardiac myocyte apoptosis, fibrosis and lipid accumulation as well as biventricular enlargement and dysfunction.<sup>19</sup> Lyon et al. used the ventricular myosin light chain-2-Cre system to ablate desmoplakin specifically from cardiac myocytes, a mouse model designated as DSP-cKO.<sup>61</sup> Following gene deletion, animals developed a dramatic phenotype characterized by biventricular dilatation, extensive myocyte loss with fibrosis and ultrastructural abnormalities at intercalated disks. DSP-cKO mice also exhibited ventricular arrhythmias that were exacerbated in response to exercise and catecholamine stimulation. Finally, the mice showed RV conduction defects and abnormal electrical wavefront propagation, presumably associated with reduction in expression of the gap junction proteins Cx40 and Cx43.<sup>61</sup> While this mouse model exhibits some major features reminiscent of biventricular AC in patients with severe desmoplakin mutations, it should be remembered that germline deletion of desmosomal proteins including plakoglobin,<sup>62</sup> desmoplakin<sup>63</sup> and

plakophilin-2<sup>64</sup> are all embryonic lethal in mice. Furthermore, the best available evidence indicates that mutant desmosomal proteins are expressed in various forms of AC in patients suggesting that disease pathogenesis may be mediated by dominant-negative effects rather than by null alleles.

The idea that exercise exacerbates the disease phenotype in AC noted above in the DSP-cKO model has also been investigated in mice with heterozygous deletion of the plakoglobin gene.<sup>65</sup> By 10 months of age, plakoglobin<sup>+/-</sup> mice show enlarged right ventricles, but endurance training (8 weeks of daily swimming) caused premature right ventricular dilatation and dysfunction at 5 to 6 months of age.<sup>65</sup>

Transgenic mice with cardiac-specific over-expression of an ARVC-causing mutant form of *DSG2* (N271S) recapitulate clinical features of the disease including premature death, spontaneous ventricular arrhythmias, cardiac dysfunction and biventricular dilatation and aneurysms.<sup>66</sup> Hearts of mice heterozygous for a *PKP2*-null allele show mild ultrastructural changes but no histological or gross anatomical abnormalities.<sup>67</sup> However, ventricular myocytes exhibit changes in  $I_{Na}$  and the  $Na^+$  channel blocker flecainide provoked ventricular arrhythmias and death in *PKP2*-deficient animals but not in controls.<sup>67</sup>

While clinical experience has suggested that exercise increases disease expression in patients with AC and disease phenotypes are intensified in some mouse models subjected to exercise, relatively little is known about the responsible molecular mechanisms. We have shown that HEK293 cells expressing two different mutations in plakoglobin (2057del2 which causes Naxos disease, or S39\_K40insS which causes a dominant form of ARVC without associated cutaneous abnormalities) show abnormal responses to short-term cyclical stretch.<sup>68</sup> Whereas normal cells increase the amount immunoreactive signal for plakoglobin and Cx43 at cell-cell junctions after even brief intervals of stretch, these responses are significantly blunted in cells expressing mutant desmosomal proteins.<sup>68</sup> It has also been shown that knockdown of *PKP2* expression in neonatal rat ventricular myocytes in vitro reduces cell-cell adhesion strength.<sup>69</sup> These studies must be viewed with caution, however. Responses in HEK cells may not be the same as in cardiac myocytes<sup>68</sup> and, as emphasized previously, phenotypes observed after genetic ablation strategies may not reflect disease profiles in patients caused by expression of mutant desmosomal proteins. Moreover, the underlying mechanisms remain poorly understood.

Most experimental studies of AC have focused on myocardial injury and molecular pathology with relatively little attention paid to potential changes in cellular electrophysiology that may contribute to the highly arrhythmogenic phenotype in AC. However, recent studies have begun to shed light on this important area. Using cultured cardiac myocytes, Sato et al. have provided some evidence that *PKP2* interacts directly with Nav1.5 and that knockdown of *PKP2* expression promotes changes in the  $I_{Na}$  and the velocity of impulse propagation.<sup>70</sup> Finally, Kim et al. generated iPS cell lines from a patient with clinical ARVC and a homozygous (c.2484C>T) mutation in *PKP2* that causes cryptic splicing with a 7-nucleotide deletion in exon 12 leading to frame-shift of the carboxy-terminal amino acids.<sup>72</sup> The derived cardiac myocytes abnormal plakoglobin nuclear translocation and decreased Wnt signaling. This important study was the first to characterize



cardiac myocytes derived from a patient with AC. Many technical limitations apply to such studies but it can be anticipated that additional insights into disease mechanisms in patients will come from further studies using this approach.

## Conclusions

AC has an unusually dramatic arrhythmogenic phenotype, which is manifest early in the natural history of the disease, often preceding the development of significant ventricular remodeling or contractile dysfunction. The identification of desmosomal gene mutations in >50% of index patients has focused attention on the possibility that abnormal cell-cell adhesion may play a critical role in myocardial injury and arrhythmogenesis. At the same time, increasing evidence has implicated abnormal Wnt signaling, perhaps related to redistribution of junctional plakoglobin to intracellular/intranuclear sites. Moreover, AC may be caused by mutations in non-desmosomal genes, and the genetic basis of the disease is undefined in a significant number of patients. It remains to be determined to what extent changes in electrical coupling contribute to the highly arrhythmogenic phenotype in AC and how gap junction remodeling interacts with other potential arrhythmogenic mechanisms in this deadly disease. One promising avenue for future investigation is more detailed elucidation of the manifold mechanisms implicated in dysregulated Wnt signaling pathways. How a mutation in a  $Ca^{+2}$  homeostasis protein such as *PLN* or a nuclear membrane protein such as *TMEM43* promotes displacement of plakoglobin from cell-cell junctions must be elucidated. The exact role of plakoglobin in the nucleus and the panel of genes whose expression it may control must be determined. The relationship between genetic, epigenetic and environmental factors, such as exercise, and the role they play in modifying disease manifestation also requires further investigation. Advances in these areas could provide new insights into arrhythmogenesis not only in AC but also in other, more common forms of heart disease, and identify much needed mechanism-based therapies to treat or prevent sudden death.

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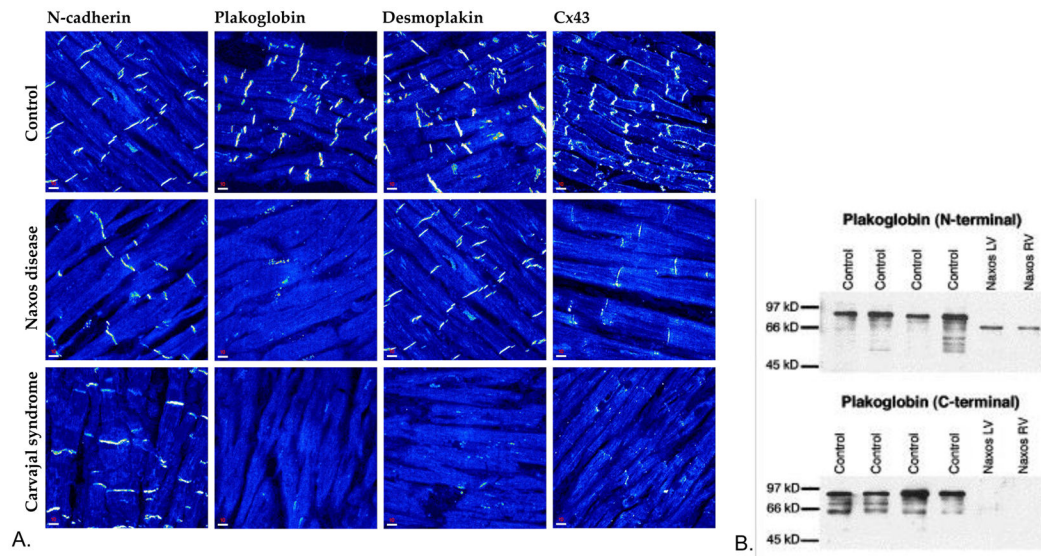
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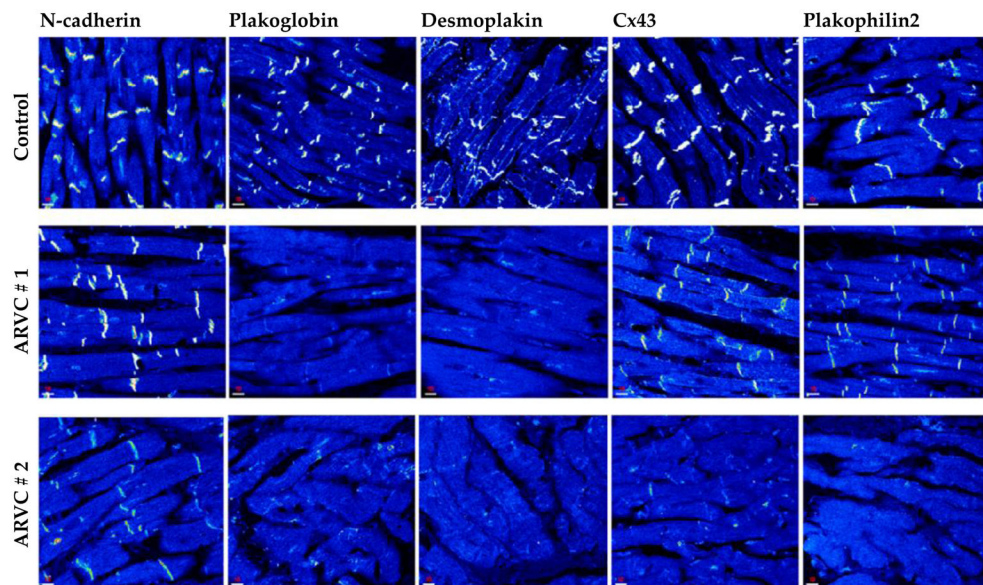


**Figure 1.**

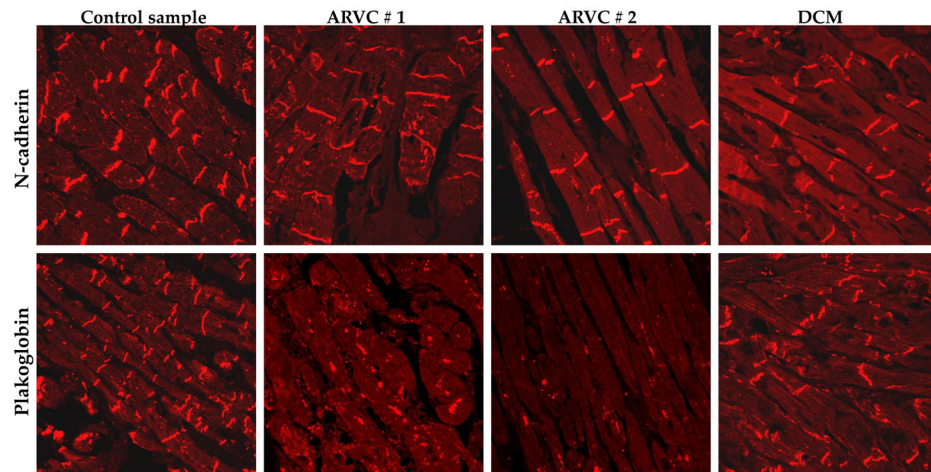
(A) Representative confocal immunofluorescence images of control myocardium and myocardium from a patient with Naxos disease and a patient with Carvajal syndrome. Specific immunoreactive signal for plakoglobin was significantly depressed in both cases compared to controls, as was signal for the major gap junction protein Cx43.

Immunoreactive signal for desmoplakin was depressed at intercalated disks in Carvajal syndrome but not in Naxos disease. Signal for the non-desmosomal adhesion protein N-cadherin was present and control-like in both cases.

(B) Western Immunoblots using an N-terminal plakoglobin antibody showed that the mutant plakoglobin form (2057del2) is expressed in left and right ventricular myocardium from a patient with Naxos disease. Mutant plakoglobin migrates at a lower molecular weight than the wildtype protein. 2057del2 plakoglobin cannot be detected when a C-terminal plakoglobin antibody is used (reproduced from Kaplan et al *Heart Rhythm* 2004; 1)<sup>40</sup>



**Figure 2.** Representative confocal immunofluorescence images of control myocardium and myocardium from two patients with autosomal dominant ARVC. Specific immunoreactive signal for plakoglobin was depressed at cell-cell junctions in the great majority of cases regardless of the underlying pathogenic mutation. Signal for desmoplakin and plakophilin2 varied, while signal for N-cadherin was always present and indistinguishable from controls. The majority of cases examined showed gap junction remodeling as evidenced by decreased junctional signal for Cx43 (reproduced from Asimaki et al. *NEJM* 2009; 360:1078).<sup>41</sup>



**Figure 3.**

Immunofluorescence images of endomyocardial biopsy samples from two patients diagnosed with ARVC and one with idiopathic dilated cardiomyopathy, all carrying the *PLN* R14del mutation, compared to a control sample. Immunoreactive signal for plakoglobin at cell-cell junctions was significantly depressed in the ARVC subjects compared to controls and the dilated cardiomyopathy patient (reproduced from Van der Zwaag et al. *Eur J Heart Fail* 2012; 14:1204).<sup>25</sup>