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Arrhythmogenic cardiomyopathy: pathology, genetics, and concepts in pathogenesis

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Abstract

Arrhythmogenic cardiomyopathy (ACM) is a rare, heritable heart disease characterized by fibro-fatty replacement of the myocardium and a high degree of electric instability. It was first thought to be a congenital disorder, but is now regarded as a dystrophic heart muscle disease that develops over time. There is no curative treatment and current treatment strategies focus on attenuating the symptoms, slowing disease progression, and preventing life-threatening arrhythmias and sudden cardiac death. Identification of mutations in genes encoding desmosomal proteins and in other genes has led to insights into the disease pathogenesis and greatly facilitated identification of family members at risk. The disease phenotype is, however, highly variable and characterized by incomplete penetrance. Although the reasons are still poorly understood, sex, endurance exercise and a gene-dosage effect seem to play a role in these phenomena. The discovery of the genes and mutations implicated in ACM has allowed animal and cellular models to be generated, enabling researchers to start unravelling it's underlying molecular mechanisms. Observations in humans and in animal models suggest that reduced cell–cell adhesion affects gap junction and ion channel remodelling at the intercalated disc, and along with impaired desmosomal function, these can lead to perturbations in signalling cascades like the Wnt/β-catenin and Hippo/YAP pathways. Perturbations of these pathways are also thought to lead to fibro-fatty replacement. A better understanding of the molecular processes may lead to new therapies that target specific pathways involved in ACM.

Keywords   Arrhythmogenic cardiomyopathy • Arrhythmogenic right ventricular cardiomyopathy • Pathology • Genetics • Pathogenesis

This article is part of the Spotlight Issue on Right Ventricle.

1. Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC), which is now considered a subform of arrhythmogenic cardiomyopathy (ACM) with right ventricular (RV) pre-dominance, is a heritable condition characterized by fibro-fatty replacement of the myocardium that predisposes patients to ventricular arrhythmias (VA), which are frequently life-threatening, and to slowly progressive ventricular dysfunction.[1–4] Structural involvement of the RV predominates,[5] although left-dominant forms of ACM are also well-recognized.[6] Patients typically present in their second to fifth decade with symptoms associated with VA.[7] Sudden cardiac death may be the presenting symptom in up to 50% of index cases.[8] The diagnosis is based on International Task Force Criteria[9] and mutations in genes encoding proteins of the cardiac desmosome are found in up to 60% cases.[1,10] Cardiac desmosomes are composed of a symmetrical group of proteins (cadherins, armadillo proteins, and plakins) that provide mechanical connections between myocytes. However, non-desmosomal genes have also been identified.[11] The current management strategies focus on lifestyle advice (restriction of physical exercise), attenuating symptoms, and slowing disease progression with anti-arrhythmic and heart failure medications, catheter ablation, and implantable cardioverter defibrillator (ICD) implantation. In cases of end-stage heart failure or refractory VA, a heart transplantation may be required.[12] Unravelling the genetic basis of ACM has led to the

generation of animal and cellular models, enabling researchers to uncover the molecular mechanisms underlying ACM and even to discover new therapies.[13]

This review will discuss the pathological findings, the genetic basis and the proposed mechanisms underlying ACM.

2. Pathological findings in ACM

2.1 Morphological features

In ACM, part of the myocardium is replaced by fibrous and fatty tissue with either localized or diffuse myocardial atrophy due to cumulative myocyte loss.[14] The pathological hallmarks of the disease, the fibro-fatty replacement and myocyte atrophy, are usually distinctly present in the RV but may also occur in the left ventricle (LV), and can be segmental or patchy. Traditionally, the typical localization in the RV was described as the 'triangle of dysplasia',[14,15] consisting of the RV inflow tract, RV outflow tract, and RV apex. However, recent cardiac magnetic resonance data[16] have revealed that limited ACM preferentially affects the basal inferior RV, with involvement of the RV apex only in advanced cases as part of global RV involvement. LV involvement has been observed in 76–84% of ACM cases,[6,14] with a pre-dilection for the thin posterolateral and posteroseptal areas.

Typically, the LV is affected to a lesser extent than the RV; however, there are disease variants characterized by pre-dominant LV involvement, these are also referred to as arrhythmogenic left ventricular cardiomyopathy (ALVC).[17]

Involvement of the ventricular septum is rare, probably because it is not a subepicardial structure. The fibro-fatty scar tissue progresses from the subepicardial muscle layer towards the endocardium, ultimately resulting in transmural lesions with focal or diffuse wall thinning (*Figure 1*). This implies the ventricular wall is weakened, especially the relatively thin, free RV wall, which may lead to typical aneurysmal dilatation.

Microscopic examination typically shows islands of surviving myocytes, with fibro-fatty tissue in between.[14,17] These changes may account for intraventricular conduction delay and re-entry circuits triggering VA. Affected cardiomyocytes show non-specific degenerative features of myofibrillar loss and hyperchromatic changes in nuclear morphology.[14,17] Cardiomyocyte death (acquired injury), by either apoptosis[18] and/or necrosis,[19] accounts for the progressive loss of the ventricular myocardium. These changes may be accompanied by inflammatory infiltrates, seen in up to 67% of hearts at autopsy.[14] Importantly, active inflammation might account for worsening of electrical instability and the onset of life-threatening arrhythmias. Whether the inflammatory cells are reactive to cell death or a primary event due to infection[20] or non-infective immune factors needs to be investigated.[21]

2.2 Clinical utility of RV endomyocardial biopsy (EMB)

RV EMB may be useful for the diagnosis of ACM, through an *in vivo* histological demonstration of fibro-fatty replacement. Moreover, EMB may provide additional information to rule out phenocopies, such as myocarditis or sarcoidosis, particularly in sporadic cases in which non-invasive evaluation remains inconclusive. The optimal EMB site is the RV free wall, which may, however, be severely thinned due to ACM.

In a normal heart, with increasing age and body weight, intramyocardial fat is, to a limited extent, present in the RV. Therefore, adipose tissue should be accompanied by replacement fibrosis and myocyte degeneration to be a sufficient morphologic diagnostic feature of ACM.[22]
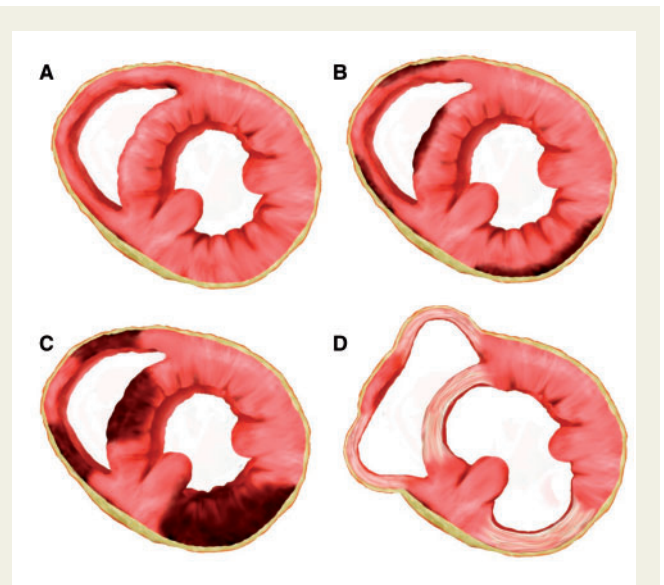
Figure 1 Development of arrhythmogenic cardiomyopathy (ACM) over time. Evidence from the *Dsg2*^{N271S} mouse model for ACM. (*A*) At birth a structurally normal heart is presence. (*B*) Early myocardial injury start on the epicardial side, extends transmurally (*C*) and is followed by wall thinning with fibrous repair and aneurysm development (*D*). Figure adapted from Basso et al.[104]

In addition to conventional histology, immunohistochemical analysis can be a valuable tool, because plakoglobin (PG) signal levels at intercalated disks can be diffusely diminished in most ACM patients, also in samples from the LV or interventricular septum, irrespective of the underlying mutation.[23] However, the reliability and validity of this test for routine clinical practice still has to be confirmed.[24]

3. Genetic basis of ACM

Clustering of ACM within families was appreciated early.[25] Recognition that the cardiac phenotype of Naxos disease, a rare, familial, cardiocutaneous condition, overlapped with familial ACM[26] was a key insight. Following the discovery that mutations in *JUP*, encoding PG, was the cause of Naxos disease,[27] the ACM-associated mutations in the desmosomal genes were rapidly unveiled, including *DSP* encoding desmoplakin,[28] *PKP2* encoding plakophilin-2,[29] *DSG2* encoding desmoglein-2,[30] and *DSC2* encoding desmocollin-2.[31]

Up to two-thirds of ACM patients harbour mutations in these desmosomal genes.[1,7] Heterozygous mutations resulting in pre-mature termination of the protein product and/or abnormal splicing in *PKP2* are the most prevalent.[10,32] Inheritance of desmosomal mutations follows an autosomal dominant pattern with age-related, incomplete penetrance and variable expressivity. However, ACM patients with multiple mutations (compound heterozygosity and digenic) are not uncommon and their occurrence ranges widely (4–21% reported in various cohorts).[7,32–34] This range is likely related to how stringently missense variants are adjudicated and how many genes are sequenced.[35] Cases with homozygous mutations are also seen.[36,37] In addition, there are pedigrees in which siblings of the index case are more likely to be affected than their parents or their parents' siblings. These phenomena

raise the suspicion that other genetic and/or environmental factors may play a modifying role.[38]

Although most reported ACM-associated pathogenic variants are in desmosomal genes (as in 95.5% of the variants reported in the ARVC Genetic Variant Database[10]), extra-desmosomal mutations have been identified in a few patients. The first of these was the p.S358L founder mutation in *TMEM43*, encoding transmembrane protein 43, which was identified in patients in Newfoundland and Europe.[39,40] Pathogenic mutations have also been reported in genes associated with other cardiomyopathies and arrhythmia syndromes including desmin (*DES*),[41] titin (*TTN*),[42] lamin A/C (*LMNA*),[43] phospholamban (*PLN*),[44] $Na_V1.5$ (*SCN5A*),[45] and Filamin C (*FLNC*).[46] Together, these discoveries reflect the clinical and genetic overlap of ACM with dilated cardiomyopathy at one phenotypic extreme[47] and with arrhythmia syndromes at the other. Supporting this concept, pathogenic ACM-associated *PKP2* missense mutations also have been identified in Brugada syndrome patients.[48]

Genes encoding proteins in the 'area composita' (composed of desmosomes, adherens junctions (AJ), ion channels, and gap junctions) have also emerged as potentially important in the pathogenesis of ACM. Mutations in *CTNNA3*, encoding αT-catenin, have been identified in families with classic ACM.[49] Recently, two families, with right-pre-dominant ACM, were found to have likely pathogenic mutations in *CDH2*, encoding cadherin-2, a calcium-dependent cell surface adhesion molecule.[50]

Mutations in transforming growth factor-beta3 (*TGFB3*)[51] and the cardiac ryanodine receptor-2 (*RYR2*)[52] genes have been described in ACM, although this association needs to be confirmed.

Finally, there are some ACM cases with no identifiable mutation. In the largest study of ACM, amongst 439 index cases, 37% had no identifiable mutation in the desmosomal genes, *PLN*, or *TMEM43*.[7] Amongst these gene-elusive cases, only one-fifth had evidence of familial disease. A recent meta-analysis confirmed a lower prevalence of family history amongst ACM patients without desmosomal mutations.[53] This raises the question whether these gene-elusive cases have a primarily monogenic disease or whether they represent an oligogenic form of ACM with unknown, low-penetrant genetic variants and/or with external factors playing a role in their disease pathogenesis. Recent research showed that gene-elusive ACM cases without a positive family history were disproportionately observed in high-level endurance athletes,[54,55] which points to exercise as a key lifestyle risk factor in these cases.

3.1 Genotype–phenotype association in ACM

Several clinically useful genotype–phenotype associations have been identified. Broadly, neither the cardiac phenotype nor clinical course differ substantially between ACM patients with and without a mutation.[7] A recent meta-analysis identified inverted anterior pre-cordial T-waves (V_{1-3}) but not structural abnormalities, epsilon waves, or arrhythmias with a left-bundle branch block morphology, as being more common amongst ACM patients with desmosomal mutations.[53] Patients with mutations do have earlier onset of ACM.[7,53,56]

In addition to an increased penetrance, carrying multiple mutations seems to be an important risk factor for malignant VA and sudden death.[57] Similarly, in 577 desmosomal, *PLN*, and *TMEM43* mutation carriers, the 4% of patients with multiple mutations had significantly earlier occurrence of malignant VA and more frequent LV dysfunction, class C heart failure, and transplantation.[32] Together these data suggest there is a gene-dosage effect in ACM.

Other associations between genotype and ACM phenotype include a higher prevalence of LV involvement and heart failure amongst ACM patients with *FLNC*, *DSP*, and *PLN* mutations.[32,46,58] The TMEM43 p.S358L founder mutation is associated with high disease penetrance and arrhythmic risk amongst male carriers.[39]

Table 1 provides an overview of the genes implicated in ACM and the yield of genetic testing. Caution is warranted as variants in ACM-related genes are also often found in the general population.[59]

3.2 Penetrance of ACM mutations

Familial ACM is characterized by incomplete age-related penetrance and significantly variable expressivity.[60] With the expansion of genetic testing for ACM, increased numbers of at-risk mutation carriers are now being identified, so that understanding the risk conveyed by the presence of an ACM-associated variant is critical. The penetrance of ACM-associated mutations is likely to be overestimated, as families reported in genetic studies will have higher than typical penetrance and more affected individuals, making them attractive for genetic research. Such families likely share additional genetic or envirnomental factors that put them at increased risk. In a report of over 500 desmosomal mutation carriers,[32] roughly only one-third met diagnostic Task Force Criteria.

Data from unselected populations with incidentally detected desmosomal variants suggest that penetrance in the general population may be considerably lower. A recent publication[61] showed that amongst 18 individuals with incidentally identified pathogenic ACM mutations and 194 cases with rare variants of uncertain significance, neither cardiac diagnoses reported in the electronic medical record nor cardiac tests evaluated by ARVC experts showed higher rates of abnormalities than the control population.

3.3 Interplay of genotype and exercise in ACM pathogenesis

While there is no clear explanation for phenotypic heterogenity in ACM, even amongst carriers of the same mutation, there is increasing evidence that exercise plays a major role in disease penetrance and arrhythmic risk. A history of participation in endurance exercise is associated with increased likelihood of disease penetrance in a dose-dependent fashion.[62] Desmosomal mutation carriers who were endurance athletes also have earlier onset of ACM, worse structural abnormalities, higher likelihood of heart failure, and greater arrhythmic risk.[62,63]

There is evidence that strongly suggests exercise is also associated with gene-elusive ACM. A study suggested that ultra-endurance athletes may develop a pre-dominantly exercise-induced form of ACM.[64] Two research groups showed that ACM patients without a desmosomal mutation had done considerably more intense exercise prior to clinical presentation than desmosomal mutation carriers.[54,55]

An emerging paradigm suggests there is a threshold for phenotypic expression of ACM depending on the relative amount of exercise undertaken.[54,65] As shown in *Figure 2*, we hypothesize that individuals born with a very high genetic risk, such as carriers of multiple mutations, require little (or perhaps no) exercise for ACM disease expression. Ultra-endurance athletes may develop a pre-dominantly exercise-induced form of ACM,[64] although we suspect only a subset of this population is susceptible.
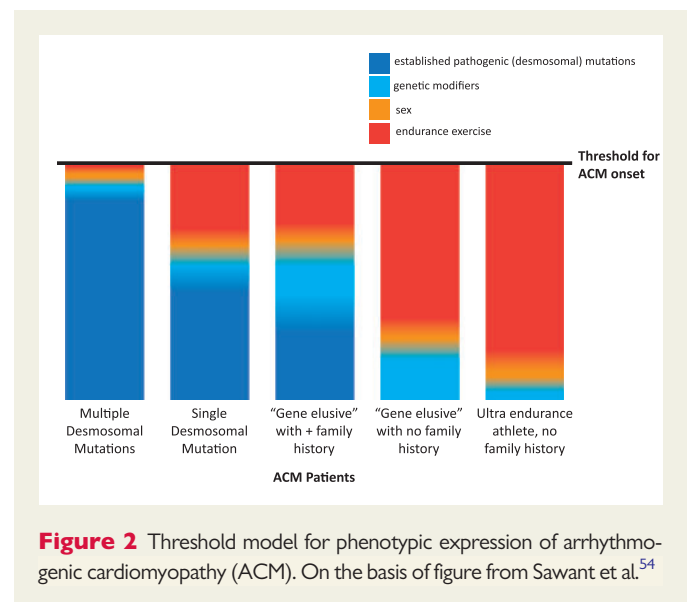
Table 1 Overview of the genes implicated in ACM and the yield of genetic testing

| Gene	Protein	% index cases	Notes	References
Desmosomal				
PKP2	Plakophilin-2	20–46	Most prevalent in the majority of ACM populations	7,29,33,43,53
DSP	Desmoplakin	3–15	Autosomal dominant inheritance associated with ACM	7,28,43,53
			Autosomal recessive inheritance associated with Carvajal Syndrome (cardiocutaneous)	
DSG2	Desmoglein-2	3–20		7,30,43,53
DSC2	Desmocollin-2	1–8		7,31,43,53
JUP	Plakoglobin	0–1 (except in Naxos, Greece)	Autosomal dominant inheritance associated with ACM	7,27,43,53,82
			Autosomal recessive inheritance	
			Associated with Naxos disease (cardiocutaneous)	
Area composite				
CTNNA3	αT-catenin	0–2	2/76 Italian probands without desmosomal mutations	49
CDH2	Cadherin-2	0–2	2/74 families without desmosomal mutations	50
Other or overlapping syndromes				
PLN	Phospholamban	0–1 (except in Dutch populations)	Dutch founder mutation	7,44,47
TMEM43	Transmembrane protein 43	0–2 (except in Canadian populations)	Canadian (Newfoundland) founder mutation	7,39,40
SCN5A	$Na_V1.5$	2		10,45
LMNA	Lamin A/C	0–4	Overlap with dilated cardiomyopathy	33,43
DES	Desmin	0–2	1 of two cases detected with pathogenic *PKP2* mutation	33,41
FLNC	Filamin C	3	7/219 Southern European ARVC patients	46
			Left-dominant cardiomyopathy with myocardial fibrosis	
TTN	Titin	0–10	Overlap with dilated cardiomyopathy	42

4. Pathogenesis

Multiple aetiopathogenic models have been proposed for ACM. The initial model explained the disease as a manifestation of an embryonic maldevelopment (dysplasia) of the RV. That ACM is not a congenital abnormality, like Uhl's disease, but a cardiomyopathy which develops over time, is supported by the pathological findings described above in Section 2.1.[14,17] This assertion is further supported by an ACM mouse model, which demonstrated that loss of the myocardium starts after birth.[19] Chronic inflammation could also contribute to the development of ACM. Experiments in mice (BALB/c strain) inoculated with Coxsackie virus B3 showed myocardial necrosis and inflammatory infiltrates with subsequent exclusive RV fibrosis.[66] While one report demonstrated that cardiotropic viruses are present in a subset of sporadic ACM cases,[20] another cohort that included almost half familial ACM cases, did not uncover any viral material in the hearts.[21] This suggests that a viral aetiology could be possible in sporadic cases, whereas a genetic substrate is more likely in familial ACM.[67]

The discovery that desmosomal gene mutations cause ACM offered important leads in understanding the mechanisms responsible for the disease. As mentioned in Section 3, desmosomes together with the AJ, gap junctions and ion channels form the area composita at the intercalated disc (ID; *Figure 3A*). This structure is important for the electromechanical coupling of cardiomyocytes and plays a role in multiple intracellular signalling cascades.[68] Firstly, mutations affecting the desmosomal proteins could lead to a decreased mechanical coupling between

Figure 2 Threshold model for phenotypic expression of arrhythmogenic cardiomyopathy (ACM). On the basis of figure from Sawant et al.[54]

the cells. Cardiomyocytes are especially subject to mechanical stress and decreased coupling can lead to detachment of the cardiomyocytes with subsequent cell death, inflammation and loss of myocardium. Ultrastructural abnormalities of the desmosomes and ID, reflecting an
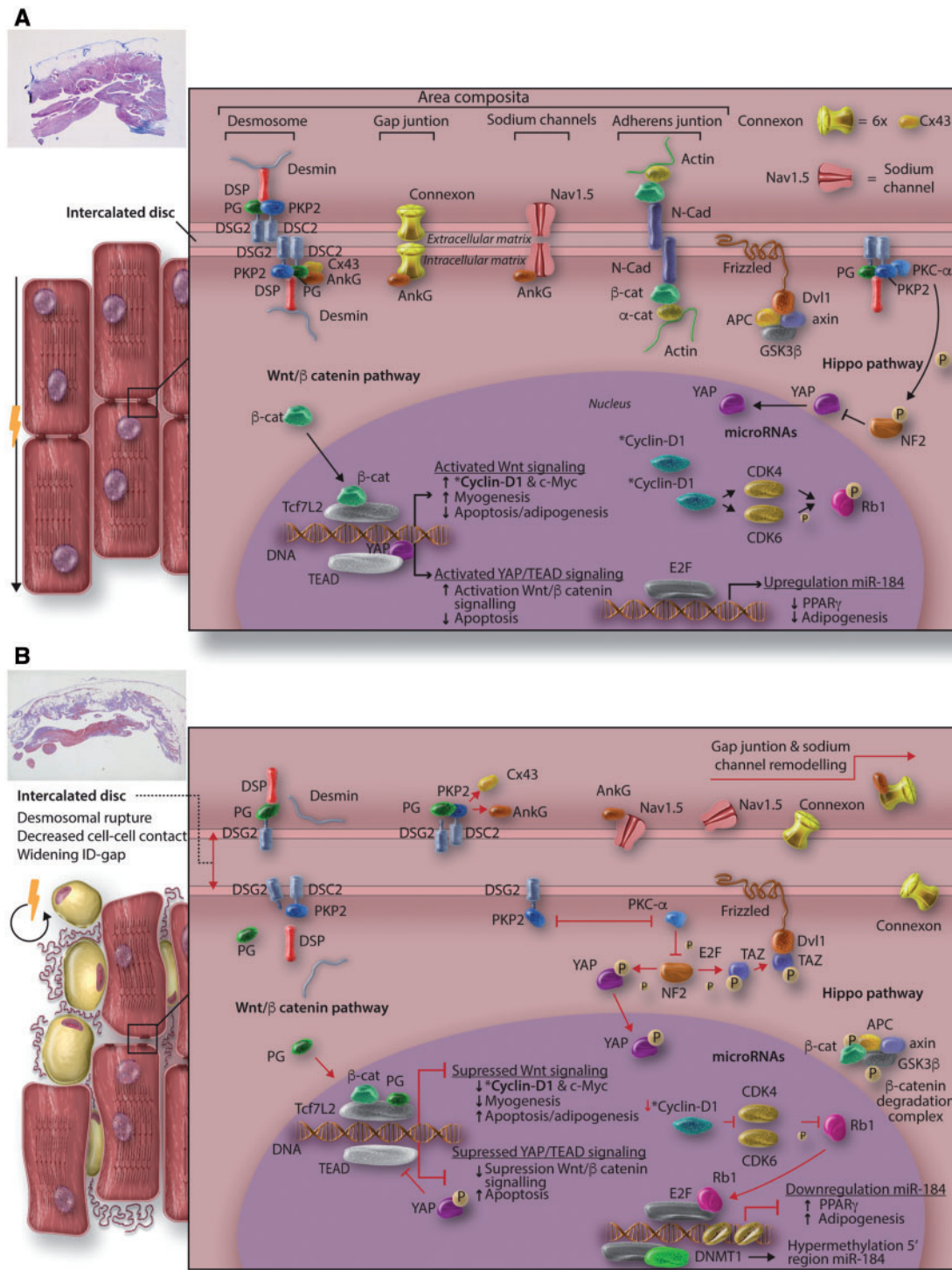
Figure 3 (*A*) Overview of the 'area composita' and (*B*) proposed remodelling of desmosomal proteins, gap junctions and ion channels at the intercalated and aberrant signalling pathways involved in arrhythmogenic cardiomyopathy substantiated by the various experimental models. Histological images of normal right ventricle (*A*) and right ventricle affected by ACM (*B*). Histological images are adapted from Basso and Pilichou et al.[3,14] Pathways are based on Corrado et al., Hu et al., Gurha et al., and Vermij *et al.*[4,68,95,96]

impaired cell–cell coupling, have been reported in hearts of ACM patients.[69] Secondly, considering the interaction between components of the desmosome, ion channels,[70] and gap junctions,[71] desmosome dysfunction could also lead to the remodelling of these proteins at the ID causing abnormal electric coupling between cardiomyocytes. Remodelling of Cx43 and cardiac voltage-gated sodium channel (Na$_V$1.5) has been demonstrated with immunofluorescence in human ACM hearts.[72] These observations suggest that gap junction or ion channel remodelling may increase susceptibility to arrhythmias. Lastly, altered signalling pathways due to remodelling at the ID could also contribute to the pathogenesis of ACM.

Most of the experimental models have focused on genetic defects in the desmosomal proteins (*Figure 3*). The major components of the desmosomes are the cadherins (DSG2 and DSC2), the armadillo proteins (PG and PKP2), and the cytolinker protein DSP.[73] The cadherins have an extracellular domain, which bind to the cadherins from adjacent cells and are important for the adhesive properties of the cell–cell contact. The intracellular domains of the cadherins bind to the armadillo proteins, which are indirectly connected to the intermediate filament desmin by DSP. In addition to a structural role in the desmosome, the armadillo proteins participate in different signalling pathways.[73]

4.1 Cell–cell coupling

Cell–cell coupling has been studied extensively in multiple ACM models. Experimental models mimicking a deficiency of one of the desmosomal proteins in a cardiac-restricted or constitutive fashion showed that a deficiency of any of these desmosomal proteins can lead to ultrastructural abnormalities of the ID and desmosomes.[74–78] Similar findings were obtained *in vitro* in cellular studies that demonstrated decreased cell–cell adhesion upon down-regulation of PKP2 or PG.[70,79] In addition, overexpression of mutations in different desmosomal proteins, simulating a dominant negative effect, also led to ultrastructural defects at the ID.[80–82] Impaired cell–cell coupling was also demonstrated to play a key role in the pathogenesis of ACM in mice overexpressing the *Dsg2* N271S mutation (*Dsg2*^{N271S}), which is homologous to the human mutation DSG2-N266S. In *Dsg2*^{N271S} mice, widening of the ID preceded the occurrence of fibrosis and necrosis. In some observations this widening co-occurred with focal lysis of the cardiomyocytes at the points of attachment to the desmosomes.[81] *Dsp* cardiac-specific knock-out mice (*Dsp*-cKO), exhibit many features seen in the human ACM phenotype (including fat deposition and arrhythmic instability). Rupture of the desmosomes, widening of the ID, and loss of myocyte adhesion was observed as an early manifestation of the phenotype.[83] Collectively, these data underline the necessity of proper desmosomal function for the stable coupling of cardiomyocytes (*Figure 3B*).

4.2 Gap junction and ion channel remodelling

The cross-talk between dysfunction of desmosomal proteins and of components of cardiac electrical function has been studied in several models. At the ID, the desmosomes, AJ, gap junctions, and ion channels interact with each other and function as one unit.[68]

PKP2 has been shown to physically interact with Cx43, ankyrin-G (AnkG) and Na$_V$1.5 *in vitro*.[70,84] Ankyrin-G is a cytoskeletal adaptor protein and is an important component of the voltage-gated sodium channel complex.[70] Silencing of PKP2 in neonatal rat ventricular myocytes led to a reduced signal for Cx43, Na$_V$1.5 and AnkG at the ID.[70,84] Although no reduced signal of Cx43 and Na$_V$1.5 was seen at the ID in heterozygous

PKP2 knock-out mice (*Pkp2*$^{+/-}$), they did show altered sodium current kinetics and were prone to ventricular tachycardia when provoked by flecainide, without having histological cardiac alterations.[48] A marked reduction of immunoreactive signals of Cx43 and Na$_V$1.5 was also seen in human induced pluripotent stem cells (hiPSCs) derived from patients with *PKP2* mutations.[13] *In vivo* mislocalization of Cx43, represented by punctate distribution instead of a continuous organization pattern of Cx43, was observed in mice overexpressing *Pkp2*^{R735X}, but only after they were subjected to exercise.[85] In addition to PKP2, DSG2 also co-immunoprecipitates with Na$_V$1.5.[81] Hearts from *Dsg2*^{N271S} mice that were studied *ex vivo* prior to the development of cardiomyopathic changes demonstrated reduced cardiac conduction velocities and increased arrhythmia inducibility, possibly mediated by a disturbed *Dsg2*-Na$_V$1.5 interaction.[81] DSC2 was also shown to physically interact with Cx43[71] and a specific *DSC2* mutation led to a decreased binding affinity for Cx43, indicating that *DSC2* mutations can alter Cx43 function. Experimental models, both *in vitro* as well as *in vivo*, modelling PG and DSP deficiency and mutations with dominant negative effect therein, demonstrated that Cx43 remodelling also occurred when these genes are affected.[13,79,80,83,86] In line with observations in *Dsg2*^{N271S} mice, heterozygous *Dsp* knock-out mice (*Dsp*$^{+/-}$) exhibited conduction delay and increased susceptibility to inducible ventricular tachycardia, without overt cardiac structural abnormalities. The observed mislocalization and reduced expression of Cx43 was noted as a possible underlying mechanism.[87]

An altered inward rectifier potassium current (I_{k1}), which is mediated by the potassium channel subunit Kir2.1, was seen in zebrafish overexpressing the mutant c.2057del2 *JUP* (PG-2057del2). Immunostaining of PDZ domain-containing synapse-associated protein-97 (SAP97) demonstrated a reduction of this protein. SAP97 mediates the trafficking of PG, Kir2.1 and Na$_V$1.5 to the ID, suggesting a possible role for these ion channels in the disease process.[13] That proteins at the area composita function as one unit is emphasized in a double knock-out mouse in which PG and β-catenin were deleted, were Cx43 remodelling preceded the highly arrhythmogenic phenotype of the mice.[88] Of note, Cx43 remodelling was not observed in a homozygous cardiac-restrictive PG deficient mouse model (Car *Pg*$^{-/-}$) with electric instability, while there was Cx43 remodelling in other Car *Pg*$^{-/-}$ mice, who did not have any electric abnormalities.[86,89] In conclusion, these studies in different cellular and mouse models support the view that the interaction of desmosomal proteins with gap junctions and ion channels at the area composita leads to conduction abnormalities and electrical instability upon disruption of desmosomal function (*Figure 3B*).

4.3 ID remodelling and signalling pathways
4.3.1 Wnt/β–catenin pathway
Suppression of the canonical Wnt/β-catenin pathway can lead to an enhanced adipogenesis. β-Catenin is an activator of Wnt signalling by activating T cell/lymphoid-enhancing binding (Tcf/Lef) transcription factors. Since PG, which is also known as γ-catenin, shares functional and structural properties with β-catenin, it is postulated that nuclear translocation of PG can interfere with this pathway by binding to a different sites on Tcf7L2 transcription factor than β-catenin does (*Figure 3B*). In cultured DSP-deficient atrial myocytes (HL-1 cells) there was an increase of PG in the nuclear fraction, with a subsequent decrease in Tcf/Lef1-mediated gene transcription followed by a supressed canonical Wnt signalling (represented by an increase in adipogenetic transcriptional regulators). It is therefore believed that this mechanism may underlie fibro-fatty

replacement in ACM. The heterozygous cardiac-specific DSP knockout (Car Dsp$^{+/-}$) mouse, with an ACM phenotype including electric instability and accumulation of fat droplets in the myocardium, showed an increase of PG in the nuclear fraction and suppressed Wnt signalling as seen in HL-cells.[90] This translocation of PG was also seen in mice overexpressing the mutant PG-2057del2, with a subsequently supressed Wnt signalling [represented by down-regulation of the Wnt target genes (c-Myc and cyclin-D1)].[91] Further support of a role of the Wnt/β-catenin pathway in ACM was demonstrated in hiPSC-CMs from a patient with a *PKP2* mutation, which showed nuclear translocation of PG and a decreased β-catenin activity.[92] A decrease in expression of the Wnt target gene cyclin-D1 was also noted in PKP2 knockdown HL-1 cells.[93] Indirect evidence of supressed Wnt signalling was shown in homozygous mice lacking exons 4–5 of DSG2 (*Dsg2*$^{exon4–5/exon4–5}$).[94] Inhibition of the glycogen synthase kinase-3 beta (GSK3β), which targets β-catenin for degradation, reversed the adverse remodelling of the desmosomal proteins and gap junctions and prevented cardiac myocyte injury and cardiac dysfunction. As GSK3β targets degradation of β-catenin, inhibition of GSK3β should lead to activation of the canonical Wnt/β-catenin pathway. The observations that inhibition of GSK3β normalizes the desmosomal protein remodelling and improves the cardiac phenotype in the *Dsg2*$^{exon4–5/exon4–5}$ mice supports the concept that *DSG2* mutations could suppress the canonical Wnt/β-catenin pathway, most likely by disrupting the desmosome complex and leading to increased nuclear translocation of PG.[94]

Whether the aberrant Wnt signalling is a common pathway in ACM is a topic of debate, since PG remodelling was not seen in another set of hearts from ACM patients.[24] Kant et al. suggested that the reduced PG immunofluorescence signal was due to epitope masking rather than remodelling. They also stated that target genes (CTGF and cyclin-D1) of the Wnt/β-catenin signalling, which should be down-regulated during suppression, were upregulated in six human hearts with ACM.[24]

4.3.2 Hippo/YAP pathway

The Hippo/YAP pathway was also shown to be involved in experimental models with different mutations. PKP2 functions as a scaffold protein for the protein kinase C alpha (PKC-α). PKC-α inactivates neurofibromin (NF2), which is located up-stream of the Hippo pathway. When NF2 is activated, it phosphorylates and then deactivates Yes-associated protein 1 (YAP), a transcription factor. Subsequently, phosphorylated YAP (pYAP) can contribute to suppression of Wnt/β-catenin signalling (*Figure 3B*).[93,95] When PKP2 is not present, PKC-α (which needs PKP2 as a scaffold) is significantly reduced in PKP2 knock-down HL-1 cells. Also, NF2 was activated and levels of increased pYAP were demonstrated. This activation of NF2 was also observed in cardiac-restricted *Dsp*$^{+/-}$ mice and mice overexpressing PG-2057del2.[93]

4.3.3 MicroRNAs

Recently a new mechanism was proposed as being involved in ACM. In knock-down PKP2 HL-1 cells, transcriptome analysis showed that microRNA-184 (miR-184) was down-regulated, although it is normally up-regulated by E2F transcription factors. Cyclin-D1 was also down-regulated, which normally deactivates retinoblastoma (RB1) protein.[96] In the case of down-regulation of cyclin-D1, RB1 levels are increased and inhibit E2F transcription factors, which leads to a decrease in levels of miR-184. However, the down-regulation was only partially explained by the diminished levels of cyclin-D1. Another factor contributing to the diminished levels appeared to be that the genomic region of miR-184 was hypermethylated by DNA (cytosine-5)-methyltransferase 1

(DNMT1). This could be due to the E2F/RB1 complex, as it has been shown that this complex can recruit DNA methyltransferases (*Figure 3B*).[97] Diminished levels of miR-184 cause an increase in expression of peroxisome proliferator-activated receptor gamma (PPARγ), which is an inducer of adipogenesis and should not normally be activated in cardiomyocytes.[96] This supports a prior discovery that hiPSC-CMs from patients with desmosome mutations require, besides normal activation of PPARα, abnormal activation of PPARγ to induce ACM features *in vitro*.[92] This down-regulation of miR-184 was confirmed in mice overexpressing PG-2057del2.[96] Of note, miR184 overexpression or down-regulation did not affect transcriptional activities of Hippo and canonical Wnt pathways. Whether this down-regulation plays a role in the other desmosomal ACM models remains to be investigated.

4.4 Calcium handling deficits

Abnormal calcium handling may also contribute to ACM as experimental models showed perturbed calcium handling in hiPSCs-CMs with the homozygous *PKP2* c.2484C>T mutation.[92] A recent study in human hearts with ACM revealed that mRNA levels for PLN, a protein involved in the intracellular calcium homeostasis, was significantly up-regulated.[98] Additionally, mutated PLN is well known to cause ACM in humans.[47] These findings indicate that abnormal calcium handling could play a role in the pathogenesis and it is important that this topic should be studied further.

4.5 Exercise

The effect of exercise has been studied retrospectively in humans with ACM and prospectively in several ACM mouse models; all studies consistently demonstrated that exercise induces or exacerbates the cardiac phenotype.[62,85,94,99,100] Mice overexpressing the mutant *Dsp* R2843H (*Dsp*^{R2483H}) showed a blunted activation of AKT1 in response to exercise. This blunted response could be due to sequestration of PG at the insoluble part of the cells.[100] The consequence of this response, including adverse cardiac remodelling, remains unknown, although it is speculated that this is due to perturbed Wnt signalling.[100] *In vitro* studies have looked at the consequences of mechanical stress in cells expressing mutated forms of PG or PKP2: the cells failed to up-regulate PG and N-cadherin when subjected to shear force.[79] In addition, cells overexpressing PG also showed increased apoptosis when subjected to uniaxial stress.[13] In conclusion, there is experimental evidence that exercise *in vivo* or induced mechanical force *in vitro* leads to an altered response in desmosomal mutations. How the altered responses eventually lead to ACM is not known but it is a topic of great interest.

4.6 Other proteins involved in ACM

Mutations in other proteins of the area composita (see Section 3) have also been associated with ACM. Recently, rare missense variants in *SCN5A* were identified in 6 out of 287 (2%) ACM patients and hiPSCs were generated to assess the functional consequences of one of these variants (p.Arg1898His). The peak sodium current density was reduced, and a reduced density of Na$_V$1.5 and N-Cadherin at the contact site of the cells was observed.[45]

Another group reported two mutations in αT-catenin protein gene as associated with ACM. This protein is important for integrating the cadherin–catenin complex. *In vitro* studies of one mutation showed a decrease of binding affinity of αT-catenin to β-catenin and PG. Immunofluorescence showed abnormal localization of αT-catenin through the cytoplasm. No translocation was observed for PG or PKP2.
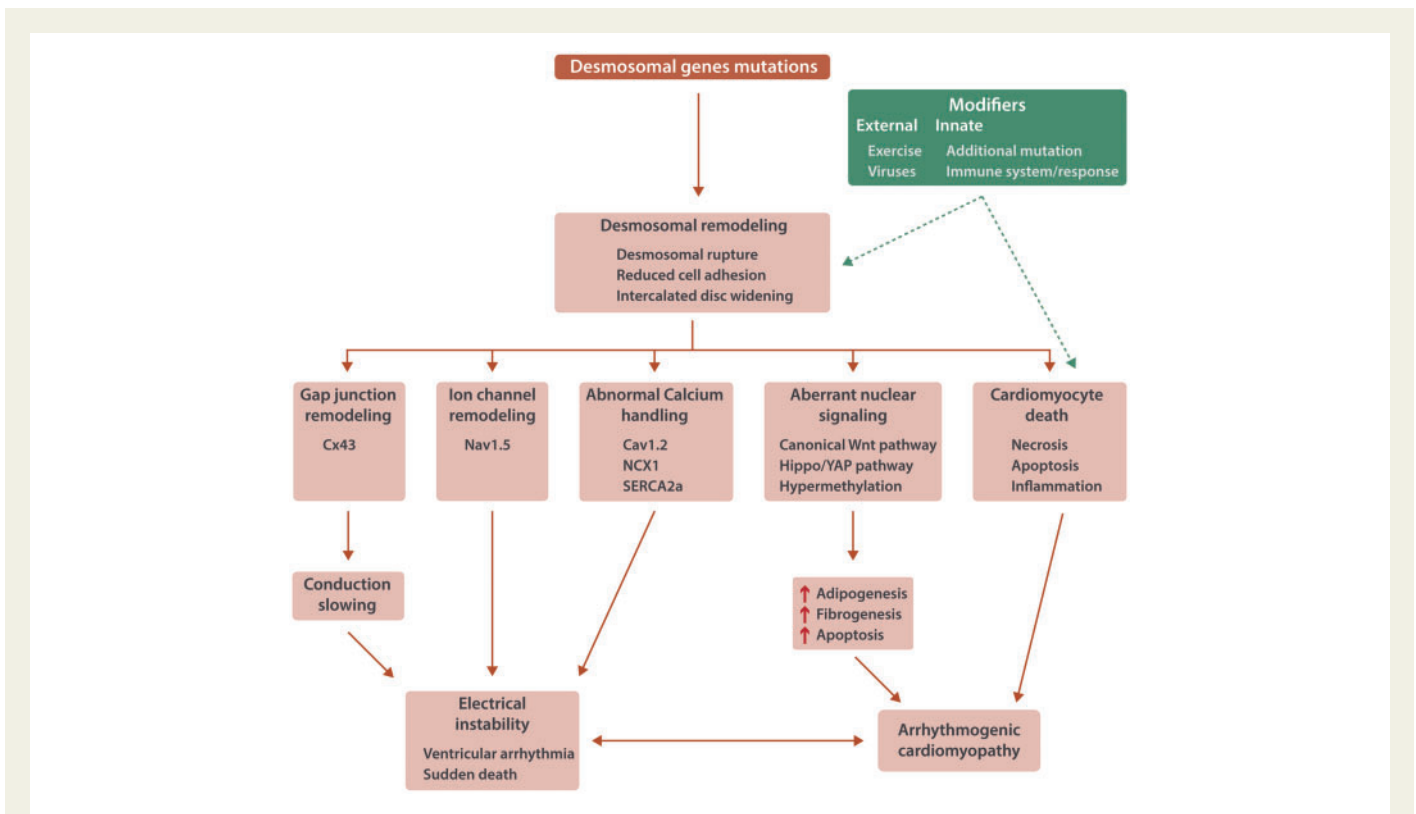
Figure 4 Proposed cellular and molecular cascades underlying arrhythmogenic cardiomyopathy, supported by evidence from experimental models. On the basis of figure from Basso *et al.*[104]

The mechanisms underlying ACM caused by mutations in this gene need further study.[49]

Two new mouse models were generated recently in which Rho-kinase inhibition before birth or deficiency of inhibitor of apoptosis-stimulating protein of p53 (iASPP) led to an ACM phenotype. Similar features to the other ACM mouse models were observed, however, they occurred in the Rho-kinase model only when subjected to inhibition before birth.[101,102] On the basis of these findings, the role of Rho-kinase inhibition and deficiency of iASPP in the pathogenesis of ACM warrants further investigation. Of note, p53 was recently found to be significantly up-regulated in ACM patients.[98]

5. Translational aspects

Besides major breakthroughs in understanding the pathophysiology, modelling the disease in experimental settings has led to the discovery of a possible pharmacological therapy.[13] Via high throughput screens of zebrafish embryos expressing the mutant PG-2057del2, the compound SB216763 improved the cardiac phenotype. This compound, a GSK3β inhibitor, prevents degradation of β-catenin and could therefore enhance the supressed canonical Wnt signalling. It also prevented desmosomal protein and gap junction remodelling in neonatal rat ventricular cardiomyocytes expressing the mutant PG-2057del2 and reversed these processes in hiPSCs with *PKP2* mutations.[13] This compound was later tested in two mice models with different genetic mutations and showed that the adverse cardiac remodelling could be prevented.[94]

New technologies also offer unique possibilities to study and model ACM. Human cardiomyocytes, derived from hiPSC-CMs that are generated from patients, capture the exact genetic background and mutation status of the patient and should therefore model the disease more accurately and in a personalized fashion. However, *in vitro* hiPSC-CMs do not mature and lack the complex environment of the heart *in vivo*. Recently, by introducing hiPSCs-CM (with two different *PKP2* mutations) into neonatal rat hearts *in vivo*, it was shown that hiPSCs-CM can mature into adult cardiomyocytes. These cells captured the disease phenotype, as was shown by ultrastructural abnormalities of the ID, increased apoptosis, and accumulation of fat.[103] This new model means we can now investigate the disease processes underlying ACM with a human and patient-specific genetic mutation in the complex environment of the mammalian heart and it also provides a possible platform for *in vivo* drug testing.

6. Conclusion

Results from experimental and human studies have yielded valuable insights into the pathogenesis of ACM. Impaired mechanical coupling seems to be a uniform finding in the models with different desmosomal mutations. In addition, gap junction and ion channel remodelling seems to play a major role, even before gross structural abnormalities occur, manifesting as electric instability. However, there are models that do capture the electric instability, but they do not show gap junction remodelling, which suggests that these processes need further study. A suppressed Wnt/β-catenin signalling, by nuclear localization of PG, is supported by models with mutations in different desmosomal proteins, although other pathways also contribute to ACM. The proposed cascades leading to ACM and supported by the experimental models are shown in *Figure 4*.

Mutations in non-desmosomal genes are also implicated in ACM; modelling these in an experimental setting could provide more information on the underlying mechanisms. Furthermore, no mutation has been found in most of the sporadic cases of ACM so far. Possible environmental factors (e.g. cardiotropic viruses or endurance exercise) or innate factors (immune system) may play a role, but these require further investigation.

Future research to improve our understanding of how genetic and non-genetic factors interact to trigger disease onset will be key to managing ACM patients. It is critical that we expand our understanding of the molecular mechanisms through which exercise interacts with expression of abnormal protein or reduced protein expression to cause the pathologic features of ACM.

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