

Molecular mechanisms of arrhythmogenic cardiomyopathy

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Abstract | Arrhythmogenic cardiomyopathy is a genetic disorder characterized by the risk of life-threatening arrhythmias, myocardial dysfunction and fibrofatty replacement of myocardial tissue. Mutations in genes that encode components of desmosomes, the adhesive junctions that connect cardiomyocytes, are the predominant cause of arrhythmogenic cardiomyopathy and can be identified in about half of patients with the condition. However, the molecular mechanisms leading to myocardial destruction, remodelling and arrhythmic predisposition remain poorly understood. Through the development of animal, induced pluripotent stem cell and other models of disease, advances in our understanding of the pathogenic mechanisms of arrhythmogenic cardiomyopathy over the past decade have brought several signalling pathways into focus. These pathways include canonical and non-canonical WNT signalling, the Hippo–Yes-associated protein (YAP) pathway and transforming growth factor- β signalling. These studies have begun to identify potential therapeutic targets whose modulation has shown promise in preclinical models. In this Review, we summarize and discuss the reported molecular mechanisms underlying the pathogenesis of arrhythmogenic cardiomyopathy.

Arrhythmogenic cardiomyopathy (ACM) is a heritable disorder characterized by palpitations, syncope and/or cardiac arrest secondary to ventricular tachycardia (VT) or fibrillation; in some patients, ventricular dysfunction and heart failure can also develop¹. Initially considered to be a developmental abnormality of the right ventricle, the disease was originally referred to as arrhythmogenic right ventricular dysplasia². With increasing awareness that its features resemble those of a progressive myocardial disorder rather than a developmental defect, ACM was subsequently named arrhythmogenic right ventricular cardiomyopathy (ARVC)³. Recognition of left ventricular involvement led to the more inclusive term ACM, which incorporates classical right ventricular, left ventricular and biventricular phenotypes⁴.

The prevalence of ACM is estimated to be between 1:1,000 and 1:5,000, depending on the population^{5–8}. The disease shows varied expressivity and reduced, age-related penetrance. Clinical symptoms typically present in the third to fourth decades of life, with arrhythmic manifestations generally preceding structural features. ACM affects adolescents infrequently and children rarely^{9,10}.

Over the past 2 decades, the genetic determinants of ACM have been identified in a subset of patients. Despite the discovery of multiple disease-causing genes, a substantial proportion of patients (35–50%^{11,12}) have

no identifiable disease-associated variant, suggesting a more heterogeneous and complex aetiology, with both polygenic and environmental factors contributing to phenotypic expression. For patients with an identifiable genetic cause, the exact biological mechanisms that underpin this diverse and pleiotropic disease remain poorly characterized. The aim of this Review is to summarize and critically discuss our current understanding of the molecular pathogenesis of ACM.

Clinical features

Early clinical manifestations are typically caused by ventricular arrhythmias and include intermittent and sustained palpitations, arrhythmic syncope and cardiac arrest, which can be the presenting feature¹³. The development of heart failure with associated symptoms typically occurs later in the disease process, although it can be an initial manifestation in a subset of patients. Features suggestive of myocardial inflammation, including chest pain, ST-segment changes on the electrocardiogram (ECG) and elevated serum troponin levels, can also occur, most commonly in desmoplakin-mediated left ventricular disease^{14,15}. Increasingly, asymptomatic relatives with variably penetrant disease can be detected by cascade family screening¹¹.

The electrocardiographic, structural and functional features of ACM reflect progressive myocardial

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Key points

- Arrhythmogenic cardiomyopathy (ACM) is a genetic disorder characterized by the risk of life-threatening arrhythmias, myocardial dysfunction and fibrofatty replacement of myocardial tissue.
- Disease-causing mutations, most commonly in genes encoding desmosomal proteins, can be identified in approximately half of patients with ACM.
- The molecular links between desmosome mutations and the pathological hallmarks of ACM — cardiomyocyte loss, fibrosis, adipogenesis, inflammation and arrhythmogenesis — are under active investigation but remain poorly defined.
- Probable pathogenic mechanisms include loss of mechanical integrity at cell–cell junctions, altered signalling pathways at intercalated discs, disruption of ion channels and gap junctions, and aberrant protein trafficking.
- The development of refined disease models and studies of the molecular pathogenesis of ACM promise to yield novel therapeutic targets and disease treatments.

involvement and vary according to the predominantly affected ventricle or ventricles¹⁶. The diagnosis of ACM can be challenging and mandates a high degree of clinical suspicion in addition to supporting diagnostic evidence. First published in 1995 and most recently updated in 2010, an International Task Force (ITF) has promulgated criteria for clinical diagnosis of ACM to inform the diagnostic process and to improve consistency across research studies¹⁷. In classical right ventricular disease, repolarization (T-wave inversion) and depolarization (terminal activation delay and ϵ -waves) abnormalities occur in the anterior precordial leads, and arrhythmias have a left bundle morphology reflecting right ventricular origin. When other electrocardiographic and structural changes are absent, it can be difficult to differentiate VT associated with ACM from benign idiopathic outflow tract VT¹⁸, a well-tolerated form of ventricular arrhythmia that is observed in patients without associated structural heart disease. VT with a superior QRS axis, thus originating in the inferior right ventricle, is less commonly benign, although it is not specific for ACM and can be seen in other diseases, such as cardiac sarcoidosis^{19,20}. A high burden of ventricular premature beats and nonsustained VT can be seen on ambulatory ECG monitoring. Structural changes rarely occur in the absence of ECG abnormalities. These changes, including right ventricular dilatation, aneurysms, regional wall motion abnormalities, fibrosis, fatty infiltration and reduced ventricular function, are best imaged using cardiac MRI with late gadolinium enhancement, which highlights fibrotic or oedematous tissue.

In left ventricular ACM, T-wave inversion is seen in the anterior and lateral leads on the ECG, and structural changes predominantly affect the left ventricle. The extent of arrhythmia, which typically originates in the left ventricle, can be incongruent with the degree of ventricular dysfunction and dilatation. Similar to right ventricular disease, myocardial thinning, local aneurysm and wall motion abnormalities can be present. Cardiac MRI can be used to identify extensive late gadolinium enhancement in an epicardial and mid-myocardial distribution, which can precede clinical features in children and young adults⁹. This pattern of enhancement can mimic acute infective myocarditis during inflammatory

phases of the disease; therefore, repeat imaging is needed to define ongoing myocardial fibrosis.

Biventricular ACM is defined by active and equal involvement of both ventricles, and patients can have features of both classical and left-dominant disease. Severe biventricular dysfunction can ensue or be evident at presentation. These patients can be diagnosed as having dilated cardiomyopathy with right ventricular involvement, although ventricular ectopy and arrhythmias originating from either ventricle are a cardinal feature¹⁶.

The role of endurance athletics as an environmental modifier has received substantial attention. First recognized in twins and small families in which more severely affected individuals were exposed to higher levels of endurance athletics, exercise has been implicated in disease severity and the risk of ventricular arrhythmias^{10,21}, although the focus has been on those individuals with plakophilin-2-mediated disease and activities with a high dynamic component, defined as exercise that typically increases oxygen uptake to >70% of the predicted maximum (category C)^{22,23}. Different studies have identified varying levels of exercise necessary to worsen the disease phenotype or increase the risk of arrhythmia. Some studies have found that recreational sports including highly dynamic category C activities do not increase the risk of arrhythmia²⁴, whereas other studies have reported increased ongoing risk of arrhythmia with fairly modest levels of exercise²⁵. Together, the studies suggest that highly dynamic exercise might increase ACM severity, but no consensus has emerged as to the level of permissible exercise or the degree to which it elevates risk.

Genetic causes

The known genetic causes of ACM are summarized in TABLE 1 and discussed below.

Desmosomal and junctional gene mutations

Early observations of familial disease clustering suggested a genetic basis for ACM²⁶. Protonotarios and colleagues recognized that individuals on the Greek island of Naxos had a form of ACM in conjunction with a cardiocutaneous syndrome²⁶. In addition to cardiac manifestations, which were 100% penetrant by adolescence²⁷, these individuals had keratoderma and woolly hair that were expressed in infancy. In 2000, genetic linkage analysis identified a homozygous truncating mutation in the *JUP* gene, encoding junction plakoglobin, as the cause of the aptly named Naxos disease. Plakoglobin is a component of desmosomes, a type of intercellular junction present in cardiac muscle and epithelia, such as the skin^{27–29}.

In parallel work, Carvajal-Huerta and colleagues reported a similar syndrome of keratoderma, dry and blister-prone skin, woolly hair and cardiomyopathy in an Ecuadorian family³⁰. Cardiac manifestations of ‘Carvajal syndrome’ predominantly involved the left ventricle and resulted in a dilated cardiomyopathy³¹. Similar to Naxos disease, genetic analysis found a causative homozygous truncating mutation in another desmosome gene, *DSP*, encoding desmoplakin.

These foundational discoveries sparked candidate-gene sequencing of other desmosome genes in patients with ACM. In addition to *JUP* and *DSP*, both truncating and missense mutations in the desmosome genes *PKP2* (encoding plakophilin 2), *DSG2* (encoding desmoglein 2) and *DSC2* (encoding desmocollin 2) have been identified in patients with ACM^{31–34} (TABLE 1). Approximately half of patients with ACM have mutations in one or more of these desmosomal genes^{35,36}. *PKP2* is the most commonly affected gene in adult cohorts^{10,32}, whereas some studies have suggested that the paediatric age group more frequently has mutations in *DSP*^{9,10}. These paediatric patients also seem to be more likely to harbour compound heterozygous mutations^{9,37}. Autosomal dominant inheritance with incomplete penetrance is the most common mode of transmission, although autosomal recessive mutations, such as those that cause Naxos disease and Carvajal syndrome, have been described.

Cardiomyocytes form structural and electrical connections via desmosomes, adherens junctions and gap

junctions, which all occur in mixed-type junctions, named area composita, located at the intercalated disc^{38,39}. One important function of desmosomes is to tether adjacent cells mechanically by joining their intermediate filaments to create a unified cytoskeletal network³⁹ (FIG. 1). The unique and overlapping structure of mixed-type junctions prompted sequencing of genes encoding other components of the intercalated disc in patients with ACM. Mutations in the adherens junction genes *CDH2* (encoding cadherin 2, also known as N-cadherin)^{40,41} and *CTNNA3* (encoding catenin- α 3)⁴² have recently been identified in patients with ACM. At adherens junctions, classical cadherins such as cadherin 2 join neighbouring cardiomyocytes through interactions with the actin cytoskeleton via catenin- α 3 and a paralogue, catenin- α 1, encoded by *CTNNA1* (REFS^{42,43}). Interestingly, this link requires either plakoglobin (also known as catenin- γ ; ~80% homology), also found in desmosomes and mutated in some patients with ACM, or its paralogue, catenin- β 1. Furthermore, catenin- α 3

Table 1 | Genes associated with ACM

Gene	Protein	Estimated frequency (%)	Features	Refs
Desmosome				
<i>PKP2</i>	Plakophilin 2	19–46	Most commonly mutated	11,35,36,55,226
<i>DSP</i>	Desmoplakin	1–16	AR mutation associated with Carvajal syndrome; C-terminal mutations associated with LV-dominant disease	9,226
<i>DSG2</i>	Desmoglein 2	2.5–10.0	Overlap with DCM phenotype	226–228
<i>DSC2</i>	Desmocollin 2	1–8	AR (without cutaneous manifestations) and AD inheritance	33,226,229,230
<i>JUP</i>	Junction plakoglobin	Rare–1	AR mutation associated with Naxos disease	43,226,231
Adherens junction				
<i>CTNNA3</i>	Catenin- α 3	Rare	Incomplete penetrance; normal plakoglobin localization	42
<i>CDH2</i>	Cadherin 2	Rare–2	No specific genotype–phenotype relationship identified	40,41
Cytoskeletal structure				
<i>LMNA</i>	Lamin A/C	Rare	More common in severe forms of ACM with a dilated phenotype and high risk of sudden cardiac death	58
<i>DES</i>	Desmin	Rare	Fully penetrant; associated with LV-dominant ACM, DCM and skeletal myopathies	45
<i>FLNC</i>	Filamin C	Rare	Associated with LV-dominant ACM and high rates of arrhythmia	67
<i>TMEM43</i>	Transmembrane protein 43	Rare	Fully penetrant; men more severely affected than women	60
<i>TTN</i>	Titin	Rare	Higher risk of supraventricular tachycardia and progression to heart failure	66,232
Ion transport				
<i>RYR2</i>	Ryanodine receptor 2	Rare	Mutations in regions of calcium channel regulation	72
<i>SCN5A</i>	Na _v 1.5	Rare–2	Prolonged QRS interval	78
<i>PLN</i>	Phospholamban	Rare	Clinical overlap with DCM	48
Cytokine				
<i>TGFB3</i>	Transforming growth factor- β 3	Rare	No specific genotype–phenotype relationship identified	79

ACM, arrhythmogenic cardiomyopathy; AD, autosomal dominant; AR, autosomal recessive; DCM, dilated cardiomyopathy; LV, left ventricle.

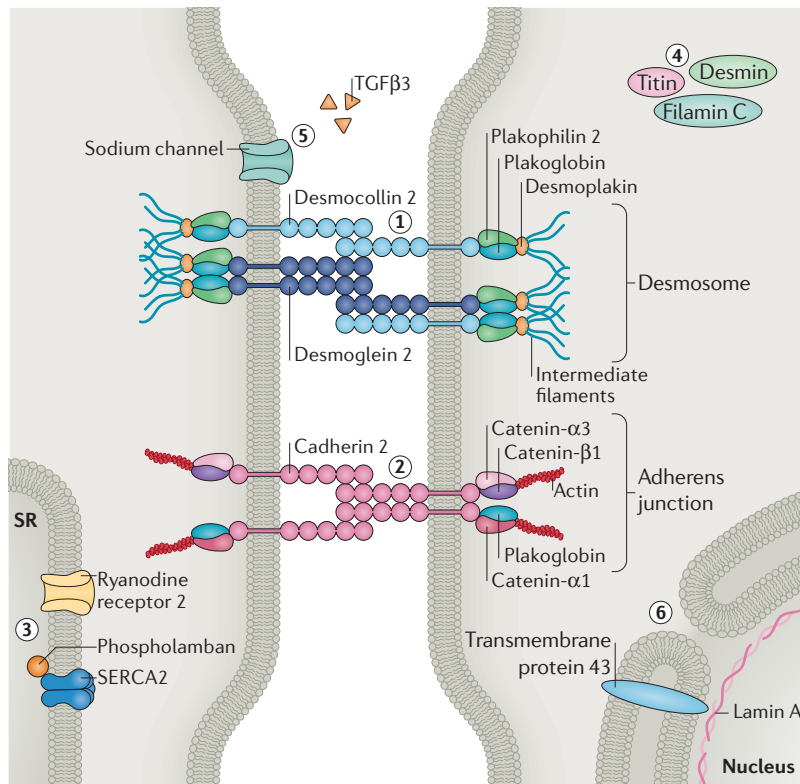


Fig. 1 | Cellular components implicated in ACM. The intercalated disc of cardiomyocytes contains the area composita, which is an intermixed architectural and signalling structure that includes components of the desmosome, adherens junction and ion channels. Mutations in cellular components of the intercalated disc, as well as in intracellular structures, have been identified in arrhythmogenic cardiomyopathy (ACM). Categories of protein in which ACM-causing mutations occur are labelled accordingly. (1) Components of the desmosome, including desmocollin 2, desmoglein 2, junction plakoglobin, plakophilin 2 and desmoplakin. (2) Components of the adherens junction, including cadherin 2 and catenin- α 3. (3) Contributors to calcium handling, including phospholamban and ryanodine receptor 2 located in the membrane of the sarcoplasmic reticulum (SR). (4) Intracellular structural proteins, including desmin, titin and filamin C. (5) The sodium channel and transforming growth factor- β 3 (TGF β 3). (6) Nuclear envelope proteins transmembrane protein 43 and lamin A. SERCA2, sarcoplasmic/endoplasmic reticulum calcium ATPase 2.

directly interacts with plakophilin 2 at the cardiomyocyte intercalated disc, which implies that this protein participates in a hybrid junction to strengthen cell–cell interactions⁴⁴. In ACM, disease-causing mutations that disrupt interactions in the area composita might destabilize and alter tethering or signalling functions of the intercalated disc.

Non-junctional gene mutations

Although desmosome gene mutations are by far the most prevalent and well validated of the genetic variants associated with ACM, mutations in non-desmosome genes have also been described. These mutations reside in genes encoding proteins with a diverse range of biological functions, including cytoskeletal architecture, calcium handling, sodium transport and cytokine signalling^{45–49}. After desmosomal genes, genes encoding cytoskeleton-associated proteins constitute the second-largest category of ACM-associated mutations. Cytoskeletal defects might alter the structural integrity and mechanotransduction

of cardiomyocytes, mirroring a proposed mechanism of desmosome mutations in ACM^{50,51}. Through candidate-gene and whole-exome sequencing, novel mutations in *DES* (encoding desmin), *LMNA* (encoding lamin A), *TMEM43* (encoding transmembrane protein 43), *TTN* (encoding titin) and *FLNC* (encoding filamin C) have been identified in patients with ACM.

Cytoskeletal proteins. Desmin is an intermediate filament expressed in cardiomyocytes and other muscle cells. These filaments act as bridges that connect Z-discs of sarcomeres to sarcolemmal costameres and desmosomes and to the nuclear envelope⁵². Through these interactions, desmin coordinates movements of neighbouring Z-discs with the nuclear and plasma membranes⁵³. Desmin mutations are associated with skeletal myopathies as well as dilated and restrictive cardiomyopathies⁴⁵. Evidence now suggests that particular desmin mutations are associated with distinct forms of ACM^{45,54,55}.

The LINC (linker of nucleoskeleton and cytoskeleton) complex tethers the nuclear envelope to the cytoskeleton. Major components of this complex, the A-type and B-type lamins, form a meshwork of intermediate filaments immediately below the inner nuclear membrane. In addition to their structural function, lamins participate in chromatin organization, DNA replication and gene expression⁵⁶. Lamin mutations result in a spectrum of clinical diseases that includes myopathies, lipodystrophy and progeria^{47,57}. Lamins were first suggested as a genetic cause of ACM by Quarta and colleagues in 2012, when they noted four unique *LMNA* mutations while genetically screening patients with ACM⁵⁸. Forleo and colleagues later identified a novel *LMNA* duplication in a family affected by a number of arrhythmogenic phenotypes, including ACM⁴⁷.

Transmembrane protein 43, also known as protein LUMA, is a conserved inner nuclear membrane protein with an otherwise poorly understood function. Transmembrane protein 43 has been shown to associate with lamins and other LINC complex components and is implicated in nuclear membrane organization⁵⁹. *TMEM43* mutations have been suspected in cases of mutation-negative muscular dystrophy. A *TMEM43*-p.S358L missense mutation was unequivocally identified as the disease-causing mutation in an extended family from Newfoundland, Canada⁶⁰. Owing to a founder effect, this mutation is a frequent cause of ACM-related heart disease in this genetically isolated population^{59–61}. Independent ACM-causing *TMEM43* mutations have also been reported in other populations⁶².

Titin is the largest human protein and functions as a bidirectional spring, generating passive stiffness in cardiomyocytes⁶³. *TTN*-truncating mutations are considered to be the most common genetic cause of dilated cardiomyopathy⁶⁴, although *TTN* is also located near a genetic locus (2q31.1-p32.2) that has been implicated in a subgroup of patients with ACM⁶⁵. Probands from 38 families with a clinical diagnosis of ACM were screened for *TTN* variants, identifying a missense mutation (*TTN*-p.T2896I) that showed strong segregation in nine confirmed or obligate patients with

ACM⁶⁶. Functional analysis of the mutant titin protein highlighted small alterations in folding kinetics that allowed for increased susceptibility to proteolysis and degradation⁶⁶. Structural impairment of the titin spring, through protease vulnerability, is now considered a potential cause of ACM.

Filamins are a family of proteins that crosslink actin filaments and anchor membrane-associated proteins to the cytoskeleton. Consequently, filamins contribute to both structural stability and membrane-triggered signal transduction⁵³. Filamin C (also known as γ -filamin) associates with Z-discs, and mutations have been linked to skeletal myopathies as well as dilated and restrictive cardiomyopathies^{67,68}. Truncating mutations in *FLNC* have been linked to a left-dominant form of ACM. Patients had a high prevalence of ventricular arrhythmias (82%) and had signs of fibrosis on either MRI or histological evaluation⁶⁷.

Calcium-handling proteins. Calcium homeostasis is critical for cardiomyocyte function. In addition to regulating excitation–contraction coupling, calcium levels also influence cardiac electrophysiology. Improper calcium handling can become a substrate for arrhythmogenesis by triggering aberrant depolarizations. Mutations in genes that regulate calcium homeostasis have also been implicated in ACM, highlighting a potential mechanism for arrhythmogenesis. Phospholamban (encoded by *PLN*) is a transmembrane protein of the sarcoplasmic reticulum that regulates calcium handling by inhibiting the activity of the sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA2; encoded by *ATP2A2*)⁶⁹. Following adrenergic stimulation, protein kinase A phosphorylates phospholamban and reduces its braking activity on SERCA2, resulting in augmented calcium handling⁶⁹. *PLN* mutations have been associated with dilated, hypertrophic and arrhythmic cardiomyopathies. A 3 bp deletion leading to removal of R14 in phospholamban has been reported to cause both dilated cardiomyopathy and ACM, probably reflecting a spectrum of disease and clinical overlap between these diagnostic categories^{70,71}.

The ryanodine receptor 2 (encoded by *RYR2*) is the major cardiomyocyte intracellular calcium-release channel. Located on the sarcoplasmic reticulum, ryanodine receptor 2 is responsible for calcium-induced calcium release into the cytosol, which generates calcium transients to trigger sarcomere contraction⁷². Genetic mapping of a cohort of four Italian families with ACM found missense mutations in the *RYR2* gene that led to amino acid substitutions in a highly conserved cytosolic region of the protein⁷². A second study undertook systematic screening of patients with ACM without an identified mutation in a desmosomal gene and found that 9% had putative *RYR2* mutations⁷³. Previously, gain-of-function mutations in *RYR2* had been associated with catecholaminergic polymorphic VT (CPVT), whereas a rare loss-of-function mutation has been implicated in an overlapping syndrome of left ventricular noncompaction and CPVT^{74,75}. Whether *RYR2* mutations cause ACM is controversial, because identification of *RYR2* mutations in ACM is likely to reflect clinical diagnostic overlap between ACM and CPVT^{12,76}.

Sodium-transport proteins. Voltage-gated sodium channels are integral for action potential initiation and propagation⁷⁷. Mutations in *SCN5A*, which encodes the pore-forming subunit of the sodium channel $\text{Na}_v1.5$, have been associated with a number of arrhythmogenic disorders including Brugada, long QT and sick sinus syndromes⁷⁷. *SCN5A* mutations have been implicated in a small number of patients with ACM. In an ACM cohort without an identified desmosomal gene mutation, whole-exome sequencing identified a rare *SCN5A* missense variant that altered channel activity⁷⁸. Furthermore, *SCN5A* sequencing in 281 patients with ACM identified five patients (1.8%) with presumed damaging mutations, either with (two patients) or without (three patients) pathogenic desmosome variants. These data suggest that *SCN5A* pathogenic variants are associated with ACM phenotypes.

Cytokine signalling proteins. Finally, the profibrotic cytokine transforming growth factor- β 3 (TGF β 3; encoded by *TGFB3*) has been associated with distinct forms of ACM. Genetic analysis of three families with ACM linked the 14q24.3 locus to the disease phenotype⁶. Within this locus, *TGFB3* is an attractive candidate gene, given the pro-inflammatory and profibrotic activities of TGF β 3. However, sequencing did not identify coding-region variants that linked to ACM. Further analysis found a rare, non-coding G>A mutation in the 5' untranslated region (UTR) of *TGFB3* that co-segregated with the disease in all affected family members and increased translation of a reporter gene⁷⁹. An isolated case of an unrelated patient with ACM was also found to have a rare variant in the 3' UTR of *TGFB3*. Nonetheless, the other two pedigrees in this study lacked *TGFB3* variants, and additional *TGFB3* variants have not been reported. Interestingly, TGF β 3 is the only secreted protein implicated in the pathogenesis of ACM so far and would represent the first evidence suggesting a mechanism involving paracrine and autocrine signalling. Overall, these data suggest that *TGFB3*, or a closely linked locus, is associated with rare cases of ACM.

Histological features

The pathological hallmark of ACM is fibrofatty replacement of myocardial tissue with associated ventricular atrophy⁴³. The ITF diagnostic criteria for ACM specify the major histological criteria as residual cardiomyocyte number <60% of normal by morphometric analysis with fibrous replacement of the right ventricular free wall myocardium¹⁷. Although fibrofatty myocardial replacement is characteristic of ACM, the requirement for this finding on endomyocardial biopsy samples was dropped from the 2010 ITF criteria to avoid false negatives that arise from imperfect sampling in endomyocardial biopsies. This modification would also include patients with cardiomyocyte loss and fibrosis but without fatty infiltration. For instance, Carvajal syndrome, caused by homozygous *DSP* mutation, features cardiomyocyte loss and fibrosis without fatty involvement^{80–82}. Moreover, fibrofatty infiltration, although a classic feature of ACM, is not unique to this disease and has also been reported in myotonic dystrophy⁸³ and myocardial infarction^{84,85}.

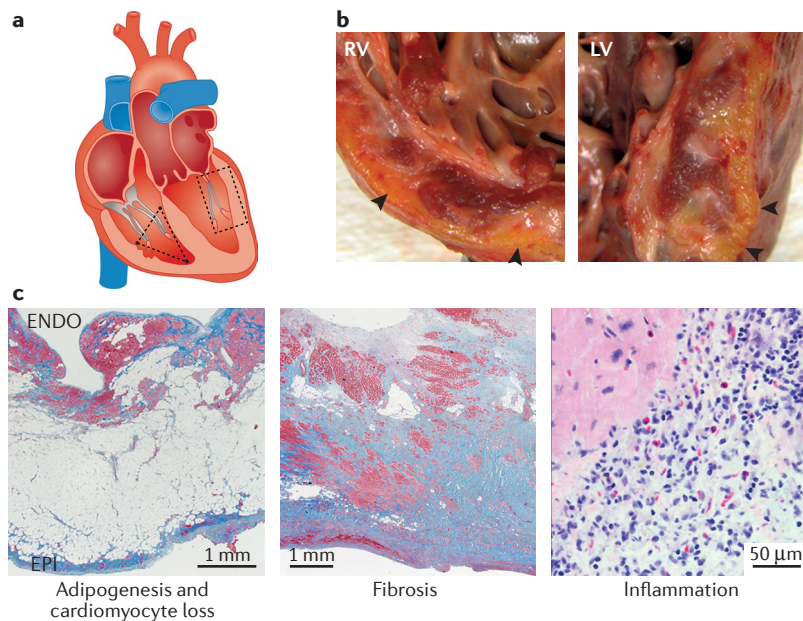


Fig. 2 | Gross and histological features of ACM. **a** | Illustration depicting the most commonly affected ventricular regions in arrhythmogenic cardiomyopathy (ACM). Right ventricular disease predominantly affects the inflow tract, apex and infundibulum, known as the triangle of dysplasia (dashed triangle). Left-dominant disease commonly affects the inferior and inferolateral walls (dashed rectangle). **b** | Gross images of the right ventricle (RV) and left ventricle (LV), highlighting epicardial fat deposition (black arrowheads). **c** | Histological features of ACM including adipogenesis and cardiomyocyte replacement (left; trichrome stain), fibrosis (middle; trichrome stain) and inflammation juxtaposed to myocardial tissue (right; haematoxylin and eosin stain). ENDO, endocardium; EPI, epicardium.

Myocardial involvement is predominantly concentrated in the right ventricle, although biventricular⁸⁶ and even predominantly left ventricular patterns are also seen, often in patients with *DSP*-truncating mutations^{31,87}. The affected chamber is typically globally, or regionally, dilated and thin-walled. The distribution of myocardial involvement varies from patchy and localized to diffuse, with localized remodelling predisposing to cardiac aneurysms. The regions most commonly affected are the right ventricular inflow tract, apex and infundibulum — an area referred to as the triangle of dysplasia² (FIG. 2).

Histologically, the disease progresses in an outside-in fashion, affecting the subepicardial tissue first and extending towards the endocardium, eventually resulting in a thinned, transmural lesion⁴³. Multifocal inflammatory infiltrates are common in ACM and often include interstitial concentrations of mononuclear cells located near necrotic or damaged cardiomyocytes⁸⁶. Inflammatory infiltrates are often observed in both ventricular free walls, even in hearts with macroscopically right-ventricle-dominant disease⁸⁶.

Disease models

The study of mechanisms underlying ACM progression requires experimental systems to model the disease. No system recapitulates all the features of the human disease, which has impeded progress in dissecting the pathogenesis of ACM. Owing to space limitations, we cannot comprehensively review all the model systems that have been

developed (TABLE 2). Instead, we discuss several major approaches and their strengths and limitations.

Animal models

Mouse models include null, conditional and mutant alleles of affected genes. Homozygous deletion of *Dsp*, *Jup* or *Pkp2* in mice is embryonically lethal during mid-gestation as a result of cardiac wall rupture, presumably owing to defects in cellular adhesion and mechanical stability^{88–90}. Although these null alleles emphasize the vital importance of desmosomes, embryonic lethality precludes disease modelling. Mice haploinsufficient for the aforementioned genes, which is more representative of genetic findings in patients with ACM, are viable and show a proclivity towards cardiac electrical abnormalities, including spontaneous, exercise-induced and/or drug (flecainide)-induced arrhythmias^{91–93}.

To circumvent embryonic lethality and promote more robust phenotypes, numerous conditional or transgenic overexpressing mouse models have been reported (TABLE 2). Most of these models display two or more features of ACM, including ventricular dysfunction, electrical abnormalities, gap junction mislocalization, myocardial cell death and inflammation and to a lesser extent subepicardial fat accumulation^{50,94–103}. The fairly short lifespan of mice also offers an opportunity to study transitions from asymptomatic or ‘concealed’ phenotypes to overt structural damage. For example, Cerrone and colleagues reported that isoprenaline-induced VTs precede severe muscle disease in mice with inducible *Pkp2* deletion⁹⁴. More severe phenotypes might also represent later stages along a continuum of disease progression. Indeed, transgenic mice expressing mutant *Dsg2* have myocardial necrosis and inflammation but subsequent to structural damage⁵⁰.

Although mouse models have provided insights, limitations do exist. A less-understood feature of ACM is the replacement of myocardial tissue with adipocytes. Unfortunately, modelling this phenomenon has proved to be a difficult endeavour in mice. Murine models of ACM do have some subepicardial adipocyte infiltration^{100,102,104}, but far less than is commonly seen in patients with ACM. This finding parallels the natural development of epicardial adipose tissue, which is abundant in the human heart and scarce in mice¹⁰⁵. Mice have been used to study the mechanisms that promote adipogenesis in ACM^{106–108}, but the extent to which these insights relate to the human disease is uncertain. Larger-animal models with epicardial adipose tissue might help to resolve the complex nature of adipogenesis in human hearts.

Cell culture models

In addition to animal models, cell culture has been used to investigate the molecular mechanisms underlying ACM. Cardiomyocytes have long been proposed to be major contributors to ACM progression given their desmosome-rich adhesions and electrical properties. Unfortunately, adult cardiomyocytes are difficult to study for >1–2 days in vitro owing to their limited proliferative capacity, low viability and phenotypic dedifferentiation in cell culture. To circumvent these difficulties, surrogate cell types have been used to study

ACM in vitro, including the HL-1 cell line, neonatal rat ventricular myocytes (NRVMs) and cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs). HL-1 cells are an immortalized atrial cardiac cell line derived from transgenic mice expressing the SV40 T-antigen under the control of the atrial natriuretic factor promoter¹⁰⁹. This atrial tumour lineage maintains a differentiated state with passaging, forms myofibrils and has nascent intercalated discs¹⁰⁹. These features make HL-1 attractive for in vitro studies, but caveats do exist. ACM is predominantly a disease of the ventricles, whereas HL-1 cells are a proliferative, tumour-derived atrial lineage that does not acquire the phenotypic hallmarks of mature ventricular cardiomyocytes and that requires adrenergic stimulation to preserve a differentiated state¹⁰⁹.

The development of hiPSC technology by Yamanaka and colleagues¹¹⁰ has provided an unprecedented opportunity to model human diseases using patient-derived cell models. Using a cocktail of reprogramming factors, patient-derived somatic cells, such as skin fibroblasts or peripheral blood mononuclear cells, are converted into hiPSCs, which can be maintained indefinitely in culture. hiPSCs are then directed to differentiate into other cell types, including cardiomyocytes^{111,112}. Genetic malleability of hiPSCs through CRISPR–Cas9 genome editing further amplifies their power for in vitro studies of human disease. Despite these strengths, at present, hiPSC-CMs also have important limitations. A major limitation is the immaturity of hiPSC-CMs, which resemble late fetal or neonatal cardiomyocytes. Given that ACM develops in mature cardiomyocytes, this limitation has been a major hurdle to the use of hiPSC-CMs to model many physiological aspects of the disease. Advances in tissue engineering, including ‘heart-on-chip’ tissues¹¹³ and electrically stimulated engineered heart tissues^{114,115}, might offer strategies to improve hiPSC-CM maturation for modelling the pathogenesis of ACM.

Pathogenesis

Large gaps remain in our understanding of the molecular pathogenesis of ACM. The major features of the disease that must be linked to pathogenic mutations are cardiomyocyte loss, fibrosis, adipogenesis, inflammation and arrhythmogenesis. We review the current knowledge on how desmosomal gene mutations cause this pathology of ACM manifestations (FIG. 3).

Cardiomyocyte loss

Cardiomyocyte loss is a characteristic feature of ACM, which results in loss of cardiac muscle and myocardial free wall thinning¹⁷. Extensive histological evaluation of 30 hearts from patients with ACM demonstrated cardiomyocyte death with surrounding patchy inflammation and fibrofatty replacement, resulting in substantial atrophy¹¹⁶. These histological data, combined with the adult presentation and progressive disease course, provided support for the degenerative model of ACM pathogenesis¹¹⁶. Furthermore, in mouse models of ACM, hearts are typically normal in early life (<2 weeks) and subsequently accumulate hallmark features of ACM^{50,101}. Taken together, the loss of cardiomyocytes

from ventricular tissue is an important step in the disease process.

Cardiomyocyte loss could result from irrevocable cellular injury, such as loss of sarcolemmal integrity or damage by inflammatory mediators, or by activation of programmed cell death pathways, such as apoptosis or necroptosis. Given the role of desmosomes in mechanical coupling, injury due to contraction against weakened intercellular junctions emerges as an obvious mechanism. Pathological examination of ACM samples has shown abnormal junctional protein expression and localization at intercalated discs, which has been recapitulated in animal and hiPSC models^{45,58,70,104,117–119}. Furthermore, study of these tissues has demonstrated loss of sarcolemmal integrity, consistent with mechanical injury^{50,51}. In cultures of epithelial or HL-1 cardiomyocyte-like cells, reduction in desmosome proteins or overexpression of some ACM-causing mutations decreased cell–cell adhesion^{120,121}. Exercise training, an environmental stress that increases mechanical stress on the heart, has been implicated in accelerating disease progression in patients^{122,123} and similar findings have been made in murine models^{102,124}. These observations are consistent with a mechanism of mechanical cardiac injury, although they could also reflect aberrant transduction of mechanical signals by mutant desmosomes.

Numerous studies have demonstrated the presence of apoptotic cells in patient samples, indicating that programmed cell death via apoptosis is likely to contribute to cardiomyocyte loss in ACM^{125–127}. Similarly, hiPSC-CM models of ACM have shown a threefold increase in apoptosis in the context of widened desmosomal gaps¹¹⁸. Mouse models with either overexpression of mutant desmoplakin or conditional *Dsp* knockout have grossly abnormal cell–cell interfaces in conjunction with increased TUNEL (TdT-mediated dUTP nick end labelling)-positive staining for apoptosis^{102,103}. Accumulation of cleaved poly(ADP-ribose) polymerase (PARP), a direct assay for apoptosis, has also been observed in the hearts of cardiomyocyte-restricted *Jup*-mutant mice⁵¹. Furthermore, evaluation of mRNA transcripts from the myocardial tissue of patients with ACM has shown significantly higher expression levels of *PERP*, which encodes a protein that is present in the desmosomes of cardiomyocytes and that contributes to the initiation of p53-dependent apoptosis^{128–131}.

Apoptosis in some models of ACM has been linked to abnormal transduction of mechanical signals. In NRVMs, overexpression of mutant plakoglobin proteins did not alter cell adhesion but promoted shear stress or cyclic-stretch-induced apoptosis^{132,133}. This finding was associated with aberrant nuclear localization of plakoglobin, which has been reported to antagonize WNT–catenin- β 1 signalling in ACM¹⁰⁰ and other contexts¹³⁴. A screen for small molecules that increase survival of a zebrafish model of Naxos disease identified SB21676, which inhibits glycogen synthase kinase 3 β (GSK3 β)¹³³. Because GSK3 β promotes catenin- β 1 degradation, one effect of its inhibition is increased WNT–catenin- β 1 signalling¹³³. Interestingly, SB21676 normalized plakoglobin localization, prevented both stress-induced and stretch-induced apoptosis and

Table 2 | Summary of animal or cell-based models used to investigate ACM

Genetic manipulation	Model	Phenotypes reported														Refs												
		Embryonic or perinatal lethality	Sudden death	RV-dominant	LV-dominant or biventricular	Wall thinning	Spontaneous arrhythmias or PVCs	Induced arrhythmias or PVCs	QRS prolongation	Cell death	Fibrosis and/or fibrotic GEP	Lipid accumulation and/or GEP	Calcification	Inflammation	Nuclear or cytosolic JUP		Upregulated or nuclear PPAR γ	Ultrastructural intercalated disc defects	Altered desmosome expression	Altered desmosome interactions	Altered cell–cell adhesion	Altered connexin 43 localization	Altered sodium current	Altered potassium current	Altered calcium handling	Altered WNT–GSK3 β signalling	Altered Hippo–YAP signalling	Altered cell metabolism
Pkp2																												
<i>Pkp2</i> ^{-/-}	Mouse	•																										90
<i>Pkp2</i> ^{+/-}	Mouse						•																•					93
<i>Pkp2</i> ^{fllox} Myh6–CreERT2	Mouse		•		•		•			•												•		•				94
AAV-cTNT- <i>Pkp2</i> R735X	Mouse		•		•																	•						233
<i>Pkp2</i> siRNA	HL-1																					•						199
<i>Pkp2</i> -C796R; <i>Pkp2</i> -C796M	HL-1																					•						234
<i>Pkp2</i> shRNA	HL-1										•					•										•	•	104
<i>Pkp2</i> shRNA	HL-1																					•						235
<i>Pkp2</i> shRNA	NRVM																					•						201
<i>Pkp2</i> siRNA	NRVM									•	•											•						144
<i>Pkp2</i> c.1841T>C	iPSC										•											•						236
<i>Pkp2</i> c.972InsT/N; <i>Pkp2</i> c.148_151delACAG/N	iPSC															•	•	•				•						118
<i>Pkp2</i> c.2484C>T; <i>Pkp2</i> c.2013delC	iPSC								•		•											•		•				237
<i>Pkp2</i> c.2484C>T	iPSC								•		•				•									•	•		•	151
<i>Pkp2</i> siRNA	Epicardial																					•						201
<i>Pkp2</i> siRNA	Epicardial										•																	238
Dsp																												
<i>Dsp</i> ^{-/-}	Mouse	•																										89
<i>Dsp</i> ^{+/-}	Mouse						•																•					92
Tg(Myh6)- <i>Dsp</i> -R2834H	Mouse				•	•			•	•	•											•						103
<i>Dsp</i> ^{fllox} Mlc2v–Cre	Mouse				•	•	•	•	•	•	•											•						102
<i>Dsp</i> ^{fllox} Myh6–Cre	Mouse	•	•		•	•	•	•	•	•	•				•										•			100
<i>dsp</i> morpholino	Zebrafish																								•	•		154
<i>Dsp</i> siRNA	HL-1										•													•				100
<i>Dsp</i> siRNA	HL-1																					•	•					239
<i>Dsp</i> siRNA; <i>Dsp</i> -N287K, N458Y, K470E, I533T, A566T	NHEK and NRVM																					•						207
Dsc2																												
Tg(Myh6)- <i>Dsc2</i>	Mouse									•		•	•															95
<i>Dsc2</i> -E102K; <i>Dsc2</i> -I345T	HL-1																					•						240
<i>Dsc2</i> -Q851fsX855; <i>Dsc2</i> -A897fsX900	HL-1 and NRVM																						•					241
<i>Dsc2</i> -Q554X	HeLa and HEK																					•						242

Table 2 (cont.) | Summary of animal or cell-based models used to investigate ACM

Genetic manipulation	Model	Phenotypes reported																Refs										
		Embryonic or perinatal lethality	Sudden death	RV-dominant	LV-dominant or biventricular	Wall thinning	Spontaneous arrhythmias or PVCs	Induced arrhythmias or PVCs	QRS prolongation	Cell death	Fibrosis and/or fibrotic GEP	Lipid accumulation and/or GEP	Calcification	Inflammation	Nuclear or cytosolic JUP	Upregulated or nuclear PPAR γ	Ultrastructural intercalated disc defects		Altered desmosome expression	Altered desmosome interactions	Altered cell–cell adhesion	Altered connexin 43 localization	Altered sodium current	Altered potassium current	Altered calcium handling	Altered WNT–GSK3 β signalling	Altered Hippo–YAP signalling	Altered cell metabolism
Dsg2																												
<i>Dsg2</i> ^{-/-}	Mouse	•																										243
<i>Dsg2</i> ^{-/-}	Mouse			•			•	•	•																			98
<i>Dsg2</i> ^{flox} Myh6–Cre	Mouse		•		•		•	•	•				•															101
Tg(Myh6)- <i>Dsg2</i> -N271S	Mouse		•		•	•	•	•	•				•	•														96
<i>Dsg2</i> siRNA; <i>Dsg2</i> -D154E, D264E, N266S, D494N, A517V, G812C, G812S, C813R, V920G	HL-1																			•								121
Jup																												
<i>Jup</i> ^{-/-}	Mouse	•																										88
<i>Jup</i> ^{+/-}	Mouse			•		•	•																					91
Tg(Myh6)- <i>Jup</i> truncated	Mouse			•		•	•			•	•			•	•					•						•	•	104
<i>Jup</i> ^{flox} Myh6–CreERT2	Mouse	•			•				•	•				•							•						•	51
Tg(<i>jup</i> c.2157del2)	Zebrafish		•						•					•	•						•	•	•				•	133
<i>Jup</i> c.2157del2 (OriNax)	Mouse	•																										244
<i>Jup</i> c.2157del2 (FuseNax)	Mouse																											244
<i>Jup</i> c.2157del2	Mouse						•		•	•				•												•		98
<i>Jup</i> S39_K40insS	HEK293																				•							245
<i>Jup</i> S39_K40insS; <i>Jup</i> c.2157del2	HEK293																				•		•					120
Tmem43																												
<i>Tmem43</i> ^{-/-}	Mouse																											246
<i>Tmem43</i> S358L	Mouse																											246
Lmna																												
<i>Lmna</i> c.418_438dup	HL-1									•																		47
Flnc																												
<i>Flnc</i> p.G1891Vfs61X	hBMC																				•						•	247
Rock2^a																												
DN-Rock2	Mouse		•		•	•	•		•																			99
Ppp1r13l^a																												
<i>Ppp1r13l</i> ^{-/-}	Mouse		•	•			•		•	•											•	•						97

Bullet points indicate a role for a given gene in a reported phenotype. ACM, arrhythmogenic cardiomyopathy; DN, double negative; flox, conditional allele; GEP, gene-expression programme; GSK3 β , glycogen synthase kinase 3 β ; hBMC, human buccal mucosa cell; HEK, human embryonic kidney cell; iPSC, induced pluripotent stem cell; JUP, junction plakoglobin; LV, left ventricle; NHEK, normal human epidermal keratinocyte; NRVM, neonatal rat ventricular myocyte; PPAR γ , peroxisome proliferator-activated receptor- γ ; PVC, premature ventricular contraction; RV, right ventricle; shRNA, short hairpin RNA; siRNA, small interfering RNA; Tg, transgenic; YAP, Yes-associated protein. ^aACM-like phenotype; no known ACM-causing mutations in these genes.

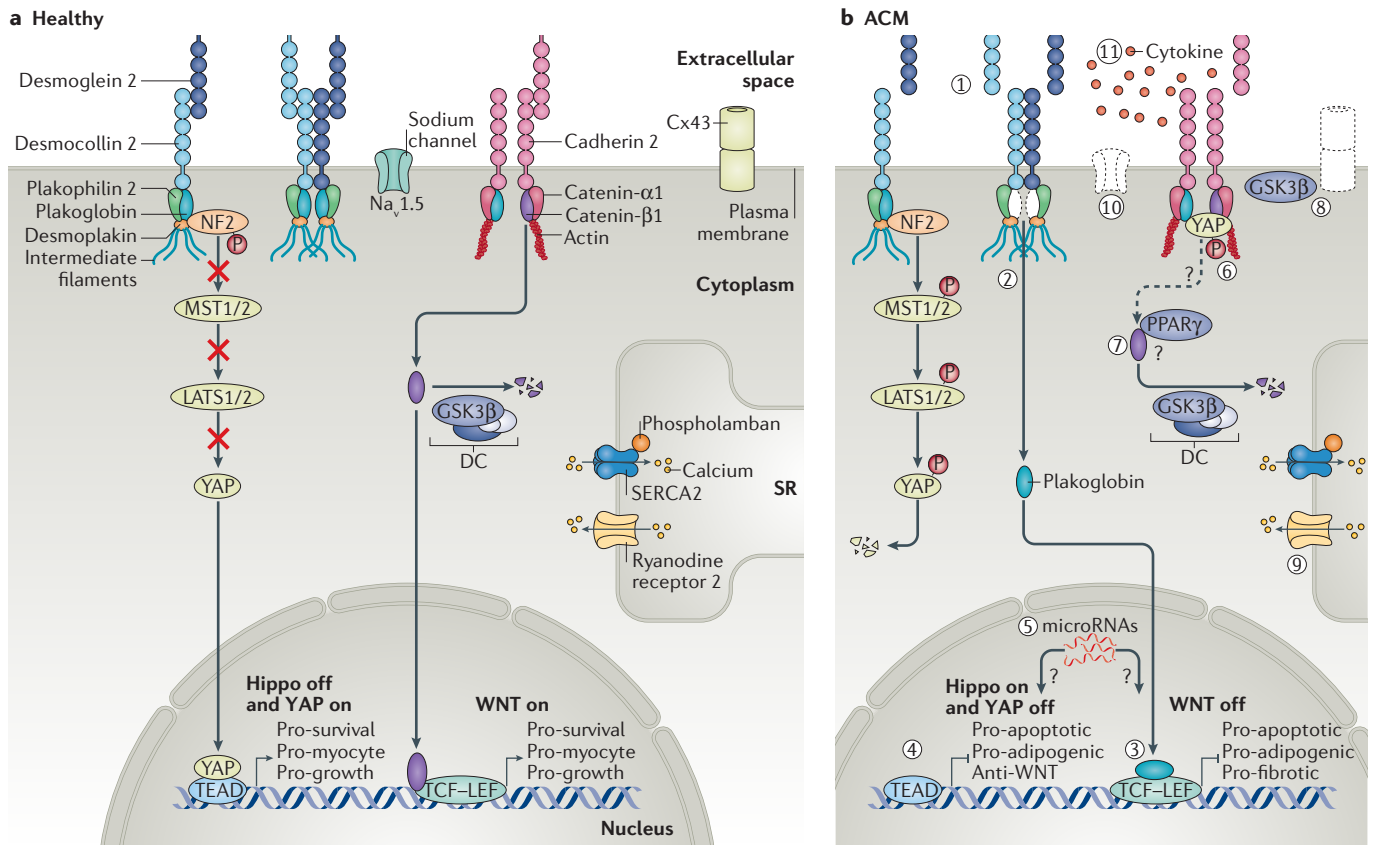


Fig. 3 | Proposed molecular mechanisms contributing to the pathogenesis of ACM. a | In the healthy cardiomyocyte, both desmosomes and adherens junctions in the area composita form strong intercellular connections with neighbouring cells. Likewise, both the gap junction, formed by connexin 43 (Cx43), and the sodium channel (Na_v1.5) are appropriately positioned as a result of coordinated trafficking and membrane tethering. Catenin-β1 has a structural function in adherens junctions as well as a role in modifying transcriptional activity through activation of WNT-dependent gene expression. Cytoplasmic catenin-β1 is quickly degraded through proteosomal targeting by the destruction complex (DC), which contains glycogen synthase kinase 3β (GSK3β). The Hippo pathway is appropriately 'off', allowing for transcription of genes that promote cardiomyocyte survival, function (pro-myocyte) and growth. Calcium flux is well regulated in the sarcoplasmic reticulum (SR) through functioning sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA2), phospholamban and ryanodine receptor 2. **b** | In arrhythmogenic cardiomyopathy (ACM), multiple signalling pathways seem to be perturbed. (1) Disruption of the desmosomes and adherens junctions leads to increased mechanical stress on the cardiomyocyte. (2) Plakoglobin can dissociate from the desmosome, further destabilizing the intercalated disc and (3) inhibiting WNT-dependent gene transcription. (4) Activation of the Hippo pathway, potentially through neurofibromin 2 (NF2), results in inhibition of gene targets and promotes a pro-apoptotic and adipogenic phenotype. (5) Likewise, microRNAs can modulate both Hippo and WNT signalling. (6) Active Hippo signalling leads to phosphorylation of Yes-associated protein (YAP), which potentially associates with both plakoglobin and catenin-β1 at the plasma membrane, sequestering catenin-β1 and further inhibiting canonical WNT signalling. (7) Increased peroxisome proliferator-activated receptor-γ (PPARγ) expression has been associated with WNT inhibition, potentially through a direct relationship that promotes catenin-β1 degradation. (8) GSK3β translocates to the plasma membrane, although the relevance of this change in localization is uncertain. (9) Dysregulation of calcium handling in the SR is thought to contribute to arrhythmogenesis in a subset of patients. (10) Abnormal shuttling and tethering of both the sodium channel and gap junction components (Cx43) have been suspected to be involved in arrhythmogenesis. (11) Increased pro-inflammatory and profibrotic cytokine production, including transforming growth factor-β1 (TGFβ1) and TGFβ3, is thought to contribute to the pathogenesis of ACM via canonical and non-canonical pathways. LATS, large tumour suppressor homologue; MST, mammalian STE20-like protein kinase; TEAD, transcriptional enhancer factor TEF.

normalized ACM phenotypes in both in vitro and in vivo models of ACM^{98,132,133}. These data suggest that aberrant mechanical signal transduction and protein trafficking in ACM, linked to increased GSK3β level, depressed WNT–catenin-β1 signalling or both, contribute to apoptotic cardiomyocyte loss in ACM.

The contribution of programmed cardiomyocyte necrosis (necroptosis) is less clear. Pathological evaluation

of samples from patients with ACM often detects dying and dead cardiomyocytes, termed necrosis¹¹⁶. A histological finding of necrosis does not clearly indicate the mechanism of death¹³⁵; however, apoptosis is traditionally considered non-inflammatory, but in many ACM samples, cardiomyocytes stained by TUNEL assay were surrounded by inflammatory cells^{116,125,127}. In a mouse model of ACM with overexpression of

a dominant-negative *Dsg2* transgene, investigators observed cardiomyocyte loss with histological and ultrastructural features of necrosis as a prominent early pathological feature. The widespread necrosis was accompanied by inflammatory infiltrates⁵⁰. A similar finding of regional necrosis with inflammation was seen in *Jup*-mutant mouse hearts at 18 days of life, which showed substantial deposition of complement and loss of sarcolemmal integrity, both of which are associated with cellular necrosis⁵¹. However, whether cardiomyocyte necrosis in ACM involves initiation of active necroptotic pathways or represents sequelae of mechanical injury has not been determined.

Fibrosis

Fibrosis is a common response to cardiomyocyte injury in the heart, and ACM is no exception. However, the mechanisms that recruit fibrofatty tissue to the damaged myocardium in ACM are poorly understood. Investigation into scar formation after myocardial infarction and diabetes-mellitus-induced cardiac fibrosis suggests a complex network of interactions between cytokines, growth factors and hormones that promote cardiac fibrosis¹³⁶. The TGF β signalling pathway is an active contributor to cardiac fibrosis^{137,138}. In canonical TGF β signalling, TGF β 1–TGF β 3 bind a series of membrane-bound receptors, which results in the phosphorylation and activation of receptor-associated SMAD transcription factors that drive expression of a profibrotic gene programme¹³⁹. TGF β stimulates fibrosis by increasing expression of extracellular matrix proteins and tissue inhibitors of matrix metalloproteinases as well as by directly inhibiting expression of matrix metalloproteinases, which break down the extracellular matrix¹³⁶. Non-canonical TGF β signalling pathways have also been identified, the most prominent of which include activation of mitogen-activated protein kinase (MAPK) signalling and generation of reactive oxygen species^{140,141}. Specifically, MAPK-dependent signalling, which utilizes a cascade of activating kinases, including MAPK kinase 7 (also known as TAK1), has been implicated in myocardial fibrosis, apoptosis and hypertrophy¹⁴².

Mutation of the 5' UTR of *TGFB3* has been associated with a familial case of ACM and resulted in a 2.5-fold increase in reporter gene expression in vitro^{79,143}. Follow-up studies to determine whether TGF β 3 expression is increased in vivo and whether it directly leads to excessive fibrosis have not yet been performed. In addition to TGF β 3, TGF β 1 has also been implicated in the ACM fibrotic phenotype. Mice expressing cardiomyocyte-restricted, loss-of-function mutant *Jup* had increased TGF β 1 expression and activation of SMAD2 signalling, with no alteration in MAPK pathways, suggesting activation of canonical TGF β signalling⁵¹. In addition, knockdown of *Pkp2* in NRVMs promoted TGF β 1-dependent expression of fibrotic genes via non-canonical TGF β –MAPK signalling¹⁴⁴. Inhibition with the TAK1-specific kinase inhibitor oxozaenol abrogated the profibrotic effect of *Pkp2* knockdown, confirming the essential role of non-canonical TGF β signalling in mediating

ACM-driven fibrosis¹⁴⁴. Taken together, these data suggest that both canonical and non-canonical TGF β signalling can have a role in inducing myocardial fibrosis in ACM.

Adipogenesis

Historically, the replacement of cardiomyocytes by adipocytes was considered a hallmark of ACM, although there is now increasing awareness that a spectrum of fatty infiltration exists. Whereas other cardiomyopathies might share a similar, or even greater, degree of fibrosis, pathological fatty infiltration is still a common histological feature of ACM^{71,116}. Of note, this fatty involvement is caused by infiltration by adipocytes (adipogenesis) rather than the presence of lipid droplets within other cell types, such as cardiomyocytes (lipogenesis)¹⁴⁵. There are two parts to dissecting adipogenesis in ACM. First, what is the cellular source of the adipocytes? Second, what are the signals that induce the formation of adipocytes in ACM?

Cellular source of adipocytes. Conceptually, candidates for the cellular origin of adipocytes in ACM include cardiomyocytes, differentiated non-myocytes (fibroblasts, endothelial cells, smooth muscle cells, epicardial cells or pre-existing adipocytes), cardiac progenitor cells and circulating progenitor cells. *Cre-loxP* genetic lineage tracing approaches in mouse models of ACM have been useful in addressing this question, with the caveat that mouse models of ACM generally do not have the robust fatty involvement seen in humans^{100,103,108}. *Nkx2-5-Cre* labels descendants of the first and second heart field as well as the proepicardium^{146,147}. *Mef2c-AHF-Cre* labels descendants of the second heart field but not the first heart field nor proepicardium¹⁴⁸, and *Myh6-Cre* is largely restricted to differentiated cardiomyocytes¹⁴⁹. *Nkx2-5-Cre* and *Mef2c-AHF-Cre*, but not *Myh6-Cre*, labelled the majority of adipocytes in mice with Cre-induced *Dsp* haploinsufficiency¹⁰⁸. These data suggest that at least a subset of adipocytes in ACM arise from second heart field progenitors.

In subsequent work, the same group showed that adult heart 'fibroadipogenic progenitors' marked by *Pdgfra* are a source of adipocytes¹⁰⁷. Interestingly, *Pdgfra*-expressing mesenchymal progenitor-like cells of the adult mouse heart were previously found to be a multipotent cell type derived from fetal epicardial progenitors¹⁵⁰. These somewhat divergent results suggest that mesenchymal progenitors from multiple embryonic origins contribute to adipogenesis in ACM. Although these data argue against a cardiomyocyte origin of adipocytes, this question remains open. hiPSC-CMs cultured under lipogenic stress form lipid droplets¹⁵¹, and one report documents transdifferentiation of ACM hiPSC-CMs to adipocytes in vitro¹⁵². In homozygous *Dsp*-floxed mice, the *Myl2-Cre* transgene (nominally specific to cardiomyocytes) labelled adipocytes in the heart¹⁰². Overall, a consensus has yet to be reached on the origin of adipocytes in ACM, even in mouse models, and findings from mice might not extrapolate directly to humans owing to interspecies variation in the extent of adipogenesis.

Adipogenesis signalling. Although work continues on the origin of adipocytes, the signals that stimulate adipogenesis downstream of ACM-causing mutations remain an active area of investigation. A fundamental question is whether these signals are autonomous to desmosome-expressing cells (that is, an intracellular signal) or involve non-autonomous signals from a desmosome-expressing cell to an adipogenic cell (that is, a paracrine signal). Information on the source of adipocytes will go a long way to answering this question about the mode of adipogenic signalling. Likewise, defining the desmosome-containing cells that generate the adipogenic signal is central to determining the mechanism. Although cardiomyocytes have long been considered the main desmosome-expressing cells in the heart, work on fibroadipogenic progenitors suggests that desmosome-expressing cardiac non-myocyte cells might also contribute to myocardial fibrofatty infiltration¹⁰⁷. Despite these uncertainties, several signalling pathways have been implicated in the adipogenic phenotype of ACM, notably WNT, Hippo–Yes-associated protein (YAP), peroxisome proliferator-activated receptor- γ (PPAR γ) and microRNA (miRNA) signalling.

WNT signalling. Currently, the largest body of evidence in ACM supports a role for WNT ligand signalling through catenin- β 1, known as canonical WNT signalling^{100,153,154}. Catenin- β 1 is a multifunctional protein with diverse cellular roles in cell–cell junctions and transcriptional regulation¹⁵⁵. At the cell membrane, catenin- β 1 and its paralogue plakoglobin are located at adherens junctions, where they link classical cadherins to the actin cytoskeleton. Of note, this function of plakoglobin is in parallel to its role as a component of the cardiac desmosome. Intracellularly, the activity of catenin- β 1 is dependent on the presence of WNT ligands. In the absence of ligand–receptor, membrane-associated signalling, intracellular catenin- β 1 is rapidly degraded by a cytoplasmic destruction complex. Binding of WNT ligands to their receptors inhibits the destruction complex, allowing intracellular accumulation of catenin- β 1. Cytoplasmic catenin- β 1 translocates to the nucleus, where it co-activates target genes through members of the TCF–LEF transcription factor family¹⁵⁶. The absence of WNT activity has a pivotal role in cellular adipogenesis and is a driving factor in the differentiation of mesenchymal stem cells and preadipocytes into adipocytes¹⁵⁷.

As a catenin- β 1 paralogue, plakoglobin has previously been shown to competitively inhibit catenin- β 1 transcriptional activity and promote its degradation^{155,158}. The discovery that mutations in plakoglobin result in a form of ACM suggested that altered canonical WNT signalling might contribute to disease pathogenesis. Interestingly, ACM-associated mutations in desmosomal genes result in the dislocation of plakoglobin from the cell membrane, freeing plakoglobin to participate in non-structural functions^{58,159,160}. In cellular and mouse models of desmoplakin depletion, nuclear localization of plakoglobin interfered with catenin- β 1 transcriptional functions and promoted the expression of adipogenic genes^{43,100}. Conversely, *Jup* knockout increased stability

and transcriptional activity of catenin- β 1, resulting in cardiomyocyte loss and fibrosis, but not adipogenesis in mouse hearts¹¹⁹. Similarly, increased plakoglobin nuclear localization increased adipogenic activity of KIT-positive cardiac progenitor cells, whereas *Jup* knockout or WNT activation inhibited adipogenesis¹⁶¹. These data highlight a possible antagonistic role of nuclear plakoglobin in canonical WNT signalling and the subsequent development of adipogenesis.

Mutations in the adherens junction protein catenin- α 3, which is mutated in a small percentage of patients with ACM⁴², might affect WNT–catenin- β 1 signalling in a similar manner. As discussed previously, catenin- α 3 can physically interact with both catenin- β 1 and plakoglobin⁴². Pathological mutations in catenin- α 3 reduce its membrane localization in cardiomyocytes and alter its interactions with catenin- β 1 and plakoglobin⁴². In epithelial cells, catenin- α 3 shows catenin- β 1-dependent nuclear translocation, resulting in inhibition of WNT-dependent transcriptional activity, although a similar mechanism has yet to be shown in cardiomyocytes¹⁶². Taken together, these data point to a common theme of diminished WNT–catenin- β 1 signalling and nuclear plakoglobin translocation in ACM. However, decreased catenin- β 1 signalling alone is unlikely to be sufficient for disease propagation because expression of loss-of-function catenin- β 1 in adult murine hearts caused a hypertrophic, rather than an adipogenic, phenotype. ACM phenotypes might require complex interactions between aberrant WNT–catenin- β 1 signalling and underlying desmosome dysfunction^{163,164}.

WNT ligands can activate catenin- β 1-independent signalling pathways, referred to as non-canonical WNT signalling. Non-canonical WNT signalling, and its effector pathway Rho GTPase (Rho)–Rho-associated protein kinase (ROCK), have been implicated in ACM-associated adipogenesis. Rho–ROCK activity has been shown to inhibit adipogenic differentiation of mesenchymal stem cells, which led to the hypothesis that inhibition of this pathway could be an important contributor to an adipogenic programme^{165,166}. Mice expressing dominant-negative ROCK (DN-ROCK) in cardiomyocytes and smooth muscle cells¹⁶⁷ had characteristic ACM features, including ventricular dilatation, dysfunction, fibrofatty histological changes, ventricular arrhythmias and sudden death⁹⁹. Furthermore, plakoglobin expression was significantly reduced and localized to the nuclei of DN-ROCK cardiomyocytes, as has previously been reported in ACM^{99,100}. The transcripts encoding the pro-adipogenic proteins PPAR γ and the non-canonical WNT5B ligand were also notably increased in DN-ROCK hearts⁹⁹; WNT5B has been shown to inhibit canonical WNT signalling by blocking catenin- β 1 nuclear localization¹⁶⁸. Rho also has a structural role in cytoskeletal and desmosomal organization; therefore, disruption of this function might be what drives plakoglobin nuclear localization and subsequent catenin- β 1 inhibition, similar to classical desmosomal mutations^{99,169,170}. This structural function provides an alternative mechanism for how inhibition of Rho–ROCK signalling could lead to an adipogenic programme in ACM. Together, these data suggest that

Rho–ROCK activity has effects on canonical and non-canonical WNT signalling in the heart, both of which promote adipogenesis.

Hippo–YAP signalling. The Hippo–YAP signalling pathway has been implicated in ACM pathogenesis and adipogenesis¹⁰⁴. This evolutionarily conserved pathway regulates organ size and cell proliferation, survival and differentiation; in many cases, activation is in response to mechanical cues or cell–cell contacts¹⁷¹. Briefly, YAP and its paralogue, WW domain-containing transcription regulator protein 1 (WWTR1; also known as TAZ), are transcriptional co-activators that stimulate the expression of genes that promote proliferation and resistance to apoptosis^{171,172}. YAP and TAZ activity is regulated at multiple levels. In the canonical regulatory pathway, known as the Hippo kinase cascade, which ultimately restrains YAP and TAZ, upstream cues activate mammalian STE20-like protein kinase 1 (MST1) and MST2, which phosphorylate and activate the kinases large tumour suppressor homologue 1 (LATS1) and LATS2. LATS1 and LATS2 phosphorylate YAP and TAZ, resulting in their nuclear exclusion and downregulation of their transcriptional targets. Among the upstream regulators of the Hippo kinases is neurofibromin 2 (NF2; also known as merlin), a multifunctional protein that links cell–cell and cell–matrix adhesions to downstream signalling pathways, including Hippo^{172,173}. Another important regulatory mechanism involves the binding of YAP to catenin- α proteins and its subsequent sequestration at the cell membrane¹⁷⁴. This mechanism regulates YAP activity independent of Hippo kinases and regulates cardiomyocyte proliferation in response to cytoskeletal tension¹⁷⁵. Interestingly, mechanotransduction by YAP, independent of Hippo kinases, regulates mesenchymal stem cell to adipocyte differentiation¹⁷⁶, suggesting additional mechanisms by which YAP might participate in pathological adipogenesis in ACM.

How do activation of Hippo and suppression of YAP promote adipogenesis? YAP interacts with catenin- β 1, and in the presence of inhibitory Hippo kinase activity, this interaction suppresses catenin- β 1 nuclear translocation¹⁷⁷. This mechanism was proposed to link Hippo activation and YAP inactivation in *Pkp2*-depleted HL-1 cells with catenin- β 1 inhibition and lipogenesis¹⁰⁴. A potential second mechanism stems from the observation that YAP directly interacts with plakoglobin in human heart protein extracts and cultured cells¹⁰⁴. The relevance of this interaction in vivo has not been experimentally determined, but in ACM, nuclear plakoglobin might inhibit the previously reported YAP-dependent activation of catenin- β 1 target genes¹⁷⁸. The Hippo–YAP and WNT–catenin- β 1 pathways are intertwined at multiple other levels. YAP and TAZ are integral components of the catenin- β 1 destruction complex, which degrades cytoplasmic catenin- β 1 in the absence of WNT ligand. Activation of WNT signalling dislodges YAP and TAZ from the destruction complex, permitting their nuclear translocation and activation of transcription¹⁷⁹. The importance of this complex crosstalk between these signalling pathways in ACM pathogenesis remains to be fully explored.

PPAR γ signalling. Activation of PPAR γ , a nuclear receptor whose functional integrity is required for adipocyte differentiation, lipogenesis and adipocyte survival¹⁸⁰, is also a candidate for pro-adipogenic signalling in ACM. Mice overexpressing cardiac-specific PPAR γ develop a dilated cardiomyopathy with increased lipid accumulation and impaired systolic function¹⁸¹. A reciprocal relationship between PPAR γ and canonical WNT signalling has been demonstrated in models of ACM, potentially via a direct interaction between PPAR γ and catenin- β 1 that promotes catenin- β 1 degradation¹⁸². *Dsp* knockdown in murine cardiomyocytes suppressed canonical WNT signalling and increased *Pparg* mRNA and PPAR γ protein expression¹⁰⁰. In addition, hiPSC-CMs derived from patients with a c.2484C>T mutation in *PKP2* had decreased catenin- β 1 activity with profound lipogenesis and increased PPAR γ expression¹⁵¹. Further investigation is needed to determine whether PPAR γ upregulation in ACM models occurs upstream, downstream or in parallel to changes in WNT–catenin- β 1 signalling and to determine whether changes in PPAR γ signalling are essential to pathogenic events in ACM.

MicroRNA signalling. miRNAs are a class of small non-coding RNAs that modulate the activity of a variety of signalling pathways through post-transcriptional effects on gene expression. Measurement of 1,078 miRNAs in 24 histologically confirmed ACM heart samples compared with controls identified 21 that were differentially expressed¹⁴². Two of these, miR-21-5p and miR-135b, are known regulators of WNT and Hippo signalling pathways in cancer¹⁴². In a separate study, *PKP2* small interfering RNA (siRNA) knockdown in HL-1 cardiomyocyte-like cells, as well as primary mesenchymal progenitor cells and two distinct mouse models of ACM, resulted in decreased expression of miR-184 (REF.¹⁸³). Subsequent pathway analysis for miR-184 identified abnormal signalling in canonical WNT, Hippo and integrin pathways, corroborating previous reports in ACM^{100,104,184}. Furthermore, miR-184 target genes associated with lipogenesis were upregulated with *PKP2* knockdown, and in vitro models of aberrant miRNA-184 expression illustrated its role as a molecular switch for adipogenic gene regulation¹⁸³.

Inflammation

Patients with ACM can develop chest pain, ST-segment changes on the ECG, elevated levels of serum troponins and increased levels of circulating cytokines¹⁸⁵, findings that are suggestive of myocardial inflammation. Inflammatory infiltrates are frequently, although not universally, observed in ACM biopsy samples. This finding does not simply reflect limited sampling of patchy disease because evaluation of 36 ACM post-mortem hearts with right ventricular fibrofatty infiltration with or without left ventricular involvement revealed inflammatory cell infiltrates containing macrophages, neutrophils and mast cells in 39% of cases¹⁸⁶. A subset also contained T cell infiltrates. When present, these infiltrates were most common in areas of fibrosis and correlated with more severe, biventricular disease.

The highly inflammatory presentation of some patients with ACM prompted speculation as to whether

the cardiomyopathy was actually a form of myocarditis¹¹⁶. Interestingly, desmosome disruption is also apparent in biopsy samples from patients with granulomatous myocarditis¹⁸⁵. The most common cause of myocarditis is infection by cardiotropic viruses¹⁸⁷. However, viral genomic material has been variably identified in patients with ACM^{188–191}. Furthermore, case reports have identified patients who were erroneously diagnosed with myocarditis instead of ACM, or vice versa, highlighting the clinical and diagnostic overlap^{185,192,193}. Although the presence of viral genomes in a subset of patients with ACM might represent a predisposition to disease progression after a viral trigger¹⁹⁰, additional cases are required to substantiate this hypothesis.

Mechanistically, identifying the events that precipitate an inflammatory environment in ACM is a similar conundrum to that of defining adipocyte recruitment. As previously discussed, patients with ACM can have cutaneous syndromes in parallel to cardiac disease. The epidermis is also prone to inflammation with abrogated desmosome expression¹⁹⁴. The similarities between the heart and epidermis in ACM raise the question of how junctional instability triggers inflammation. Conceivably, progressive loss of myocardial tissue by necroptosis could elicit inflammatory infiltrates. Mice with cardiac-restricted inactivation or overexpression of wild-type or mutant desmosomal cadherins^{50,95,96,101} had neutrophil and macrophage infiltration into calcified patches of necrotic tissue. This recruitment occurred subsequent to overt structural damage and cell death⁵⁰, perhaps arguing for a secondary response to tissue damage.

In NRVMs, overexpression of the truncated form of plakoglobin found in patients with Naxos disease (*Jup*^{2157del2}) stimulates the secretion of multiple cytokines, including IL-6 and tumour necrosis factor (TNF)^{133,195}. Mutant plakoglobin also sensitizes NRVMs to mechanically induced cell death, albeit apoptotic death^{133,195}. Interestingly, recombinant TNF, IL-6 and IL-7 are also sufficient to cause abnormal plakoglobin localization in wild-type NRVMs¹⁸⁵. Together, these findings suggest a scenario in which desmosomal dysfunction stimulates the production of an inflammatory milieu, possibly fostering inflammatory cell recruitment and further weakening junctional stability. Although an attractive hypothesis, it has yet to be verified whether myocardial inflammation is a primary insult to disease mutations or merely a secondary response to cardiomyocyte death.

Arrhythmogenesis

Fatal arrhythmia is among the most-feared complications of ACM. In late-stage hearts, extensive, patchy cardiomyocyte loss and fibrofatty infiltration creates a myocardial substrate that is highly vulnerable to arrhythmia. However, during the early ‘concealed’ disease phase, ACM hearts without grossly abnormal myoarchitecture can still be highly arrhythmogenic^{13,92,96}. In this section, we discuss the mechanisms that might account for this risk of lethal arrhythmia in the absence of gross structural abnormalities.

Conduction of the cardiac impulse requires gap junctions, specialized intercellular structures formed by the connexin family of proteins that allow the passage of ions

and small molecules between cells¹⁹⁶, and the cardiac sodium channel Na_v1.5, which is responsible for the rapid upstroke of the action potential. Connexin 43 (Cx43; also known as gap junction-α1 protein), the predominant connexin expressed in the ventricular myocardium^{197,198}, localizes to the intercalated discs of mature cardiomyocytes. Ischaemic heart disease and heart failure cause reduced Cx43 expression and aberrant localization to the lateral surfaces of cardiomyocytes, which contribute to conduction disturbances and risk of arrhythmia in these conditions¹⁹⁷. In ACM myocardium from patients and animal models, perturbations in Cx43 expression and localization are reproducibly found, even in myocardial regions that were not grossly abnormal^{102,159,160,199–203}. Desmosome integrity is required for normal Cx43 protein levels, given that dose-dependent knockdown of *Dsp* in NRVMs resulted in parallel decreases in Cx43 expression without effects on cadherin 2, plakophilin 2 or other proteins of the intercalated disc¹⁰². Similar to Cx43, Na_v1.5 is preferentially localized at the intercalated disc and is reduced in the myocardium of patients with ACM and murine models of ACM^{92,96,159}. Plakophilin 2 and Na_v1.5 are present in the same protein complex, and *Pkp2* knockdown reduced Na_v1.5 levels and sodium current density²⁰⁴. The cytoskeletal adaptor protein ankyrin 3, which is required for Na_v1.5 trafficking²⁰⁵, also interacts with plakophilin 2, and *PKP2* depletion reduced ankyrin 3 levels²⁰⁶. Altered Na_v1.5 interaction with ancillary molecules, such as ankyrin 3, is likely to explain the abnormal Na_v1.5 gating observed in *PKP2* knockdown^{204,206}. Therefore, intercalated disc targeting and function of Cx43 and Na_v1.5, which are critical for cardiac impulse conduction, require intact desmosomes.

Multiple potential mechanisms link desmosome integrity to Cx43 expression and localization. Appropriate Cx43 localization at intercalated discs requires trafficking along microtubules. Desmosomes regulate the local organization and stability of microtubules through desmoplakin, although desmoplakin does not directly interact with microtubules. Instead, microtubule-associated protein RP/EB family member 1 (EB1), a microtubule-binding protein that regulates microtubule dynamics and protein association with microtubule plus ends, interacts with desmoplakin, and loss of *Dsp* or *Mapre1* (which encodes EB1) impairs localization of Cx43 (REFS^{207,208}). ACM-associated *Dsp* mutations, particularly those in the amino terminus, blocked EB1 interaction and decreased Cx43 signal intensity at points of cell–cell contact²⁰⁷. Na_v1.5 also requires microtubule-mediated trafficking for appropriate localization to the membrane and intercalated disc, and desmosome disruption by *Pkp2* knockdown similarly impaired its localization to microdomains at intercalated discs via EB1 (REFS^{209,210}). The PDZ-domain-containing protein synapse-associated protein 97 (SAP97; also known as disks large homologue 1 (DLG1)), which is required for normal Na_v1.5 targeting, has also been implicated in abnormal Na_v1.5 function in ACM¹³³. SAP97 expression and localization are disrupted in NRVMs by the overexpression of *Jup*^{2157del2} and in myocardial samples from patients with ACM. SAP97 knockdown itself impaired targeting of Na_v1.5 and plakoglobin but interestingly did not affect Cx43.

These data indicate that desmosomes organize Cx43 and Na_v1.5 delivery to intercalated discs via EB1-based and microtubule-based mechanisms. In ACM, disassembly of desmosomes disrupts trafficking of these important proteins, creating an arrhythmogenic substrate.

GSK3 β is a kinase that regulates WNT–catenin- β 1 and a range of other cellular processes²¹¹. Discovery that a GSK3 β inhibitor, SB216763, ameliorated features of ACM¹³³ suggested that GSK3 β is critical for the pathogenesis of ACM. Interestingly, GSK3 β is mislocalized to the intercalated disc in the myocardium of patients with ACM and in cellular and murine disease models of ACM⁹⁸. Moreover, in both cell and animal models of ACM, SB216763 normalized the expression and localization of GSK3 β as well as plakoglobin, Cx43 and SAP97. SB216763-treated mice with ACM were protected from cardiac fibrosis, inflammation and ventricular ectopy. Further work is required to determine the mechanism of GSK3 β mislocalization and to understand how GSK3 β activity contributes to arrhythmogenesis in ACM.

Dysfunctional calcium handling has also been implicated in arrhythmogenesis in ACM. Transcriptome analysis of conditional-*Pkp2*-knockout mice highlighted altered gene expression of proteins involved in intracellular calcium homeostasis⁹⁴. Functional studies showed that *Pkp2*-knockout cardiomyocytes had increased amplitude and prolongation of calcium transients as well as a propensity for early and delayed afterdepolarization events, which predispose to arrhythmia^{94,212}. A similar increase in after-transient events was observed with patient-derived (*DSG2*-mutant) hiPSC-CMs, emphasizing the functional effects of altered calcium handling in the context of desmosomal mutations²¹³. Consistent with calcium-handling abnormalities in patients with ACM, mutations in *RYR2* and *PLN* have been found in individuals with ACM phenotypes^{70,72}. Furthermore, *PLN* mRNA and protein were upregulated in patients with a wide spectrum of ACM mutations¹²⁹. Together, these data suggest that disordered calcium handling occurs in ACM and is likely to contribute to the propensity for arrhythmia.

Karmouch and colleagues have explored the cellular source of arrhythmias by inactivating a conditional *Dsp* allele with Cre recombinase driven by *Cspg4* regulatory elements²¹⁴. *Cspg4*-CreER recombined *Dsp* in the atrioventricular node and the His–Purkinje system but not in the chamber myocardium. These conduction system cells express desmosomal proteins, and *Dsp* inactivation in these cells caused sinus bradycardia, high-grade atrioventricular block, nonsustained VT and sudden death²¹⁴. The study points out the essential role of desmosomes in the central conduction system. However, its relevance to patients with ACM requires further study because patients with ACM do not typically develop conduction block, the presumed cause of death in this mouse model, and patients with ACM do not generally have homozygous *DSP* loss of function.

Conclusions

ACM is an important cause of sudden cardiac death and inherited arrhythmia. Currently, treatment of ACM is largely empirical, with the aim of preventing life-threatening arrhythmias and progressive heart failure.

Gaining an understanding of the fundamental mechanisms that drive the pathological features of ACM provides an opportunity to develop targeted therapeutics (FIG. 3). The central role of desmosome mutations in the majority of patients with ACM highlights the critical cardiac function of these structures as both structural elements that preserve cardiomyocyte integrity in the face of billions of cycles of contraction and relaxation as well as mechanotransduction signalling hubs.

Reduced canonical WNT–catenin- β 1 signalling and mislocalized GSK3 β seem to be central components of the disease, as shown by promising results from initial preclinical studies of SB216763, a small-molecule inhibitor of GSK3 β and activator of canonical WNT signalling^{98,133}. However, targeted GSK3 β deletion in cardiac fibroblasts caused fibrogenesis and ventricular dysfunction, highlighting some of the potential undesirable effects of systemic GSK3 β inhibition^{215,216}. Nevertheless, the discovery of SB216763 is an inspiring example of how development of disease models and mechanistic understanding can yield promising new therapeutic approaches.

An alternative therapeutic approach for ACM that has been initiated targets cardiac remodelling via the well-established renin–angiotensin–aldosterone system (RAAS). In other forms of heart disease, inhibition of the RAAS has emerged as an effective therapeutic intervention to halt maladaptive remodelling, particularly in the setting of heart failure and after myocardial infarction^{217,218}. RAAS activation has been shown to have direct effects on extracellular matrix production²¹⁹ and cardiomyocyte proliferation²²⁰ while also stimulating the activity of the profibrotic cytokine TGF β 1 (REFS^{221,222}). Undesirable fibrosis is an important pathological feature that is shared among heart failure, myocardial infarction and ACM. This observation led to the hypothesis that modification of the RAAS axis, and probably TGF β signalling in parallel, could have a similar role in ACM-driven fibrosis. The BRAVE study²²³ was designed to address the effects of the angiotensin-converting-enzyme inhibitor ramipril in ACM, with planned initiation in January 2019. Ramipril was specifically chosen because this drug has been reported to normalize PPAR γ expression, and increased PPAR γ activity been implicated in ACM pathogenesis^{224,225}. Together with SB216763, our growing mechanistic understanding of ACM is beginning to identify potential treatment avenues.

Despite our evolving understanding of the pathway perturbations that contribute to ACM, a causative genetic lesion has been identified in approximately only half of patients with a clinical diagnosis. The cause in the remaining patients remains undetermined, as are the reasons for the considerable variation in disease penetrance and expression. The development of multiple cellular and animal models has accelerated our understanding of disease pathogenesis, but large knowledge gaps remain. Doubtless, further study of the pathogenesis of ACM will reveal disease mechanisms that are important not only for ACM but also for other forms of heart disease.

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