HHS Public Access

Author manuscript

Nat Rev Cardiol. Author manuscript; available in PMC 2019 November 21.

Published in final edited form as:

Nat Rev Cardiol. 2019 September ; 16(9): 519–537. doi:10.1038/s41569-019-0200-7.

Molecular mechanisms of arrhythmogenic cardiomyopathy

Karyn M. Austin[1], **Michael A. Trembley**[1], **Stephanie F. Chandler**[1], **Stephen P. Sanders**[2], **Jeffrey E. Saffitz**[3], **Dominic J. Abrams**[1], **William T. Pu**[1,4,*]

[1]Basic and Translational Cardiovascular Research and Inherited Arrhythmias Programs, Department of Cardiology, Boston Children's Hospital, Boston, MA, USA.

[2]Departments of Cardiology, Cardiac Surgery and Pathology, Boston Children's Hospital, Boston, MA, USA.

[3]Department of Pathology, Beth Israel Deaconess Medical Center, Boston, MA, USA.

[4]Harvard Stem Cell Institute, Cambridge, MA, USA.

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 develop[1].

[*] wpu@pulab.org.

Author contributions

K.M.A., M.A.T., S.F.C. and W.T.P. researched data for the article, and K.M.A., M.A.T. and W.T.P. discussed its content. K.M.A., M.A.T., S.F.C., D.J.A. and W.T.P. wrote the manuscript, and K.M.A., M.A.T., S.P.S., J.E.S., D.J.A. and W.T.P. reviewed and edited it before submission.

Competing interests

The authors declare no competing interests.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Reviewer information

Nature Reviews Cardiology thanks M. Delmar, A. J. Marian and the other anonymous reviewer(s), for their contribution to the peer review of this work.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Initially considered to be a developmental abnormality of the right ventricle, the disease was originally referred to as arrhythmogenic right ventricular dysplasia[2]. 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 phenotypes[4].

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 feature[13]. 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 screening[11].

The electrocardiographic, structural and functional features of ACM reflect progressive myocardial involvement and vary according to the predominantly affected ventricle or ventricles[16]. 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 studies[17]. 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

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

VT[18], 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 adults[9]. 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 feature[16].

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 arrhythmia[24], whereas other studies have reported increased ongoing risk of arrhythmia with fairly modest levels of exercise[25]. 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.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Desmosomal and junctional gene mutations

Early observations of familial disease clustering suggested a genetic basis for ACM[26]. Protonotarios and colleagues recognized that individuals on the Greek island of Naxos had a form of ACM in conjunction with a cardiocutaneous syndrome[26]. In addition to cardiac manifestations, which were 100% penetrant by adolescence[27], 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 family[30]. Cardiac manifestations of ‘Carvajal syndrome’ predominantly involved the left ventricle and resulted in a dilated cardiomyopathy[31]. Similar to Naxos disease, genetic analysis found a causative homozygous truncating mutation in another desmosome gene, *DSP,* encoding desmoplakin.

These foundational discoveries sparked candidategene 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 network[39] (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-α3)[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-α3 and a paralogue, catenin-α1, 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-β1. Furthermore, catenin-α3 directly interacts with plakophilin 2 at the cardiomyocyte intercalated disc, which implies that this protein participates in a hybrid junction to

strengthen cell–cell interactions[44]. 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 envelope[52]. Through these interactions, desmin coordinates movements of neighbouring Z-discs with the nuclear and plasma membranes[53]. Desmin mutations are associated with skeletal myopathies as well as dilated and restrictive cardiomyopathies[45]. 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 expression[56]. 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 ACM[58]. Forleo and colleagues later identified a novel *LMNA* duplication in a family affected by a number of arrhythmogenic phenotypes, including ACM[47].

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 organization[59]. *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, Canada[60]. 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 populations[62].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript

Titin is the largest human protein and functions as a bidirectional spring, generating passive stiffness in cardiomyocytes[63]. *TTN*-truncating mutations are considered to be the most common genetic cause of dilated cardiomyopathy[64], although *TTN* is also located near a genetic locus (2q31.1-p32.2) that has been implicated in a subgroup of patients with ACM[65]. 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 ACM[66]. Functional analysis of the mutant titin protein highlighted small alterations in folding kinetics that allowed for increased susceptibility to proteolysis and degradation[66]. 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 transduction[53]. 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 evaluation[67].

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 handling[69]. *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 contraction[72]. 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 protein[72]. 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* mutations[73]. 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 propagation[77]. Mutations in *SCN5A,* which encodes the pore-forming subunit of the sodium channel $Na_v1.5$, have been associated with a number of arrhythmogenic disorders including Brugada, long QT and sick sinus syndromes[77]. *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 activity[78]. 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-β3 (TGFβ3; 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 phenotype[6]. Within this locus, *TGFB3* is an attractive candidate gene, given the pro-inflammatory and profibrotic activities of TGFβ3. 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 gene[79]. 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, TGFβ3 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 atrophy[43]. 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 myocardium[17]. 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 dystrophy[83] and myocardial infarction[84,85].

Myocardial involvement is predominantly concentrated in the right ventricle, although biventricular[86] and even predominantly left ventricular patterns are also seen, often in

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

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 dysplasia[2] (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 lesion[43]. Multifocal inflammatory infiltrates are common in ACM and often include interstitial concentrations of mononuclear cells located near necrotic or damaged cardiomyocytes[86]. Inflammatory infiltrates are often observed in both ventricular free walls, even in hearts with macroscopically right-ventricle-dominant disease[86].

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 midgestation 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* deletion[94]. 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 damage[50].

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

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

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 mice[105]. 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 promoter[109]. This atrial tumour lineage maintains a differentiated state with passaging, forms myofibrils and has nascent intercalated discs[109]. 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 state[109].

The development of hiPSC technology by Yamanaka and colleagues[110] 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’ tissues[113] 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,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

fibrosis, adipogenesis, inflammation and arrhythmogenesis. We review the current knowledge on how desmosomal gene mutations cause this panoply 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 thinning[17]. Extensive histological evaluation of 30 hearts from patients with ACM demonstrated cardiomyocyte death with surrounding patchy inflammation and fibrofatty replacement, resulting in substantial atrophy[116]. These histological data, combined with the adult presentation and progressive disease course, provided support for the degenerative model of ACM pathogenesis[116]. 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 gaps[118]. 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 mice[51]. 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

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

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-β1 signalling in ACM[100] and other contexts[134]. A screen for small molecules that increase survival of a zebrafish model of Naxos disease identified SB21676, which inhibits glycogen synthase kinase 3β (GSK3β)[133]. Because GSK3β promotes catenin-β1 degradation, one effect of its inhibition is increased WNT–catenin-β1 signalling[133]. Interestingly, SB21676 normalized plakoglobin localization, prevented both stress-induced and stretch-induced apoptosis and 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 necrosis[116]. A histological finding of necrosis does not clearly indicate the mechanism of death[135]; 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 infiltrates[50]. 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 necrosis[51]. 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 fibrosis[136]. The TGFβ signalling pathway is an active contributor to cardiac fibrosis[137,138]. In canonical TGFβ signalling, TGFβ1–TGFβ3 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 programme[139]. TGFβ 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 matrix[136]. Non-canonical TGFβ 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 hypertrophy[142].

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 TGFβ3 expression is increased in vivo and whether it directly leads to excessive fibrosis have not yet been performed. In addition to TGFβ3, TGFβ1 has also been implicated in the ACM fibrotic phenotype. Mice expressing cardiomyocyte-restricted, loss-of-function mutant *Jup* had increased TGFβ1 expression and activation of SMAD2 signalling, with no alteration in MAPK pathways, suggesting activation of canonical TGFβ signalling[51]. In addition, knockdown of *Pkp2* in NRVMs promoted TGFβ1-dependent expression of fibrotic genes via non-canonical TGFβ–MAPK signalling[144]. Inhibition with the TAK1-specific kinase inhibitor oxozeaenol abrogated the profibrotic effect of *Pkp2* knockdown, confirming the essential role of non-canonical TGFβ signalling in mediating ACM-driven fibrosis[144]. Taken together, these data suggest that both canonical and non-canonical TGFβ 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 proepicardium[148], and *Myh6*–Cre is largely restricted to differentiated cardiomyocytes[149]. *Nkx2–5*–Cre and *Mef2c*–AHF–Cre, but not *Myh6*–Cre, labelled the majority of adipocytes in mice with Cre-induced *Dsp* haploinsufficiency[108]. 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 adipocytes[107]. Interestingly, *Pdgfra*-expressing mesenchymal progenitor-like cells of the adult mouse heart were previously found to be a

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

multipotent cell type derived from fetal epicardial progenitors[150]. 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 droplets[151], and one report documents transdifferentiation of ACM hiPSC-CMs to adipocytes in vitro[152]. In homozygous Dsp-floxed mice, the *Myl2–Cre* transgene (nominally specific to cardiomyocytes) labelled adipocytes in the heart[102]. 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 signaling—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 infiltration[107]. 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 (PPARγ) and microRNA (miRNA) signalling.

WNT signaling—Currently, the largest body of evidence in ACM supports a role for WNT ligand signalling through catenin-β1, known as canonical WNT signalling[100,153,154]. Catenin-β1 is a multifunctional protein with diverse cellular roles in cell–cell junctions and transcriptional regulation[155]. At the cell membrane, catenin-β1 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-β1 is dependent on the presence of WNT ligands. In the absence of ligand–receptor, membrane-associated signalling, intracellular catenin-β1 is rapidly degraded by a cytoplasmic destruction complex. Binding of WNT ligands to their receptors inhibits the destruction complex, allowing intracellular accumulation of catenin-β1. Cytoplasmic catenin-β1 translocates to the nucleus, where it co-activates target genes through members of the TCF-LEF transcription factor family[156]. 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 adipocytes[157].

As a catenin-β1 paralogue, plakoglobin has previously been shown to competitively inhibit catenin-β1 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

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

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-β1 transcriptional functions and promoted the expression of adipogenic genes[43,100]. Conversely, *Jup* knockout increased stability and transcriptional activity of catenin-β1, resulting in cardiomyocyte loss and fibrosis, but not adipogenesis in mouse hearts[119]. Similarly, increased plakoglobin nuclear localization increased adipogenic activity of KITpositive cardiac progenitor cells, whereas *Jup* knockout or WNT activation inhibited adipogenesis[161]. 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-α3, which is mutated in a small percentage of patients with ACM[42], might affect WNT–catenin-β1 signalling in a similar manner. As discussed previously, catenin-α3 can physically interact with both catenin-β1 and plakoglobin[42]. Pathological mutations in catenin-α3 reduce its membrane localization in cardiomyocytes and alter its interactions with catenin-β1 and plakoglobin[42]. In epithelial cells, catenin-α3 shows catenin-β1-dependent nuclear translocation, resulting in inhibition of WNT-dependent transcriptional activity, although a similar mechanism has yet to be shown in cardiomyocytes[162]. Taken together, these data point to a common theme of diminished WNT–catenin-β1 signalling and nuclear plakoglobin translocation in ACM. However, decreased catenin-β1 signalling alone is unlikely to be sufficient for disease propagation because expression of loss-of-function catenin-β1 in adult murine hearts caused a hypertrophic, rather than an adipogenic, phenotype. ACM phenotypes might require complex interactions between aberrant WNT–catenin-β1 signalling and underlying desmosome dysfunction[163,164].

WNT ligands can activate catenin-β1-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 cells[167] had characteristic ACM features, including ventricular dilatation, dysfunction, fibrofatty histological changes, ventricular arrhythmias and sudden death[99]. 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 PPARγ and the non-canonical WNT5B ligand were also notably increased in DN-ROCK hearts[99]; WNT5B has been shown to inhibit canonical WNT signalling by blocking catenin-β1 nuclear localization[168]. 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-β1 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

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

canonical and non-canonical WNT signalling in the heart, both of which promote adipogenesis.

Hippo–YAP signaling—The Hippo–YAP signalling pathway has been implicated in ACM pathogenesis and adipogenesis[104]. 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 contacts[171]. 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 LAT2 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 membrane[174]. This mechanism regulates YAP activity independent of Hippo kinases and regulates cardiomyocyte proliferation in response to cytoskeletal tension[175]. Interestingly, mechanotransduction by YAP, independent of Hippo kinases, regulates mesenchymal stem cell to adipocyte differentiation[176], 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-β1, and in the presence of inhibitory Hippo kinase activity, this interaction suppresses catenin-β1 nuclear translocation[177]. This mechanism was proposed to link Hippo activation and YAP inactivation in *Pkp2-depleted* HL-1 cells with catenin-β1 inhibition and lipogenesis[104]. A potential second mechanism stems from the observation that YAP directly interacts with plakoglobin in human heart protein extracts and cultured cells[104]. 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-β1 target genes[178]. The Hippo–YAP and WNT–catenin-β1 pathways are intertwined at multiple other levels. YAP and TAZ are integral components of the catenin-β1 destruction complex, which degrades cytoplasmic catenin-β1 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 transcription[179]. The importance of this complex crosstalk between these signalling pathways in ACM pathogenesis remains to be fully explored.

PPARγ signaling—Activation of PPARγ, a nuclear receptor whose functional integrity is required for adipocyte differentiation, lipogenesis and adipocyte survival[180], is also a candidate for pro-adipogenic signalling in ACM. Mice overexpressing cardiac-specific PPARγ develop a dilated cardiomyopathy with increased lipid accumulation and impaired

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

systolic function[181]. A reciprocal relationship between PPARγ and canonical WNT signalling has been demonstrated in models of ACM, potentially via a direct interaction between PPARγ and catenin-β1 that promotes catenin-β1 degradation[182]. *Dsp* knockdown in murine cardiomyocytes suppressed canonical WNT signalling and increased *Pparg* mRNA and PPARγ protein expression[100]. In addition, hiPSC-CMs derived from patients with a c.2484C>T mutation in *PKP2* had decreased catenin-β1 activity with profound lipogenesis and increased PPARγ expression[151]. Further investigation is needed to determine whether PPARγ upregulation in ACM models occurs upstream, downstream or in parallel to changes in WNT–catenin-β1 signalling and to determine whether changes in PPARγ signalling are essential to pathogenic events in ACM.

MicroRNA signaling—miRNAs are a class of small noncoding 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 expressed[142]. Two of these, miR-21–5p and miR-135b, are known regulators of WNT and Hippo signalling pathways in cancer[142]. 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 regulation[183].

Inflammation

Patients with ACM can develop chest pain, ST-segment changes on the ECG, elevated levels of serum troponins and increased levels of circulating cytokines[185], 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 cases[186]. 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 myocarditis[116]. Interestingly, desmosome disruption is also apparent in biopsy samples from patients with granulomatous myocarditis[185]. The most common cause of myocarditis is infection by cardiotropic viruses[187]. 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

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

with ACM might represent a predisposition to disease progression after a viral trigger[190], 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 expression[194]. 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 death[50], 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 NRVMs[185]. 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 cells[196], 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 conditions[197]. 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

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

parallel decreases in Cx43 expression without effects on cadherin 2, plakophilin 2 or other proteins of the intercalated disc[102]. 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 density[204]. The cytoskeletal adaptor protein ankyrin 3, which is required for Na_v1.5 trafficking[205], also interacts with plakophilin 2, and *PKP2* depletion reduced ankyrin 3 levels[206]. 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 contact[207]. 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 ACM[133]. 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.

GSK3β is a kinase that regulates WNT–catenin-β1 and a range of other cellular processes[211]. Discovery that a GSK3β inhibitor, SB216763, ameliorated features of ACM[133] suggested that GSK3β is critical for the pathogenesis of ACM. Interestingly, GSK3β is mislocalized to the intercalated disc in the myocardium of patients with ACM and in cellular and murine disease models of ACM[98]. Moreover, in both cell and animal models of ACM, SB216763 normalized the expression and localization of GSK3β 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 GSK3β mislocalization and to understand how GSK3β activity contributes to arrhythmogenesis in ACM.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

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 homeostasis[94]. 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 mutations[213]. 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 mutations[129]. 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 elements[214]. *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 death[214]. 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-β1 signalling and mislocalized GSK3β seem to be central components of the disease, as shown by promising results from initial preclinical studies of SB216763, a small-molecule inhibitor of GSK3β and activator of canonical WNT signalling[98,133]. However, targeted GSK3β deletion in cardiac fibroblasts caused fibrogenesis and ventricular dysfunction, highlighting some of the potential undesirable effects of systemic GSK3β 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.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

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 production[219] and cardiomyocyte proliferation[220] while also stimulating the activity of the profibrotic cytokine TGFβ1 (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 TGFβ signalling in parallel, could have a similar role in ACM-driven fibrosis. The BRAVE study[223] 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 PPARγ expression, and increased PPARγ 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.

Acknowledgements

M.A.T. is supported by the NIH (T32HL07572). D.J.A. is supported by the AHA (16CSA28750006). W.T.P. is supported by the NIH (UG3 HL141798) and the AHA (16CSA28750006) and by charitable donations from the Boston Children's Heart Center. The Inherited Cardiac Arrhythmia Program (S.F.C., D.J.A. and W.T.P.) is generously supported by the Mannion and Roberts families.

References

1. Sen-Chowdhry S & McKenna WJ Reconciling the protean manifestations of arrhythmogenic cardiomyopathy. Circ. Arrhythm. Electrophysiol 3, 566–570 (2010). [PubMed: 21156776]
2. Marcus FI et al. Right ventricular dysplasia: a report of 24 adult cases. Circulation 65, 384–398 (1982). [PubMed: 7053899]
3. Thiene G, Nava A, Corrado D, Rossi L & Pennelli N Right ventricular cardiomyopathy and sudden death in young people. N. Engl. J. Med 318, 129–133 (1988). [PubMed: 3336399]
4. Sen-Chowdhry S, Morgan RD, Chambers JC & McKenna WJ Arrhythmogenic cardiomyopathy: etiology, diagnosis, and treatment. Annu. Rev. Med 61, 233–253 (2010). [PubMed: 20059337]
5. Peters S, Trummel M & Meyners W Prevalence of right ventricular dysplasia-cardiomyopathy in a non-referral hospital. Int. J. Cardiol 97, 499–501 (2004). [PubMed: 15561339]
6. Rampazzo A et al. The gene for arrhythmogenic right ventricular cardiomyopathy maps to chromosome 14q23–q24. Hum. Mol. Genet 3, 959–962 (1994). [PubMed: 7951245]
7. Corrado D et al. Trends in sudden cardiovascular death in young competitive athletes after implementation of a preparticipation screening program. JAMA 296, 1593–1601 (2006). [PubMed: 17018804]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

8. Thiene G, Corrado D & Basso C Arrhythmogenic right ventricular cardiomyopathy/dysplasia. Orphanet J. Rare Dis 2, 45 (2007). [PubMed: 18001465]
9. Bauce B et al. Clinical phenotype and diagnosis of arrhythmogenic right ventricular cardiomyopathy in pediatric patients carrying desmosomal gene mutations. Heart Rhythm 8, 1686–1695 (2011). [PubMed: 21723241]
10. Te Riele ASJM et al. Arrhythmogenic right ventricular dysplasia/cardiomyopathy in the pediatric population: clinical characterization and comparison with adult-onset disease. JACC Clin. Electrophysiol 1, 551–560 (2015). [PubMed: 29759408]
11. Groeneweg JA et al. Clinical presentation, long-term follow-up, and outcomes of 1001 arrhythmogenic right ventricular dysplasia/cardiomyopathy patients and family members. Circ. Cardiovasc. Genet8, 437–446 (2015). [PubMed: 25820315]
12. Lazzarini E et al. The ARVD/C genetic variants database: 2014 update. Hum. Mutat 36, 403–410 (2015). [PubMed: 25676813]
13. Corrado D et al. Arrhythmogenic right ventricular dysplasia/cardiomyopathy: need for an international registry. Study Group on Arrhythmogenic Right Ventricular Dysplasia/ Cardiomyopathy of the Working Groups on Myocardial and Pericardial Disease and Arrhythmias of the European Society of Cardiology and of the Scientific Council on Cardiomyopathies of the World Heart Federation. Circulation 101, E101–E106 (2000). [PubMed: 10725299]
14. Sen-Chowdhry S et al. Left-dominant arrhythmogenic cardiomyopathy: an under-recognized clinical entity. J. Am. Coll. Cardiol 52, 2175–2187 (2008). [PubMed: 19095136]
15. Martins D et al. Myocardial inflammation detected by cardiac MRI in arrhythmogenic right ventricular cardiomyopathy: a paediatric case series. Int. J. Cardiol 271, 81–86 (2018). [PubMed: 29885824]
16. Sen-Chowdhry S et al. Clinical and genetic characterization of families with arrhythmogenic right ventricular dysplasia/cardiomyopathy provides novel insights into patterns of disease expression. Circulation 115, 1710–1720 (2007). [PubMed: 17372169]
17. Marcus F I. et al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the Task Force Criteria. Eur. Heart J 31, 806–814 (2010). [PubMed: 20172912]
18. Shimizu W Arrhythmias originating from the right ventricular outflow tract: how to distinguish 'malignant' from 'benign'? Heart Rhythm 6, 1507–1511 (2009). [PubMed: 19695964]
19. Zhang L, Liu L, Kowey PR & Fontaine GH The electrocardiographic manifestations of arrhythmogenic right ventricular dysplasia. Curr. Cardiol. Rev 10, 237–245 (2014). [PubMed: 24827798]
20. Abdelhamid MA & Diab HS The arrhythmic burden in patients with sarcoidosis. Is it a real concern? Egypt. J. Chest Dis. Tuberc 65, 311–317 (2016).
21. James CA et al. Exercise increases age-related penetrance and arrhythmic risk in arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated desmosomal mutation carriers. J. Am. Coll. Cardiol 62, 1290–1297 (2013). [PubMed: 23871885]
22. Maron BJ et al. Eligibility and disqualification recommendations for competitive athletes with cardiovascular abnormalities: Task Force 3: hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy and other cardiomyopathies, and myocarditis: a scientific statement from the American Heart Association and American College of Cardiology. Circulation 132, e273–e280 (2015). [PubMed: 26621644]
23. Mitchell JH, Haskell W, Snell P & Van Camp S P Task Force 8: classification of sports. J. Am. Coll. Cardiol 45, 1364–1367 (2005). [PubMed: 15837288]
24. Ruwald A-C et al. Association of competitive and recreational sport participation with cardiac events in patients with arrhythmogenic right ventricular cardiomyopathy: results from the North American multidisciplinary study of arrhythmogenic right ventricular cardiomyopathy. Eur. Heart J 36, 1735–1743 (2015). [PubMed: 25896080]
25. Mazzanti A et al. Arrhythmogenic right ventricular cardiomyopathy: clinical course and predictors of arrhythmic risk. J. Am. Coll. Cardiol 68, 2540–2550 (2016). [PubMed: 27931611]
26. Protonotarios N et al. Cardiac abnormalities in familial palmoplantar keratosis. Br. Heart J 56, 321–326 (1986). [PubMed: 2945574]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

27. Protonotarios NI, Tsatsopoulou AA & Gatzoulis KA Arrhythmogenic right ventricular cardiomyopathy caused by a deletion in plakoglobin (Naxos disease). Card. Electrophysiol. Rev 6, 72–80 (2002). [PubMed: 11984022]
28. McKoy G et al. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). Lancet 355, 2119–2124 (2000). [PubMed: 10902626]
29. Coonar AS et al. Gene for arrhythmogenic right ventricular cardiomyopathy with diffuse nonepidermolytic palmoplantar keratoderma and woolly hair (Naxos disease) maps to 17q21. Circulation 97, 2049–2058 (1998). [PubMed: 9610536]
30. Carvajal-Huerta L Epidermolytic palmoplantar keratoderma with woolly hair and dilated cardiomyopathy. J. Am. Acad. Dermatol 39, 418–421 (1998). [PubMed: 9738775]
31. Norgett EE et al. Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma. Hum. Mol. Genet 9, 2761–2766 (2000). [PubMed: 11063735]
32. Gerull B et al. Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. Nat. Genet 36, 1162–1164 (2004). [PubMed: 15489853]
33. Heuser A et al. Mutant desmocollin-2 causes arrhythmogenic right ventricular cardiomyopathy. Am. J. Hum. Genet 79, 1081–1088 (2006). [PubMed: 17186466]
34. Pilichou K et al. Mutations in desmoglein-2 gene are associated with arrhythmogenic right ventricular cardiomyopathy. Circulation 113, 1171–1179 (2006). [PubMed: 16505173]
35. den Haan AD et al. Comprehensive desmosome mutation analysis in north americans with arrhythmogenic right ventricular dysplasia/ cardiomyopathy. Circ. Cardiovasc. Genet 2, 428–435 (2009). [PubMed: 20031617]
36. Fressart V et al. Desmosomal gene analysis in arrhythmogenic right ventricular dysplasia/ cardiomyopathy: spectrum of mutations and clinical impact in practice. Europace 12, 861–868 (2010). [PubMed: 20400443]
37. Xu T et al. Compound and digenic heterozygosity contributes to arrhythmogenic right ventricular cardiomyopathy. J. Am. Coll. Cardiol 55, 587–597 (2010). [PubMed: 20152563]
38. Vite A & Radice GL N-Cadherin/catenin complex as a master regulator of intercalated disc function. Cell Commun. Adhes 21, 169–179 (2014). [PubMed: 24766605]
39. Vermij SH, Abriel H & van Veen TAB Refining the molecular organization of the cardiac intercalated disc. Cardiovasc. Res 113, 259–275 (2017). [PubMed: 28069669]
40. Mayosi BM et al. Identification of cadherin 2 (CDH2) mutations in arrhythmogenic right ventricular cardiomyopathy. Circ. Cardiovasc. Genet 10, e001605 (2017). [PubMed: 28280076]
41. Turkowski KL, Tester DJ, Bos JM, Haugaa KH & Ackerman MJ Whole exome sequencing with genomic triangulation implicates CDH2-encoded N-cadherin as a novel pathogenic substrate for arrhythmogenic cardiomyopathy. Congenit. Heart Dis 12, 226–235 (2017). [PubMed: 28326674]
42. van Hengel J et al. Mutations in the area composita protein αT-catenin are associated with arrhythmogenic right ventricular cardiomyopathy. Eur. Heart J 34, 201–210 (2013). [PubMed: 23136403]
43. Hoorntje ET et al. Arrhythmogenic cardiomyopathy: pathology, genetics, and concepts in pathogenesis. Cardiovasc. Res 113, 1521–1531 (2017). [PubMed: 28957532]
44. Goossens S et al. A unique and specific interaction between alphaT-catenin and plakophilin-2 in the area composita, the mixed-type junctional structure of cardiac intercalated discs. J. Cell Sci 120, 2126–2136 (2007). [PubMed: 17535849]
45. Bermúdez-Jiménez FJ et al. The novel desmin mutation p. Glu401Asp impairs filament formation, disrupts cell membrane integrity and causes severe arrhythmogenic left ventricular cardiomyopathy/ dysplasia. Circulation 137, 1595–1610 (2017). [PubMed: 29212896]
46. Xiong Q et al. Arrhythmogenic cardiomyopathy in a patient with a rare loss-of-function KCNQ1 mutation. J. Am. Heart Assoc 4, e001526 (2015). [PubMed: 25616976]
47. Forleo C et al. Clinical and functional characterization of a novel mutation in lamin a/c gene in a multigenerational family with arrhythmogenic cardiac laminopathy. PLOS ONE 10, e0121723 (2015). [PubMed: 25837155]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

48. van der Heijden JF & Hassink RJ The phospholamban p. Arg14del founder mutation in Dutch patients with arrhythmogenic cardiomyopathy. Neth. Hear: J 21, 284–285 (2013).
49. Tamargo J TGF03 mutations cause arrhythmogenic right ventricular dysplasia type 1 and open the door to understanding the biological role of TGFβ3 (where there's a will, there's a way). Cardiovasc. Res 96, 188–190 (2012). [PubMed: 22878021]
50. Pilichou K et al. Myocyte necrosis underlies progressive myocardial dystrophy in mouse dsg2-related arrhythmogenic right ventricular cardiomyopathy. J. Exp. Med 206, 1787–1802 (2009). [PubMed: 19635863]
51. Li D et al. Restrictive loss of plakoglobin in cardiomyocytes leads to arrhythmogenic cardiomyopathy. Hum. Mol. Genet 20, 4582–4596 (2011). [PubMed: 21880664]
52. Stroud MJ Linker of nucleoskeleton and cytoskeleton complex proteins in cardiomyopathy. Biophys. Rev 10, 1033–1051 (2018). [PubMed: 29869195]
53. Sequeira V, Nijenkamp LLAM, Regan JA & van der Velden J The physiological role of cardiac cytoskeleton and its alterations in heart failure. Biochim. Biophys. Acta 1838, 700–722 (2014). [PubMed: 23860255]
54. Lorenzon A et al. Desmin mutations and arrhythmogenic right ventricular cardiomyopathy. Am. J. Cardiol 111, 400–405 (2013). [PubMed: 23168288]
55. Klauke B et al. De novo desmin-mutation N116S is associated with arrhythmogenic right ventricular cardiomyopathy. Hum. Mol. Genet 19, 4595–4607 (2010). [PubMed: 20829228]
56. Burke B & Stewart CL The nuclear lamins: flexibility in function. Nat. Rev. Mol. Cell Biol 14, 13–24 (2013). [PubMed: 23212477]
57. Le Dour C et al. Decreased WNT/β-catenin signalling contributes to the pathogenesis of dilated cardiomyopathy caused by mutations in the lamin a/C gene. Hum. Mol. Genet 26, 333–343 (2017). [PubMed: 28069793]
58. Quarta G et al. Mutations in the Lamin A/C gene mimic arrhythmogenic right ventricular cardiomyopathy. Eur. Heart J 33, 1128–1136 (2012). [PubMed: 22199124]
59. Rajkumar R, Sembrat JC, McDonough B, Seidman CE & Ahmad F Functional effects of the TMEM43 Ser358Leu mutation in the pathogenesis of arrhythmogenic right ventricular cardiomyopathy. BMC Med. Genet 13, 21 (2012). [PubMed: 22458570]
60. Merner ND et al. Arrhythmogenic right ventricular cardiomyopathy type 5 is a fully penetrant, lethal arrhythmic disorder caused by a missense mutation in the TMEM43 gene. Am. J. Hum. Genet 82, 809–821 (2008). [PubMed: 18313022]
61. Christensen AH, Andersen CB, Tybjaerg-Hansen A, Haunso S & Svendsen JH Mutation analysis and evaluation of the cardiac localization of TMEM43 in arrhythmogenic right ventricular cardiomyopathy. Clin. Genet 80, 256–264 (2011). [PubMed: 21214875]
62. Baskin B et al. TMEM43 mutations associated with arrhythmogenic right ventricular cardiomyopathy in non-Newfoundland populations. Hum. Genet 132, 1245–1252 (2013). [PubMed: 23812740]
63. Granzier H, Wu Y, Siegfried L & LeWinter M Titin: physiological function and role in cardiomyopathy and failure. Heart Fail. Rev 10, 211–223 (2005). [PubMed: 16416044]
64. Gigli M et al. A review of the giant protein Titin in clinical molecular diagnostics of cardiomyopathies. Front. Cardiovasc. Med 3, 21 (2016). [PubMed: 27493940]
65. Rampazzo A et al. ARVD4, a new locus for arrhythmogenic right ventricular cardiomyopathy, maps to chromosome 2 long arm. Genomics 45, 259–263 (1997). [PubMed: 9344647]
66. Taylor M et al. Genetic variation in titin in arrhythmogenic right ventricular cardiomyopathy-overlap syndromes. Circulation 124, 876–885 (2011). [PubMed: 21810661]
67. Ortiz-Genga MF et al. Truncating FLNC mutations are associated with high-risk dilated and arrhythmogenic cardiomyopathies. J. Am. Coll. Cardiol 68, 2440–2451 (2016). [PubMed: 27908349]
68. Kiselev A et al. De novo mutations in FLNC leading to early-onset restrictive cardiomyopathy and congenital myopathy. Hum. Mutat 39, 1161–1172 (2018). [PubMed: 29858533]
69. MacLennan DH & Kranias EG Phospholamban: a crucial regulator of cardiac contractility. Nat. Rev. Mol. Cell Biol 4, 566 (2003). [PubMed: 12838339]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

70. van der Zwaag PA et al. Phospholamban R14del mutation in patients diagnosed with dilated cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy: evidence supporting the concept of arrhythmogenic cardiomyopathy. Eur. J. Heart Fail 14, 1199–1207 (2012). [PubMed: 22820313]
71. Sepehrkhouy S et al. Distinct fibrosis pattern in desmosomal and phospholamban mutation carriers in hereditary cardiomyopathies. Heart Rhythm 14, 1024–1032 (2017). [PubMed: 28365402]
72. Tiso N et al. Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). Hum. Mol. Genet 10, 189–194 (2001). [PubMed: 11159936]
73. Roux-Buisson N et al. Prevalence and significance of rare RYR2 variants in arrhythmogenic right ventricular cardiomyopathy/dysplasia: results of a systematic screening. Heart Rhythm 11, 1999–2009 (2014). [PubMed: 25041964]
74. Priori SG et al. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. Circulation 103, 196–200 (2001). [PubMed: 11208676]
75. Roston TM et al. A novel RYR2 loss-of-function mutation (I4855M) is associated with left ventricular non-compaction and atypical catecholaminergic polymorphic ventricular tachycardia. J. Electrocardiol 50, 227–233 (2017). [PubMed: 27646203]
76. Moncayo-Arlandi J & Brugada R Unmasking the molecular link between arrhythmogenic cardiomyopathy and Brugada syndrome. Nat. Rev. Cardiol 14, 744–756 (2017). [PubMed: 28703223]
77. Rook MB, Evers MM, Vos MA & Bierhuizen MFA Biology of cardiac sodium channel Nav1.5 expression. Cardiovasc. Res 93, 12–23 (2012). [PubMed: 21937582]
78. Te Riele ASJM et al. Multilevel analyses of SCN5A mutations in arrhythmogenic right ventricular dysplasia/cardiomyopathy suggest non-canonical mechanisms for disease pathogenesis. Cardiovasc. Res 113, 102–111 (2017). [PubMed: 28069705]
79. Beffagna G et al. Regulatory mutations in transforming growth factor-beta3 gene cause arrhythmogenic right ventricular cardiomyopathy type 1. Cardiovasc. Res 65, 366–373 (2005). [PubMed: 15639475]
80. Kaplan SR et al. Structural and molecular pathology of the heart in Carvajal syndrome. Cardiovasc. Pathol 13, 26–32 (2004). [PubMed: 14761782]
81. Prompona M, Kozlik-Feldmann R, Mueller-Hoecker J, Reiser M & Huber A Images in cardiovascular medicine. Magnetic resonance imaging characteristics in Carvajal syndrome (variant of Naxos disease). Circulation 116, e524–e530 (2007). [PubMed: 17998464]
82. Kolar AJO, Milroy CM, Day PF & Suvarna SK Dilated cardiomyopathy and sudden death in a teenager with palmar-plantar keratosis (occult Carvajal syndrome). J. Forensic Leg. Med 15, 185–188 (2008). [PubMed: 18313016]
83. Pelargonio G, Dello Russo A, Sanna T, De Martino G & Bellocci F Myotonic dystrophy and the heart. Heart 88, 665–670 (2002). [PubMed: 12433913]
84. Samanta R, Pouliopoulos J, Thiagalingam A & Kovoor P Role of adipose tissue in the pathogenesis of cardiac arrhythmias. Heart Rhythm 13, 311–320 (2016). [PubMed: 26277495]
85. Baroldi G, Silver MD, De Maria R, Parodi O & Pellegrini A Lipomatous metaplasia in left ventricular scar. Can. J. Cardiol 13, 65–71 (1997). [PubMed: 9039067]
86. Corrado D et al. Spectrum of clinicopathologic manifestations of arrhythmogenic right ventricular cardiomyopathy/dysplasia: a multicenter study. J. Am. Coll. Cardiol 30, 1512–1520 (1997). [PubMed: 9362410]
87. Castelletti S et al. Desmoplakin missense and non-missense mutations in arrhythmogenic right ventricular cardiomyopathy: genotype-phenotype correlation. Int. J. Cardiol 249, 268–273 (2017). [PubMed: 28527814]
88. Ruiz P et al. Targeted mutation of plakoglobin in mice reveals essential functions of desmosomes in the embryonic heart. J. Cell Biol 135, 215–225 (1996). [PubMed: 8858175]
89. Gallicano GI et al. Desmoplakin is required early in development for assembly of desmosomes and cytoskeletal linkage. J. Cell Biol 143, 2009–2022 (1998). [PubMed: 9864371]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

90. Grossmann KS et al. Requirement of plakophilin 2 for heart morphogenesis and cardiac junction formation. J. Cell Biol 167, 149–160 (2004). [PubMed: 15479741]

91. Kirchhof P et al. Age- and training-dependent development of arrhythmogenic right ventricular cardiomyopathy in heterozygous plakoglobin-deficient mice. Circulation 114, 1799–1806 (2006). [PubMed: 17030684]

92. Gomes J et al. Electrophysiological abnormalities precede overt structural changes in arrhythmogenic right ventricular cardiomyopathy due to mutations in desmoplakin-A combined murine and human study. Eur. Hear: J 33, 1942–1953 (2012).

93. Cerrone M et al. Sodium current deficit and arrhythmogenesis in a murine model of plakophilin-2 haploinsufficiency. Cardiovasc. Res 95, 460–468 (2012). [PubMed: 22764151]

94. Cerrone M et al. Plakophilin-2 is required for transcription of genes that control calcium cycling and cardiac rhythm. Nat. Commun 8, 106 (2017). [PubMed: 28740174]

95. Brodehl A et al. Transgenic mice overexpressing desmocollin-2 (DSC2) develop cardiomyopathy associated with myocardial inflammation and fibrotic remodeling. PLOS ONE 12, e0174019 (2017). [PubMed: 28339476]

96. Rizzo S et al. Intercalated disc abnormalities, reduced Na+ current density, and conduction slowing in desmoglein-2 mutant mice prior to cardiomyopathic changes. Cardiovasc. Res 95, 409–418 (2012). [PubMed: 22764152]

97. Notari M et al. iASPP, a previously unidentified regulator of desmosomes, prevents arrhythmogenic right ventricular cardiomyopathy (ARVC)-induced sudden death. Proc. Natl Acad. Sci. USA 112, E973–E981 (2015). [PubMed: 25691752]

98. Chelko SP et al. Central role for GSK3β in the pathogenesis of arrhythmogenic cardiomyopathy. JCI Insight 1,85923 (2016). [PubMed: 27170944]

99. Ellawindy A et al. Rho-kinase inhibition during early cardiac development causes arrhythmogenic right ventricular cardiomyopathy in mice. Arterioscler. Thromb. Vasc. Biol 35, 2172–2184 (2015). [PubMed: 26315406]

100. Garcia-Gras E et al. Suppression of canonical Wnt/beta-catenin signaling by nuclear plakoglobin recapitulates phenotype of arrhythmogenic right ventricular cardiomyopathy. J. Clin. Invest 116, 2012–2021 (2006). [PubMed: 16823493]

101. Kant S, Holthöfer B, Magin TM, Krusche CA & Leube RE Desmoglein 2-dependent arrhythmogenic cardiomyopathy is caused by a loss of adhesive function. Circ. Cardiovasc. Genet 8, 553–563 (2015). [PubMed: 26085008]

102. Lyon RC et al. Connexin defects underlie arrhythmogenic right ventricular cardiomyopathy in a novel mouse model. Hum. Mol. Genet 23, 1134–1150 (2014). [PubMed: 24108106]

103. Yang Z et al. Desmosomal dysfunction due to mutations in desmoplakin causes arrhythmogenic right ventricular dysplasia/cardiomyopathy. Circ. Res 99, 646–655 (2006). [PubMed: 16917092]

104. Chen SN et al. The hippo pathway is activated and is a causal mechanism for adipogenesis in arrhythmogenic cardiomyopathy. Circ. Res 114, 454–468 (2014). [PubMed: 24276085]

105. Yamaguchi Y et al. Adipogenesis and epicardial adipose tissue: a novel fate of the epicardium induced by mesenchymal transformation and PPARγ activation. Proc. Natl Acad. Sci. USA 112, 2070–2075 (2015). [PubMed: 25646471]

106. Lombardi R et al. Nuclear plakoglobin is essential for differentiation of cardiac progenitor cells to adipocytes in arrhythmogenic right ventricular cardiomyopathy. Circ. Res 109, 1342–1353 (2011). [PubMed: 22021931]

107. Lombardi R et al. Cardiac fibro-adipocyte progenitors express desmosome proteins and preferentially differentiate to adipocytes upon deletion of the desmoplakin gene. Circ. Res 119, 41–54 (2016). [PubMed: 27121621]

108. Lombardi R et al. Genetic fate mapping identifies second heart field progenitor cells as a source of adipocytes in arrhythmogenic right ventricular cardiomyopathy. Circ. Res 104, 1076–1084 (2009). [PubMed: 19359597]

109. Claycomb WC et al. HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte. Proc. Natl Acad. Sci. USA 95, 2979–2984 (1998). [PubMed: 9501201]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

110. Takahashi K et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861–872 (2007). [PubMed: 18035408]
111. Burridge PW et al. Chemically defined generation of human cardiomyocytes. Nat. Methods 11, 855–860 (2014). [PubMed: 24930130]
112. Zhang J et al. Functional cardiomyocytes derived from human induced pluripotent stem cells. Circ. Res 104, e30–e41 (2009). [PubMed: 19213953]
113. Wang G et al. Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies. Nat. Med 20, 616–623 (2014). [PubMed: 24813252]
114. Mannhardt I et al. Human engineered heart tissue: analysis of contractile force. Stem Cell Rep. 7, 29–42 (2016).
115. Ronaldson-Bouchard K et al. Advanced maturation of human cardiac tissue grown from pluripotent stem cells. Nature 556, 239–243 (2018). [PubMed: 29618819]
116. Basso C et al. Arrhythmogenic right ventricular cardiomyopathy. Dysplasia, dystrophy, or myocarditis? Circulation 94, 983–991 (1996). [PubMed: 8790036]
117. Asimaki A & Saffitz JE Remodeling of cell-cell junctions in arrhythmogenic cardiomyopathy. Cell Commun. Adhes 21, 13–23 (2014). [PubMed: 24460198]
118. Caspi O et al. Modeling of arrhythmogenic right ventricular cardiomyopathy with human induced pluripotent stem cells. Circ. Cardiovasc. Genet 6, 557–568 (2013). [PubMed: 24200905]
119. Li J et al. Cardiac tissue-restricted deletion of plakoglobin results in progressive cardiomyopathy and activation of {beta}-catenin signaling. Mol. Cell. Biol 31, 1134–1144 (2011). [PubMed: 21245375]
120. Huang H, Asimaki A, Lo D, McKenna W & Saffitz J Disparate effects of different mutations in plakoglobin on cell mechanical behavior. Cell Motil. Cytoskeleton 65, 964–978 (2008). [PubMed: 18937352]
121. Schlipp A et al. Desmoglein-2 interaction is crucial for cardiomyocyte cohesion and function. Cardiovasc. Res 104, 245–257 (2014). [PubMed: 25213555]
122. Sawant AC et al. Exercise has a disproportionate role in the pathogenesis of arrhythmogenic right ventricular dysplasia/cardiomyopathy in patients without desmosomal mutations. J. Am. Heart Assoc 3, e001471 (2014). [PubMed: 25516436]
123. Bosman LP et al. Predicting arrhythmic risk in arrhythmogenic right ventricular cardiomyopathy: a systematic review and meta-analysis. Heart Rhythm 15, 1097–1107 (2018). [PubMed: 29408436]
124. Gergek M et al. Cardiomyocyte hypertrophy in arrhythmogenic cardiomyopathy. Am. J. Pathol 187, 752–766 (2017). [PubMed: 28183531]
125. Mallat Z et al. Evidence of apoptosis in arrhythmogenic right ventricular dysplasia. N. Engl. J. Med 335, 1190–1196 (1996). [PubMed: 8815941]
126. Kavantzas NG, Lazaris AC, Agapitos EV, Nanas J & Davaris PS Histological assessment of apoptotic cell death in cardiomyopathies. Pathology 32, 176–180 (2000). [PubMed: 10968390]
127. Valente M et al. In vivo evidence of apoptosis in arrhythmogenic right ventricular cardiomyopathy. Am. J. Pathol 152, 479–484 (1998). [PubMed: 9466574]
128. Franke WW et al. Transmembrane protein PERP is a component of tessellate junctions and of other junctional and non-junctional plasma membrane regions in diverse epithelial and epithelium-derived cells. Cell Tissue Res. 353, 99–115 (2013). [PubMed: 23689684]
129. Akdis D et al. Myocardial expression profiles of candidate molecules in patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia compared to those with dilated cardiomyopathy and healthy controls. Heart Rhythm 13, 731–741 (2016). [PubMed: 26569459]
130. Ihrie RA et al. Perp is a mediator of p53-dependent apoptosis in diverse cell types. Curr. Biol 13, 1985–1990 (2003). [PubMed: 14614825]
131. Marques MR, Ihrie RA, Horner JS & Attardi LD The requirement for perp in postnatal viability and epithelial integrity reflects an intrinsic role in stratified epithelia. J. Invest. Dermatol 126, 69–73 (2006). [PubMed: 16417219]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

132. Hariharan V et al. Arrhythmogenic right ventricular cardiomyopathy mutations alter shear response without changes in cell–cell adhesion. Cardiovasc. Res 104, 280–289 (2014). [PubMed: 25253076]
133. Asimaki A et al. Identification of a new modulator of the intercalated disc in a zebrafish model of arrhythmogenic cardiomyopathy. Sci. Transl Med 6, 240ra74 (2014).
134. Alaee M, Nool K & Pasdar M Plakoglobin restores tumor suppressor activity of p53R175H mutant by sequestering the oncogenic potential of β-catenin. Cancer Sci. 109, 1876–1888 (2018). [PubMed: 29660231]
135. Fink SL & Cookson BT Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. Infect. Immun 73, 1907–1916 (2005). [PubMed: 15784530]
136. Leask A Getting to the heart of the matter: new insights into cardiac fibrosis. Circ. Res 116, 1269–1276 (2015). [PubMed: 25814687]
137. Dobaczewski M et al. Smad3 signaling critically regulates fibroblast phenotype and function in healing myocardial infarction. Circ. Res 107, 418–428 (2010). [PubMed: 20522804]
138. Khalil H et al. Fibroblast-specific TGF-β-Smad2/3 signaling underlies cardiac fibrosis. J. Clin. Invest 127, 3770–3783 (2017). [PubMed: 28891814]
139. Leask A TGFbeta, cardiac fibroblasts, and the fibrotic response. Cardiovasc. Res 74, 207–212 (2007). [PubMed: 16919613]
140. Jain M et al. Mitochondrial reactive oxygen species regulate transforming growth factor-β signaling. J. Biol. Chem 288, 770–777 (2013). [PubMed: 23204521]
141. Yu L, Hébert MC & Zhang Y E. TGF-beta receptor-activated p38 MAP kinase mediates Smad-independent TGF-beta responses. EMBO J. 21, 3749–3759 (2002). [PubMed: 12110587]
142. Zhang H et al. Profiling of differentially expressed microRNAs in arrhythmogenic right ventricular cardiomyopathy. Sci. Rep 6, 28101 (2016). [PubMed: 27307080]
143. Rampazzo A et al. Arrhythmogenic right ventricular cardiomyopathy type 1 (ARVD1): confirmation of locus assignment and mutation screening of four candidate genes. Eur. J. Hum. Genet 11, 69–76 (2003). [PubMed: 12529708]
144. Dubash AD et al. Plakophilin-2 loss promotes TGF-β1/p38 MAPK-dependent fibrotic gene expression in cardiomyocytes. J. Cell Biol 212, 425–438 (2016). [PubMed: 26858265]
145. Stadiotti I et al. Arrhythmogenic cardiomyopathy: the guilty party in adipogenesis. J. Cardiovasc. Transl Res 10, 446–454 (2017). [PubMed: 28983804]
146. Moses KA, DeMayo F, Braun RM, Reecy JL & Schwartz RJ Embryonic expression of an Nkx2–5/Cre gene using ROSA26 reporter mice. Genesis 31, 176–180 (2001). [PubMed: 11783008]
147. Zhou B, von Gise A, Ma Q, Rivera-Feliciano J & Pu WT Nkx2–5- and Isl1-expressing cardiac progenitors contribute to proepicardium. Biochem. Biophys. Res. Commun 375, 450–453 (2008). [PubMed: 18722343]
148. Verzi MP, McCulley DJ, De Val S, Dodou E & Black BL The right ventricle, outflow tract, and ventricular septum comprise a restricted expression domain within the secondary/anterior heart field. Dev. Biol 287, 134–145 (2005). [PubMed: 16188249]
149. Agah R et al. Gene recombination in postmitotic cells. Targeted expression of Cre recombinase provokes cardiac-restricted, site-specific rearrangement in adult ventricular muscle in vivo. J. Clin. Invest 100, 169–179 (1997). [PubMed: 9202069]
150. Chong JJH et al. Adult cardiac-resident MSC-like stem cells with a proepicardial origin. Cell Stem Cell 9, 527–540 (2011). [PubMed: 22136928]
151. Kim C et al. Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. Nature 494, 105–110 (2013). [PubMed: 23354045]
152. Dorn T et al. Interplay of cell-cell contacts and RhoA/ MRTF-A signaling regulates cardiomyocyte identity. EMBO J. 37, e98133 (2018). [PubMed: 29764980]
153. Lorenzon A et al. Wnt/β-catenin pathway in arrhythmogenic cardiomyopathy. Oncotarget 8, 60640–60655 (2017). [PubMed: 28948000]
154. Giuliodori A et al. Loss of cardiac Wnt/β-catenin signalling in Desmoplakin-deficient AC8 zebrafish models is rescuable by genetic and pharmacological intervention. Cardiovasc. Res 114, 1082–1097 (2018). [PubMed: 29522173]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

155. Zhurinsky J, Shtutman M & Ben-Ze'ev A Plakoglobin and beta-catenin: protein interactions, regulation and biological roles. J. Cell Sci 113, 3127–3139 (2000). [PubMed: 10954412]
156. Niehrs C The complex world of WNT receptor signalling. Nat. Rev. Mol. Cell Biol 13, 767–779 (2012). [PubMed: 23151663]
157. Laudes M Role of WNT signalling in the determination of human mesenchymal stem cells into preadipocytes. J. Mol. Endocrinol 46, R65–R72 (2011). [PubMed: 21247979]
158. Salomon D et al. Regulation of beta-catenin levels and localization by overexpression of plakoglobin and inhibition of the ubiquitin-proteasome system. J. Cell Biol 139, 1325–1335 (1997). [PubMed: 9382877]
159. Noorman M et al. Remodeling of the cardiac sodium channel, connexin43, and plakoglobin at the intercalated disk in patients with arrhythmogenic cardiomyopathy. Heart Rhythm 10, 412–419 (2013). [PubMed: 23178689]
160. Asimaki A et al. A new diagnostic test for arrhythmogenic right ventricular cardiomyopathy. N. Engl. J. Med 360, 1075–1084 (2009). [PubMed: 19279339]
161. Lombardi R et al. Nuclear plakoglobin is essential for differentiation of cardiac progenitor cells to adipocytes in arrhythmogenic right ventricular cardiomyopathy. Circ. Res 109, 1342–1353 (2011). [PubMed: 22021931]
162. Daugherty RL et al. α-Catenin is an inhibitor of transcription. Proc. Natl Acad. Sci. USA 111, 5260–5265 (2014). [PubMed: 24706864]
163. Grigoryan T, Wend P, Klaus A & Birchmeier W Deciphering the function of canonical Wnt signals in development and disease: conditional loss- and gain-of-function mutations of beta-catenin in mice. Genes Dev. 22, 2308–2341 (2008). [PubMed: 18765787]
164. Chen X et al. The β-catenin/T-cell factor/lymphocyte enhancer factor signaling pathway is required for normal and stress-induced cardiac hypertrophy. Mol. Cell. Biol 26, 4462–4473 (2006). [PubMed: 16738313]
165. Cristancho AG & Lazar MA Forming functional fat: a growing understanding of adipocyte differentiation. Nat. Rev. Mol. Cell Biol 12, 722–734 (2011). [PubMed: 21952300]
166. McBeath R, Pirone DM, Nelson CM, Bhadriraju K & Chen CS Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Dev. Cell 6, 483–495 (2004). [PubMed: 15068789]
167. Li L, Miano JM, Cserjesi P & Olson EN SM22 alpha, a marker of adult smooth muscle, is expressed in multiple myogenic lineages during embryogenesis. Circ. Res 78, 188–195 (1996). [PubMed: 8575061]
168. Kanazawa A et al. Wnt5b partially inhibits canonical Wnt/beta-catenin signaling pathway and promotes adipogenesis in 3T3-L1 preadipocytes. Biochem. Biophys. Res. Commun 330, 505–510 (2005). [PubMed: 15796911]
169. Godsel LM et al. Plakophilin 2 couples actomyosin remodeling to desmosomal plaque assembly via RhoA. Mol. Biol. Cell 21, 2844–2859 (2010). [PubMed: 20554761]
170. Schlessinger K, Hall A & Tolwinski N Wnt signaling pathways meet Rho GTPases. Genes Dev. 23, 265–277 (2009). [PubMed: 19204114]
171. Yu F-X, Zhao B & Guan K-L Hippo pathway in organ size control, tissue homeostasis, and cancer. Cell 163, 811–828 (2015). [PubMed: 26544935]
172. Karaman R & Halder G Cell junctions in Hippo signaling. Cold Spring Harb. Perspect. Biol 10, a028753 (2018). [PubMed: 28600393]
173. Halder G, Dupont S & Piccolo S Transduction of mechanical and cytoskeletal cues by YAP and TAZ. Nat. Rev. Mol. Cell Biol 13, 591–600 (2012). [PubMed: 22895435]
174. Schlegelmilch K et al. Yap1 acts downstream of alpha-catenin to control epidermal proliferation. Cell 144, 782–795 (2011). [PubMed: 21376238]
175. Vite A, Zhang C, Yi R, Emms S & Radice GL α-Catenin-dependent cytoskeletal tension controls Yap activity in the heart. Development 145, dev149823 (2018). [PubMed: 29467248]
176. Dupont S et al. Role of YAP/TAZ in mechanotransduction. Nature 474, 179–183 (2011). [PubMed: 21654799]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

177. Imajo M, Miyatake K, Iimura A, Miyamoto A & Nishida E A molecular mechanism that links Hippo signalling to the inhibition of Wnt/β-catenin signalling. EMBO J. 31, 1109–1122 (2012). [PubMed: 22234184]
178. Heallen T et al. Hippo pathway inhibits Wnt signaling to restrain cardiomyocyte proliferation and heart size. Science 332, 458–461 (2011). [PubMed: 21512031]
179. Azzolin L et al. YAP/TAZ incorporation in the β-catenin destruction complex orchestrates the Wnt response. Cell 158, 157–170 (2014). [PubMed: 24976009]
180. Lefterova MI, Haakonsson AK, Lazar MA & Mandrup S PPARγ and the global map of adipogenesis and beyond. Trends Endocrinol. Metab 25, 293–302 (2014). [PubMed: 24793638]
181. Son N-H et al. Cardiomyocyte expression of PPARgamma leads to cardiac dysfunction in mice. J. Clin. Invest 117, 2791–2801 (2007). [PubMed: 17823655]
182. Liu J, Wang H, Zuo Y & Farmer SR Functional interaction between peroxisome proliferator-activated receptor gamma and beta-catenin. Mol. Cell. Biol 26, 5827–5837 (2006). [PubMed: 16847334]
183. Gurha P, Chen X, Lombardi R, Willerson JT & Marian AJ Knockdown of plakophilin 2 downregulates miR-184 through CpG hypermethylation and suppression of the E2F1 pathway and leads to enhanced adipogenesis in vitro. Circ. Res 119, 731–750 (2016). [PubMed: 27470638]
184. Quang KL et al. Loss of cardiomyocyte integrin-linked kinase produces an arrhythmogenic cardiomyopathy in mice. Circ. Arrhythm. Electrophysiol 8, 921–932 (2015). [PubMed: 26071395]
185. Asimaki A et al. Altered desmosomal proteins in granulomatous myocarditis and potential pathogenic links to arrhythmogenic right ventricular cardiomyopathy. Circ. Arrhythm. Electrophysiol 4, 743–752 (2011). [PubMed: 21859801]
186. Campuzano O et al. Arrhythmogenic right ventricular cardiomyopathy: severe structural alterations are associated with inflammation. J. Clin. Pathol 65, 1077–1083 (2012). [PubMed: 22944624]
187. Pollack A, Kontorovich AR, Fuster V & Dec GW Viral myocarditis-diagnosis, treatment options, and current controversies. Nat. Rev. Cardiol 12, 670–680 (2015). [PubMed: 26194549]
188. Heim A, Grumbach I, Stille-Siegener M & Figulla HR Detection of enterovirus RNA in the myocardium of a patient with arrhythmogenic right ventricular cardiomyopathy by in situ hybridization. Clin. Infect. Dis 25, 1471–1472 (1997). [PubMed: 9431399]
189. Grumbach IM et al. Coxsackievirus genome in myocardium of patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy. Cardiology 89, 241–245 (1998). [PubMed: 9643269]
190. Bowles NE, Ni J, Marcus F & Towbin JA The detection of cardiotropic viruses in the myocardium of patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy. J. Am. Coll. Cardiol 39, 892–895 (2002). [PubMed: 11869858]
191. Calabrese F et al. No detection of enteroviral genome in the myocardium of patients with arrhythmogenic right ventricular cardiomyopathy. J. Clin. Pathol 53, 382–387 (2000). [PubMed: 10889821]
192. Belkaya S et al. Autosomal recessive cardiomyopathy presenting as acute myocarditis. J. Am. Coll. Cardiol 69, 1653–1665 (2017). [PubMed: 28359509]
193. Tanawuttiwat T, Sager SJ, Hare JM & Myerburg RJ Myocarditis and ARVC/D: variants or mimics? Heart Rhythm 10, 1544–1548 (2013). [PubMed: 23773988]
194. Li D, Zhang W, Liu Y, Haneline LS & Shou W Lack of plakoglobin in epidermis leads to keratoderma. J. Biol. Chem 287, 10435–10443 (2012). [PubMed: 22315228]
195. Asimaki A, Kléber AG, MacRae CA & Saffitz JE Arrhythmogenic cardiomyopathy - new insights into disease mechanisms and drug discovery. Prog. Pediatr. Cardiol 37, 3–7 (2014). [PubMed: 25506190]
196. Kleber AG & Saffitz JE Role of the intercalated disc in cardiac propagation and arrhythmogenesis. Front. Physiol 5, 404 (2014). [PubMed: 25368581]
197. Severs NJ et al. Remodelling of gap junctions and connexin expression in heart disease. Biochim. Biophys. Acta 1662, 138–148 (2004). [PubMed: 15033584]

198. Jongsma HJ & Wilders R Gap junctions in cardiovascular disease. Circ. Res 86, 1193–1197 (2000). [PubMed: 10864907]
199. Fidler LM et al. Abnormal connexin43 in arrhythmogenic right ventricular cardiomyopathy caused by plakophilin-2 mutations. J. Cell. Mol. Med 13, 4219–4228 (2009). [PubMed: 18662195]
200. Kaplan SR et al. Remodeling of myocyte gap junctions in arrhythmogenic right ventricular cardiomyopathy due to a deletion in plakoglobin (Naxos disease). Heart Rhythm 1, 3–11 (2004). [PubMed: 15851108]
201. Oxford EM et al. Connexin43 remodeling caused by inhibition of plakophilin-2 expression in cardiac cells. Circ. Res 101, 703–711 (2007). [PubMed: 17673670]
202. Chen X, Chen L, Chen Z, Chen X & Song J Remodelling of myocardial intercalated disc protein connexin 43 causes increased susceptibility to malignant arrhythmias in ARVC/D patients. Forens. Sci. Int 275, 14–22 (2017).
203. Siragam V et al. TMEM43 mutation p. S358L alters intercalated disc protein expression and reduces conduction velocity in arrhythmogenic right ventricular cardiomyopathy. PLOS ONE 9, e109128 (2014). [PubMed: 25343256]
204. Sato PY et al. Loss of plakophilin-2 expression leads to decreased sodium current and slower conduction velocity in cultured cardiac myocytes. Circ. Res 105, 523–526 (2009). [PubMed: 19661460]
205. Lowe JS et al. Voltage-gated Nav channel targeting in the heart requires an ankyrin-G dependent cellular pathway. J. Cell Biol 180, 173–186 (2008). [PubMed: 18180363]
206. Sato PY et al. Interactions between ankyrin-G, plakophilin-2, and connexin43 at the cardiac intercalated disc. Circ. Res 109, 193–201 (2011). [PubMed: 21617128]
207. Patel DM, Dubash AD, Kreitzer G & Green KJ Disease mutations in desmoplakin inhibit Cx43 membrane targeting mediated by desmoplakin-EB1 interactions. J. Cell Biol 206, 779–797 (2014). [PubMed: 25225338]
208. Shaw RM et al. Microtubule plus-end-tracking proteins target gap junctions directly from the cell interior to adherens junctions. Cell 128, 547–560 (2007). [PubMed: 17289573]
209. Agullo-Pascual E et al. Super-resolution fluorescence microscopy of the cardiac connexome reveals plakophilin-2 inside the connexin43 plaque. Cardiovasc. Res 100, 231–240 (2013). [PubMed: 23929525]
210. Agullo-Pascual E et al. Super-resolution imaging reveals that loss of the C-terminus of connexin43 limits microtubule plus-end capture and Nav1.5 localization at the intercalated disc. Cardiovasc. Res 104, 371–381 (2014). [PubMed: 25139742]
211. Lal H, Ahmad F, Woodgett J & Force T The GSK-3 family as therapeutic target for myocardial diseases. Circ. Res 116, 138–149 (2015). [PubMed: 25552693]
212. Song Z, Ko CY, Nivala M, Weiss JN & Qu Z Calcium-voltage coupling in the genesis of early and delayed afterdepolarizations in cardiac myocytes. Biophys. J 108, 1908–1921 (2015). [PubMed: 25902431]
213. El-Battrawy I et al. Electrical dysfunctions in human-induced pluripotent stem cell-derived cardiomyocytes from a patient with an arrhythmogenic right ventricular cardiomyopathy. Europace 20, f46–f56 (2018). [PubMed: 29566126]
214. Karmouch J et al. A distinct cellular basis for early cardiac arrhythmias, the cardinal manifestation of arrhythmogenic cardiomyopathy, and the skin phenotype of cardiocutaneous syndromes. Circ. Res 121, 1346–1359 (2017). [PubMed: 29018034]
215. Lal H et al. Cardiac fibroblast glycogen synthase kinase-3β regulates ventricular remodeling and dysfunction in ischemic heart. Circulation 130, 419–430 (2014). [PubMed: 24899689]
216. Kapoor M et al. GSK-3beta in mouse fibroblasts controls wound healing and fibrosis through an endothelin-1-dependent mechanism. J. Clin. Invest 118, 3279–3290 (2008). [PubMed: 18802478]
217. Konstam MA et al. Effects of the angiotensin converting enzyme inhibitor enalapril on the long-term progression of left ventricular dysfunction in patients with heart failure. SOLVD Investigators. Circulation 86, 431–438 (1992). [PubMed: 1638712]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

218. Greenberg B et al. Effects of long-term enalapril therapy on cardiac structure and function in patients with left ventricular dysfunction. Results of the SOLVD echocardiography substudy. Circulation 91, 2573–2581 (1995). [PubMed: 7743619]
219. Schnee JM & Hsueh WA Angiotensin II, adhesion, and cardiac fibrosis. Cardiovasc. Res 46, 264–268 (2000). [PubMed: 10773230]
220. Schorb W et al. Angiotensin II is mitogenic in neonatal rat cardiac fibroblasts. Circ. Res 72, 1245–1254 (1993). [PubMed: 8495553]
221. Campbell SE & Katwa LC Angiotensin II stimulated expression of transforming growth factor-beta1 in cardiac fibroblasts and myofibroblasts. J. Mol. Cell. Cardiol 29, 1947–1958 (1997). [PubMed: 9236148]
222. Gray MO, Long CS, Kalinyak JE, Li HT & Karliner JS Angiotensin II stimulates cardiac myocyte hypertrophy via paracrine release of TGF-beta 1 and endothelin-1 from fibroblasts. Cardiovasc. Res 40, 352–363 (1998). [PubMed: 9893729]
223. Morel E et al. Blockade of the renin-angiotensin-aldosterone system in patients with arrhythmogenic right ventricular dysplasia: A double-blind, multicenter, prospective, randomized, genotype-driven study (BRAVE study). Clin. Cardiol 41,300–306 (2018). [PubMed: 29574980]
224. Cernecka H et al. Ramipril restores PPARβ/δ and PPARγ expressions and reduces cardiac NADPH oxidase but fails to restore cardiac function and accompanied myosin heavy chain ratio shift in severe anthracycline-induced cardiomyopathy in rat. Eur. J. Pharmacol 791, 244–253 (2016). [PubMed: 27592051]
225. Djouadi F et al. A potential link between peroxisome proliferator-activated receptor signalling and the pathogenesis of arrhythmogenic right ventricular cardiomyopathy. Cardiovasc. Res 84, 83–90 (2009). [PubMed: 19497962]
226. Xu Z et al. Genotype-phenotype relationship in patients with arrhythmogenic right ventricular cardiomyopathy caused by desmosomal gene mutations: a systematic review and meta-analysis. Sci. Rep 7, 41387 (2017). [PubMed: 28120905]
227. Gehmlich K et al. Novel missense mutations in exon 15 of desmoglein-2: role of the intracellular cadherin segment in arrhythmogenic right ventricular cardiomyopathy? Heart Rhythm 7, 1446–1453 (2010). [PubMed: 20708101]
228. Posch MG et al. A missense variant in desmoglein-2 predisposes to dilated cardiomyopathy. Mol. Genet. Metab 95, 74–80 (2008). [PubMed: 18678517]
229. Al-Sabeq B, Krahn AD, Conacher S, Klein GJ & Laksman Z Arrhythmogenic right ventricular cardiomyopathy with recessive inheritance related to a new homozygous desmocollin-2 mutation. Can. J. Cardiol 30, 696.e1–696.e3 (2014).
230. Lorenzon A et al. Homozygous desmocollin-2 mutations and arrhythmogenic cardiomyopathy. Am. J. Cardiol 116, 1245–1251 (2015). [PubMed: 26310507]
231. Karmouch J, Protonotarios A & Syrris P Genetic basis of arrhythmogenic cardiomyopathy. Curr. Opin. Cardiol 33, 276–281 (2018). [PubMed: 29543670]
232. Brun F et al. Titin and desmosomal genes in the natural history of arrhythmogenic right ventricular cardiomyopathy. J. Med. Genet 51, 669–676 (2014). [PubMed: 25157032]
233. Cruz FM et al. Exercise triggers ARVC phenotype in mice expressing a disease-causing mutated version of human plakophilin-2. J. Am. Coll. Cardiol 14, 1438–1450 (2015).
234. Kirchner F et al. Molecular insights into arrhythmogenic right ventricular cardiomyopathy caused by plakophilin-2 missense mutations. Circ. Cardiovasc. Genet 5, 400–411 (2012). [PubMed: 22781308]
235. Cerrone M et al. Missense mutations in plakophilin-2 cause sodium current deficit and associate with a Brugada syndrome phenotype. Circulation 129, 1092–1103 (2014). [PubMed: 24352520]
236. Ma D et al. Generation of patient-specific induced pluripotent stem cell-derived cardiomyocytes as a cellular model of arrhythmogenic right ventricular cardiomyopathy. Eur. Heart J 34, 1122–1133 (2013). [PubMed: 22798562]
237. Wen JY et al. Maturation-based model of arrhythmogenic right ventricular dysplasia using patient-specific induced pluripotent stem cells. Circ. J 79, 1402–1408 (2015). [PubMed: 25971409]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

238. Matthes SA et al. Plakophilin-2 and the migration, differentiation and transformation of cells derived from the epicardium of neonatal rat hearts. Cell Commun. Adhes 18, 73–84 (2011). [PubMed: 21985446]
239. Zhang Q et al. Silencing of desmoplakin decreases connexin43/Nav1.5 expression and sodium current in HL-1 cardiomyocytes. Mol. Med. Rep 8, 780–786 (2013). [PubMed: 23877755]
240. Beffagna G et al. Missense mutations in desmocollin-2 N-terminus, associated with arrhythmogenic right ventricular cardiomyopathy, affect intracellular localization of desmocollin-2 in vitro. BMC Med. Genet 8, 65 (2007). [PubMed: 17963498]
241. Gehmlich K et al. A novel desmocollin-2 mutation reveals insights into the molecular link between desmosomes and gap junctions. Heart Rhythm 8, 711–718 (2011). [PubMed: 21220045]
242. Gerull B et al. Homozygous founder mutation in desmocollin-2 (DSC2) causes arrhythmogenic cardiomyopathy in the Hutterite population. Circ. Cardiovasc. Genet 6, 326–327 (2013).
243. Eshkind L et al. Loss of desmoglein 2 suggests essential functions for early embryonic development and proliferation of embryonal stem cells. Eur. J. Cell Biol 81, 592–598 (2002). [PubMed: 12494996]
244. Zhang Z et al. Normalization of Naxos plakoglobin levels restores cardiac function in mice. J. Clin. Invest 125, 1708–1712 (2015). [PubMed: 25705887]
245. Asimaki A et al. A novel dominant mutation in plakoglobin causes arrhythmogenic right ventricular cardiomyopathy. Am. J. Hum. Genet 81, 964–973 (2007). [PubMed: 17924338]
246. Stroud MJ et al. Luma is not essential for murine cardiac development and function. Cardiovasc. Res 114, 378–388 (2017).
247. Begay RL et al. Filamin C truncation mutations are associated with arrhythmogenic dilated cardiomyopathy and changes in the cell-cell adhesion structures. JACC Clin. Electrophysiol 4, 504–514 (2018). [PubMed: 30067491]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

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.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

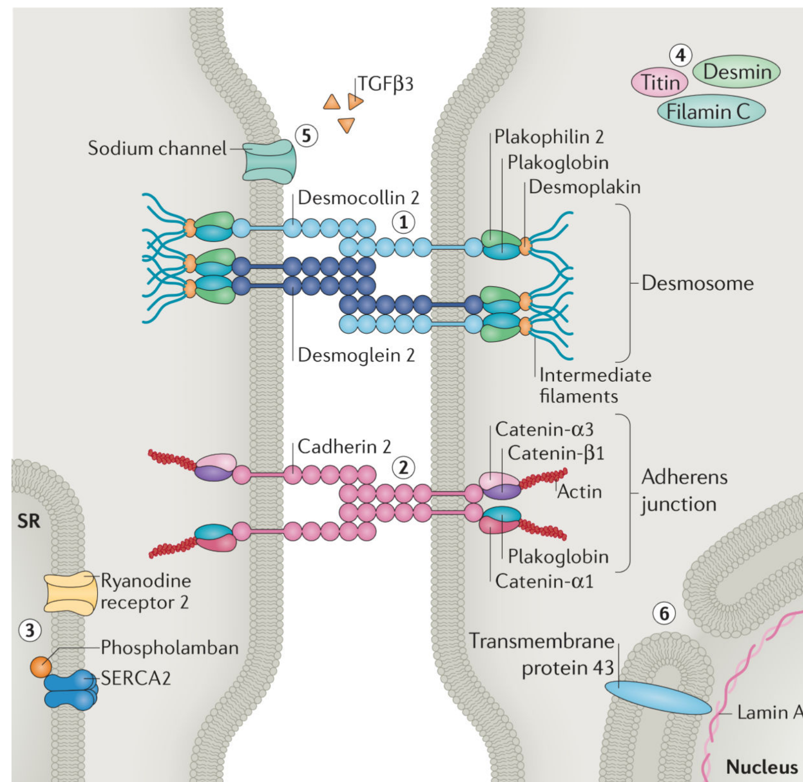

Fig. 1 |. Cellular components implicated in ACM.

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-α3. (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-β3 (TGFβ3). (6) Nuclear envelope proteins transmembrane protein 43 and lamin A. SERCA2, sarcoplasmic/endoplasmic reticulum calcium ATPase 2.

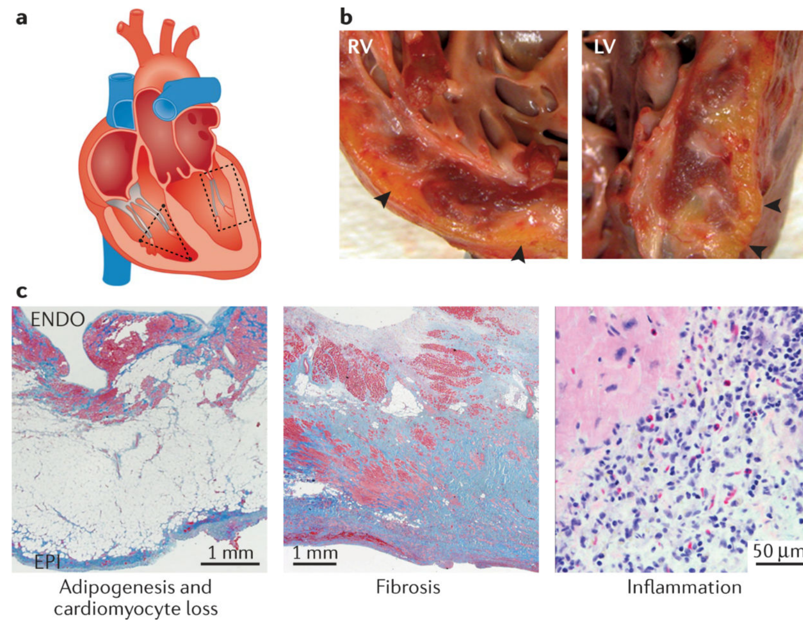

Fig. 2 |. Cross and histological features of ACM.

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.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

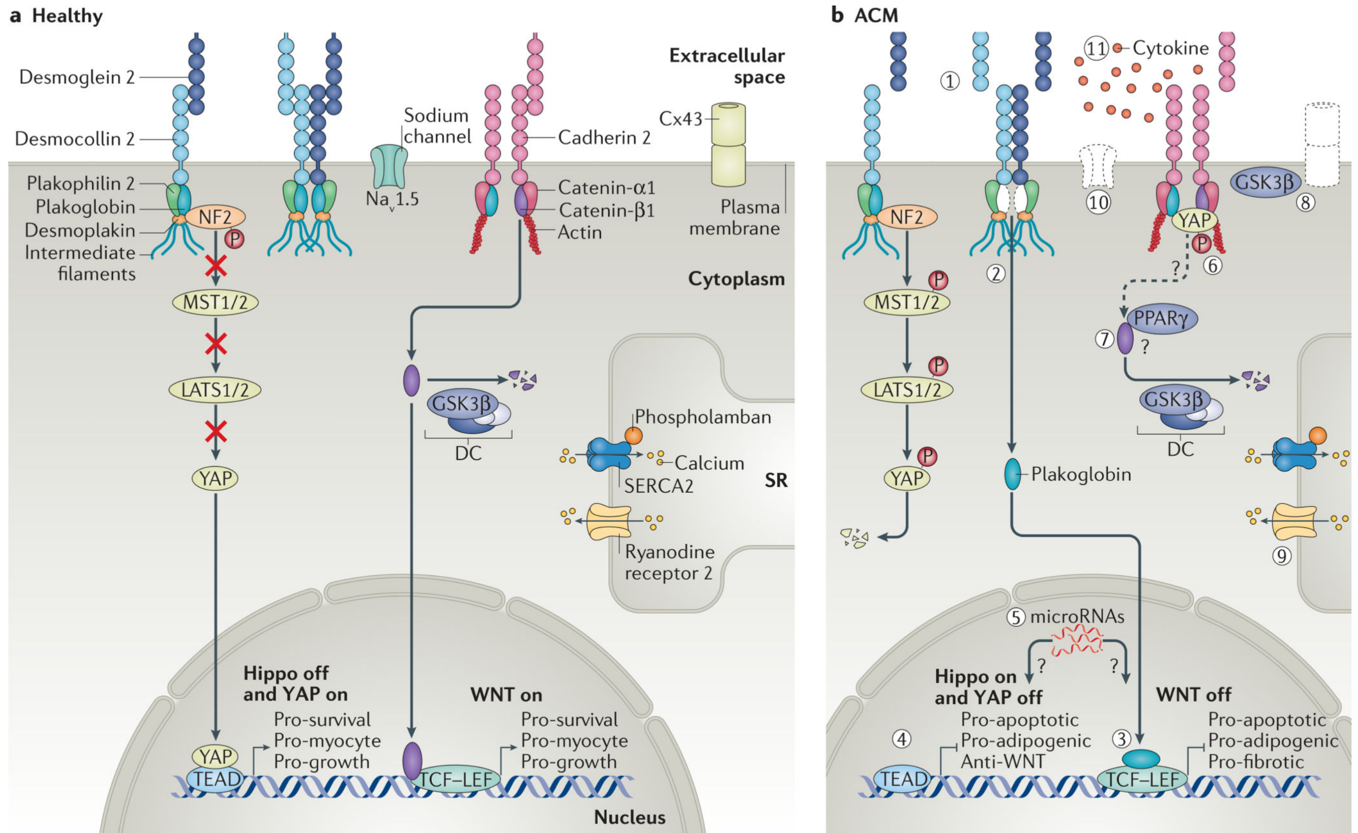

Fig. 3 |. Proposed molecular mechanisms contributing to the pathogenesis of ACM.

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. (ii) Increased pro-inflammatory and profibrotic cytokine production, including transforming grown 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.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

Table 1 |

Genes associated with ACM

Gene	Protein	Estimated frequency (%)	Features	Refs
Desmosome				
PKP2	Plakophilin 2	19–46	Most commonly mutated	11,35,36,55,226
DSP	Desmoplakin	1–16	AR mutation associated with Carvajal syndrome; C-terminal mutations associated with LV-dominant disease	9,226
DSG2	Desmoglein 2	2.5–10.0	Overlap with DCM phenotype	226–228
DSC2	Desmocollin 2	1–8	AR (without cutaneous manifestations) and AD inheritance	33,226,229,230
JUP	Junction plakoglobin	Rare–1	AR mutation associated with Naxos disease	43,226,231
Adherens junction				
CTNNA3	Catenin-α3	Rare	Incomplete penetrance; normal plakoglobin localization	42
CDH2	Cadherin 2	Rare–2	No specific genotype–phenotype relationship identified	40,41
Cytoskeletal structure				
LMNA	Lamin A/C	Rare	More common in severe forms of ACM with a dilated phenotype and high risk of sudden cardiac death	58
DES	Desmin	Rare	Fully penetrant; associated with LV-dominant ACM, DCM and skeletal myopathies	45
FLNC	Filamin C	Rare	Associated with LV-dominant ACM and high rates of arrhythmia	67
TMEM43	Transmembrane protein 43	Rare	Fully penetrant; men more severely affected than women	60
TTN	Titin	Rare	Higher risk of supraventricular tachycardia and progression to heart failure	66,232
Ion transport				
RYR2	Ryanodine receptor 2	Rare	Mutations in regions of calcium channel regulation	72
SCN5A	$Na_v1.5$	Rare–2	Prolonged QRS interval	78
PLN	Phospholamban	Rare	Clinical overlap with DCM	48
Cytokine				
TGFB3	Transforming growth factor-β3	Rare	No specific genotype–phenotype relationship identified	79

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

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

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 PPARγ	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–GSK3β signalling	Altered Hippo–YAP signalling	Altered cell metabolism	
Pkp2																												
Pkp2$^{-/-}$	Mouse	•																										90
Pkp2$^{+/-}$	Mouse							•														•						93
Pkp2^{flox} Myh6–CreERT2	Mouse			•		•		•			•											•		•				94
AAV-cTNT-*Pkp2* R735X	Mouse			•		•															•							213
Pkp2 siRNA	HL-1																				•							199
Pkp2-C796R; Pkp2-C796M	HL-1																	•										214
Pkp2 shRNA	HL-1											•				•									•	•		104
Pkp2 shRNA	HL-1																					•						215
Pkp2 shRNA	NRVM																				•							201
Pkp2 siRNA	NRVM										•	•						•										144
Pkp2 c.1841T>C	iPSC											•						•										216
Pkp2 c.972InsT/N; *Pkp2* c.148_151delACAG/N	iPSC															•	•	•			•							110
Pkp2 c.2484C>T; *Pkp2* c.2013delC	iPSC									•		•										•		•				217
Pkp2 c.2484C>T	iPSC									•		•			•									•	•		•	152
Pkp2 siRNA	Epicardial																				•							203
Pkp2 siRNA	Epicardial											•																218
Dsp																												
Dsp$^{-/-}$	Mouse	•																										89
Dsp$^{+/-}$	Mouse							•													•							92
Tg(Myh6)-Dsp-R2834H	Mouse				•	•				•	•	•					•											105
Dsp^{flox} Mlc2v–Cre	Mouse				•	•	•	•	•		•	•					•				•							102
Dsp^{flox} Myh6–Cre	Mouse	•	•		•	•	•	•		•	•	•			•										•			100
dsp morpholino	Zebrafish																•								•	•		154
Dsp siRNA	HL-1											•				•									•			100
Dsp siRNA	HL-1																				•	•						239
Dsp siRNA; Dsp-N287K, N458Y, K470E, I533T, A566T	NHEK and NRVM																				•							207
Dsc2																												
Tg(Myh6)-Dsc2	Mouse										•		•	•														95
Dsc2-E102K; Dsc2-I345T	HL-1																	•										240
Dsc2-Q851fsX855; Dsc2-A897fsX900	HL-1 and NRVM																		•									241
Dsc2-Q554X	HeLa and HEK																	•										242
Dsg2$^{-/-}$	Mouse	•																										243
Dsg2$^{-/-}$	Mouse				•				•	•	•										•							98
Dsg2^{flox} Myh6–Cre	Mouse			•		•			•	•	•		•	•			•				•							101
Tg(Myh6)-Dsg2-N271S	Mouse		•		•	•	•		•	•	•		•	•								•						96
Dsg2 siRNA; Dsg2-D154E, D264E, N266S, D494N, A517V, G812C, G812S, C813R, V920G	HL-1																			•								121
Jup																												
Jup$^{-/-}$	Mouse	•															•											88
Jup$^{+/-}$	Mouse			•			•	•																				91
Tg(Myh6)-Jup truncated	Mouse			•		•	•				•	•			•	•				•					•	•		104
Jup^{flox} Myh6–CreERT2	Mouse	•			•					•	•			•			•				•				•			53
Tg(jup c.2157del2)	Zebrafish		•							•				•	•						•	•	•		•			133
Jup c.2157del2 (OriNax)	Mouse	•																										244
Jup c.2157del2 (FuseNax)	Mouse																											244
Jup c.2157del2	Mouse						•			•	•				•						•				•			98
Jup S39_K40insS	HEK293																	•			•							245
Jup S39_K40insS; *Jup* c.2157del2	HEK293																	•		•								130
Tmem43																												
Tmem43$^{-/-}$	Mouse																											246
Tmem43 S358L	Mouse																											246
Lmna																												
Lmna c.418_438dup	HL-1										•																	67
Flnc																												
Flnc p.G1891Vfs61X	hBMC																	•			•							247
Rock2[a]																												
DN-Rock2	Mouse		•		•	•	•				•						•											99
Ppp1r13l[a]																												
Ppp1r13l$^{-/-}$	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; GSK3β, glycogen synthase kinase 3β; 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; PPARγ, 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. [a]ACM-like phenotype; no known ACM-causing mutations in these genes.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript