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Molecular Mechanisms in the Pathogenesis of Arrhythmogenic Cardiomyopathy^{*,**}

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Abstract

The article is based on work presented in the Distinguished Achievement Award lecture at the Society for Cardiovascular Pathology meeting in Seattle, WA in March, 2016. It reviews our current understanding of mechanisms responsible for a highly arrhythmogenic, non-ischemic cardiomyopathy. It highlights the armamentarium of powerful methods available to the experimental pathologist in efforts to define how complex cardiovascular diseases work. It concludes with acknowledgment of the need for a far more detailed approach as to how we categorize human disease, a task for which pathologists are especially well positioned.

Keywords

arrhythmogenic cardiomyopathy; desmosomes; protein trafficking; gap junctions; ion channels; zebrafish models; buccal mucosa

Introduction

Arrhythmogenic cardiomyopathy (ACM) is a familial non-ischemic cardiomyopathy first described clinically [1] and pathologically [2] in the 1980s. It is a leading cause of sudden death in the young and especially in athletes [2–4,6,7]. It has a dramatic and complex clinical and pathologic phenotype. Arrhythmias occur early in the disease, during the so-called “concealed phase”, before the onset of ventricular remodeling and contractile dysfunction. With time, progressive myocardial degeneration ensues with replacement by fibro-fatty scar tissue [1–4]. The disease typically affects the right ventricle most severely but both ventricles usually exhibit pathological abnormalities [3,4]. The right ventricular

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preponderance is likely related to different biomechanical responses of the ventricles to exercise [6,7].

ACM is caused, in most cases, by mutations in genes that encode desmosomal proteins [3–5]. Mutations in all known desmosomal genes (encoding the desmosomal cadherins desmocollin-2 and desmoglein-2, and intracellular linker proteins plakophilin-2, plakoglobin and desmoplakin) have been implicated in ACM [3–5]. Mutations in other genes including some previously linked to dilated cardiomyopathy, such as phospholamban [8], or hypertrophic cardiomyopathy, such as titin [9], have also been reported in subjects who fulfill diagnostic criteria for ACM. This likely reflects the increasing recognition of clinicopathological overlap among the non-ischemic cardiomyopathies as well as persistent challenges in clinical diagnosis in this complex spectrum of heart muscle diseases.

In most cases, ACM is inherited as an autosomal dominant trait with variable penetrance [3–5]. Digenic, compound heterozygous and recessive forms have been reported and are typically associated with more severe clinical phenotypes [10–12]. Still, ACM is generally regarded as a “monogenic” cardiomyopathy in which the disease can be reasonably attributed to specific pathologic variants within single genes. Desmosomal proteins have traditionally been thought to fulfill structural roles in cell-cell adhesion, but they also interact with diverse cell signaling pathways [13]. Thus, disease alleles in ACM likely exert deleterious effects through multiple mechanisms. The fact that arrhythmias arise during the concealed phase implicates mechanisms such as may be at play in the ion channelopathies, but the progressive development of myocardial damage, fibro-fatty scarring and inflammation points to mechanisms of cardiac myocyte injury that go beyond those of the ion channelopathies. And, the fact that exercise increases disease penetrance and risk of adverse events [6,7] strongly suggests that mechanical stimuli somehow interact with genetic factors to determine disease expression.

The overarching goal of our work is to explain how mutations in desmosomal genes cause ACM. Ultimately, we want to help develop truly mechanism-based therapies to prevent sudden death and myocardial damage in this lethal human heart disease. As detailed in this review, we have found that glycogen synthase kinase-3 β (GSK3 β) plays a role in the pathogenesis of ACM [14]. We have also identified a small molecule, annotated as an inhibitor of GSK3 β [15], that has a remarkable ability to prevent and/or reverse the full disease phenotype (arrhythmias, exercise-induced sudden death, ventricular myocyte injury and apoptosis, inflammation, and contractile dysfunction) in various experimental models of ACM, and in human iPSC-cardiac myocytes derived from patients with ACM [14,16]. Although much of our work and several representative figures included in this review involve models based on expression of a mutant form of plakoglobin (an allele implicated in only a small minority of ACM patients), the basic disease pathway involving GSK3 β has been observed consistently in diverse models including those involving expression of mutant plakophilin-2 and desmoglein-2, as well as in cardiac myocytes derived from iPSCs from ACM patients with plakophilin-2 mutations [14,16]. The ACM disease mechanism appears to include a defect in forward trafficking of ion channel proteins to the intercalated disc in ventricular myocytes that may be responsible for establishing conditions conducive to abnormal impulse propagation and arrhythmias [14,16]. Whether abnormal protein

trafficking also contributes to myocyte injury is unknown, but the fact that a single small molecule is able to prevent both the arrhythmia and myocardial injury features of the ACM phenotype suggests a mechanistic connection.

Why is ACM so arrhythmogenic?

There is something inherently arrhythmogenic about the basic disease mechanism in ACM. Whereas overall survival free of cardiac death or transplantation is better in patients with ACM compared with dilated cardiomyopathy (DCM), the proportion of sudden deaths is much greater in ACM (>80%) than DCM (~30%) patients [17]. Moreover, arrhythmias in the hypertrophic and dilated cardiomyopathies arise in hearts that exhibit significant structural remodeling (myocyte injury/death and fibrosis) [17]. By contrast, arrhythmias in ACM typically occur as an early manifestation of disease in hearts that show no apparent gross or microscopic features of remodeling [3,4]. While there has not been a previous report of a desmosomal mutation in a sudden death victim with a normal heart at autopsy, molecular autopsies have only recently included analysis of desmosomal genes. Thus, it remains an open question whether ACM can cause sudden death in a structurally normal heart.

We have gained insights into mechanisms responsible for the early arrhythmogenic clinical phenotype in ACM through study of patient myocardium. For example, one of the first changes seen in the hearts of individuals with ACM disease alleles is remodeling of gap junctions in ventricular myocardium, shown by a marked reduction in the amount of immunoreactive signal for Cx43, the major ventricular gap junction protein, at intercalated discs (Figure 1A) [18,19]. Similar changes in Cx43 distribution occur in experimental models of ACM (Figure 1B) [14,16]. In addition, immunoreactive signal for Nav1.5, the pore-forming protein of the cardiac Na^+ current (I_{Na}), is reduced at intercalated discs in patients with ACM [20], and patch clamping studies in experimental models have shown a marked (>75%) reduction in I_{Na} (Figure 1C) [16]. Because the extent of cell-cell electrical coupling at gap junctions and peak Na^+ current density are both major determinants of electrical impulse propagation in myocardium [21], it is likely that the combination of gap junction remodeling and reduced I_{Na} contribute to the highly arrhythmogenic phenotype in ACM. By themselves, these changes may be sufficient to explain the greatly increased number of premature ventricular complexes seen on Holter monitoring in ACM patients [3,4]. Subsequent myocardial damage and remodeling, superimposed on the underlying conduction defect, likely produces an even more arrhythmogenic substrate responsible for the characteristic ventricular tachycardias of right ventricular origin (left bundle branch block morphology with superior axis) [3,4].

A transgenic model of ACM in zebrafish

In collaboration with Calum MacRae, we created a model of ACM in zebrafish with cardiac myocyte-specific expression of a mutant form (2157del2) of the desmosomal protein plakoglobin (γ -catenin) that causes a highly penetrant form of ACM in patients [16]. The purpose was to enable a high-throughput chemical screen to identify small molecules that reverse the disease. Mutant fish developed a dramatic phenotype characterized by marked

ventricular dilatation, poor contractile function, and premature death (Figure 2). After mating the ACM fish with a reporter line that expresses luciferase as a marker of heart failure (driven by the natriuretic peptide b promoter), we screened a library of small molecules looking for those that reduced luminescence, thereby, indicating rescue of the heart failure phenotype. One molecule in particular, SB216763, showed a remarkable ability to prevent ACM in the fish model [16]. This compound has been annotated as an inhibitor of GSK3 β and, thus, is an activator of the canonical Wnt/ β -catenin signaling pathway [15]. This was of particular interest because we had previously reported that patients with ACM exhibit reduced signal for the desmosomal protein plakoglobin (γ -catenin) at intercalated discs (Figure 3) [19]. Furthermore, loss of plakoglobin signal in desmosomes and its nuclear accumulation have been implicated in competitive inhibition (by nuclear γ -catenin) of the canonical Wnt/ β -catenin signaling [22,23]. Interestingly, loss of plakoglobin signal at intercalated discs also occurs in cardiac sarcoidosis (a known phenocopy of ACM [24]) and giant cell myocarditis, both forms of granulomatous myocarditis [25]. Potential mechanistic links remain undefined. However, as detailed below, SB216763 has proven useful in elucidating mechanisms driving both the arrhythmia and myocardial injury phenotypes in ACM.

Effects of SB216763 on the disease phenotype in experimental models of ACM

We and others have developed experimental models of ACM, both in vitro and in vivo, that exhibit features of the human disease [14,16,22]. These features include three major components: *myocardial damage* (necrotic/fibrotic lesions, myocyte apoptosis), *electrophysiological abnormalities* (action potential remodeling and ventricular arrhythmias) and *inflammation* (inflammatory infiltrates and expression of pro-inflammatory cytokines). And, as illustrated in Figures 1 and 3, the ACM disease phenotype also includes characteristic changes in the subcellular distribution of key junctional and regulatory proteins in ventricular myocytes. Having identified SB216763 as a small molecule that rescues the heart failure phenotype in fish, we turned to mammalian models of ACM to determine if this drug candidate is capable of mitigating the arrhythmia and/or myocardial injury phenotypes, the two key components of disease expression in patients.

We first tested the ability of SB216763 to reverse changes in subcellular distribution of proteins and cellular electrophysiology in monolayers of neonatal rat ventricular myocytes that expressed two different desmosomal mutations (2157del2 plakoglobin or 1851del123 plakophilin-2) known to cause ACM in patients. Exposure of cultured myocytes to SB216763 for only 12 to 24 hours restored the normal subcellular distribution of key intercalated disc proteins including plakoglobin and Cx43 (Figure 4) [14,16]. These large shifts in subcellular distribution of intercalated discs proteins occurred with no apparent change in the total cellular content for each protein (Figures 4) [16]. SB216763 also increased the cardiac Na⁺ current, I_{Na}, to near control levels, and normalized the distribution of Nav1.5 (Figure 5) [14,16]. Taken together, these observations implicate a protein trafficking defect as a potential mechanism in ACM. In further support of this mechanism, we found that the distribution of the MAGUK protein, SAP97, a chaperone involved in

transport of Nav1.5 to the cell surface, is also altered in ACM models and is rapidly normalized in response to SB216763 (Figure 5) [16].

Tests of drug efficacy are limited in studies of cultured cells in vitro. Accordingly, we also characterized the effects of SB216763 in two different mouse models of ACM, with either cardiac myocyte-specific transgenic expression of 2157del2 plakoglobin or homozygous knock-in of a mutant form of the gene encoding the desmosomal cadherin, desmoglein-2. In both models, we observed remarkable salutary effects [14]. SB216763 restored the normal distribution of intercalated disc proteins (as seen in culture cells), but it also prevented development of myocardial lesions (necrosis, fibrosis and inflammation) (Figure 6A) and stopped the progressive deterioration of ventricular contractile function seen in untreated mice [14]. And, it significantly reduced the number of premature ventricular beats and runs of non-sustained ventricular tachycardia (Figure 6B) [14].

Role of GSK3 β in the pathogenesis of ACM

The results of studies described above provide strong evidence that both the myocardial injury and arrhythmia phenotypes in ACM share a common disease mechanism and, further, that SB216763 acts to correct a protein trafficking defect that prevents key desmosomal and ion channel proteins from accumulating normally at cardiac myocyte intercalated discs. How such a protein trafficking defect arises is unknown and the extent to which it may contribute to myocyte injury is also unclear. Although SB216763 is annotated as an inhibitor of GSK3 β [15], there is no direct proof at the current time that abnormal activation of GSK3 β or aberrant Wnt/ β -catenin signaling is necessary or sufficient to cause the disease phenotype. Nevertheless, it appears that expression of GSK3 β is required to produce the disease phenotype, at least in vitro. To explore this relationship, we used a specific shRNA to knock-down GSK3 β expression in neonatal rat ventricular myocytes and then introduced disease alleles [14]. Ablation of GSK3 β had no effect on plakoglobin and Cx43 distribution in normal cells. By contrast, abnormal redistribution of these cell-cell junction proteins was prevented when GSK3 β was first knocked down in myocytes expressing mutant desmosomal proteins. Whether GSK3 β expression is required to produce the arrhythmia and myocardial injury components of the disease phenotype requires a similar approach in intact mouse models of ACM.

Expression of the ACM disease phenotype in patient buccal mucosa cells

The advent of genomics has revealed levels of complexity and variability on a scale vastly greater than our traditional categorization of human disease. This has led to recognition of a glaring mismatch in efforts to characterize genotype-phenotype relationships. A recent expert commentary on this dilemma points out that “unless we attempt to move our assessment of disease more proximately in the pathobiological chain, we will not achieve the change in resolution that is necessary to elucidate the genetic architecture of cardiovascular traits” [26].

It comes as no surprise that we tend to focus almost exclusively on the heart to diagnose and quantify heart disease. Virtually all available diagnostic modalities involve direct

interrogation and/or imaging of cardiac structure/function, or assessment of biomarkers derived directly from the myocardium. There is little doubt, however, that various forms of human heart disease include phenotypes expressed beyond the heart. In the case of the “desmosomal disease” ACM, we know that desmosomes occur in many tissues and organs (e.g., smooth muscle cells and intestinal epithelium), but desmosomal gene mutations cause overt clinical phenotypes only in heart and skin, organs that bear a significant mechanical burden. Whereas rare recessive desmosomal gene mutations can cause cardio-cutaneous syndromes (with heart *and* skin phenotypes) [11,12,27], the great majority of ACM patients with autosomal dominant disease alleles have no clinically apparent skin phenotype. Nevertheless, this does not mean that ACM is not demonstrably manifest outside of the heart.

We have been motivated to look beyond the heart in studies of ACM, mainly because it is so difficult to obtain myocardial tissue from probands, not to mention family members who may have inherited disease alleles but show no clinical evidence of disease. One possibility is to study cardiac myocytes from patient-derived iPSCs. We and others have taken advantage of this approach but it remains expensive and time consuming [16,28]. In a search for an inexpensive and readily accessible surrogate to study ACM, we have turned to the buccal mucosa, which can be easily and safely obtained by rubbing the inside of the cheek with a swab and smearing the collected cells on slides for microscopic and molecular analysis. Using this approach, we have found that buccal mucosa cells from ACM patients exhibit changes in the distribution of cell junction proteins similar to those seen in the heart (Figure 7) [29]. Similar reductions occur in buccal mucosa cells of many family members who carry disease alleles but show no clinical evidence of heart disease (and in whom analysis of endomyocardial biopsies is not feasible) [29]. Analysis of buccal swabs may, therefore, be useful in future studies of disease mechanisms in probands, identification of disease modifiers in family members, and drug screens to identify new therapies in ACM.

Conclusion

ACM has a remarkably complex clinical phenotype that encompasses the major causes of morbidity and mortality in human heart disease: sudden death due to ventricular tachyarrhythmias driven by disturbances in cardiac cellular electrophysiology; heart failure due to myocardial injury, apoptosis and inflammation; and abnormal responses to overload states such that strenuous exercise greatly increases disease penetrance and risk of adverse clinical events. The fact that most ACM patients have mutations in genes encoding desmosomal proteins provides an entry point into a basic understanding of the responsible mechanisms. New insights into such mechanisms implicate derangements in forward trafficking of key cell-cell junction proteins potentially related to abnormal Wnt signaling. Much remains to be learned, however, about how mutations in proteins that form cell-cell adhesion organelles give rise to such a complex clinical phenotype, and why some individuals who inherit disease alleles show no evidence of the disease. ACM thus provides a rich backdrop for the study of fundamental aspects of cardiac myocyte signaling, homeostasis and pathobiology.

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References

1. Marcus FI, Fontaine GH, Guiraudon G, Frank R, Laurenceau JL, Malergue C, Grogogeat Y. Right ventricular dysplasia: a report of 24 adult cases. *Circulation*. 1982; 65:384–398. [PubMed: 7053899]
2. Thiene G, Nava A, Corrado D, Rossi L, Pennelli N. Right ventricular cardiomyopathy and sudden death in young people. *N Eng J Med*. 1988; 318:129–133.
3. Corrado D, Link M, Calkins H. Arrhythmogenic right ventricular cardiomyopathy. *New Eng J Med*. 2017; 376:61–72. [PubMed: 28052233]
4. Basso C, Bauce B, Corrado D, Thiene G. Pathophysiology of arrhythmogenic cardiomyopathy. *Nat Rev Cardiol*. 2012; 9:223–33.
5. Refaat MM, Hotait M, London B. Genetics of sudden cardiac death. *Curr Cardiol Rep*. 2015; 17:606. [PubMed: 26026997]
6. James CA, Bhonsale A, Tichnell C, Murray D, Russell SD, Tandri H, Tedford RJ, Judge DP, Calkins H. Exercise increases age-related penetrance and arrhythmic risk in arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated desmosomal mutation carriers. *J Am Coll Cardiol*. 2013; 62:1290–1297. [PubMed: 23871885]
7. Rojas A, Calkins H. Present understanding of the relationship between exercise and arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Trends Cardiovasc Med*. 2015; 25:181–188. [PubMed: 25666490]
8. van der Zwaag PA, van Rijsengen IA, Asimaki A, Jongbloed JD, van Veldhuisen DJ, Wiesfeld AC, Cox MG, van Lochem LT, de Boer RA, Hofstra RM, Christiaans I, van Spaendonck-Zwarts KY, Lekanna dit Deprez RH, Judge DP, Calkins H, Suureijer AJ, Hauer RN, Saffitz JE, Wilde AA, van der Berg MP, van Tintelen JP. 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*. 2012; 14:1199–1207. [PubMed: 22820313]
9. Taylor M, Graw S, Sinagra G, Barnes G, Slavov D, Brun F, Pinamonti B, Salcedo EE, Sauer W, Pyxaras S, Anderson B, Simon B, Bogomolovas J, Labiet S, Granzier H, Mestroni L. Genetic variation in titin in arrhythmogenic right ventricular cardiomyopathy-overlap syndromes. *Circulation*. 2011; 124:876–885. [PubMed: 21810661]
10. Rigato I, Bauce B, Rampazzo A, Zorzi A, Pilichou K, Mazzotti E, Migliore F, Marra MP, Lorenzon A, De Bortoli M, Calore M, Nava A, Daliento K, Gregori D, Illiceta S, Thiene G, Basso C, Corrado D. Compound and digenic heterozygosity predicts lifetime arrhythmic outcome and sudden cardiac death in desmosomal gene-related arrhythmogenic right ventricular cardiomyopathy. *Circ Cardiovasc Genet*. 2013; 6:533–542. [PubMed: 24070718]
11. McKoy G, Protonotarios N, Crosby A, Tsatsopoulou A, Anastasakis A, Coonar A, Norman M, Baboonian C, Jeffery S, McKenna WJ. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). *Lancet*. 2000; 355:2119–2124. [PubMed: 10902626]
12. Norgett EE, Hatsell SJ, Carvajal-Huerta L, Cabezas JC, Common J, Purkis PE, Whittock N, Leigh IM, Stevens HP, Kelsell DP. Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma. *Hum Mol Genet*. 2000; 9:2761–2766. [PubMed: 11063735]
13. Broussard JA, Getsios S, Green KJ. Desmosome regulation and signaling in disease. *Cell Tissue Res*. 2015; 360:501–512.
14. Chelko SP, Asimaki A, Andersen P, Bedja D, Amat-Alarcon N, DeMazumder D, Jasti R, MacRae CA, Leber R, Kleber AG, Saffitz JE, Judge DP. Central role for GSK3 β in the pathogenesis of arrhythmogenic cardiomyopathy. *JCI Insight*. 2016; 1(5) pii: e85923.

15. Coghlan MP, Culbert AA, Cross DA, Corcoran SL, Yates JW, Pearce NJ, Rausch OL, Murphy GJ, Carter PS, Roxbee Cox L, Mills D, Brown MJ, Haigh D, Ward RW, Smith DG, Murray KJ, Reith AD, Holder JC. Selective small molecule inhibitors of glycogen synthase kinase-3 modulate glycogen metabolism and gene transcription. *Chem Biol.* 2000; 7:793–803. [PubMed: 11033082]
16. Asimaki A, Kapoor S, Plovie E, Karin Arndt A, Adams E, Liu Z, James CA, Judge DP, Calkins H, Churko J, Wu JC, MacRae CA, Kléber AG, Saffitz JE. Identification of a new modulator of the intercalated disc in a zebrafish model of arrhythmogenic cardiomyopathy. *Sci Transl Med.* 2014; 6:240ra74.
17. Sen-Chowdhry, McKenna WJ. Sudden death from genetic and acquired cardiomyopathies. *Circulation.* 2012; 125:1576–1576.
18. Kaplan, Sr, Gard, JJ., Protonotarios, N., Tsatsopoulou, A., Spiliopoulou, C., Anastasakis, A., Squarcioni, CP., McKenna, WJ., Thiene, G., Basso, C., Brousse, N., Fontaine, G., Saffitz, JE. Remodeling of myocyte gap junctions in arrhythmogenic right ventricular cardiomyopathy due to a deletion in plakoglobin (Naxos disease). *Heart Rhythm.* 2004; 1:3–11. [PubMed: 15851108]
19. Asimaki A, Tandri J, Huang H, Halushka MK, Gautam S, Basso C, Thiene G, Tsatsopoulou A, Protonotarios N, McKenna WJ, Calkins H, Saffitz JE. A new diagnostic test for arrhythmogenic right ventricular cardiomyopathy. *New Eng J Med.* 2009; 360:1075–84. [PubMed: 19279339]
20. Noorman N, Hakin S, Kessler E, Groeneweg JA, Cox MG, Asimaki A, van Rijen HV, van Stuijvenberg L, Chkourko H, van der Heyden NMA, Vos MA, de Jonge N, van der Smagt JJ, Dooijes D, Vink A, de Weger RA, Varro A, de Bakker JM, Saffitz JE, Hund TJ, Mohler PJ, Delmar M, Hauer RN, van Veen TA. Remodeling of the cardiac sodium channel, connexin43, and plakoglobin at the intercalated disk in patients with arrhythmogenic cardiomyopathy. *Heart Rhythm.* 2013; 10:412–419. [PubMed: 23178689]
21. Kléber AG, Rudy Y. Basic mechanisms of cardiac impulse propagation and associated arrhythmias. *Physiol Rev.* 2004; 84:431–488. [PubMed: 15044680]
22. Garcia-Gras E, Lombardi R, Giocondo MR, Willerson JT, Schneider MD, Khoury DS, Marian AJ. Suppression of canonical Wnt/beta-catenin signaling by nuclear plakoglobin recapitulates phenotype of arrhythmogenic right ventricular cardiomyopathy. *J Clin Invest.* 2006; 116:2012–21. [PubMed: 16823493]
23. Lombardi R, da Graca Cabreira-Hansen M, Bell A, Fromm RR, Willerson JT, Marian AJ. Nuclear plakoglobin is essential for differentiation of cardiac progenitor cells to adipocytes in arrhythmogenic right ventricular cardiomyopathy. *Circ Res.* 2011; 109:1342–53. [PubMed: 22021931]
24. Philips B, Madhavan S, James CA, te Riele AS, Murray B, Tichnell C, Bhonsela A, Nazarian S, Judge DP, Calkins H, Tandri H, Chang A. Arrhythmogenic right ventricular dysplasia/cardiomyopathy and cardiac sarcoidosis: distinguishing features when the diagnosis is unclear. *Circ Arrhythm Electrophysiol.* 2014; 7:230–236. [PubMed: 24585727]
25. Asimaki A, Tandri H, Duffy ER, Winterfield JR, Mackey-Bojack S, Picken MM, Cooper LT, Wilber DJ, Marcus FI, Basso C, Thiene G, Tsatsopoulou A, Protonotarios N, Stevenson WG, McKenna WJ, Gautam S, Remick DG, Calkins, Saffitz JE. Altered desmosomal proteins in granulomatous myocarditis and potential pathogenic links to arrhythmogenic right ventricular cardiomyopathy. *Circ Arrhythm Electrophysiol.* 2011; 4:743–52.
26. MacRae CA, Vasan RS. The future of genetics and genomics – closing the phenotype gap in precision medicine. *Circulation.* 2016; 133:2634–2639. [PubMed: 27324359]
27. Carvajal-Huerta L. Epidermolytic palmoplantar keratoderma with woolly hair and dilated cardiomyopathy. *J Am Acad Dermatol.* 1998; 39:418–421. [PubMed: 9738775]
28. Kim C, Wong J, Wen J, Wang C, Spiering S, Kan NG, Forcales S, Puri PL, Leone TC, Marine JE, Calkins H, Kelly DP, Judge DP, Chen HS. Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. *Nature.* 2013; 494:105–110. [PubMed: 23354045]
29. Asimaki A, Protonotarios A, James CA, Chelko SP, Tichnell C, Murray B, Tsatsopoulou A, Anastasakis A, te Riele A, Kléber A, Judge DP, Calkins H, Saffitz JE. Characterizing the molecular pathology of arrhythmogenic cardiomyopathy in patient buccal mucosa cells. *Circ Arrhythm Electrophysiol.* 2016; 9:e003688. [PubMed: 26850880]

Highlights

Arrhythmogenic cardiomyopathy is a non-ischemic familial heart muscle disease.

It has a complex phenotype of arrhythmias and myocardial injury and inflammation.

Abnormal forward trafficking of ion channel proteins to intercalated discs of cardiac myocytes, related to aberrant Wnt signaling, plays a role in disease pathogenesis.

Buccal mucosa cells may be useful as a surrogate for cardiac myocytes in studies of disease pathogenesis.

C

Figure 1.

A,B: Immunohistochemical staining of human myocardium (A) and cultured neonatal rat ventricular myocytes (B). In A, abundant immunoreactive signals for the adhesion molecule N-cadherin and the major ventricular gap junction protein Cx43 localize to intercalated discs in a normal control heart (upper panels), whereas signal for Cx43 is greatly reduced at cell-cell junctions in hearts from representative ACM patients (middle and lower panels). In B, Cx43 signal in control myocytes is seen as numerous punctate clusters at cell-cell junctions, but is greatly reduced in myocytes expressing 2157del2 plakoglobin which causes arrhythmogenic cardiomyopathy in patients with Naxos disease. **C:** Reduced sodium current in ventricular myocytes isolated from zebrafish expressing 2157del2 plakoglobin (Naxos) compared to control myocytes. **A** is reproduced from Asimaki et al [19]; **B** and **C** are reproduced from Asimaki et al [16].

Figure 2.

Cardiac myocyte-specific expression of 2157del2 plakoglobin in zebrafish produces marked cardiomegaly due to ventricular dilatation, associated with heart failure and premature death. A,A': control fish and isolated heart; B,B': mutant fish and isolated heart. Reproduced from Asimaki et al [16].

Figure 3.

Abundant immunoreactive signals for the adhesion molecule N-cadherin and the desmosomal protein plakoglobin localize to intercalated discs in a normal control heart (upper panels), whereas signal for plakoglobin is greatly reduced at cell-cell junctions in hearts from representative ACM patients (middle and lower panels). Reproduced from Asimaki et al [19].

Figure 4.

Exposure of neonatal rat ventricular myocytes expressing 2157del2 plakoglobin (PG) to SB216763 (SB2) corrects the abnormal subcellular distribution of plakoglobin and Cx43 seen by immunohistochemistry (upper panels). Such changes in subcellular distribution occur with no apparent change in the total cellular content of plakoglobin, Cx43 or β -catenin as shown in the western blot (lower panel). Endogenous (wildtype) plakoglobin in both control (Con) and mutant (Mut) cells is marked by antibodies that recognize epitopes in both the C-terminus (C-term Ab) and N-terminus (N-term Ab) of plakoglobin. 2157del2 plakoglobin is recognized by antibodies against the V5 epitope tag and the N-terminal epitope, but not the C-terminal epitope reflecting truncation of the C-terminus in the mutant. Exposure to SB216763 (SB2) has no apparent effect on plakoglobin content but does increase total Cx43 content in both control and mutant cells, consistent with the known ability of the canonical Wnt/ β -catenin pathway to stimulate Cx43 gene expression. Reproduced from Asimaki et al [16].

Figure 5.

SB216763 (SB2) normalizes peak Na⁺ current (I_{Na}) in zebrafish ventricular myocytes expressing 2157del2 plakoglobin (PG) (upper panel). It also restores the normal subcellular distribution of SAP97 and Nav1.5 in neonatal rat ventricular myocytes expressing 2157del2 PG (middle panels), but such changes in subcellular distribution occur with no apparent change in the total amount of SAP97 or Nav1.5 in the cells (lower panel). Reproduced from Asimaki et al [16].

Figure 6.

A. Mice with cardiac myocyte-specific transgenic expression of 2157del2 plakoglobin (PG) develop ventricular lesions characterized by fibrosis and inflammation. Intraperitoneal injection of SB216763 prevents formation of such lesions. **B.** These ACM mice also develop numerous premature ventricular beats and short runs of ventricular tachycardia as shown in the electrocardiograms and the white bars in the graph. Treatment with SB216763 (black bars) greatly reduces these arrhythmias. Reproduced from Chelko et al [14].

Figure 7.

Immunohistochemistry of buccal mucosa cells obtained from a normal subject (Control) and two ACM patients - one with a mutation in plakophilin-2 (*PKP2*) and another with a mutation in desmoplakin (*DSP*). Control cells show bright signals at the cell surface for the desmosomal proteins plakoglobin, plakophilin-1 and desmoplakin, and the gap junction protein Cx43. Both ACM patients show reduced cell surface signals for plakoglobin and Cx43, as seen in the hearts of ACM patients (see Figures 1 and 3). The patient with the *PKP2* mutation also shows reduced signal for plakophilin-1, and the patient with the *DSP* mutation shows reduced signal for desmoplakin. Reproduced from Asimaki et al [29].