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## Genetics of and pathogenic mechanisms in Arrhythmogenic Right Ventricular Cardiomyopathy --Manuscript Draft--

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<b>Abstract:</b>	Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited heart disease, associated with a high risk of sudden cardiac death. ARVC has been termed a 'disease of the desmosome' based on the fact that in many cases it is caused by mutations in genes encoding desmosomal proteins at the specialised intercellular junctions between cardiomyocytes, the intercalated discs. Desmosomes maintain the structural integrity of the ventricular myocardium and are also implicated in signal transduction pathways. Mutated desmosomal proteins are thought to cause detachment of cardiac myocytes by the loss of cellular adhesions and also affect signalling pathways, leading to cell death and substitution by fibrofatty adipocytic tissue. However, mutations in desmosomal proteins are not the sole cause for ARVC as mutations in non-desmosomal genes were also implicated in its pathogenesis. This review will consider the pathology, genetic basis, mechanisms of pathogenesis, clinical diagnostics and treatment options for ARVC.
<b>Response to Reviewers:</b>	<p>We are grateful for the extremely insightful comments from Reviewer 1 and have addressed all his/her comments.</p> <p>We are similarly grateful for the constructive criticism of Reviewer 2 and have addressed most of his/her comments. We did remain with the name ARVC, since the main audience of this journal are biophysicists rather than clinicians, who will find consistency with the cited literature more relevant than the usage of up to date clinical terminology. All other changes and additions were made as suggested.</p>

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# Genetics of and pathogenic mechanisms in Arrhythmogenic Right Ventricular Cardiomyopathy

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## Abstract

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited heart disease, associated with a high risk of sudden cardiac death. ARVC has been termed a 'disease of the desmosome' based on the fact that in many cases it is caused by mutations in genes encoding desmosomal proteins at the specialised intercellular junctions between cardiomyocytes, the intercalated discs. Desmosomes maintain the structural integrity of the ventricular myocardium and are also implicated in signal transduction pathways. Mutated desmosomal proteins are thought to cause detachment of cardiac myocytes by the loss of cellular adhesions and also affect signalling pathways, leading to cell death and substitution by fibrofatty adipocytic tissue. However, mutations in desmosomal proteins are not the sole cause for ARVC as mutations in non-desmosomal genes were also implicated in its pathogenesis. This review will consider the pathology, genetic basis, mechanisms of pathogenesis, clinical diagnostics and treatment options for ARVC.

Key words: desmosomes, arrhythmogenic right ventricular cardiomyopathy, desmoglein, desmocollin, desmoplakin, plakoglobin, plakophilin, sudden cardiac death

## Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a rare (1:2000-1:5000) inherited cardiac condition (Pilichou et al. 2016). Sudden cardiac death can often be the first presentation of the disease in previously asymptomatic individuals, making this condition life-threatening and difficult to diagnose (Thiene et al. 1998). It affects young people and athletes, as strenuous exercise can exacerbate the incidence of arrhythmias and cause sudden cardiac death. Of note, 20% of non-exercise-related sudden cardiac deaths in young people in the Europe and US were attributed to ARVC (Shen et al. 1995).

Diagnosis is based on 'Task Force Criteria', a scoring system that takes into account structural and electrical abnormalities, family history of the disease and genetic findings, and is divided into major and minor criteria (Marcus et al. 2010). This results in the classification of 'definite', 'borderline' or 'possible' diagnosis of ARVC. By taking the diagnosis of a first degree relative into consideration, the criteria are more stringent for cases of familial ARVC.

The histological hallmark of ARVC is the substitution of cardiac myocytes by fibrofatty tissue (Sen-Chowdhry et al. 2005). ARVC is commonly an autosomal dominant inherited disease with variable clinical manifestations (Corrado et al. 2017). Mutations in desmosomal genes, specifically desmoplakin, plakophilin-2 and desmoglein-2 were found, in addition to non-desmosomal genes encoding for transforming growth factor (TGF)  $\beta$ 3, human ryanodine receptor (RyR) 2 and the transmembrane protein (TMEM) 43 (Herren et al. 2009).

Treatment aims both at delaying the progression of heart failure and the prevention of arrhythmic events. This is achieved by general lifestyle changes, pharmacological

1 interventions such as beta-blockers or anti-arrhythmic drugs, by surgical means  
2 including catheter ablation and implantable cardioverter defibrillator (ICD) or ultimately  
3 by heart transplantation (Corrado et al. 2017).  
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## 10 **Pathology**

11 ARVC was initially categorised as a dysplasia based on histological findings (hence  
12 also referred to as ARVD), but closer analysis led to the characterisation of the disease  
13 as being a genetically predisposed cardiomyopathy (Basso et al. 1996; Nava et al.  
14 1988). Defining pathological characteristics of ARVC include cardiac myocyte  
15 depletion and substitution with fibrofatty tissue (Sen-Chowdhry et al. 2005). Fibrous  
16 scar tissue replacement of cardiac myocytes occurs initially in the epicardium of the  
17 heart but over time, infiltrates transmurally into the endocardium, resulting in thinning  
18 of the right ventricular walls. In ARVC, the three areas of the heart affected most  
19 include the anterior infundibulum, the apex of the right ventricle and the inferior wall of  
20 the right ventricle. These areas, which are collectively coined the 'triangle of dysplasia',  
21 appear especially susceptible to stress and stretch and had the most fibrofatty  
22 replacement of myocardial tissue and the thinnest walls of the right ventricle (Gerull et  
23 al. 2004; Marcus et al. 1982). Although coined 'arrhythmogenic right ventricular  
24 cardiomyopathy', the disease is not limited solely to the right ventricle, as fibrofatty  
25 replacement of the left ventricular myocardium may also occur (Beffagna et al. 2005).  
26 Although it is less common, myocyte degeneration may also be seen predominantly  
27 in the left ventricle and the interventricular septum (Beffagna et al. 2005). Hence the  
28 term 'arrhythmogenic cardiomyopathy' (AC) has been proposed (Basso et al. 2018).  
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## Genetics of ARVC

### *Mutations in genes encoding for desmosomal proteins*

Desmosomes are integral cellular adhesion structures found at junctions between adjacent cardiomyocytes called intercalated discs (Vermij et al. 2017). A simplified scheme of a desmosome is depicted in Figure 1. Desmosomal cadherins – desmoglein (DSG)-2 and desmocollin (DSC)-2 - are transmembrane proteins that link neighbouring cardiomyocytes via homophilic or heterophilic interactions. Their cytoplasmic domains interact with the armadillo proteins, plakoglobin and plakophilin. These two proteins link to desmoplakin, which binds strongly to intracellular intermediate filaments, which are composed of desmin in cardiomyocytes (reviewed in (Kottke et al. 2006). These cellular adhesions are essential for the heart to be able to withstand mechanical stresses caused by the contractile cycle. Additionally, desmosomes are implicated in intercellular signalling cascades (reviewed in (Broussard et al. 2015) and perturbation of these signalling pathways could contribute to the development of ARVC. More recently the exclusive molecular composition of desmosomes versus adherens junctions, which are actin-anchoring contacts at the intercalated disc, was challenged and the concept of the Area composita was proposed, which suggests a more dynamic distribution of proteins between these two types of cell-cell contact (Franke et al. 2006).

ARVC is most commonly inherited autosomal dominantly with incomplete penetrance and variable phenotypic expressivity, although it can also be recessively inherited (reviewed in (Herren et al. 2009). There are 8 autosomal dominant forms of the disease (Beffagna et al. 2005; Rampazzo et al. 1994) and 50% of these cases are the result of mutations in the five genes *JUP*, *DSP*, *PKP2*, *DSG2* and *DSC2* encoding the

desmosomal proteins plakoglobin, desmoplakin, plakophilin 2, desmoglein 2 and desmocollin 2, respectively (Marcus et al. 2010). Together with mutations in genes coding for non-desmosomal proteins, 15 disease genes were identified so far (Table 1).

**Naxos disease and Carvajal Syndrome:** Naxos disease and Carvajal syndrome are both known as cardiocutaneous disorders as, in addition to cardiomyocyte dysfunction they are accompanied by skin disorders (Protonotarios and Tsatsopoulou 2004). The first mutation in a desmosomal protein that was unequivocally linked with the ARVC-implicated autosomal recessive Naxos disease was a homozygous frameshift-causing deletion mutation at chromosomal locus 17q21 for the *JUP* gene, encoding plakoglobin (McKoy et al. 2000). Homozygous mutations in the *DSP* gene encoding desmoplakin at locus 6p24 cause the recessively-inherited Carvajal syndrome, which is also characterised by woolly hair, keratoderma and ARVC with a prominent left ventricular manifestation (Norgett et al. 2000; Rampazzo et al. 2002).

**Plakophilin-2 (*PKP2*):** Mutations in the *PKP2* gene encoding plakophilin-2, the main cardiac plakophilin, at locus 12p11 are a common cause of ARVC. It was shown that mutations in the *PKP2* gene were present in 27% of 120 probands of Western European descent (Gerull et al. 2004), and all were ultimately diagnosed with ARVC. Furthermore, it appears that the nucleotide residue 235 is a mutational hotspot as 6 cases of nonsense mutations occurred at this position (Mertens et al. 1996). An additional study further validated the correlation between *PKP2* mutations and the

1 prevalence of ARVC as 43% of a cohort of 58 ARVC cases contained *PKP2* mutations  
2 (Dalal et al. 2006).  
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8 **Desmoglein-2 (*DSG2*):** Mutations in the *DSG2* gene encoding desmoglein-2 at locus  
9 18q12 were reported in ARVC patients (Pilichou et al. 2006). Desmoglein-2 is  
10 synthesised as a pro-protein, which is post-translationally cleaved by the protease  
11 furin, at an R-X-K-R motif, into its fully functional form. However, mutations in residues  
12 R48H and R45Q appear to interfere with this cleavage, thus preventing the formation  
13 of the active form of desmoglein-2 (Awad et al. 2006). Dieding and colleagues used  
14 atomic force microscopy (AFM) based single molecule force spectroscopy (SMFC)  
15 and cell-cell adhesion assays to study the kinetic properties of wildtype desmoglein-2  
16 and compared it to two ARVC-associated mutants (p.D105E and p.V343I; (Dieding et  
17 al. 2017). While D105E, which is closely located to a calcium ion coordination site,  
18 seemed to lead to stronger binding and an increased lifetime, potentially affecting the  
19 dynamics of desmosomal turnover, no significant differences were found in these  
20 molecular assays for the V343I mutant, indicating it may be more of a modifier (Dieding  
21 et al. 2017).  
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46 **Desmoplakin (*DSP*):** As discussed above, recessive mutations in the gene that  
47 encodes desmoplakin at locus 6p24, give rise to Carvajal disease (Norgett et al. 2000).  
48 Autosomal dominant mutations in the *DSP* gene can give rise to ARVC without  
49 cutaneous involvement (Rampazzo et al. 2002). It was suggested that mutations  
50 resulting in the truncation of the desmoplakin protein due to a premature incorporation  
51 of a stop codon, result in a more severe phenotypic expression of the disorder (Lopez-  
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1 Ayala et al. 2014). However, it should be noted that desmoplakin mutations appear to  
2 lead to a higher frequency of left ventricular dysfunctional forms of the disease  
3 (Castelletti et al. 2017). The mutant form of desmoplakin has a high tendency to  
4 dimerise, hence aggregates are likely to be found in desmosomal protein complexes  
5 (Sen-Chowdhry et al. 2005).  
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16 **Plakoglobin (JUP):** Autosomal dominant mutations in JUP coding for plakoglobin are  
17 rare contributors to ARVC and apart from Naxos disease so far only few cases were  
18 reported, e.g. JUP p. S39\_K40insS (Asimaki et al. 2007), which leads to the insertion  
19 of a serine into the N-terminus of plakoglobin. In this case, only the heart is affected  
20 and there are no signs of a skin or hair phenotype.  
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32 **Desmocollin-2 (DSC2):** Autosomal dominant mutations in *DSC2* at 18q12.1 have  
33 been reported to be causative for ARVC (Beffagna et al. 2007; Gehmlich et al. 2011).  
34 Both missense and truncating mutations were described. For some of the missense  
35 mutations, trafficking defects, i.e. the failure of the protein to locate to the  
36 desmosomes, were suggested as the pathogenic mechanism. In contrast, a common,  
37 very C-terminal truncation variant *DSC2* p.A897KfsX4 affects only the DSC2a isoform  
38 and is now thought to be clinically silent (De Bortoli et al. 2010).  
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53 Since multiple desmosomal protein mutations have been found to be linked with  
54 causing ARVC, it could be proposed that these all lead to the deregulation of one final  
55 common pathway via the disruption of cellular adhesions between cardiac myocytes  
56 (Vatta et al. 2007), resulting in a loss of plakoglobin from desmosomes (Asimaki et al.  
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2009). The latter was also suggested as a diagnostic feature for the disease. However, more recent evidence showed that the disappearance of the plakoglobin signal from the intercalated disc in an ARVC mouse model and samples from human patients is seen just with one antibody but not with others specific for plakoglobin, suggesting that epitope masking may have contributed to this interpretation and that plakoglobin is retained to a certain extent also in disease (Kant et al. 2016). With more and more mutations being structurally mapped (for a review see (Al-Jassar et al. 2013), it has become clear that a one fits all explanation is maybe too optimistic, since proven disease causing missense mutations may have several consequences, such as affecting stability, protein-protein interaction, trafficking as well as signalling roles of a desmosomal protein.

#### *Mutations in genes encoding for non-desmosomal proteins*

In addition to desmosomal mutations, also mutations in genes encoding non-desmosomal proteins were reported as causes of ARVC. These include – among others – TGF- $\beta$ 3, cardiac RyR2 and TMEM43 (Herren et al. 2009).

**Transforming growth factor- $\beta$ 3 (TGFB3):** The transforming growth factor- $\beta$ 3 (TGF- $\beta$ 3) gene encodes a cytokine (Beffagna et al. 2005) that plays a role in regulating cellular adhesions, and was found to be involved in the classic autosomal dominant form of the disease, ARVD1 (Sen-Chowdhry et al. 2005; Sporn and Roberts 1992). Direct sequencing of genomic DNA highlighted two mutations, which were localised to regulatory 3' untranslated region (UTR) and 5' UTR regions of the TGF- $\beta$ 3 gene that may have a role in the disorder. Studies suggest that UTR mutations, which induce

1 TGF- $\beta$ 3 overexpression may also be associated with the distinctive fibrosis observed  
2 of the cardiac myocardium in ARVC (Beffagna et al. 2005). More recently another link  
3 between TGF $\beta$  signalling and desmosomes was established, when it was  
4 discovered that reduced expression of *PKP2* in cultured cardiomyocytes activated  
5 TGF $\beta$ 1 and p38-mitogen activated protein kinase signalling with the final readout  
6 of increased expression of profibrotic genes (Dubash et al. 2016).  
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18 **Ryanodine receptor 2 (*RYR2*):** Another non-desmosomal gene that was linked to  
19 ARVC is ryanodine receptor 2 (*RYR2*; (Tiso et al. 2001), mapping to chromosomal  
20 locus 1q42--q43 and encoding a large calcium ion channel responsible for eliciting  
21 excitation-contraction coupling in the heart (Lehnart et al. 2008). 4 heterozygous  
22 missense mutations were identified in the gene, classified as arrhythmogenic right  
23 ventricular dysplasia 2 (ARVD2). Although structural abnormalities in ARVD2 are very  
24 limited, the disease is thought to be allelic with familial catecholaminergic polymorphic  
25 ventricular tachycardia as there are many phenotypic overlaps (Sen-Chowdhry et al.  
26 2005). Comparing the two similar phenotypic ryanodine receptor-induced disorders  
27 allows for a better understanding of the disease-causing mechanism of ARVC.  
28 Calcium-induced calcium release from the sarcoplasmic reticulum into the cytosol is  
29 induced by the cardiac ryanodine receptor 2. Therefore, RyR2 mutations may result in  
30 the leakage of calcium ions from the sarcoplasmic reticulum due to defective channels  
31 (Laitinen et al. 2001). This consequently contributes to dysfunctional cardiac  
32 excitability in response to sympathetic stimulation, giving rise to arrhythmias induced  
33 by strenuous exercise, as is observed in ARVC (Lehnart et al. 2008).  
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**Transmembrane protein 43 (*TMEM43*):** Missense mutations in the *TMEM43* gene which encodes the transmembrane protein 43 (also called Luma) were described to cause the ARVD5 form of this cardiomyopathy. A potentially deleterious missense mutation in *TMEM43* (S358L) was observed in all ancestral haplotypes across 15 families with ARVD5 (also termed ‘Newfoundland’ mutation). It should be noted that the *TMEM43* gene contains a response element for peroxisome proliferator-activated receptor- $\gamma$ , PPAR $\gamma$  (Merner et al. 2008). PPAR $\gamma$  is an adipocyte growth factor and may be associated with the differentiation of fibroadipocyte progenitor cells into myocardial adipocytes – thus offering an explanation for the phenomenon of fibrofatty infiltration of the cardiac myocardium (Corrado et al. 2017). However, two recent mouse models, a knock out and a knock-in of the S358L missense mutation, display no cardiac abnormalities (Stroud et al. 2018). Moreover, the recent work establishes *TMEM43* as a nuclear envelope protein (Stroud et al. 2018).

**Sodium voltage-gated channel alpha subunit, 5 (*SCN5A*):** Mutations in the gene *SCN5A*, which encodes the sodium voltage-gated channel alpha subunit, 5 (Nav1.5), found in myocardial cells were also hypothesised as being significant in causing ARVC (Te Riele et al. 2017). A similar cardiomyopathy, Brugada Syndrome, arises due to impairment of the same sodium channel, and shares many phenotypic features with ARVC, in particular the high frequency of ventricular tachycardia and sudden cardiac death (Stokoe et al. 2007).

There are multiple reports of variants identified in further genes in patients with ARVC (see Table 1). The affected proteins localise to various cellular compartments and

1 pathogenic mechanisms are largely unknown. A further confounding factor is the  
2 phenotypic overlap of ARVC with other inherited cardiac diseases, for example  
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4 predominantly left ventricular forms of ARVC share a similarity with dilated  
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6 cardiomyopathy.  
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## 10 11 12 13 **Pathogenic Processes** 14 15

16 The two main pathogenic mechanisms that are observed at the histological level are  
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18 apoptosis and inflammation. Apoptosis leads to programmed cardiomyocyte death  
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20 and contributes to the observed progressive atrophy of the myocardium (Thiene et al.  
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22 2000). Features of apoptotic cells include chromatin condensation, nuclear  
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24 fragmentation and the presence of protease CPP-32 (also called caspase-3; (Herren  
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26 et al. 2009). In a study using TUNEL assay to identify apoptotic cardiomyocytes, 7 out  
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28 of 20 cases of ARVC displayed characteristics of apoptosis (Thiene et al. 1998).  
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30 Immunohistological investigation of the expression of CPP-32 in right ventricles of  
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32 normal subjects and ARVC patients revealed high levels of CPP-32 in cardiomyocytes  
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34 of ARVC patients, whereas unaffected individuals had cardiomyocytes with  
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36 undetectably low levels of the protease (Mallat et al. 1996). Apoptosis was also  
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38 documented in a transgenic mouse model with a mutation in the desmosomal protein  
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40 desmoglein-2 (Pilichou et al. 2009).  
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49 Inflammation is another mechanism thought to contribute to the pathological features  
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51 of ARVC. Inflammation may arise following myocardial degeneration as histology has  
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53 revealed that patchy inflammatory infiltrates of T-lymphocytes were present in 50% of  
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55 ARVC patients studied (Campuzano et al. 2012). It was speculated that the fibrofatty  
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57 replacement of the myocardium occurs as a healing process in response to the  
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1 inflammatory disease such as chronic myocarditis (Thiene et al. 1991) and that  
2 inflammation is a trigger of ARVC in genetically-predisposed individuals (Thiene et al.  
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4 2000). In support of this, a study involving BALB/c mice subjected to Coxsackie virus  
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6 led to selective right ventricular myocarditis and wall thinning, which is consistent with  
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8 the predominant right ventricular involvement observed in the pathology of ARVC  
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10 (Matsumori and Kawai 1980). However, Coxsackie virus infection can also upregulate  
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12 the expression of miR-21 which leads to reduced desmin protein levels, the  
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14 cytoskeletal filament to which desmosomes attach (see Figure 1), which might again  
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16 lead to a destabilisation of desmosomes (Ye et al. 2014).  
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22 Both apoptosis and inflammation may only be secondary mechanisms that are  
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24 ultimately be due to a compromised resistance of the cardiac tissue to mechanical  
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26 stress due to impaired function of desmosomes. Experiments on cultured cells  
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28 provided evidence for mutated plakophilin-2 directly affecting the assembly and  
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30 stability of desmosomes (Hall et al. 2009). This may lead to cell detachment and  
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32 induce necrotic and apoptotic events.  
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37 From a histological point of view it has been a mystery for a long time, why a disease  
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39 that is caused by mutated proteins that make up a subcellular structure such as the  
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41 desmosome that is found throughout the heart is predominantly observed in the right  
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43 ventricle, which is usually exposed to less mechanical stress. Recent research has  
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45 highlighted that the underlying cause for this might be due to differential embryonic  
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47 origin of parts of the heart, since it was shown that cardiac progenitor cells from the  
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49 epicardium can be stimulated to transdifferentiate into adipocytes upon nuclear  
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51 translocation of plakoglobin (Garcia-Gras et al. 2006; Lombardi and Marian 2010).  
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53 Since these cardiac stem cells are predominantly derived from the secondary heart  
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55 field, which contributes more to the right ventricle than the left ventricle (Kelly et al.  
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2014), this suggests that the higher adogenic potential may be due to a differential embryonic origin.

## Pathological events at the cellular level

### *Plakoglobin and Wnt signalling pathways*

The desmosomal armadillo protein plakoglobin may play a role in the pathogenesis of ARVC. Under normal conditions, plakoglobin is exclusively found at the cell-cell contacts (intercalated discs). However, plakoglobin is also known as  $\gamma$ -catenin and is related to  $\beta$ -catenin, a well-known factor in the classical Wnt signalling pathway (Zhurinsky et al. 2000). In a cardiac cell line, the HL-1 cells, suppression of desmoplakin expression leads to a nuclear translocation of plakoglobin and suppression of the Wnt/ $\beta$ -catenin signalling pathway by binding to the Tcf/Lef1 transcription factors (Garcia-Gras et al. 2006). A similar nuclear concentration of plakoglobin was also demonstrated for whole tissue extracts from hearts from desmoplakin-deficient mice (Garcia-Gras et al. 2006). However, since only 15% of the nuclei in heart tissue are from cardiomyocytes (Soonpaa et al. 1996), it cannot be excluded that the reported effect on Wnt signalling at the tissue level occurs mainly in non-cardiomyocytes. Nuclear plakoglobin was also detected in neonatal cardiomyocytes that expressed a truncated version (2057del2), but was not really obvious in the nuclei of iPSC-derived cardiomyocytes from ARVC patients (Asimaki et al. 2014), despite the well-known immature status of this kind of cells. Glycogen synthase kinase (GSK)  $3\beta$ , which has a suppressive role in the canonical Wnt signalling pathway, shows differential subcellular distribution in mouse models for ARVC (Chelko et al. 2016). In control mice, GSK $3\beta$  was localised to the cytoplasm,

1 however in mice with an excision of exon 4 and 5 from the desmoglein 2 gene, GSK3 $\beta$   
2 was found abnormally redistributed to the intercalated disc (Chelko et al. 2016). There  
3  
4 is also potential crosstalk between  $\beta$ -catenin and the Hippo signalling pathway in  
5  
6 ARVC: Following molecular remodelling of the intercalated disc, it was discovered that  
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8 levels of protein kinase C  $\alpha$ , a signalling molecule which localises to the intercalated  
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10 disc upon interactions with desmosomal plakophilin 2, decreased (Chopra et al. 2011).  
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12 As a result, neurofibromin-2 (or Merlin) becomes activated and in turn, phosphorylates  
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14 and inactivates the effector of the Hippo pathway, Yes-associated protein, YAP (Zhou  
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16 and Hanemann 2012). YAP is then free to bind to  $\beta$ -catenin and  $\gamma$ -catenin  
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18 (plakoglobin) and therefore drives adipogenesis in myocardial tissue. The Hippo  
19  
20 pathway has a number of functions but notably, plays a role in the regulation of cell  
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22 differentiation and maintenance of tissue homeostasis (Yu and Guan 2013).  
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24 Significantly, activation of the Hippo pathway has a negative feedback effect on the  
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26 canonical Wnt pathway, thus reinforcing the hypothesis that the two pathways might  
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28 be implicated in the pathogenesis of ARVC (Chen et al. 2014).  
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#### 46 *Trafficking*

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49 Since potentially fatal arrhythmias are one of the main characteristics of ARVC, it is  
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51 important to determine which histological or cellular changes lead to this detrimental  
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53 phenotype. On one hand it may be caused by cardiomyocyte death and fibro-fatty  
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55 replacement interfering with coordinated conduction, on the other hand it has become  
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57 clear that also more subtle alterations in ion channel expression levels and localisation  
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are observed at the cellular level. A lack of interaction of a Dsc2a mutant with connexin-43, which leads to its altered phosphorylation and decreased presence (Gehmlich et al. 2011) and dysregulation of Nav1.5 sodium channels in *Pkp2* knockdown mice (Cerrone et al. 2012) were described. Immunohistochemical analysis of heart tissue from ARVC patients also showed decreased signals for plakoglobin, connexin-43 and Nav1.5, suggesting that this remodelling may be a general phenomenon, contributing to a vulnerability to arrhythmias (Noorman et al. 2013). Trafficking of desmosomal proteins is not very well understood, but for desmoplakin an involvement of microtubules was demonstrated as it was shown that disease mutations in desmoplakin do interfere with its binding to EB1, a microtubule binding protein (Huang et al. 2014). In addition to its interaction with Desmocollin 2, connexin-43 was also shown to bind to ankyrin-G and plakophilin (Sato et al. 2011). Since plakophilin is required for the transcription of RyR, ankyrin-B and other proteins involved in the control of calcium cycling (Cav1.2, triadin, calsequestrin2) (Cerrone et al. 2017), signalling crosstalk from the intercalated disc to the nucleus obviously contributes to the development of an ARVC phenotype. Interestingly, in a recent screen using a zebrafish model of ARVC, a drug, SB216763, was identified (Asimaki et al. 2014), which changed the subcellular localisation of SAP97 (also known as DLG-1), a protein known to be involved in the trafficking of Nav1.5. SB216763 can act as an inhibitor of GSK3 $\beta$  and seemed to increase expression levels of SAP97 as well as preserve its plasma membrane location. In ARVC model systems (neonatal rat cardiomyocytes expressing mutant plakoglobin and iPSC-derived cardiomyocytes from human ARVC patients) treated with this drug, this was accompanied by connexin-43 and Nav1.5 being retained at cell-cell contacts (Asimaki et al. 2014). This suggests

that it may be possible to interfere with subcellular trafficking issues that appear to be a hallmark of ARVC and thus lead to a functional improvement.

## Outlook and further perspectives

ARVC is an inherited cardiac disease with a wide phenotypic spectrum. Diagnosis can be challenging, especially hindered by the incomplete penetrance. Therefore, the first manifestation of the disease can be life-threatening arrhythmias, which may result in sudden cardiac death. The majority of familial cases show autosomal dominant inheritance. Even though the majority of mutations are found in desmosomal genes, also mutations in non-desmosomal proteins were reported to cause ARVC. Next generation sequencing and its wider use in clinical practice will pave the way for a “molecular diagnosis” of ARVC, however the evaluation of identified variants can be challenging, since presumed disease causing missense mutations may also be present in the control population (Andreasen et al. 2013). It is also becoming more and more apparent that compound and digenic heterozygosity of desmosomal gene mutations contribute to the disease (Xu et al. 2010). Recently it was suggested that the ARVD/C database might be a useful tool for practitioners (Lazzarini et al. 2015), listing 1,400 variants in 12 ACM-related genes, both desmosomal and non-desmosomal ones (as of 2014). However, also in this database the majority of variants are classified as of ‘unknown significance’, highlighting the dilemma of assigning causality to variants identified in individuals. The analysis of patient material and in particular the thorough study of animal and cellular disease models and *in vitro* functional studies have provided insights into the disease mechanisms of ARVC.

1 However, a deeper understanding of aberrant signalling in the presence of  
2 desmosomal impairment is required to develop novel therapies to combat myocardial  
3 remodelling and conduction defects.  
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Figure 1: **Schematic drawing of a desmosome:** Intercellular connections between two cardiomyocytes (only partially shown) are mediated by the transmembrane proteins, the desmosomal cadherins Desmocollin 2 (Dsc2) and Desmoglein 2 (Dsg2). The link to the cytoskeleton, the intermediate filaments composed of desmin, is mediated by a complex consisting of Desmoplakin, Plakoglobin and Plakophilin-2. Dsc2 also interacts with the gap junction protein connexin-43. PM stands for plasma membrane.

Table 1: Reported disease genes for ARVC.

Gene	Encoded Protein	Subcellular localisation	Chromosomal locus	Reference
<i>JUP</i>	Plakoglobin	Desmosome	17q21.2	(McKoy et al. 2000)
<i>DSP</i>	Desmoplakin	Desmosome	6p24.3	(Rampazzo et al. 2002)
<i>PKP2</i>	Plakophilin-2	Desmosome	12p11.21	(Gerull et al. 2004)
<i>DSG2</i>	Desmoglein-2	Desmosome	18q12.1	(Pilichou et al. 2006)
<i>DSC2</i>	Desmocollin-2	Desmosome	18q12.1	(Syrris et al. 2006)
<i>CDH2</i>	N-Cadherin	Area composita	18q12.1	(Mayosi et al. 2017)
<i>LMNA</i>	Lamin A/C	Nuclear envelope	1q22	(Quarta et al. 2012)
<i>DES</i>	Desmin	Intermediate filament	2q35	(van Tintelen et al. 2009)
<i>CTNNA3</i>	Alpha-T-catenin	Area composita	10q21.3	(van Hengel et al. 2013)
<i>PLN</i>	Phospholamban	Sarcoplasmic reticulum	6q22.31	(van der Zwaag et al. 2012)
<i>RYR2</i>	Ryanodine receptor 2	Sarcoplasmic reticulum	1q43	(Tiso et al. 2001)
<i>TGFB3</i>	Transforming growth factor $\beta$ 3	Growth factor	14q24.3	(Beffagna et al. 2005)
<i>TTN</i>	Titin	Sarcomere	2q31.2	(Taylor et al. 2011)
<i>SCN5A</i>	Sodium voltage-gated channel alpha subunit 5	Sodium channel Intercalated disk	3p22.2	(Te Riele et al. 2017)
<i>TMEM43</i>	Transmembrane protein 43	Nuclear envelope	3p25.1	(Merner et al. 2008)

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# Cardiomyocyte 1

# Cardiomyocyte 2

