ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY

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# LIST OF ABREVIATIONS

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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ARVC</td>
<td>Arrhythmogenic right ventricular cardiomyopathy</td>
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<tr>
<td>DSP</td>
<td>Desmoplakin</td>
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<tr>
<td>SCD</td>
<td>Sudden cardiac death</td>
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<tr>
<td>VT</td>
<td>Ventricular tachycardia</td>
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<td>VF</td>
<td>Ventricular fibrillation</td>
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<tr>
<td>CE-CMR</td>
<td>Contrast-enhanced cardiac magnetic resonance</td>
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<tr>
<td>ID</td>
<td>Intercalated disc</td>
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<tr>
<td>AJs</td>
<td>Adherens junctions</td>
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<td>Gjs</td>
<td>Gap junctions</td>
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1. INTRODUCTION

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is caused by a structural and functional abnormality of the right ventricle. It is responsible for about 10-20% of sudden cardiac deaths in young adults and athletes as the disease is often associated with ventricular tachycardia or ventricular fibrillation. Increasing knowledge about the pathophysiology will permit an early diagnosis of the disease and also reduce transition to heart failure among patients.

2. CLINICAL ASPECTS

2.1 CLINICAL MANIFESTATION

The most characteristic clinical presentation of patients with ARVC includes syncope, ventricular tachycardia (VT), palpitations, cardiac arrest or sudden cardiac death (SCD). Because of the high 10-year lethality of 30% when untreated and the early onset of the disease, an early clinical diagnosis and treatment is essential for patients with ARVC. The primary reason for a clinician to suspect an ARVC is ventricular tachycardia in young adults. Chest pain mimicking a myocardial infarction or a myocarditis and an increase in myocardial enzyme release are nonspecific clinical symptoms and can distract from the diagnosis of ARVC.

2.2 DIAGNOSING ARVC

To ensure a high sensitivity and specificity in diagnosing ARVC while including the heterogeneous clinical presentation, a task force was formed in 2010 to develop diagnostic criteria. Six different parameters are evaluated to categorize patients such as global or regional dysfunction and structural alterations, tissue characterization, depolarisation or conduction abnormalities, repolarisation abnormalities, arrhythmias and family history.

Clinical presentation of patients with a diagnosed ARVC can be divided up into three clinical phases: (1) the subclinical phase where structural abnormalities may not be present (concealed disease), even though SCD can occur, (2) the classical phase with ventricular arrhythmias, syncope, palpitations and structural abnormalities (overt disease), (3) he advanced phase with severe structural progression, dilatation and systolic dysfunction (end-stage disease).

In the case of suspected ARVC a routine diagnostic work-up should start with non-invasive techniques such as family history collection, 12-lead ECG, 24h ECG and echocardiography. In
the event of inconclusive evaluation further examination is required. Invasive techniques include contrast-enhanced cardiac magnetic resonance (CE-CMR), angiography, endomyocardial biopsy and electroanatomical mapping. Intramyocardial fibrosis and structural changes and abnormalities detected via CE-CMR supports the diagnosis of ARVC (Figure 1). Electroanatomical mapping allows recognizing abnormal low-voltage areas caused by loss of electrically active myocardium also referred to as electroanatomical scar. It is only performed in selected cases, due to the invasive procedure, primarily to differentiate between idiopathic RV outflow tachycardia and ARVC and it is also used to guide catheter ablation.

Figure 1: Electroanatomical map from one control subject (A) with normal bipolar voltages compare to one patient with ARVC (B) with low amplitude electrical active regions. Voltages are colour coded where purple represents amplitude > 1.5mV which is considered as electroanatomic normal myocardium and red represents amplitude < 0.5mV which is considered as an electroanatomical scar. Right, Cardiac magnetic resonance image with dilated right ventricle and an aneurysm in the subtricuspid region (arrows). LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle. Adapted from Delmar M. (Circ Res. 2010) and Corrado D (Heart. 2009)

3. GENES RELATED TO ARVC

Mutations of genes encoding desmosomal proteins have been discovered in more than 40% of ARVC patients. (Table I) Mutations in PKP2 are most frequently observed in ARVC (see below for description of desmosomal proteins). Regardless of PKP2 mutation, the expression of Cx43, which is the gap junction channel connection protein found in IDs is often decreased. This suggests that desmosomal gene mutations may also influence the amount and distribution of
other proteins in the intercalated disc (ID), including gap junction proteins, cardiac ion channels and adherens junction. As well, genetic testing should not override clinical judgement because it is not unequivocal in which manner and how severely these genes affect the development of the disease.

Table I: Genes associated with ARVC (Basso C, Nat Rev Cardiol 2012)

<table>
<thead>
<tr>
<th>gene</th>
<th>chromosome locus</th>
<th>protein</th>
<th>mode of inheritance</th>
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<tr>
<td>Desmosomal gene</td>
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<tr>
<td>JUP</td>
<td>17q21</td>
<td>Junction plakoglobin</td>
<td>AR/AD</td>
</tr>
<tr>
<td>DSP</td>
<td>6p24</td>
<td>Desmoplakin</td>
<td>AR/AD</td>
</tr>
<tr>
<td>PKP2</td>
<td>12p11</td>
<td>Plakophilin-2</td>
<td>AR/AD</td>
</tr>
<tr>
<td>DSG2</td>
<td>18q12</td>
<td>Desmoglein-2</td>
<td>AD</td>
</tr>
<tr>
<td>DSC2</td>
<td>18q12</td>
<td>Desmocollin-2</td>
<td>AR/AD</td>
</tr>
<tr>
<td>Extradesmosomal gene</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>RYR2</td>
<td>1q42-q43</td>
<td>Ryanodine receptor2</td>
<td>AD</td>
</tr>
<tr>
<td>TGFBI</td>
<td>14q23-q24</td>
<td>Transforming growth factor β3</td>
<td>AD</td>
</tr>
<tr>
<td>TMEM43</td>
<td>3p25</td>
<td>Transmembrane protein 43 (protein LUMA)</td>
<td>AD</td>
</tr>
<tr>
<td>DES</td>
<td>2q35</td>
<td>Desmin</td>
<td>AD</td>
</tr>
<tr>
<td>TTN</td>
<td>2q31</td>
<td>Titin</td>
<td>AD</td>
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4. DESMOSOME

Desmosome is one type of cell junction molecular complexes. Cell junctions are specialized connective region between two cells or between a cell and the extracellular matrix. Cell junctions have two main functions. One is attaching cells to their neighbour cells or extracellular matrix. The other function is mediating the passage of chemical or electrical signals from one interacting cell to its partner. There are several types of cell junctions. In particular, cardiac junction at the intercalated disc (ID) consists of adherens junctions (AJs), desmosomal junctions, and gap junctions (GJs). (Figure 2)

![Figure 2: Structure of intercalated disc (Noorman M JMCC 2009)](image)

Each desmosomal plaque consists of a thick outer dense plaque and a translucent inner dense plaque. Intercellular space is between 20-35 nm wide. (figure-3-A) Desmosome is built up of three parts such as desmosomal cadherins, armadillo family members, and the plakin family
members. (figure-3-B). Desmosomal cadherins are cell-adhesion proteins, which bridge adjacent cells. Proteins such as desmogleins (DSG1-4) and desmocolins (DSC1-3) are essential for Ca2+-dependent cell-cell adhesions. DSG2 and DSC2 are the main desmosomal cadherins expressed in cardiac IDs. Armadillo family members are associative proteins which can (1) link desmosomal cadherins and plakin family members and (2) cluster desmosomal proteins during the formation of desmosomes. PKP2, PKP4, and PG are expressed in cardiac muscle. Especially, PKP2 has a function as a scaffold for protein kinase Ca, regulating the association of the desmoplakin with intermediate filaments. Plakin family members such as desmoplakin (DSP), plectin, envoplakin, and periplakin link the desmosome to cytoskeletal proteins. DSP is the most abundant component in the desmosome. DSP has 2 major subtypes, DSPI and DSPII. DSPI predominates in cardiac myocytes.

Figure 3: Desmosome structure: (A) electron microscopy, (B) desmosomal components (Brooke M, J Pathol 2012)

5. PHYSIOPATHOLOGY

The exact pathomechanism of ARVC is still the subject of many ongoing studies. What seems to be clear is that the pathogenesis of ARVC can be explained by the combination of altered mechanical coupling and altered signaling pathway of the cardiomyocytes.

5.1 CELL COUPLING

It is not unequivocal in which manner and how severely discovered genetic alterations affect the development of the disease. The disease subtypes may coexist within families where the
members have different degrees of malformation, so other modifier factors need to be considered that can have an impact on risk stratification. However all genetic alterations ultimately lead to is a final common pathway that is responsible for the pathologic manifestations.

The identified mutations all affect genes that express proteins important for desmosomal function, so the dysfunction of the desmosome seems to have a major part in pathogenesis. The protein that is absent at cardiac junctions in nearly every case of ARVC is plakoglobin. This reduced plakoglobin signal seems to be relatively specific for ARVC thus it represents an opportunity for diagnostic tests and also suggest that it might have a pivotal role in the disease pathogenesis although the exact mechanism remains unclear (figure 4).

Figure 4: Detachment of cardiomyocytes due to the absence of plakoglobin (MacRae, JCI 2006)

The main question considering the pathomechanism is: how does a desmosomal, therefore a mechanical problem lead to arrhythmias? Two main phenomena can be used to make the connection here.

The first reason can be derived from the fact that mechanical and electrical coupling mechanisms at the intercalated disks are codependent, which can be suspected from the highly organized structure of adhesive connections (AJs, GJs, desmosomes). This morphological association also plays a major part in the disease pathogenesis. Though ARVC is considered a desmosomal disease, mutations that affect the number and intracellular localization of desmosomes influence the amount and distribution of other ID proteins, including GJ proteins and cardiac ion channels. Multiple studies have indicated that gap junction formation requires the presence of neighbouring mechanical junctions, probably to provide stability for docking and/or assembly of functional GJ channels. This function is required during both embryogenesis of the heart and remodeling that occurs after myocardial infarction. In most cases of ARVC, gap junction remodeling occurs, which can be detected by reduced Cx43 signal at the IDs. This redistribution of GJs is a consistent feature of ARVC. However, this process occurs diffusely and is also observed in the left ventricle and the interventricular septum. Though it is not sure to
which extent it contributes to the disease pathogenesis. However, it is indisputable that the abnormality of GJ localization and function can cause a serious impairment of cardiac electrical functions since gap junctions are responsible for normal propagation of electric current amongst cardiomyocytes.

The second connection that can be made between the desmosomal dysfunction and arrhythmias is that abnormal cell-cell adhesion invokes mechanic stress which injures the cardiomyocytes and promotes necrosis. The significant myocyte loss in either ventricle can cause an inflammatory response. The myocardium has a limited regenerative capacity so this inflammation cannot be fully repaired, a fibrofatty scar will take the place of the muscle. Both the inflammation and the fibrofatty islands result in the loss of electrically active myocardium hence creating potential sources of ventricular arrhythmia. This second hypothesis would confirm why strenuous exercise worsens the progression of the disease, as it produces extra mechanical stress on the cardiac cells. In addition, this pathomechanism also explains why the disease manifestation occurs in the right ventricle (RV): its thin walls and distensibility, which is necessary in the adaptation to variable preload, makes it more susceptible. However, even considering this it’s still unclear why the disease is mostly restricted to the RV.

5.2 CELL SIGNALLING

While the role of desmosomes in cell-cell adhesion is well known and has been shown to cause cardiomyocytes to malfunction in ARVC, more recent research suggests that desmosomes are also involved in cell signaling, which might be another key to understand the physiopathology of ARVC. Desmosomal components can translocate to other subcellular compartments, participate in signaling pathways, and thereby influence transcriptional regulation of genes involved in proliferation and differentiation. Some desmosomal proteins, especially plakoglobin, have been found to act as nuclear signaling molecules, regulating gene expression through the canonical Wnt signaling pathway.

Studies have shown that in ARVC, mutated proteins fail to localize at intercellular junctions, although they are clearly expressed in the tissue. This has led scientists to wonder whether these proteins were redistributed to other locations within the cell. In Naxos disease, which is caused by a mutation of the JUP gene, the mutant protein, plakoglobin, fails to localize normally at intercellular junctions. In Carjaval disease, which is caused by a mutation in the desmoplakin gene, both desmoplakin and its binding partner plakoglobin fail to localize normally at intercellular junctions. This shows that a mutant desmosomal protein may alter the expression or cellular distribution of other, non-mutated desmosomal proteins.
It also highlights the key role of plakoglobin in ARVC. Though ARVC can be caused by a various set of genetic mutations, affecting different genes that encode different proteins, all of these mutations might –directly or through other desmosomal proteins– have an effect on the cellular distribution of plakoglobin. This protein might thus be the key to altered signaling pathways in ARVC.

Suppression of desmoplakin expression leads to a redistribution of plakoglobin in the nuclear department, and a reduction of canonical Wnt signaling (figure 5). Plakoglobin is also known as γ-catenin, because it is functionally and structurally similar to β-catenin (they share an 85% sequence identity). Plakoglobin is known to compete with β-catenin at multiple cellular levels, suppressing the canonical Wnt pathway that uses β-catenin as an effector. The canonical Wnt pathway, also known as Wnt/β-catenin pathway, is a signaling pathway involved in many physiological mechanisms such as body axis patterning, cell proliferation, cell migration… More relevantly, it takes part in cell differentiation during the embryonic development, allowing pluripotent stem cells to differentiate first into endoderm and mesoderm cells, then into more specialized cells such as cardiomyocytes. Three types of Wnt signaling pathways have been characterized; the one described here, the canonical Wnt signaling pathway, is the only one that involves β-catenin. The Wnt pathway takes its name from the Wnt ligand, which enables β-catenin to act as a transcriptional coactivator.

Figure 5: Wnt pathway enabling β-catenin to act as a transcriptional cofactor (Eisenmann 2005)
β-catenin is usually localized in the cytosol, where its level is kept low by a complex of proteins called a destruction complex that binds to β-catenin to destroy it. The Wnt ligand disrupts this complex by binding to it. β-catenin thus accumulates in the cytosol and is eventually translocated into the nucleus, where it acts as a transcriptional coactivator for transcription factors that belong to the TCF/LEF family. The Wnt signaling pathway is regulated by the cytosolic levels of β-catenin through a negative feedback loop. The redistribution of plakoglobin in the cytosol in ARVC mimics high level of β-catenin, inhibiting the Wnt pathway.

Although the Wnt pathway is ubiquitous and well conserved through evolution, the specific genes it regulates are cell-type and context dependent. In cardiomyocytes, Wnt signalling inhibits adipogenic factors and activates myogenesis. Inhibition of the Wnt pathway leads to adipogenesis and fibrogenesis instead of myogenesis, causing the replacement of cardiomyocytes by fibro-fatty tissue. The involvement of desmosomes in cell coupling, and cell signalling through the Wnt pathway, provides an explanation as to the cell necrosis and fibro-fatty replacement phenotype in ARVC, and thus allows a better understanding of the physiopathology of this disease.

However, the key molecular targets to develop therapy based on specific ARVC pathophysiology remain unclear and there is still a long way to go before research can have an active implication in the clinical aspects of the disease, especially treatment. This is why current management mostly revolves around risk stratification and secondary prevention.

6. CURRENT MANAGEMENT

6.1 RISK STRATIFICATION

Due to ARVC being an inherited disease in about 50% of the cases, the diagnosis in one index patient causes consequences in the patient care of the family members. Since the pathomechanism is not yet fully understood clinical risk stratification is needed to improve the patient care. The development of good and reliable criteria for risk stratification in ARVC was considered problematic for a long time because of the heterogeneity of clinical symptoms. In 2013, Bhonsale et al proposed a new approach in risk stratification in patients with ARVC associated desmosomal mutations using pedigree interpretation, ECG and occurrence of premature ventricular complexes (PVC) to evaluate the probability of sustained arrhythmias (Figure 6).
6.2 TREATMENT OPTIONS

Ideal management and treatment of ARVC would be based on the understanding of the molecular mechanisms. Animal models tried to reproduce the human phenotype and gave new insight to the disease etiology and pathogenesis but the contribution to clinical treatment and management is still limited. Until this day there is no possibility to treat the underlying condition and the most important objective in patient care diagnosed with ARVC is to prevent sudden cardiac death.

The management of ARVC is adjusted to the different clinical stages of the patient. In the early concealed phase lifestyle advice and routine checkups play an important role. It has been discovered that intense physical exercise promotes the progression from the concealed to the overt phase thus patients should refrain from such activities. The current research indicates that asymptomatic patients do not benefit from a prophylactic treatment. However in the overt phase antiarrhythmic drugs are the first-line therapy while a significant reduction in VT or VF could not be proven for β-blockers. The use of an implantable cardioverter defibrillator (ICD) is highly recommended for patients with a high risk of SCD and improves the long term prognosis and survival rate. The individual risk stratification for ICD implantation needs to be accurate in order to reduce inappropriate interventions and complications as well as reducing potential psychological repercussions mainly in younger patients. Improvements in electroanatomical mapping of the heart lead to a progression from the palliative use of catheter ablation to a
possible treatment option for patients suffering from drug refractory incessant VT or frequent VT after ICD implantation and may evolve to an early treatment option in patients with VT. As a final therapeutic option heart transplantation can be considered in case of refractory right heart failure and/or untreatable ventricular arrhythmias.

7. CONCLUSION

To conclude, the heterogeneous clinical presentation of ARVC is probably related to the diversity of the pathomechanisms involved. In order to be able to make an early diagnosis both clinical and genetic aspects have to be considered to reduce mortality among people at risk. An existing mutation in more than one of the related genes may be required for clinical disease expression. Additional potential disease-causing genes are currently under investigation, facilitated by the advance of next-generation sequencing techniques. In addition, while abnormalities of IDs and especially the desmosome are central to ARVC pathomechanism, it is largely unknown which mutations are directly involved in the functional abnormalities of IDs and how they lead to ARVC. What seems to be sure is that although the basis of the disease derives from a mechanical dysfunction it also affects the electrical functions of the cardiomyocytes and some cell signalling pathways involved in the specialisation of the cell. This causes the two main manifestations of the disease: the arrhythmias and the fibrofatty tissue replacement. Due to the genetic origin and the complexity of the pathomechanism, clinical treatment to alter the progression of ARVC is tricky. Therefore, the current management focuses on risk stratification and preventing sudden cardiac death using implantable cardioverter defibrillator (ICD).

Further studies are essential to understand this disease, especially developing cellular models by using human iPS cells and some other stem cells since obtaining cardiac samples from early stages of human ARVC hearts is difficult and mouse models of ARVC remain inconclusive for finding clinically feasible therapy.
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REFERENCES

Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, et al. (2011) HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). Europace 13: 1077–1109


Calum A. MacRae, Walter Birchmeier and Ludwig Thierfelder : Arrhythmogenic right ventricular cardiomyopathy: moving toward mechanism JCI 2006;116(7):1825–1828.


ABSTRACT

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited disease with heterogeneous clinical and genetic presentation with the highest prevalence in athletes and people under the age of 35. Characterized by fibrofatty replacement of the heart muscle mainly in the right ventricle, the clinical presentation can vary from ventricular tachycardia or ventricular fibrillation to cardiac arrest, heart failure and sudden cardiac death. In the 2010 revised diagnostic criteria, detection of a mutation linked to ARVC in a first-degree relative is now a major diagnostic criteria and emphasizes the genetic alterations which can be found in about 50% of the cases.

Most of the mutations found in ARVC patients are in genes of desmosomal proteins, PKP-2 being the most frequent. Desmosomes are intercellular adhesions junctions which, in cardiac myocytes, reside within intercalated disks (IDs). Plakoglobin encoded by PKP-2, as well as other desmosomal proteins fulfill roles both as structural proteins in cell–cell adhesion junctions and as signalling molecules. The dysfunction of normal cellular adhesion leads to mechanical and electrical dysfunctions which promote early cardiomyocyte death and arrhythmias. The disruption of the Wnt signalling pathway induces adipogenesis and fibrogenesis that replace the normal myocardium, mainly in the right ventricle. The aim of this paper is to review the clinical aspects, genetic background and physiopathological pathways that lead to ARVC and some prospects of treatment that are still under development.