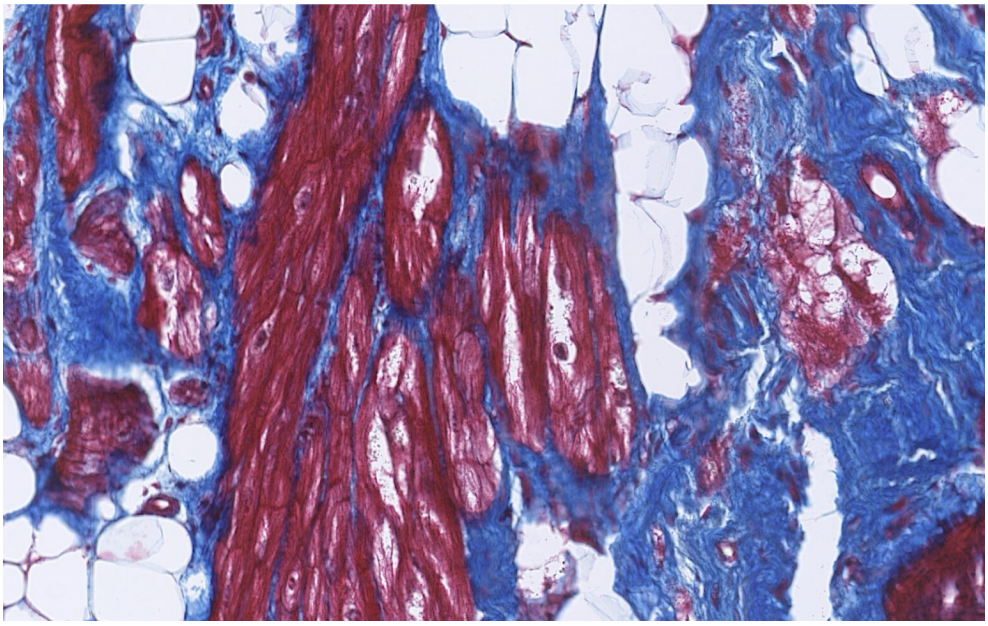
The role of Plakophilin-2 in the pathogenesis and progression of ACM and treatment strategies

A literature review



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Abstract

Arrhythmogenic Cardiomyopathy (ACM), also known as arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a rare hereditary disease of the heart, where the connective, and fatty tissue is deposited in the muscle tissue of the ventricular myocardium primarily of the right but also left ventricle. ACM is mainly caused by mutations in the desmosome proteins such as in Plakophilin-2. At the moment, more than 200 mutations leading to ACM are known, whereas most of them are point mutations, but also frameshift and splicing variations have been reported. Treatment options at the moment mainly aim to prevent disease progression and increase quality of life. However, new studies have provided promising results for working with e.g. proteasome inhibitors, for certain mutations, isogenic induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs), to restore the function of malfunctioning desmosomes and CRISPR/Cas9 to better understand the disease.

This review explains and discusses the effects of mutations in desmosome proteins like plakophilin-2, and the treatment options for ACM.

Keywords: AVC, ARVD, ARVC, PKP2, treatments

Introduction and General Information on the topic

With a prevalence of 1:2500 to 1:5000 affected people worldwide, ARVC/D is a rare autosomal dominant hereditary disease of the heart, leading to palpitation, caused by ventricular arrhythmia, syncope, dyspnoea, leg swelling, and atypical chest pain. In the worst case, it leads to sudden cardiac death.[1,2] ARVC or ARVD stands for arrhythmogenic right ventricular cardiomyopathy or dysplasia. This is usually caused by mutations in the desmosome proteins, which result in the cell adhesion molecules not functioning properly. Consequently, connective, and fatty tissue is deposited in the muscle tissue of the ventricular myocardium of the right ventricle. However, as this disease not only affects the right ventricle but also occurs in the left ventricle, it has generally been summarised as arrhythmogenic cardiomyopathy, ACM, since this year. [1,3] Most of these diseases are linked to mutations in the desmosomes. Desmosomes are cell structures that among others anchor the ends of the heart muscle fibres together so that the cells are not pulled apart when the individual fibres contract and can withstand the strain. Desmosome proteins are made up of a group of non-classical cadherins (Desmoglein and Desmocollin), which are linked to intermediate filaments as well as armadillo proteins (Plakoglobin and Plakophilin). Specifically, cardiomyocytes desmosomes are made up of five proteins: Desmoglein-2, Desmocollin-2, Plakophilin-2, plakoglobin and desmoplakin (Figure 1[4]). If the desmosomes do not function properly, e.g. due to mutations, the tissue cannot be held

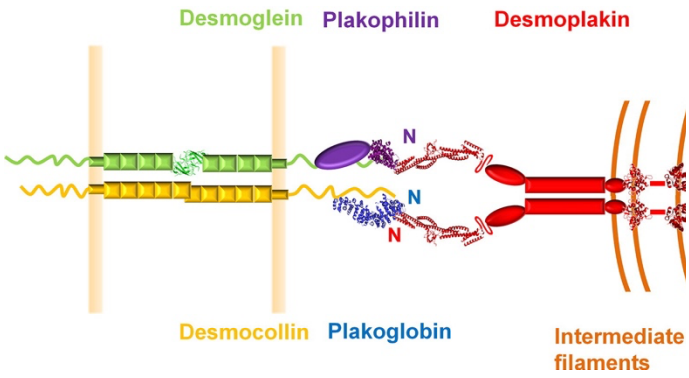

Figure 1 – Architectural Unit of the Desmosome [4]

Desmoglein and desmocollin have a single transmembrane domain and an intracellular anchor to secure their position in the cell membrane and can bind to plakoglobin. Desmoplakin mediates attachment to the intercellular filament of the desmosome structure and is anchored by plakoglobin and plakophilin. Plakoglobin and plakophilin mediate attachment to the intercellular filament and to cell membrane proteins. Anchoring of desmoplakin and intermediate filament to the desmosome structure.

together and cannot withstand forces.[4] This review explains and discusses the effects of mutations in desmosome proteins like plakophilin-2, its mutations, and the treatment options for ACM.

Function of the desmosome proteins

As briefly mentioned above and seen in Figure 1, desmosomes are a build-up of multiple proteins that form the overall desmosome and the desmosome intermediate filament complex, making sure that the desmosomes are anchored into the cytoplasm and the cells are connected to each other. Desmosomes are of crucial importance for tissues exposed to mechanical stress as they provide structural and mechanical stability. The formation of desmosomes is induced by two members of the cadherin superfamily, desmogleins and desmocollins, which then mobilise other proteins such as plakoglobin and plakophilin. These protein complexes attract yet another cytoskeletal system, the intermediate filaments.[5] Figure 2[6] gives an overview of the desmosomes in the cardiac tissue. Part A shows the anatomy of the heart – in ACM the most affected ventricle is the right one. Part B shows the intercalated discs of the cardiac tissue and how this is characterised by adherens junctions, gap junctions and desmosomes. Each of them provides a specific function to the tissue. For example, adherens junctions give structure and provide additional support to the tissue.[7] Together the junctions in the heart work to transport the impulses for the heart muscle to contract throughout the tissue. Part C shows a close-up of the desmosomes. The cell-to-cell adhesion is primarily dependent on the components that together make up the desmosome complex.[6] The last part of this picture, D, shows what would

happen if the desmosomes do not connect anymore, due to mutations or other influences, leading to an infiltration of the cardiac tissue with fatty and fibrous tissue.

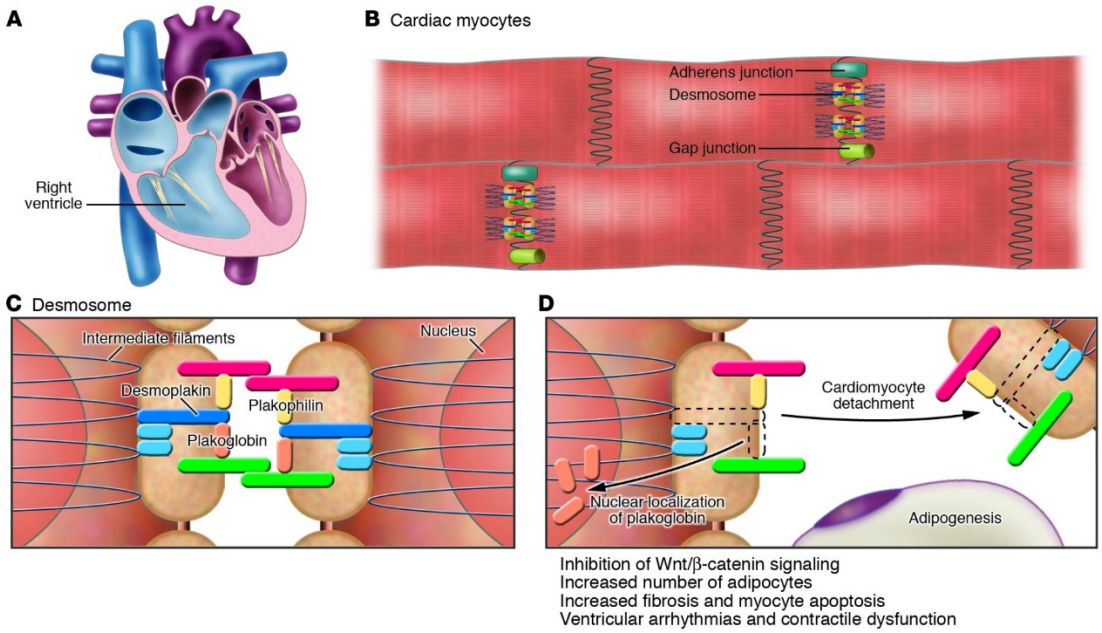

Figure 2 – Funktion of Desmosomes [6]

In part A of the image the heart is shown, indicating where the right ventricle is since in most cases ACM affects the right ventricle first. Part B shows how the intercellular discs are characterized by adherens junctions, desmosomes, and gap junctions. As mentioned before desmosomes offer support to the cells to withstand forces. Part C is a close-up of how the Desmosomes are built up since they play an important role in cell-to-cell adhesion. It shows the intermediate filaments, how it is attached to the desmosome complex and how desmoplakin, plakoglobin and plakophilin work together to build the desmosome complex. In part D it is shown what would happen if the desmosomes would break down and not hold together anymore, due to mutations leading to an infiltration of the cardiac tissue by fibro-fatty tissue.

Mutations in desmosome proteins

For this review, the focus will lay on Plakophilin-2 (PKP2) since most mutations relate to this gene and therefore change the protein function of Plakophilin-2.

The *PKP2* gene, located in 12p11.21, and with an exon count of 15 is next to the heart tissue, also often expressed in the colon and the skin tissue.[8]

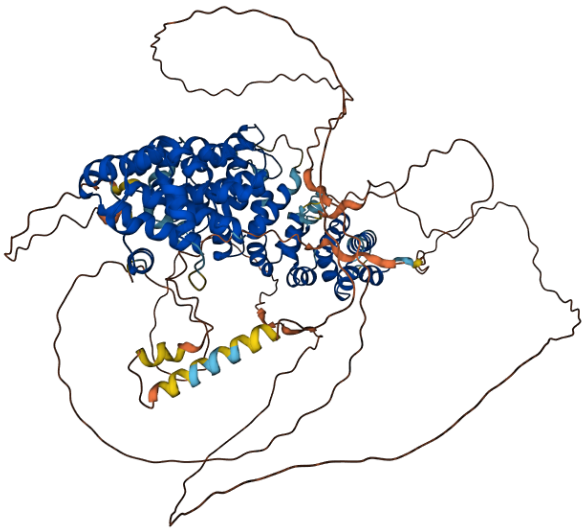

Figure 3 – Protein structure of Plakophilin-2 [11]

This image shows the quaternary structure of the Plakophilin-2 protein. PKP2 is one of the proteins forming the desmosomal plaque, needed for cell adhesion.

Up to now, over 200 mutations in the *PKP2* gene have been reported, the majority of which have been categorised as point mutations, but also frameshift and splicing variants have been recorded.[9,10]

PKP2 encodes for the eponymous Plakophilin-2 protein (Figure 3 – Protein structure of Plakophilin-[11]).

Plakophilin-2 is an armadillo protein, made up of 881 amino acids, featuring an N-terminal head domain, followed by eight arm repeat domains and a C-terminal tail. Two splice variants of PKP2 are known - PKP2a and PKP2b. Those two distinguish themselves by a 44 amino acid insertion between arm repeats two and three.

PKP2 possesses a flexible insert between arm repeats five and six, leading to a structural bend. Next to that, PKP2 engages with other desmosomal armadillo proteins and cadherins, contributing to lateral stabilizing force interactions within the desmosomal intermediate filament complex.[12]

Additionally, its function encompasses directing the desmoplakin to adherend junctions, and extensive interactions with beta-catenin and within the nucleus it is presumed to have a negative regulatory role for RNA polymerase III, influencing protein synthesis and growth control. PKP2 also plays a role in calcium handling.[12]

Next to that, PKP2 oversees the dynamics of focal adhesion turnover, influencing alterations in focal adhesion dimensions, cell adhesion, and cell spreading, potentially mediated by the transcriptional modulation of beta integrins. It is essential for the propagation of cardiac sodium currents and the maintenance of electrical synchrony in cardiac myocytes and plays a crucial role in establishing desmosomal connections between cells in cardiomyocytes, contributing significantly to the proper formation of the heart, particularly during trabeculation and the development of atrial walls. The disruption of desmosome-cell junctions results in the mislocalization of DSP and DSG2, leading to the disturbance of cell-to-cell adhesion and the arrangement of intermediate filaments.

Furthermore, it exerts regulatory control over profibrotic gene expression in cardiomyocytes, achieved through the modulation of DSP expression and subsequent activation of downstream TGFB1 and MAPK14/p38 MAPK signalling pathways.[13]

Generally very little is known about how PKP2 exactly influences ACM since multiple factors are involved, but new studies have shown, that a result of a PKP2 mutation leads to increased desmosomal protein degradation (as seen in Figure 4).[14] This would lead to a lost connection between the cells. As a consequence, the heart tissue is falling apart, causing infiltration with fibro-fatty tissue and ultimately leaving the heart with less muscle tissue to pump provoking arrhythmia or in the worst case sudden cardiac death. It is therefore certain that abnormalities in PKP2 are leading to abnormalities in the heart tissue, due to desmosomal destabilization and

among others malformation of the heart especially during trabeculation and the development of atrial walls.[13,15]

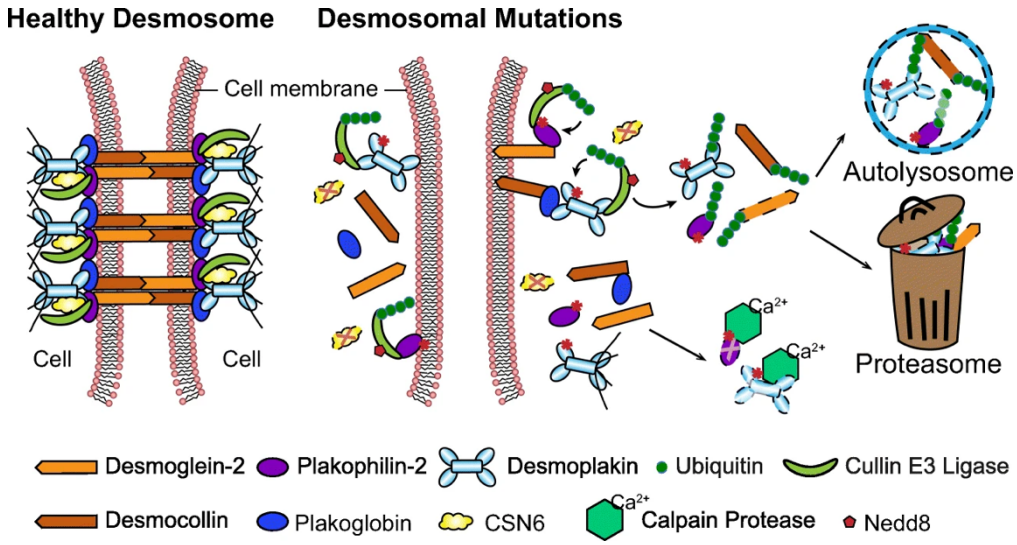

Figure 4 – Buildup of Desmosomes [16]

This figure shows how the desmosomes are built up when healthy and how malfunctioning desmosomes, e.g. caused by mutations would look like. Furthermore, it shows how desmosomal proteins like PKP2 work together to build the desmosomes and connect the cells. When the desmosomes lose their function and therefore cannot hold tissue together anymore, they get tagged with Ubiquitin and will therefore be recognized by the proteosomes and the proteins will be degraded.

Treatments for ACM

Treating ACM is generally aimed to prevent disease progression, reduce mortality and improve quality of life. Next to lifestyle changes, pharmacological therapy (e.g. beta-blockers, flecainide, propafenone, and sotalol), the placement of an implantable cardioverter defibrillator, a catheter-based ablation or a heart transplant are options.[17]

Treatment variations differ depending on the symptoms and history. However, since lots of times, the cause of the ACM is unknown, the treatment options are limited. The most common treatment is the use of Beta-Blockers, but also this is limited to the symptoms.[17]

As for now research on how to gain back the loss of function is ongoing. The use of isogenic induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs) shows that the function of the malfunctioning desmosomes could be restored.[18] However, nothing is known about the long-term effect and since ACM is influenced by multiple factors such as genetics, epigenetics and environmental factors, it is unknown if replacing the tissue will over time address all aspects of the disease. Another approach that is currently being researched also working with pluripotent stem cells is the use of the CRISPR/Cas9 system. Using the CRISPR/Cas9 system, researchers have utilised the ability to modify induced pluripotent stem cell (iPSC) lines. Specifically, the genes associated with PKP2 and DSG2 were intentionally knocked out using CRISPR/Cas9. This approach has created an isogenic human in vitro model system that faithfully reproduces the key features of arrhythmogenic cardiomyopathy (ACM). Thus, this provides a platform for studying ACM, providing insights into disease mechanisms and potential therapeutic strategies.[19]

Conclusion

ACM is a rare hereditary disease of the heart that is caused by mutations of the desmosome proteins, among other things. To date, over 200 mutations of the PKP2 gene alone are known. The malfunction of the desmosomal proteins such as PKP2 leads to the disconnection of the cells, protein degradation of the desmosomal proteins as well as fibro-fatty tissue replacement – ultimately leading to the heart muscle not being able to pump anymore.[14] The fact that so little is known about the development of this disease, despite ongoing research, means that treatment options are still limited. The most used treatment at present is the use of beta-blockers and implantable cardioverter defibrillators, but these are not suitable for every clinical picture of ARVD. The treatment options are therefore usually based on treating the symptoms instead of the actual cause of the disease and can vary from patient to patient. However, new studies have shown, that a certain mutation of the PKP2 gene degrades those desmosomal proteins. This protein degradation can be prevented with the help of medication, such as proteasome inhibitors and results in the maintenance and restoration of desmosome function. However, this needs to be further tested and verified, as protein degradation is fundamentally an important process in cells, and if it is completely suppressed, it can lead to other problems. Next to that the use of CRISPR/Cas9 and iPS-CMs to research and treat AMC are researched as well and might give further insights into treatments and tissue replacements.

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