Skin–Heart Connection
What Can the Epidermis Tell Us About the Myocardium in Arrhythmogenic Cardiomyopathy?

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The importance of desmosomes in the human heart is evidenced by arrhythmogenic cardiomyopathy (AC), an inherited disease in which mutations in desmosomal proteins can lead to fatal ventricular arrhythmias and sudden death. The disease is pathologically characterized by right, left-, or biventricular cardiomyopathy with progressive replacement of myocardium with fatty and fibrous tissue and mainly transmitted as an autosomal dominant inheritance pattern. A similar cardiac phenotype is found in recessive cardiocutaneous disorders called Naxos disease and Carvajal syndrome, where AC is combined with palmoplantar keratoderma and woolly hair. The common structures affected in those diseases are desmosomes, membrane-bound adhesive junctions that are abundantly expressed in the myocardium and stratified epithelia where they maintain tissue integrity under high mechanical forces. Desmosomes consist of Ca²⁺-dependent adhesion molecules of the cadherin family, which span the extracellular space to connect adjacent cells, and linker proteins of the plakin and catenin families, which form intracellular assemblies linking the desmosomal cadherins to intermediate filaments of the cytoskeleton.

Mechanistic insights of desmosomal function in health and disease have been gathered from experiments either in epithelial cell lines or other artificial expression systems and transgenic mice. Human primary keratinocyte cultures derived from patients via skin biopsies, an easy and less invasive approach, might be an alternative and more personalized approach for mechanistic studies with focus not only on skin defects but also on the heart because keratinocytes also express all isoforms of cardiac desmosomal proteins.

Because the heart is difficult to access, there are obviously major limitations for studying patient-specific cardiac expression patterns of desmosomal proteins in the tissue of interest. Tissue is usually only available postmortem, associated with a questionable quality caused by degradation or as endomyocardial biopsies obtained from the right ventricular septum with limited material and procedure-related risks for patients. To overcome the dilemma, Rasmussen et al did ask if changes in cardiac expression profiles of the mutant desmosomal proteins are mirrored in the epidermis given that cardiac isoforms are also abundantly expressed in epithelial desmosomes. In this issue of Circulation Cardiovascular Genetics, Rasmussen et al provided insights into a novel model using primary keratinocyte cultures of patients with AC carrying plakophilin-2 (PKP2) truncation mutations. For proof of concept, myocardial expression of desmosomal proteins obtained from cardiac biopsies was compared with expression profiles of the same proteins in cultured keratinocytes derived from patient’s skin biopsies. They convincingly showed in cardiac tissue and keratinocyte cultures that PKP2 mutations causing a premature stop codon lead to decreased levels of PKP2 expression, suggesting haploinsufficiency as the underlying genetic mechanism. More importantly, they also proved that keratinocytes mimic expression changes observed in the heart.

AC is characterized by genetic complexity. Mutations in 5 desmosomal genes (JUP, PKP2, DSC2, DSG2, and DSP) are known to cause AC accounting for 50% to 70% of the genetic causes of the disease. Complicating issues are that 6% to 15% of individuals carry multiple mutations (digenic and compound heterozygous) explaining to some extent more severe clinical phenotypes. Moreover comprehensive genetic approaches led to the identification of many variants of unknown significance, which are neither clearly pathogenic nor benign but might act potentially as genetic modifiers. Missense variants are more often classified as variants of unknown significance, whereas frameshift, splice site, or nonsense mutations are likely, but not exclusively, pathogenic. Rasmussen et al not only focused on expression profiles of carriers with premature stop codon mutations in PKP2 but also compared expression profiles of digenic cases and demonstrated potential influences of additional uncertain missense variants on expression in some of their probands. For instance, a comprehensive expression analysis of an explanted heart from a patient carrying a PKP2 and desmoglein-2 (DSG2) truncation mutation indicated haploinsufficiency of both proteins by different protein quantification methods. However, additional changes in the expression of other desmosomal proteins and the gap junction protein connexin 43 were observed differentially depending on the quantification method. Interestingly, previously reported reduced expression patterns of plakoglobin (JUP) and connexin 43 in patient tissue of AC detected by immunohistochemistry could not be observed in this patient, whereas Western blotting showed slightly diminished protein amounts. Overall those results underline that desmosomal cardiomyopathies are often associated with a complex mode of inheritance and a highly variable pattern of protein expression, limiting the value of protein expression changes.
observed in small tissue pieces of few patients by applying a single semiquantitative method with high technical variations.

The clear advantage of Rasmussen et al. is the complementary approach of different quantification methods, which has been used for expression profiling in cardiac biopsy samples and keratinocyte cultures. Proteins were quantified using 3 methods: Western blotting, nanoliquid chromatography tandem mass-spectrometry, and immunohistochemistry. Although sample sizes and tissue amounts specifically of cardiac biopsy samples were limited and in most of the experiments statistics could not be applied, the application of complementary quantification methods and sufficient numbers of wild-type controls make the results more plausible when compared with previously reported approaches. Of interest, it should be noted that there was an extensive variability of wild-type PKP2 protein expression even between the control samples. In perspective, because protein and RNA expression studies by Rasmussen et al. were mainly focused on PKP2 and DSG2 in keratinocyte cultures, an extension to a more comprehensive expression analysis would provide even more insights in to other cardiac and epidermal expressed desmosomal components and their regulation in disease. Despite the changes in cardiac expressed isoforms in cardiac tissue of PKP2 mutation carriers were also present in their recessive forms in Naxos disease and Carvajal syndrome, respectively. However, recently, mechanistic studies of PKP2 mutations have provided insights into the pathophysiology of AC by showing that PKP2 deficiency results in disturbed sodium channel function as a potential substrate for arrhythmias. In humans, the minority of described mutations in PKP2 are missense mutations, whereas 90% of the identified mutations lead to premature stop codons, suggesting haploinsufficiency as the predominant genetic mechanism. In accordance with previous observations demonstrating PKP2 protein instability in vitro, Rasmussen et al. demonstrated in cardiac tissue and keratinocyte cultures of several patients diminished RNA and protein expression of PKP2 highlighting haploinsufficiency as the common mechanism in those patients. More importantly, abnormalities observed in cardiac tissue of PKP2 mutation carriers were also present in their keratinocytes, suggesting that epidermis and myocardium mirroring each other. Those findings are also consistent with previous observations in carriers of DSP and DSG2 mutations shown by the same group.

The major insight provided by the study of Rasmussen et al. is that patient-specific keratinocyte cultures, suggesting a promising tool for detecting abnormalities in desmosomal expression patterns. In fact, can the skin may be able to help understanding the pathogenesis of AC in the heart? Although intercellular junctions are differentially organized in cardiac and epithelial tissues, the mirroring of expression patterns of patients with AC might provide further evidence that studies conducted in epithelial systems may help in understanding cardiac desmosomes in health and disease. Despite potential limitations at the technical and biological level, there is the opportunity of using individualized keratinocytes in complementation with other model systems, such as induced pluripotent stem cell–derived cardiomyocytes for understanding cardiac desmosome pathology and more importantly for implementing screening tools focusing on drug discovery in a simplified but patient-specific manner.

Despite those exciting perspectives for novel insights into the mechanisms of AC, there is still the clinical dilemma of diagnosing the disease. Studies on cardiac biopsy tissue demonstrated substantial variability in the diagnostic yield of specific expression patterns, such as JUP and connexin 43, detected by immunohistochemistry. However, skin biopsies that are less invasive and useful for primary keratinocyte cultures and fibroblast-derived induced pluripotent stem cells might provide a promising alternative in the future, not only for research but also for diagnostic purposes.

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None.

**References**


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