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# Arrhythmogenic Cardiomyopathy

## Pathophysiology Beyond Cardiac Myocytes

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In the current issue of the *Circulation Research*, Karmouch et al<sup>1</sup> present a provocative study attributing a pivotal role in the pathogenesis of arrhythmogenic cardiomyopathy (ACM) to the loss of DSP (desmoplakin) gene in a subpopulation of the cells of the conduction system. This hypothesis is in sharp departure from the current view on the disease, and it opens the question on whether the data reported in mice with a selective expression of the genetic defects in the specialized cells of the conduction system replicate the clinical phenotype found in patients.

ACM is a genetic disease of the heart muscle caused, in most instances, by mutations in genes encoding for desmosomal proteins that is predominantly inherited as an autosomal dominant trait.<sup>1</sup> Two syndromic recessive forms of the disease have been described and both present abnormalities in skin<sup>2</sup> and heart. Carvajal Syndrome<sup>3</sup> presents a striate type of keratoderma, is associated with a cardiomyopathy with preferred involvement of the left ventricle, and is associated with DSP mutations. Occasionally, Carvajal is inherited as a dominant disease that also presents hypodontia.<sup>4</sup> Naxos disease<sup>5</sup> is associated with mutations in the plakoglobin gene and manifests diffuse keratoderma and right ventricular cardiomyopathy. Occasionally, Naxos disease with dominant inheritance has been reported.

Karmouch et al<sup>1</sup> investigate the recessive form of ACM caused by mutations in the DSP gene that manifests with palmo-plantar keratosis, wooly hair, and dilatation of both ventricular chambers with fibro-fatty infiltration and life-threatening arrhythmias.<sup>3</sup> There is general consensus that cardiac manifestations of the disease are the consequence of DSP mutations that alter the function of cardiac myocytes (CMs). The assumption that mutations in cardiac genes affect CMs is not unique to ACM; rather, it is the paradigm in the study of most inherited cardiomyopathies and channelopathies, and, therefore, reading the study of Karmouch et al,<sup>1</sup> it is reasonable to wonder on which bases the authors explore the effect of mutations beyond CMs.

Data in support of the view that non-CMs are implicated in arrhythmogenesis come from studies originating in the electrophysiology field demonstrating the role of fibroblasts and endothelial cells<sup>2</sup> in the modulation of heart rhythm. These observations confirm that cells that have traditionally been considered innocent bystanders are electrically coupled with CMs and interfere with their physiological and pathological responses. The most explored interaction between non-CMs and myocytes concerns cardiac fibroblasts.<sup>6</sup> For a long time, these cells have been considered merely as a structural support for CMs and only

recently has it been recognized that they regulate cell-to-cell communication through the release of cytokines, growth factors, miRNAs,<sup>7</sup> and metabolites.<sup>8</sup> Germane to the implication of fibroblasts in cellular electrophysiology has been the discovery that they are electrically coupled to CMs and contribute to impulse propagation.<sup>9</sup>

More recently, a groundbreaking study by Hulsmans et al<sup>10</sup> demonstrated that macrophages that populate the heart express connexin 43 and are electrically connected to cells of the distal portion of the atrioventricular node. This study unexpectedly demonstrates that, when connected to nodal myocytes, macrophages depolarize in synchrony with beating nodal cells and, when coupled with resting cells, their electrotonic load shortens nodal action, potentially facilitating faster rate of conduction. Interestingly, this study also showed that the depletion of macrophages causes atrioventricular block, opening a fascinating question on whether macrophages are key players in determining atrioventricular blocks in conditions such as myocardial ischemia or inflammatory diseases such as sarcoid or myocarditis.

This short overview confirms the existence of a solid rationale to the hypothesis by Karmouch et al<sup>1</sup> that loss of DSP in non-CMs may contribute to the cardiocutaneous manifestations of the recessive form of ACM. The idea that the phenotype of the ACM variant caused by DSP mutations cannot be explained solely by the lack of this desmosomal protein in ventricular myocytes originates from the observation by Garcia-Gras et al<sup>11</sup> team that their DSP knockout mouse model engineered to selectively delete the DSP gene in CMs only partially replicates the human phenotype in genetically modified mice. As stated by Karmouch et al,<sup>1</sup> cardiac arrhythmias in the myocyte-specific DSP-deficient mice occurs in the context of cardiac dysfunction, but not independent of cardiac dysfunction. The association between overt structural abnormalities and arrhythmias is at variance with what occurs in humans, where arrhythmias may precede extensive fibrosis and ventricular dilatation. This difference is not trivial because arrhythmias that originate in a structurally intact heart are likely to be caused by different electrophysiological mechanisms and to respond to different treatments, when compared with arrhythmic episodes generated in the presence of fibrosis and contractile dysfunction.

In searching for an explanation for this phenotypic discrepancy, Karmouch et al<sup>1</sup> hypothesize that the loss of DSP in non-CMs might be needed for the generation of arrhythmias. Critical to the development of the hypothesis has been the evidence, obtained from public databases, that chondroitin sulfate proteoglycan<sup>4</sup> is expressed in a fraction of cells of the cardiac conduction system and in keratinocytes, that is, in cells located in tissues (heart, muscle, and skin) where the phenotype of the disease manifests. This evidence raised the bold speculation that chondroitin sulfate proteoglycan<sup>4</sup> may serve as a shared molecular link between early arrhythmias and skin phenotype.<sup>1</sup>

To test this hypothesis, the authors develop a mouse model with postnatal inducible deletion of DSP under the transcriptional regulation of the chondroitin sulfate proteoglycan<sup>4</sup> locus to cause a selective loss of DSP in cells of the cardiac conduction system that expresses chondroitin sulfate proteoglycan<sup>4</sup>. This model manifests cutaneous

abnormalities typical of the human disease, such as wooly hair and palmo-plantar keratosis combined with an arrhythmic phenotype, supporting the conclusion of the authors that the involvement of cells of the conduction system is a contributor to arrhythmogenesis in this model of ACM. These data may complement the study by Lombardi et al<sup>12</sup> that provided an interesting hypothesis for the origin of the presence of fatty tissue in ACM. The authors provided evidence that, in analogy with the skeletal muscle, the myocardium presents a specific group of cells called fibro-adipogenic progenitors that express platelet-derived growth factor receptor- $\alpha$ . In the heart, most of fibro-adipogenic progenitors follow the fibroblasts lineage; however, a minority of them take the adipogenic pathway and express the adipogenic transcription factor CCAAT/enhancer-binding protein  $\alpha$ . Of note, mice with conditional heterozygous deletion of DSP present an increase of myocardial fibroadipogenesis, suggesting that the mutation in the desmosomal protein DSP acts as activator of fibroadipogenesis in ACM. The global picture provided by the present study<sup>1</sup> and the data of Lombardi et al<sup>12</sup> would therefore attribute the origin of pivotal manifestations of ACM, that is, adipogenesis and arrhythmias, to the loss of DSP in a selected group of non-CM cardiac cells.

Before we can fully embrace this view, we need to decide whether we are ready to conclude that the pivotal role attributed to DSP deletion in selected cells of the cardiac conduction system may cause clinical arrhythmias in cardiocutaneous ACM patients based on the data presented. Although the hypothesis is fascinating and the data are suggestive, we are not quite ready to jump to the conclusion that the mice with DSP deletion in cardiac conduction tissue are the cause of the life-threatening arrhythmias that cause sudden death in patients.<sup>13</sup> Further investigations are needed.

From a clinical standpoint, it is known that tachyarrhythmias in ACM are predominantly represented by monomorphic ventricular tachycardias<sup>13</sup>; these arrhythmias are usually inducible with programmed electric stimulation, and the underpinning mechanism is reentry facilitated by ventricular fibrosis. However, when we look at the arrhythmias that develop in the mouse model by Karmouch et al,<sup>1</sup> we observe that the majority of the arrhythmic episodes are bradyarrhythmias such as atrioventricular blocks and sinus pauses. Furthermore, the episodes of ventricular tachycardias are not the rapid monomorphic ventricular tachycardias that are seen in patients. Specifically, the spontaneous monomorphic ventricular tachycardia shown in Figure 4B, bottom,<sup>1</sup> is slower than the sinus rhythm in Figure 4B, top.<sup>1</sup> Thus, it resembles an idioventricular rhythm rather than a ventricular tachycardia, that is, a slower ventricular rhythm that, at variance with the clinically observed ventricular tachycardia is not life-threatening. Similarly, when Karmouch et al<sup>1</sup> use pacing to induce arrhythmias, a rapid polymorphic ventricular tachycardia occurs that is also not typical of ACM patients who usually develop monomorphic ventricular tachycardias.<sup>9</sup> This latter point is not surprising because these mice, devoided from fibro-fatty infiltration in the ventricle, lack the anatomic substrate for the development of a rapid reentrant rhythm.

Nevertheless, it would be quite interesting to cross these mice with targeted and inducible loss of DSP in a portion of the cardiac conduction cells with a line of mice that presents partial loss of DSP in the CMs and present fibro-fatty infiltration in the absence of overt

heart failure.<sup>11</sup> It is likely that offspring presenting the combination of loss of DSP in cardiac conduction cells in the presence of fibrosis may develop reentrant ventricular tachycardia, thus recapitulating the clinical phenotype of the disease and supporting the hypothesis of the authors.

For now, Karmouch et al<sup>1</sup> are to be congratulated for this contribution to the field; their work is a tribute to the importance of animal models in dissecting the complexity of clinical phenotypes, and it demonstrates once more the value of interaction between clinical scientists and basic scientists to advance the mechanistic understanding of human diseases for the benefit of patients.

## **ARTICLE INFORMATION**

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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### **Disclosures**

None.

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