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Reduced sodium channels in human ARVC

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In recent years, the relatively uncommon disorder of arrhythmogenic right ventricular cardiomyopathy (ARVC) has received a lot of attention from basic and clinical investigators. Histological characterization of ARVC hearts is notable for patchy fibrofatty replacement of the myocardium, and the syndrome can result in both heart failure and ventricular arrhythmias. Slightly more than half of the patients with ARVC have mutations in desmosomal proteins¹ residing in the intercalated disc that connects adjacent cardiomyocytes. As a genetic disorder associated with improper assembly and dysfunction of critical intercalated disc proteins, ARVC is an important model that may also help us deduce how proteins of the intercalated disc arrive and are maintained in normal and diseased hearts. Where there is interest, however, there is also disappointment. ARVC genotype to phenotype correlations are not well understood, nor is the mechanism of ventricular arrhythmogenesis associated with ARVC. As a result, ARVC diagnostic and clinical criteria are based more on clinical gestalt than underlying pathogenesis.

The current study by Noorman et al² examined 5 post-mortem and 15 biopsy heart samples of patients with ARVC. Immunocytochemistry was performed, revealing a reduction of Nav1.5, connexin 43 (Cx43), and plakoglobin (PG) at the intercalated disc. While the finding of decreased Cx43 and PG is supportive of previous reports, the finding of reduced Nav1.5 is both novel and highly interesting. Together, the results add tantalizing data to our understanding of the mechanism underlying ARVC arrhythmogenesis, as well as another piece to the puzzle that is the cardiac intercalated disc.

ARVC is most commonly associated with mutations in plakophilin 2 (PKP2) and other desmosomal proteins,¹ while 30%–40% of the ARVC cases have no known mutations. At present, the scientific community has not identified a direct role for desmosomal protein mutations on cardiac electrophysiology. Furthermore, while a detailed analysis of ARVC hearts reveals subtle involvement of muscle tissue that appears healthy, ARVC hearts typically have a progressive patchwork replacement of muscle that characteristically involves the right ventricle more than the left ventricle.³ This patchy and inhomogeneous involvement of heart muscle, despite a uniform genotype, indicates a combination of numerous genetic background, cellular, physiologic, and environmental factors are involved in generating a phenotype that is perplexingly removed from the original genotype.

It is important to note that known ARVC genotypes apply to the cellular cytoskeleton that not only affects cellular survival but also regulates ion channel localization. In the current study,² PG and Cx43 are found to be incorrectly localized, consistent with previous reports highlighting Cx43 as an important component of ARVC arrhythmogenesis. Trafficking of Cx43 occurs via the cellular cytoskeleton and is both microtubule and actin dependent.⁴⁻⁶ Specificity of delivery to the intercalated disc region includes, at a minimum, the adherens junctions structure and microtubule plus-end tracking proteins EB1 and p150(glued).^{4,5} How can the repeated observation that Cx43 is mislocalized in ARVC hearts be reconciled with what is known of the cytoskeleton delivery apparatus. Adherens junctions are not affected by ARVC.^{2,7} It is not known if EB1 binding to microtubules is affected, as occurs in the general oxidative stress environment of the failing heart,⁵ and while actin is known to be involved,⁶ the contribution of actin to destination specificity is unclear. What is clear is that existing studies have just scratched the surface of cytoskeleton-based trafficking of ion channels. PG and other desmosomal proteins could be involved in trafficking or organizing protein complexes at the intercalated disc that stabilize Cx43 channels once in the plasma membrane. A better understanding of the cell biology of the cytoskeleton and cardiomyocyte intercalated disc is essential to translate ARVC genotype to phenotype.

With much unknown about how ARVC genotypes result in ventricular arrhythmogenesis and a decrease in Cx43 at intercalated discs providing, at best, a partial answer, the findings of Noorman et al² are especially intriguing. Their report that Nav1.5 is decreased along with Cx43 in human myocardium suggests that a powerful and dangerous arrhythmogenic combination may be at work in these patients. Modeling studies have established that propagation in ventricular muscle is much less tolerant of reduced sodium channel availability than that of gap junctions.⁸ The spatial inhomogeneities within ARVC hearts and thus inhomogeneous reductions in sodium channel and gap junctions at intercalated discs will result in both slowed conduction and unidirectional block. Secondary effects on transverse conduction may provide partial rescue of conduction.⁹ However, if indeed both gap junctions and sodium channels are reduced in patches of still viable ventricular myocardium that also includes regions of fibrofatty replacement, the debate should not be how ventricular arrhythmias originate but why they do not occur more often.

It should be mentioned that the study by Noorman et al² consisted exclusively of immunohistochemistry data without supportive electrophysiology or biochemistry. However, there are previous data from animal models and cell lines that support interaction between Cx43, PG, and Nav1.5. For instance, data exist that Cx43 may help organize Nav1.5 channels at the intercalated disc.¹⁰ In addition, while it is not well understood how sodium channels are trafficked, Sato et al¹¹ have identified that PKP2 associates with Nav1.5 and PKP2 knockdown reduces sodium current and action potential propagation velocity. Thus, it is possible that sodium channel reduction may be not just a symptom but a consequence of intercalated disc protein remodeling involving Cx43, PKP2, PG, and other scaffold proteins.

One of the important findings of the study by Noorman et al² is that biopsy of ARVC hearts and testing for Cx43, PG, and Nav1.5 may provide risk assessment. As argued above, there is little doubt that the combination of reduced gap junction coupling together with reduced

Nav1.5 will increase arrhythmia risk. It is therefore possible that the tissue-based evidence of reductions of these proteins at the intercalated disc could fit into a clinical decision algorithm for either device or pharmacologic intervention. However, there are multiple large hurdles to overcome before the results of the current study can be used as precedent to perform risk assessment-related heart biopsies of clinical patients. Validation over several hundred patients is necessary and only after a careful standardization of both tissue processing and imaging. Furthermore, it is not clear that results are specific to patients with ARVC. For instance, if Cx43 reduction directly contributes to reduced Nav1.5 at the intercalated disc, then any condition that reduces Cx43 will result in reduced Nav1.5. Furthermore, the patchy nature of ARVC penetration in hearts may necessitate multiple biopsies or biopsies from areas other than right ventricular septum regions, which would prohibitively increase procedural risk. These reservations should not be interpreted as the discouragement of the use of human heart sampling to further both our understanding of mechanism and improve clinical care. Just as some medical centers routinely perform diagnostic biopsy on hearts with idiopathic dilated cardiomyopathy, studies such as those by Noorman et al² begin to strengthen the argument to biopsy idiopathic arrhythmia syndromes as well.

It is also worth examining the plasma of these patients. A recent study found that BIN1, a protein that lines T tubules and is important for calcium channel trafficking,¹² is reduced in the plasma of patients with ARVC who have heart failure and predicts ventricular arrhythmia.¹³ Despite this study's focus on patients with ARVC, it is doubtful that plasma BIN1 levels provide specificity to ARVC hearts; rather, they reflect the biochemistry and arrhythmia sequelae of generally diseased cardiomyocytes. Blood vs heart muscle sampling is safer but also requires establishing specificity of the blood protein to the heart vs other organs. The examination of plasma-based exosomes for ARVC-related cardiac proteins may be a future area of interest, connecting ARVC pathogenesis to prognostic tests.

Despite the ability to positively identify genotypes in a majority of patients with ARVC, the association of genotype to phenotype remains mechanistically elusive. The study by Noorman et al² brings us one step closer to understanding how cytoskeletal deficiencies result in a lethal arrhythmic and heart failure syndrome by identifying the powerful arrhythmogenic combination of reduced Nav1.5 together with reduced Cx43. The results also highlight the importance of experiments with human patient samples, with the results providing not just mechanistic insight but the data that will ultimately lead to improved diagnosis, prognosis, and therapy.

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