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Deciphering the pecking order of HCN4 expression in the developing heart: lessons from chicken

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In the last decade, many studies focusing on genetic determinants of cardiac conduction system (CCS) formation have been conducted. Findings from these studies indicate that regulation of myocardial differentiation into either a conductional or working myocardial phenotype is a multifactorial process during which several factors from different gene families contribute to the formation of the different elements of this complex system.^{1–5} A number of molecular markers have been identified and used to delineate the elements of the developing CCS.^{2,3} Among these, of particular interest is the hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channel family. HCN channels carry an inward, depolarizing current known as pacemaker current or “funny” current (I_f) that underlies cardiac pacemaker activity. Four genes encoding HCN channels have been identified, HCN1, HCN2, HCN3 and HCN4.⁶ Among the four HCN isoforms, HCN4 is the most abundant isoform in adult SA node in multiple species including human⁷ and it contributes to ~70–80% of total HCN mRNA as well as I_f current in man.⁷ Consistent with this, loss-of-function mutations in HCN4 have been associated with inherited sinus bradycardia in man,⁸ pointing to the functional importance of HCN4 in maintaining normal sinus rhythm. While conventional HCN4 global deletion in mice leads to embryonic lethality,⁹ studies in HCN4-deficient embryos showed significantly reduced contraction rates and immature action potential in HCN4 deficient embryos comparing to wildtype.¹⁰ These findings indicate HCN4 is critical for normal cardiac development and is essential for the generation of mature pacemaker potential in the emerging SA node during embryonic stage.

In the elegant study reported in this issue of *Heart Rhythm*, Vicente-Steijn, et al.,¹¹ used sophisticated techniques to study the expression of HCN4 into the developing chicken heart. The goal of their study was to identify the key regions required to maintain the CCS as determined by HCN4 localization. Using *in situ* hybridization, the authors assessed cardiac HCN4 mRNA expression and localization during sequential developmental stages in the chick embryo. In general, their findings of HCN4 expression pattern throughout development in the chick embryo is consistent with a previous study in mice with a few distinctions.⁴ One distinct finding from the current study is that HCN4 was expressed in the early heart tube at a very early stage in development. A few other genes that are linked to myocardial and CCS development have also been described in the early heart tube.^{12,13} The primary heart tube presents a nodal-like functional phenotype in that it shows automaticity, slow conduction of the electrical signal and a peristaltic-like contraction pattern.^{1,14} Considering the functional relevance of the ion channel HCN4, the wide expression of HCN4 in primary heart tube suggests that the entire myocardium has the capacity to generate the electrical impulse necessary for the peristaltic contractions at this stage.^{11,14}

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Another important finding from the current study is the expression of HCN4 in regions other than the SA node in the chick.^{2,11} It was demonstrated by the authors that HCN4 mRNA was expressed in some of the second heart field-derived myocardium such as the internodal myocardium encompassing the right venous valve (the future terminal crest), the left venous valve, as well as the myocardium that will develop around the pulmonary veins (PV). This is of special interest because in recent years, clinical and preclinical studies have demonstrated that atrial fibrillation (AF) and ectopic atrial tachycardia may originate from specific anatomic sites, such as the PV,¹⁵ the ostia of the coronary sinus and of the caval veins,^{16,17} and the terminal crest.¹⁸ Transient staining of the monoclonal antibody HNK1, originally used as a marker of neural crest cells during embryologic development, is observed in the myocardium surrounding the PV and in the coronary sinus.⁵ P cells, transitional cells and Purkinje cells have also been identified in human PVs.¹⁹ Interestingly, these cells were mainly found in the PVs of subjects with AF. More recent reports also support abnormal automaticity or enhanced pacemaker activity in the PVs, with or without infusion of medication or pacing manoeuvres.²⁰ These findings have raised the question why these structures, which do not belong to the mature CCS, are able to generate or sustain arrhythmias. Extensive studies have been performed to look for answers from the embryonic development of the CCS. Findings suggest an embryonic background of AF originating from the PV and caval veins.^{5,11,21} Taking all together, the HCN4 expression patterns during development may indeed, delineate potential arrhythmogenic sites in the adult heart, as postulated by Vicente-Steijn and his colleagues.¹¹

The chicken embryo is extensively used as an experimental model for studying cardiovascular development and function, including angiogenesis, heart formation, vascular tone, and hemodynamic and neurohumoral control.²² Despite the fact that the earliest stage of pacemaker activity was recorded in the unbeating heart in a chick embryo over 40 years ago,²³ the expression of HCN4 throughout chick heart development was previously undetermined. The current study systematically evaluated expression of HCN4 throughout development of the CCS in chick embryos, and has identified HCN4 as a marker of the developing CCS in the chick. These findings have significantly improved our understanding regarding the distribution of HCN4 and how its expression relates to pacemaking activity in this model.

As stated by the authors, although HCN4 appears to be the predominant isoform expressed in the developing CCS, HCN1 is also present during development. Furthermore, HCN2 has also been implicated in pacemaking currents in other model systems.²⁴ It would be of great interest to determine the distribution of other HCN family members and the role of different HCN isotype throughout the developmental stages during heart development in this model system. At least one of such studies has been done in mice.³ Other limitations, as correctly pointed out by the authors, include that the data refers only to mRNA expression patterns and therefore no conclusions on protein levels or functional repercussions can be made. A logical extension of this study would be to determine whether HCN4 mRNA expression will translate to changes in I_f and pacemaking activity.

In spite of the limitations, the sophisticated data generated by the authors regarding HCN4 expression in different stages of chick development are important and have substantial implications in our understanding of cardiogenesis, in particular CCS formation. Additionally, the study has generated important data for supporting further investigation of the potential association between arrhythmogenic sites with specific anatomic embryologic sites.

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