Is Pitx2 Growing Up?

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In this issue of Circulation: Cardiovascular Genetics, Kirchhof et al present an interesting study on the expression of the developmental transcription factor Pitx2c in adult heart and its potential role in atrial fibrillation (AF). AF is the most common sustained cardiac arrhythmia, with a prevalence that increases with age and affects 1% to 2% of the general population. Despite recent advances in AF research, underlying pathophysiology is not fully understood. In general, initiation and perpetuation of AF needs a trigger for its onset and a vulnerable substrate for its maintenance. Focal ectopic activity can act as a trigger to initiate AF on the basis of a single- or multiple-circuit reentry. Especially, cardiomyocyte sleeves around the pulmonary veins have been shown as an origin of focal activity. Underlying mechanisms are early and delayed after depolarizations as a result of disturbances in depolarizing inward (Na\(^+\) or Ca\(^2+\)) or repolarizing outward (K\(^+\)) currents.

Once initiated, AF itself induces structural and electric remodeling, which stabilizes reentry and AF maintenance. Structural remodeling due to signaling through angiotensin, profibrotic cytokines like transforming growth factor-\(\beta\), or microRNAs leads to upregulation of extracellular matrix proteins (eg, collagen) and downregulation of matrix-degrading enzymes (eg, matrix metalloproteinase), resulting in progressive atrial fibrosis. Electric remodeling is caused by expression changes in ion channel subunits. AF induces calcium overload and increases activity of protein phosphatases, leading to downregulation of depolarizing L-type calcium channels and upregulation of repolarizing potassium channels. Measurable substrate of these changes in atrial electric properties is the shortening of action potential duration (APD), leading to shortening of refractory period and enabling reentry mechanisms. In most cases, therefore, AF is self-terminating (paroxysmal) in the beginning, developing to more sustained forms (persistent and permanent) over time based on progressive remodeling processes.

Several risk factors for initiation and maintenance of AF have been described, including age, sex, hypertension, ischemic heart disease, congestive heart disease, valvular disease, and hyperthyroidism. Recently, genetic factors associated with AF raised

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attention. On the one hand, there are rare genetic variants that exert strong effects and show a clear phenotype. Gain- or loss-of-function mutations in ion channel genes favor AF by changing depolarizing and repolarizing ion currents, resulting in shortening of APD, conduction slowing, promotion of after depolarizations, and ectopic firing.

On the other hand, common genetic variants (single-nucleotide polymorphisms [SNPs]) act with a weaker effect and a less-clear phenotype. Genome-wide association studies identified 3 genetic loci on chromosomes 4q25, 16q22, and 1q21 associated with AF. The most significantly associated SNP at chromosome 1q21 maps to intron 1 of the KCNV3 gene, which encodes a calcium-activated potassium channel potentially involved in AF pathophysiology, by shortening of APD in pulmonary veins. The SNPs at chromosome 16q22 map to intron 1 of a gene encoding the zinc finger homeobox protein 3 (ZFHX3), which has not been shown to be involved in cardiac arrhythmias until now.

The SNPs most significantly associated with AF are located at chromosome 4q25 and map to a large intergenic region without any known genes (“genomic desert”). However, recent fine-mapping results demonstrated localization of regulatory elements at this locus. The closest gene in the region is PITX2, a member of the pituitary homeobox (Pitx) family of transcription factors that play an important role in morphogenesis in early development. Pitx2 is expressed in vertebrates, birds, zebrafish, and chordates, indicating its broad conservation among species. Until now, 3 isoforms have been described: Pitx2a, 2b, and 2c, with Pitx2c being the predominant isoform expressed in the heart. Pitx2 is a key mediator of developmental signaling cascades involving factors like Lefty, Nodal, Shox2, Nkx2.5, and Tbx3.

Apart from Axenfeld-Rieger syndrome, which includes ocular and facial malformations, mutations in Pitx2 have been described in cardiac malformations like double-outlet right ventricle or arterial transposition. Pitx2 is crucial for left-right asymmetry, differentially regulating left atrial identity and ventricular asymmetrical remodeling programs. This functional role is supported by reports showing that Pitx2 knockout mice that die before birth present with right atrial isomerism and atrial and ventricular septal defects. Additionally, Pitx2 plays essential roles in regulating the development of the cardiac conduction system and the pulmonary myocardium.

All cardiomyocytes of the developing heart initially possess pacemaker properties, but only a small proportion of cells differentiate into pacemaker cells that form the cardiac conduction system, including the sinus node. Pitx2 represses Shox2, a transcription factor expressed in sinus node precursors that result in downregulation of a nodal gene program (ie, HCN4, Cav3.1) and upregulation of Nkx2.5 that induces a gene program characteristic for a working myocardium phenotype (ie, Cx40, Cx43, Nppa, Kir2.1). Because upstream Lefty1 restricts Pitx2
expression to left atrium Shox2 expression, consequently, development of the sinus node is only repressed in the left atrium. In the right atrium, missing Pitx2 results in Shox2 upregulation that blocks Nkx2.5 but induces Tbx3, which is necessary for sinus node development (Figure).16,18 Interestingly, in 2007, Mommersteeg et al17 demonstrated (without any knowledge of the genome-wide association study data mentioned previously) that Pitx2 is required for the formation and identity of the pulmonary myocardium. Pulmonary veins contain myocardial sleeves that have been confirmed as a source of electric activity triggering AF. They could show that pulmonary myocardium does not originate from atrial myocytes but from pulmonary mesenchymal cells differentiating to cardiomyocytes at the atrial-vein border zone and forming myocardial sleeves by rapid proliferation. Pitx2-deficient mice develop normal pulmonary veins but without any pulmonary myocardium because Pitx2 is required for the differentiation of the primary cell population.

Wang et al18 identified a potential role of Pitx2 in atrial arrhythmia. They investigated Pitx2null+/− mice that are heterozygous for a Pitx2 null allele that removes all isoform function and could show that these mice—unless no overt electrophysiological properties except for QRS prolongation at baseline have been depicted—are more susceptible to pacing-induced atrial arrhythmias. They could show that Pitx2 is expressed predominantly in the left atrium and pulmonary veins of newborn mice, with decreasing expression over time and genes involved in sinus node formation like Shox2 and Tbx3 being upregulated in Pitx2null+/− mice. They hypothesized that Pitx2 is an inhibitor of a pacemaker gene program in the left atrium that triggers ectopic activity as the underlying arrhythmogenic mechanism.

So far, studies on Pitx2 associated with AF have focused on its role in triggering AF onset by inducing arrhythmogenic foci during development. However, nothing is known about Pitx2 in adults or its potential role in maintaining AF. Kirchhof et al1 address this question.

Kirchhof et al1 show that Pitx2c is expressed in adult humans and mice predominantly in the left atrium, but it was detected in the right atrium and ventricles as well. Interestingly, in their study, Pitx2c is downregulated in left and right atrial tissue of patients with AF. To further examine the role of Pitx2c in AF pathophysiology, they performed a study on heterozygous Pitx2c+/− mice. These mice show a downregulation of Pitx2c by 37% without any compensatory upregulation of other Pitx2 isoforms, making it a suitable animal model for Pitx2c research. Using echocardiography on Pitx2c+/− mice, the investigators could demonstrate that cardiac dimensions and ejection fraction, as parameters for cardiac morphology and function, are not altered, with the exception of a slight, but significant increase in pulmonary flow velocity. Narrowing of the pulmonary valve is suspected to be the morphological substrate because the right ventricular outflow tract showed regular dimensions. Besides, they could not reveal any ultrastructural changes like interstitial fibrosis, differences in cell size, or sinus node morphology in Pitx2c+/− mice. In addition, electrophysiological studies on these mice revealed no differences in resting heart rate, atrial conduction velocity, or atrial activation patterns compared with wild-type mice. Spontaneous arrhythmias occurred at an equal level in both genotypes. At short-paced cycle lengths, however, Pitx2c+/− mice showed a significant APD shortening. Finally, the investigators show that Pitx2c+/− hearts were more susceptible to AF during programmed stimulation with
A single premature beat. Perfusion with β-adrenergic orci-
preline abolished this effect and normalized APD. Using
microarray analysis, they revealed differentially expressed
genes in Pitx2c−/− mice, including genes involved in calcium
handling, cell adhesion, gap junction biology, and ion chan-
nels. Of note, the investigators report left atrial Tbx3 and
Shox2 being upregulated in Pitx2c−/− mice.

In summary, Kirchhof et al17 present a new potential role of
Pitx2c in the adult heart by shortening of the APD as a
pathophysiological hallmark of sustained AF. However, there
are still some questions on Pitx2c. Apart from direct targeting of
transcription factors like Shox2 (Figure), it remains unclear
whether Pitx2c is a causal factor of reported gene expression
changes (e.g., potassium channels) potentially being involved in
APD shortening. Development of pulmonary myocardial
sleeves, which have been described as an origin of ectopic activity
inducing AF, has been reported to depend on Pitx2c signaling,17 but nothing is known about the role of Pitx2c in
adult pulmonary myocardium. Besides, Mueller-Hoecker et al19
showed that there are remarkable anatomic differences between
pulmonary myocardium in mice and humans, indicating possible
differences in Pitx2c expression and function in mice and
humans.

In the end, Pitx2 grew out of a purely developing factor
into an interesting, but still challenging and mysterious player
humans.

Disclosures

None.

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