Almost a decade ago, the deCODE (geCODE genetics Inc, Reykjavik, Iceland) consortium published the first genome-wide analysis of common gene variants associated with atrial fibrillation (AF). They found a gene variant on chromosome 4q25, ≈150 kB upstream of the PITX2 gene, to be the most important risk variant predisposing to AF.1 This initial observation has since been replicated and refined: We know of at least 6 independent single nucleotide variants, located at a 25 to 200 kB distance from the PITX2 gene, which modify the risk for AF.2 Furthermore, the risk allele of the initial variant is associated with recurrent AF in several clinical settings.

See Article by Gore-Panter et al

The initial observation has added a new dimension to our understanding of the mechanisms causing AF. Several research groups took this initial finding and studied the function of PITX2, a transcription factor with a known function in the development of heart and lungs, in the adult heart and specifically in the left atrium. Several important observations have been reported: PITX2c is expressed in the adult human and murine left atrium,3 whereas it is virtually absent in other parts of the adult heart. Genetic modifications that reduce left atrial PITX2 mRNA levels predispose mouse hearts to inducible AF.3–5 Reducing PITX2 levels alters the expression of several relevant left atrial genes,6 and the effects of PITX2 on gene regulation are partially mediated by the miR-17 to miR-92 complex.7 Preliminary analyses suggest that PITX2 regulates ion channel genes in the left atrium,5,8 underpinning the observation that reduced atrial PITX2 levels shorten the atrial action potential and cause membrane potential depolarization.3,9 On the basis of these insights and the impact of gene variants on chromosome 4q25 for AF, the genetic predisposition to AF has been identified as a promising candidate to develop personalized approaches to AF management.10

In this issue of Circulation Arrhythmia and Electrophysiology, Gore-Panter et al11 describe a new regulator of PITX2c expression in the left atrium: They identified a long noncoding RNA adjacent to the PITX2 gene (Pitx2 adjacent noncoding RNA [PANCR]). PANCR positively correlates with PITX2c mRNA expression in human patient left atrial appendage samples and knock down of PANCR in H9-derived cardiomyocytes depletes cells of PITX2c. Furthermore, the alteration in gene expression induced by a reduction in PANCR was remarkably consistent with that caused by knock down in PITX2c. The authors used one of the largest collections of human left atrial appendages available for research purposes, harvesting the fruits of an excellent local effort to collect such tissue and to combine them with adequate and correct clinical information.

The authors discuss their puzzlement at the lack of correlation between single nucleotide polymorphism status and PITX2 or PANCR mRNA levels in their data set. Nine years after the initial description of these gene variants, we do not have clear data demonstrating an association between left atrial PITX2 levels and single nucleotide polymorphism status.12,13 But is this really surprising? All tissue samples available to Gore-Panter et al11—similar to what is available to other research groups—were obtained from patients requiring open-heart surgery for severe heart disease (usually coronary artery bypass grafting and valve surgery). It is entirely unknown how atrial PITX2 (and PANCR) expression is altered by pressure overload, diabetes mellitus, hypoxia/ischemia, inflammation, autonomic dysfunction, or by the stress of open-heart surgery. Most carriers of the AF gene variants are middle-aged or old before they develop AF, suggesting that the genetic predisposition precipitates AF in atria exposed to additional second hit stressors such as oxidative free radicals, metabolic dysfunction, fibro-fatty infiltration, autonomic dysregulation, pressure overload, and hypertrophic signaling.14 Thus, modulation of atrial PITX2 (and PANCR) expression by such stressors or other unidentified factors is likely and needs to be characterized. Equally important, the human left atrial tissue available is almost invariably taken from the left atrial appendage, a portion of the left atrium with a distinct embryological origin. Other areas of the left atrium, for example the posterior left atrium containing the insertion points of the pulmonary veins, have a different anatomical structure and embryological origin, suggesting a different gene expression pattern and ultimately a different function. Interestingly, it is the posterior left atrial wall that is enriched for PITX2 expression in humans.15 Furthermore, the genetic predisposition to AF may lead to another type of AF (called polygenic AF by...
some) than AF found in patients undergoing open-heart surgery (AF associated with severe cardiac disease). The report by Gore-Panter et al adds PACNR as another major modifier of atrial PITX2 expression to the list of potential confounders in such association analyses. As known from clinical medicine, associations can generate hypotheses, but experiments involving gene-modifying interventions are needed to test the functional relevance of a gene (including transcription factors and noncoding RNAs).

Gore-Panter et al show that PACNR, a long, noncoding RNA, is expressed in the human left atrium and that it is a potent regulator of PITX2 expression. This is important information that can help to guide the quest to understand the mechanisms mediating the genetic predisposition to AF. Their report also illustrates the clear need for well-planned and executed experiments assessing the functional effects of genetic dysregulation in interaction with atrial stressors leading to AF.

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Pitx2 Adjacent Noncoding RNA: A New, Long, Noncoding Kid on the 4q25 Block
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