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EDITORIAL

PITX2: a master regulator of cardiac channelopathy in atrial fibrillation?

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This editorial refers to 'Pitx2c increases in atrial myocytes from chronic atrial fibrillation patients enhancing I_{Ks} and decreasing $I_{Ca,L}$' by M. Pérez-Hernández *et al.*, pp. 431–441.

This editorial refers to 'Pitx2 impairs calcium handling in a dose-dependent manner by modulating Wnt signalling' by E. Lozano-Velasco *et al.*, pp. 55–66.

The pathogenesis of atrial fibrillation (AF), the most common sustained cardiac arrhythmia, involves multiple factors including dysfunction of a variety of ion channels, dysregulation of calcium handling proteins, developmental defects, etc.[1] Emerging evidence has shown that genetic factors can increase the risk of AF. For example, genome-wide association studies (GWASs) have identified a number of single-nucleotide polymorphisms (SNPs) that are associated with AF.[2–5] Among these AF-associated SNPs, two (rs2200733 and rs10033464) located on chromosome 4q25 are most significant and have been repeatedly found in AF patients of various ethnicities.[2–4]

It has been postulated that these 4q25 variants can increase AF susceptibility by modulating the activity of paired-like homeodomain transcription factor 2 (PITX2), since they are located in the vicinity (~150 000 bp) of the *cis*-regulatory region of PITX2.[2–4] Consistently, independent groups have demonstrated that the deficiency of PITX2c (the cardiac-specific isoform) predisposes to AF development in mice.[6,7] However, it remains controversial whether and how human 4q25 variants regulate PITX2 transcription and whether the level of PITX2 is affected in AF patients.

Previous experimental work has revealed that PITX2 itself plays a crucial role in left atrium (LA)–right atrium (RA) patterning during cardiac development, and that the lack of PITX2c can alter LA–RA asymmetry leading to malformation of the pulmonary veins.[6] The latter is a well-known site for ectopic activity promoting spontaneous AF induction.[8] More recently, utilizing next generation sequencing and microarray techniques, PITX2c was shown to potentially regulate a variety of ion transporters and gap junction proteins that are crucial for pacemaking and cardiac conduction.[7,9] Lozano-Velasco *et al.*[10] and Pérez-Hernández *et al.*[11] have separately investigated the impact of PITX2c on ion channels and calcium handling proteins.

First, consistent with the majority of publications to date, Lozano-Velasco *et al.* demonstrated that down-regulation of PITX2 is arrhythmogenic (*Figure 1*, left panel). They utilized two distinct *Pitx2* loss-of-function models, a conditional mouse line (Sox2-Cre-Pitx2) and a previously established atrial-specific knockout line (Nppa-Cre-Pitx2), to demonstrate potential transcriptional changes in key calcium handling proteins. This study revealed alterations in mRNA levels of calcium handling proteins such as SERCA2a, calsequestrin-2, and phospholamban; a reduction in $I_{Ca,L}$ current density; and an increase in sarcoplasmic reticulum calcium content in Nppa-Cre-Pitx2 mice, but not in Sox2-Cre-Pitx2 mice. These differences explain the different phenotype in basal electrophysiology, as the former mouse line developed atrial arrhythmias spontaneously whereas the latter mouse strain exhibits normal sinus rhythm. Another novel finding from this study is that Wnt signalling may play a pivotal role in directing such differences, since (i) PITX2c is a negative regulator of Wnt11 via Wnt8a and (ii) overexpression of Wnt11 increases gene transcription of SERCA2a, calsequestrin-2, and phospholamban, similar to the effect of loss-of-function *Pitx2c*.

On the other hand, the study by Pérez-Hernández *et al.*[11] has raised an alternative hypothesis that up-regulation of *PITX2* is also associated with AF arrhythmogenesis (*Figure 1*, right panel). In this study, the investigators used freshly isolated human atrial cardiomyocytes from patients with long-standing persistent (chronic) AF (cAF) and HL-1 cells (murine atrial myocyte-derived cultured cells). In contrast to prior studies, they found an increased level of *PITX2c* mRNA in atrial cardiomyocytes of cAF patients. The expression levels of *PITX2c* correlated positively with I_{Ks} current density and negatively with $I_{Ca,L}$ current density, respectively. The inward $I_{Ca,L}$ and outward I_{Ks} are major currents that modulate phase 2 of the action potential of cardiomyocytes, and APD shortening is a hallmark of atrial electrical remodelling facilitating the induction and maintenance of AF. However, the putative correlation between PITX2c expression and APD was not determined in this study. Despite this limitation, this study provided the first evidence of *PITX2c* expression in atrial cardiomyocytes from cAF patients. Moreover, Pérez-Hernández *et al.* demonstrated that PITX2c can directly

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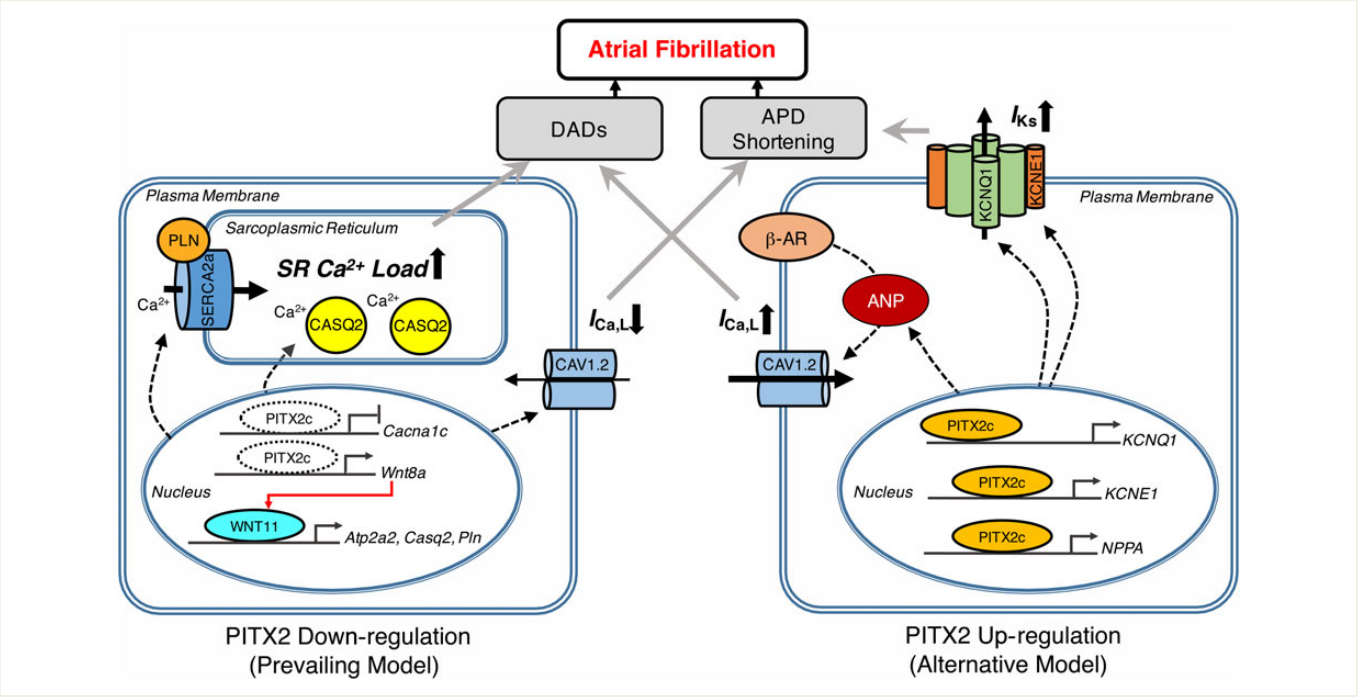

Figure 1 Schematic representation of arrhythmic mechanisms associated with PITX2. In the prevailing model (left, Lozano-Velasco study), down-regulation of *PITX2c* (i) reduces the gene expression of α-subunit of L-type Ca^{2+} channel ($I_{Ca,L}$), a hallmark of electrical remodeling in AF (ii) increases the expression of Ca^{2+} handling proteins via Wnt signaling, which ultimately leads to Ca^{2+} overload in sarcoplasmic reticulum (SR). In an alternative model (right, Pérez-Hernández study), up-regulation of *PITX2c*, observed in chronic AF patients, can enhance the transcription of genes encoding the subunits of slow delayed rectifying K^+ channel (I_{Ks}) and atrial natriuretic peptide (ANP). The latter is responsible for the augmented $I_{Ca,L}$ in response to β-adrenergic stimulation. These cellular events will ultimately (i) promote the development of delayed afterdepolarizations (DADs), or (ii) abbreviate the action potential duration (APD), both of which are the well-established mechanisms contributing to pathogenesis of atrial fibrillation.

modulate the transcription of *KCNQ1* and *KCNE1* (genes encoding I_{Ks} channel) and that overexpression of PITX2c increases the I_{Ks} current in HL-1 cells. Although PITX2c does not exert direct transcriptional modulation on $I_{Ca,L}$ subunits, Pérez-Hernández *et al.* showed that PITX2c can affect the function of $I_{Ca,L}$ indirectly via an atrial natriuretic peptide-mediated transcriptional activity. The essential findings from these two studies are highlighted in *Figure 1*.

Data comparisons between humans and mice have been inconsistent at times. For example, the level of PITX2c in AF patients is either unaltered,[12] increased in Pérez-Hernández *et al.*'s study,[11] or decreased in a previous study by Dr. Franco's group.[13] However, all data from animal models have shown consistently that deficiency of PITX2c increases the susceptibility to AF. A possible explanation is that human atrial cardiomyocytes were obtained from right atrial appendages of AF patients, which may not reflect properly the entire atrial landscape since PITX2c expression is higher in the LA. In addition, the history (duration and recurrence rate) of AF and clinical variability (age, comorbidities, drug therapy, etc.) among the patients may also complicate the results, although the higher PITX2 levels in cAF might be a compensatory mechanism counteracting the proarrhythmic effects of downregulated PITX2 at early stages. Finally, it remains to be established—for example in transgenic mouse models—whether increased PITX2c expression as observed by Pérez-Hernández *et al.*[11] is causally linked to AF pathogenesis.

Despite the differences between these two studies, both groups have linked arrhythmia mechanisms attributed to PITX2 with other established molecular determinants of AF. With the findings from both cAF patients and conditional knockout mice, the role of PITX2 in AF arrhythmogenesis has been expanded to the adulthood stage.[9–11,14] Finally, although we have witnessed a rapid discovery of genetic variants that could increase the risk of AF, it remains unclear whether and how these variants modulate nearby genes (directly vs. indirectly).[12] Perhaps, with the assistance of cutting-edge technologies, such as Crispr gene-editing, iPS cells, and AAV-mediate gene transfer, future studies can focus on rapid validation of the causative variants associated with AF development. Even if these common variants revealed by GWASs are truly not causative genetic determinants, they may potentially act as genetic modifiers of other rare mutations or variants to alter the atrial electrophysiology.[15] Last but not least, it deserves investigation to determine whether any of these common variants could serve as the genetic biomarker of AF.

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