In this issue of Circulation Research, Tessari et al\(^1\) investigate the role of an interesting gene, Pitx2, which acts in breaking symmetry in early development and has subsequent roles in differentiation of the inflow and outflow segment of the heart. Already almost 10 years ago, the transcription factor Pitx2 was demonstrated by several groups to be expressed asymmetrical in the early embryo and present in the left-sided plate mesoderm only.\(^2\)\(^-\)\(^4\) It is a target gene of Nodal and is kept from leaking to the right side of the midline barrier by Lefty1. The Nodal–Lefty–Pitx2 module is conserved in vertebrates, although the earliest phases in breaking symmetry show similarities, as well as differences, among zebrafish, birds, and mammals.\(^5\) The Pitx2 gene is also described in chordates and echinoderms, proving its evolutionary ancestry. The Pitx2c variant seems to be cardiogenic and triggers a cascade involving Nkx, Gata, and Hand. These genes are important for the early phase of cardiac development, starting with the bilateral cardiogenic fields and their fusion to form the cardiac tube. The next stage, rightward cardiac looping, is reported to be either dependent\(^6\) or independent of Pitx2.\(^7\) Recently, the involvement of the second heart field, contributing to both the arterial pole (anterior and secondary heart field) and the venous pole (posterior heart field), has gained importance. Therefore, many other genes governed or cofactored by Pitx2 can be added to the list of cardiac determinants, or at least as determinants of specific parts of the heart. These include the second heart field gene Isl1;\(^8\) the anterior heart field gene Tbx1;\(^9\) Pdga and Vegfr2;\(^10\) Tbx2, Tbx3, sonic hedgehog, Wnt11 and o-catenin;\(^11\) and others. It is small wonder that Pitx2 mutants present syndrome-like malformations involving other organs as well. Consistent with this, humans with Pitx2 mutations have Rieger syndrome, a disorder that involves multiple organ systems.\(^8\)\(^,\)\(^11\)

Tessari et al\(^1\) demonstrate that cardiac asymmetry results in chamber specification and that myocardial Pitx2 expression regulates left atrial identity and, as a consequence, ventricular asymmetrical remodeling programs. Aspects of differentiation and proliferation have been addressed but did not yet resolve the exact mechanisms in myocardial development and differentiation. Pitx2\(^-\) mice die before birth. These embryos do not show randomization of the atria but right atrial isomerism pointing toward abolishment of the left program. Franco and Campione\(^12\) describe that Pitx2 expression delineates the remodeling of the downstream parts of the heart, including the left atrioventricular canal, the inner curvature, the ventral face of the interventricular communication, and the adjacent parts of the left and right ventricular wall. This has now been substantiated by elegant studies using myocardium-specific Pitx2 knockouts.\(^1\) Animals carried on until adulthood and their cardiac anomalies could be studied providing the bridge for clinical interesting phenotypes using, eg, echocardiography. Clinical relevance may be extrapolated to arrhythmias that are present in many patients, because sequence variations close to Pitx2 have been described in patients with atrial fibrillation and atrial flutter.\(^13\)

During cardiac development, the specific role of Pitx2c has been dissected using loss-of-function experiments, ectopic expressions, and analysis of the results of complex interactions with other genes. Franco and Campione\(^12\) described Pitx2-mediated signaling during cardiogenesis in 3 cell types, the myocardium, cardiac neural crest, and pharyngeal arch mesenchyme. Impaired Pitx2 function in the myocardium resulted in malformations such as double outlet right ventricle and transposition of the great arteries, whereas impaired Pitx2 cardiac neural crest presented with persistent truncus arteriosus.\(^12\) Pitx2 in cardiac neural crest is dispensable but required for crosstalk with second heart field–derived myocardium and splanchnic mesoderm for proper outflow tract development, as well as aortic arch formation. More recently, it has been proven that hemodynamics resulting from Pitx2-induced morphological changes during normal development of the outflow tract are responsible for the asymmetrical remodeling of the pharyngeal arch arteries.\(^10\) In mice, this results in the prenatal persistence of the left-sided ductus arteriosus, whereas the right-sided ductus arteriosus will regress by increased apoptosis caused by the diminished protective effect of flow and ensuing shear stress.\(^14\) Here, we have to realize the important differences between species, because zebrafish maintain a symmetrical pharyngeal arterial system, whereas in birds, a right-sided aortic arch is the rule and a bilateral symmetrical ductus arteriosus persists until hatching, differing again from mammals with a left-sided aorta and only 1 left-sided ductus arteriosus before birth.

Pitx2 is required for determination of left–right identity of the sinoatrial region and suppresses a left-sided sinoatrial node transcriptional program and represses the proliferation of cells in the left sinus venosus.\(^15\)\(^,\)\(^16\) The participation of another early cardiac transcription factor, Nkx2.5, which

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interacts with Pitx2c, is important because the sinoatrial node and atrioventricular node display different levels of Nkx2.5, implying that distinct regulatory mechanisms control their formation and function.

Interestingly, the Nkx2.5-negative sinoatrial node is maintained on the right side, whereas the left-sided sinus venosus myocardium forming a putative left-sided sinoatrial node,17 as well as the myocardium surrounding the pulmonary vein, becomes Nkx2.5-positive.18 Left–right asymmetry of the dorsal atrial wall is regulated by yet other genes including the short stature gene Shox2 and podoplanin. Shox2 has a critical function in the posterior heart field.19 Mutant mice show a marked hypoplasia of the posterior heart field myocardium on both the left and right side and present with an upregulation of Nkx2.5, as well as Cx40 and -43, in the sinoatrial node.18 Zebrafish Shox2 antisense morpholino-treated embryos develop severe sinus bradycardia and intermittent sinus exit block. The Nkx2.5-negative myocardium shows a peculiar interaction with podoplanin, which is expressed in the developing conduction system,18 as well as in the coelomic lining and the epicardium-derived structures.20 The epicardium-derived cells, which are symmetrical in origin in mice but asymmetrical in birds,5 have a major influence on ventricular myocardial differentiation, and it can be questioned whether abnormal epicardial to myocardial interaction may be instrumental in the ventricular cardiac abnormalities observed in the myocardial-specific knockout mice.1 It is clear that the pulmonary myocardium, which expresses both podoplanin18 and the conduction system–linked CCS-LacZ gene,21 is directed by Pitx2c, because knockouts lack pulmonary myocardium altogether.22 The close anatomic relation with the transient left sinoatrial nodal region makes atrial arrhythmias originating in this area conceivable.18 Recently, DNA variants downstream of the Pitx2 gene have been associated with atrial fibrillation and typical atrial flutter in a population-based genetic study.13 Disturbance of the left–right asymmetry may influence both right- and left-sided second heart field–derived atrial structures because atrial flutter is mostly a right-sided phenomenon. It is necessary to study the correlation of Pitx2c expression with posterior heart field–derived smooth muscle cells that line the pulmonary veins as Campione and colleagues have done so excellently for the myocardial cells. Smooth muscle cells extend from the pulmonary veins to line the inside of the body of the left atrium, whereas they are lacking in the right atrium, which is supplied by the cardinal veins (Figure).23

We conclude that Pitx2, although still a teenager since its discovery, brings us many challenges not only in the basic research of the factors governing atrial situs but also in the complex ventricular remodeling in which isomerism does not exist. The first links to human malformations seem to be emerging, and provocative aspects in translational research are to be expected.

Figure. a, Inside view of the heart (adapted from Douglas et al23). The vessel wall tissue is in red, myocardium in blue, and atrial appendages (LAA and RAA) in brown. b, Section (mouse, embryonic day 18.5) with the atrial myocardial marker myosin light chain (MLC) 2a. Boxes depict magnifications in the right (RA) (c) and left (LA) atrium (d). c, In the right atrium, vascular smooth muscle wall (1A4 staining) is absent. d, In the left atrium, vascular wall is observed throughout the entire left atrial wall. IAS indicates interatrial septum; IVC, inferior caval vein; LCV, left cardinal vein; MV, mitral valve; PV, pulmonary vein; SVC, superior caval vein; TV, tricuspid valve; RVV, right venous valves; LCV, left venous valves; RCV, right cardinal vein; CS, coronary sinus.
References


KEY WORDS: Pitx2 ■ left-right asymmetry ■ cardiac morphogenesis ■ second heart field

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