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RESEARCH HIGHLIGHT

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Laterality, heterotaxy, and isolated congenital heart defects

The genetic basis of the segmental nature of the heart

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Abstract

To date, the role of *NODAL* in normal and abnormal L-R asymmetry has been well established. In a recent paper, mutations of this gene have been reported in heterotaxy but also in transposition with D- or L-ventricular loop. The effects of *NODAL* and other laterality genes can be recognized separately in all three cardiac segments: for topology and septation of the atria, for ventricular looping, and for spiralization and alignment of the great arteries.

Keywords Laterality defects, Laterality genes, Heterotaxy, Congenital heart disease, Nodal

Background

In a recent issue of this Journal, Dardas et al. published an interesting paper on several *NODAL* gene mutations associated with different types of congenital heart defects (CHD) [1]. In their study, the authors analyzed a large cohort of cases ($n=321$), including heterotaxy but also transposition of the great arteries (TGA) with or without left ventricular loop, demonstrating rare variants of *NODAL* in 33 subjects.

The essential role of *NODAL* gene in normal and abnormal left–right (L-R) asymmetry in the embryo is well established [1], and previous reports have shown *NODAL* mutations in cases of heterotaxy, TGA, and congenitally corrected TGA (CCTGA) [2] and also in anatomically corrected malposition of the great arteries [3].

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However, the paper of Dardas et al. confirms that rare *NODAL* gene variants are associated with heterotaxy and may also contribute to the development of other types of CHD with normal visceroatrial situs (solitus) but with inverted position of the ventricles (L-loop) and inverted position (transposition) of the great arteries (TGA).

It is noteworthy that other laterality and ciliary genes (*ZIC3*, *CFC1*, *GDF1*, *DNAH9*, *DNAH5*) have been reported in association with heterotaxy and TGA with situs solitus. Furthermore, interestingly, the *NODAL* gene is highly conserved in vertebrates but is also present in snails with the role of shell spiralization. The same spiralization that NODAL-signaling pathway determines in the great arteries of the vertebrate heart [4].

In the following sections, we will discuss the separate effects of the Nodal gene-signaling pathway on the atrial, ventricular and great arteries segments of the heart.

Atrial and atrioventricular canal (AVC) septation

In 1995, the revelation of early asymmetric expression of three genes, *ACTIVIN*, *SHH*, and *NODAL*, represents the most important discovery for understanding the genetics of L-R asymmetry in vertebrates. The NODAL-signaling pathway activates an asymmetric left-sided expression of *PITX2*, inducing a morphologic specification of left-sided body organs and of heart segments. This genetic

pathway, due to a clockwise rotation of primary cilia at the left/right organizer, generates an asymmetric leftward flow, long before the appearance of morphological asymmetries.

In the atria, the normal expression of *PITX2* at the level of the left atrium and atrial septum, but not of the right atrium, induces position, specific morphology, and septation of the atria. However, the mechanism linked to *PITX2* is not the only one involved in atrial septation. In fact, the "dorsal mesenchymal protrusion," an extracardiac tissue, has been reported to be an important determinant of AVC development. This extracardiac cell population arises from the posterior segment of the second heart field and grows toward the atrial surface and toward the posteroinferior and dorsal endocardial cushion. This structure closes the primary atrial foramen forming the atrioventricular junction. To date, we know that sonic hedgehog (SHH), one of the early genes involved in L-R lateralization, is widely expressed in the dorso-mesenchymal protrusion and is therefore critical in the AVC formation [5]. *SHH* may be involved not only in heterotaxy but also in Ellis-Van Creveld, Noonan, and Down syndromes. In all these genetic conditions, a unifying pathogenetic role of *SHH* acting on the dorso-mesenchymal protrusion may explain the recurrent presence of AVC without heterotaxy [5].

Ventricular loop

The bending of the ventricles, better known as ventricular loop, has been one of the most studied embryological events, but is still among the least understood.

The heart is the first organ to break bilateral morphological embryological symmetry with an early asymmetrical rightward looping of the ventricles. However, experiments in mice have failed to demonstrate an exclusive role of known laterality genes in ventricular looping, which appears to be *NODAL* dependent but *PITX2* independent [6]. Probably, in addition to *NODAL/PITX2* genes, other signaling pathways are involved in the ventricular bending. It has been suggested that an intrinsic mechanism of ventricular cells chirality, perhaps mainly of the left ventricle or extrinsic forces, drives the right ventricular loop [6]. The asymmetric *NODAL/PITX2* signaling could enhance an existing skeleton-based intrinsic mechanism of chirality, thus influencing the direction of the ventricular loop.

Great arteries spiralization

More than 20 years ago, it was suggested that the parallel and non-spiral morphology of the great arteries in TGA might be a phenotypic sign of laterality defect [7]. In the subsequent years, this hypothesis has been supported by studies on familial recurrence and molecular biology [8].

The embryological rotational movement of the myocardial outflow induces the spiral morphology of the cardiac outflow tract and the correct alignments of the great arteries. Moreover, the absence of Pitx2 in knock out mice can affect the outflow tract, causing parallel course and failure of the great arteries rotation and finally TGA [9].

In addition, recent contributions have shown that the polarization of pulmonary veins and the asymmetry of aortic arches could also be influenced by ciliary and laterality genes.

These observations not only are of genetic and nosographic value but also could have important clinical, therapeutic, and prognostic implications. Indeed, the Pittsburgh group has shown that patients with TGA and CCTGA may experience respiratory symptoms similar to those observed in children with heterotaxy, due to ciliary dysfunction [10].

Defining the pathogenesis will be useful in disease treatment and precision medicine in the field of CHD as well.

Conclusions and perspectives

The effects of lateralization genes can be found separately in all three cardiac segments: for the topology and septation of the atria and AVC, for the ventricular looping, and for the spiralization and alignment of the great arteries.

Defects in these genetic pathways can cause the complete form of heterotaxy but also CHD with established viscero atrial situs solitus (or inversus) and discordance between cardiac segments and/or between ventricular chambers and great arteries.

It is evident that the early development and the segmental complexity of the heart make it susceptible to L-R asymmetry abnormalities. The laterality genes are probably involved in far more CHDs than is currently known [9].

Future studies are required to explain the precise effects of laterality genes on various heart segments for normal and abnormal alignment of cardiac chambers and great arteries. Moreover, they may clarify why an apparently equal genetic defect can cause such different segmental cardiac phenotypes [1–3].

Certainly, the understanding and diagnosis of lateralization patterns cannot be guided only by atrial morphology and the definition of isomerism. Complex CHD deserve a complex and specific approach rather than a simplistic and rigid nomenclature. Perhaps nowadays atrial situs by itself is not sufficient to define cardiac situs; rather, it is more accurate to also consider ventricular and great artery situs, which may be independent of each other.

The potential independent development within the same genetic pathway is the new way to understand complex CHD with discordance or malposition of heart segments and great arteries.

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