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Review

Xenopus as a model organism for birth defects—Congenital heart disease and heterotaxy

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ABSTRACT

Congenital heart disease is the leading cause of birth defects, affecting 9 out of 1000 newborns each year. A particularly severe form of congenital heart disease is heterotaxy, a disorder of left–right development. Despite aggressive surgical management, patients with heterotaxy have poor survival rates and severe morbidity due to their complex congenital heart disease. Recent genetic analysis of affected patients has found novel candidate genes for heterotaxy although their underlying mechanisms remain unknown. In this review, we discuss the importance and challenges of birth defects research including high locus heterogeneity and few second alleles that make defining disease causality difficult. A powerful strategy moving forward is to analyze these candidate genes in a high-throughput human disease model. *Xenopus* is ideal for these studies. We present multiple examples demonstrating the power of *Xenopus* in discovering new biology from the analysis of candidate heterotaxy genes such as GALNT11, NEK2 and BCOR. These genes have diverse roles in embryos and have led to a greater understanding of complex signaling pathways and basic developmental biology. It is our hope that the mechanistic analysis of these candidate genes in *Xenopus* enabled by next generation sequencing of patients will provide clinicians with a greater understanding of patient pathophysiology allowing more precise and personalized medicine, to help patients more effectively in the future.

Contents

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1. Birth defects

Birth defects, or structural malformations of the body that occur during embryological development, have a massive impact on the health and welfare of children. Approximately 8 million children are born with serious birth defects each year—roughly 6% of births worldwide [1]. Importantly, an additional, untold number of birth defects lead to stillbirths and miscarriages, which can have a devastating effect on parents hoping to start a family [2,3]. In the United States, during the first year of life, birth defects are the leading cause of pediatric hospitalizations [4], medical expenditures [5], and death [6]. Further, birth defects rank as a leading cause of death among children aged 1–4 years (#2 cause of death), 5–14 years (#3) and 15–24 years (#6) [7]. Therefore, given the impact birth defects have on children, there is a pressing need to improve diagnosis and treatment. Unfortunately, for the vast majority of birth defects, we lack a molecular understanding of the pathophysiology. Advances in human genomics are offering exciting avenues to address the causes of birth defects, but challenges remain prompting clinicians, developmental biologists, and geneticists to urge the NIH to address the problem of birth defects and their impact on child health [8]. Here, we outline some of these challenges and strategies to integrate patient driven gene discovery with a powerful disease model, *Xenopus*.

2. Congenital heart disease

Of the birth defects, congenital heart disease (CHD) is the most common and the most life threatening. CHD affects 9 in every 1000 live births and 1.3 million newborns annually worldwide [9]. CHD is associated with high rates of morbidity and mortality in those affected and typically requires surgical intervention early in life [10]. Finally, the care costs for patients with CHD in the United States exceeds 1.75 billion dollars annually [11].

Recent medical and surgical advances have permitted a greater number of infants with complex CHD to survive to adulthood. There were at least 117,000 CHD adult survivors living in the U.S. in 2000; this number has increased greatly over the past fifteen years and is predicted to rise by approximately 5% per year [12,13]. Coincident with increasing survival for children with CHD is the recognition of the associated medical and surgical disorders they may harbor. These range from hemodynamic instability and arrhythmias to infertility, pulmonary disease and significant neurodevelopmental disorders [14,15].

Emerging data suggest that both the rate and type of CHD vary significantly across the world, ranging from 6.1/1000 live births in the U.S. to 9.3/1000 in Asia. Although access to health care may contribute to global differences in CHD, both environmental and genetic factors most certainly play a role [9]. In the age of molecular medicine, identifying those genetic mechanisms responsible for CHD is a priority for both physicians and developmental biologists. To do so, *Xenopus* is an outstanding model and has made major contributions to our understanding of cardiac development [16,17].

3. Heterotaxy

Heterotaxy (Htx) is a cause of 3% of CHD occurring in 1 in 10,000 newborns, and leads to a particularly severe form [18,19]. Heterotaxy is an abnormal development of the left–right (LR) axis, which leads to incorrect position and organization of the internal organs. The heart, whose function depends on its LR asymmetry, can be severely affected by abnormal development of the LR axis [18], and patients with Htx are at high risk for increased post-operative and respiratory complications, arrhythmias, and complications due to other congenital malformations [20,21]. Positioning of the internal organs can be divided into three categories: *Situs solitus* or a normal positioning of the internal organs; *situs inversus*, in which the organs are a mirror image; and finally, *situs ambiguous*, in which there is no clear specificity of the organs along the LR axis. Htx falls within the category of *situs ambiguous*.

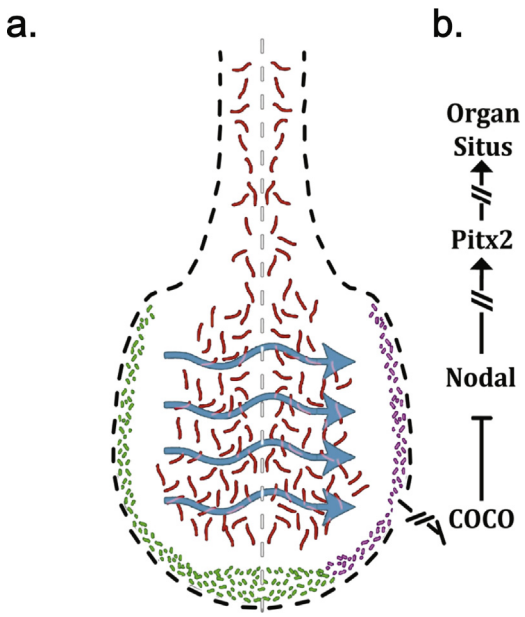

Fig. 1. Ciliary flow and signaling in the gastrocel roof plate (GRP) of the frog.
● The black dashed line outlines the GRP. The red lines represent the inner motile cilia, and the green and purple represent the outer immotile cili The blue arrows show the leftward fluid flow across the GRP that is produced by the motile cilia and sensed by the immotile cili.
● *coco* is initially expressed bilaterally. Ciliary flow reduces the expression of *coco* on the left side. Coco inactivates Nodal on the left, which leads to *pitx2c* expression on the left side of the embryo and ultimately plays a role in organ *situs* and asymmetric development.

Studies in many developmental model organisms have identified a conserved left–right (LR) patterning program that determines proper cardiac situs. At the end of gastrulation, asymmetric development begins at the left–right organizer (LRO; mouse node, zebrafish Kuppfer's vesicle, and gastrocoel roof plate [GRP] in frog; shown in Fig. 1), which forms in the dorsal posterior region of the embryo [22]. In cells of the LRO, inner motile monocilia (red cilia) beat to create a leftward flow of extracellular fluid (blue arrows) [23,24]. According to the two-cilia model, immotile cilia (green and purple cilia) on surrounding cells act as sensors, detecting the flow (purple cilia) driven by motile cilia (red cilia) and eventually translating it into asymmetric gene expression [25,26]. *Coco* (CERL2), a nodal antagonist, is one of the earliest genes that is asymmetrically expressed [27,28]. *Coco* expression leads to nodal inhibition and decreased phosphorylated Smad2 on the right [29]. Phosphorylated Smad2 eventually activates *pitx2* on the left side of the embryo, which is an essential step in organ *situs* determination [29,30]. *Pitx2* is also involved in the organogenesis of the heart, gut and lungs [31,32]. Following activation of these developmental pathways, cardiac precursor cells in the lateral plate mesoderm fuse at the midline to form a straight heart tube [33,34]. This central region and first heart field will eventually form the left ventricle. One end of the tube will become the outflow tracts and atria, and the other end will become the right ventricle and inflow tracts. This linear tube eventually loops to the right, establishing cardiac asymmetry. A complex signaling network is essential for this asymmetry to occur [35].