Developmental structure-function insights from $Tbx5^{del/+}$ mouse model of Holt-Oram syndrome

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IN THIS ISSUE of the American Journal of Physiology-Heart and Circulatory Physiology, Zhou et al. (32) describe impaired diastolic ventricular function measured by high-resolution (30 MHz) ultrasound and evidence for atrial septal defect (ASD) based on in vivo ultrasound and postmortem magnetic resonance imaging and anatomic examination in a $Tbx5^{del/+}$ mouse model for Holt-Oram syndrome. $Tbx5^{del/+}$ mice and control littersmates were studied at 1, 2, 4, and 8 wk of age. A significant correlation between left ventricular $Tbx5$ gene expression and left ventricular diastolic dysfunction and right ventricular dilation was noted at 2 wk of age. This innovative study highlights several important issues related to the search for primary mechanisms for congenital cardiovascular (CV) malformations, the dynamic relationship structure and function during CV morphogenesis, and the correlation of data derived from targeted genetic animal models with clinical syndromes.

This study serves as an excellent example of the value of a combined analysis of both cardiac function and structure in evaluating CV phenotype in targeted genetic animal models of congenital heart disease. The goal of defining the primary mechanisms responsible for congenital CV malformations has been a driving force in CV research for over one hundred years (25). Major advances in our understanding of CV morphogenesis have occurred based on improved imaging and morphometric techniques (1, 14, 22, 27–29), the careful analysis of fate maps (24), ablation and chimera experiments (12), and the analysis of explant cell-cell and cell-matrix interactions (6), to name only a few key developments. Many of the genetic cascades that regulate normal CV morphogenesis have been identified over the past 20 years, leading to the generation of numerous targeted genetic animals, primarily mice, zebra fish, and frogs, that are useful in identifying and confirming these developmental mechanisms (7, 16, 30).

Animal models that target specific genetic cascades have become standard reagents in the investigation of development and disease. One of the challenges in targeting the key genes and proteins involved in CV morphogenesis is the tendency for lethal phenotype in homozygous deficient animals and for subtle or absent phenotypes in heterozygous animals. The subtle phenotypes of heterozygous animals, as in the $Tbx5^{del/+}$ mouse model for Holt-Oram syndrome (5), are most likely to provide insights into human malformations (4). For example, the haplotype deletion $Nkx2.5^{del/+}$ mouse recapitulates the ASD and conduction defects noted in humans with $Nkx2.5$ mutations (10, 17, 20). In the current study, it is important to note that although all 12 $Tbx5^{del/+}$ mice were found to have left ventricular diastolic dysfunction and altered $Tbx5$ gene expression, only 8 of 12 mice were noted to have ASDs by direct inspection. These findings are consistent with the variable cardiac phenotype of patients with Holt-Oram syndrome, where ASD was found in 60.8% of patients and more complex congenital cardiac defects were found in 17.5% (23).

To date there is no correlation between $Tbx5$ genotype and phenotype in Holt-Oram syndrome patients (3, 15), and $Tbx5$ mutations have also been noted in patients with congenital heart disease independent of Holt-Oram syndrome (19). Developmental studies show a uniform $Tbx5$ gene expression throughout the developing myocardium (13), with later restriction to posterior sinoatrial segments of the heart. In the current study (32), left ventricular $Tbx5$ gene expression correlated with measures of altered ventricular diastolic function in $Tbx5^{del/+}$ mice. Thus it is possible that there are more subtle changes in ventricular structure and function in patients with Holt-Oram syndrome that will be shown to correlate with genotype.

Understanding the genotype-phenotype variations in clinical populations and in targeted genetic animals requires recognition of the dynamic adaptation that occurs between genetic cascades and CV structure and function in the embryo and fetus. The quantitative measurement of CV function during the period of primary cardiac morphogenesis has rapidly progressed to include the characterization of CV function in a wide range of species (fly, fish, frog, chick, rat, mouse, and human) from shortly after the onset of cardiac activity through the completion of gestation. For the mouse embryo, quantitative measures include the invasive measurement of blood pressure, blood velocity, and chamber dimensions as well as the increasing availability of noninvasive measures to define both embryonic function and structure (see, e.g., Refs. 8, 11, 18, 31). Relevant to the current data described by Zhou et al. (32), primary changes in ventricular filling characteristics (9, 26) can generate permanent changes in CV structure and function that include both obvious changes in ventricular phenotype (21) and subtle changes in myofiber architecture (27). Thus the current study provides data to suggest that the primary event in this model is altered ventricular $Tbx5$ gene expression that results in left ventricular diastolic dysfunction and raises the possibility that changes in atrial septal morphogenesis (2), as well as flow-mediated changes in tricuspid valve diameter and right ventricular dimensions, may be secondary events.

The current study by Zhou et al. (32) validates the utility of chamber-specific, quantitative, high-resolution echocardiographic imaging in characterizing the primary and secondary changes in CV structure and function present in the $Tbx5^{del/+}$ mouse model of Holt-Oram syndrome. The underlying mechanisms by which temporally and spatially specific reductions in myocardial $Tbx5$ expression during embryogenesis alter CV structure, function, adaptation, and final CV phenotype are yet to be identified.
REFERENCES