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Abstract Topic:- Prenatal, perinatal and developmental genetics

Abstract Title:- Screening and Functional Analysis of Birth Defect Varinats

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Aims:-Projecting from most vertebrate cells, cilia play a central role either as a mechanical motor to create an extracellular fluid flow or as a specialized signaling center during embryogenesis and organogenesis. Thus, ciliary dysfunction can result in a constellation of congenital disabilities ranging from axis malformation and infertility to cerebral and skeletal abnormalities, collectively known as ciliopathies. We focus on deciphering the molecular mechanisms underlying the ciliopathy, heterotaxy (HTX). HTX is a most severe form of congenital heart disease (CHD) where left-right (LR) asymmetry is not established correctly, leading to malformations of internal organs, such as the heart, lung, spleen, liver, and gut.

Methods:- Advances in human genomics have identified many potential disease-causing genes and variants from CHD/HTX patients. However, determining if the candidate gene and the variant are pathogenic remains challenging because most variants have no second alleles and are either completely novel or have no known role in HTX. To address these challenges, we first screen the candidate disease genes using a high-throughput loss of function (LOF) screen using CRISPR/Cas9 in the Xenopus system to eliminate false positives quickly. We then perform the function analysis of new proteins in embryogenesis to improve our understanding of the underlying disease mechanisms and explain the patient phenotype.

Results:- The pediatric cardiac genomics consortium (PCGC) recently identified rare inherited and de novo variants from thousands of CHD patients. 272 patients presented a classic HTX phenotype. From this group, we prioritized 30 putative LOF or damaging variants for the LOF screen. We discovered that 35-40% of the new genes and variants recapitulated the patient phenotype using a CRISPR/Cas9-based LOF approach, and ~50-60% of likely pathogenic genes have a role in the assembly or function of motile cilia in the left-right organizer and the multiciliated cells of the airway.

Conclusions:- Our study is innovative because we are leveraging the advances in human sequencing to accelerate the exploration of fundamental mechanisms critical for embryogenesis and to use that knowledge to explain the genetic burden in congenital malformations at the molecular level.

Keywords:- birth defects, functional genomics, congenital heart defects, CRISPR, cilia