Unraveling the genomic basis of congenital heart disease

Dawood Darbar[1,2]

[1]Division of Cardiology, Departments of Medicine and Pharmacology, University of Illinois at Chicago, Chicago, Illinois, USA. [2]Department of Medicine Jesse Brown Veterans Administration Medical Center, Chicago, Illinois, USA.

The genetic, epigenetic, and environmental etiologic basis of congenital heart disease (CHD) for most heart anomalies remains unexplained. In this issue of the *JCI*, Lahm et al. performed the largest genome-wide association study (GWAS) to date of European individuals with CHD and clinical subtypes. The comprehensive meta-analysis included over 4000 patients and 8000 controls and uncovered common genetic variants that associated with cardiac anomalies. Lahm and colleagues performed single-cell analysis of induced pluripotent stem cells and heart cells, revealing a role for *MACROD2*, *GOSR2*, *WNT3*, and *MSX1* in the developing heart. This study advances our understanding of the genetic basis of common forms of CHD.

Congenital heart disease as a genetic disease

Congenital heart disease (CHD), a structural abnormality of the heart and great vessels that is present at birth, is the most common congenital anomaly worldwide, with an incidence of nearly 10–12 per 1000 live births (1, 2). It represents a major global health burden with its associated morbidity and increased mortality, as CHD causes over 200,000 deaths annually worldwide. Despite the development of newer genomic technologies, including next-generation sequencing that reveals copy number variants and SNPs, providing important insights into the underlying genetic basis of CHD, epidemiological studies identify a genetic or environmental cause in fewer than one-third of cases (3).

Historically, CHDs represent a broad array of heart defects classified into cyanotic heart disease, left-sided obstruction defects, septation anomalies, and others. Cyanotic heart defects include tetralogy of Fallot, transposition of the great arteries (TGA), tricuspid and pulmonary atresia, Ebstein's anomaly, double-outlet right ventricle, and persistent truncus arteriosus. In contrast, left-sided obstruction defects consist of hypoplastic left heart syndrome, mitral and aortic valve stenosis, and aortic anomalies including aortic coarctation. The third main type of CHD includes septation of the atria (atrial septal defects [ASDs]) and ventricles (ventricular septal defects [VSDs]) and atrioventricular septal defects (AVSDs). Bicuspid aortic valve and patent ductus arteriosus (PDA) constitute other types of CHD that do not fit into one of the three main categories. Most genetic causes of syndromic CHD, responsible for approximately 20% of all cases, are the result of chromosomal aneuploidy, chromosomal translocations and deletions, or single gene defects. Traditional linkage analyses and positional cloning approaches have identified a number of uncommon syndromic forms of CHD such as Holt-Oram syndrome, consisting of ASDs, VSDs, and conduction system defects caused by a single gene mutation in the T-box transcription factor gene *TBX5* (4). Subsequently, mutations in *NKX2–NKX5* were identified in kindreds with congenital ASDs and atrioventricular (AV) block and a diverse array of CHDs including VSDs and Ebstein's anomaly (5). However, it is only over the past decade that advances in genomic technologies have enabled us to identify the genetic basis of the more common forms of CHD. Next-generation sequencing has not only identified both common and rare genetic variants contributing to CHD susceptibility but has also uncovered a striking complexity (6). Although the underlying mechanisms have not been entirely worked out, emerging studies suggest that CHD is a genetic disease determined by diverse genetic (both monogenic and complex) and environmental mechanisms.

Uncovering common genetic variation

Although we have known for a long time that genetic factors play an important role in the development of CHD and identified single gene mutations in uncommon syndromic forms, the contribution of common genetic variation in the etiology of CHD is poorly understood. In this issue of the *JCI*, Lahm, Jia, and colleagues performed a comprehensive meta-analysis of a GWAS in over 4000 European patients with CHD (defined according to the Society of Thoracic Surgeons) and clinical subtypes and over 8000 controls. The researchers identified one risk variant associated with all CHD phenotypes and multiple other loci/SNPs in patients with TGA, anomalies of thoracic arteries and veins (ATAV), and septal defects (7). Functional characterization of the identified SNPs mapped to candidate genes using human and murine induced pluripotent stem cells (iPSCs) and single-cell RNA-Seq of developing murine and human hearts showed important roles for *MACROD2*, *GOSR2*, *WNT3*, and *MSX1* at all stages of heart development (7).

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This was the largest GWAS meta-analysis of European individuals performed for the five categories of CHD across the major clinical subgroups. This GWAS, combined with the observed functional association of the related genes during murine and human heart development, strongly supports the idea that *MACROD2*, *GOSR2*, *WNT3*, and *MSX1* play a vital role in cardiac development. Importantly, knockout mouse models of the genes related to the identified SNPs failed to demonstrate cardiac phenotypes, suggesting a multigenic etiology for most forms of CHD. Although there is considerable overlap in the sequence of cardiac development between humans and mice, Lahm, Jia, and colleagues used single-cell transcriptomic analysis to identify shared genes across the two species in the four different cardiac cell types, with cardiomyocytes possessing the most species-shared genes. The analysis also showed that the optimal overlap for each cell type occurred at different time points during heart development between humans and mice (7). Furthermore, functional characterization of the identified SNPs in both species reinforced the important role they play during cardiac development.

Lahm, Jia, and colleagues identified a cluster of SNPs that mapped to *MACROD2*, a gene that has not previously been associated with CHD. However, a chromosomal imbalance is frequently encountered in patients with congenital heart anomalies, and microdeletions in *MACROD2* have been implicated in chromosomal instability, albeit in cancers. The high expression of MACROD2 in human embryonic cardiac cells raises the possibility that it may act as a transcriptional regulator. The investigators also identified three SNPs mapping to *GOSR2* that were associated with ATAV (7). While GOSR2 is important for the movement of macromolecules between Golgi compartments, a role for *GOSR2* in cardiac development has not previously been reported. Importantly, heart anomalies in the ATAV subgroup, which include coarctation of the aorta and PDA, all share a common origin in the aortic sac and emerging aortic arches during embryonic development. Furthermore, a potential interaction between *GOSR2* and *WNT3* during cardiac development of human embryonic stem cells was previously reported (8). Importantly, Lahm, Jia, and colleagues also identified a SNP (rs185531658) that associated with septal defects and CHD risk in general, with *YTHDC2*, an RNA helicase involved in meiosis, being the nearest gene. The study went on to identify SNPs previously associated with ASDs in a Chinese cohort and confirms the importance of potential functional interactions between *STX18* and *MSX1* and the development of ASDs (7).

Conclusions

Lahm and colleagues should be congratulated not only for identifying many candidate genes for CHD but also for uncovering the importance of common genetic variation in the etiology of congenital heart anomalies (7). However, the study also raises a number of intriguing questions that need addressing in future studies. First, the underlying mechanisms by which *MACROD2*, *GOSR2*, *WNT3*, and *MSX1* cause structural heart defects remain unknown. While it is possible that these genes may act as transcriptional regulators, precisely how they lead to CHD requires investigation. Future research should focus on identifying the specific targets and the underlying cellular mechanisms by which candidate genes cause CHD. Second, this study (7) and others have uncovered a striking complexity to the genetics of CHD that remains unexplained. One potential explanation is the two-hit hypothesis, whereby the interaction of a single gene mutation with susceptibility SNPs or environmental factors plays an important role in the pathogenesis of CHD. Studies have shown that defective interactions between the zinc-finger transcription factor–encoding gene *GATA4* and *NKX2-NKX5* and *GATA4* and *TBX5* lead to CHD caused by *GATA4* mutations (9, 10). Finally, although transcription factors are important in cardiac development, the epigenetic regulation of CHD, i.e., factors that modify the structure of chromatin and histones, remains unclear and may provide important insights into the complexity of common congenital heart defects.

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Address correspondence to: Dawood Darbar, 840 S. Wood St., 920S (MC 715), University of Illinois at Chicago, Chicago, Illinois 60612, USA. Phone: 1.312.413.8870; Email: darbar@uic.edu.

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