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Congenital Heart Disease Entering a New Era of Human Genetics

Benoit G. Bruneau, Deepak Srivastava

De Novo Mutations in Histone-Modifying Genes in Congenital Heart Disease

Zaidi et al

Nature. 2013;498:220–223.

Congenital heart disease (CHD) remains a leading cause of morbidity and mortality in childhood and is the most common human birth defect, affecting nearly 1% of all live births worldwide. The morphogenetic events that are disrupted during cardiogenesis that lead to CHD are now partially understood, as are many of the molecular networks that guide normal heart development.^{1–4} Studies of rare Mendelian forms of familial CHD, as well as CHD associated with stereotypic syndromes, have revealed numerous single-gene mutations that cause CHD.^{1–5} However, mutations in these genes are infrequent in the more common sporadic form of CHD. Despite epidemiological evidence for an inherited component in sporadic CHD, the contribution of inherited variants or de novo mutations in the setting of CHD has been unclear. A recent landmark article in *Nature*⁶ begins to tackle this question by using modern genetic approaches and suggests that roughly 10% of sporadic CHD cases have de novo mutations that contribute significantly to the disease process.

The ability to perform DNA sequencing at a reasonable cost now allows the interrogation of rare, as well as common, variants in large populations. Although many complex traits have been studied using whole-genome or exome sequencing, a rate-limiting step in the CHD field has been acquisition of sufficient numbers of cases for meaningful statistical analysis. To address this, a National Heart, Lung, and Blood Institute–funded consortium of centers was established to recruit patients and parents into common studies to reveal the genetic underpinnings of CHD (Pediatric Cardiac Genomics Consortium).⁷ In the first report from this group, Zaidi et al⁶

sequenced the entire protein-coding exome from 362 trios of patients with a variety of sporadic, more complex CHDs and their unaffected parents, as well as a similar number of trios without CHD. Although inherited variants may contribute, the consortium focused on de novo mutations present in an index case but absent in both unaffected parents, with the notion that the new genetic variant may be contributing to the appearance of CHD. Using strict statistical criteria, they identified many de novo mutations that were predicted to be damaging to the open reading frame, in many cases causing premature truncation, frameshifts, or splicing abnormalities. Genes expressed at high levels in the heart (as assessed by RNA sequencing of mouse embryonic hearts) had a higher rate of de novo mutations than those expressed at lower levels, increasing confidence that the affected genes could be involved in heart development. In total, 28 genes harboring de novo mutations in the CHD probands were implicated with high confidence, particularly considering the comparison with sequencing of the control trios.

Remarkably, the set of de novo variants that Zaidi et al⁶ identified with a high degree of statistical confidence were mainly in genes encoding proteins that are related to histone modifications, which generally function to regulate gene expression.⁸ In particular, 5 of the genes identified encode proteins that participate in the writing, erasing, or reading of a particular histone modification, trimethylation of lysine 4 of histone H3 (H3K4me3). This modification is largely associated with transcriptionally active genes, and thus its regulation is critical for most cellular processes. The 5 genes included *MLL2*, which catalyzes the deposition of H3K4me3, *WDR5*, which is part of the MLL complex, *CHD7*, which recognizes the histone modification, and *KDM5A* and *KDM5B*, both involved in removing the methylation mark. Additional genes involved in other histone modifications or other aspects of chromatin remodeling were also identified. Mapping of histone modifications in cardiac differentiation has pinpointed the timing and location of H3K4me3 and other histone modifications,^{9,10} and it is clear that many aspects of cardiac differentiation (and by extension development) would be affected by abnormally regulated H3K4me3. Thus, the results from Zaidi et al⁶ implicate potential disease-causing de novo mutations in the epigenetic machinery involved in controlling gene expression during development.

Because epigenetic modifications are used to quantitatively regulate transcription, the findings of Zaidi et al⁶ reinforce a theme involving gene dosage that has emerged from years of more traditional human genetic studies with CHD. The majority of previously identified single-gene causes of CHD involve heterozygous mutations of transcription factors, typically resulting in haploinsufficiency of central developmental regulators. Given that cardiogenesis seems to

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be exquisitely sensitive to gene dosage, it follows that mutations in genes more broadly affecting quantitative levels of transcriptional output through epigenetic regulation would contribute to CHD.

An intriguing question is why the mutations identified in this study cause cardiac-specific defects. The genes pinpointed function in all cell types and broadly regulate gene expression from the earliest stages of development and throughout adulthood. Indeed, somatic mutations in some of the same genes are considered to be causative forces for many cancers. *MLL2* mutations were previously found to be associated with a syndrome (Kabuki syndrome) that included CHD,¹¹ among a constellation of other defects, and on re-evaluation of the patient with *MLL2* mutations, Zaidi et al⁶ indeed found features that correspond with some of the notable facial features of Kabuki syndrome. On the contrary, the patient bearing the *CHD7* mutation had no other features typical of CHARGE association, previously known to be caused by mutations in *CHD7*. The authors conclude by suggesting that environmental perturbation might modulate the expressivity of CHD-associated mutations or could even modulate the pathways that they regulate. Identifying the specific genomic targets of the chromatin remodeling factors and the effect that the CHD-associated mutations have on the epigenomic status of these target loci will be required to establish what pathways they regulate and to assess the degree to which these might be disrupted in the setting of disease.

Although this study represents an important step forward in the study of CHD, many questions remain. For example, sequencing limited to the exome precluded the investigation of noncoding regulatory elements that are the key modulators of gene expression. Future studies will likely involve whole-genome sequencing as the cost differences narrow and improved algorithms allow deeper analysis of noncoding regions. Similar to many other genome-wide association studies, a recent genome-wide association study of CHD identified 2 loci associated with secundum atrial septal defect that likely involve noncoding variants.¹² An interesting conclusion from this study was that lumping all CHDs together provided less power than focusing on specific lesions, suggesting that future sequencing efforts may be more powerful if performed on more homogeneous populations of CHD. Finally, it will be critical to understand the genetic contribution of rare and common inherited variants from unaffected parents, although the ability to have confidence in the cause and effect relationship of such variants remains challenging.

Nevertheless, the likely mechanism by which many of these mutations result in CHD is abnormal gene regulation resulting in defective heart development. Future studies will aim to provide experimental evidence implicating the identified mutations in functional consequences, supplementing what is currently a statistical argument. To this end, collaboration with the closely linked National Heart, Lung and Blood Institute–funded Cardiovascular Development Consortium will allow functional assessment of variants in in vivo animal

models and human induced pluripotent stem cell models of disease. Ultimately, we hope that association of genetic variants with CHD and subsequent mechanistic understanding will lead to new therapeutic approaches that can be tested in the National Heart, Lung and Blood Institute–funded Pediatric Heart Network established to coordinate clinical trials across multiple centers. The 3 intertwined consortia focused on CHD are collectively known as the Bench to Bassinet program and will eventually lead to sorely needed new therapeutic approaches based on disease mechanisms. Future expansion of the number of centers involved would bring larger numbers of patients to bear, which will be critical to increase the power of genetic studies, particularly for the discovery of relatively rare variants that might be associated with CHD. The model displayed by Zaidi et al⁶ provides hope that we will finally begin to understand the complex genetics underlying this all too common disease that causes lifelong suffering for children and their families.

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Disclosures

None.

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