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Beyond Gene Panels: Whole Exome Sequencing for Diagnosis of Congenital Heart Disease

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Driven by next generation sequencing (NGS), rapid advances in our knowledge of inherited cardiovascular diseases have greatly improved our ability to make molecular diagnoses. Focused gene panels covering select sets of genes implicated in a specific disease are the mainstay of clinical genetic testing. Panel testing has proven to be successful in the diagnosis and management of Mendelian diseases attributable to a single gene such as cystic fibrosis or a limited number of genes as in the case of several cardiomyopathies, channelopathies, and connective tissue disorders. While gene panels have diagnostic rates surpassing 70% for some inherited cardiovascular diseases, the utility of gene panels has not translated as effectively to the detection of diseases characterized by more complex genetic architecture such as congenital heart disease.

Congenital heart disease (CHD), a heterogeneous group of structural cardiac malformations, is the most common human birth defect and the leading cause of neonatal death due to a congenital illness.[1] Hundreds of genes have been identified as having confirmed or putative pathogenicity in causing syndromic and non-syndromic CHD.[2, 3] For familial, non-syndromic cases of CHD, the diagnosis rate for gene panels inclusive of over 50 CHD-associated genes is 31-46% at best,[4, 5] a far cry from the diagnosis rate of 75% achieved by a 17-gene panel for Long QT syndrome.[6] Moreover, the diagnostic yield for non-familial (sporadic), non-syndromic CHD is even slimmer. While approximately 20% of the genetic attributable risk for sporadic CHD has been uncovered to date,[7] the genetic cause of the vast

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majority of CHD still remains a mystery. As a result of the complex genetic structure of CHD, commercial gene panels aimed at diagnosing the cause of CHD are incomplete and highly variable in terms of which genes have been selected for inclusion (Table 1). Given the vast number of genes potentially involved, and our rapidly changing understanding of disease, envisioning a gene panel which is both broad and specific enough to detect the cause of CHD in a majority of individuals, especially those with sporadic disease, is a difficult and perhaps unnecessary task. Thus, unbiased screening methods, such as whole exome sequencing (WES), represent an attractive approach for studying the genetic contributions to CHD by casting a broader net for discovery.

In this issue of *Circulation: Genomic and Precision Medicine*, Szot et al.[8] present a genetic study harnessing the power of WES to identify CHD-associated gene variants in 30 unrelated families with at least one family member affected by CHD. Probands, their parents, and any other available affected family members underwent WES. The authors began their analysis with a more targeted approach by evaluating variants in 90 “high-confidence” genes known to cause human CHD, a list that the authors will make public and continually update. They utilized conservative criteria and only included genes that had been previously identified as a monogenic cause of CHD in at least three families or in at least two separate publications. Of note, candidate genes suspected to cause CHD based solely on mouse data, gene expression, protein function, or non-cardiac phenotypes were excluded from this list. In contrast to this approach, another recent study by Li et al.[9] generated a more comprehensive candidate list of over 1700 candidate genes in their WES analysis of patients with left-sided obstructive lesions, including genes involved in known critical signaling pathways and genes identified through animal studies (mouse, zebrafish), regardless of their prior identification in cases of human CHD. The range from 90 to 1700 candidate CHD genes highlights the tremendous variability in gene selection criteria inherent to targeted candidate gene approaches to genetic discovery.

In the second part of their analysis, Szot et al. take an unbiased approach and perform a “Comprehensive Screen” in which the WES data from the 30 families were further analyzed for additional variants not identified through the high confidence CHD gene list. In both analyses, variants that segregated with disease were further evaluated based on: 1) a minor allele frequency (MAF) in the Exome Aggregation Consortium (ExAC) and the 1000 Genomes Project databases, 2) predicted effects on protein function based on the Combined Annotation Dependent Depletion (CADD) and PolyPhen-2 scoring schema, and 3) The American College of Medical Genetics and Genomics (ACMG) classification criteria. Using their dual approach of interrogating high confidence genes as well as an unbiased screen, the authors achieved an overall diagnostic rate of 29% of sporadic cases, nearly triple the ~10% diagnostic rate of other recent studies.[10] For familial cases, they achieved an overall diagnostic rate of 56%, a significant jump from the 31-46% yield in other familial studies which restricted their analysis to known CHD genes.[4, 11] The authors thus offer evidence for the utility of taking an unbiased approach using WES. Additionally, the authors highlight another significant advantage of WES over panel testing; as more causal or contributing variants are discovered, findings from WES can be re-analyzed. It is reasonable to believe that WES data generated today could be re-analyzed in 10 years and provide actionable information in an even greater proportion of patients.

While WES significantly broadens the horizon of variant discovery and can be revisited clinically to unveil previously unseen associations, identifying the full spectrum of genetic causality in CHD still remains an elusive target, to which the authors do an admirable treatment of the limitations of their study. Analysis of the exome may reveal functional variants changing protein structure or function, but these data still only encompass 1% of the entire genome. For many diseases with complex genetic architecture such as coronary artery disease, most disease-associated variants exist outside of the exome in the non-coding regions of the genome.[12, 13] Additionally, the study does not account for structural variation which, as the authors acknowledge, accounts for roughly 25% of CHD associated with a genetic syndrome[14] and 10% of sporadic CHD.[15]

The authors also acknowledge an often unrecognized limitation of WES-based studies, which is the approach to genetic discovery through a Mendelian lens to uncover singly causative genetic variants. It has become increasingly apparent that the genetic architecture of complex diseases such as CHD may be polygenic, in which genetic risk is spread broadly across the genome and single variants which do not confer significant risk for disease in isolation may combine with other variants to cause disease. The predictive value of polygenic risk scores depends on precise estimates of risk for individual variants which can only be obtained from large-scale population studies, studies which are challenging to construct for rare and heterogeneous diseases such as CHD. As polygenic risks are described and validated, the tools used in clinical genetic testing will accordingly require further adaptation.

In conclusion, the study by Szot et al. represents an important contribution to the field of cardiac genetics and for diseases with a complex genetic landscape such as CHD by highlighting the need to move away from incomplete gene panels and towards unbiased comprehensive approaches such as WES. They provide an excellent framework that could be adapted to a clinically useful tool for analyzing WES data and identifying likely pathogenic variants. We are fortunate to be practicing in an era in which cost-effective clinical tests for exome and structural variation are available to essentially all patients with CHD in the developed world. Nonetheless, the appropriate use of such tests is limited largely by our understanding of the genetic basis of CHD. By evaluating more patients with comprehensive testing, we will move closer to identifying the primary genetic determinants of risk for CHD in the population and in the clinic.

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Table 1

Commercially Available Gene Panels for Congenital Heart Disease

Company	Panel Name	Number of Genes	Weblink
Baylor (John Welsh Cardiovascular Diagnostic Laboratory)	Panel B: Congenital Heart Disease	117	https://www.bcm.edu/departments/pediatrics/research/labs/diagnostic-testing/testing-services/genetic-testing-disease
Blueprint Genetics	Congenital Structural Heart Disease Panel	28	https://blueprintgenetics.com/tests/panels/cardiology/congenital-structural-heart-disease-panel/
Center for Genomics and Transcriptomics (CeGaT)	Congenital Heart Defects Panel	44	https://www.cegat.de/en/diagnostics/diagnostic-panels/cardiac-diseases/#gene-sets
Center for Genomics and Transcriptomics (CeGaT)	RASopathies Panel	23	https://www.cegat.de/en/diagnostics/diagnostic-panels/cardiac-diseases/#gene-sets
Centogene	Congenital Heart Defects Panel	12	https://www.centogene.com/centogene/centogene-test-catalogue-detail.php?test=NGS&ID=442906fe&search=Panel
Centogene	Heterotaxy Panel	11	https://www.centogene.com/centogene/centogene-test-catalogue-detail.php?test=NGS&ID=e53f87ed&search=Panel&disease=Heterotaxy_panel
Connective Tissue Gene Tests	Congenital Heart Disease NGS Panel	15	http://ctgt.net/panel/congenital-heart-disease-ngs-panel
Fulgent	Congenital Heart Defect NGS Panel	113	https://www.fulgentgenetics.com/congenital-heart-defect
Health in Code	Congenital Heart Diseases Panel	114	http://www.healthincode.com/servicios/genetica-medica/paneles/cardiopatias-congenitas?locale=en_US
Invitae	Congenital Heart Defects and Heterotaxy Panel	89	https://www.invitae.com/en/physician/tests/04201/#info-panel-assay_information
Invitae	RASopathies Panel	18	https://www.invitae.com/en/physician/tests/04151/
MGZ Medical Genetics Center	Congenital Heart Disease	47	https://www.mgz-muenchen.com/multi-gene-panels/panel/congenital-heart-disease-entire-panel.html
Partners HealthCare	Congenital Heart Disease Panel A	3	http://personalizedmedicine.partners.org/Laboratory-For-Molecular-Medicine/Ordering/Congenital-Heart-Defects/CHD-PanelA.aspx