JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
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PUBLISHED BY ELSEVIER INC.

VOL. 64, NO. 23, 2014
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2014.09.048

Targeted Next-Generation Sequencing Identifies Pathogenic Variants in Familial Congenital Heart Disease

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ABSTRACT

BACKGROUND Many genes have been implicated in the development of congenital heart disease (CHD). Next-generation sequencing offers opportunities for genetic testing but is often complicated by logistic and interpretative hurdles.

OBJECTIVES This study sought to apply next-generation sequencing technology to CHD families with multiple affected members using a purpose-designed gene panel to assess diagnostic potential for future clinical applications.

METHODS We designed a targeted next-generation sequencing gene panel for 57 genes previously implicated in CHD. Probands were screened in 16 families with strong CHD histories and in 15 control subjects. Variants affecting protein-coding regions were classified in silico using prediction programs and filtered according to predicted mode of inheritance, minor allele frequencies, and presence in databases such as dbSNP (Single Nucleotide Polymorphism Database) and ESP (Exome Sequencing Project). Disease segregation studies were conducted in variants identified in CHD cases predicted to be deleterious and with minor allele frequencies $<0.1\%$.

RESULTS Thirteen potential disease-causing variants were identified in 9 families. Of these, 5 variants segregated with disease phenotype, revealing a likely molecular diagnosis in 31% of this cohort. Significant increases in the number of "indels, nonsense, and splice" variants, as well as variants classified as "probably damaging" were identified in CHD cases but not in control subjects. Also, there was a significant increase in the total number of "rare" and "low" frequency variants (minor allele frequencies <0.05) in the CHD cases.

CONCLUSIONS When multiple relatives are affected by CHD, a gene panel–based approach may identify its cause in up to 31% of families. Identifying causal variants has implications for clinical care and future family planning. (J Am Coll Cardiol 2014;64:2498–506) © 2014 by the American College of Cardiology Foundation.

The most common noninfectious cause of death in infants, congenital heart disease (CHD) affects approximately 6 to 8 per 1,000 live births (1). The majority of cases (~80%) occur as sporadic events, but in some, multiple family members are affected. The high heritability of CHD, estimated to be between 0.6 and 0.7 (2) suggests a strong genetic component and numerous genes have been linked to

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Manuscript received May 22, 2014; revised manuscript received August 18, 2014, accepted September 16, 2014.

syndromic and nonsyndromic forms of CHD (3). The majority of these genes encode transcription factors, although genes in other categories have also been implicated, such as those for structural proteins.

Next-generation sequencing (NGS) enables rapid analysis of large amounts of genetic information. The last few years have produced an explosion of research using NGS technology, especially exome sequencing, for novel gene discoveries in many genetic diseases. Although one cannot dispute the advantage of exome sequencing for gene discovery through its unbiased approach, issues relating to coverage, analysis, and storage of large amounts of data and reporting of incidental findings complicate its use in the clinical setting (4). In comparison, limiting the capture regions to only those known to be associated with the disease(s) of interest mitigates some of these issues, making it a valuable and arguably more suitable approach in the diagnostic arena (5). Coverage, both in terms of depth as well as capture of on-target regions, is far greater, and being able to supplement missed NGS regions with Sanger sequencing further ensures high confidence in the results. Furthermore, issues relating to reporting of incidental findings can be avoided almost entirely as only disease-relevant genes are screened. Numerous disease-targeted gene panels are clinically available, including panels for hereditary cancers (6), metabolic disorders (7), cardiomyopathies (8), and aortopathies (9).

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NGS technology has been applied to the study of both familial and sporadic forms of CHD (10,11). A recent publication by Zaidi et al. (12) used exome sequencing analysis in parent-offspring trios to compare variants in 362 cases with severe sporadic CHD to 264 control subjects. They identified a significant excess of protein-altering, deleterious de novo mutations in known heart-expressing genes (odds ratio: 7.5), implicating several hundred genes that collectively may factor into ~10% of sporadic CHD cases. Although these findings, as well as similar results relating to the contribution of rare and de novo copy number variants (13,14), greatly advance our understanding of sporadic forms of this disease, they do not resolve the cause of most CHD cases. Moreover, this information has little clinical relevance as it is not directly applicable to families with CHD.

In this study, we set out to design the first NGS CHD gene panel comprising genes previously linked to structural heart disease to assess this tool's diagnostic potential in families with multiple affected individuals and an apparently Mendelian pattern of inheritance. Identifying the possible cause of CHD in such families would have significant clinical relevance in terms of recurrence risk estimates and family planning.

METHODS

STUDY PARTICIPANTS. Ethical approval for this study was obtained from the Sydney Children's Hospitals Network Human Research Ethics Committee (approval number CHW/2006/123). Individuals with structural CHD and family histories of CHD with an apparently Mendelian inheritance pattern were selected from the Kids Heart Research DNA (deoxyribonucleic acid) Bank. Families were excluded if CHD cases were unable to be confirmed via echocardiography and/or if they already had a definite or tentative genetic diagnosis. Participating families included a proband with CHD, an immediate family member affected by CHD, and a minimum of 1 other family member (immediate or extended) with CHD (mean number of affected family members = 4). In most cases, the proband was selected for deoxyribonucleic acid (DNA) analysis but in cases where DNA quantity was inadequate, an immediate family member affected with the same or similar phenotype was selected for analysis. A total of 16 CHD cases were included in this study, with the number limited by the size of the targeted NGS capture kit used.

Control subjects. To distinguish between possible disease-causing variants and normal population variations, 15 healthy control subjects from the Kids Heart Research DNA Bank were screened using the CHD panel. All control subjects had no self-reported history of CHD within 3 generations. Principal component analysis was performed to ensure the control subjects and CHD cases were ethnically matched using 646 bi-allelic single nucleotide polymorphism positions that were present in HapMap (International HapMap Project) samples. After projecting our data onto 415 HapMap samples, no separation was evident between cases and control subjects, suggesting appropriate matching. Online Figure 1 shows the results of the principal component analysis.

CHD PANEL DESIGN. Harnessing information from various fields, 57 genes were included in the CHD panel. (For a full list of genes included in the panel, see Online Table 1.) We used the web application Sure Design (Agilent Technologies, Santa Clara, California) to create a custom SureSelect target enrichment library of the 57 selected genes. Target parameters were manipulated to optimize coverage of all coding exons, 5′ and 3′ untranslated regions, as well as

ABBREVIATIONS AND ACRONYMS

CHD = congenital heart disease
CI = confidence interval
DNA = deoxyribonucleic acid
ELN = elastin gene
MAF = minor allele frequency
NGS = next-generation sequencing