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EDITORIAL COMMENT

Next-Generation Sequencing in Congenital Heart Disease

Do New Brooms Sweep Clean?*

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The familial reverberation of heart disease has long captured the curiosity of clinicians. Interestingly, the observation that some "morbid predisposition" is at the heart of the problem in some families was actually made before Mendel's laws were known (1). Indeed, a precise genotype-phenotype prediction of several rare syndromes that are now well-known clinical entities—such as Holt-Oram or Char syndromes—predates the arrival of modern genetic technologies. Over the years, such cases, in particular those with a familial basis, were instrumental for 2 reasons: 1) for epidemiological investigations, they offered a means to estimate the burden of disease attributable to genetic factors; and 2) with the advent of more sophisticated genetic tools, they provided a gateway to dissect the molecular basis of congenital heart disease (CHD).

Over the last decade, tremendous progress was made in the area of genetic technologies. This includes genotyping, calling of copy number variants, and next-generation sequencing (NGS) of the entire coding part of the genome, called whole-exome sequencing (WES). As a consequence, we better understand the genetic basis of numerous forms of syndromic and nonsyndromic CHD (2), although the proportion of cases explained by these studies remains fairly small. For example, copy number variants are thought to explain 5% to 20% of selected CHD, and WES studies implicate de novo mutations in 10% of sporadic severe cases (3–5).

In this issue of the *Journal*, Blue et al. (6) report the results of screening a targeted panel of candidate genes in a cohort of 16 families with a strong history of CHD. The 57 genes of this panel were selected due to their known involvement in human CHD or on the basis of suggestive evidence from mouse studies. The investigators compared these sequencing results to those from 15 healthy control subjects as well as data from the 1,000 Genomes Project (which is sequencing the genomes of a multitude of people to develop a comprehensive resource of human genetic variation) and the ESP (Exome Sequencing Project) of the National Heart, Blood, and Lung Institute, totaling more than 6,000 control subjects. Variants identified as damaging that also segregated with disease in individual families were considered to be disease causing. The investigators thus identified a pathogenic variant in 5 of 16 (31%) of the families studied, at the same time identifying a possible syndromic cause in 3 of these 5 families on the basis of the genes identified. Additionally, they found variants of unknown significance in 25% of the families studied, as well as an excess of several types of rare genetic variation in the familial CHD cohort versus control subjects.

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Although some findings of this study must be interpreted cautiously, there are several grains of truth for the pediatric and adult congenital cardiologist here, as well as questions that will take center stage in the field throughout the coming years. First, and most importantly: how many cases were really resolved using this approach? One could argue that in hindsight, the results in several of the families who received a definitive genetic diagnosis on the basis of this study may have been predicted via clinical examination alone. This includes the family exhibiting a *TFABP2* mutation (Char syndrome), the 2 families

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with *TBX5* mutations (Holt-Oram syndrome), and the family with an *ELN* mutation (supravalvular aortic stenosis). However, in reality, many of these families present minimal clinical phenotypes, exhibit decreased penetrance, or variable phenotypes. These commonly observed phenomena, which hamper rapid and easy phenotype recognition, were also present in this study, corresponding to a clinical reality that can be more easily resolved now using molecular diagnostics. To do this will require careful, step-by-step correlation of genotypes with phenotypes; two processes that contrast starkly at the level of throughput given that NGS technologies generate lists of putative variants of unprecedented scale, whereas phenotyping continues to be a more time-consuming task.

At the same time, we have to keep in mind that only a small minority of CHD follows clear Mendelian transmission patterns, and that the fraction of disease explained in this study shrinks further if the denominator becomes the entire pediatric cardiac patient population. Extrapolating from other NGS studies in complex disease traits, we can anticipate that WES alone will not fully determine the causes of several forms of CHD, at least not with the prevailing analysis paradigm that WES will always detect causal coding variants. As a case in point, applying WES to a multiplex family with bicuspid aortic valve and other cardiovascular malformations failed to identify a single strong coding variant, despite a family structure similar to the many families that have greatly facilitated deciphering the Mendelian basis of disease with NGS technologies over the last 4 years (7). Noncoding variants and oligogenic inheritance likely contribute to those cases that are not readily explained by rare genetic variants predicted to have strong effects. The major challenge here will be to provide consistent, standardized distinctions between benign variants and causal mutations, and to develop these approaches into truly personalized tools that ideally solve the vast majority of cases at genetic level: only pathogenic variants will be useful variants.

From a technical standpoint, WES has already superseded targeted gene panels and affords an unbiased, genome-wide interrogation of coding variants. However, this technology's obvious advantages come at a considerable expense at the analytical level. WES automatically generates many incidental findings; results in the clinical setting are easier to interpret if a clear genetic model can be applied for analysis. Also, WES is inferior to targeted gene panels in terms of depth of coverage, because NGS technologies require that multiple sequencing reads overlap to provide greater confidence of genotype calls. Therefore, WES is currently recommended only as a follow-up when other investigations, including targeted gene panels, fail to yield results. While these technologies mature, studies like the one by Blue et al. (6) are important to establish which types of CHD can be explained by specific gene sets in defined clinical settings. Further development of content, yield, and indications for tractable CHD gene sets will require larger studies, ideally with biological samples and medical history for family members. When such information is available, genetic models can be applied to facilitate interpretation of sequencing results: for instance, for sporadic, severe phenotypes, analysis would be performed assuming a de novo mutation, whereas studies like the one by Blue et al. (6) would require cosegregation of alleles with the phenotype (4).

The current study also raises the question whether pediatric cardiology has the resources to deal with the progress made in the genomics arena. Historically, our field has progressed more through a mechanical than a mechanistic paradigm. This study is 1 of several that now shift this stance and point toward the need of properly trained pediatric cardiologists with a knowledge base and procedural competence commensurate with the possibilities the new technologies afford. Genomic tools and insights are evolving rapidly and fellowship programs and continuing medical education must adapt quickly to these realities. Beyond the training of candidates for investigative careers, core curricula must change rapidly to keep pace with the progress in the genomics arena and to fulfill its promises of improved patient care.

Finally, will results from this study change patient management? For several patients in the study presented here, the results are already actionable. Mutations in *TBX5* may influence the lifetime risk for atrioventricular block, and carrier status for a highly penetrant causal mutation offers insights into recurrence risk. Related examples are diagnosis and care for patients and families with heritable arrhythmias and cardiomyopathies, which have undergone significant changes with the advent of genetic diagnoses, or Marfan syndrome, where novel therapeutic targets have emanated from mechanistic insight (8). As a "new broom," NGS is rapidly becoming a very important tool to uncover those leads that hold the potential to improve patient care.

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