A heritable basis for congenital heart disease was probably first suggested more than a century ago by a report of 3 cyanotic siblings. In the early 1960s, Zetterqvist in Sweden and Pitt in Australia convincingly demonstrated autosomal dominant inheritance in 2 large, extended families who had atrial septal defects (ASDs) and tetralogy of Fallot, respectively. Of note, Pitt ascertained the diagnosis in 215 of 275 descendants of one man spanning 6 generations, many of whom he personally examined! In contrast, it took at least 2 dozen people to identify a missense mutation of α-cardiac actin (ACTC1) as the cause of ASDs in the Swedish family. Their work, published in 2008, used linkage analysis, positional cloning, and Sanger DNA sequencing to diagnose one genetic mutation. Just 8 years later, White, Garg, and colleagues report in this issue their whole-exome sequencing (WES) analyses of 9 families. They identify the likely mutation in 3 families who had either ASDs or patent ductus arteriosus. Their stated goal was to demonstrate the clinical utility of WES in familial congenital heart disease. More broadly, their results highlight how far the field has come and how far it has to go in bringing genetics to pediatric cardiology.

WES evaluates the ≈1% of the genome that encodes proteins. If a person’s disease is caused by a mutation in the exome, the sequence variant should be present in the WES output file of ≈5×10^7 nucleotides. A well-considered experimental design and bioinformatic pipeline are needed to filter the most plausible candidates from thousands of irrelevant variants. The investigators began by selecting families that demonstrate Mendelian patterns of a specific congenital heart defect, such as tetralogy of Fallot. Whether requiring the same defect in every affected family member increases the chance of finding a mutation is uncertain. Mutations of the same gene are generally associated with pleiotropic cardiac phenotypes due at least in part to the effect of modifier genes. Variants that were shared by all affected members of a family and that fit the expected inheritance pattern, for example, autosomal dominant or homozygous recessive, were then passed to the next filtering step.

Close relatives share a large number of irrelevant variants, so common variants were excluded from further analysis. Common was defined as occurring in >1% of the general population. The filter relies on the premise that negative selection eliminates variants that have a deleterious effect on reproductive fitness. The assumption is reasonable, although the optimal threshold could be higher or lower than 1%. Deleterious variants that are associated with incomplete penetrance or mild defects would likely be more frequent than ones that have strong or severe effect. The population frequencies of the variants that the authors found for familial ASD and patent ductus arteriosus were <1 in 100000. Interestingly, our back-of-the-envelope calculations suggest that this observed frequency roughly explains the incidence of ASD and patent ductus arteriosus in humans, ≈560 per million live births, assuming a largely monogenic basis for the defects, complete penetrance, and ±100 relevant genes.

Next, the authors selected variants among 69 genes known to be involved in the pathogenesis of human congenital heart disease. The number of genes is certain to be higher. One recent analysis suggested 392; many more genes remain to be discovered. The narrow focus on 69 genes provides a conservative estimate of the diagnostic yield, 33%, if WES were applied in the clinical setting today. Inclusion of novel genes should increase the diagnostic yield significantly.

The last filter selected variants suspected to have a deleterious effect on protein function. The establishment of pathogenicity is arguably the most challenging scientific problem and most practical clinical question. Mutations that cause premature termination of the protein sequence, a frameshift, or disruption of a splice site are likely to be deleterious. The effect of a mutation that alters one amino acid in a protein is more difficult to judge. To predict the pathogenicity of a mutation, algorithms typically rely on bioinformatic variables, such as the degree of conservation of the sequence across species and the frequency of polymorphisms in humans. Because no algorithm is perfectly sensitive and specific, investigators typically prioritize variants based on the consensus of several methods. Following this general strategy, the authors identified suspect variants in 3 of the 9 families.

The real challenge is the independent validation of candidate variants. Validation strategies come in 2 flavors. Statistical or dry-lab approaches can broadly implicate variants that affect genes in a common pathway. For example,
a recent, large WES study implicated de novo mutations of histone-modifying genes in the pathogenesis of sporadic, isolated congenital heart disease. In contrast, wet-lab approaches assess the functional effect of a specific mutation in a biological assay. Here, the authors tailored experiments to the gene and its known functions. For example, GATA4 is a transcription factor, so they compared the ability of wild-type and the mutant GATA4 to transactivate a heterologous promoter construct.

The definitive biological assay would be recapitulation of the cardiac phenotype in an animal model that carries the suspect variant. Zebrafish and mouse are the usual model species. Fish do not have a 4-chambered heart, but mutants can be generated relatively efficiently and inexpensively. Mutations affecting cardiac developmental pathways that are shared across species commonly cause an analogous phenotype in the 2-chambered fish heart. Mice do have a 4-chambered heart, but mutants cost much more time and money to engineer and study. In this regard, the recently developed CRISPR-Cas9 genome engineering method significantly lowers the barrier to validation. We suggest that the analysis in engineered mice of a relatively large number of variants discovered by WES may be worthwhile to test the underlying bioinformatic assumptions. Knockout mouse models, which can implicate the function of a gene in cardiac development, do not properly address the effect of missense mutations that can cause a gain or loss of function or affect one of several functions of a gene.

The genetics of congenital heart disease has come a long way since Zetterqvist, Pitt, and others established a heritable basis for congenital heart disease. Mutations affecting cardiac developmental pathways that are shared across species commonly cause an analogous phenotype in the 2-chambered fish heart. Mice do have a 4-chambered heart, but mutations cost much more time and money to engineer and study. In this regard, the recently developed CRISPR-Cas9 genome engineering method significantly lowers the barrier to validation. We suggest that the analysis in engineered mice of a relatively large number of variants discovered by WES may be worthwhile to test the underlying bioinformatic assumptions. Knockout mouse models, which can implicate the function of a gene in cardiac development, do not properly address the effect of missense mutations that can cause a gain or loss of function or affect one of several functions of a gene.

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**Key Words:** Editorials  ■  congenital heart disease  ■  family study  ■  gene mutation  ■  exome