Next-Generation Sequencing for Clinical Diagnostics

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Yang et al.\textsuperscript{1} report in the \textit{Journal} the results of using whole-exome sequencing to make a molecular diagnosis in 62 of 250 cases (25%) sequenced by the clinical sequencing laboratory at the Baylor College of Medicine, which is accredited by the College of American Pathologists and certified through the Clinical Laboratory Improvement Amendments program. This level of success is a marked improvement over that obtained by testing single genes or gene panels currently used in standard care. The exome is the portion of the genome known to encode proteins (approximately 1% of the human genome\textsuperscript{2}). Whole-exome sequencing has the advantage of reduced cost and analysis of a much smaller data set than that obtained by sequencing the entire genome. The other advantage of whole-exome sequencing is that current knowledge about functional genetic variation is largely limited to the coding sequences of genes (i.e., exons).

Previous reports have shown the usefulness of whole-exome sequencing\textsuperscript{3-6}; however, the large collection of clinical cases reported by Yang et al. provides a multifaceted view of the use of next-generation sequencing in clinical practice. Conceptually, the strategy of next-generation sequencing is simple: read (sequence) the DNA, identify variations in the DNA, compare these variations against a catalogue of disease-causing variants, and decide whether a diagnosis can be confidently made. The 25% success rate described by Yang et al. is consistent with that described by others,\textsuperscript{6,7} which suggests that the diagnostic yield is limited by the capabilities of current tools and the extent of our knowledge.

There are several types of limits: the authors note a lack of knowledge related to mendelian disease, regions of the genome that are missed by sequencing, and a lack of noncoding data as possibilities. The major limitation on making a diagnosis is very likely to be a lack of knowledge about what genes and variants do, resulting in a very large number of variants of uncertain significance. Any clinician who has received a clinical laboratory report from an exome-sequencing laboratory will have noted that variants of uncertain significance constitute the largest category of variants in many reports.

Indeed, a major challenge of interpreting genetic sequence is the determination of whether any given variant is pathogenic.\textsuperscript{8,9} The American College of Medical Genetics and Genomics (ACMG) has recommended a series of interpretative categories and definitions of sequence variations.\textsuperscript{9} Five categories are commonly used to classify variants: known pathogenic variants, those that are likely to be pathogenic, those of unknown significance, those that are likely to be benign, and benign variants. These categories are not the problem. The problem is how to determine which variants are pathogenic and which are benign, particularly for rare diseases that may be unique and found in a single patient. Data sharing will help, but the development of other criteria related to function is going to be critical and not necessarily easy. For example, once mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR) were identified as a cause of disease, many CFTR variants were described. Indeed, nearly 2000 CFTR variants have been described, but only a few dozen have been shown to cause cystic fibrosis,\textsuperscript{10} leaving in question the pathogenicity of many other CFTR variants. Approximately 12% of the CFTR variants that are known to be pathogenic are associated with heterogeneous clinical phenotypes, illustrating the need for detailed clinical workups.

What data should be returned to patients and ordering physicians? This is also a gray area. Recent guidelines developed by the ACMG for returning secondary (incidental) findings have accelerated the debate about who should determine what data are returned.\textsuperscript{11} The factors of patient autonomy, physician liability, and clinical-laboratory guidelines can be at odds with one another and will require additional study.

One of the most encouraging findings of Yang et al. is that 126 of the 129 claims sent to insurance companies were paid. I would like to know what percentage of the bill was paid, but nonetheless, the percentage of claims paid is im-
Spinal Bracing in Adolescent Idiopathic Scoliosis

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Before the introduction of the poliomyelitis vaccination, paralysis due to poliovirus infection was a leading cause of spinal deformity in children and young adults. The severe, stiff spinal deformities associated with poliovirus infection were complicated by pulmonary dysfunction; spinal bracing, which was often ineffective in correcting the deformity or preventing the progression of scoliosis, also exacerbated restrictive pulmonary disease, pulmonary hypertension, and cor pulmonale. Today, coronal spinal deformity in children and young adults is nearly always adolescent idiopathic scoliosis: a flexible, three-dimensional, spinal curvature affecting primarily girls, beginning in adolescence, and very rarely leading to clinically significant cardiopulmonary disease. When children with idiopathic scoliosis reach skeletal maturity with a primary curve of less than 40 degrees, the curvature usually stabilizes and there are few or no long-term clinical effects. However, if the primary curve progresses to more than 50 degrees during adolescence, consequences include not only frequent cosmetic dissatisfaction but also increased risks of progressive deformity.

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