A lot of nexts

Next-generation sequencing, databases, and neurologists

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Whole-exome sequencing (WES) was featured prominently in the first issue of *Neurology® Genetics*,[1] and this technology again contributed to identification of a homozygous *AMPD2* mutation as the cause of a neurodevelopmental syndrome published in this issue.[2] A different approach to analysis of a large number of exons is described by Tian and collaborators[3] and discussed by Bönnemann and colleagues,[4] both in this issue.

Tian et al. examined all coding exons and at least 20 bp of flanking intronic sequences of 236 genes implicated in neuromuscular disease. In contrast to WES, their technical approach did not attempt to capture all human exons, but instead relied on targeted capture of 4,815 coding exons solely in those 236 preselected genes. As discussed in detail in the accompanying editorial,[4] this approach comes with distinct advantages and disadvantages. Targeted capture of a smaller set of exons ensures higher and more even coverage, a technical problem that still plagues WES. They achieved average coverage of their targeted exons more than an order higher than commonly seen in WES. Despite the ability to detect sequence substitutions and small deletions very well using this approach, this technology is still challenged in the detection of larger scale copy number variations or repeat mutations.

Even with the focus on known neuromuscular genes, Tian et al. were successful in uncovering the underlying mutations in >80% of their 39 cases. Although the precise percentages will vary with phenotypic selection of patients, geographic region, ethnicity, and practice setting, we will see these technologies move into the practice setting rapidly.

As Foley et al.[4] point out, application of these novel technologies to clinical practice will be limited less by technical challenges than by challenges in the interpretation of findings. They go through 5 patient-based scenarios to illustrate these points. Of their 5 scenarios, the greatest challenge is scenario 4, the interpretation of variants of unknown significance. Unfortunately, variants of unknown significance are too often interpreted by clinicians and patients alike as the disease-causing mutation, when in fact it may just represent a rare or even private benign DNA variant.

Although several databases have been available in the past to check allele frequencies and help distinguish rare benign variants from disease-causing mutations, the Exome Aggregation Consortium (ExAC) has now provided a framework to understand exome variation at a scale that is unprecedented.[5] The newest release (version 0.3) of the ExAC data and browser includes data from 60,706 individuals spanning >9M sites and >10M variants. Surprisingly, nonsynonymous substitutions occurring at a frequency of less than 1 in 100,000 alleles are quite common in human genomes. It is important to recognize, however, that information contributed to ExAC is from individuals who have survived into adulthood, but not from normal controls. Thus, rare alleles causing late-onset neurologic diseases are certainly present in the database (just check out variants in *LRRK2* or *SCNA*).

In addition to many other mutations, Tian et al. report a heterozygous $CACNA1S^{Arg1554Trp}$ change in a patient with a periodic muscle disease. This amino acid substitution allele is found in 20 of 121,404 (haploid) exomes. Is it a rare benign variant or are the 20 individuals included in the ExAc database nonpenetrant or very mildly affected? In the end, the neurologist will play an important role in conjunction with the medical geneticist to interpret genetic variation in the context of the patient's phenotype and family history.[3,6] We need to make sure that our next-generation neurologists receive the appropriate training to deal with these complexities.

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