Targeted NGS or exome? Consider the clinical context

William Check, PhD

December 2017—American writer Maile Meloy published a short story collection in 2009 titled *Both Ways Is the Only Way I Want It*. Molecular pathology laboratory directors faced with the variety of next-generation sequencing diagnostic panels might feel similarly. As the main character in Meloy's title story asks, "What kind of fool wanted it only one way?"

However, the clinical laboratory is much different from Meloy's fictional world. Depending on patient mix and the lab's financial and technical resources and personnel expertise, selecting one NGS platform rather than another can be the wisest move. "There are benefits to both targeted NGS and more comprehensive exome and genome analyses. The decision to lean one way or the other is mainly dependent on the clinical context," says Linnea M. Baudhuin, PhD, co-director of the Personalized Genomics Laboratory at Mayo Clinic, Rochester, Minn. Dr. Baudhuin gave a presentation at this year's annual scientific meeting of the American Association for Clinical Chemistry, in the session, "Pros and Cons of Targeted vs. Comprehensive Genetic Testing," and spoke with CAP TODAY recently.

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She presented two cases from her own laboratory to illustrate the benefits and shortcomings of targeted NGS testing versus the use of exomes. She focused on inherited (germline) disorders, such as cardiomyopathies and aortopathies, rather than somatic diseases. For tumor (somatic) sequencing, targeted NGS is generally the best approach at this time, she said, because of the need for a fast turnaround and greater depth of coverage.

In case No. 1, Dr. Baudhuin described a 35-year-old male commercial airline pilot who came in for a routine physical examination, during which an abnormal ECG with prolonged QTc (470 to 560 msec) was discovered. Neither the pilot's physical history nor his family history was remarkable. He was healthy with no unexplained syncope. In 2011 an expert consensus group recommended genetic testing for LQTS in such patients.

In Dr. Baudhuin's laboratory, an LQTS targeted panel for 13 genes involved in LQTS identified a likely pathogenic variant in *KCNH2*. In comparing this with an off-the-shelf exome reagent, she noted that exome analysis would not have detected this variant in this patient, due to poor coverage in this region of the gene. Exome sequencing is prone to incomplete coverage over the exome, particularly in regions with highly repetitive stretches of DNA. In fact, Dr. Baudhuin said, "There's no such thing as 'whole exome' sequencing. Most exome sequencing platforms really cover only 85 to 90 percent of the exome." Data have shown that 50 percent of exons have lower than 30× average coverage. "We are looking for at least 40× coverage for the most part," she said. Thus, because of low-coverage regions, "exome sequencing can miss critically important regions or variants."

Over time, Dr. Baudhuin predicts, NGS will move from targeted panels to exome sequencing to genome sequencing. But cost must come down, exome reagents have to provide better coverage, and variant classification and interpretation tools and skills must become more effective.

Dr. Baudhuin presented as an example the evaluation of a 270-gene targeted panel for primary immunodeficiencies (PID) compared with exome. “We wanted to see whether we could implement a PID panel from an exome reagent compared to a targeted capture panel that we designed,” Dr. Baudhuin tells CAP TODAY. “What we found is that we need our own panel.”

In this work two of her colleagues compared two Agilent products: a specific panel limited to preselected genes (SureSelect Custom) versus a broad-coverage exome capture reagent consisting of about 4,600 genes (SureSelect Focused Exome). Focused Exome missed several regions due to low depth of coverage. Overall, five percent of variants detected in targeted panels were not found via exome sequencing, which also missed homologous regions (supplemental Sanger sequencing was used for those in the targeted panel). Further, the targeted panel was advantageous in terms of time, cost, and ease of interpretation.

“We anticipate having something like Focused Exome in the future,” Dr. Baudhuin says. “Eventually we will need to. As associations with new genes get discovered, we will want to add them to our disease-focused panels. Exome panels already contain these genes that may be extraneous now but will later become pertinent.”

Using one reagent would provide an advantage in efficiency. “Otherwise we have to go through validating and revalidating when new medically relevant genes are discovered. With an exome reagent, however,” she says, “we only need to validate once and unmask genes of interest.” Right now using one broad exome reagent still has many coverage issues: “We have to boost genes and regions that are not adequately covered, which could become unwieldy.”

A second exome reagent, Medical Exome, is noncommercial and publicly available. Like Focused Exome, it has about 4,000 genes. It was developed by an academic consortium under the auspices of the National Center for Biotechnology Information.

Similar to their work with primary immunodeficiencies, Dr. Baudhuin's laboratory evaluated Focused Exome coverage of about 130 cardiovascular disease genes. "Many genes had less than 40× coverage" with the large exome reagent, Dr. Baudhuin says, adding, "This is unacceptable."

Their initial survey was done on the Illumina HiSeq 2500. "We will look at it on the HiSeq 4000, which should get us better coverage, but we don't know for sure if it will," Dr. Baudhuin says. Like a race team souping up its car's engine, Dr. Baudhuin and her colleagues can enhance the panel's performance: "We may have to boost this reagent for regions that don't have high enough coverage, and we can backfill some regions with Sanger sequencing if we still have unacceptable coverage." But this highlights one of the issues with the exome when the aim is to look at specific genes. Whether these performance modifications will enable the large exome panel to take the checkered flag remains an open question.

For case No. 2, Dr. Baudhuin described a two-year-old female Caucasian with developmental delay/regression, autism spectrum disorder, seizure disorder, hearing loss, spina bifida, and other abnormalities. These features did not suggest a specific diagnosis. Neither parent was affected. All previous genetic tests were normal.

Dr. Baudhuin reviewed the decision process for choosing a direct genetic test. A single-gene test would probably not be helpful because the multisystem nature of this child's condition potentially implicated many genes. A multigene panel could be helpful, although three such panels could potentially be used—one each for developmental delay, hearing loss, and epilepsy, with overlapping genes among the panels. For complete coverage, all of these panels would have to be done. "Collectively, these three tests would probably cost about the same as exome," Dr. Baudhuin said. An exome panel would offer "an enormous number" of candidate genes and the possibility of identifying novel pathogenic genes. Even so, Dr. Baudhuin said, "The exome may miss important gene regions, and we may not receive a definitive answer." In this case, an exome panel was ultimately used to analyze the proband and both parents—a classic trio analysis. "This helps to filter the data to determine where variants are coming from or if they are de novo" and the cis- or trans- nature of recessive variants.

No likely causative variants were found. Two possibly causative variants were detected, both heterozygous variants of unknown significance (VUS), and a homozygous variant was found in a gene of unknown significance (GUS). "So was a genetic cause detected with exome sequencing in this case?" Dr. Baudhuin asked. "Yes, no, or maybe? I would say the correct answer would be maybe." The uncertainty, she said, comes from the VUSs and GUSs.

Both variants of unknown significance were inherited from the father. One is associated with an autosomal dominant disorder. "The dad did not have any features of this disorder so it's unlikely that it's causative," she said, but there's a chance it's nonpenetrant in the father. With the second VUS, which is associated with an autosomal recessive disorder, if it is causative of the patient's symptoms, which is unknown, "that would make the patient just a carrier."

The variant in the gene of unknown significance was homozygous, meaning one allele in the girl came from each parent. "Some studies have shown that this gene is involved in a phenotype that overlaps strongly with this little girl's," Dr. Baudhuin said. "But again, it's really just a lot of unknowns here."

Variant classification clearly requires expertise. "Right now we have lab-based genetic counselors in a lot of labs. They spend a lot of their time on variant classification. That was not the case five or more years ago before expanded genetic testing through NGS became so much more widely utilized," she says.

In a case like this one, where the outcome is uncertain, misinterpretation and unnecessary, even harmful actions may be taken if communication with the family isn't clear. "That's the danger of reporting variants of uncertain significance," Dr. Baudhuin notes. "As clear as you try to be in your report, it can be construed as positive in that family. You could get false attribution of risk or non-risk, and they could stop doing important screenings."

Technically, she says, if you can do a panel, you can do an exome. “You need technical expertise to run the test, but if you have personnel and funds, you can do it.” However, she cautions against underestimating the demands of the so-called back end: “Once you get data off the instrument, you need bioinformatics specialists who know how to handle data and filter it, and you need a group of experts to classify variants.” It’s an important and likely underappreciated area, she says. “It also tends to be the most expensive part of the test. It takes up the most time.”

Incidental findings are those that are not related to the patient’s clinical phenotype (there were none in the prior case). The American College of Medical Genetics and Genomics has identified 59 important genes causative of inherited disorders, such as hereditary cancer, cardiomyopathies, or long QT syndromes, which need to be reported to patients when found incidentally.

Incidental findings are more likely with exome sequencing, where thousands of genes are interrogated, but they can occur in targeted panels as well. “For example,” Dr. Baudhuin said, “if you’re doing a targeted panel for ataxia, you could detect a heterozygous variant in the *ATM* gene, which would confer a significantly increased risk for breast cancer.”

“Oftentimes, for incidental findings you’re going to have an opt-out option,” Dr. Baudhuin said. “The genetic counselors will give the patient and their parents the option of getting a report that does not have any incidental findings on it. But if that’s the case, then you have to have two workflows in your laboratory—one for everything and one for the opt-out—and that’s a significant undertaking.” As is the review and interpretation of the 59 genes and the work related to confirming the incidental finding. “But it is recommended that we report incidental findings in probands undergoing whole exome.”

Dr. Baudhuin did not present a case in which exome sequencing proved definitive, but many such cases exist, she says. “Just look at the yield for diagnostic odyssey.” Between 25 percent and 30 percent of such cases—cases in which extensive testing has not revealed an underlying basis for a genetic condition—are solved by exome sequencing, according to published results (Lazaridis KN, et al. *Mayo Clin Proc.* 2016;91[3]:297–307; Lee H, et al. *JAMA.* 2014;312[18]:1880–1887; Yang Y, et al. *N Engl J Med.* 2013;369[16]:1502–1511).

Diagnostic odyssey most often occurs in a patient who has a nonspecific phenotype. “We have a good proportion of cases with nonspecific phenotypes,” she says. For this reason, more practices seem to be moving to exome sequencing as a frontline test. “A few years ago it was recommended that all possible genetic testing be done before considering exome,” Dr. Baudhuin says. “But I think now people are choosing exome sequencing more readily than they were before.” In her view, this movement comes from the perspective that exome sequencing in the right patient population is a good test. “There are many complex developmental disorders in which it makes more sense to do exome testing first,” she says, “rather than ordering a bunch of panels.”

Over the long term, next-generation sequencing will move from targeted panels to exome sequencing to genome sequencing, Dr. Baudhuin predicts. “We need a few things to happen for our practice to move in that direction.”

First, the cost must come down. The cost of the large targeted panels ranges from $1,000 to $5,000, with most falling into the $1,000 to $2,500 range, she says. For clinical exome sequencing, the range is $5,000 to $10,000. Second, the technology has to improve such that exome reagents provide better coverage. Third, variant classification and interpretation tools and skills have to become more effective and streamlined. “When those things come together,” she says, “we will see a lot more exome and genome testing because they are so much more encompassing.”
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William Check is a writer in Ft. Lauderdale, Fla.