In next-gen sequencing, panel versus exome

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January 2016—As next-generation sequencing takes its place in clinical laboratory medicine, a difference is developing between its use when there is a defined phenotype, as with hereditary oncology syndromes or hereditary cardiovascular disorders, and its use in diagnosing hereditary developmental disorders. In oncology, targeted panels remain the optimal mode of application. In medical genetics, NGS is moving beyond panels to whole exome sequencing and perhaps soon even to whole genome sequencing. A recent Association for Molecular Pathology workshop on the clinical utility of genomes versus exomes versus targeted panels spotlighted how decisions are being made for one or the other in diagnosing inherited disorders.

Heidi L. Rehm, PhD, an associate professor of pathology at Brigham and Women's Hospital and Harvard Medical School, addressed the laboratory component of the topic. Her main message: In genetic testing for Mendelian disorders, it is not a question of panels or exomes. Rather, the challenge is how to combine the two to support the most useful services. To do that, Dr. Rehm told CAP TODAY in an interview, "we may actually decide to offer defined panels which we interpret off of an exome platform where the rest of the data are available for reflex testing or research."

Dr. Rehm says it is much less costly to offer many rare disease tests by doing them on the same technical platform. "We are moving in that direction. To do that," she says, "we have to ensure a very high-quality exome backbone and ensure that clinically relevant genetic regions are covered fully for each indication." Much of her presentation was about how to achieve that.

Dr. Rehm, who is also director of the Partners HealthCare laboratory for molecular medicine and clinical director of the Broad Institute Clinical Research Sequencing Platform, says she and colleagues are likely to move to whole genome sequencing in the future. "It is inevitable. The quality of data coming from PCR-free whole genome sequencing is impressive. We have data at the Broad on this." She doesn't know yet when the transition will take place: "It is a cost question. My guess is within the next two years." Balancing genome versus panel testing will entail the same strategy as exome versus panel testing.

Robert Nussbaum, MD, chief medical officer at Invitae, speaking at the same workshop, addressed the question, How is a clinician to decide? Dr. Nussbaum was a clinician for many years and is now Holly Smith professor of medicine emeritus in the Department of Medicine and Institute for Human Genetics, University of California, San Francisco. He sounded a Heraclitean note. "Technologies are in flux. I can stand up here and say anything I want," he said, "but it will all change by next year. This is more of a problem for clinicians because they don't fundamentally understand the technical aspects of sequencing."

Dr. Nussbaum focused on patient choice and cost as driving forces for choosing between exomes and panels.

Workshop organizer D. Brian Dawson, PhD, of Mayo Clinic, says the views the speakers presented were "more because of the structure of what I had asked them to present. I asked them to speak on areas that we are all dealing with right now: Is it better to do targeted panels or to use a whole exome sequencing backbone?"

"From that standpoint, they presented strengths and weaknesses of both approaches"—which is helpful to hear for those who are just getting into whole exome sequencing or targeted panels using next-generation sequencing, says Dr. Dawson, co-director of Mayo's molecular genetics laboratory and an associate professor of laboratory medicine and pathology and medical genetics.

In choosing between panels and whole exome sequencing (WES) for rare inherited disease testing, several factors are important, Dr. Rehm told attendees. First is certainty of diagnosis. Exome analysis is best suited

to conditions with multiple clinical features that have no clear diagnosis, such as neurodevelopmental and other neurological presentations. Gene panel testing is best suited to patients with a clear diagnosis and for which panel testing yields a reasonable detection rate, such as cardiomyopathy, retinal disease, and hearing loss.

She showed data from two 2014 publications, one from Baylor and one from UCLA, on clinical findings with WES among patients referred for genetic identification of rare Mendelian disorders (Lee H, et al. *JAMA.* 2014;312:1880–1887; Yang Y, et al. *JAMA.* 2014;312:1870–1879). Both groups showed that "Exome testing is a good primary test for cases with unusual presentations for which no panel is available or for which currently available tests are very low yield," Dr. Rehm tells CAP TODAY. For pediatric neurologic presentations, for example, "There is no really good targeted panel that gives you a high-yield answer."

A second factor in choosing a testing modality is the analytical performance of exomes versus panels. Completeness and depth of coverage are critical for good analytical performance. "Whole exomes are not whole," Dr. Rehm says. Using 51 genes for inherited cardiomyopathy as an example, analysis in the Partners laboratory for molecular medicine showed that with a panel, less than one percent of exons were not fully covered (0.7 percent of base pairs had less than 20× coverage), in contrast to 15 percent of exons not being fully covered with standard exome capture (3.7 percent base pairs had less than 20× coverage). However, with probe supplementation of its hybrid capture, Dr. Rehm's laboratory was able to improve its exome platform coverage to less than one percent of exons requiring Sanger fill-in, enabling panel-based analysis of an exome backbone to be a high-quality, cost-effective first approach for many indications.

Dr. Rehm

Another consideration is whether the type of variant known to cause the disorder is reliably detected by next-generation sequencing, which is the underlying technique for both panels and exomes. "Most labs supplement" NGS assays, she says. Typical supplements are Sanger sequencing as fill-in for incomplete coverage, add-on triplet expansion assays for such conditions as Fragile X and spinocerebellar ataxias, inversion/breakpoint assays for factor VIII, and add-on copy number variant assays for genes known or likely to be subject to deletions and duplications. Copy number variants still present a challenge to NGS, she notes.

Analysis of results with the hearing loss panel illustrates some of these problems. "The two most common genes require supplemental testing," one for a noncoding deletion and one to differentiate pseudogene variants, Dr. Rehm says. In addition, 30 percent of pathogenic variants are copy number variants.

Detecting all clinically relevant variants can require a complex algorithm, as illustrated in an article on detection of germline mutations in the DNA mismatch repair gene PMS2 that underlie Lynch syndrome (Fig. 1 in: Li J, et al. *J Mol Diagn.* 2015;17:545–553). Fortunately, Dr. Rehm says, "We usually don't need such a complex algorithm for most diseases."

Gene homology can also confound NGS-based methods. A pathogenic gene can be homologous with a pseudogene, which is the case with PMS2. In other conditions there may be more than one functional gene, such as hereditary cardiomyopathy and spinal muscular atrophy (SMN1 and SMN2). Dr. Rehm's colleague, Diana Mandelker, MD, PhD, has identified 286 homologous genes of medical relevance.

A fourth consideration in test choice is clinical experience. A hearing loss panel produces an inconclusive result in almost 60 percent of cases. In one patient Dr. Rehm described, a known pathogenic variant was found but it is not associated with profound hearing loss, which the patient had. Dr. Rehm recommended reflex to broader panel

testing, which identified two novel pathogenic variants in MYO7A, a gene for Usher syndrome (deafness and retinitis pigmentosa). Diagnosis of Usher syndrome was confirmed through electroretinography testing. A laboratory director must be not only experienced enough to recognize this type of situation but also willing and able to pursue novel genes or variants.

Secondary findings can also present problems. “In our experience a lot of physicians don’t want to deal with secondary findings. It’s information irrelevant to the care of their patient for the indication they showed up with. On the other hand,” she says, “that clinician perspective is not necessarily in the best interest of the patient, who may very well want or need to know secondary findings. It’s a bit of a conundrum sometimes to know how much information to return.” In practice, she adds, “In individual cases we rely on the physician to order the best test for each patient.”

Cost is an important issue. Dr. Rehm noted that insurers may cover panel tests but not exomes.

A further complication is that new disease genes are being discovered at a steady rate. Over the past four years, 915 new disease genes have been reported (Chong JX, et al. *Am J Hum Genet.* 2015;97:199–215). “Updating and revalidating panel tests is costly and time-consuming.”

“Can we make it simpler as a lab industry?” she asks. This is possible, in her view, using panels on an exome backbone. In this approach, the technical platform is the same for all tests but the genes analyzed and reported are distinct. The entire exome is sequenced, but the analysis pipeline only returns variants in the genes relevant to the condition for which the clinician ordered testing. Tests are offered to physicians as classic disease panels. Such an approach is less costly to validate and quicker to update through analysis pipeline modification. On the negative side, coverage may be lower per gene and variable costs (per test) are higher, though some of this may be offset by lower fixed costs.

Dr. Rehm showed that incomplete coverage with WES can be largely overcome by adding more capture probes. Coverage rose from less than 95 percent to more than 99.5 percent on several of her laboratory’s exome-based panels.

Over several months in 2014, Dr. Rehm and her colleagues compared WES to panels for 160 patients with genetic sendouts. They assessed clinical sensitivity and cost of the physician-ordered test versus using a panel-based analysis of their exome assay.

Results were mixed. There were a number of cases where exome analysis could improve clinical sensitivity and costs would be lower. However, copy number detection was critical for a number of tests ordered, and the added clinical sensitivity provided by exome assay did not save costs for small panels covering the most common causes.

Dr. Rehm says they are moving some panel tests to exome backbone near term (for example, cardiomyopathy, pulmonary) but leaving others on panels (such as hearing loss due to CNVs and RASopathies due to rapid turnaround time requirements). Meanwhile, they are validating their exome algorithm for detecting CNVs.

For panels performed on an exome backbone, Dr. Rehm does not think the recommendation of the American College of Medical Genetics and Genomics to report secondary findings on 56 disease-related genes applies. “When a clinician orders a panel test, such as an 80-gene panel for hearing loss, in my opinion you are only ordering analysis of genes on that panel. So there is no issue of secondary findings.” Where it is relevant, she says, is if a physician orders a full exome test and the whole exome is sequenced and analyzed.

Dr. Nussbaum agrees that a defined phenotype is needed to consider using a panel. He agrees, too, that exome or genome sequencing is preferred for undiagnosed disorders and for resolving a diagnostic odyssey.

Dr. Nussbaum

He showed additional data for the efficacy of panels in complex disorders. A publication from Ambry Genetics using WES in 500 unselected families with undiagnosed genetic conditions showed that a positive or likely positive result in a characterized gene was identified in 30 percent of patients (152/500) (Farwell KD, et al. *Genet Med.* 2015;17:578–586). Data from this study also underscored the importance of keeping up with newly identified pathogenic genes: Genes characterized within the past two years accounted for 23 percent of positive findings. "Rapid progress in human genetics means panels are often chasing a moving target," Dr. Nussbaum said. Moreover, adding genes to a panel means revalidating it.

Turning to the issue of secondary findings, Dr. Nussbaum showed results from two surveys of patients undergoing diagnostic WES. In one study, 187 of 200 individuals (93.5 percent) chose to receive one or more categories of secondary findings. In a second study, parents of children undergoing WES were most receptive to learning about variants that predispose to disorders treatable or preventable in childhood.

Dr. Nussbaum noted that dilemmas regarding secondary findings are mostly avoided with panels, which return few, if any, such results. Of course, additional information that parents might want is also not available.

"I've been talking to a lot of payers lately," Dr. Nussbaum said. "Payers are concerned about generating downstream costs from secondary findings." Not surprisingly, payers are most comfortable with panels that stay within guidelines for specific indications. As Dr. Rehm had said, payers are less comfortable with exomes because of their higher expense.

Invitae's method for genetic analysis is intermediate in complexity, between isolated panels and WES with reporting of only indicated genes. Exons for all genes for all panels that Invitae offers—about 600 genes—are captured in one step. However, in any given assay the company investigates and annotates only those genes relevant to the individual patient's condition. "We sequence exons for all genes we capture," Dr. Nussbaum explained, "but we only work up the relevant ones." As for depth of coverage: "If we don't have at least 50× coverage of all bases, we redo the sequencing."

Dr. Nussbaum said in an interview that clinicians are frustrated over lack of consistency. "The genetic testing industry is terribly fragmented, with many different billing policies, as well as dozens and dozens of payers, each with different and inconsistent policies on coverage of genetic testing. When I see a patient and decide a genetic test is warranted, either I or one of my colleagues has to spend as much or more time than I spent with the patient figuring out what insurance the patient has, what their coverage policy is, what the out-of-pocket cost will be to the patient, and, often, ultimately coming to the conclusion the patient is either going to have to pay an exorbitant bill or forego what I think is a valid, warranted genetic test." He ends up having to tell the patient, "I think this is medically necessary but you can't have it unless you pay thousands of dollars."

"There is no other area of clinical medicine that has to deal with this," he says.

Mayo Clinic for now is mainly developing targeted panels for a variety of disorders, Dr. Dawson says. Mayo currently offers whole exome sequencing through the Center for Individualized Medicine but is preparing to offer whole exome sequencing in the Department of Laboratory Medicine and Pathology through the efforts of a team led by Matthew J. Ferber, PhD, and Eric W. Klee, PhD. "We will start with trio analysis, mainly for diagnostic odyssey cases," Dr. Dawson says. "We have looked at trying to develop panels out of whole exome sequencing. We were just not real happy with depth of coverage." He says there may be a way to do that down the line.

“We are discussing it.”

Where they are offering subpanels based on large gene panels, the Mayo laboratory is not including the genes for which the ACMG recommends reporting secondary findings unless those genes are already a part of the targeted panel due to the diseases of interest.

In Mayo’s molecular genetics laboratory, a minimum coverage of 100× is currently the goal for inherited disease target panels. “If we go lower than 100×,” Dr. Dawson says, “we need to be transparent about that to our clinicians.” Where mosaicism is known to be a possible cause, high depth of coverage is mandatory. “Some of the literature suggests that in some cases even small insertions and deletions need depth of coverage greater than 100× to detect them routinely.”

Commenting on cost, Dr. Dawson says, “Certainly with large panels we know exactly what genes we will be looking at, and we know the cost of the bioinformatic component up front.” With whole exome sequencing, that can change during the investigation. Then, too, “Some targeted panels are getting CPT codes. In that situation certain genes must be included, so hopefully you know about that ahead of time.”

Limitations of the chemistry are preventing them from moving to whole exome or whole genome platforms. “What ends up happening,” he says, “is that the more sequences you do, the more the depth of coverage decreases. For a targeted panel, you can have much higher depth of coverage for specific genes of interest. And depth of coverage impacts our ability to detect genetic alterations.”

On the optimistic side, Dr. Dawson adds, “We are getting pretty close to reliably detecting copy number variants with some algorithms.” The methods that have been optimized for coverage, however, may not be the best for determining CNVs. “Better algorithms plus enhanced sequencing should allow us to start calling copy number variants soon.”

Dr. Biesecker

Leslie G. Biesecker, MD, chief and senior investigator in the Medical Genomics and Metabolic Genetics Branch of the National Human Genome Research Institute, National Institutes of Health, cautions about so-called virtual panels. In these panels, the entire exome is examined but only genes related to an individual patient’s condition are reported. “The clinician may say, ‘I ordered a virtual panel on intellectual disability, so there is no reason to look at colon cancer susceptibility genes,’ even though they are on ACMG’s list of variants to be considered as secondary findings,” says Dr. Biesecker, who attended the AMP workshop and spoke with CAP TODAY recently. “I think this will turn out badly. It won’t be taken kindly to by patients who suffer adverse outcomes in the future because of the absence of that information.” (Dr. Biesecker explicitly excludes from this caution the approaches of Dr. Rehm and Dr. Nussbaum.)

Ultimately, interpretation is the real challenge. Even with the best-designed methods it is still possible for laboratories to disagree on the interpretation of variants. “There can be legitimate reasons for labs to come to somewhat different conclusions about a variant,” Dr. Biesecker says. “It is overly simplistic to say that everyone’s categorization should be the same. There will be edge cases, and it is reasonable that labs might differ in interpretation of those cases.”

It is important for laboratories contemplating NGS for Mendelian disorders to understand that there is nothing

automatic about this process. “There is an assumption that you can turn the NGS crank and variants will fall out and be apparent,” Dr. Biesecker says. “However, there is a lot of genetics and genomics behind that process. And that level of expertise is essential to the process. Many elements can be systematized and automated. On the other hand, the interpretive part will always be essential, and it is critical that labs have the expertise that equips them to make that determination.”

Dr. Rehm said it’s now known that variants were not always interpreted in the most accurate and consistent manner. She supported this with reference to data from ClinVar showing that of the nearly 13,000 variants in ClinVar with at least two submitters, 17 percent were interpreted differently (Rehm HL, et al. *N Engl J Med.* 2015;372:2235–2242). The problem is that it’s difficult to generate a standard for “truth.”

“If I want to compare how well my lab is doing in interpreting variants and making calls, there is no easy way for me to do that,” she says. “If I want to know whether my NGS calls are valid, I can compare them to Sanger data. But there is no gold standard for the interpretive part.”

What can help is for laboratories to share data and evidence. “Two groups can look at the same data and come to different conclusions, just as clinicians sometimes differ in a diagnosis,” Dr. Rehm says. “There is subjectivity to the process. It is an imperfect science. On the other hand, in some cases groups differ not because of expert opinion but because of access to data. If both groups had access to the same evidence, they might have come to the same conclusion.” She encourages laboratories to share data and communicate.

“This was a great session,” Dr. Biesecker says. “It brought up a number of challenges and showed how genomics differs from a lot of other kinds of testing. We really have to come to grips with the comprehensiveness of genomic interrogation, and there is resistance to that.”

Like change, comprehensiveness is also more of a challenge to clinicians, he says. “Most geneticists are generalists; we are disease agnostic.” As a result, comprehensiveness doesn’t perturb geneticists, he says. “It gets tougher with people who are disease or organ specialists—oncologists or cardiologists. They are focused on their field of interest. They may become perplexed or overwhelmed about other diseases that genomics can tell you about.” For instance, a cardiologist may not feel as comfortable about managing inherited cardiomyopathies as ischemic heart disease.

As a result, ordering clinicians may not want laboratories to include all data from a genetic test in a report. “They may want to shift responsibility to labs,” Dr. Biesecker cautions. “Labs need to be mindful that there is a tendency on the part of some people to make this somebody else’s problem and should be wary about accepting that kind of request.

“There is a balance to be struck and that’s a hard problem.”
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