What UCLA learned in seven years of exome sequencing

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May 2019—Never go it alone without the input of the ordering clinician, and the diagnostic yield is better than expected. Those are two of the five lessons UCLA learned in its first seven years of clinical whole exome sequencing, said Wayne W. Grody, MD, PhD, in a session at last year's Association for Molecular Pathology meeting.

UCLA Medical Center was an early adopter of next-generation sequencing. "Our group decided to focus first on heritable or germline variants for patients with undiagnosed or very rare diseases," said Dr. Grody, director of UCLA's molecular diagnostic laboratories and Clinical Genomics Center.

Stanley F. Nelson, MD, co-director of the Clinical Genomics Center, ran the high-throughput sequencing core for research purposes in the Department of Human Genetics. "We brought the clinical laboratory infrastructure to get CLIA certification for part of that laboratory, so we hit the ground running," said Dr. Grody, who is also a professor in the medical genetics and molecular diagnostics divisions, Departments of Pathology and Laboratory Medicine, Pediatrics, and Human Genetics, UCLA School of Medicine.

Dr. Grody pointed to the published American College of Medical Genetics and Genomics/AMP standards and guidelines for interpreting sequence variants (Richards S, et al. *Genet Med.* 2015;17[5]:405–424) and warned against becoming overconfident in interpretation. A study published in 2016 of the performance of the ACMG-AMP variant-interpretation guidelines among nine expert laboratories revealed only a 34 percent concordance across laboratories (Amendola LM, et al. *Am J Hum Genet.* 2016;98[6]:1067–1076).

"That doesn't bother me too much, because I really feel with clinical genome interpretation as practiced in medicine, a big portion of it is art as well as science," Dr. Grody said. Even with ClinVar, for variants with two or more submitters, there is a classification discrepancy of about 17 percent. "This is of more than academic interest."

Dr. Grody briefly reviewed the case of "the first, and to my knowledge only, lawsuit based on misclassification of a missense variant." Christian Millare, of South Carolina, underwent next-generation sequencing as an infant. A missense variant was found on the *SCN1A* gene but reported as a variant of unknown significance (VUS), and the pediatric neurologist did not act on it.

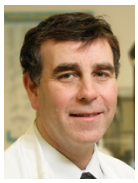
"*SCN1A* is the gene for Dravet syndrome, a very severe congenital form of epilepsy that is known not to respond to typical anticonvulsant agents like Dilantin," Dr. Grody said. It is treated with its own drugs, which have to be given in combination.

Christian went on to have seizures and did not respond to the anticonvulsant medications he received; he died at age three in status epilepticus. His mother sued (*Amy Williams, et al v Quest/Athena*) on the basis that if the variant had been called likely pathogenic, which is how it was subsequently reclassified, it would have been acted on and her son would have been prescribed the proper drugs and may have survived.

"Some of this reflects not only changing classifications, which are going to be with us forever, but misunderstanding by clinicians of what a VUS is," Dr. Grody said. "It doesn't mean it's benign and it doesn't mean it's nonactionable. We only report VUSs that are in some form actionable." The case raises important issues, he said, around the responsibilities of labs and clinicians to stay current and understand the appropriate translation of genomic sequence results to clinical care.

UCLA has a clinical genomics board that meets weekly to review cases and variants. After filtering out 25,000 of the variants that are likely benign, "we end up with a couple hundred to go through," Dr. Grody said. The board

consists of molecular/genomic laboratory directors and technical staff, genomic bioinformaticists, genetic counselors, clinical geneticists, pediatricians, residents and fellows, the specialists who have been seen, and the ordering clinicians, "who really know the patient's phenotype better than we would."



Dr. Grody

For internal UCLA cases, the clinicians are in the meetings and able to go through the variants in real time with the rest of the group. If the case is an outside referral, the ordering clinician is invited to join via web-based access.

"This is important because no one can know the whole genome, no one can know all those genes, all those diseases," Dr. Grody said. "You could argue that it takes an expert center for every gene, every disease, but we can't do that when we have 22,000 genes and a much smaller number of sequencing laboratories. We have to aggregate them somehow."

One of UCLA's first cases was a 17-year-old girl who had ataxia since age two and carried a longstanding diagnosis of juvenile ALS. When she arrived at UCLA Medical Center, however, the neurologists doubted the diagnosis of juvenile ALS. "They didn't know what it was, so they ordered the exome sequence," Dr. Grody said. "We found a homozygous, nonsense variant [E359X]—the parents were consanguineous—in a gene none of us had ever heard of before: *AAAS*, which is achalasia-addisonianism-alacrima syndrome," also known as Triple A-S syndrome.

"None of those three symptoms was in the medical note, at least not from UCLA," except for the ataxia, which is also a feature of Triple A-S syndrome, Dr. Grody said. The pediatric neurologist was there, though, and confirmed that the patient had undergone years of motility studies for the esophageal symptoms of achalasia. She also found in the patient history a noted absence of tears (alacrima).

If the clinician had not been there to answer questions, "I may have not reported this at all, or buried it somewhere, because it didn't really seem to fit the known phenotype," Dr. Grody said. "It's just a fact that if you're in an academic medical center, unlike a commercial entity, the laboratory is one degree of separation at most from the treating physician. Sometimes it's zero degrees because we have the person in the room."

Dr. Grody shared a second exome sequencing case, one he said was recent and caused "a great deal of angst" owing to its ethical dilemma: A young girl presenting with a rare tumor that raised suspicion of a familial cancer syndrome. Exome sequencing revealed a known pathogenic variant in the *P53* gene, diagnostic of Li-Fraumeni syndrome. But "there was also an incidental finding, if you can call it that": The father's DNA sample showed no relation to the child. "This compromises the informativeness of the exome report since it could not be determined whether the mutation was inherited or de novo," meaning it did not come from the mother, he said. "And the real father could be at risk of cancer himself, without knowing it." Yet there was resistance among the clinicians about including this finding in the report because of potential adverse effect on the family and because parentage testing had not been requested.

Lessons learned

- *Communication with the ordering clinician is essential*
- Diagnostic yield is better than expected
- Trio cases are much easier and more fruitful than singletons
- WES has better diagnostic yield than gene panel testing
- Insurance coverage remains problematic

Unexpected paternity results are nothing new in genetic medicine, but they are more frequent in "trio" exome sequencing, Dr. Grody said, which becomes a de facto paternity test and affects how the results are interpreted. The dilemma generated debate between the lab personnel and the clinical team, all of whom wanted what they felt was best for the family. "It's the first case in about 20 years

in which I actually consulted with our hospital ethics committee," Dr. Grody said, noting that he usually is able to solve most ethical problems himself. "But this one was really difficult, and sure enough, the ethics committee also found it difficult." The solution, a compromise of sorts, was to provide "some tactful genetic counseling" and was possible only because "all of us were on the same page—the genetic counselors, the oncologists, the laboratory directors, all of us involved with the patient."

The outcome of the case prompted UCLA to change its informed consent form to include a statement that unexpected family relationship results will be discussed and reviewed, and to perform a survey of other clinical genomics laboratories to compare approaches (Eno C, et al. *Genet Med.* 2019;21[4]:861–866).

The need for communication with the ordering clinician is first among the lessons learned from UCLA's first seven years of clinical whole exome sequencing, Dr. Grody said. There have been many other cases at UCLA in which the presence of the clinical expert in the room "made all the difference in which variants were reported or not reported." He cited some of the exome diagnoses that depended on clinical expertise: hereditary ataxias (e.g. SCA types 1–35), skeletal dysplasias (e.g. *COL2A1*), osteogenesis imperfecta type XIV (*TMEM388*) versus child abuse, immunodeficiency-14 (*PIK3CD*), hemophagocytic lymphohistiocytosis (*PRF1*), disorders of sexual development (e.g. *SRD5A2*), visceral myopathy (*ACTG2*), and autoinflammatory disorders (e.g. *MEFV*).

Of the ataxia cases, Dr. Grody said: "I don't know the clinical subtle differences between them—age of onset and what part of the body is ataxic. But we have neurology experts in the room who will be familiar with SCA type 23, for example, and say, 'There's no way that fits the phenotype.' So we will not report it."

"It doesn't mean they are absolutely right all the time," he added, "but as a result of this, our reports probably have fewer VUSs on them than many others, both commercial and academic laboratories."
A recent case of multiple fractures and potential child abuse was an example of when not to recommend whole exome sequencing. "If you think it's osteogenesis imperfecta, I would do the panel for that. If you open it up to all the genes, there's going to be a VUS somewhere that a defense lawyer will grab ahold of and say, 'See, it's genetic,'" Dr. Grody said.

Sequencing might have been ordered for the patient had the clinical expert not been in the room to say that the VUS for osteogenesis imperfecta type XIV was of a different age onset than the patient's age and affected different parts of the body. "There was no way any of us in the laboratory could know all this."

Another lesson learned: The diagnostic yield from whole exome sequencing has been better than expected, between 27 and 57 percent. "I think everyone nationwide and worldwide has found this," Dr. Grody said, for which he credits the honing and refining of the interpretation. The diagnostic yield has proved better than that for gene panel testing. "There was some argument about that," he noted. "There are always new gene discoveries for the genes that are not in panels, and different laboratories will have different panels, even for something well known like hypertrophic cardiomyopathy."

The UCLA laboratory has also found that trio cases are much easier and more fruitful than singletons. "Certainly, we have learned that if you can get the parents, it's a much easier interpretation, and you can at least tell if a sequence variant is de novo, or not inherited from either parent," he said. "You can cancel out some parental variants since they're healthy." UCLA's oldest clinical whole exome sequencing patient so far is a 98-year-old woman. "I did ask for parents but unfortunately they were not available," he joked. "We actually did find a pathogenic variant, though."

Insurance coverage has remained a problem because insurers are more willing to pay for a gene panel than for whole exome sequencing. "They understand what panels are but say exome sequencing is investigational," Dr. Grody said. "We admit [to insurers] it's expensive but say it can finally put an end to the diagnostic odyssey that has cost much more than the $4,000 for the exome. But they don't always see it that way."

Should whole exome sequencing be a first- or second-tier test? "If it's going to end the odyssey," Dr. Grody asks, "why not end it sooner and save even more money?" He described a case in which earlier exome sequencing

would have led to an earlier diagnosis of the cause of congenital seizures in an infant and spared the patient unnecessary neurosurgery.

Like other medical centers, UCLA is dipping cautiously into whole genome sequencing, Dr. Grody said. While WGS has advantages over whole exome sequencing—better overall coverage, no exon-capture step, and the chance to identify mutations outside the coding regions, it is more expensive and Dr. Grody remains unconvinced of the return on investment. UCLA is one of seven clinical site locations of the Undiagnosed Diseases Network (UDN), which presented and published data last year (Splinter K, et al. *N Engl J Med.* 2018;379:2131–2139). Patients may receive UDN grant support for whole genome sequencing if their whole exome sequencing was negative and inconclusive. Results presented at a July 2018 UDN consortium meeting failed to impress, though. "Of those [132] undiagnosed patients who had already gone through exome sequencing, which is a lot of them, only 13 percent benefited by going on to genome sequencing," Dr. Grody said.

"We can't go too deep into the introns, and I think RNA-Seq and similar techniques help you interpret the genome, but it requires the correct tissue sample," he said of WGS drawbacks. "Blood will not always work if it's a myopathy or a brain disorder. The gene may not even be expressed in lymphocytes, or if it is, it might have an alternate splice pattern from what it is in the affected tissue."
"There are no simple answers to this," Dr. Grody said.

He was struck by one piece of data: 11 percent of patients admitted to UDN sites were diagnosed by a simple reading of the medical records. "It was just a matter of getting experts in the room with different perspectives than the referring center, or putting disparate pieces of evidence from different subspecialists together."

Identifying druggable mutation targets in malignant tumors is one of the clinical applications of NGS. "I am finding it less and less acceptable to have this artificial division between germline and somatic applications of genetic testing, and especially NGS," Dr. Grody said, referring to early "turf battles" between genetics and pathology. "As we now can access the whole genome in both types of specimens, it's time for this artificial division to go away."

Noninvasive prenatal screening or testing for aneuploidies and other genetic disorders, adopted largely by the commercial sector and less by academic centers, is another application and likely the highest volume. "I found it interesting that free DNA in the mother's blood has become an attractive target at about the same time as free tumor DNA in cancer patients," Dr. Grody said. "Both tried cells first—circulating tumor cells, circulating fetal cells. Neither of those worked as well as the cell-free DNA. And there are cases where circulating DNA from an occult maternal malignancy is picked up in a routine noninvasive prenatal screen" (Osborne CM, et al. *Prenat Diagn.* 2013;33[6]:609–611).

"Again, it's an example of genetics and oncology coming together."

NGS for expanded carrier screening for rare recessive disorders has also been driven almost exclusively by the commercial sector. "I have some strong feelings about this, that it's gone a little too out of control," Dr. Grody said, sharing his view in 2016 in an editorial titled "Where to draw the boundaries for prenatal carrier screening" (Grody WW. *JAMA*. 316[7]:717–719). "I think the ease of sequencing," he told AMP attendees, "has let in too many genes and disorders that we know nothing about, that are incredibly rare, let alone what the variants mean or the natural history." This "creates a big genetic counseling burden and a burden on the couple to make an irreversible decision about termination."

Prenatal testing is one of three clinical applications of NGS that are being proposed or studied now. Of the potential of sequencing the prenatal exome, Dr. Grody said he has been "scared to go there these last seven years" but is becoming more open. "If I do it, it's going to be very limited with set restrictions. It's got to be an abnormal ultrasound—there would be some other reason you would think the fetus has a problem." He would report only the pathogenic variants.

He predicts that whole exome and whole genome screening will someday be commonplace for all newborns, noting that several NIH-funded newborn sequencing studies are underway. "It's what we do with the data that's up for

grabs, whether it will be stored until the patient reaches the age of consent." It casts a broader net than tandem mass spectrometry, and it is applicable to disorders not ideally amenable to MS-MS. "There are some metabolic disorders, like Wilson disease, that don't show up with that technique, but you'd find a mutation at the DNA level."

In addition, there should be fewer technical false-positive and false-negative results because DNA is unaffected by such things as low birth weight, prematurity, concurrent illness, and maternal abnormalities. And knowing the variant may point to mutation-specific cofactor therapies, such as BH4 in PKU, and new genotype-specific protein and molecular therapies.

The likely disadvantages are numerous. "The genotype-phenotype correlation for disorders like PKU are not that great," he said, even among siblings with the same variants. Interpreting VUSs and reporting incidental findings are challenging, and the need for trio testing to improve interpretation would increase costs and logistical burdens. Large deletions, mutations in noncoding regions, and some splicing defects could be missed, and there would be difficulty in predicting the clinical effect of compound heterozygous variants, especially for multimeric enzymes (e.g. phenylalanine hydroxylase).

"I think the advantage of newborn screening for abnormal metabolites is it's actually a sign of the disease; it's not a prediction of a future disease," he said.

The questions raised by the last potential application of NGS, wellness screening ("a terrible name but that's what it's been called") seem to outnumber the benefits, Dr. Grody said. "What would you sequence in them? Should it be exome or genome? Should it be the 59 genes in the ACMG panel? That panel was made for incidental findings, not for primary findings, which is what this would be."

In a healthy young adult, there would be no phenotype to guide interpretation, "so all 20,000 variants that don't match the reference genome are fair game."

There is also the potential for employment discrimination and poor customer satisfaction. "We know from exome sequencing of symptomatic patients that about two percent will have a reportable incidental finding if you follow just the 59 genes on the ACMG list." For healthy people, it should be the same, he said, which means that 98 percent of those who paid out of pocket would get a negative result. "It's not even really negative; it doesn't mean they're never going to get heart disease or breast cancer. We just ruled out the few genes we know about."

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