News & Analysis

Medical News & Perspectives

Exome Sequencing Comes to the Clinic

Ricki Lewis, PhD

Vincent Pieterse was so eager to enter the world on December 16, 2002, that he couldn't wait for the hospital. His startled father, Marc, delivered the boy in the family's home in a small village in the southern Netherlands. Vincent seemed healthy as a baby, but at school he acquired labels of nonverbal learning disability and mild autism. By age 8, his learning disability, autism, hypotonia, extra teeth, and elastic skin prompted a pediatrician to gently suggest the possibility of a genetic syndrome. But tests for select neurological diseases and chromosomal abnormalities were negative, and no relatives shared symptoms with Vincent.

When the diagnostic odyssey ruled out gene after gene, Marc read about exome sequencing and realized that the approach could provide information on many genes, perhaps identifying a treatable problem. Sequencing would also reveal whether Vincent had a new (*de novo*) mutation or whether his parents had passed the condition on. Finally, in 2012, on Marc's persistence, Joris Veltman, PhD, an investigator at Radboud University in Nijmegen who was conducting an exome sequencing study of children with severe intellectual disability (de Ligt J et al. *N Engl J Med*. 2012;367[20]:1921-1929), agreed to sequence the exomes of the boy and his parents.

Sequencing Vincent's exome, the 1% of the genome that encodes protein and accounts for 85% of inherited disease, uncovered a *de novo* mutation in a gene that encodes a specific ribosomal protein, but no

information indicated how the mutation could be pathogenic. "So I looked at the literature, and found a publication on Diamond-Blackfan anemia (DBA)," Marc recalled. Based on the publication (Lipton JF, Ellis SR. *Curr Opin Pediatr*. 2010;22[1]:12-19), some of Vincent's symptoms matched the ribosomopathy DBA, but he didn't have anemia and his mutation had never been associated with DBA.

Marc contacted the lead author of the publication, Jeffrey Lipton, MD, PhD, from Schneider Children's Hospital in New Hyde Park, NY, and by fall 2014, the researchers found that Vincent's RNA processing was normal. But Marc, convinced that his son had DBA, emailed a dozen researchers studying the disorder, including Alyson MacInnes, PhD, from the Sanquin Research and Landsteiner Laboratory in Amsterdam, who began coordinating studies of Vincent's ribosomes in several model systems. MacInnes was intrigued by Vincent's case history, mutation, and the sequence of events.

"Marc's email turned a corner into the future of genetic research. Instead of a patient going to the clinic with a list of features, getting a diagnosis, doing the exome sequencing, and then enlisting a researcher to figure out the molecular mechanism, it's going in reverse. Now the patient, armed with exome data, contacts the researcher, who figures out the mechanism, and then with the clinician is able to determine the diagnosis," she said.

Robert Marion, MD, pediatrician and chief of the division of genetics at The Children's Hospital at Montefiore, agreed. "It used to be we'd look at a kid and come up with a differential diagnosis based on the features, and then we'd test. Now we do testing first."

The Rise of Exome Sequencing

Because Vincent's ribosomes aren't abnormal in the way that they are in other DBA cases, his father suspects that the boy likely has a novel yet related ribosomopathy. On his physician's approval, Vincent takes leucine, an essential amino acid now being tested in a US multicenter clinical trial to determine whether it safely and effectively reduces the need for red blood cell transfusions in patients with DBA (http://1.usa.gov/1z5BQ5k). In cases like Vincent's, exome sequencing may lead clinicians to a diagnosis, or at least point them in the right direction.

Exome sequencing grew out of microarray technology used to detect specific genes, begun a decade ago. Tens of thousands of people have now had their exomes sequenced, but researchers needed more information on the degree to which sequencing is clinically useful. In 3 recent studies, investigators tried to determine the percentage of cases that could be resolved and the types of clinical presentations most likely to be diagnosed by exome sequencing.

Researchers from the University of California, Los Angeles, Clinical Genomics Center reported a 25% diagnostic rate among a cohort of 814 patients (Lee H et al. *JAMA*. 2014;312[18]:1880-1887). Investigators with the Department of Molecular and Human Genetics at the Baylor College of Medicine reported a similar diagnostic rate in a study of 2000 participants (Yang Y et al. *JAMA*. 2014;312[18]:1870-1879). Collectively, the 2 studies reported a higher rate of diagnosis for mother-father-child trios (31%), patients with developmental delay (41%), and patients with specific neurological findings (36.1%).

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All of the patients in the third study, from the Center for Pediatric Genomic Medicine at Children's Mercy Kansas City, were children with neurodevelopmental disorders (Soden S et al. *Sci Transl Med*. 2014;6[265]:265ra168). This study reported a diagnostic rate of nearly 40% (33 of 85 cases) using exome sequencing. In the same cohort, whole genome sequencing yielded a 73% (11 of 15) diagnostic rate among very young acutely ill children.

The study by Yang and colleagues reported that only 5% of secondary findings (not related to presenting symptoms) led to a treatment decision. Actionable outcomes for the primary, symptom-driven diagnoses were reported as "relatively few," which might be because most of the detected variants were novel or recently discovered. The smaller and more selective study of 119 patients by Soden and colleagues reported actionable outcomes or the underlying pathophysiology in 49% of patients.

While the vast majority of diagnostic outcomes resulting from exome sequencing have not lead to treatments, there have been notable exceptions. This was the case for a young Wisconsin boy who was near death from inflammatory bowel disease in 2010 when one of the first exome sequencing experiments was compassionately accelerated. He had an atypical presentation of X-linked inhibitor of apoptosis deficiency, which was dissolving his intestines.

"We narrowed down 16 124 variants to 8 using bioinformatics," recalled Howard Jacob, PhD, director of the Medical College of Wisconsin's Human and Molecular Genetics Center, who led the project. An allogeneic hematopoietic progenitor cell transplant, standard treatment for the deficiency, restored the boy's health (Worthey EA et al. *Genet Med*. 2011;13[3]:255-262). The cost of sequencing and analyzing his exome exceeded $75 000; the price now is $4000 to $6000, Jacob said.

Interpreting Exomes

Two dozen laboratories and companies currently provide whole exome sequencing and analysis. "We're getting samples from community hospitals, but the majority of exome sequencing is still done through academic tertiary care centers," said Jacob. A family practitioner might request exome sequencing when single-gene tests, chromosome tests, and copy number variant panels are normal, he added.

Patients or their parents may present "raw," uninterpreted exome data. For $1095, a customer can buy a buccal swab kit from Gene By Gene (https://www.genebygene.com/#), send in saliva, and 10 weeks later receive an incomprehensible string of DNA bases as results.

"The data are useless," Marion said, "unless you are a scientist with the ability to understand which genes are important in the clinic and which ones not." Otherwise, making sense of raw exome sequence is like trying to reconstruct the story of Moby Dick from a pile of word-sized pieces cut from the novel. The challenge lies in determining which gene variants, among thousands, could account for the clinical phenotype.

Detailed documentation of clinical features prior to exome sequencing is key, as it can help to subsequently narrow down possible candidates from the gene variants identified. Helpful tools for matching phenotypes to genotypes include Phenomizer (http://compbio.charite.de/phenomizer/) and the Human Phenotype Ontology (http://www.human-phenotype-ontology.org/), which compute differential diagnoses and identify possible candidate genes. Another tool, ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), detects signatures in a DNA sequence that suggest pathogenicity, such as mutations that disrupt protein conformation or halt protein production.

However, sorting through gene variants is time consuming. "Genetics experts at the laboratories search a huge wealth of information for nuggets. Are variants clinically actionable so you can use them to make decisions in patient care?" said Joy Larsen Haidle, MS, president of the National Society of Genetic Counselors (http://nsgc.org/p/cm/ld/fid=164) who practices at the Humphrey Cancer Center in Minneapolis. A colleague of Marion's with an undiagnosed arrhythmia is analyzing his own 40 000 gene variants. "It took 6 months for this physician-scientist in his spare time to find half a dozen hits," Marion said. Particularly vexing are "variants of uncertain significance" that have not yet been associated with disease and can confuse and alarm patients.

Genetic counselors can help when sequencing reveals secondary findings, Larsen Haidle said. The American College of Medical Genetics and Genomics lists disorders deemed reportable to patients because they are actionable (http://www.ncbi.nlm.nih.gov/clinvar/docs/acmg/). Patients can choose during the informed consent process whether to receive such findings as well as discoveries of carrier status for recessive diseases. Secondary findings arise in up to 10% of sequenced exomes.

When exome results don't lead to a diagnosis, full *genome* sequencing may help, said Soden. This was the case in the recent

Science Translational Medicine study by Soden and colleagues wherein exome sequencing failed to produce a diagnosis for 2 sisters presenting with hypoglycemia, hypotonia, finger contractures, and dysmorphic features. Although exome sequencing did not reveal a diagnosis, "we still felt strongly that there was something to find," Soden recalls. Whole genome sequencing subsequently identified a variant in the gene *MAGEL2*.

Exome sequencing did not identify this variant in *MAGEL2* because the gene is mired in a GC-rich stretch of DNA, a feature that disrupts sequencing and contributes to the 5% of genes that exome sequencing misses. Exome sequencing generally cannot detect copy number variants, repetitive DNA sequences such as trinucleotide repeats associated with Huntington disease, long insertion or deletion variants, aneuploidy, or epigenetic alterations (Biesecker LG, Green RC. *N Engl J Med*. 2014;370[25]:2418-2425). The overall error rate of exome sequencing is 0.1% to 0.6% but can be even greater for rare or unique variants (Wall JD et al. *Genome Res*. 2014;24[11]:1734-1739).

Who Pays?

Insurance coverage for exome sequencing is on a case-by-case basis, and appeals often are required. "It's frustrating for the physician because the situation feels out of control. We're the ones sitting in the room with the family saying 'We're sorry, but your insurance won't cover even standard gene tests, let alone exome sequencing,'" said Soden.

Having genetic expertise on a team helps. "If a clinical geneticist says, 'I need to do testing' for a child with a group of abnormalities that don't fit, insurers may cover that," Marion said. Exome sequencing can be economical, he added. A test panel for 62 autism genes costs $5500, whereas exome sequencing covers 20 000 genes for about the same price.

When insurance says no, families still can find funding. Clinical geneticists may enroll patients in clinical trials or in programs like the New York Genome Center (http://www.nygenome.org/) or the National Institutes of Health's Undiagnosed Diseases Program (http://rarediseases.info.nih.gov/research/pages/27/undiagnosed-diseases-program). The Rare Genomics Institute (RGI) also matches families with clinical trials. "If all else fails, we help with crowdfunding," said president Jimmy Lin, MD, PhD, referring to web pages on the RGI site (http://raregenomics.org/) set up to raise funds for particular families.

The Future

DNA-based tests are certified through the Clinical Laboratory Improvement Act and 1988 Amendments and the College of American Pathologists. However, the US Food and Drug Administration is evaluating guidelines to regulate all laboratory-developed tests as medical devices. This would add requirements for evidence of clinical validity, external review, manufacturing standards, and adverse event reporting (Deverka PA et al. *JAMA*. 2014;312[18]:1857-1858; Evans JP, Watson MS. *JAMA*. 2015;313[7]:669-670).

Current studies indicate that neurological syndromes are perhaps most appropriate for exome sequencing, and the hit rate in general will increase as more of the human genome is annotated. Meanwhile, physicians are learning how to use the new information. They're attending workshops, taking continuing education courses, and analyzing their own exomes and genomes.

Pioneered on rare diseases, exome and genome sequencing already are moving into preventive medicine. Genome sequencing in newborns and the feasibility of replacing current newborn screening tests with exome and genome sequencing is the subject of an early April conference in Kansas City (childrensmercy.org/NewbornGenomics). Marion predicts that genome sequencing of all newborns will be routine in 5 to 10 years. "Pediatricians will get readouts of every mutation and be asked to predict a newborn's health for the rest of his or her life," he said. ■

Correction: This article was corrected online April 28, 2015, for a misspelling in the institution name for one of the individuals quoted.