TESTING UPDATE CONTINUED

Rehm adds. Currently, at least four U.S.-based labs, including hers, offer hearing loss panel tests.

However, the guidelines also point to drawbacks with panel tests. Those tests that use disease-targeted exon capture focused on specific genes may only sequence a subset of the genes known to cause hearing loss, and there is limited knowledge of which genes are involved in hearing loss.

Richard J.H. Smith, MD, Director of the Molecular Otolaryngology and Renal Research Laboratories at the Iowa Institute of Human Genetics at University of Iowa in Iowa City, is a proponent of panel testing for hearing loss. His lab offers a test that covers 90 genes known to cause hearing loss, which he suggests over any initial single-gene test.

Dr. Smith says because panel tests offer more depth of coverage in genes associated with hearing loss, they are superior to WES, which looks at 20,000 genes and may miss parts of them. "Each gene gets read more times with a panel [test]," says Dr. Smith. "There are no blank spots. There are always some blank spots with exome sequencing."

Also new in the guidelines is a broader focus on diagnosing all causes of hearing loss, including pathogens and environmental factors, plus hearing loss with onset at all ages, says first author Raye Alford, PhD, Associate Professor in the Bobby R. Alford Department of Otolaryngology – Head and Neck Surgery at Baylor College of Medicine in Houston. Among these causes are noise exposure, congenital cytomegalovirus and rubella infections, premature birth, and mothers' use of certain drugs during pregnancy.

"Given the challenges that can exist in distinguishing between syndromic and nonsyndromic forms of hearing loss, all children and adolescents showing hearing loss without a known etiology, e.g., confirmed *GJB2* mutations or documented congenital cytomegalovirus (CMV) infection, should be evaluated for syndromic conditions by a clinical geneticist," the guidelines say.

Reference

Alford RL, Arnos KS, Fox M, Lin JW, Palmer GC, Pandya A, Rehm HL, Robin NH, Scott DA, Yoshinaga-Itano C; ACMG Working Group on Update of Genetics Evaluation Guidelines for the Etiologic Diagnosis of Congenital Hearing Loss, for the American College of Medical Genetics and Genomics Professional Practice and Guidelines Committee. 2014. Guideline for the clinical evaluation and etiologic diagnosis of hearing loss. Genet Med 16(4):347-355 PMID: 24651602.

DOI: 10.1002/ajmg.a.36643

RESEARCH UPDATE

MUTATIONS IN *NGLY1* GENE LINKED WITH NEW GENETIC DISORDER

Parents' reports of children's symptoms help facilitate the discovery

An alliance between genetics researchers and parents has resulted in discovery of a culprit gene and identification of a new, rare disorder that causes developmental delays, abnormal movements, liver problems, and lack of tear production in affected children.

Writing in the March 20 online issue of *Genetics in Medicine,* researchers describe mutations in the *NGLY1* gene that cause deficiency of the enzyme N-glycanase 1, which helps break down defective proteins so their components can be reused [Enns et al., 2014].

They write that children with NGLY1 deficiency have various abnormalities in movement, including a combination of muscle contractions that cause abnormal, tremulous movements, plus developmental delays, liver issues, and lack of tear production. Their paper describes eight affected children, but a total of 14 cases have been identified thus far.

The *NGLY1* discovery process—which lasted about one year—is remarkable both for its speed and the involvement of families. A combination of genomic sequencing, blog entries written by the father of an affected child, and frequent open communication between researchers and parents facilitated the effort.

Key Evidence from a Father's Blog

Matt Might, PhD, a computer science professor at the University of Utah in Salt Lake City, helped lead researchers to discover the cause of other children's symptoms when he began writing in his blog about the medical odyssey spurred by his son Bertrand's puzzling symptoms, some of which were life-threatening and included seizures, leukodystrophy, intractable multifocal epilepsy, peripheral neuropathy, liver fibrosis, gastroesophageal reflux disease, osteopenia, cortical visual impairment, and movement disorder.

When Bertrand was four and a half years old, Duke University researchers via whole genome sequencing and extensive analysis discovered that he had two different mutations causing a lack of the NGLY1 enzyme, a finding Dr. Might noted in his blog.

Meanwhile, Matthew Bainbridge, PhD, of Baylor College of Medicine in

Houston was also searching for possible causes of similar symptoms suffered by three-year-old Grace Wilsey when he came upon a paper detailing use of genomic sequencing in 12 undiagnosed patients, one of whom was found to have an apparently causative *NGLY1* mutation [Need et al., 2012].

Internet searching led Dr. Bainbridge to Dr. Might's blog, which revealed Bertrand didn't produce tears when he cried. Dr. Bainbridge contacted Grace's mother, Kristen Wilsey, who confirmed that Grace only had tears when she was especially upset. "Only a parent would know about the tears," Dr. Bainbridge notes. "This was a big moment."

Biochemical testing revealed that Grace produced very little NGLY1 enzyme, prompting teams at Duke and Baylor to compare findings. Meanwhile, the Mights and Wilseys began to work together to help researchers understand the disease, using social media to find other affected families.

The Study

Thanks to those efforts, authors from several centers were able to describe NGLY1 deficiency, an autosomal recessive disorder of the endoplasmic reticulum–associated degradation pathway. Their paper details how they reviewed the medical records of eight children and sequenced patients' and parents' exomes in most families, while other families had exome sequencing at other institutions.

The majority of the eight patients studied carried the nonsense mutation c. 1201A>T (p.R401X), which appears to be associated with a more severe phenotype. The researchers note that it was the most commonly detected deleterious allele and homozygous in five of eight children. Two children who did not carry this particular mutation had milder features.

The disease's most important features are elevated alpha-fetoprotein and liver enzymes in infancy that decrease to near normal levels in early childhood. Other features include accumulation in liver-cell cytoplasm of a substance with staining properties similar to glycogen, and symptoms include movement disorder, peripheral neuropathy, and absent tears resulting in inflamed eyelids and corneal ulceration.

Parents' Perspective

In a commentary accompanying the paper in *Genetics in Medicine*, Dr. Might and Grace's father, Matt Wilsey, encourage researchers to make better use of next-generation sequencing (NGS), the Internet, and social media when seeking genetic causes of disorders.

Many families of children described in the paper found Dr. Might's blog after NGS revealed their children's *NGLY1* mutation and then connected with one another via the blog, he notes. Since the paper's publication, Mr. Wilsey has written about Grace and Bertrand on Facebook and Twitter.

Their commentary also lists best practices that aided the search for the *NGLY1* mutation, including collaboration across institutions, disciplines, patients, and parents. But this isn't always easy, says first author Gregory M. Enns, MD, Associate Professor of Pediatrics and Director of the Biochemical Genetics Program at Stanford University in Stanford, California.

"Researchers hold their cards close to make sure both that information is correct and that they get recognition," adds Dr. Enns. A related suggestion is that researchers "move fast and break things," meaning they should take risks to make discoveries, even if their ideas or assays fail.

Dr. Might and Mr. Wilsey also stress the importance of listening to ideas and effective strategies from parents around helping their children cope with symptoms related to NGLY1 deficiency. One parent shared information about a compound that spurred improvements in a child's quality of life. Another parent told researchers and physicians about a novel fluorescent assay that has become an important tool in functional analysis of the gene and another formed a multi-institutional network of researchers.

Stanford researchers regarded the Wilseys' involvement so highly that they actually gave the family genomic data and included them in review meetings with geneticists and bioinformatics specialists.

Mr. Wilsey likened the process of NGLY1 deficiency discovery to a three-legged stool supported by clinicians, researchers, and parents and to the animated family movie *Ratatouille*. It tells the story of a sewer rat that learns fine cooking due to an alliance with a young kitchen worker at a famous Paris restaurant. Mr. Wilsey made kitchen magnets that read, "Anyone can cook" for the team working on *NGLY1* discovery. "I sent those to remind the team that a great idea can come from anywhere at any time and to be open to serendipity," he explains.

Photo courtesy of Matt and Kristen Wilsey.

Kristen and Matt Wilsey, shown with their daughter, Grace, have helped researchers identify a genetic disorder by sharing details about their girl's condition and symptoms.

References

Enns, GM, Shashi V, Bainbridge M, Gambello M, Zahir F, Bast T, Crimian R, Schoch K, Platt J, Cox R, Bernstein J, Scavina M, Walter R, Bibb A, Jones M, Hegde M, Graham B, Need A, Oviedo A, Schaaf C, Boyle S, Butte A, Chen R, Clark M, Haraksingh R, Cowan T, FORGE Canada Consortium, He P, Langlois S, Zoghbi H, Snyder M, Gibbs R, Freeze HH, Goldsein DB. 2014. Mutations in NGLY1 cause an inherited disorder of the endoplasmic reticulum-associated degradation pathway. Genet Med Mar 20. doi: 10.1038/gim.2014.22. [Epub ahead of print]

Need AC, Shashi V, Hitomi Y, Schoch K, Shianna KV, McDonald MT, Meisler MH, Goldstein DB. 2012. Clinical application of exome sequencing in undiagnosed genetic conditions. J Med Genet 49(6):353-361.

DOI: 10.1002/ajmg.a.36644