

HUMAN GENETICS

Affordable 'Exomes' Fill Gaps in a Catalog of Rare Diseases

The couple had four healthy babies, but tragedy struck as the children became teenagers: Three began to lose their sight. Now in their 30s, two of the sisters and their only brother have lost most peripheral vision; one is legally blind from retinitis pigmentosa, the disease all three inherited. Although doctors tested them for more than 50 genetic mutations linked to the disease, they came up empty. Then late last year, geneticists at the University of Miami in Florida tried a new test, one that searched for mutations by sequencing the siblings' entire protein-coding DNA.

By July, the family had an answer: The three affected siblings had inherited two copies of a defect in a gene called *DHDDS*. It codes for an enzyme that may be involved in tacking sugar groups to the light-sensing protein rhodopsin; when researchers blocked *DHDDS* in zebrafish, the fish became partially blind. The family members, who for years worked to raise money to study inherited blindness, "were overwhelmed when they learned that we found the gene," says geneticist Stephen Züchner of the University of Miami. He presented the study this week at the American Society for Human Genetics (ASHG) meeting in Washington, D.C.

The study adds to a flurry of reports this year on new genes for Mendelian disorders: rare diseases caused by a defect in a single gene. The work takes advantage of cheap, next-generation DNA-sequencing technologies that make it possible, for a few thousand dollars, to sequence the 1% of the genome that codes for proteins, known as exons. This so-called exome sequencing has "reinvigorated the field of Mendelian disorders," says Jay Shendure of the University of Washington, Seattle, who gave an overview at the ASHG meeting. Researchers studying rare diseases are experiencing a "euphoria," says Eric Green, director of the National Human Genome Research Institute (NHGRI) in Bethesda, Maryland.

For decades, geneticists have had only one way to find the genes underlying Mendelian disorders: studying families. By analyzing inheritance patterns of genetic markers, they could pinpoint the disease gene. But this so-called linkage approach doesn't work when few affected family members can be found or the disease is extremely rare, often because the mutation was not inherited but occurred spontaneously. As a result, of the nearly 7000

known or suspected Mendelian disorders identified based on clinical features, less than half have been linked to a gene (see table).

That is changing thanks to exome sequencing. Researchers use an off-the-shelf kit to separate out the exon-coding DNA in a sample, then feed the DNA into the new sequencing machines. This yields a list of perhaps 20,000 mutations. The researchers then filter out data such as changes that don't alter amino acids in proteins or mutations commonly found in other people.

In August 2009, Shendure and colleagues reported as a proof of principle that by sequencing the exomes of four unrelated patients with the same disease, Freeman-Sheldon syndrome, they found the known underlying gene. A string of reports of new Mendelian disease mutations have followed,



Mystery solved. Exome sequencing found the mutation that led to blindness in three children in the Lidsky family of Miami. The approach is uncovering the cause of other single-gene disorders.

some in known genes but also 10 or so novel disease genes, says Shendure. These include, for example, the first gene for Kabuki syndrome, an extremely rare disorder involving deformities and retardation. (In a couple of cases, the researchers used whole-genome sequencing, which costs eight to 10 times as much as exome sequencing, but the genes could have been found with just the exome, Shendure notes.) "It's solving things that we've been looking at for a long time," says molecular geneticist Joris Veltman of Radboud University Nijmegen Medical Centre in the Netherlands, whose group has found several novel genes—and "you don't have to be a big genome institute."

Some mutations point toward treatments. Yale University geneticist Richard Lifton's team last year reported that a Turkish infant with failure to thrive who had been diagnosed with an inherited kidney disorder instead had a known defect in a gene coding for an intestinal chloride transporter, leading to chronic diarrhea; the boy was put on oral rehydration therapy. Züchner's group is exploring whether drops of the chemical made by the *DHDDS* enzyme can restore some sight in the blind siblings.

Other discoveries reveal new insights about human biology. For example, as reported online in *Nature* in August, Lifton's group found a gene called *WDR62*, which when mutated severely alters how the brain develops.

Exome sequencing isn't perfect, however. The kits used in most published work capture only about 75% of all 20,000 genes, although newer versions are more comprehensive. The approach also misses structural changes and noncoding DNA that can influence gene regulation. The exome could soon be eclipsed by whole-genome sequencing, which is getting ever cheaper, geneticists say.

It's an open question whether exome sequencing can ferret out rare mutations that underlie complex, common diseases. Answers may come soon: Tens of thousands of exomes from people with common diseases are now pouring out of sequencing machines, Shendure notes.

But the technique's success for Mendelian disorders is undisputed. One sign is that NHGRI plans to fund a center next year that would find the genes underlying 40 to 50 Mendelian diseases a year and coordinate samples for studying others. Medical geneticist David Rosenblatt of McGill University in Montreal, Canada, spoke out passionately at the ASHG meeting in support of such a systematic effort to find the genes behind Mendelian diseases. "We should put the pins in the map ... and in the next 2 years find them all," Rosenblatt said. —JOCELYN KAISER

MENDELIAN DISORDERS

	Number
Gene known	2893
Gene unknown	1771
Suspected disorders	1977
Total	6641