New Gene Test Reported for Isolated Cleft Lip and Palate

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Researchers report they now can predict whether some parents are more likely than others to have a second child with the "isolated" form of cleft lip and palate, one of the world's most common birth defects, according to results of a study published last month in the *New England Journal of Medicine*.

The authors say their latest gene test applies to about 12 percent of isolated cleft lip and palate, or babies born with clefts only and no other birth defects. Last year, the authors and their colleagues reported that mutations in another gene account for about 2 percent of all cases of isolated clefts, meaning researchers in the field now can collectively screen for about 15 percent of isolated cleft lip and palate, an impossibility just a few years ago. Isolated clefts account for 70 percent of all cleft lip and palate.

In the latest paper, the scientists report a so-called "haplotype" gene test, one of the first of its kind in medicine. A haplotype is the sum of several recurring variations in the usual DNA sequence of a species that are spaced out, like signposts, along a gene or chromosome. In this case, they found that distinct combinations of sequence variations in and around the gene IRF6 correlated with an increased chance that a child would be born with a cleft. IRF6, which encodes a gene-activating protein called a transcription factor, plays a role during development in orchestrating the normal formation of the lips, palate, skin, and genitalia.

"This study shows that we've reached a point where it's possible to take blood samples from parents, test certain genes, and determine whether their risk for a second child with cleft lip or palate is, say, 1 or 20 percent," said Jeffrey Murray, M. D., a scientist at the University of Iowa and the senior author on the study. "Now is the time to begin thinking about how best to apply these types of tests clinically and ensure that they truly benefit the families and their children."

According to Murray, roughly one in every 600 babies in the United States is born with the isolated, also called "non-syndromic," cleft lip and palate. Though the condition is usually correctable with several surgeries, families undergo tremendous emotional and economic hardship during the process, and children often require many other services, including complex dental care and speech therapy.

The challenge has been how to move the field forward. Isolated clefts arise during fetal development from a dynamic and still poorly understood interplay of genes, diet, and environmental factors, and current research tools cannot adequately cut through the complexity.

One productive inroad has been to isolate genes linked to "syndromic" cleft lip and palate, hoping some might also play a role in causing the isolated condition. Syndromic cleft lip and palate refers to babies born with clefts that are accompanied with other birth defects. There are over 150 of these syndromes, and, collectively, they account for about 30 percent of all cleft lip and palate.

Two years ago, Murray and colleagues Brian Schutte and Shinji Kondo hit the jackpot when they found the IRF6 gene plays a role in causing Van der Woude syndrome (VWS), the most common of the syndromic conditions. The discovery marked a potentially important lead because, about 15 percent of people with VWS have malformations that are clinically indistinguishable from isolated cleft lip and palate, suggesting the gene might be involved in both types of clefting.

While studying the structure of IRF6, the group noticed a sequence variation that they thought might play a role in causing isolated clefts. Such variations, called single nucleotide polymorphisms, or SNPs, occur about every 1,000 bases in our DNA and are generally considered to be harmless.

What interested them about this specific SNP is it caused an amino acid change, substituting an isoleucine for the normal valine, precisely where the IRF6 protein attaches to other substrates. They reasoned the isoleucine insertion might somehow hamstring the protein's normal biological activities during tissue and organ development. Fueling their suspicions was the fact that the normal valine is tightly conserved from fish to humans, meaning if the valine was trivial, species along the evolutionary ladder might have altered it with greater frequency.

"The change wasn't going to cause the condition by itself, because we already had found it in lots of people who didn't have clefts," said Theresa Zucchero, a member of Murray's lab and the lead author on the paper. "But maybe it would be found more frequently. Alternatively, if there is another variation near this SNP, you might also be more likely to have a cleft."

To test their hypothesis, Zucchero and colleagues turned to their collaborators in Europe, South America, and Asia, providing a pool of 1,968 families — or about 8,000 people — in 10 countries with a history of isolated clefts. Rarely do research studies have such a broad international flavor, but, as Murray noted, the rate of isolated clefts in some parts of the world, such as the Philippines, Brazil,

and China, are even higher than in the United States. “We wanted to see whether the variation could be found across multiple ethnic and ancestral groups,” he said. “Or, was it confined to a single population?”

The researchers found that the isoleucine variation was indeed present at a low but measurable level in all of the populations. This allowed the group to ask the next question: Was their original hypothesis correct?

To their complete surprise, the answer was no. “What we found is that the valine was over transmitted in those with clefts,” said Murray. “That was actually a puzzle and remains so a little bit because it’s both the ancestral and common sequence, which is found in 97 percent of Europeans.”

“What we strongly believe is happening is the valine serves as a marker for some other mutation nearby within the gene that’s really doing the deed,” he continued. “In a sense, the valine is hitchhiking with the actual mutation.”

At this point, the researchers stepped back and looked more broadly at the gene and flanking regions of the chromosome. They identified a total of 36 SNPs, both inside and outside the gene, and nine of these variations seemed to be associated with clefting. “These individual variations, or SNPs can be assembled into a haplotype,” said Mary Marazita, Ph.D., a statistician at the University of Pittsburgh and a major contributing author on the paper. “What we found is that a particular haplotype is overtransmitted in some families with isolated clefts, suggesting a predictive association with the birth defect, and this was true in the populations that we analyzed from The Phillippines, Denmark, and the United States.”

Based on a detailed analysis of 1,316 families, the scientists estimated that the risk of parents with this haplotype having a second child with isolated cleft lip and palate is about 12 percent. As the researchers noted, their estimate is based on their analysis of the families and cannot be generalized to the broader public.

“For a complex trait like cleft lip and palate, this is a nice step forward because there may be dozens of genes that contribute to the condition,” said Murray.

Murray said the mutation that causes the cleft remains unidentified. The group is currently tracking it in a 140,000 base pair, or unit of DNA, surrounding the gene. The region contains an estimated 150 SNPs, one of which is likely to be the mutation.

The countries involved in this study were: United States, Denmark, Brazil, Colombia, India, the Philippines, Japan, China, and Vietnam. In addition, the researchers collaborated with Estudio Colaborativo Latino Americano de Malformaciones Congenita, a multinational birth defects registry in South America.

The article is titled, “Interferon Regulatory Factor 6 (IRF6) Gene Variants Confer Risk for Isolated Cleft Lip and Palate.” The research was supported in part by the National Institute of Dental and Craniofacial Research and the National Institute of Environmental Health Sciences, part of the National Institutes of Health.