Researchers find genes directly linked to cleft lip and palate

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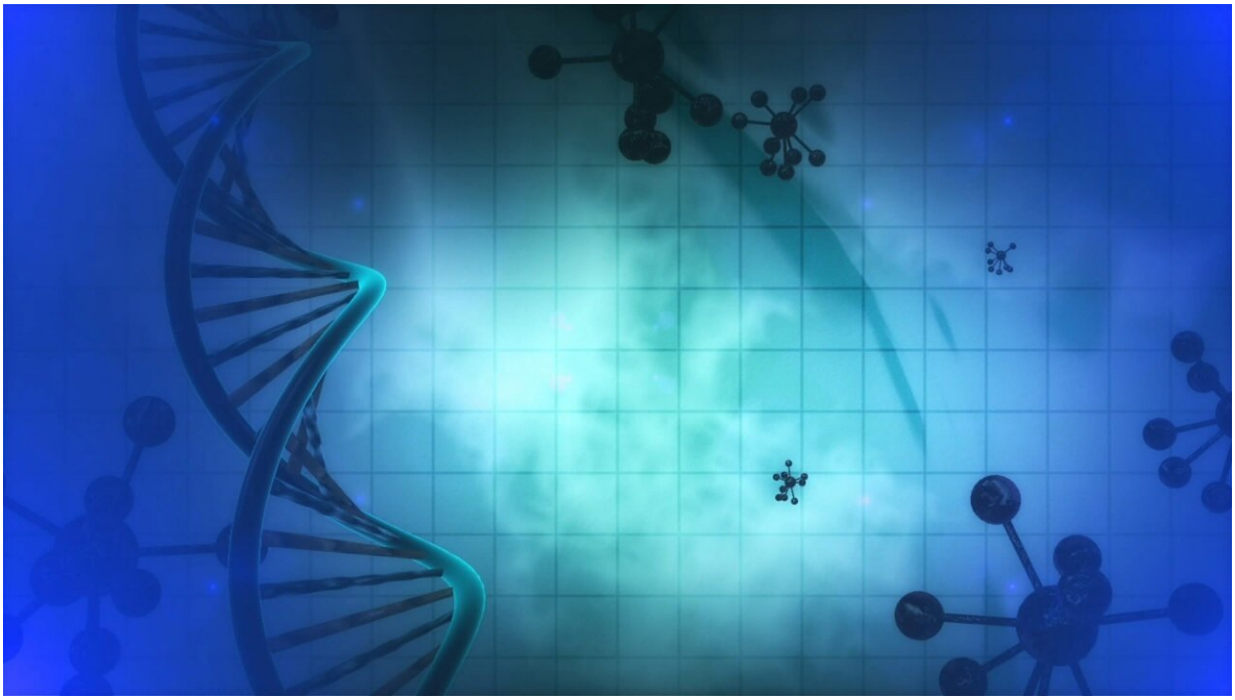

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Researchers are finding more genes directly associated with cleft lip and palate.

In a new study, a research team led by the University of Iowa identified three genes that when deleted cause cleft lip or palate, a facial deformation that occurs in about 1 in every 1,600 babies born in the

United States, according to the U.S. Centers for Disease Control and Prevention.

The team identified the genes by executing a high-resolution search through the genomes of more than 1,000 patients with cleft lip/palate, a repository stemming from Iowa's lengthy involvement with studying cleft lip or palate disorder worldwide.

Cleft lip and cleft palate are birth defects that occur when a baby's lip or mouth do not form properly during pregnancy. Children with a cleft lip or palate alone often have problems with feeding and speaking clearly and can develop ear infections.

In patients with the disorder, the researchers found small sections in the genome that were deleted or duplicated, known as copy number variants. Within the deleted sections, the researchers looked for genes that were rare even in people with the disorder. This is important because by searching for gene losses that are rare in people with the disorder—and even rarer or nonexistent in everyone else—it would mean those losses should have a central role in clefting, rather than simply contributing to the disorder.

The researchers then confirmed the genes' direct association with cleft lip or palate by reducing their function in two species, African clawed frogs and zebrafish. Each species developed signs of clefting when the target genes' function was reduced.

"We've found, and validated in experiments with vertebrates, three genes that are directly associated with this disorder," says John Manak, professor in the Department of Biology at Iowa and the study's corresponding author. "It's going to take a long time before we can do anything about it in humans, but now we've added several key genes driving the disorder. Eventually, if you know the genetics behind cleft

lip and palate, and the step-by-step process of how you build a face, then you might figure out how to intervene to prevent the defect."

The causes of orofacial clefts among most infants are unknown but are thought to arise from changes in genes and perhaps outside factors.

The researchers analyzed DNA from patients with clefting in the United States and in the Philippines. The extensive patient group comes courtesy of Jeffrey Murray, professor in the Stead Family Department of Pediatrics at Iowa; Sandra Daack-Hirsch, professor in the College of Nursing; and many others who traveled for years to the Philippines to enroll patients with clefts and their family members to gather samples and information about the disorder as part of surgical missions sponsored by Operation Smile.

"The families who generously enrolled in this study were hopeful that this work would someday lead to improved prevention or treatment of cleft lip/palate," Murray says, "and this work is a landmark step in that direction."

With DNA from those patients, Manak used a technique called comparative genomic hybridization to seek out deleted sections of the DNA in the patient pool with the disorder compared with a control group that did not have clefting. From there, he sought to find deleted genes that were so rare in the clefting group that less than 1% of the 1,102 patients surveyed had them.

"I wanted to identify those incredibly rare mutations that are driving this disorder, because mutations that do bad things are reduced in frequency in the population," says Manak, who is affiliated with the Stead Family Department of Pediatrics and the Interdisciplinary Graduate Program in Genetics at Iowa.

"In other words, a copy number loss where all you need is that gene deletion, and you get the disorder. That's exciting because it defines some really key genes in the clefting pathway. Of course, we also needed to verify that our candidate genes were actually expressed in the face and made sense for taking part in craniofacial development, before we were fully confident in our results."

The researchers used this analysis to find three genes: COBLL1, RIC1, and ARHGEF38.

When the team reduced function of these genes in embryos of African clawed frogs and zebrafish, each species showed signs of altered facial development. The experiments with the frogs, in particular, were important, because they're more closely aligned in an evolutionary way with humans than are zebrafish, and the experiments with the frogs produced facial features that resembled human clefts.

Lisa Lansdon, who earned her doctoral degree in genetics from Iowa in 2018 and is the study's first author, says the research was the primary focus of her thesis. She also oversaw a group of undergraduates who helped perform the analysis.

"It was very exciting to get to see the study evolve from the early design phases to ultimately discover new genes that were supported as being important for craniofacial development when we tested them in fish and frogs," says Lansdon, who currently is clinical assistant professor at the University of Missouri-Kansas City School of Medicine.

The findings build upon a previous study led by Manak, published in 2018, in which he used the same gene-hunting techniques in a smaller group of people with cleft lip or palate to find one gene directly linked to the disorder, called ISM1. He validated that gene's role in clefting in experiments with clawed frogs, as in this study.

"A highlight for me is the strategy we employed in both studies, looking for gene deletions that are rare in our disease cohort that were even rarer or absent in our controls," Manak says. "People haven't generally thought along those lines. It's a lot easier to just sequence genes and then look for more traditional mutations that alter the function of a gene."

He's also excited because the genes likely are important in facial development generally.

"There are multiple pathways and genes and interactions between many different cell types, so we need to identify all these components in order to understand how a face gets put together," Manak says.

The study, "Genome-wide analysis of copy number variation in humans with cleft lip and/or cleft palate identifies COBLL1, RIC1, and ARHGEF38 as clefting genes," was published Dec. 8 in the *American Journal of Human Genetics*.

More information: Lisa A. Lansdon et al, Genome-wide analysis of copy-number variation in humans with cleft lip and/or cleft palate identifies COBLL1, RIC1, and ARHGEF38 as clefting genes, *The American Journal of Human Genetics* (2022). DOI: 10.1016/j.ajhg.2022.11.012

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