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Researchers Use New Sequencing Strategies To Discover Rare Inherited Illness Rapidly

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(PhysOrg.com) -- A team of researchers from the National Human Genome Research Institute (NHGRI) has demonstrated a new technical strategy that promises to rapidly determine the genetic cause for very rare inherited illnesses. Relying on inexpensive, high-speed sequencing and a newly developed ability to capture pieces of the genome that encode genes, the team diagnosed an extremely rare X chromosome-linked cleft palate syndrome known to affect just two families.

The disorder, called TARP (talipes equinovarus, atrial septal defect, robin sequence, persistent left superior vena cava), is caused by a mutation in a gene called RBM10.

This is the first example of uncovering a gene defect on the X chromosome by analyzing DNA samples from unaffected carriers. In this case, the DNA came from the mothers of the two affected families. DNA was unavailable from any of the affected male infants because they died before, or soon after, birth. TARP syndrome is 100 percent lethal in males.

The findings were published in the May 14 issue of the American Journal of Human Genetics.

"This study demonstrates the feasibility of using new sequencing technologies to uncover causative genes for thousands of rare diseases, an effort that historically has been costly and arduous," said the paper's senior author Leslie G. Biesecker, M.D., chief of NHGRI's Genetic

Disease Research Branch. "It is also gratifying to know that the two families known to be affected by TARP syndrome finally have answers about what causes the devastating disorder that has afflicted their families for decades."

Humans have 46 chromosomes, which contain all of a person's genes and DNA. Two of these chromosomes, the sex chromosomes, determine a person's gender. Both of the sex chromosomes in females are called X chromosomes. Males have an X and a Y chromosome. The X chromosome holds a prominent place in the study and understanding of human disease. If a gene on the X chromosome is defective, it will cause an illness in males inheriting that defect, because the Y chromosome does not carry corresponding genes to compensate. Hundreds of genes have been mapped to the X chromosome, though the X chromosome contains only 4 percent of all human genes. It accounts for almost 10 percent of inherited diseases, which doctors also call Mendelian disorders. These so-called X-linked disorders include red-green color blindness, hemophilia, numerous forms of mental retardation and Duchenne muscular dystrophy.

TARP syndrome was originally described in 1970. It affected a single family. Subsequently, it was mapped to the X chromosome by a group that included NHGRI researchers in 2003. However, the mapping work only narrowed the search to a region containing 28 million base pairs, or letters of DNA sequence, on the X chromosome that contained more than 200 genes.

At the time, sequencing all of those genes to identify and validate variants was a daunting and costly task. So the samples were returned to the refrigerator until recently, when NHGRI researchers identified a second family with TARP syndrome. Meanwhile, new sequencing technologies drove down the cost of sequencing DNA, and another technique was developed for capturing exons. Exons are stretches of

DNA that ultimately encode portions of proteins. All the exons in the genome are called the exome. Until recently, it was not possible to sequence the exome on a single chromosome.

Several months ago, a kit specifically designed to capture and sequence only the exons along the X chromosome prompted the researchers to try again. NHGRI's researchers used the kit to sequence all of the exons on the X chromosomes of the mothers from the two families. Similar kits are currently available or being developed to target the exons on each of the other 22 chromosomes, as well as the Y sex chromosome.

In the original family, the researchers found an insertion of single base pair, a mutation called a single frame-shift mutation, and in the second family the substitution of a single base pair, a mutation called a single nonsense mutation, both within the RBM10 gene, or the RNA binding motif 10 gene. This gene is a member of the larger RBM gene family, but only mutations in a few of its family member genes are known to cause human disorders.

The loss of function in RMB10 in TARP syndrome indicates that the gene is critical for normal development. In their paper, the authors suggest that understanding the role of this gene in early embryo development will lead to a better understanding of inherited disorders that affect the face, heart and limbs.

The chance of finding two rare variants in the same gene in two families is small, so the researchers immediately moved into the lab to validate their findings by inserting the mutated gene into a mouse model. In the mice with the mutated gene, the gene was turned on in the jaw and limb, both critical areas affected in TARP syndrome.

"There are about 2,500 of these rare, inherited disorders, and the cause of the great majority of them is unknown," said Dr. Biesecker. "With the

help of these new technologies, biomedical researchers can potentially start making major inroads into finding the genes that cause such diseases."

Before the discovery, the women in the two families who may be carriers of the gene for TARP syndrome had put any reproductive plans on hold, fearful of having a child with the disorder. Now that the gene has been discovered, it is possible that a genetic test could be developed to indicate whether or not they carry the mutation. Those who are carriers can then make reproductive decisions based on the information. Furthermore, the information could potentially be used for pre-implantation genetic diagnosis, or embryo screening, to select an embryo with the mutated RBM10 gene.

"Studying the function of genes in rare diseases — both those we already know something about and the one-third of genes in the human genome we don't yet — can lead to a better understanding of their larger biological function and role in other human diseases," said James C. Mullikin, Ph.D., a co-author of the study and acting director of the NIH Sequencing Center in Rockville, Md., where the sequencing work was done. "For instance, further studies of the RBM10 gene may give researchers further insight into more common forms of cleft palate."

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