MedicalXpress

Genetic cause for CLOVES syndrome identified

May 31 2012

Using advanced technologies for rapidly sequencing and analyzing DNA from clinical and pathologic samples, a multidisciplinary research team consisting of geneticists, pathologists and surgeons at Boston Children's Hospital has identified the genetic basis for CLOVES syndrome, a rare congenital malformation and overgrowth disorder.

The discovery raises the hope that, for the first time, it will be possible to develop targeted medical treatments capable of delaying, reversing or possibly preventing CLOVES's debilitating consequences. Importantly, it also demonstrates the potential of advanced DNA sequencing technologies for identifying the underlying molecular roots of malformation disorders that are genetic but not hereditary.

The team—led by Matthew Warman, MD, director of the Orthopedic Research Laboratories at Boston Children's, and Kyle Kurek, MD, of the hospital's department of Pathology, and members of the hospital's Vascular Anomalies Center—reported the discovery today in the online edition of the *American Journal of Human Genetics*.

Some 90 children worldwide have been diagnosed with CLOVES (which stands for Congenital Lipomatous Overgrowth, Vascular malformations, Epidermal nevis, Spinal/skeletal anomalies/scoliosis) since 2006, when the condition was first characterized by Boston Children's Ahmad Alomari, MD, and investigators at the National Institutes of Health. Alomari co-directs the Vascular Anomalies Center with Steven Fishman, MD, and John Mulliken, MD; all three are authors on the paper.

The clinical features of CLOVES—in general a combination of fatty growths in the torso, vascular and skin anomalies, overgrowth in or deformities of limbs or extremities and spinal problems such as scoliosis—can vary greatly from child to child. Presently there is no cure for CLOVES, only surgical treatments aimed at alleviating symptoms or managing the syndrome's progression.

Until now, the exact nature of the genetic defect or defects that cause CLOVES has remained a mystery.

"CLOVES is dynamic, presenting itself in new ways all the time, even within the same patient," said Fishman, who with Alomari and others in the Vascular Anomalies Center has treated numerous children with CLOVES. "With this discovery we are optimistic that it will now be possible to develop treatments that take less of a shotgun approach and which could prevent the syndrome's progression."

The researchers started from the assumption that CLOVES is genetic but not inherited, because the syndrome always appears sporadically and is never passed from affected parents to their children; nor do the parents of affected children show signs of the syndrome.

"We suspected that a mutation in a single gene would be the cause, but in the beginning we weren't sure if the mutation would affect the gene's coding sequence or genetic regions that determine how a gene's expression is regulated." said Warman. "We also did not know whether the mutation would be the same across patients."

To identify the disease-causing mutation, Warman, Kurek and their colleagues used massively parallel (also known as next generation) sequencing technologies to read and compare the full exomes (all protein-coding gene sequences) of affected and unaffected tissues from several CLOVES syndrome patients treated in the Vascular Anomalies Center at

Boston Children's.

The team found that between six and 60 percent of cells in each individual's affected tissues contained mutations in a gene called PIK3CA, a component of a key molecular pathway regulating cell division and growth. Even though the precise mutations differed slightly between the patients, each mutation—a simple replacement of one DNA base for another, altering the structure of the protein PIK3CA encodes—has the effect of activating the pathway in the absence of external signals promoting growth.

The mutations were absent in the unaffected tissues tested.

Based on their findings, Warman and his colleagues determined that CLOVES is the result of a somatic mosaic mutation—a mutation that appears only in a portion of an individual's cells, rather than being present throughout his or her entire body.

"These are point mutations that likely arise spontaneously in a single cell during embryonic or fetal development, and which are passed on only to cells derived from that original mutant cell," Warman explained. "The presence of a large percentage of unmutated cells within affected tissues suggests that there is a kind of innocent bystander effect occurring, where unmutated cells respond to abnormal signals produced by cells carrying the mutation and contribute to the syndrome's malformations and overgrowths."

Both Fishman and Warman credit the interdisciplinary environment at Boston Children's with making the team's breakthrough possible.

"Gene discovery in rare conditions like CLOVES requires a combination of circumstances nearly unique to Children's: doctors who classified CLOVES as a new condition; tissues from the large number of CLOVES

patients referred to the Vascular Anomalies Center because of our experience with unusual vascular and overgrowth conditions; and world-class molecular genetic expertise," Fishman said. "The methods Drs. Warman and Kurek developed to find these mutations are very exciting and could help many other children with sporadically occurring diseases that are not hereditary but likely genetic."

"This project represents the perfect marriage of surgery, pathology and genetics," said Warman. "It was only through the combined efforts of multiple specialists—the surgeons in the Vascular Anomalies Center who had the foresight to save for future genetic studies the tissues resected from their patients, the pathologists who could tease apart affected and unaffected tissues from within the lesions, and the geneticists with the tools to sequence and compare unaffected and affected genomes—that this breakthrough was possible.

"Having now found these driving mutations in CLOVES," he continued, "we have a good starting point from which to both develop models to understand how mutations in PIK3CA cause malformation and overgrowth and to determine which drugs and other therapies can be used safely and successfully to improve the lives of individuals with CLOVES and other conditions with similar clinical characteristics, such as Klippel-Trenaunay syndrome."

Provided by Children's Hospital Boston