Double-teaming a whole-genome hunt

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By inspecting the sequence of all 3 billion "letters" that make up the genome of a single person affected with a rare, inherited disorder, a Johns Hopkins and Duke University team ferreted out the single genetic mutation that accounts for the disease.

Reporting their results in the June 17 issue of PLoS Genetics, the team says an altered version of the gene PTPN11 is the cause of metachondromatosis, a disorder characterized by bony growths, often on the hands and feet.

The study, the scientists say, demonstrates that new, whole-genome sequencing technology can efficiently and accurately lead investigators to the identification genes that cause Mendelian diseases — those caused by mutations in a single gene and passed on according to classic genetic patterns.

The traditional way of collaring a Mendelian disease-causing gene entails time-consuming and labor-intensive genetic analyses of numerous related individuals across generations. Known as "linkage," this approach depends on collecting families, especially large families with multiple affected members. This can be difficult and time consuming and often does not have sufficient resolution to identify the responsible gene. In fact, the definitive catalog of genes and disorders maintained at Johns Hopkins, Online Mendelian Inheritance in Man (OMIM), lists more than 1,500 disorders for which linkage studies have identified a large genomic region but have failed to pinpoint the responsible gene.

The failures were often due to the absence of enough related and affected individuals to provide linkage evidence of sufficient strength and resolution to identify the genes responsible for rare inherited diseases. Another weakness of linkage studies is that an affected individual may be so mildly affected that they are erroneously classified as unaffected, thereby skewing study results.

Success for metachondromatosis came when researchers combined a linkage study of 11 family members (five affected with metachondromatosis and six unaffected) with the whole-genome sequencing of one affected member. The linkage study identified likely regions of the genome where the suspect mutation could be found, considerably reducing the fraction of the genome that could contain the disease. Focusing on these regions, the team identified a mutation in PTPN11 that was sure to cause loss of function of the gene product.

"This whole-genome study, which took only two months, got us to a place where otherwise we wouldn't have arrived very quickly, if ever," says David Valle, M.D., Henry J. Knott Professor and director of the Institute of Genetic Medicine, Johns Hopkins University School of Medicine. "It's a great example of the power of a broad, agnostic approach."

By comparing the one whole genome of the affected individual with eight non-affected control genomes as well as to a database of single-letter variations known to occur in more than one percent of the population, and to other sequencing data, the researchers came up with a list of 100 possible candidate genes. These were analyzed in light of the linkage evidence which, although modest, allowed the team to narrow its search for variants to just a fraction of the genome and narrowed the list to half a dozen genes. Then they combed the literature to understand what was known of the biological function of these candidates, looking for any that might be involved in bone development.

Nara Sobreira, a graduate student in human genetics at Johns Hopkins and a lead author of the study, found that lots had been published about one of the six genes, PTPN11. Mutations in this gene made it hyperactive, causing Noonan syndrome, a genetic disorder that prevents normal development in various parts of the body, including the skeleton.

This newly discovered mutation or altered version involved a so-called "deletion" in which a piece of the genetic code is missing and likely to cause a loss of function of the gene, disabling its ability to manufacture normal protein, Sobreira explains. She said that gave credibility to the possibility that PTPN11 was responsible for metachondromatosis, which gives rise to different physical characteristics.

To confirm their suspicions, the team first checked to see if all affected members of the family in the linkage studies had the mutation and if all unaffected members didn't. The answer was yes.

The next and final assurance needed to prove that this gene was responsible for metachondromatosis was to find the same mutation of the same gene in an affected person unrelated to the family originally studied. The Hopkins team located a second family already seeking treatment at the Greenburg Center for Skeletal Dysplasias and confirmed that mutations, causing a loss of function of the PTPN11 gene, caused metachondromatosis.

"This discovery has given us clues about the molecular basis of other genetic diseases for which a cause remains unknown and that are not benign like this one," Sobreira says.

More information: PLoS Genetics: www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1000991

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