Customer case study
Field of study: Rare disease

Oxford Scientists Uncover Rare Mutations in Neurological Disorder

At the Oxford Biomedical Research Center, scientists used Ingenuity Variant Analysis from QIAGEN in a family study to pinpoint unknown mutations causing abnormal brain development.

Thanks to scientists at the University of Oxford, a family with a history of conceiving babies with severe brain malformations has a new path toward having a healthy child. The program that helped them could make a difference for many other families in similar situations as well. The university's Biomedical Research Center (BRC) has performed exome sequencing on some 20 families as part of a brain malformation study. Most affected individuals have microcephaly or related phenotypes, says Alistair Pagnamenta, a postdoc who has focused on neurological disorders since joining the center in 2010.

Pagnamenta was the lead author on a recent paper in *Human Molecular Genetics* reporting results for a family that had three pregnancies terminated due to the detection of abnormalities including polymicrogyria, a brain malformation that can lead to severe developmental and motor problems. He and his colleagues conducted exome sequencing and used a clever linkage analysis approach to track down the genetic variant causing the brain abnormalities. One important ingredient in this study, and others at the center, was Ingenuity® Variant Analysis™ from QIAGEN Bioinformatics.

Finding the Culprit

In this project, the UK-based family was directed to the Oxford BRC by their local genetics service. In addition to the three pregnancy terminations, the unaffected parents had also endured three early miscarriages, possibly due to the same condition. Polymicrogyria is a brain anomaly where poor organization of neurons results in an increased number of small folds in the cortex, instead of a smaller number of large folds to maximize the brain's surface area. The condition can lead to intellectual disability, muscle weakness or paralysis, seizures, and more.

One challenge Pagnamenta and his colleagues faced early on was limited access to DNA samples from the three fetuses. With those precious samples as well as DNA from the parents, the team performed exome sequencing on all five individuals. Using Ingenuity Variant Analysis, they generated a list of genes already known to be associated with polymicrogyria and quickly determined that in this family, none of them were faulty. They would have to search for a novel gene.

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But whole exome data from five people is a lot of DNA to comb through, so the team focused the search for a causal variant on genomic regions where all three fetuses had inherited the same chromosome segments from each parent. Targeting these identical-by-descent regions allowed the scientists to narrow their search to just 8.6 percent of the genome. "Then we searched within those regions and looked at all the variants present — how many of them were rare, how many were predicted deleterious," Pagnamenta says. Within that subset of variants, they scanned for homozygous and compound heterozygous mutations.

The inheritance pattern for the variant of interest was unknown going into the project, Pagnamenta notes. "We thought it was most likely to be a recessive condition because there were multiple affected fetuses and the parents were unaffected," he says. "But we couldn't be absolutely sure. It could also have been germline mosaicism — a *de novo* mutation that was present in all three fetuses but not in DNA from the parents' blood."

Testing both modes of inheritance was another way that Ingenuity Variant Analysis proved to be a handy tool. "Having this software was quite useful because you can change the order that all the filtering is performed, and you can very quickly switch from the recessive mechanism to this *de novo* parental mosaicism model," Pagnamenta says.

The team's analysis turned up one very strong candidate that matched the expected autosomal recessive inheritance mode — compound heterozygous mutations in *PI4K* on chromosome 22, one causing a premature stop and the other a missense substitution at a conserved location. The variants affect "an enzyme that's part of a well-known signaling pathway," Pagnamenta says. "Mutations in other components of this pathway were known to cause related brain malformations."

As the scientists learned more about this gene, they found there was an active research community interested in this particular signaling pathway. They worked with specialists who were able to functionally test the missense mutation and confirm that the variants changed activity of the enzyme.

The scientific findings were made all the more important by what they meant for the family, which can now use modern approaches such as preimplantation diagnosis together with this variant data to conceive a healthy child. Usha Kini, chief investigator for the project, notes that "this is very useful information for the family."

Other Uses

Pagnamenta and his colleagues use Ingenuity Variant Analysis for a number of projects, including other family studies as well as a large case-control study looking at genetic associations of drug response. "It saves a lot of time that we would spend going through the literature and compiling gene lists," he says.

The application's functionality and ease of use are other reasons the team relies on it.

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"It's very quick to change filter settings and find something you may have missed," Pagnamenta says. Those filters prove useful for more pragmatic reasons as well. When the team submitted the manuscript of this family study for publication, one reviewer asked whether they had considered a different way of looking at the data. That might have been tedious, but with Ingenuity Variant Analysis, they simply pulled up the study data and applied new filters. They also used the application to create a supplementary table listing all rare, deleterious variants found in the family so readers of the paper could see the full results. "Having this software makes it very easy to manage this," Pagnamenta says.

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