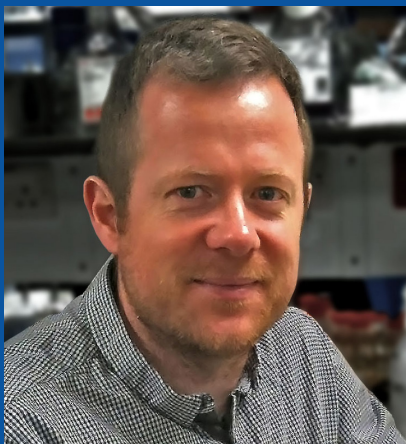


In London, Rare Disease Specialists Uncover Novel Pediatric Syndrome

Scientists at University College London's Institute of Child Health use Ingenuity Variant Analysis to find causal mutations in children with the rarest of diseases. In one new study, they identified a novel syndrome that explained undiagnosed cases in three unrelated families.



In the world of rare disease, genomics has been transformational. Nobody sees this more clearly than Hywel Williams, manager of a translational genomics center dedicated to studying the genetic basis of uncharacterized and ultra-rare diseases in children. Williams and his team work with clinicians whose patients are often the most hopeless of cases — they have usually been bounced around from one specialist to another on an unsuccessful diagnostic odyssey.

But when these patients get to Great Ormond Street Hospital in London, there is cause for hope. Affiliated with the University College London and its Institute of Child Health, the hospital is the largest pediatric research and clinical facility in Europe. Williams heads up the Centre for Translational Omics (<http://www.ucl.ac.uk/ich/services/lab-services/gosgene>), more commonly known as GOSgene, which functions in partnership with hospital clinicians to identify genetic variants responsible for patients' conditions. GOSgene was established by UCL professor Philip Beales and is funded by the hospital's Biomedical Research Centre.

In one study just published in the *American Journal of Human Genetics*, Williams collaborated with scientific and clinical experts and identified a novel syndrome in three separate families. Each family had a previously undiagnosed disease thought to be unique; the identification of a syndrome linking these families' conditions offers a promising research path for understanding the syndrome and diagnosing it in other affected individuals.

An integral part of that work was performed with Ingenuity Variant Analysis from QIAGEN. GOSgene was an early adopter of the web-based application when it first came out in 2012 and has used it as a key component in its analysis pipeline ever since. "It's absolutely essential to everything that we do," Williams says.

'LOST IN THE SYSTEM'

GOSgene's focus on rare childhood diseases offers Williams and his team an opportunity to have a real impact on the lives of patients. While they do not work directly with the patients — clinicians handle that part — they manage the critical research that goes into each case.

Those patients are often in dire straits. They are all children, and usually have made several other stops before landing at Great Ormond Street Hospital. "These families are just lost in the system," Williams says. "They tend to have fairly heterogeneous phenotypes, so they get seen by multiple clinical experts. But their disease isn't a specific entity, so nobody can really treat them holistically." Because the diseases are rare, there's typically no funding for researchers or clinicians to elucidate them or to help affected patients.

The samples sent to GOSgene for analysis are characterized by unique phenotypes — only seen in a single family, or at most two or three families — or diverse phenotypes that cannot be linked to just one disease. "These samples and conditions are so rare they can't be tested in a normal clinical laboratory," Williams says. GOSgene is funded specifically to perform gene identification in

cases like this, so the group is able to make inroads in situations where other teams might be stymied.

Williams and his group work closely with clinicians on these cases. They usually perform exome sequencing to identify the genetic underpinnings of each disease. "Because they tend to be the very rare and undiagnosed, we're generally looking for novel genes," he says, noting that nearly half of the genes they find are indeed new.

A common problem faced by families in this situation is misdiagnosis. After seeing so many specialists, and with phenotypes that could be linked to a number of diseases, several patients wind up with a diagnosis that doesn't match the underlying biology. A key role for Williams' team is correcting these inaccurate calls. "In about half of the cases we sequence, we've actually been able to find a known mutation within a gene, which can lead to a proper diagnosis," he says. Having this information can help families get better treatment as well as support, particularly for diseases with patient foundations, charities, or official funding channels.

"These parents are desperate for any kind of answer," Williams says. "Just knowing there's a genetic mutation or lesion can help them understand there's a reason why their child or family member is this way and they don't have to blame themselves."

Because of the broad array of phenotypes treated at the hospital, GOSgene handles a number of diverse cases. Williams says that since the program began in 2010, the team has looked at more than 80 separate clinical phenotypes — and most of those are represented in ongoing projects. For all of them, the team relies on Ingenuity Variant Analysis to flag genes of interest and accelerate interpretation of exome sequence results.

'NOT SNX14 AGAIN'

In one study, which was recently described in a paper entitled "Mutations in SNX14 Cause a Distinctive Autosomal-Recessive Cerebellar Ataxia and Intellectual Disability Syndrome," the GOSgene crew began with one sample from a patient in Portugal. The individual came from a consanguineous family and had a range of symptoms including microcephaly, hearing loss, and intellectual disability. Williams' team sequenced the exome of the patient, expecting to find an autosomal recessive mutation. Using standard filters for call quality and frequency with Ingenuity Variant Analysis, they were directed to a homozygous nonsense mutation in a gene that looked like a strong candidate.

When Williams dug deeper into the gene, Sorting Nexin 14 (SNX14), he found that the mutation was located in a microdeletion region associated with a known copy number variation. He suspected that the variant might be syndromic rather than unique to this one patient. In a meeting with other scientists and clinicians, he presented the data. "One of my colleagues said, 'Oh, not SNX14 again,'" Williams recalls. The same gene had been implicated in a previous family study; as Williams and his colleague compared notes, they realized the phenotypes of affected individuals in these families overlapped significantly.

Still, despite seeing deletions in the same region in both families, Williams and his team couldn't be sure that the mutation was affecting the gene. He went back to the BAM files and analyzed them again. "There was a large deletion that knocked out nine exons in the middle of this gene," he said. "Suddenly we had two families with overlapping phenotypes and loss of function mutations in this gene. That's when we realized that this is highly likely to be a new syndrome."

"By using the filtering in Ingenuity Variant Analysis, we were able to find the gene which then led to the identification of this syndrome."

Hywel Williams

Since then, clinical geneticists at the Great Ormond Street Hospital located a third family with another mutation in that gene that causes the same syndrome. "By using the filtering in Ingenuity Variant Analysis, we were able to find the gene which then led to the identification of this syndrome," Williams says. "The effort also highlights the importance of working in a group and sharing your preliminary data with your colleagues."

FROM SORT TO FILTER

When GOSgene first started up in 2010, the kind of analysis that led to the SNX14 discovery would not have been possible. The team began its foray into exome analysis with the same tool many scientists try for variant processing: Excel. "They'd try to annotate the Excel file, and they'd use the sort function to try and gain an understanding of what's going on," Williams says. "They quickly realized once you go beyond a handful of samples it becomes completely unmanageable."

The team has been using Ingenuity Variant Analysis ever since. "It's essential to our analysis pipeline. All of our samples go into Variant Analysis," Williams says. "It completely revolutionizes the way you can do gene identification."

"At the click of a button I can look back at all the old analyses. That's really powerful for spotting overlaps between samples."

Hywel Williams

One of the most important features for GOSgene's work is the application's ability to quickly filter out variants that are already listed in public databases so the team can focus on rare and

novel mutations. Williams and his group focus on frameshift, nonsense, missense, and splice site mutations and use the biological context feature in Variant Analysis to rapidly home in on the most likely causal variants. When there is a strong candidate, "it fairly shouts out from the screen," he says.

A big draw for Williams is that the application is "so user-friendly," he adds. "You upload your VCF and everything just goes into the cloud. You can access it anytime and you can share it if you want to." That's particularly important to his team, since they routinely share results with clinicians working on the same cases. Williams also makes frequent use of the ability to reanalyze data with

different filters and parameters. Ingenuity Variant Analysis saves each analysis, so anyone on the team can go back to any analysis at any time. "At the click of a button I can look back at all the old analyses. That's really powerful for spotting overlaps between samples," Williams says.

For the GOSgene group, any tool that helps find answers for patients is invaluable. "We've run almost 650 exomes now. There's no way we could've done the amount of work that we've done without Variant Analysis," Williams says.

For more information on GOSgene, visit <http://www.ucl.ac.uk/ich/services/lab-services/gosgene>

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