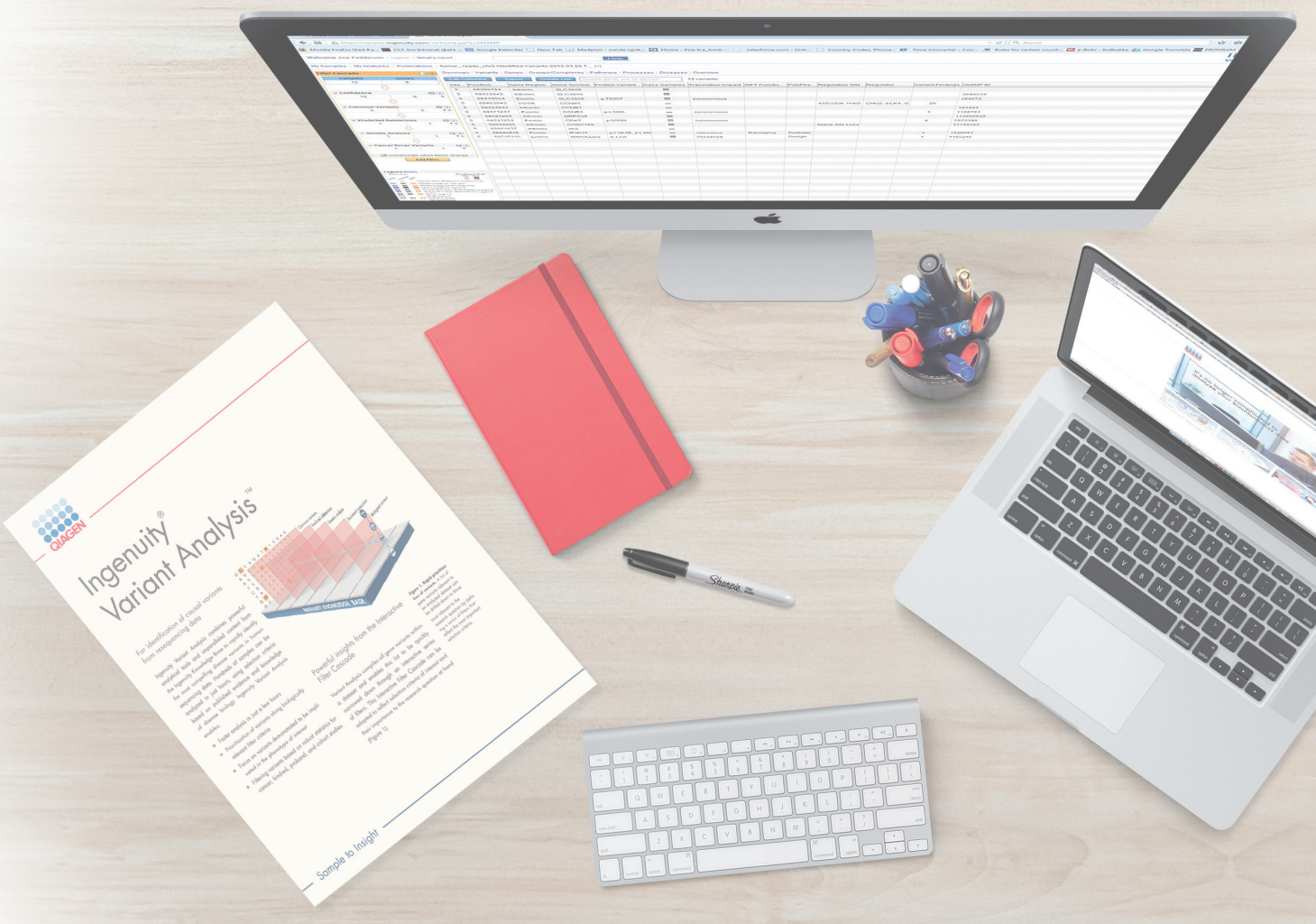
QIAGEN

Customer case study — Genome-wide variation

Impact of Human Variation on Disease



Sample to Insight

Rajini Haraksingh aims for a broad understanding of the complexities of human variation. Along the way, she has uncovered some key genetic causes of disease. She relies on Ingenuity Variant Analysis to ask better questions of her data with simplicity and speed.

For Rajini Haraksingh's scientific career, timing was everything. During the course of her PhD, advances in genome sequencing and variant mapping technologies made it possible to study all of the genetic variants in large numbers of individuals and develop a deeper understanding of normal human genetic variation. Haraksingh gained a strong interest in learning everything she could about the interactions between genes and other DNA elements, and how they work together as a system to produce our phenotypically diverse species.

Now a postdoctoral fellow in Alexander Urban's lab at Stanford School of Medicine, Haraksingh focuses on the functional implications of copy number variants (CNVs) and other structural variation. This expertise in the fundamental elements of the genome allows her to dive into any disease or condition and make important connections. "I try to understand the interplay between different levels of gene expression and gene regulation," she says. "How does the entire set of genomic content work together to create a functioning cell, a functioning system, and ultimately a functioning being?"

In one recent project, she performed exome sequencing and copy number variant analysis on a cohort with sensorineural hereditary hearing loss, finding novel loci associated with the condition. One important tool in her arsenal was QIAGEN's Ingenuity Variant Analysis, which helped her to quickly filter out benign variants and home in on the ones that were likely causative.

Haraksingh also uses the web application in her nonprofit work with the Rare Genomics Institute, an organization that enables patients

to take advantage of genomic solutions for diseases that defy diagnosis. "I'm not an expert in any particular disease," she says. "I come at this from the angle of trying to understand the variation in the genome, and what that means for developing different diseases."

The Whole System

The child of two academics, Haraksingh says that she always expected to land in the same world. "I really loved the intellectual freedom that came with their jobs," she says. "I like to understand everything at its most fundamental level and I'm not satisfied until that's what I get." Her early studies of genetics and biochemistry introduced her to the concept of the genome as a system. "I'm fascinated by how this one entity can basically create everything that we need to make a cell and the whole human system function," Haraksingh adds.

She pursued a PhD in Mike Snyder's lab, moving with him from Yale University to Stanford, because his genome-wide approach appealed to her far more than dedicating her career to understanding a single gene. When Haraksingh arrived at the lab, little was known about copy number variation, making it an appealing opportunity. "This whole new area was wide open, so I decided to work on methods to map and refine copy number variation in humans," she recalls. "Those projects and many others in the field ended up giving us much better resolution in this form of variation." Since copy number variation studies were so new at the time, there was no immediate way to determine which CNVs were pathogenic. "Before we could figure out which of these variants cause disease, we needed to characterize the full extent of normal copy number variation," she says.

Hearing Loss

As Haraksingh and her colleagues charted human variation, they looked for opportunities to elucidate the function of certain CNVs. "In trying to figure out what the functional implications of this variation might be, we started to look at various disease models to see if any of this variation can account for disease phenotypes," she says.

Her postdoc work, which largely continues her PhD focus, included a study of samples from patients with sensorineural hereditary hearing loss. Earlier work by her colleagues had revealed that taste receptors and olfactory receptors were copy number variable, so Haraksingh theorized that hearing might be another sense affected by CNVs. "It was known that copy number variants were enriched in genes involved in sensory perception and interaction with the environment," she says.

Using patient samples collected by Stanford pathology professor Iris Schrijver, Haraksingh and her team performed exome sequencing of families and isolated cases, and genome-wide CNV variant mapping on some 300 cases and controls to learn what they could about the complex process of hearing. Both technical approaches were important. Haraksingh says, "One of our most impor-

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tant conclusions was that in order to discover novel contributors to complex disease, we really need to use multiple complementary strategies.”

The project led to a publication in BMC Genomics in which lead author Haraksingh and her collaborators report a novel gene and a novel copy number variant linked to the phenotype. The experimental work yielded thousands of variants that under other circumstances would have required a Herculean effort to interpret using a number of external databases. “It’s extremely challenging to work with these databases. You’re constantly downloading and moving around large data sets,” she says. “It’s really messy and it takes a long time.”

Fortunately for Haraksingh, she was spared the endless hours that such a process would have required. “We came across Ingenuity Variant Analysis and it was a godsend,” she says, noting that the application is connected to those external databases and can easily query them in one fell swoop. “It turned what was an extremely frustrating experience into one that was actually really fun.”

Haraksingh also found it handy to be able to ask all sorts of questions with the application, use multiple pipelines, and get answers back immediately. “You only have to upload your data once, and Variant Analysis allows you to manipulate it, query all of the external databases, and run all the algorithms you want to use — all at the click of a button,” she says. Some of those questions led Haraksingh to delve into curated biological pathways for samples that lacked any known mutation related to hearing loss. She posited that the answer might be a variant of a gene located in the same pathway as the known mutations. “That was really helpful because we were able to find likely causative mutations based on that pathway analysis for several patients,” she says.

Ingenuity Variant Analysis even enabled Haraksingh to do something she never would have been able to otherwise: reanalyze her data over time. For her, that meant adding to her data set several weeks after her initial upload, and then reanalyzing all of it to learn whether the new data changed anything. “If I had to do this by hand, there’s no way I would reanalyze the data. It would just take way too long,” she says. But she thinks most scientists would benefit from reanalyzing data — even if they haven’t added new information — months or even years after their first analysis. “Knowledge is growing so quickly around what we know about genomes,” Haraksingh says. Being able to include more recent literature findings and database entries could shed new light on an old data set. “It’s really worth your while to reanalyze data a few years later, because you might be able to say a lot more from the same data,” she adds.

Clinical Direction

Beyond her work at Stanford, Haraksingh has also used Ingenuity Variant Analysis for projects through the Rare Genomics Institute, a volunteer-based organization using genomic technologies to try to get answers for patients

who have been on diagnostic odysseys. RGI enables patients to obtain genome or exome sequencing, often supported by crowdfunding. But there's still a lot of data interpretation required to get useful information back to patients. Often, the standard analysis performed by sequencing centers is not sufficient for understanding these rare and particularly challenging cases.

"As the director of RGI's Science 2.0 initiative, I lead a team of 12 researchers with the mission of analyzing the genomic and medical data from our patients to try to figure out what's going on," Haraksingh says. Ultimately, the volunteers write an in-depth research report for each patient that is used to refer the case to outside specialists or garner knowledge for future research from RGI's global network of scientists.

Ingenuity Variant Analysis is one tool they've used for RGI patients, and Haraksingh says that its ease of use makes the application a natural fit for volunteers who come from other backgrounds. "It allows people who aren't experts in genomics to quickly ask questions about the data without having to worry about putting all the components together to make the best informatics pipelines," she says. "Variant Analysis is so intuitive and easy to use that they're able to pick it up quickly."

Ultimately, Haraksingh hopes that the deep analysis she can provide to patients will yield progress, if not the exact answer, for each case — be it a new genetic lead or connecting the patient to a specialist who can provide more insight. "It's been extremely gratifying to apply my expertise in the nonprofit sector," she says.

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