

Perspective

From Whole-Genome to Focused Analysis: Homing in on the Right Genes

Determining gene function in autoimmune disease.

Autoimmune disease, when the body considers itself the enemy and attacks its own cells, affects millions of people—and the incidence rates are increasing in developed countries. These individuals have overactive immune systems that are waging a destructive battle against connective tissue, the digestive system, nerves, or muscle. They will most likely be treated with therapeutic tools that could best be described as molecular sledgehammers that pound out-of-control immune systems into submission, often draining the patient's vitality and quality of life in the process. Unfortunately, many suffer these negative side effects without deriving any benefit at all.

Seeing the need to improve the clinical outcomes for these patients, Dr. Annette Lee, Assistant Director Genotyping Research at the Feinstein Institute for Medical Research, is taking on autoimmune disease. She is focusing on rheumatoid arthritis (RA), a painful, progressive, and debilitating autoimmune disorder that affects about 1% of the world's population. Lee's far-reaching goals are to determine the genes responsible for disease onset and progression, to develop clinical tools that can predict responsiveness to existing therapies, and, ultimately, to support the creation of new, more effective treatments. These are large tasks, and by relying on Illumina's whole-genome and focused analysis platforms, Lee and her team are getting closer to the solutions.

HOMING IN ON THE RIGHT GENES

In 2005, Lee and colleagues acquired their own Illumina BeadArray™ Reader. Their first project was processing samples for Dr. Richard Duerr and his group, who were studying irritable bowel disease. Together, they published the first genome-wide association study (GWAS) using Illumina's whole-genome genotyping arrays. With that success under her belt, Lee and her team quickly moved on to their own work with RA. Using Illumina's Infinium® Whole-Genome DNA Analysis BeadChips, they scanned the genomes of healthy and sick individuals to identify single-nucleotide polymorphisms (SNPs) that are unique to ill patients. Like many groups studying complex genetic disorders, her team uncovered thousands of associated genetic variants across a large number of genes.

Lee needed to do follow-up studies to home in on the genes most likely to be at the root of the disease, and she needed the assays to be affordable. Her team designed an iSelect® HD Genotyping BeadChip with approximately 12,000 custom SNPs. They populated their custom array with more than 4,000 high-interest SNPs identified in their GWAS, 7,000 admixture markers for determining ethnic groups, rare variants discovered from resequencing experiments, and SNPs associated with other autoimmune diseases identified by the larger research community. Lee says, "We just moved these validated SNPs to a more fo-



Dr. Annette Lee is currently Assistant Director Genotyping Research at the Feinstein Institute for Medical Research.

cused panel which we could use to screen lots of samples and screen them fast. With iSelect, we were able to get the information that we wanted at a cost that we could afford.”

Besides enabling more cost-effective and speedy throughput for focused analysis, Lee’s iSelect array allowed her to ask more discrete questions and reanalyze her data in many different ways. Depending on how she chose to stratify her population—whether she looked at people who smoked, were on birth control, or familial history—different genes looked important. However, Lee uncovered important trends. “So far, the genes that we’ve pulled out can have a function and pathology in the disease. Most are somehow involved in signaling in T-cells or B-cells, which gives us confidence that what we see is real,” she explains.

Now that she had a favorite subset of likely genes, Lee further refined her investigation down to hundreds of SNPs using Illumina’s VeraCode® Technology. On going with the low- to mid-plex platform she says, “To do a 384-plex with VeraCode was significantly less expensive than other assays. And it’s fast. The BeadXpress® Reader allows us to screen up to 288 samples a day.” Quickly running thousands of samples from diverse populations, Lee confirmed the involvement of her selected genes in RA. In addition, the flexibility of VeraCode technology on the BeadXpress Reader allowed her to design extremely low-plex allele-specific primer extension (ASPE) assays and expand her investigations into other areas of interest. Using a custom-designed 16-plex ASPE assay, her group is genotyping patients who suffer from two or more autoimmune diseases that lack GWAS information. They are targeting SNPs highly associated with RA, lupus, and irritable bowel disease, hoping to discover whether or not these genes are involved in other

common and rare immune diseases. With her ASPE assay up and running Lee says, “The VeraCode Assay Design Tool was great. You just put your sequences in and you get all the information you need to run the assay. The assay is easy and flexible in the number of SNPs you target and the number of samples that you do. It has really worked out well for us.”

Lee sums up her experience using Illumina’s genetic analysis platforms for her RA studies: “I think Illumina has every tool that you could possibly want. There are genome-wide assays that you can use out of the box. At the same time, the assays can be customized. Having that flexibility to do the big picture and then narrow it down is one of Illumina’s strong points. Other companies offering genetic analysis platforms don’t have this capability. That’s one of the main reasons that we stay with Illumina.”

NEXT STEPS

Lee is beginning to look at the functional consequences of the genetic variation she has uncovered. For her, the involved genes make sense given their pathways, but now she wants to know how they are functioning in the big picture. “All we know is that the genes are important. Now we’re looking at how they are expressed. We are looking at how the genes function in relation to other cells. What do they secrete? What other signals do they take from other cells? We’re trying to work out the biology so we’re shifting gears a little and trying to tease out what’s important. I don’t want to say we’ve found all the genes, but we’ve looked at the top hits and then we keep drilling down. We are certainly closer to finding the answers.”

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ADDITIONAL INFORMATION

To learn more about Illumina’s genetic analysis solutions, please visit www.illumina.com.

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