Unmasking Autoimmune Diseases Using Genomics

Researchers at Australian National University are performing human whole-genome and whole-exome sequencing on HiSeq X Ten™ and HiSeq® 2500 Systems to identify variants associated with lupus and other autoimmune disorders.

Introduction

Our immune systems operate in seemingly mysterious ways. On one hand, the immune cells in our bodies are programmed to fight off foreign invaders. Paradoxically, a person's immune cells can also turn against normal cells, treating them as alien and unfamiliar, and causing autoimmune disorders.

Carola Vinuesa, MD, PhD, has been studying immunological disorders for 16 years, with a focus on autoimmune diseases, such as lupus. Using Sanger sequencing, she discovered a gene family critical for immune regulation and tolerance: Roquin and its paralog, Roquin 2.[1,2] She and her research team at Australian National University's John Curtin School of Medical Research have made important discoveries regarding 2 specific families of T-cells involved in the control of autoimmunity: T follicular helper (Tfh) cells, and T follicular regulatory (Tfr) cells.[3–5]

Today, Prof. Vinuesa is the head of the Department of Immunology and Infectious Disease at the John Curtin School of Medical Research. She is also the Co-Director of the Centre for Personalized Immunology (CPI), which aims to understand the genetic causes of autoimmunity to refine diagnosis and improve treatment. Her goal is to use genetic tools, including next-generation sequencing (NGS), to understand the genetic differences between patients to identify more effective and personalized treatments based on an individuals' genome.

iCommunity spoke with Prof. Vinuesa about how she has used the HiSeq X Ten and HiSeq 2500 Systems to uncover the genetics behind lupus (also known as systemic lupus erythematosus, SLE) and other autoimmune disorders, and her efforts in supporting gender equality in science.

Q: What sparked your interest in studying autoimmune disorders?
Carola Vinuesa (CV): Early in my research career, one of my main interests became understanding germinal center reactions that control the quality of antibodies that the body produces. One of the least understood questions is how quality control fails during antibody production, and that intrigued me.

During my first postdoc, I set up a large screening program for chemically (N-ethyl-N nitrosourea or ENU) induced mouse mutations to identify which genes were causing lupus-like phenotypes, which led to my discovery of a new family of genes, Roquin. That led me to study pathways involved in autoimmune disease, and we made several interesting findings associated with

a clinical problem for which there are currently no curative treatments. However, given the tremendous clinical and pathway heterogeneity in lupus and other autoimmune diseases, it became clear that we needed to identify the molecular or genetic differences and stratify patients according to defective pathways, in order to find effective therapies.

Q: Before NGS, what cell and molecular biology tools did you use to identify gene variants associated with disease?
CV: We identified Roquin using conventional mapping techniques. We outcrossed affected mice carrying the disease-causing mutation with mice of a different genetic background, using polymorphic markers to identify the region linked with disease. After we narrowed the interval to less than 1 Mb of the genome, we performed Sanger sequencing of individual exons. We identified a mutation in a gene that was then unknown, Roquin.

To translate our findings into human disease, we set up the APOSLE (Australian Point mutation in SLE) cohort with the help of many clinicians throughout Australia. First, we performed candidate gene sequencing to identify mutations in Roquin and related genes. A limitation of that technique was the enormous time and effort to sequence many exons of individual genes in over 300 subjects. Roquin alone has 20 exons. That prevented us from sequencing many genes.

Carola Vinuesa, MD, PhD, is head of the Department of Immunology and Infectious Disease at Australian National University's John Curtin School of Medical Research, and Co-Director of the Centre for Personalized Immunology in Canberra, Australia.

We then used High Resolution Melting Analysis (HRMA) to identify mutations without having to sequence entire exons. However, we could still only focus on a few genes, so that approach wasn't going to be sufficiently comprehensive.

We started looking at copy number variation using genome-wide human SNP arrays. This technique allowed us to process tens of patients in one round and identify some copy number variations associated with lupus that we are currently following up. However, none of these technologies could interrogate large numbers of genes for rare mutations.

"Exome sequencing enables us to identify rare and novel gene variants in subjects, which we then test for their ability to cause disease."

Q: How are you using NGS to study lupus?
CV: We started performing whole-exome sequencing (WES) using the HiSeq 2500 System in 2010. WES enables us to identify rare and novel gene variants in subjects, which we then test for their ability to cause disease.

We've been sequencing the exomes and genomes of subjects in the APOSLE cohort. We focus on extreme or severe phenotypes, including consanguineous cases, multiplex families, and patients with early disease onset, to enrich for those cases caused by one or few rare gene variants exerting strong effects. Although rare variants might explain only a few cases, they can be extremely informative for understanding disease pathogenesis.

We sequence the genomes of trios or several affected subjects from multiplex families. We run the sequences through our in-house–developed bioinformatics pipeline and prioritization algorithms, which narrow the list of potential candidate genes that could be causing the disease. We then introduce one or several putative disease-causing mutations into mice using CRISPR-Cas9 and see if those mice develop autoimmunity. Not all human syndromes can be recapitulated in mice, and some molecules signal differently in mice and humans. However, when a mutation from a human subject is introduced into a mouse, and the mouse begins developing lupus-like signs, we can conclude that the mutation the subject carries contributes to the disease.

Q: How has the HiSeq 2500 System performed in your laboratory?
CV: My bioinformatics colleague, Matt Field, codeveloped our sequencing pipeline and believes that one of the best features of the HiSeq 2500 System is its flexibility. It offers custom run modes capable of maximizing sequence content when we prioritize coverage or minimizing run time when we prioritize speed. It also offers low cost per base pair. With its flexibility, rapid turnaround time, and high-throughput capabilities, the HiSeq 2500 System is a perfect platform for variant discovery in human disease. We routinely use exome data from the HiSeq 2500 System and are always impressed with the quality of the sequence.

"With its flexibility, rapid turnaround time, and high-throughput capabilities, the HiSeq 2500 System is a perfect platform for variant discovery in human disease."

Q: What is the value of whole-genome sequencing (WGS) in your studies?
CV: We found that occasionally we missed mutations with WES because the coverage wasn't quite good enough. We decided to perform WGS because it provides deeper and more even coverage, even in exonic regions, reducing false negatives. It's the result of differences in the capture kits.

Q: Why did you choose the HiSeq X Ten System for WGS?
CV: Using the HiSeq X Ten System for WGS is more cost-effective and faster than the HiSeq 2500 System. Typically, we are sending out batches of 20–50 samples for WGS, with a turnaround time of 3–6 weeks.

We don't have a HiSeq X Ten System in our lab. We've been sending our WGS samples to Macrogene, and some lately to Novogene, because we have established a China-Australia Centre for Personalized Immunology (CACPI) at Shanghai's Renji Hospital. We might start sending WGS samples to the Garvan Institute in the near future for Australian-based cohorts.

"Using the HiSeq X Ten System for WGS is more cost-effective and faster than the HiSeq 2500 System."

Q: How do you analyze the variants?
CV: We've developed our own variant database, which now contains more than 8 million variants from 300 subjects, including CPI patients with lupus and other autoimmune deficiency diseases. We can scan and pull out variants based on many different criteria, such as frequency, prediction of damage, subject cohort, mode of inheritance, etc. By including subjects' clinical information, we are aiming to increase the power of this database.

Q: Out of the > 8 million variants in your variant database, how do you prioritize which ones to study?
CV: We use our own prioritization tool to identify which variants to study. It's disease-specific, so we have different criteria, depending on the type of immune disorder. We are looking specifically for rare and damaging variants, with our pipeline providing the frequencies from dbSNP and from the Exome Aggregation Consortium (ExAC) data set. We score damage using

3 functional annotation tools (PolyPhen, SIFT, and CADD[*]). We also take into account variants that have been assigned clinical significance, and the relevance of the genes to immune function and disease according to several different criteria and sources, including genome-wide association study (GWAS) data. We have developed an algorithm that takes all these factors into consideration and ranks the variants. Our system isn't perfect, but we are identifying some variants that we believe have the potential to become clinically important.

Q: What are the next steps in your lupus and autoimmune disease research?
CV: Regarding lupus, we've had a few cases where two or more rare variants appear to be involved in disease presentation. We want to use robust tools to investigate the possibility of genetic epistasis. We are generating mouse models carrying one or several mutations so that we can interrogate the functional significance of these variants alone and in combination. Deciphering how the mutations that we identify in subjects can cause changes in protein or cell function is essential for understanding disease pathogenesis. Ultimately, It will enable us to make more accurate diagnoses, and develop more effective and targeted therapies.

We're using a similar approach in studying other immune diseases. For autoimmune diseases, as in some complex diseases, we believe that we will find an entire spectrum of variants: from a mix of common variants with weak effects acting together, to a few or single rare variants with strong effects causing that disease.

"A molecular diagnosis for lupus would enable us to provide patients with more targeted therapies."

Q: What will the molecular diagnosis of autoimmune diseases enable?
CV: There are currently broad diagnoses of lupus. However, we believe that it is not just one disease, but rather several different diseases that all present similarly in the clinic and that are caused by different genes or pathways. If we can stratify patients into subdiagnoses or subgroups according to certain defective molecular pathways, then we might be able to identify the different treatment options that could be beneficial.

At the moment, there isn't a specific treatment for lupus. We tend to use broad immunosuppressants, such as steroids, to control disease. However, we do have an enormous array of immunomodulatory drugs, including many monoclonal antibodies, that could potentially be used. Yet, we don't understand the genetics of why some patients are responders, while others are

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[*]PolyPhen—predicts the possible impact of an amino acid substitution on the structure and function of a human protein.

SIFT—predicts whether an amino acid substitution affects protein function

CADD—scores the deleteriousness of single nucleotide variants and insertions/deletions in the human genome

not. In the future, if we can understand the molecular basis for lupus and drug responders, we will be able to offer more targeted therapies.

For instance, we found a rare homozygous mutation in *TREX1* in a little girl with cerebral lupus, which causes an exaggerated Type 1 interferon response.[6] There are Type 1 interferon blockers on the market, but not every lupus case has this type of signature. Identifying the molecular cause could point to therapies that might be useful in a particular subset of cases, but not others.

Q: What do you think needs to happen to make personalized medicine a reality?
CV: For some diseases, it's becoming obvious that we will need a molecular diagnosis to know what type of treatment will be most effective. For complex diseases, there's some skepticism about whether it's as simple as a particular mutation or set of mutations leading to a specific diagnosis.

I think teaching the clinical utility of genomics in medical school could be helpful in making personalized medicine more commonplace. It's surprising that genomic medicine has penetrated so little into medical training and clinical practice. Currently, our center runs a yearly class in personalized immunology for clinicians, and a 2-day school for post-graduate students on how to use online tools to uncover putative disease-causing gene variants in human genomes that have been run through our pipeline. Hopefully it won't be long until genomic medicine is integrated into the medical curriculum.

Finally, a drop in the cost of sequencing could make a significant difference in practice and make these diagnostic procedures more commonplace.

Q: You're passionate regarding the issue of gender equality in research. How are you involved with this effort?
CV: Until I had children, I didn't feel like there was a problem being a woman in science. I thought I could perform scientific research as well as anybody. During my first pregnancy, Istruggled to find time to write the grant applications to support myself and my research, and that was very stressful. I've seen my colleagues and postdocs go through the same tough periods. They suddenly had a several-year publication gap and that's enough to prevent you from getting the next grant or fellowship. Eventually, many women have to leave the system altogether.

We invest significantly in the many bright women scientists who deserve a chance. It is a real pity that they can be so easily left out of this super-competitive system when their science slows down during their pregnancies and early years of childrearing. I'm now a part of the National Health and Medical Research Council (NHMRC) Women in Science working group, which has studied initiatives that could have significant impact and enhance funding for women scientists.[7]

There are some encouraging new steps that have been approved, which I can't disclose at the moment, which will hopefully make a significant difference for women applying for funding. These steps

will be announced in late 2016. I've advocated strongly in Australian grant and fellowship panels for women. I also started the Gender Equity Committee at the John Curtin School of Medical Research.[8] We've already established the first fully funded fellowship for women returning from maternity leave. I also started a gender equity prize for women scientists going on maternity leave to help fund childcare or other needs that might help their productivity.

Learn more about the Illumina systems mentioned in this article:

HiSeq X Series, www.illumina.com/systems/hiseq-x-sequencing-system.html

HiSeq 2500 System, www.illumina.com/systems/hiseq_2500_1500.html

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