NGS Identifies Rare Disease Variants in Infants with Undiagnosed Disease

Cardiologist teams with pediatricians to perform landmark NGS study that leads to a national clinical diagnostic exome sequencing platform in Singapore.

Introduction

After receiving his MD from the National University of Singapore (NUS), cardiologist Roger Foo underwent higher specialist training at the University of Cambridge, and started research into the epigenetic regulation of gene expression in heart failure. The power of next-generation sequencing (NGS) became apparent as Dr. Foo began using NGS systems to perform RNA-Seq and ChIP-Seq on patient heart muscle samples. It was clear that clinical researchers throughout the world were using NGS more broadly for the identification of disease-associated DNA variants and as an integral part of clinical translation studies. When he returned to Singapore, Dr. Foo became a vocal advocate for the broader use of NGS in the clinic.

Founding a cardiac genetic service for Mendelian-associated monogenic cardiac diseases in adults provided the first step to raise awareness of the value of NGS among his peers. He proceeded to collaborate with his pediatric colleagues through a Biomedical Research Council (BMRC)-funded Rare Undiagnosed Disease program to make clinical exome sequencing available to children. About 1% of all babies born in Singapore have potential genetic conditions.[1] Some are immediately recognizable by pediatricians, including trisomies and less complex syndromes. Others are more complicated congenital conditions, including those with developmental delay or intellectual disability, that might become apparent only in the weeks and months after birth. Rare undiagnosed diseases provided the perfect entry point to bring NGS into more routine use for clinical translation in Singapore. Together with pediatricians from Singapore National University Hospital (NUH) Kids and KK Women's and Children's Hospital, he established the Singapore Undiagnosed Diseases Research program for Kids (SUREKids). Using NGS to sequence babies with rare undiagnosed disease provides the SUREKids team with an opportunity to find a possible genetic diagnosis for these children's conditions.

iCommunity spoke with Dr. Foo about SUREKids, the results that have been obtained so far, building an NGS analysis and data storage infrastructure within Singapore, and growing Singapore government support for NGS clinical studies.

Q: What's your medical background?
Roger Foo (RF): I'm a cardiologist and became interested in basic research when I was a Research Fellow at the University of Cambridge and a Consultant Physician at Addenbrooke's Hospital, the university teaching hospital at Cambridge. The heart is a special organ whose genes change expression as it undergoes stress. My research at Cambridge focused on gene expression control and epigenetics, using NGS to perform RNA-Seq and ChIP-Seq on heart muscle samples.

Q: Was NGS used extensively in Singapore?
RF: In 2012, the use of NGS in Singapore was limited to research studies. In contrast, I had seen the use of NGS to sequence patient samples in Cambridge, before I transitioned back to working in Singapore.

I realized that Singapore could easily translate the use of NGS to the clinic, and I became an advocate of expanding its use. Rare pediatric disease offered an entry point to familiarize clinicians about the benefits of NGS. I'm not a pediatrician, but I see cardiac patients with rare Mendelian disorders in my cardiac genetic clinic at NUS.

Q: How was SUREKids formed and what is its focus?
RF: My medical training was in Singapore and I knew many physicians in the system. There are two state sector pediatric centers in Singapore, Singapore NUHKids and KK Women's and Children's Hospital. The pediatric consultants in these hospitals had not worked together on NGS projects before, so this was an exciting opportunity to join forces.

We received a BMRC grant from the Singapore Agency for Science, Technology and Research (A*STAR). SUREKids was formed in 2015 with the objective of using NGS to develop a clinical exome panel for the Singapore population. We're sequencing trios that include the patients and both parents. We're performing whole-exome sequencing (WES) with HiSeq™ 2500 and NextSeq™ 550 Systems to identify disease-associated variants in Singapore children with rare undiagnosed disease, with the two hospitals sharing the data.

Roger Foo, MD is a Professor in the Department of Medicine at the National University of Singapore.

There is information that WES cannot provide, so we are complementing it with other tests. Long insertions/deletions and translocations might not be seen with WES. As a result, patient samples are analyzed with chromosomal arrays and, if necessary, cytogenetics testing before WES is performed.

> "...we've performed WES on 300 trios consisting of 100 infants and their parents. We identified rare genetic disease variants in 38 cases that had puzzled doctors for several months or sometimes nearly a year."

Q: Before SUREKids, how were children with rare disease diagnosed?
RF: Parents of children with undiagnosed rare disease knew that their children weren't thriving, but they didn't know why. Only parents who could afford it could have samples sent to Australia or the United States for genetic analysis, with the hope that they would obtain the results quickly enough for clinical decisions to be made. At least at NUH, not many more than 10 samples per year were sent overseas because of the high cost.

Q: Did you need to create a special team to perform sequencing of SUREKids samples?
RF: The sequencing is performed at the Genomic Institute where researchers have extensive wet lab experience performing NGS and bioinformatics skills for various research studies. However, our goal was to create NGS clinical diagnostic level tests in a College of American Pathologists (CAP)-certified environment. We established processes and created the documented workflows necessary to obtain CAP-certification of this new NGS laboratory.

The lab, called Polaris, is located within the Genomics Institute. Patient samples are recorded, barcoded, and anonymized in the hospital clinical laboratories, and then follow a CAP-certified pathway to Polaris where WES is performed.

Q: Who is responsible for bioinformatic analysis of SUREKids data?
RF: The bioinformatics side of the project was the fun part of the challenge. Bioinformaticians with experience analyzing NGS research data are typically not familiar with clinical phenotypes and scenarios. We needed to train a special set of people for the hospitals to transition from research-focused to clinical-focused bioinformatics. NUHKids and KK Women's and Children's hospitals saw the value of developing clinical bioinformatics capabilities in their hospitals and it contributed to gaining their support for the project. As a result of the SUREKids project, the hospitals jointly developed the bioinformatics expertise and training. Many bioinformaticians with basic research skills were also eager to obtain clinical expertise and have the job security of working in a hospital, so it ended up a win-win situation for everyone.

Both hospitals now have their own clinical bioinformatics teams. We call them variant curators. They are focused on integrating their analysis using sequencing results and the clinical terms and phenotype descriptions of infants and children with undiagnosed rare disease. The variant curators are now part of the Clinical Genomics Multi-Disciplinary Teams at both medical centers. We call these MDTs in the clinical world.

An important benefit of the SUREKids project is that sequencing and analysis are now being performed in Singapore rather than outside the country. For the first time, government money is being spent to support a local clinical NGS activity.

Q: How do you choose whether to perform WES or WGS on a sample?
RF: The A*STAR grant provided us with enough money to only perform some WES. Thankfully, the Genome Institute director saw the value of supporting SUREKids and he provided supplemental funding, especially to pump-priming our experience and collaboration. We used the funds whenever we felt that there was something to be gained from performing WGS and sequencing the exonic and intronic regions more deeply. We need more experience now to analyze whole genomes, and hope to increase our proficiency in that area during the next phase of the project. WGS would enable us to detect other types of mutations responsible for rare disease that WES cannot, such as insertions/deletions, substitutions, translocations, inversions, or frameshifts.

We've also created a rare disease registry. The effort was led by one of our consultants who is a pediatrician and a geneticist. It was an opportunity for the two hospital bioinformatics teams to work together on a data pipeline to populate the registry as children are diagnosed.

> "As a result of the SUREKids project, the hospitals jointly developed the bioinformatics expertise and training."

Q: What have you achieved in the SUREKids project so far?
RF: During the last three years, we've performed WES on 300 trios consisting of 100 infants and their parents. We identified rare genetic disease variants in 38 cases that had puzzled doctors for several months, or sometimes, nearly a year.

In at least five cases, the variant data resulted in patient management changes. In several instances, the variant data confirmed that a bone marrow transplant would be beneficial. Bone marrow transplants had been considered for these cases. However, bone marrow transplants are expensive and the physician teams hadn't been confident it was the correct next step. When WES identified gene variants associated with

immune-deficiency conditions, the teams went ahead and performed bone marrow transplants and the patients made full recoveries.

In several cases, we discovered new rare disease mutations and uploaded the genetic and phenotypic information to the Matchmaker Exchange.[2]

> "One of the benefits of the SUREKids project is that all the sequences are uploaded into Singapore national databases...that will provide the basis for personalized medicine and personalized therapeutics studies in the future."

Q: What has the identification of rare disease variants meant for the parents of children with undiagnosed rare disease?
RF: Identification of these variants lifts the clouds of uncertainty in their lives. In several instances, the parents finally had a name for the disease that their child had suffered from since birth. It enabled them to use Facebook and other social media to locate families in other countries whose children were suffering from the same disease. It was comforting for them to make those connections and feel like they weren't so alone.

The information also helps parents decide if they want to have another baby. Over two-thirds of the infants that we tested possessed *de novo* mutations, making the risk for having another child with the disease no higher than it would be for any other conception. That's helpful for parents to know. The information is also important for parents with babies possessing a homozygous or compound heterozygous condition.

We've received several very moving letters of gratitude from parents who participated in the SUREKids project. It's been a gratifying experience for all of us.

Q: What are the next steps for the SUREKids project?
RF: We achieved our initial objective of creating a clinical exome panel. Our next goal is to add WGS to our trio testing on a routine basis.

NUS, the University of Cambridge, and University of California, Berkeley have established a tripartite agreement called the Global Alliance. In 2017, the medical centers of these universities established a Global Alliance grant and invited medical researchers within their organizations to apply. Pediatricians from the three medical centers applied for the grant to extend the SUREKids research project to include WGS. We were thrilled when we were awarded the grant and have begun performing WGS on trio samples.

Q: Under the Global Alliance grant, will you be performing WGS on newborns in general, or newborns with potentially rare disease?
RF: The Global Alliance is an NUH effort to continue rare disease research in the neonatal intensive care unit (NICU). Its goal is to create a WGS analysis workflow that provides a 48-hour turnaround time. in addition to gaining WGS analysis experience, we'll share the data to identify new rare disease variants.

A positive outcome of the SUREKids project was the realization of the need for a Singapore national supercomputer to support clinical sequencing analysis. In 2016, the Singapore National Supercomputing Centre added a supercomputer for precision medicine. It will enable us to store WGS data securely, as we gain expertise in whole-genome analysis. It is likely that nearly 90% of the sequencing data stored there will be from Illumina NGS systems.

> "We achieved our initial objective of creating a clinical exome panel. Our next goal is to add WGS to our trio testing on a routine basis."

Q: What changes need to occur before NGS can be used more extensively in a clinical setting?
RF: Currently, NGS is not reimbursed in Singapore. We've communicated routinely with the Singapore Ministry of Health about the value of NGS and clinicians' desire to have it more readily available to all patients. Our activities contributed to the national genome sequencing policy that was published in September 2018. The real-world experiences from the SUREKids project and lobbying by parent groups were instrumental in achieving this milestone.

There are now clear guidelines being developed about the elements that must be in place for a clinical laboratory performing NGS. These include genetic counselors on staff to advise patients and parents when an NGS test is requested, policies for how data are reported to patients or their parents, and how incidental findings are handled. The policy document has been reviewed by the Ministry of Law. It would be a dream come true if the Singapore government made NGS subsidizable or reimbursable.

Q: What are the next steps in your team's rare disease research?
RF: One goal is to perform more WGS and sharpen our whole-genome bioinformatics capabilities. My lab has many postdoctoral research fellows, and students, with most of them working on cardiology, cardio biology, or cardio epigenetics research projects. Recently, I'm paying more attention to rare disease analytics, working together with Polaris to advance more genomics tests to the clinic.[3] WGS will enable us to reassess some of the unsolved SUREKids cases and possibly discover new rare disease variants located outside of exonic regions.

Learn more about the products and systems mentioned in this article:

HiSeq 2500 System, www.illumina.com/systems/sequencing-platforms/hiseq-2500.html

NextSeq 550 System, www.illumina.com/systems/sequencing-platforms/nextseq.html

References

1. Tan KH, Tan TY, Tan I, et al. Birth Defects in Singapore: 1994-2000. *Singapore Med J*. 2005; 46: 545-552.
2. Matchmaker Exchange. Genomic discovery through the exchange of phenotypic and genotypic profiles. www.matchmakerexchange.org/. Accessed November 9, 2018.
3. Polaris. The right drug to the right person at the right time. www.a-star.edu.sg/polaris/ABOUT-US. Accessed December 3, 2018.

Illumina, Inc. • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com