

Improving transplantation success with genotyping

Introduction

Dr. Brendan Keating is one of the founders and leaders of the International Genetics and Translational Research in Transplantation Network (iGeneTRiN). Working in a consortium with Dr. Folkert Asselbergs of the University Medical Center Utrecht in the Netherlands and Dr. Ajay Israni of the University of Minnesota, they aim to improve transplantation success through the discovery of immunological markers while gaining a deeper understanding of the genomic factors that specifically contribute to graft rejection and other transplant complications. Ultimately, the network aims to apply discoveries of the genomic underpinnings of graft rejection to the clinic in order to improve transplantation success. iGeneTRiN was initially focused on genomic studies in kidney, liver, heart, and lung transplants, but has now begun pilot studies in hematopoietic stem cell transplantation. The first stages of the work, which involved the development and testing of high-density genotyping arrays covering over 780,000 variants, were recently published in *Genomic Medicine* and the journal *Transplantation*. To learn more about the work that iGeneTRiN is doing to accelerate transplantation research, visit www.igenetrain.org

Affymetrix: Tell us how iGeneTRiN was originally formed.

Keating: We began conducting a number of transplantation genome-wide association studies (GWAS) at the University of Pennsylvania and Children's Hospital of Philadelphia and were aware of a few other ongoing studies in the Netherlands and the United Kingdom. We knew that the phenotypes we were looking at were very complex and would require increased statistical power through the aggregation of a large number of transplant GWAS samples. So we started to reach out within National Institutes of Health (NIH), searching for awards that had been given to different research groups doing similar work, because we knew they would be in the same situation. That's how we came across an NIH U01 grant for the DeKAF study that Dr. Ajay Israni and his colleagues were conducting on long-term deterioration of kidney allograft function. At that time, it was the largest transplant genomics study in the US. (Dr. Israni is now one of the leaders of iGeneTRiN.) Then we learned about a group based out of Mount Sinai Hospital, led by Professor Barbara Murphy, who was also running a kidney transplant GWAS. We reached out to these groups in addition to others across Europe until we came up with about 8,000 samples with existing GWAS data. We began collectively discussing the study metrics for our respective studies and exploring the development of a customized GWAS array tailored specifically for transplantation research. We formed the consortium in 2012, and one of the first things we did was to design the transplant GWAS chip. The consortium has grown rapidly, and we now have data for over 35,000 organ donors and recipients.

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(Division of Transplantation) in the Perelman School of Medicine at the University of Pennsylvania. Dr. Keating studies miRNA, mRNA, and DNA of heart, liver, lung, and kidney transplant donors and recipients. The goal of his work is to deliver individualized treatment of immunosuppression therapies post-transplant, and predict genetic signals that may underpin graft rejection and complications of rejection. He is the Principal Investigator of GWAS for solid organ transplant studies at Children's Hospital of Philadelphia. Dr. Keating is also one of the founders and leaders of the International Genetics and Translational Research in Transplantation Network (iGeneTRiN). This consortium is dedicated to improving transplant outcomes by better understanding the genetics and complications of transplant rejection.



Affymetrix: So what exactly are you looking for?

Keating: Our studies are looking for links between gene variants and five common transplant outcomes: (1) survival of the transplanted organ, (2) acute organ rejection, (3) new onset diabetes after transplantation (NODAT), which is a metabolic side-effect of immunosuppression therapy, (4) other adverse events due to immunosuppressive therapy, and (5) delayed function of the transplanted organ. Currently, selecting donors whose human leukocyte antigen (HLA) genes are a good match with transplant recipients via HLA molecular typing is the standard of care. However, we know that HLA matching does not guarantee success and that there are other transplant-relevant genes involved. These other genes are now known to include those encoding so-called killer-cell immunoglobulin-like receptors (KIR), as well as “natural killer” immune cells, which interact with HLA molecules and which some studies suggest impact outcomes of a grafted organ. Genes that influence the body’s metabolism of immunosuppressive drugs are also now recognized as likely factors in transplant complications. Further study into the role of KIRs and all other regions across the entire genome are a major goal of the consortium’s efforts.

Affymetrix: Why is it so important to have a large data set for this work?

Keating: Because transplantation phenotypes and outcomes are very complex, with a lot of co-variables. You can imagine the condition of a donor’s organ is dependent on a spectrum of factors, including cause of death, harvesting conditions, age, health, and lifestyle of the donor. Factors attributable to the donor organ are thought to account for about 40% of the graft lifetime. About 20% is attributable to HLA compatibility between the donor and recipient, while the remaining 40% is due to non-HLA factors. Therefore we need very large sets of well-harmonized phenotypes. The consortium has contributed huge value in terms of the number of subjects and rejection outcomes. The genetic datasets we put together in the iGeneTRaIN project are by far the largest ever assembled in transplant genomics.

Affymetrix: How important was it to you to include HLA markers in the array design?

Keating: HLA has been the most classically studied region in transplantation. We know from epidemiological and functional studies that HLA is very important so we went to great lengths to add in as many markers in those regions as practically possible. But again, we think that non-HLA components are equally important; we just have never had the tools with which to discover them.

Affymetrix: Which tools do you need to analyze the HLA regions?

Keating: There are four methods for the analysis in the HLA region. The first is straightforward genetic association using genotypes. But some of our transplant population, particularly liver transplant subjects, have not had conventional HLA typing performed so we only have blood matching.

The second and third methods are amino acid imputation techniques. One is Axiom® HLA Analysis, which was developed by Peptide Groove LLP and the Wellcome Trust Centre for Human Genetics at the University of Oxford, and the other imputation technique is called SNP2HLA. The fourth method is conventional HLA typing. We are in the process of evaluating these four methods in our studies.

Affymetrix: What types of genetic markers have you found to be the most difficult to cover in the array design?

Keating: Copy number variant (CNV) regions were the trickiest to cover. Usually, these CNV regions have more deletions and duplications and can be very hard to mark. Everyone may have slightly different duplications and deletions so you’ve got to try and get markers that can be used in conjunction with other markers in the region. Typically, we try to saturate the regions so that we can pick up where the duplication and break points are located.

This is very reliant on the quality of the DNA we have to work with. We see an effect we call “waviness” because we are dealing with varying signal intensities. In the most simplistic of models, if there is a two-copy deletion, we should not get a signal. With a one-copy deletion, we see half the signal, and then with a duplication, we observe an increase in signal. If the DNA quality is very good, we tend to see signal dips and increases with better clarity, but if the DNA quality is poor, we get more waviness in general. In certain regions, the intensity varies more than in other regions, generally making CNVs the trickiest.

Affymetrix: Is it challenging to call copy number variations and polymorphisms?

Keating: Yes, it is, but they are very important. One of the main reasons we are looking at these CNV regions is for loss of function (LOF). If you have a one-copy deletion, you may have a LOF variant in the other, meaning there is one functional and one non-functional copy. CNVs do make up a lot of these LOFs.

Affymetrix: How did you come to choose Affymetrix as a technology partner?

Keating: Because we were looking at so many variants, the Axiom® high-throughput genotyping technology from Affymetrix was a natural fit. We also wanted to make the array evolvable. For example, researchers from different countries might want to swap out different single-nucleotide polymorphisms (SNPs). So the fact that the arrays from Affymetrix are so easy to modify is important. The Axiom® platform is much more malleable and customizable than other technologies we have worked with before. The technology performed very well with the difficult to genotype HLA markers. We look forward to using Axiom® HLA Analysis software from Affymetrix, which offers analysis of 11 classical loci at 4-digit resolution. We also had a lot of support from Affymetrix when it came to building in CNV probes, in particular for the LOF polymorphisms that we think are very important in transplantation.

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The customized array we developed is comprised of tailored content for deeper capture of variants across HLA, KIR, pharmacogenomic, and metabolic loci that are important in transplantation. This is the first large-scale investigation to include non-HLA genetic determinants of clinical outcomes following organ transplantation.

The first stages of the work involved the development and testing of high-density genotyping arrays covering over 780,000 variants. Affymetrix contributed an additional set of ~350,000 SNPs for Caucasian-European and non-European populations to improve the mean coverage achieved in major ethnicities, including African and Asian. These SNPs were chosen with the goal of creating a comprehensive overlap with existing data generated at the UK Biobank using UK Biobank Axiom® Array and Axiom® Biobank Array from Affymetrix, enabling joint or meta-analyses of samples genotyped along with other conventional GWAS platforms. We are also very happy that Affymetrix has commercialized this array, making it available to more researchers.

Affymetrix: In what other areas of research do you see this array being useful?

Keating: We hope it might eventually be used in stem cell population studies as well as by HLA labs. There are LOF variants that are important in graft-versus-host disease (GVHD). It can be thought of as opposite of typical rejection, where the recipient's immune machinery attacks the allograft. In GVHD, stem cells from the donor attack recipient's cells. In stem cell transplants, many components of GVHD are not yet well understood. It's important to survey LOF so we have gone to great lengths to engineer as much LOF content on the transplant array as possible.

Affymetrix: So what's next?

Keating: We are putting together primary GWAS across the four solid organs: kidney, heart, liver, and lung. The first phenotype we're doing is a time-to-first-biopsy proven rejection. This is a pathology-graded rejection that is actually treated clinically. Then we are also looking at graft survival, time to graft loss, and time to death. We are looking at very specific phenotypes such as NODAT, which is a significant complication that results primarily from immunosuppressant drugs. It affects about 12% of recipients in the first year post-transplant. These people can also develop *de novo* hypertension, elevated triglycerides, and LDL. Another thing we are looking at is skin cancer. In some studies, up to 20% of recipients get skin cancer post-transplant. This is primarily because they are immunosuppressed, so benign skin lesions can become cancerous. Several research groups within iGeneTRiN are investigating this.

We're doing a very large, unique study on LOF. For every gene in the genome, we're looking at the number of functional copies; in the simplest form, there are zero, one, or two functional copies. For example, we hypothesize that if the donor has one or two functional copies of a given gene expressed ubiquitously or in an organ of interest, and if the recipient has zero functional copies, then the immune system of the recipient, which has never been exposed to that gene, might have a higher likelihood of precipitating rejection. So if we can stratify those patients that have a higher chance of rejection, we may be able to adjust medication and monitoring accordingly.

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Affymetrix: What's the most exciting aspect of this work for you?

Keating: We are taking sera from transplanted individuals with LOF associations, and hybridizing these longitudinal samples pre- and post-rejection onto peptide arrays. In some patients, if we can see the *de novo* antibodies from the LOF variants and if we can go from genotype all the way to phenotype. The hope is that we can actually intervene once we know the precipitating genetic underpinning that's causing the rejection, or at least develop better monitoring systems. This is further down the road, but it is very exciting.

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