

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 (iGeneTRAiN). 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, Dr. Keating aims 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, iGeneTRAiN aims to apply discoveries of the genomic underpinnings of graft rejection to the clinic in order to improve transplantation success. The network was initially focused on genomic studies in kidney, liver, heart, and lung transplants, but has also 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 published in *Genomic Medicine* and *Transplantation*.

Learn more about the work that iGeneTRAiN is doing to accelerate transplantation research at igenetrain.org

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Customer profile

Brendan Keating, DPhil, is a faculty member of the Department of Surgery (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 the Children's Hospital of Philadelphia. Dr. Keating is also one of the founders and leaders of iGeneTRAiN. This consortium is dedicated to improving transplant outcomes by better understanding the genetics and complications of transplant rejection.



Thermo Fisher Scientific: Tell us how iGeneTRaIN was originally formed.

Keating: We began conducting a number of transplantation genome-wide association studies (GWAS) at the University of Pennsylvania and the 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 the 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 study that Dr. Ajay Israni and his colleagues were conducting on long-term deterioration of kidney allograft function (DeKAF). At that time, it was the largest transplant genomics study in the US. (Dr. Israni is now one of the leaders of iGeneTRaIN.) 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.

Thermo Fisher Scientific: 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 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, 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 studies into the role of KIRs and all other regions across the entire genome are a major goal of the consortium's efforts.

Thermo Fisher Scientific: 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 covariates. 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 data sets we put together in the iGeneTRaIN project are by far the largest ever assembled in transplant genomics.

Thermo Fisher Scientific: 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.

Thermo Fisher Scientific: 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 Applied Biosystems™ 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.

Thermo Fisher Scientific: 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.

Thermo Fisher Scientific: 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 single nucleotide variant in the other, leading to a 2 copy LOF.

Thermo Fisher Scientific: How did you come to choose technology from Thermo Fisher Scientific?

Keating: Because we were looking at so many variants, the Applied Biosystems™ Axiom™ high-throughput genotyping technology from Thermo Fisher Scientific 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 Thermo Fisher Scientific 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 Thermo Fisher Scientific, which offers analysis of 11 classical loci at 4-digit resolution. We also had a lot of support from Thermo Fisher Scientific when it came to building in CNV probes, in particular for the LOF polymorphisms that we think are very important in transplantation.

The customized array we developed consists of tailored

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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. Thermo Fisher Scientific 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 populations. These SNPs were chosen with the goal of creating a comprehensive overlap with existing data generated at the UK Biobank using the Applied Biosystems™ UK Biobank Axiom™ Array and Axiom™ Biobank Array from Thermo Fisher Scientific, enabling joint or meta-analyses of samples genotyped along with other conventional GWAS platforms. We are also very happy that Thermo Fisher Scientific has commercialized this array, making it available to more researchers.

Thermo Fisher Scientific: 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 the 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.

Thermo Fisher Scientific: 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 iGeneTRaIN 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

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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.

Thermo Fisher Scientific: 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, we can see the *de novo* antibodies downstream from the LOF variants, and thus we can go from genotype all the way to the phenotype of having an allo-antibody produced against such genetic lesions. 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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