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Concepts of Genomics in Kidney Transplantation

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Abstract

Purpose of review—Identification of genetic variants to aid in individualized treatment of solid organ allograft recipients would improve graft survival. We will review the current state of knowledge for associations of variants with transplant outcomes.

Recent findings—Many studies have yet to exhibit robust and reproducible results, however, pharmacogenomic studies focusing on cytochrome P450 (CYP) enzymes, transporters and HLA variants have shown strong associations with outcomes and have relevance towards drugs used in transplant. Genome wide association study data for the immunosuppressant tacrolimus have identified multiple variants in the *CYP3A5* gene associated with trough concentrations. Additionally, *APOL1* variants had been shown to confer risk to the development of end stage renal disease in African Americans.

Summary—The field is rapidly evolving and new technology such as next generation sequencing, along with larger cohorts, will soon be commonly applied in transplantation to understand genetic association with outcomes and personalized medicine.

Keywords

Kidney transplant; genomics; pharmacogenomics; genome wide association studies; APOL1; sequencing

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Compliance with Ethical Guidelines

Conflict of Interest

William Oetting, Pamala Jacobson, Casey Dorr, Rory Remmel, Arthur Matas, and Ajay Israni declare no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Introduction

Although new drug development and improvement in patient care have resulted in better short-term transplant allograft survival, there has been little improvement in long-term outcomes. Defining genetic associations with posttransplant outcomes has the potential to improve donor-recipient matching, selection of immunosuppressive treatment regimens and/or drug dosing, and posttransplant care. For example, identifying genetic variants associated with increased risk of acute rejection (AR) and/or chronic graft dysfunction (CGD), could lead to trials of immunosuppressive protocols for individuals with those variants.

Genetic variants in the donor and recipient have long been studied to better define transplant risk. Initial genetic variants found to impact transplant outcomes were alleles associated with Major Histocompatibility Complex (MHC) antigens, also called Human Leukocyte Antigens (HLA).¹ The response to this was to attempt better HLA matches between donor and recipient. Unfortunately, waiting for an optimal match between donor and recipient results in a balance between the risk associated with increased wait time and the risk associated with increased mismatches.²

As the different alleles of the MHC antigens were being studied, variants in additional candidate genes, including those which were thought to modulate immune function were being evaluated. In most studies, AR was the phenotype investigated. For more than 2 decades studies reported significant associations between AR and individual candidate variants,^{3,4} though other outcomes were studied.⁵ In most cases the p-values were modest, as were the effect sizes. These associations, for the most part, have not stood up to scrutiny. Oetting et al, in a large cohort of kidney transplant recipients, studied 23 genetic variants previously reported to have a significant association with AR; only one variant was found to be significant in the validation set.⁶ There are a number of reasons that the findings reported in many of these early studies have not been able to be validated; a) they were underpowered⁷ and in some, cohorts of 100 recipients or smaller were studied; b) when multiple variants are being genotyped, correction for multiple tests was not always done; and c) there were differences in defining the phenotype between the original study and the validation studies.⁷

In an effort to overcome the limitations of candidate gene approaches, genome wide association studies (GWAS) were used to test known variants.^{8,9} There have been few GWAS reports for transplant outcomes. For those variants that have been reported, similar problems with validation have emerged as was found in the candidate gene approach. In an attempt to validate results of an earlier GWAS reporting two variants associated with death-censored graft survival, Pihlstrøm was unable to find a significant association with either variant.¹⁰ In the latest GWAS reported by Ghisdal et al., using a DNA pooling approach, rs10765602 in the *CCDC67* gene and rs7976329 in the *PTPRO* gene, associated with AR, were significant in both a discovery and a validation cohort but have not yet been validated by other investigators.

Other transplant phenotypes have been studied including new onset diabetes after transplant (NODAT) for which a recent meta-analysis in kidney allograft recipients identified three associated variants.^{11,12} Genetic variants have also been associated with respiratory infection and primary graft dysfunction but, again, most were underpowered case studies; future studies will require larger cohorts.¹³ Several studies have analyzed telomere length and its association with age and have shown that shorter telomeres are associated with worse transplant outcome^{14,15,16} yet there are other studies that have not found these associations.¹⁷

As noted above, previous attempts to identify and then validate genetic risk factors have been complicated by a number of aspects including differences in study populations, underpowered cohorts, variation in study design, differing clinical interpretation of outcomes and varying use of statistical methods. There is a need for large prospective multicenter studies to identify and validate these alleles before they are translated into clinical trials.¹⁸ Most likely, multiple gene variants will cumulatively identify the genetically associated variation for the many transplant outcomes.¹⁹ These studies will be complicated though by the fact that individuals will have differing combinations of these risk alleles requiring gene-gene interaction studies and even larger well-described cohorts. Additionally, there will likely be different variants for each organ type, and for the donor and the recipient. However, once we have identified a set of alleles with strong statistical power to predict outcome, clinicians will be able to identify at-risk individuals and provide risk tailored treatment. Identification of these genes will also help us better understand the pathways associated with transplant outcomes providing a better understanding of mechanisms and lead to even better treatment trials.

Pharmacogenomics in Transplantation

Pharmacogenomics is the study of how genes affect a person's response to drugs. It is a rapidly growing and maturing field and has already had clinical implementation in areas such as psychiatry, oncology and cardiology. There are to date 17 published pharmacogenomic guidelines for 38 drugs (<https://www.pharmgkb.org/page/cpic>). The NIH Pharmacogenomics Research Network (PGRN) supports the development of these guidelines through the work of the Clinical Pharmacogenetic Implementation Consortium (CPIC) which reviews and rates the strength of the studies, and authors the guidelines. In most cases, individuals who carry risk variants require either non-standard doses due to altered pharmacokinetics and clinical outcomes or are at higher risk of drug-related adverse effects. Most of the important variants to date that have been identified are in genes mainly for drug metabolizing enzymes, drug transporters and specific HLA alleles. For immunosuppressants, clinical guidelines are available for azathioprine and more recently for tacrolimus. Table 1 provides examples of CPIC guidelines for medications used in transplantation.

Pharmacogenomic studies for the immune suppressants, cyclosporine, tacrolimus, mycophenolate, sirolimus and azathioprine have primarily evaluated variants in drug metabolizing enzymes and transporters.²⁰ The pharmacogenomics data for tacrolimus is strong and the presence of cytochrome P450 3A4/5 variants (*CYP3A4* and *CYP3A5* genes)

profoundly affects tacrolimus pharmacokinetics and dose.²¹ Randomized trials of genotype-directed tacrolimus dosing have recently been conducted in primarily Caucasian kidney transplant populations.^{22–24} One trial demonstrated improvement in achieving therapeutic blood concentrations on days 3 and 10 posttransplant, with fewer dose adaptations in the genotype-directed dosing group²³ whereas a similar trial did not.²² There was no difference in delayed graft dysfunction, AR, CGD or toxicity between the groups. However, these studies had a number of limitations: they enrolled low immunologic risk patients and low numbers of patients with the functional *CYP3A5*1* allele who would benefit most from genotype-directed dosing; they targeted higher trough concentrations than typically used in the United States, and they did not consider important variants that have been recently identified.

In the past several years there has been growing recognition that there is more genetic variation in the *CYP3A4* and *CYP3A5* genes present in individuals with African ancestry relative to Caucasians.^{25–27} A recent GWAS of tacrolimus trough concentrations in African American kidney transplant recipients identified large effects of three common *CYP3A5* variants (*CYP3A5*3*, **6* and **7*) on tacrolimus metabolism.²⁸ The *CYP3A5*3*, a cryptic splice junction variant, is a loss of function (LoF) variant in intron 3 of the *CYP3A5* gene which generates splice variants containing stop codons leading to nonsense mediated decay of the *CYP3A5* mRNA²⁹, protein truncation, resulting in an absence of CYP3A5 enzyme activity. The *CYP3A5*6* and **7* variants also result in a reduced or loss of enzyme function. One of these 3 variants is carried by 50% of African Americans; and two LoF variants by 26%. Individuals carrying two of these variants (i.e. **3/*3*, **3/*7*, **3/*6*) have profoundly reduced or complete absence of CYP3A5 enzyme activity and a slow metabolism phenotype. African Americans who do not carry these variants are assumed to carry the *CYP3A5*1* allele and express high amounts of active CYP3A5 enzyme which confers a rapid metabolism phenotype, resulting in rapid tacrolimus clearance. African Americans, who more frequently carry a *CYP3A5*1* allele and have higher risk of poorer outcomes,^{30,31} may benefit most from genotype-directed dosing.

The majority of Caucasians (90%) carry two LoF variants (mainly *CYP3A5 *3/*3*) and therefore have a nearly complete absence of CYP3A5 activity. The allele frequency for the active *CYP3A5*1* variant in Caucasians is only 5.6%. The *CYP3A5*6* or **7* alleles rarely occur in Caucasians. Therefore most Caucasians and many African Americans are lacking or have reduced CYP3A5 activity and are dependent on the CYP3A4 enzyme for the metabolism of tacrolimus and other drugs. The *CYP3A4* genetic variant, *CYP3A4*22*, is also a LoF variant that occurs more often in Caucasians and is well-known to influence tacrolimus metabolism. Individuals who lack CYP3A5 enzyme and also carry a *CYP3A4*22* variant are at high risk for elevated tacrolimus concentrations and thus toxicity. The last few years have brought understanding that multiple variants along with well-known clinical factors influence tacrolimus metabolism. Recently more precise tacrolimus dosing models which account for genotype and clinical factors such as age, drug-drug interactions, and time posttransplant have been developed for African American recipients and may improve genotype directed dosing accuracy.³² These variants may also influence the efficacy and/or toxicity of the many other CYP3A substrate drugs used in transplantation. This will

likely be a focus of future research since over 50% of marketed drugs are dependent on the CYP3A4 and/or CYP3A5 enzymes for metabolism.³³

The use of pharmacogenomics strives to reduce intra-individual variation in drug pharmacokinetics and improve efficacy. Furthermore, high intra-patient variability of tacrolimus concentrations has led to worse outcomes.^{34–37} Recent data showed that high pharmacokinetic variability in tacrolimus troughs is associated with increased risk of moderate to severe fibrosis and tubular atrophy.³⁸ Although many factors contribute to variability including adherence, early variability is related to differences in metabolism. Thus, better outcomes likely will occur with proper tacrolimus dosing and less intra-patient variability.

Donor Variants

Few studies have found robust donor genetic variants associated with transplant outcomes. However, variants in the *Apolipoprotein L1 gene (APOLI)* have been associated with worse outcomes in the recipients.³⁹ Studies have focused on the G1 and G2 alleles of APOLI. The *APOLI* G1 allele is defined by two variants (rs73885319; rs60910145) and the G2 allele which is an insertion/deletion (rs71785313) variant. In the US population, the *APOLI* variants are found predominantly in individuals with recent African ancestry. In one study of African Americans, 20% to 22% carried a G1 variant, 3% to 15% carried a G2 variant while approximately 10% to 15% of African Americans carry two *APOLI* kidney risk alleles.⁴⁰ The high frequency of these G1 and G2 alleles of *APOLI* in populations of African descent are thought to be due to positive selection being protective against African sleeping sickness caused by the *Trypanosoma brucei rhodesiense*.³⁹

It is now apparent that the variants in *APOLI* help explain the increased risk of end-stage renal disease in African Americans compared to Caucasians^{41,42} and as well as poor deceased donor kidney transplant outcomes.⁴¹ Several single center and multi-center retrospective studies have shown an association of deceased donor *APOLI* risk variants with worse allograft survival.^{41,43,44} In the largest multi-center study, 1,153 deceased donor kidney transplants from 624 African American donors showed that two APOLI risk variants were associated with increased risk of allograft failure (adjusted HR of 2.5, $p < 0.0001$).⁴¹ A recent case report highlighted two kidney transplant recipients who developed glomerular disease whose donors carried two *APOLI* risk variants.⁴⁵

Although there is an association between *APOLI* alleles and kidney transplant outcomes, no effect has been observed after liver transplant. Dorr et al. reported on 639 African American liver transplant patients, where 47% of the subjects had one *APOLI* risk allele and 14% had two risk alleles, and that these alleles do not impact liver transplant outcomes.⁴⁶ Although, not all transplant types have been studied yet, it seems these *APOLI* alleles cause most risk in kidney transplantation.

The mechanism through which *APOLI* risk variants impact allograft survival is not known and the biopsy phenotype of *APOLI* related allograft loss is not well-described. Since not every recipient of a kidney with *APOLI* risk variants undergoes premature allograft loss, a

second hit hypothesis has been postulated. How these second hits such as viral infections interact with *APOL1* risk variants to increase risk of allograft loss is not understood. Therefore, the NIH is planning a large, national, prospective observational study (APOL1 Long-Term Kidney Transplantation Outcomes Network [APOLLO]) to answer these questions.

Identifying Rare Variants

While GWAS are successful in identifying known variants associated with outcomes⁴⁷ DNA sequencing is quickly becoming state of the art for identification of risk variants.^{48–50} Whole exome, targeted, or whole genome sequencing has been employed to detect low-frequency variants associated with outcomes in transplantation. Larger transplant data sets will be required and alternative study designs, such as extreme phenotyping sampling (EPS) will be needed to identify rare variant associations with studied outcomes. This type of design allows for sequencing of smaller sample sizes and lower costs.⁵⁰ There are now data emerging that these low-frequency variants are impactful particularly towards drug therapy. One example where sequencing is applied is for the CYP genes. Common variants in the *CYP3A4*, *CYP3A5*, P450 oxidoreductase (*POR*) and cytochrome b5(*CYB5A*) alleles that constitute the P450 complex that is responsible for tacrolimus oxidative metabolism, along with clinical factors strongly account for about 50% of the variability in tacrolimus troughs in African-American transplant recipients,²⁸ but less in white European Americans. It is likely that remaining genetic variability yet to be identified is due to low-frequency variants, environmental factors such as diet, and epigenetics. It may be possible to use EPS strategies to identify low-frequency genetic variants associated with the very highest and lowest tacrolimus concentrations. Since these two groups of subjects have the most extreme phenotypes, they are more likely to carry low-frequency genetic variants. Sequences from the subjects can then be aligned to the human reference genome in dbSNP and rare variants identified with tools such as Genome Analysis Tool Kit (GATK).^{51,52} For instance, next generation sequencing (NGS) of extreme phenotypes has recently identified low-frequency variants in a variety of diseases including lung cancer,⁵³ chronic obstructive pulmonary disease,⁵⁴ diabetic retinopathy,⁵⁵ chronic *Pseudomonas aeruginosa* infection in cystic fibrosis,⁵⁶ severe iron overload,⁵⁷ and hypercholesterolemia.⁵⁸ Rare variants were associated with LDL cholesterol levels using NGS, with differing frequencies in African Americans compared to European Americans.⁵⁹ As with common variants, low-frequency variants will require validation.

Conclusion

As we progress in the future with clinical research, transplantation is becoming more personalized and tailored for the individual patient. In the past, transplant donors and recipients had a lower level of HLA mismatch, but immunosuppression was often given at standard doses, with hope for optimal outcomes. Now, as we further understand the impact of genetics variation in donor tissues and the recipient's genetic disposition to immune suppressant response, we and others are implementing dosing strategies aimed to improve outcomes.^{32,60,61} We are continually discovering new genetic variants associated with immune suppressant metabolism, associated with specific outcomes, and that are enriched in

different populations. Part of our future goal is to understand, as best possible, the entire genomic landscape of transplantation. We believe that the future of transplantation will be improved as we further understand the role of these genetic variants, gene-gene, and gene-environment interactions. Thus, we see transplantation as becoming much more personalized along with improved precision therapies.

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Table 1

Selected Guidelines from the Clinical Pharmacogenetics Implementation Consortium

Drug	Gene	Risk variant alleles or genotypes	Recommendation
allopurinol ^{62,63}	<i>HLA-B</i>	<i>HLA-B*58:01</i>	Contraindicated in individuals with one or more <i>HLA-B*58:01</i> variant alleles due to increased risk of allopurinol-induced severe cutaneous adverse reaction.
azathioprine ^{64,65}	<i>TPMT</i>	Intermediate enzyme activity- <i>*1/*2, *1/*3A, *1/*3B, *1/*3C, *1/*4.</i> Low or deficient enzyme activity <i>*3A/*3A, *2/*3A, *3C/*3A, *3C/*4, *3C/*2, *3A/*4.</i>	Intermediate activity - start at 30–70% of target dose. Low or deficient - consider an alternate agent or extreme dose reduction of azathioprine.
clopidogrel ^{66,67}	<i>CYP2C19</i>	Intermediate metabolizer <i>*1/*2, *1/*3, *2/*17</i> (~18–45% of patients) Poor metabolizer <i>*2/*2, *2/*3, *3/*3</i> (~2–15% of patients)	Intermediate or poor activity - alternative antiplatelet therapy (if no contraindication); e.g., prasugrel, ticagrelor.
citalopram/escitalopram ⁶⁸	<i>CYP2C19</i>	Ultrarapid metabolizer <i>*17/*17, *1/*17</i> (~5–30% of patients). Poor metabolizer <i>*2/*2, *2/*3, *3/*3</i> (~2–15% of patients).	Ultra-rapid - Consider an alternative drug not predominantly metabolized by <i>CYP2C19</i> . Poor - Consider a 50% reduction of recommended starting dose and titrate to response or select alternative drug not predominantly metabolized by <i>CYP2C19</i> .
phenytoin ⁶⁹	<i>HLA-B</i>	<i>HLA-B*15:02</i>	Increased risk of phenytoin- induced SJS/TEN in individuals with one or more alleles.
phenytoin ⁶⁹	<i>CYP2C9</i>	Intermediate metabolizer <i>*1/*3, *1/*2</i> (~8% of patients) Poor metabolizer <i>*2/*2, *3/*3, *2/*3</i> (~1% of patients)	Intermediate - consider 25% reduction of recommended starting maintenance dose. Subsequent doses should be adjusted according to therapeutic drug monitoring and response. Poor - Consider 50% reduction of recommended starting maintenance dose. Subsequent maintenance doses should be adjusted according to therapeutic drug monitoring and response.
simvastatin ^{70,71}	<i>SLCO1B1</i>	Intermediate function <i>*1a/*5, *1a/*15, *1a/*17, *1b/*5, *1b/*15, *1b/*17.</i> Low function <i>*5/*5, *5/*15, *5/*17, *15/*15, *15/*17, *17/*17</i>	Intermediate myopathy risk, prescribe a lower dose or consider an alternative statin (e.g. pravastatin or rosuvastatin); consider routine CK surveillance. High myopathy risk, prescribe a lower dose or consider an alternative statin (e.g. pravastatin or rosuvastatin); consider routine CK surveillance.
tacrolimus ⁶¹	<i>CYP3A5</i>	Extensive metabolizer <i>*1/*1</i> Intermediate metabolizer <i>*1/*3, *1/*6, *1/*7</i>	Extensive or intermediate - Increase starting dose 1.5 to 2 times recommended starting dose. Total starting dose should not exceed 0.3mg/kg/day. Use therapeutic drug monitoring to guide dose adjustments.
voriconazole ⁷²	<i>CYP2C19</i>	Ultrarapid metabolizer <i>*17/*17</i> (~2–5% of patients). Rapid metabolizer <i>*1/*17</i> (~2–30% of patients). Poor metabolizer <i>*2/*2, *2/*3, *3/*3</i> (~2–15% of patients).	Ultrarapid and rapid at risk for low blood concentrations. Choose an alternative agent that is not dependent on <i>CYP2C19</i> metabolism as primary therapy in lieu of voriconazole. Such agents include isavuconazole, liposomal amphotericin B, and posaconazole. Poor metabolizer are at risk for adverse events. Choose an alternative agent that is not dependent on <i>CYP2C19</i> metabolism as primary therapy in lieu of voriconazole. Such agents include isavuconazole,

Drug	Gene	Risk variant alleles or genotypes	Recommendation
			liposomal amphotericin B, and posaconazole. In the event that voriconazole is considered to be the most appropriate agent, based on clinical advice, for a patient with poor metabolizer genotype, voriconazole should be administered at a preferably lower than standard dosage with careful therapeutic drug monitoring.

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