Curr Transpl Rep (2017) 4:116–123
DOI 10.1007/s40472-017-0153-x

KIDNEY TRANSPLANTATION (M HENRY, SECTION EDITOR)

Concepts of Genomics in Kidney Transplantation

William S. Oetting[1] · Casey Dorr[2,3] · Rory P. Remmel[4] · Arthur J. Matas[5] · Ajay K. Israni[2,3] · Pamala A. Jacobson[1]

Published online: 24 May 2017

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

This article is part of the Topical Collection on *Kidney Transplantation*

✉ Pamala A. Jacobson
jacob117@umn.edu

[1] Department of Experimental and Clinical Pharmacology, University of Minnesota, 7-151 Weaver-Densford Hall, 308 Harvard Street SE, Minneapolis, MN 55455, USA

[2] Minneapolis Medical Research Foundation, Minneapolis, MN, USA

[3] Department of Medicine, Hennepin County Medical Center, University of Minnesota, Minneapolis, MN, USA

[4] Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN, USA

[5] Department of Surgery, University of Minnesota, Minneapolis, MN, USA

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 (HLAs) [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 two 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 underpowered [7], 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 (GWASs) 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 [14–16], 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 Pharmacogenetics 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 [32]. The pharmacogenomic data for tacrolimus is strong, and the presence of cytochrome P450 3A4/5 variants (*CYP3A4* and *CYP3A5* genes) profoundly affects tacrolimus pharmacokinetics and dose [33]. Randomized trials of genotype-directed tacrolimus dosing have recently been conducted in primarily Caucasian kidney transplant populations [34–36]. One trial demonstrated improvement in achieving therapeutic blood concentrations on days 3 and 10 posttransplant, with fewer dose adaptations in the genotype-directed dosing group [35], whereas a similar trial did not [34]. 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

Table 1 Selected guidelines from the Clinical Pharmacogenetics Implementation Consortium

Drug	Gene	Risk variant alleles or genotypes	Recommendation
Allopurinol [20, 21]	*HLA-B*	*HLA-B*58:01*	Contraindicated in individuals with one or more HLA-B*58:01 variant alleles due to increased risk of allopurinol-induced severe cutaneous adverse reaction
Azathioprine [22, 23]	*TPMT*	Intermediate enzyme activity-**1/*2, *1/*3A, *1/*3B, *1/*3C, *1/*4* Low or deficient enzyme activity **3A/*3A, *2/*3A, *3C/*3A, *3C/*4, *3C/*2, *3A/*4*	Intermediate activity—start at 30–70% of target dose Low or deficient—consider an alternate agent or extreme dose reduction of azathioprine
Clopidogrel [24, 25]	*CYP2C19*	Intermediate metabolizer **1/*2, *1/*3, *2/*17* (~18–45% of patients) Poor metabolizer **2/*2, *2/*3, *3/*3* (~2–15% of patients)	Intermediate or poor activity—alternative antiplatelet therapy (if no contraindication), e.g., prasugrel and ticagrelor
Citalopram/escitalopram [26]	*CYP2C19*	Ultrarapid metabolizer **17/*17, *1/*17* (~5–30% of patients) Poor metabolizer **2/*2, *2/*3, *3/*3* (~2–15% of patients)	Ultrarapid—consider an alternative drug not predominantly metabolized by CYP2C19 Poor—consider a 50% reduction of recommended starting dose and titrate to response or select alternative drug not predominantly metabolized by CYP2C19
Phenytoin [27]	*HLA-B*	*HLA-B*15:02*	Increased risk of phenytoin-induced SJS/TEN in individuals with one or more alleles
Phenytoin [27]	*CYP2C9*	Intermediate metabolizer **1/*3, *1/*2* (~8% of patients) Poor metabolizer **2/*2, *3/*3, *2/*3* (~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 [28, 29]	*SLCO1B1*	Intermediate function **1a/*5, *1a/*15, *1a/*17, *1b/*5, *1b/*15, *1b/*17* Low function **5/*5, *5/*15, *5/*17, *15/*15, *15/*17, *17/*17*	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 [30]	*CYP3A5*	Extensive metabolizer **1/*1* Intermediate metabolizer **1/*3, *1/*6, *1/*7*	Extensive or intermediate—increase starting dose 1.5 to 2 times recommended starting dose. Total starting dose should not exceed 0.3 mg/kg/day. Use therapeutic drug monitoring to guide dose adjustments
Voriconazole [31]	*CYP2C19*	Ultrarapid metabolizer **17/*17* (~2–5% of patients) Rapid metabolizer **1/*17* (~2–30% of patients) Poor metabolizer *2/*2, *2/*3, *3/*3 (~2–15% of patients)	Ultrarapid and rapid at risk for low blood concentrations. Choose an alternative agent that is not dependent on CYP2C19 metabolism as primary therapy in lieu of voriconazole. Such agents include isavuconazole, liposomal amphotericin B, and posaconazole Poor metabolizer is at risk for adverse events. Choose an alternative agent that is not dependent on CYP2C19 metabolism as primary therapy in lieu of voriconazole. Such agents include isavuconazole, 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

concentrations than typically used in the USA, 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 [37–39]. 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 [40•]. 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 non-sense mediated decay of the *CYP3A5* mRNA [41], 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 three 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 [42, 43], 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 [44••]. 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 [45].

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 [46–49]. Recent data showed that high pharmacokinetic variability in tacrolimus troughs is associated with increased risk of moderate to severe fibrosis and tubular atrophy [50]. 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* (*APOL1*) have been associated with worse outcomes in the recipients [51]. Studies have focused on the G1 and G2 alleles of APOL1. The *APOL1* G1 allele is defined by two variants (rs73885319 and rs60910145) and the G2 allele which is an insertion/deletion (rs71785313) variant. In the US population, the *APOL1* 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 *APOL1* kidney risk alleles [52]. The high frequency of these G1 and G2 alleles of *APOL1 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* [51].

It is now apparent that the variants in *APOL1* help explain the increased risk of end-stage renal disease in African Americans compared to Caucasians [53••, 54] as well as poor deceased donor kidney transplant outcomes [53••]. Several single-center and multi-center retrospective studies have shown an association of deceased donor *APOL1* risk variants with worse allograft survival [53••, 55, 56•]. In the largest multi-center study, 1153 deceased donor kidney transplants from 624 African American donors showed that two APOL1 risk variants were associated with increased risk of allograft failure (adjusted HR of 2.5, $p < 0.0001$) [53••]. A recent case report highlighted two kidney transplant recipients who developed glomerular disease whose donors carried two *APOL1* risk variants [57].

Although there is an association between *APOL1* 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 *APOL1* risk allele and 14% had two risk alleles, and that these alleles do not impact liver transplant outcomes [58]. Although not all transplant types have been studied yet, it

seems that these *APOL1* alleles cause most risk in kidney transplantation.

The mechanism through which *APOL1* risk variants impact allograft survival is not known, and the biopsy phenotype of *APOL1*-related allograft loss is not well described. Since not every recipient of a kidney with *APOL1* 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 GWASs are successful in identifying known variants associated with outcomes [59•], DNA sequencing is quickly becoming state of the art for identification of risk variants [60–62]. 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 [62]. 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 [40•], 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) [63, 64]. For instance, next-generation sequencing (NGS) of extreme phenotypes has recently identified low-frequency variants in a variety of diseases including lung cancer [65], chronic obstructive pulmonary disease [66], diabetic retinopathy [67], chronic *Pseudomonas aeruginosa* infection in cystic fibrosis [68], severe iron overload [69], and hypercholesterolemia [70]. Rare variants were associated with LDL cholesterol levels using NGS, with differing frequencies in African Americans compared to European Americans [71•]. 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 genetic 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 [30, 44••, 72]. 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.

Compliance with Ethical Standards

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.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Sheldon S, Hasleton PS, Yonan NA, Rhaman AN, Deiraniya AK, Campbell CS, et al. Rejection in heart transplantation strongly correlates with HLA-DR antigen mismatch. Transplantation. 1994;58:719–22.
2. Zachary AA, Leffell MS. HLA mismatching strategies for solid organ transplantation—a balancing act. Front Immunol. 2016;7:575. doi:10.3389/fimmu.2016.00575.
3. Kruger B, Schroppel B. Genetic variations and transplant outcomes. Nephron Clin Pract. 2011;118:c49–54. doi:10.1159/000320904.

4. Stojanova J, Pouche L, Picard N. Genetic polymorphisms in the immune response: a focus on kidney transplantation. Clin Biochem. 2016;49:363–76. doi:10.1016/j.clinbiochem.2015.07.016.
5. Chand S, McKnight AJ, Borrows R. Genetic polymorphisms and kidney transplant outcomes. Curr Opin Nephrol Hypertens. 2014;23:605–10. doi:10.1097/MNH.0000000000000068.
6. Oetting WS, Schladt DP, Leduc RE, Jacobson PA, Guan W, Matas AJ, et al. Validation of single nucleotide polymorphisms associated with acute rejection in kidney transplant recipients using a large multi-center cohort. Transpl Int. 2011;24:1231–8. doi:10.1111/j.1432-2277.2011.01359.x.
7. Stegall MD, Park WD, Dierkhising R. Genes and transplant outcomes: the search for "associations". Transplantation. 2014;98:257–8. doi:10.1097/TP.0000000000000140.
8. Israni A, Leduc R, Holmes J, Jacobson PA, Lamba V, Guan W, et al. Single-nucleotide polymorphisms, acute rejection, and severity of tubulitis in kidney transplantation, accounting for center-to-center variation. Transplantation. 2010;90:1401–8. doi:10.1097/TP.0b013e3182000085.
9. Ghisdal L, Baron C, Lebranchu Y, Viklicky O, Konarikova A, Naesens M, et al. Genome-wide association study of acute renal graft rejection. Am J Transplant. 2017;17:201–9. doi:10.1111/ajt.13912.
10. Pihlstrom HK, Mjoen G, Mucha S, Haraldsen G, Franke A, Jardine A, et al. Single nucleotide polymorphisms and long-term clinical outcome in renal transplant patients: a validation study. Am J Transplant. 2017;17:528–33. doi:10.1111/ajt.13995.
11. Chand S, McKnight AJ, Shabir S, Chan W, McCaughan JA, Maxwell AP, et al. Analysis of single nucleotide polymorphisms implicate mTOR signalling in the development of new-onset diabetes after transplantation. BBA Clin. 2016;5:41–5. doi:10.1016/j.bbacli.2015.12.004.
12. Benson KA, Maxwell AP, McKnight AJ. A HuGE review and meta-analyses of genetic associations in new onset diabetes after kidney transplantation. PLoS One. 2016;11:e0147323. doi:10.1371/journal.pone.0147323.
13. Ruttens D, Vandermeulen E, Verleden SE, Bellon H, Vos R, Van Raemdonck DE, et al. Role of genetics in lung transplant complications. Ann Med. 2015;47:106–15. doi:10.3109/07853890.2015.1004359.
14. Oetting WS, Guan W, Schladt DP, Wildebush WA, Becker J, Thyagarajan B, et al. Telomere length of recipients and living kidney donors and chronic graft dysfunction in kidney transplants. Transplantation. 2014;97:325–9. doi:10.1097/01.TP.0000436705.87898.88.
15. Kloda K, Domanski L, Kwiatkowska E, Safranow K, Drozd A, Ciechanowicz A, et al. BICD1 and chromosome 18 polymorphisms associated with recipients' telomere length affect kidney allograft function after transplantation. Transplant Proc. 2016;48:1451–5. doi:10.1016/j.transproceed.2015.10.086.
16. Melk A, Ramassar V, Helms LM, Moore R, Rayner D, Solez K, et al. Telomere shortening in kidneys with age. J Am Soc Nephrol. 2000;11:444–53.
17. Kloda K, Domanski L, Mierzecki A. Telomere length assessment for prediction of organ transplantation outcome. Future or failure: a review of the literature. Med Sci Monit. 2017;23:158–62.
18. International Genetics & Translational Research in Transplantation Network (iGeneTRAiN). Design and implementation of the international genetics and translational research in transplantation network. Transplantation. 2015;99:2401–12. doi:10.1097/TP.0000000000000913.
19. Oetting WS, Jacobson PA, Israni AK. Transplant rejection and risk: in search of the genetic dark matter. J Gastrointestin Liver Dis. 2013;22:125–6.
20. Hershfield MS, Callaghan JT, Tassaneeyakul W, Mushiroda T, Thorn CF, Klein TE, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for human leukocyte antigen-B genotype and allopurinol dosing. Clin Pharmacol Ther. 2013;93:153–8. doi:10.1038/clpt.2012.209.
21. Saito Y, Stamp LK, Caudle KE, Hershfield MS, McDonagh EM, Callaghan JT, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for human leukocyte antigen B (HLA-B) genotype and allopurinol dosing: 2015 update. Clin Pharmacol Ther. 2016;99:36–7. doi:10.1002/cpt.161.
22. Relling MV, Gardner EE, Sandborn WJ, Schmiegelow K, Pui CH, Yee SW, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing: 2013 update. Clin Pharmacol Ther. 2013;93:324–5. doi:10.1038/clpt.2013.4.
23. Relling MV, Gardner EE, Sandborn WJ, Schmiegelow K, Pui CH, Yee SW, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. Clin Pharmacol Ther. 2011;89:387–91. doi:10.1038/clpt.2010.320.
24. Scott SA, Sangkuhl K, Gardner EE, Stein CM, Hulot JS, Johnson JA, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy. Clin Pharmacol Ther. 2011;90:328–32. doi:10.1038/clpt.2011.132.
25. Scott SA, Sangkuhl K, Stein CM, Hulot JS, Mega JL, Roden DM, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. Clin Pharmacol Ther. 2013;94:317–23. doi:10.1038/clpt.2013.105.
26. Hicks JK, Bishop JR, Sangkuhl K, Muller DJ, Ji Y, Leckband SG, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2D6 and CYP2C19 genotypes and dosing of selective serotonin reuptake inhibitors. Clin Pharmacol Ther. 2015;98:127–34. doi:10.1002/cpt.147.
27. Caudle KE, Rettie AE, Whirl-Carrillo M, Smith LH, Mintzer S, Lee MT, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C9 and HLA-B genotypes and phenytoin dosing. Clin Pharmacol Ther. 2014;96:542–8. doi:10.1038/clpt.2014.159.
28. Wilke RA, Ramsey LB, Johnson SG, Maxwell WD, McLeod HL, Voora D, et al. The clinical pharmacogenomics implementation consortium: CPIC guideline for SLCO1B1 and simvastatin-induced myopathy. Clin Pharmacol Ther. 2012;92:112–7. doi:10.1038/clpt.2012.57.
29. Ramsey LB, Johnson SG, Caudle KE, Haidar CE, Voora D, Wilke RA, et al. The Clinical Pharmacogenetics Implementation Consortium guideline for SLCO1B1 and simvastatin-induced myopathy: 2014 update. Clin Pharmacol Ther. 2014;96:423–8. doi:10.1038/clpt.2014.125.
30. Birdwell KA, Decker B, Barbarino JM, Peterson JF, Stein CM, Sadee W, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for CYP3A5 genotype and tacrolimus dosing. Clin Pharmacol Ther. 2015;98:19–24. doi:10.1002/cpt.113.
31. Moriyama B, Obeng AO, Barbarino J, Penzak SR, Henning SA, Scott SA, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC(R)) guideline for CYP2C19 and voriconazole therapy. Clin Pharmacol Ther. 2016; doi:10.1002/cpt.583.
32. Picard N, Bergan S, Marquet P, van Gelder T, Wallemacq P, Hesselink DA, et al. Pharmacogenetic biomarkers predictive of the pharmacokinetics and pharmacodynamics of immunosuppressive drugs. Ther Drug Monit. 2016;38(Suppl 1):S57–69. doi:10.1097/FTD.0000000000000255.
33. Rojas L, Neumann I, Herrero MJ, Boso V, Reig J, Poveda JL, et al. Effect of CYP3A5*3 on kidney transplant recipients treated with tacrolimus: a systematic review and meta-analysis of observational studies. Pharmacogenomics J. 2015;15:38–48. doi:10.1038/tpj.2014.38.

34. Shuker N, Bouamar R, van Schaik RH, Clahsen-van Groningen MC, Damman J, Baan CC, et al. A randomized controlled trial comparing the efficacy of Cyp3a5 genotype-based with body-weight-based tacrolimus dosing after living donor kidney transplantation. Am J Transplant. 2016;16:2085–96. doi:10.1111/ajt.13691.
35. Thervet E, Loriot MA, Barbier S, Buchler M, Ficheux M, Choukroun G, et al. Optimization of initial tacrolimus dose using pharmacogenetic testing. Clin Pharmacol Ther. 2010;87:721–6. doi:10.1038/clpt.2010.17.
36. Pallet N, Etienne I, Buchler M, Bailly E, Hurault de Ligny B, Choukroun G, et al. Long-term clinical impact of adaptation of initial tacrolimus dosing to CYP3A5 genotype. Am J Transplant. 2016;16:2670–5. doi:10.1111/ajt.13788.
37. Bains RK. African variation at cytochrome P450 genes: evolutionary aspects and the implications for the treatment of infectious diseases. Evol Med Public Health. 2013;2013:118–34. doi:10.1093/emph/eot010.
38. Bains RK, Kovacevic M, Plaster CA, Tarekegn A, Bekele E, Bradman NN, et al. Molecular diversity and population structure at the cytochrome P450 3A5 gene in Africa. BMC Genet. 2013;14:34. doi:10.1186/1471-2156-14-34.
39. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet. 2001;27:383–91. doi:10.1038/86882.
40.• Oetting WS, Schladt DP, Guan W, Miller MB, Remmel RP, Dorr C, et al. Genomewide association study of tacrolimus concentrations in African American kidney transplant recipients identifies multiple CYP3A5 alleles. Am J Transplant. 2016;16:574–82. doi:10.1111/ajt.13495. **CYP3A5 variants (*3, *6 and *7) were identified in AA recipients to be significantly associated with tacrolimus troughs. The three variants and clinical factors account for 53.9% of the observed variance**.
41. Busi F, Cresteil T. CYP3A5 mRNA degradation by nonsense-mediated mRNA decay. Mol Pharmacol. 2005;68:808–15. doi:10.1124/mol.105.014225.
42. Taber DJ, Egede LE, Baliga PK. Outcome disparities between African Americans and Caucasians in contemporary kidney transplant recipients. Am J Surg. 2016; doi:10.1016/j.amjsurg.2016.11.024.
43. Taber DJ, Hunt KJ, Fominaya CE, Payne EH, Gebregziabher M, Srinivas TR, et al. Impact of cardiovascular risk factors on graft outcome disparities in black kidney transplant recipients. Hypertension. 2016;68:715–25. doi:10.1161/HYPERTENSIONAHA.116.07775.
44.•• Sanghavi K, Brundage RC, Miller MB, Schladt DP, Israni AK, Guan W, et al. Genotype-guided tacrolimus dosing in African-American kidney transplant recipients. Pharmacogenomics J. 2015; doi:10.1038/tpj.2015.87. **This study is the first to develop an African American specific genotype-guided tacrolimus dosing model to personalize therapy**.
45. Pelkonen O, Turpeinen M, Hakkola J, Honkakoski P, Hukkanen J, Raunio H. Inhibition and induction of human cytochrome P450 enzymes: current status. Arch Toxicol. 2008;82:667–715. doi:10.1007/s00204-008-0332-8.
46. Borra LC, Roodnat JI, Kal JA, Mathot RA, Weimar W, van Gelder T. High within-patient variability in the clearance of tacrolimus is a risk factor for poor long-term outcome after kidney transplantation. Nephrol Dial Transplant. 2010;25:2757–63. doi:10.1093/ndt/gfq096.
47. Ro H, Min SI, Yang J, Moon KC, Kim YS, Kim SJ, et al. Impact of tacrolimus intraindividual variability and CYP3A5 genetic polymorphism on acute rejection in kidney transplantation. Ther Drug Monit. 2012;34:680–5. doi:10.1097/FTD.0b013e3182731809.
48. Hsiau M, Fernandez HE, Gjertson D, Ettenger RB, Tsai EW. Monitoring nonadherence and acute rejection with variation in blood immunosuppressant levels in pediatric renal transplantation. Transplantation. 2011;92:918–22. doi:10.1097/TP.0b013e31822dc34f.
49. Prytula AA, Bouts AH, Mathot RA, van Gelder T, Croes LK, Hop W, et al. Intra-patient variability in tacrolimus trough concentrations and renal function decline in pediatric renal transplant recipients. Pediatr Transplant. 2012;16:613–8. doi:10.1111/j.1399-3046.2012.01727.x.
50. Vanhove T, Vermeulen T, Annaert P, Lerut E, Kuypers DR. High intrapatient variability of tacrolimus concentrations predicts accelerated progression of chronic histologic lesions in renal recipients. Am J Transplant. 2016; doi:10.1111/ajt.13803.
51. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. Science. 2010;329:841–5. doi:10.1126/science.1193032.
52. Limou S, Nelson GW, Kopp JB, Winkler CA. APOL1 kidney risk alleles: population genetics and disease associations. Adv Chronic Kidney Dis. 2014;21:426–33. doi:10.1053/j.ackd.2014.06.005.
53.•• Freedman BI, Pastan SO, Israni AK, Schladt D, Julian BA, Gautreaux MD, et al. APOL1 genotype and kidney transplantation outcomes from deceased African American donors. Transplantation. 2016;100:194–202. doi:10.1097/TP.0000000000000969. **The largest multi-center, retrospective study showing increased risk of allograft loss in recipients of deceased donors with APOL1 risk variants**.
54. Freedman BI, Langefeld CD, Lu L, Divers J, Comeau ME, Kopp JB, et al. Differential effects of MYH9 and APOL1 risk variants on FRMD3 association with diabetic ESRD in African Americans. PLoS Genet. 2011;7:e1002150. doi:10.1371/journal.pgen.1002150.
55. Freedman BI. APOL1 and kidney disease: new insights leading to novel therapies. Am J Kidney Dis. 2015;66:9–11. doi:10.1053/j.ajkd.2015.05.005.
56.• Reeves-Daniel AM, JA DP, Bleyer AJ, Rocco MV, Murea M, Adams PL, et al. The APOL1 gene and allograft survival after kidney transplantation. Am J Transplant Off J Am Soc Transplant Am Soc Transplant Surg. 2011;11:1025–30. doi:10.1111/j.1600-6143.2011.03513.x. **This manuscript shows that renal transplants from African American donors with 1 or 2 APOL1 risk alleles have more rapid renal failure than those without the risk alleles**.
57. Shah PB, Cooper JE, Lucia MS, Boils C, Larsen CP, Wiseman AC. APOL1 polymorphisms in a deceased donor and early presentation of collapsing Glomerulopathy and focal segmental glomerulosclerosis in two recipients. Am J Transplant Off J Am Soc Transplant Am Soc Transplant Surg. 2016;16:1923–7. doi:10.1111/ajt.13748.
58. Dorr CR, Freedman BI, Hicks PJ, Brown WM, Russell GB, Julian BA, et al. Deceased-donor apolipoprotein L1 renal-risk variants have minimal effects on liver transplant outcomes. PLoS One. 2016;11:e0152775. doi:10.1371/journal.pone.0152775.
59.• Oetting WS, Schladt DP, Guan W, Miller MB, Remmel RP, Dorr C, et al. Genomewide association study of tacrolimus concentrations in African American kidney transplant recipients identifies multiple CYP3A5 alleles. Am J Transplant Off J Am Soc Transplant Am Soc Transplant Surg. 2016;16:574–82. doi:10.1111/ajt.13495. **This manuscript identifies common variants in African Americans that affects tacrolimus blood concentrations**.
60. Zhou YJ, Wang Y, Chen LL. Detecting the common and individual effects of rare variants on quantitative traits by using extreme phenotype sampling. Genes (Basel). 2016;7 doi:10.3390/genes7010002.
61. Barnett IJ, Lee S, Lin X. Detecting rare variant effects using extreme phenotype sampling in sequencing association studies. Genet Epidemiol. 2013;37:142–51. doi:10.1002/gepi.21699.

62. Li D, Lewinger JP, Gauderman WJ, Murcray CE, Conti D. Using extreme phenotype sampling to identify the rare causal variants of quantitative traits in association studies. Genet Epidemiol. 2011;35:790–9. doi:10.1002/gepi.20628.
63. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr Protoc Bioinformatics. 2013;43:11.10.1–33. doi:10.1002/0471250953.bi1110s43.
64. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20:1297–303. doi:10.1101/gr.107524.110.
65. Liu Y, Kheradmand F, Davis CF, Scheurer ME, Wheeler D, Tsavachidis S, et al. Focused analysis of exome sequencing data for rare germline mutations in familial and sporadic lung cancer. J Thorac Oncol. 2016;11:52–61. doi:10.1016/j.jtho.2015.09.015.
66. Bruse S, Moreau M, Bromberg Y, Jang JH, Wang N, Ha H, et al. Whole exome sequencing identifies novel candidate genes that modify chronic obstructive pulmonary disease susceptibility. Hum Genomics. 2016;10:1. doi:10.1186/s40246-015-0058-7.
67. Shtir C, Aldahmesh MA, Al-Dahmash S, Abboud E, Alkuraya H, Abouammoh MA, et al. Exome-based case-control association study using extreme phenotype design reveals novel candidates with protective effect in diabetic retinopathy. Hum Genet. 2016;135:193–200. doi:10.1007/s00439-015-1624-8.
68. Emond MJ, Louie T, Emerson J, Chong JX, Mathias RA, Knowles MR, et al. Exome sequencing of phenotypic extremes identifies CAV2 and TMC6 as interacting modifiers of chronic *Pseudomonas aeruginosa* infection in cystic fibrosis. PLoS Genet. 2015;11:e1005273. doi:10.1371/journal.pgen.1005273.
69. McLaren CE, Emond MJ, Subramaniam VN, Phatak PD, Barton JC, Adams PC, et al. Exome sequencing in HFE C282Y homozygous men with extreme phenotypes identifies a GNPAT variant associated with severe iron overload. Hepatology. 2015;62:429–39. doi:10.1002/hep.27711.
70. Peloso GM, Rader DJ, Gabriel S, Kathiresan S, Daly MJ, Neale BM. Phenotypic extremes in rare variant study designs. Eur J Hum Genet. 2016;24:924–30. doi:10.1038/ejhg.2015.197.
71.• Lange LA, Hu Y, Zhang H, Xue C, Schmidt EM, Tang ZZ, et al. Whole-exome sequencing identifies rare and low-frequency coding variants associated with LDL cholesterol. Am J Hum Genet. 2014;94:233–45. doi:10.1016/j.ajhg.2014.01.010. **This study studied extreme LDL cholesterol levels and detected genetic variants associated with the levels. They used next generation sequencing in in over 3,000 individuals in a study of over 50,000 subjects**.
72. Passey C, Birnbaum AK, Brundage RC, Oetting WS, Israni AK, Jacobson PA. Dosing equation for tacrolimus using genetic variants and clinical factors. Br J Clin Pharmacol. 2011;72:948–57. doi:10.1111/j.1365-2125.2011.04039.x.