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### Title

Genetic Variants Associated With Immunosuppressant Pharmacokinetics and Adverse Effects in the DeKAF Genomics Genome-wide Association Studies.

### Permalink

<https://escholarship.org/uc/item/7cr3d97g>

### Journal

Transplantation, 103(6)

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### Publication Date

2019-06-01

### DOI

10.1097/TP.0000000000002625

Peer reviewed



# HHS Public Access

Author manuscript

*Transplantation*. Author manuscript; available in PMC 2020 June 01.

Published in final edited form as:

*Transplantation*. 2019 June ; 103(6): 1131–1139. doi:10.1097/TP.0000000000002625.

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**Clinical Trial Notation:** NCT00270712 and NCT01714440.

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There is no conflict of interest with this co-author and the work presented in this paper.

Support was received by the National Institutes of Health NIAID Genomics of Transplantation (5U19-AI070119).

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Participated in research design

Participated in the writing of the paper

Participated in the performance of the research

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There is no conflict of interest with this co-author and the work presented in this paper.

Support was received by the National Institutes of Health NIAID Genomics of Transplantation (5U19-AI070119).

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Support was received by the National Institutes of Health NIAID Genomics of Transplantation (5U19-AI070119).

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Support was received by the National Institutes of Health NIAID Genomics of Transplantation (5U19-AI070119).

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There is no conflict of interest with this co-author and the work presented in this paper.

No direct support was received by this co-author

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No direct support was received by this co-author

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There is no conflict of interest with this co-author and the work presented in this paper.

Support was received by the National Institutes of Health NIAID Genomics of Transplantation (5U19-AI070119)

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There is no conflict of interest with this co-author and the work presented in this paper.

No direct support was received by this co-author

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## Genetic Variants Associated with Immunosuppressant Pharmacokinetics and Adverse Effects in the DeKAF Genomics Genome Wide Association Studies.

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There is no conflict of interest with this co-author and the work presented in this paper.

No direct support was received by this co-author

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Support was received by the National Institutes of Health NIAID Genomics of Transplantation (5U19-AI070119)

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No direct support was received by this co-author

Support for this project was received by the National Institutes of Health Genomics of Transplantation (5U19-AI070119), ARRA supplement (5U19-AI070119) and DeKAF (5U01-AI058013).

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There is no conflict of interest with this co-author and the work presented in this paper.

Support for this project was received by the National Institutes of Health Genomics of Transplantation (5U19-AI070119) and ARRA supplement (5U19-AI070119).

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There is no conflict of interest with this co-author and the work presented in this paper.

Support was received by the National Institutes of Health NIAID Genomics of Transplantation (5U19-AI070119).

### CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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## Abstract

**Background:** The immunosuppressants tacrolimus and mycophenolate are important components to the success of organ transplantation, but are also associated with adverse effects such as nephrotoxicity, anemia, leukopenia and new onset diabetes after transplant (NODAT). In this report, we attempted to identify genetic variants which are associated with these adverse outcomes.

**Methods:** We performed a genome-wide association study (GWAS), using a genotyping array tailored specifically for transplantation outcomes containing 722,147 SNPs, and two cohorts of kidney allograft recipients, a discovery cohort and a confirmation cohort, to identify and then confirm genetic variants associated with immunosuppressant pharmacokinetics and adverse outcomes.

**Results:** Several genetic variants were found to be associated with tacrolimus trough concentrations. We did not confirm variants associated with the other phenotypes tested although several suggestive variants were identified.

**Discussion:** These results show that adverse effects associated with tacrolimus and mycophenolate are complex and recipient risk is not determined by a few genetic variants with large effects with but most likely are due to many variants, each with small effect sizes, and clinical factors.

## Introduction

The transplantation of kidney allografts into recipients with end stage kidney disease is currently the best treatment to optimize patient health and quality of life. Though there has been a continual improvement in graft survival in the first year after transplantation, the degree of improvement has decreased in recent years and long term outcomes have not improved as quickly and have shown little improvement in the last two decades.<sup>1</sup> Reasons for the loss of graft function over time has been difficult to determine. Management of both early and late acute rejection (AR) events are thought to be critical to the improvement of transplant outcomes.<sup>2</sup>

An important component in the transplantation of kidney allografts is the use of immunosuppressants, such as tacrolimus (TAC) and mycophenolate mofetil (MMF), to

reduce the risk of acute rejection (AR) and subsequent chronic graft dysfunction and graft loss. Though immunosuppressants greatly increase the length of graft life, there are several adverse outcomes associated with these drugs, some of which can occur in high frequency.<sup>3</sup> Mycophenolic acid (MPA), a metabolite of MMF, has been associated with several adverse outcomes. MPA-related anemia occurs in 15 to 60% of recipients and MPA-related leukopenia occurs in 10 to 45% of recipients, but neither of these outcomes has been consistently associated with variation in MPA trough plasma concentrations or area under the curve (AUC).<sup>4,5</sup> Calcineurin inhibitor (CNI)-related nephrotoxicity occurs in up to 35% of recipients and it has been proposed that all recipients using CNIs eventually develop histological lesions consistent with toxicity in their allografts.<sup>6</sup> A review of 12 studies showed that the risk of CNI-related new onset diabetes after transplantation (NODAT) ranges from 2 to 50%.<sup>7</sup> Though there are several associated risk factors for NODAT, the biological basis is currently unknown.<sup>8</sup> Additionally, there is a high degree of variability of immunosuppressant pharmacokinetics between individuals and optimization of trough concentrations is critical to the reduction of associated adverse outcomes and reducing the risk of rejection.

It has been hypothesized that genetic variation plays a role in an individual's risk for immunosuppressant drug adverse outcomes.<sup>9</sup> Identification of these genetic variants could aid in the individualization of immunosuppressant selection and dosing of kidney allograft recipients leading to better outcomes. Variation in the drug metabolizing enzymes cytochrome P450 3A4 (CYP3A4) and CYP3A5 have been associated with variation in TAC trough concentrations.<sup>10,11</sup> There have been attempts to associate candidate variants with adverse outcomes associated with the use of immunosuppressants, but few have been validated, possibly due in part due to small sample sizes in the initial discovery cohort resulting in spurious findings.<sup>12-15</sup> An attempt to identify genetic variants associated with long-or short-term allograft survival using a genome wide association study (GWAS) only identified the HLA region.<sup>16</sup>

We developed two cohorts of kidney allograft recipients to identify genetic variants associated with TAC trough blood concentrations and immunosuppressant adverse effects. Our initial GWAS cohort was the Deterioration of Kidney Allograft Function (DeKAF) Genomics study (n = 2,339) and was used to identify variants associated with these drug phenotypes.<sup>17</sup> A second cohort, Genomics of Kidney Transplantation (GEN-03; n = 874), was created to confirm the findings of the initial DeKAF GWAS study.

## Materials and Methods

### Discovery and Confirmation Cohorts

Two prospective, observational, multicenter cohorts were used in this study; a discovery cohort used to identify genetic variants associated with TAC trough blood concentrations and immunosuppressant adverse effects and a confirmation cohort used to validate those variants identified in the discovery cohort. Participants were included if they had end stage renal dysfunction undergoing kidney or simultaneous kidney-pancreas transplant. Participants were enrolled at the time of transplant. Signed informed consents were approved by the Institutional Review Boards at each of the enrolling centers. The design of the

discovery cohort, (DeKAF Genomics from 7 enrolling centers, transplanted from 2005 to 2011, [www.clinicaltrials.gov](http://www.clinicaltrials.gov) NCT00270712) along with cohort characteristics has been previously reported.<sup>17–19</sup> The confirmation cohort (Genomics of Kidney Transplantation (GEN-03) study from 5 enrolling centers, transplanted from 2012 to 2016, [www.clinicaltrials.gov](http://www.clinicaltrials.gov) NCT01714440), was studied for the same clinical phenotypes as the DeKAF Genomics cohort. Only the European American (EA) and African American (AA) recipients were analyzed in this study. Recipients identified as EA and AA were determined using principal component analysis with the GWAS genotypes. The discovery cohort consisted of 1,948 EA and 391 AA kidney allograft recipients. The confirmation cohort consisted of 698 EA and 176 AA kidney allograft recipients. Clinical information was obtained from medical records. Clinical data were collected at the time of transplant and regularly through the course of the transplant and maintained in a central database.

### Definition of Phenotypes

**TAC pharmacokinetics**—Adult recipients receiving TAC with clinically measured TAC trough concentrations in the first 6 months post-transplant for therapeutic drug monitoring were eligible for analysis of TAC pharmacokinetics. Trough concentrations were dose normalized prior to analysis (ng/ml per total daily dose in mg). When available, two trough concentrations were obtained from the medical record in the first 8 weeks and two concentrations per month in months 3, 4, 5 and 6 for a maximum of 24 trough concentrations per subject. Doses were adjusted by the transplant center, based on trough concentrations, to reach institution-specific trough goals. TAC troughs were measured at each center, approximately 12-hours following the last dose, at steady state with the current dose. Generally, troughs of 8–12 ng/mL were targeted for the first 3 months and 6–10 ng/mL for 3–6 months post-transplant. A median (range) of 18 (1–24) troughs were obtained for each subject in the first 6 months post-transplant. CNI doses were adjusted for toxicity and high or low trough concentrations by center-specific preferences.

**CNI-related acute nephrotoxicity**—Recipients receiving TAC or cyclosporine for any period of time between days 7 and 180 post-transplant were eligible for analysis of CNI-related acute nephrotoxicity. Acute nephrotoxicity was defined as any rise in serum creatinine (SCr) that resulted in a lowering of the CNI dose, discontinuation of the CNI, and/or switching to an alternate CNI within 14 days after the rise, followed by any reduction in the SCr within 14 days after the last of these changes. Additionally, if a biopsy was obtained in conjunction with the rise in SCr, the primary biopsy diagnosis must not rule out CNI nephrotoxicity. An elevated CNI trough was not required for a diagnosis of nephrotoxicity. Recipients were followed for nephrotoxicity for the first 6 months post-transplant.

**MPA-related anemia**—Adult recipients receiving MPA maintenance at the time of transplant were eligible for evaluation of MPA-related anemia. MPA-related anemia was defined as the use of an MPA product (Cellcept, Myfortic or generic) for at least 14 days before a hemoglobin level less than 10 g/dL occurred resulting in a clinical intervention. Clinical interventions were a MPA dose reduction lasting more than or equal to 2 weeks, discontinuation for 2 weeks and/or initiation of erythropoietin therapy within 30 days of

the onset of anemia. Anemia was considered not to be MPA-related if the patient had an active case of bleeding or antibody administration or a diagnosis of AR within 2 weeks of anemia onset. The time to anemia was calculated from first MPA use to the date of the first respective hemoglobin level less than 10 g/dL.

**MPA-related leukopenia**—Adult recipients receiving MPA maintenance at the time of transplant were eligible for evaluation of MPA-related leukopenia. MPA-related leukopenia was defined as the use of an MPA product (Cellcept, Myfortic or generic) at least 14 days before a white blood cell (WBC) count less than 3,000 cells/mm<sup>3</sup> that resulted in a clinical intervention. Clinical interventions were a dose reduction lasting more than or equal to 2 weeks, discontinuation for more than or equal to 2 weeks and/or initiation of granulocyte colony stimulating factor or granulocyte-macrophage colony stimulating factor therapy within 30 days of the onset of the leukopenia. The leukopenia was considered not to be MPA-related if the subject had concurrent sepsis, an active CMV infection, or if the low WBC count was within 2 weeks after antibody administration or acute rejection. The time to leukopenia was calculated from first MPA use to the date of the first respective WBC less than 3,000 cells/mm<sup>3</sup>.

**CNI-related New Onset Diabetes After Transplant (NODAT)**—All recipients receiving CNI maintenance at the time of transplant, not receiving glucose lowering drugs and did not receive a pancreas transplant at baseline were eligible for NODAT evaluation. CNI-related NODAT was defined as the initiation of new glucose lowering therapy (insulin or oral hypoglycemic) within 6 months post-transplant.

**Genotyping**—Details of genotyping, genotyping data quality control, imputation and the determination of racial clusters using principle components (PCs) can be found in the supplementary information.<sup>19–28</sup> Genotyping was conducted as previously described<sup>19</sup> using a custom genome-wide genotyping tool, the Affymetrix Axiom Transplant Array, which was tailored with content for transplantation outcomes.<sup>20</sup>

**Statistical Analysis for Individual Phenotypes**—The initial GWAS used measured and imputed SNPs and was performed using the discovery cohort for each phenotype, adjusting for recipient age, sex and the 4 top ancestry PCs and adjusting for transplant center in mixed effect longitudinal models and stratifying by transplant center in Cox proportional hazards models. EA and AA races were evaluated separately for each phenotype. SNPs were coded using an additive genetic model. Variants were considered potentially associated with the phenotype and then tested in the confirmatory cohort if the p-value was less than  $1 \times 10^{-6}$ , had a minor allele frequency (MAF) greater than 0.05, and the imputation info score was 0.8. For all phenotypes tested, significant associations in the confirmatory cohort were determined using a p-value of 0.05 with a Bonferonni correction, which was different for each phenotype due to the different number of variants tested for each phenotype and cohort. Analyses were conducted with SAS version 9.4 (SAS Institute, Cary, NC) and R software version 3.3.

Dose-normalized TAC troughs in the first 6 months were analyzed using a mixed effects longitudinal model with a spline at day 9, as previously described.<sup>19</sup> The analyses were

adjusted for transplant center, age, gender, and 4 PCs. Total daily dose-normalized TAC troughs were natural log transformed to ensure normal distribution of model residuals. For dose-normalized TAC troughs, the analysis was adjusted for the known loss-of-function (LoF) variants *CYP3A5*\*3 (rs776746), \*6 (rs10264272), \*7 (rs41303343), and *CYP3A4*\*22 (rs35599367) for the EA cohort and rs776746, rs10264272 and rs41303343 for the AA cohort. This was done to remove the large number of SNPs in high LD with these variants on chromosome 7.<sup>11,19</sup>

The time to TAC-related nephrotoxicity in the first 6 months was determined using a Cox proportional hazards model for the discovery and the confirmatory cohorts. For the EA confirmatory cohort, the analysis was stratified by transplant center and adjusted for age, prior kidney transplant, gender, donor gender and the first 4 PCs. TAC-related nephrotoxicity was not analyzed in AA cohort due to the low number of events.

The time to cyclosporine-related nephrotoxicity in the first 6 months was determined using a Cox proportional hazards model for the discovery and the confirmatory cohorts. The EA confirmatory analysis was stratified by transplant center and adjusted for age, prior kidney transplant, gender, donor gender and the first 4 PCs. Cyclosporine-related nephrotoxicity was not analyzed in AA cohort due to the low number of events.

The time to MPA-related anemia in the first 6 months was analyzed using a Cox proportional hazards model for the discovery and the confirmatory cohorts. For the EA confirmatory cohort, the anemia analysis was stratified by transplant center and adjusted for recipient age and gender, prior kidney transplant, donor gender and the first 4 PCs. MPA-related anemia was not analyzed in AA cohort due to the low number of events.

The time to MPA-related leukopenia in the first 6 months was determined using a Cox proportional hazards model for the discovery and the confirmatory cohorts. For the EA and AA confirmatory cohorts, the analysis was stratified by transplant center and adjusted for recipient age and gender, prior kidney transplant, donor gender and the first 4 PCs.

The time to NODAT in the first 6 months was analyzed using a Cox proportional hazards model for the discovery and the confirmatory cohorts. For the EA and AA confirmatory cohorts, the analysis was stratified by transplant center and adjusted for age, gender and the first 4 PCs.

## Results

A comparison of the demographic and clinical factors between the discovery and the confirmation cohorts for EA and AA recipients are found in Table 1. Significant differences (p-value <0.002) between the discovery and confirmation EA cohorts included the cause of end stage kidney disease where the confirmation cohort had a lower incidence of diabetes and more glomerular disease (p-value <1×10<sup>-4</sup>), the panel reactive antibodies where the confirmation cohort had higher incidence of a greater than zero value (p-value <1×10<sup>-4</sup>), antibody induction where the confirmation cohort had fewer individuals given IL-2 blockers and a higher number given monoclonal antibodies (p-value <1×10<sup>-4</sup>) and calcineurin inhibitor type where the confirmation cohort had higher TAC use compared to cyclosporine



( $p$ -value  $<1 \times 10^{-4}$ ). The only significant differences between the discovery and conformation AA cohorts were a higher use of TAC compared to cyclosporine ( $p$ -value  $<1 \times 10^{-4}$ ).

The phenotypes tested in each cohort as well as the observed event rates for each phenotype are shown in Tables 2 and 3. For TAC pharmacokinetics, EA and AA cohorts were tested separately. The number of individuals tested, troughs and doses for each cohort are found in Table 2. The tested phenotypes and event rates are shown in Table 3. The rate of adverse outcomes in the EA discovery cohort were 6.7% for MPA-related anemia, 6.1% for CNI-related NODAT, 16.1% for TAC-related nephrotoxicity, 21.1% for CSA-related nephrotoxicity and 17.7% for MPA-related leukopenia. For the AA discovery cohort, only MPA-related leukopenia and CNI-related NODAT were tested due to the low number of events for the other phenotypes.

For dose-normalized TAC troughs in the EA discovery cohort, the Manhattan and qq plots are shown in Figures S1A and S1B in the supplementary pages. 9 variants met criteria for confirmation after adjustment for the known functional variants rs776746, rs41303343, rs10264272, and rs35599367 and are shown in Table S1A. When not adjusting for the 4 functional variants only rs776746 ( $p=3.84 \times 10^{-97}$ ) and rs35599367 ( $p=6.03 \times 10^{-18}$ ) were found to be significant. In the confirmation cohort only rs776746 ( $p=9.5 \times 10^{-34}$ ) and rs35599367 ( $p=2.8 \times 10^{-7}$ ) remained significant (Table S1B). Additionally, after adjusting for time, time spline, transplant center, age group, donor age group, GFR group, weight group, diabetes, gender, donor gender, steroid use, CCB use, ace inhibitor use, antiviral use, antibody induction, SPK, deceased/living donor, and first 4 PCs, only rs776746 ( $p=2.6 \times 10^{-32}$ ) and rs35599367 ( $p=1.3 \times 10^{-7}$ ) were significant (Table S1C).

For the dose-normalized tacrolimus troughs in the AA discovery cohort, the Manhattan and qq plots are shown in Figures S1C and S1D in the supplementary pages. 17 variants were identified for validation after adjustment for the known variants rs776746, rs10264272, and rs41303343 and are shown in Table S2A. When not adjusting for the 3 functional variants, all three were found to be significant (Table S2A). The results for each variant was rs776746 ( $p=5.424 \times 10^{-35}$ ), rs10264272 ( $p=3.47 \times 10^{-9}$ ) and rs41303343 ( $p=3.60 \times 10^{-27}$ ). In the confirmation cohort only the variants, rs776746 ( $p=6.7 \times 10^{-10}$ ), rs10264272 ( $p=3.3 \times 10^{-5}$ ) and rs41303343 ( $p=4.1 \times 10^{-8}$ ) remained significant when not adjusting for these variants (Table S2B). After adjusting for time, time spline, transplant center, age group, donor age group, GFR group, weight group, diabetes, gender, donor gender, steroid use, CCB use, ace inhibitor use, antiviral use, antibody induction, SPK, deceased/living donor, first 4 PCs, rs776746, rs10264272, and rs41303343 rs776746 and rs41303343 remained significant (Table S2C).

The Manhattan and qq plots from the EA discovery cohort for the time to cyclosporine and TAC-related nephrotoxicity, the time to mycophenolate-related anemia, the time to mycophenolate-related leukopenia, and the time to CNI-related NODAT are shown in Figures S2A to S2J in the supplementary pages. The GWAS results for these phenotypes can be found in Table S3. All variants identified in the discovery cohort with a  $p$ -value less than  $1 \times 10^{-6}$  and a MAF of greater than 0.05 are shown. Results of the confirmation of these

variants identified in the discovery GWAS are shown in Table 4S. For all variants tested, none remain significant after taking into account multiple-testing.

The Manhattan and qq plots from the AA discovery cohort for the time to MPA-related leukopenia and the time to TAC-related nephrotoxicity are shown in Figures S3A to S3D in the supplementary pages. The GWAS results for these phenotypes are shown in Table S5. All variants identified in the discovery cohort with a p-value less than  $1 \times 10^{-6}$  and a MAF of greater than 0.05 are shown. Results of the confirmation of these variants identified in the discovery GWAS are shown in Table S6. For the time to MPA-related leukopenia, there were no variants found to be statistically significant in the AA confirmatory cohort after multiple-testing correction. For the time to CNI-related NODAT, there were 56 significant variants identified in the AA discovery cohort (Table S5). In the confirmation cohort for NODAT (Table S6), two suggestive variants were identified (a true association is  $p < 9.0 \times 10^{-4}$ ). The variant rs62262402 (discovery cohort  $p = 9.47 \times 10^{-7}$  and confirmation cohort  $p = 2.7 \times 10^{-3}$ ) is located on chromosome 3 within the *adenylate cyclase 5 (ADCY5)* gene. A second variant, rs77260117 (discovery cohort  $p = 8.81 \times 10^{-7}$  and confirmation cohort  $p = 6.8 \times 10^{-3}$ ), is located on chromosome 12 but it is not adjacent to any loci associated with a known function.

## Discussion

A key to successful solid organ transplantation is the immunosuppressants used to prevent AR. The most common immunosuppressants used in transplant are TAC and MMF, both with a narrow therapeutic range. There is significant variability in TAC trough concentrations across patients, even when similar doses are administered. There are multiple reasons for variability and genetic variants, which affect hepatic and gastrointestinal metabolism, are critical factors with guidelines and publications on how to personalize therapy using these variants.<sup>29,30</sup> There is also high variability in CNI- and MPA-related toxicities however there are no reliable predictive markers to identify those individuals at high risk. This study sought to identify genomic markers associated with TAC metabolism and several immunosuppressant related adverse effects.

We have developed a large study of kidney allograft recipients with GWAS data for evaluation of immunosuppressant phenotypes. This study includes a discovery cohort and a confirmation cohort to identify and validate genetic variants associated with these outcomes. A comparison of these two cohorts showed that they are similar in clinical characteristics. Genetic variants for several immunosuppressant associated toxicity and pharmacokinetic outcomes were first identified in the discovery cohort and then retested in a smaller confirmation cohort.

In our analysis of EA allograft recipients, one variant within the *CYP3A5* gene and one within the *CYP3A4* gene were strongly associated with variation in TAC trough concentrations. We have previously reported these two LoF variants in a GWAS analysis.<sup>31</sup> The LoF variants were associated with higher TAC troughs due to a lower rate of metabolism of TAC. We did not identify any additional common variants in the genome significantly associated with TAC troughs showing that these two functional variants are the

only common polymorphisms associated with TAC trough variation in the EA population with significance and large effect sizes.

In our analysis of AA allograft recipients, we identified three LoF variants within the *CYP3A5* gene which were strongly associated with variation in TAC trough concentrations. As was shown in the EA cohort, these LoF variants are the only common polymorphisms associated with TAC trough variation in the AA population with significance and large effect sizes. There were two variants suggestive for CNI-related NODAT risk. One variant, rs62262402 in *ADCY5*, has been previously associated with type 2 diabetes and may present a possible pathway associated with this outcome (32). The occurrence of NODAT in our discover cohorts was low (EA: 6.1%; AA; 10.9%) and therefore these variants we identified should be evaluated in additional cohorts.

There have been a few studies attempting to associate genetic variants with NODAT after kidney transplantation.<sup>33–35</sup> There have been reports that variants in the peroxisome proliferator-activated receptor  $\alpha$  (*PPAR $\alpha$* ) and P450 oxidoreductase (*POR*) genes are associated with increased risk for NODAT, but other studies do not validate these associations.<sup>36,37</sup> A recent case control study evaluating variants in kidney transplant recipients identified variants in the voltage-gated K<sup>+</sup> channel (*KCNQ1*) gene, matrix metalloproteinase-2 (*MMP2*) gene and the glutathione peroxidase (*GPX1*) gene along with clinical factors have been reported to be associated with NODAT risk.<sup>38–41</sup> A recent Swiss study identified rs2114592 in the SP110 nuclear body protein (SP110) as conveying a 9.9 times higher risk for NODAT.<sup>42</sup> This variant was not significant in their analysis of a non-transplant white population with type 2 diabetes and the investigators hypothesized a gene-environment interaction may be present where immunosuppressants may unmask the gene effect. This variant was also not significant in our study, however, the Swiss cohort had a higher incidence (21.8% vs. 6.1%) of NODAT and possibly different immune suppression protocols therefore our work does not rule out the possibility of an effect of this variant. NODAT is a complex phenotype and it is possible that multiple genes, clinical factors and varying immunosuppression protocols are important which will require exceptionally large cohorts to study. Studies have also used varying definitions of NODAT which further complicate comparing the published data. Two GWASs have been used to study NODAT. Several variants were identified as being associated with NODAT, but these were not found to be significant in this study.<sup>43,44</sup>

Other investigators have attempted to associate genetic variants with MPA-related toxicities, such as a variant in *CYP2C8* (rs11572076) and two variants in *IMPDH1* (rs2228075, rs2278294), which were associated with lower risk of leukopenia, and the *UGT2B7* variant rs7438135 associated with increased risk of anemia.<sup>45–47</sup> Variants have also been previously reported to be associated with CNI-related nephrotoxicity including functional variants in *CYP3A4*, *CYP3A5* and *ABCB1*<sup>48,49</sup> and variants in aldosterone synthase with interstitial fibrosis.<sup>50</sup> Our study could not replicate the association with some of these variants and for others the variant was not present on our GWAS panel. Many of these studies used a small sample size and differing definitions of the toxicity making direct comparisons difficult.

There are several possible reasons why we did not identify genetic variants associated with nephrotoxicity, anemia and leukopenia outcomes. First, we acknowledge that defining phenotypes such as nephrotoxicity and MPA related hematologic toxicity is difficult. For these reasons it was important to include a confirmation cohort to validate variants identified in the discovery cohort. Second, variants which impact risk for complex outcomes typically have very small effect sizes and our cohorts may not have sufficient statistical power to detect them. Additionally, it is difficult to know if a specific drug is causative for a specific phenotype. This has been a common theme for GWAS and in many cases expansion of the cohort size has eventually led to the identification of variants which impact the outcome being tested. Second, it may be that rarer variants, or other types of variants such as insertion/deletions or HLA alleles, impact the risk for these outcomes and require a different testing platform (eg, DNA sequencing) and a larger cohort to be identified. For future studies we are working with additional investigators to expand the number of recipients to increase the statistical power. The formation of the iGeneTRAiN consortium was created for this purpose.<sup>22</sup> Additionally, we did not have pharmacokinetic data for MPA or the CNI at the time of the toxicity event and blood concentrations may have been transiently elevated and contributed to the acute toxicity observed.

The outcomes studied in this report are important to the wellbeing of transplant allograft recipients and identifying those factors which increase the risk of these adverse outcomes need to be identified so that their incidence can be reduced in the transplant population resulting in better graft health and survival.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGMENTS

The authors wish to thank the research subjects for their participation in this study. We acknowledge the dedication and hard work of our coordinators at each of the DeKAF Genomics clinical sites: University of Alberta, Nicoleta Boboccea, Tina Wong, Adrian Geambasu and Alyssa Sader; University of Manitoba, Myrna Ross and Kathy Peters; University of Minnesota, Mandi DeGrote, Monica Myers and Danielle Berglund; Hennepin County Medical Center, Lisa Berndt; Mayo Clinic, Tom DeLeeuw; University of Iowa, Wendy Wallace and Tammy Lowe; University of Alabama, Jacquelin Vaughn, Valencia Stephens and Tena Hilario. We also acknowledge the dedicated work of our research scientists Marcia Brott and Amutha Muthusamy. This study was supported by NIH/NIAID grants 5U19-AI070119 and 5U01-AI058013.

## ABBREVIATIONS PAGE

<b>TAC</b>	tacrolimus
<b>MMF</b>	mycophenolate mofetil
<b>MPA</b>	Mycophenolic acid
<b>AR</b>	acute rejection
<b>NODAT</b>	new onset diabetes after transplantation
<b>AUC</b>	area under the curve

<b>CNI</b>	Calcineurin inhibitor
<b>SNPs</b>	single nucleotide polymorphisms
<b>GWAS</b>	genome-wide association studies
<b>DeKAF</b>	Deterioration of Kidney Allograft Function
<b>EA</b>	European-Americans
<b>AA</b>	African Americans
<b>HWE</b>	Hardy-Weinberg Equilibrium
<b>LD</b>	linkage disequilibrium
<b>PCs</b>	principal components
<b>IBD</b>	identity by descent
<b>LoF</b>	loss-of-function

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**Table 1.**

Demographic and clinical characteristics of the DeKAF and GEN03 study cohorts.

Characteristic	Caucasians DeKAF Genomics		African Americans DeKAF Genomic		P-value	GEN-03	P-value
	GEN-03	P-value	GEN-03	P-value			
% (no. of participants):	73.62 (1948)	26.38 (698)	68.96 (391)	31.04 (176)			
<b>Recipient Gender % (no.):</b>							
Female	36.91 (719)	38.25 (267)	36.83 (144)	42.05 (74)	0.53	42.05 (74)	0.24
Male	63.09 (1229)	61.75 (431)	63.17 (247)	57.95 (102)		57.95 (102)	
<b>Age at enrollment in years:</b>							
Mean (SD)	50.42 (14.52)	50.27 (14.86)	46.78 (11.96)	48.03 (12.09)	0.82	48.03 (12.09)	0.25
<b>Cause of End Stage Kidney Disease % (no.):</b>							
Missing	(1)	(0)	(0)	(0)	<.0001	(0)	0.015
Diabetes	28.61 (557)	21.35 (149)	25.83 (101)	18.75 (33)		18.75 (33)	
Glomerular disease	22.96 (447)	29.66 (207)	17.14 (67)	23.86 (42)		23.86 (42)	
Hypertension	6.88 (134)	5.30 (37)	38.11 (149)	37.50 (66)		37.50 (66)	
Other	22.24 (433)	21.06 (147)	12.79 (50)	7.95 (14)		7.95 (14)	
Polycystic kidney disease	15.82 (308)	16.76 (117)	4.35 (17)	6.82 (12)		6.82 (12)	
Unknown	3.49 (68)	5.87 (41)	1.79 (7)	5.11 (9)		5.11 (9)	
<b>Donor Status: % (no.)</b>							
Missing	(1)	(0)	(0)	(0)	0.018	(0)	0.79
Deceased	33.69 (656)	28.80 (201)	69.31 (271)	68.18 (120)		68.18 (120)	
Living	66.31 (1291)	71.20 (497)	30.69 (120)	31.82 (56)		31.82 (56)	
<b>Donor age in years mean (SD):</b>	41.58 (13.63)	42.99 (13.51)	36.60 (13.91)	38.19 (14.60)	0.019	38.19 (14.60)	0.22
<b>Donor Gender: %(no.)</b>							
Missing	(2)	(0)	(5)	(0)	0.76	(0)	0.76
Female	53.39 (1039)	52.72 (368)	44.04 (170)	45.45 (80)		45.45 (80)	
Male	46.61 (907)	47.28 (330)	55.96 (216)	54.55 (96)		54.55 (96)	
<b>Cold Ischemia Time: % (no.)</b>							
Missing	(117)	(0)	(71)	(0)	0.94	(0)	0.76
<= 24 h	96.34 (1764)	96.28 (672)	77.81 (249)	78.98 (139)		78.98 (139)	
>24 h	3.66 (67)	3.72 (26)	22.19 (71)	21.02 (37)		21.02 (37)	

Characteristic	Caucasians DeKAF Genomics	GEN-03	P-value	African Americans DeKAF Genomic	GEN-03	P-value
<b>Prior Kidney Transplant: % (no.)</b>						
Missing	(1)	(0)	0.94	(0)	(0)	0.62
No Prior Transplants	83.92 (1634)	83.81 (585)		90.03 (352)	88.64 (156)	
Prior Transplant	16.08 (313)	16.19 (113)		9.97 (39)	11.36 (20)	
<b>Dialysis in the first 14 days post-transplant: % (no.)</b>						
No Dialysis	92.86 (1809)	93.55 (653)	0.54	86.45 (338)	78.41 (138)	0.016
Dialysis	7.14 (139)	6.45 (45)		13.55 (53)	21.59 (38)	
<b>Panel Reactive Antibodies: % (no.)</b>						
Missing	(6)	(0)	<.0001	0.0	(0)	0.25
Zero %	49.02 (952)	38.25 (267)		58.06 (227)	52.84 (93)	
Greater than Zero	50.98 (990)	61.75 (431)		41.94 (164)	47.16 (83)	
<b>T or B Cell Crossmatch: % (no.)</b>						
Missing	(40)	(0)	0.21	(1)	(1)	0.082
Negative	94.18 (1797)	92.84 (648)		93.59 (365)	97.14 (170)	
Positive	5.82 (111)	7.16 (50)		6.41 (25)	2.86 (5)	
<b>Plasmapheresis Prior to Transplant: % (no.)</b>						
Missing	(178)	(0)	0.90	(14)	(0)	0.020
No Plasmapheresis	97.23 (1721)	97.13 (678)		98.67 (372)	95.45 (168)	
Plasmapheresis	2.77 (49)	2.87 (20)		1.33 (5)	4.55 (8)	
<b>HLA mismatches: % (no.)</b>						
Missing	(3)	(17)	0.20	(0)	(1)	0.85
Greater than Zero	86.63 (1685)	88.55 (603)		94.12 (368)	93.71 (164)	
Zero	13.37 (260)	11.45 (78)		5.88 (23)	6.29 (11)	
<b>Antibody Induction: % (no.)</b>						
Missing	(8)	(0)	<.0001	(0)	(0)	0.26
Combination	2.58 (50)	2.01 (14)		2.56 (10)	2.27 (4)	
IL-2 blockers	25.21 (489)	20.77 (145)		8.95 (35)	10.23 (18)	
Monoclonal	10.67 (207)	18.05 (126)		35.81 (140)	33.52 (59)	
None	4.07 (79)	0.0 (0)		2.56 (10)	0.0 (0)	
Polyclonal	57.47 (1115)	59.17 (413)		50.13 (196)	53.98 (95)	

Characteristic	Caucasians DeKAF Genomics	GEN-03	P-value	African Americans DeKAF Genomic	GEN-03	P-value
<b>Smoking status: % (no.)</b>						
Missing	(139)	(0)	0.88	(12)	(0)	0.75
Current	8.18 (148)	8.17 (57)		12.14 (46)	13.64 (24)	
Past	35.32 (639)	36.39 (254)		23.75 (90)	25.57 (45)	
Never	56.50 (1022)	55.44 (387)		64.12 (243)	60.80 (107)	
<b>Preemptive Transplant: % (no.)</b>						
Missing	(1)	(0)	0.43	(0)	(0)	0.023
Not Preemptive	62.51 (1217)	64.18 (448)		94.12 (368)	88.64 (156)	
Preemptive	37.49 (730)	35.82 (250)		5.88 (23)	11.36 (20)	
<b>Steroid Use at Day 14 Post-Transplant: % (no.)</b>						
Missing	(114)	(0)	0.6514	(51)	(0)	0.1677
On Steroids	61.72 (1132)	60.74 (424)		56.76 (193)	63.07 (111)	
Off Steroids	38.28 (702)	39.26 (274)		43.24 (147)	36.93 (65)	
<b>Calcineurin Inhibitor Type: % (no.)</b>						
Missing	(114)	(0)	<.0001	(51)	(0)	<.0001
Both*	0.11 (2)	0.0 (0)		0.29 (1)	0.0 (0)	
Cyclosporine	28.08 (515)	8.45 (59)		16.47 (56)	2.27 (4)	
None	2.02 (37)	1.86 (13)		4.12 (14)	1.14 (2)	
Tacrolimus	69.79 (1280)	89.68 (626)		79.12 (269)	96.59 (170)	
<b>Simultaneous Pancreas Kidney Transplant: % (no.)</b>						
Missing	(1)	(0)	0.0645	(0)	(0)	0.1804
Non-SPK	93.48 (1820)	95.42 (666)		96.16 (376)	98.30 (173)	
SPK	6.52 (127)	4.58 (32)		3.84 (15)	1.70 (3)	
<b>Prior Non-kidney Transplants: % (no.)</b>						
Missing	(1)	(0)	0.0922	0.0 (0)	(0)	0.0772
No Prior Transplants	87.26 (1699)	89.68 (626)		95.91 (375)	97.73 (172)	
Prior Transplant	12.74 (248)	10.32 (72)		4.09 (16)	2.27 (4)	
<b>Cytomegalovirus Recipient/Donor Status: % (no.)</b>						
Missing	(69)	(13)	0.0265	(14)	(0)	0.3969
Recipient(-)/Donor(-)	26.18 (492)	29.49 (202)		8.75 (33)	5.68 (10)	

Characteristic	Caucasians		African Americans	
	DeKAF Genomics	GEN-03	DeKAF Genomic	GEN-03
Recipient(+)	54.18 (1018)	48.18 (330)	79.05 (298)	80.11 (141)
Recipient(-)/Donor(+)	19.64 (369)	22.34 (155)	12.20 (46)	14.20 (25)

\* Patients were converted to the other CNI and did not receive both TAC and cyclosporine concomitantly

**Table 2.**

Tacrolimus pharmacokinetics.

	DeKAF Cohort	Count	Mean	Std	# Troughs or Doses	GEN03 Count	Mean	Std	# Troughs or Doses
TAC troughs	EA	1,363	8.7 mg/ml	3.4	23,697	609	8.6 mg/ml	2.9	11,008
	AA	299	7.0 mg/ml	3.8	5,007	171	7.5 mg/ml	3.1	3,330
TAC dose	EA	1,363	6.2 mg/ml	3.8	23,697	609	5.9 mg/ml	3.8	11,008
	AA	299	8.3 mg/ml	4.2	5,007	171	9.9 mg/ml	5.2	3,330

**Table 3.**

Immunosuppressant adverse effects phenotypes and event rates.\*

Outcome	Pop	DeKAF Count	# with Outcome	Percent	RPY	GEN03 Count	# with Outcome	Percent	RPY
TAC-related nephrotoxicity	EA	1,352	218	16.1%	0.42	609	108	17.7%	0.43
CSA-related nephrotoxicity	EA	475	100	21.1%	0.64	63	19	30.2%	1.00
MPA-related anemia	EA	1,785	120	6.7%	0.15	438	25	5.7%	0.12
MPA-related leukopenia	EA	1,785	315	17.7%	0.41	657	108	16.4%	0.38
CNI-related NODAT	AA	338	79	23.4%	0.59	171	50	29.2%	0.72
	EA	1,235	75	6.1%	0.13	487	23	4.7%	0.10
	AA	256	28	10.9%	0.24	94	6	6.4%	0.14

\* Censored at 180 days post-transplant

Pop - Population

RPY - Rate per Person Year