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Genetic and Clinical Determinants of Early, Acute Calcineurin Inhibitor-Related Nephrotoxicity: Results from a Kidney Transplant Consortium

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Abstract

Background—Calcineurin inhibitor (CNI)-related acute nephrotoxicity is a common complication of transplantation. Clinical factors and elevated CNI levels are associated with nephrotoxicity; however, they do not completely explain the risk. Genetics factors may also predispose individuals to nephrotoxicity.

Methods—We enrolled 945 kidney recipients into a multicenter, prospective study. DNA was genotyped for 2,724 single nucleotide polymorphism (SNP) using a customized chip. Cox models, unadjusted and adjusted for clinical factors, examined the association between SNPs and time to early CNI-related acute nephrotoxicity in the first six months posttransplant.

Results—Cyclosporine was associated with a 1.49 hazard (95% CI, 1.04–2.14) of acute nephrotoxicity relative to tacrolimus. Acute nephrotoxicity occurred in 22.6% of cyclosporine and 19.8% of tacrolimus recipients. The median (inter-quartile range) daily dose and trough concentration at time of nephrotoxicity were 400mg (400–500mg) and 228ng/ml (190–272ng/ml) in the cyclosporine group, and 6 mg (4–8mg) and 12.6ng/ml (10.2–15.9ng/ml) in the tacrolimus group, respectively. In single-SNP adjusted analysis, nine SNPs in the XPC, CYP2C9, PAX4, MTRR and GAN genes were associated with cyclosporine nephrotoxicity. In a multi-SNP analysis, SNPs from the same genes remained significant after adjusting for the clinical factors, showing that the SNPs are jointly and independently predictive of cyclosporine nephrotoxicity. No SNPs were associated with tacrolimus nephrotoxicity.

Conclusion—We identified SNPs potentially associated with early, acute cyclosporine-related nephrotoxicity. Identifying risk SNPs prior to transplantation provides an opportunity for personalization of immunosuppression by identifying those who may benefit from CNI-avoidance or minimization, or assist in selecting CNI type. These SNPs require independent validation.

Keywords

tacrolimus; cyclosporine; pharmacogenetics; nephrotoxicity; calcineurin inhibitor

Introduction

Nephrotoxicity is a well-established adverse effect of the calcineurin inhibitors (CNI) and a major clinical problem.(1) Acute nephrotoxicity occur in 17 to 50% of kidney transplant recipients who receive CNIs; however, these estimates vary widely given differences in toxicity definitions, followup times, population studied and target CNI blood levels.(2–6) Attempts to reduce nephrotoxicity have resulted in years of investigation into CNI

minimization, avoidance or withdrawal regimens in kidney transplantation.(7–16) Data from these investigations are controversial and a clear approach to reducing CNI related renal damage remains debated and largely unsolved.

Not all patients who receive CNIs develop nephrotoxicity and to date there are no reliable clinical predictors to prospectively identify those at risk. CNI nephrotoxicity is managed by lowering the CNI dose and in severe cases discontinuing the CNI.(17) However, lowering CNI exposure is associated with an increased risk of acute rejection and potentially subclinical immunologic injury.(5, 17–27) A rejection event then increases an individual's risk of chronic graft dysfunction or graft loss.(28–31) Thus a reliable predictive marker of toxicity risk prior to CNI initiation is needed. Preemptive strategies that protect the kidney, or CNI free or minimization protocols could then be used to individualize immunosuppression in at risk patients.

The association between single nucleotide polymorphisms (SNP) and CNI related nephrotoxicity, slowly declining renal function over time while on a CNI, or kidney biopsy suggestive of CNI toxicity has been previously explored after kidney transplantation. These studies provide conflicting conclusions likely due to varying definitions of CNI toxicity, small sample sizes, evaluation of small numbers of candidate SNPs (mostly CYP3A and ABCB1), differing times of follow-up, varying transplant types or lack of distinction between early and late CNI nephrotoxicity.(32–49) As a result the data are contradictory and limit the insights into potential new mechanisms of toxicity and guidance in clinical management. Therefore, we conducted this study to define associations between early, acute CNI related nephrotoxicity in the first 6 months posttransplant and recipient SNPs in a large prospective kidney transplant population.

Results

Patients and CNI-Related Acute Nephrotoxicity

Patient demographics and characteristics are shown in Table 1. The hazard of CNI-related nephrotoxicity was higher for individuals on cyclosporine than for individuals on tacrolimus, HR (95% CI) = 1.49 (1.04–2.14). Nephrotoxicity developed in 22.6% (73/323) of cyclosporine users and 19.8% (137/692) of tacrolimus users (Table 2). In the 73 patients developing cyclosporine related-nephrotoxicity, dose reduction occurred in 71, one was switched to tacrolimus and in one the cyclosporine was discontinued. In the 137 patients developing tacrolimus related-nephrotoxicity, the tacrolimus dose was reduced in 126, tacrolimus was discontinued in 9 and no other CNI was initiated, one was switched to cyclosporine and one patient was dose reduced and then switched to cyclosporine.

The median (inter-quartile range) serum creatinine (SCr) at time of nephrotoxicity was 2.0mg/dl (1.7–2.5) in the cyclosporine group and 1.7mg/dl (1.5–2.2) in the tacrolimus group (Table 2). The median (inter-quartile range) CNI daily dose and trough concentration at time of nephrotoxicity was 400mg (400 – 500mg) and 228ng/ml (190–272ng/ml) in the cyclosporine group, and 6mg (4–8 mg) and 12.6 ng/ml (10.2 – 15.9ng/ml) in the tacrolimus group, respectively. The Scr rose a median (inter-quartile range) of 0.4mg/dL (0.3–0.5 mg/dL) or 25% above baseline in the cyclosporine and 0.4mg/dL (0.2–0.5 mg/dL) or 30% above baseline in the tacrolimus treated patients to the time of an CNI dose reduction, discontinuation or conversion to another agent. Less than 10% of individuals with a nephrotoxicity event were biopsied.

Clinical Factors and SNPs Associated with Acute Tacrolimus Related Nephrotoxicity

Increasing proximal tacrolimus troughs ($p=1\times 10^{-31}$) were associated with a higher hazard of nephrotoxicity whereas the antiviral prophylaxis ($p=0.002$) and prior kidney transplantation

($p=0.0017$) were each associated with a lower hazard. All clinical factors used in the adjusted single SNP analyses are shown in Table 3. In the multivariate clinical factor model, every increase in proximal tacrolimus trough of 1 ng/ml was associated with a hazard ratio (95% CI) of 1.22 (1.18–1.26) for nephrotoxicity. However, with and without adjustment for clinical factors no SNPs were associated with tacrolimus related nephrotoxicity after accounting for an false discovery rate (FDR) of 20%.

Clinical Factors and SNPs Associated with Acute Cyclosporine Related Nephrotoxicity

Proximal cyclosporine troughs ($p=1.8 \times 10^{-6}$), recipient age at time of transplant (quadratic effect, $p=0.017$) and recipient weight at time of transplant ($p=0.034$) were each associated with an increased hazard of nephrotoxicity. Proximal trough was trough obtained prior to and nearest to toxicity onset but no greater than 2 weeks of onset. All clinical factors used in the adjusted single SNP analyses are shown in Table 3. In the multivariate clinical factor model, every increase in proximal cyclosporine trough of 100 ng/ml was associated with a hazard ratio (95% CI) of 1.59 (1.32–1.93) for cyclosporine related nephrotoxicity. Important SNPs, with and without adjustments for clinical factors, are shown in Table 4. Hazard ratios, 95% CI's and p -values for each SNP were similar between the unadjusted and adjusted analyses. In the adjusted analysis, nine SNPs in the XPC, CYP2C9, PAX4, MTRR and GAN genes were associated with cyclosporine nephrotoxicity after accounting for an FDR of 5%. The SNPs in the XPC and MTRR genes were associated with 57–59% lower hazard of developing nephrotoxicity. SNPs in CYP2C9, PAX4 and GAN genes were associated with a 2.09 to 3.55 times greater hazard of toxicity. Two additional SNPs, from ADH4 and UGT1A1, were significant at an FDR of 20%.

To assess whether these SNPs were independent predictors of nephrotoxicity, a multiple-SNP model was developed from the significant SNPs from Table 4 and the previous clinical factors in Table 3. Of the two SNPs in the MTRR gene, only one was included (rs1802059) due to high linkage disequilibrium (LD) ($r^2=0.96$ among African Americans [AA], $r^2=0.99$ among non-African Americans [non-AA]). There was also high LD between the four CYP2C9 SNPs ($r^2>0.66$ among AA, $r^2=1.0$ among non-AA) and only one (rs1057910) of the 4 CYP2C9 SNPs was included. The top SNPs observed in the single SNP analyses remained significant at the 5% level in the multiple SNP analysis (Table 5). The directions of the associations were consistent with that in the single-SNP analysis.

Discussion

In this analysis we specifically addressed potential SNPs associated with early, acute CNI related nephrotoxicity in the first 6 months posttransplant. Prior studies have evaluated the relationship between SNPs and nephrotoxicity extending later posttransplant; however, later toxicity is highly confounded with chronic graft dysfunction and other changes.(32–34, 37, 41, 42, 48, 49) Data suggest that in kidney recipients rises in SCr after 2 years posttransplant may be related to antibody injury and not CNI toxicity.(50) In the previous genetic studies it is possible that the identified SNPs were related to mechanisms underlying chronic graft dysfunction and not CNI toxicity. Therefore, we intentionally used a highly specified early time period and a common clinical definition of acute toxicity.

CNI related acute nephrotoxicity is a common clinical problem and we observed a 19.8% and 22.6% incidence which responded to dose modifications or discontinuation while receiving tacrolimus and cyclosporine, respectively. Early nephrotoxicity frequently results in a reduction in the CNI dose and in serious cases a discontinuation of therapy which may result in subclinical immunologic graft injury and/or an acute rejection episode. Identification of at risk patients before transplantation provides the opportunity to select

patients which might benefit most from CNI sparing, avoidance or minimization protocols or identify patients requiring more aggressive posttransplant monitoring.

We found a strong association between increasing CNI troughs and nephrotoxicity which is consistent with other studies.(5, 17, 19, 25, 51, 52) Every increase in tacrolimus trough by 1 ng/mL increased the hazard of early nephrotoxicity by 22% ($p=1.0\times 10^{-31}$) even after adjusting for clinical factors. Every increase in cyclosporine trough by 100 ng/mL increased the hazard by 59% ($p=1.8\times 10^{-6}$) after adjustment. The median cyclosporine and tacrolimus troughs at time of nephrotoxicity were 228 and 12.6 ng/ml, respectively. The cyclosporine trough is within the therapeutic range whereas the tacrolimus trough is slightly higher. Therefore, levels of tacrolimus may more readily discern the risk of toxicity than cyclosporine. This also suggests that CNI troughs goals to minimize toxicity might be less than these values; however, given our study objectives and design we cannot establish a therapeutic goal. Antiviral prophylaxis and prior kidney transplant were associated with protection from tacrolimus nephrotoxicity. Whereas very young and older aged recipients, and increasing body weight were associated with increased cyclosporine nephrotoxicity.

We found nine SNPs in five genes (CYP2C9, XPC, PAX4, MTRR, GAN) associated with cyclosporine related nephrotoxicity after adjustment for clinical factors. We identified CYP2C9*3 (rs1057910) and three other CYP2C9 SNPs in high LD with CYP2C9*3 to be associated with 3.55 times greater hazard of nephrotoxicity. Endothelial cells express CYP2C and 2J enzymes. Arachidonic acid is metabolized by CYP2B, 2C, and 2J enzymes to produce multiple epoxyeicosatrienoic acids (EETs). EETs function primarily in the renal and cardiovascular systems, and regulate renal, pulmonary and cardiac function, and vascular tone. EETs activate smooth muscle resulting in hyperpolarization and vasodilatation, have anti-inflammatory activity, stimulate angiogenesis and have mitogenic effects in the kidney.(53) Acute cyclosporine nephrotoxicity is well-known to be associated with renal and vasculature constriction. We hypothesize that recipient's with CYP2C9*3, a well-known low activity enzyme,(54, 55) have reduced formation of specific EET(s) resulting in loss of vasodilatory effects. This loss of protective EET's enhances cyclosporine vasoconstriction of the glomerular arterioles, decreased renal plasma flow and glomerular filtration rate.(56) A previous study in liver transplant recipients found an increased risk of chronic CNI nephrotoxicity in patients with CYP2C8*3, and in vitro this SNP markedly reduced EET formation.(35) We evaluated CYP2C8*3 in our study and it was not associated with acute toxicity. CYPs differ in the EETs that they produce (57), therefore, it's possible that importance of CYP SNPs differs between acute and chronic nephrotoxicity.

We also found that an MTRR variant (rs1802059) was associated with protection from cyclosporine nephrotoxicity. MTRR gene is 5-methyltetrahydrofolate-homocysteine methyltransferase reductase, which encodes for the enzyme which catalyzes remethylation of homocysteine to methionine and is a regulator of plasma homocysteine concentrations. (58, 59) High homocysteine levels and MTRR variants have been associated with cardiovascular disease, Downs Syndrome, spina bifida, and neurologic diseases.(60–63) Prior studies of MTRR and MTHFR SNPs have demonstrated inconsistent associations in transplant recipients with cardiovascular disease, impact on graft and recipient survival.(64–68) MTRR SNPs may alter the regulation of the homocysteine-methionine protein biosynthesis pathway thereby reducing protein damage, cell death and immune activation.

GAN, XPC and PAX SNPs were also associated with cyclosporine related toxicity. GAN encodes for gigaxonin, a cytoskeletal BTB/Kelch protein, which plays a role in neurofilament architecture. GAN mutations have been associated with nucleoside reverse transcriptase inhibitor (stavudine) induced peripheral neuropathy in mice.(69) The XPC (xeroderma pigmentosum, complementation group C) gene is involved in recognition of

bulky DNA adducts in nucleoside excision repair and DNA damage recognition. Patients with XPC variations have increased sensitivity to vinorelbine and cisplatin.(70) PAX4 (paired box) genes are tissue specific transcription factors involved in fetal development, cancer growth, pancreatic islet development and differentiation of insulin producing cells. (71, 72) PAX4 has been associated with longevity.(73) The associations of GAN, XPC and PAX4 with cyclosporine nephrotoxicity may related to alterations in tubular epithelial cell response to CNI induced epithelial mesenchymal changes and promotion of fibroblast formation.

No SNPs on our panel were associated with tacrolimus related nephrotoxicity. The SNPs we associated with cyclosporine toxicity were not identified in the individuals receiving tacrolimus suggesting differing mechanisms of nephrotoxicity. Even though >2700 SNPs were evaluated in our study, it is possible that the SNPs associated with toxicity were not included on our candidate panel. Genome wide association studies are needed to extend exploration to other variants and genes.

Some limitations in our study deserve discussion. Calcineurin inhibitor nephrotoxicity was determined by a clinical diagnosis (rise in SCr which responded to a CNI dose reduction or discontinuation) and not a biopsy. This was an observational study and biopsies were obtained at the discretion of the treating center. In fact, we found that very few biopsies were obtained at time of CNI-related acute nephrotoxicity and a clinical diagnosis of early acute toxicity is common practice. We did not study donor genotypes which may provide additional genetic risks.

Through this analysis we have gained a better understanding of the clinical and genetic risk factors of CNI nephrotoxicity. We identified genes that may underlie the mechanisms of cyclosporine mediated toxicity. Knowledge of these risk factors and SNPs might also guide choice of CNI. These SNPs need validation in an independent cohort. The long term goal is to develop pretransplant markers that individualize immunosuppressive regimens where each individual has the least toxicity and the greatest likelihood of long term kidney function.

Methods and Material

Patient Recruitment and Data Collection

This is a multi-center, prospective study of transplant outcomes to identify SNPs, adjusting for clinical factors, associated with early, acute CNI related nephrotoxicity in kidney transplantation. Recipients (n=945) were recruited from the first 1000 enrolled from six centers of the prospective arm of the Long-term Deterioration of Kidney Allograft Function (DeKAF) study (2006–2008). DeKAF is characterizing the causes of late allograft failure and is registered at www.clinicaltrials.gov (NCT00270712).(50, 74, 75) Recipients were eligible if they were undergoing kidney or simultaneous kidney-pancreas transplantation, ≥18 years of age and to receive tacrolimus or cyclosporine during the first 6 months posttransplant. Subjects were enrolled at time of transplant and signed informed consents approved by the Institutional Review Boards.

All participants received a CNI-based immunosuppressive regimen with mycophenolate, steroids (sparing and non-sparing), with or without antibody induction per transplant center preference at baseline. Patients on steroid sparing typically received 5 days of treatment and all were off by day 14. Donor and recipient characteristics, race, SCr, CNI trough concentration, CNI dose and dosing interval changes, concomitant use of nephrotoxic drugs and acute rejection information were obtained from the medical record. CNI dosing, dose

adjustments and trough goals of cyclosporine and tacrolimus were determined by the treating center.

CNI-related Acute Nephrotoxicity

CNI-related acute nephrotoxicity was defined as any rise in SCr that resulted in a lowering of the CNI dose, discontinuation of 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 diagnosis on biopsy must not rule out CNI nephrotoxicity. An elevated CNI level was not required for a diagnosis of nephrotoxicity.

Genotyping

Pretransplant recipient DNA was isolated from lymphocytes obtained from blood after RBC lysis. Genotyping was conducted with a customized Affymetrix GeneChip (Affymetrix, Santa Clara, CA).^(76, 77) Additional variants were genotyped using SNPlex (Applied Biosystems, Foster City, CA) and Sequenom (Sequenom, San Diego, CA). The variants were within genes in pathways associated with immunity, cell cycle, signaling, growth, proliferation, differentiation, movement, structure and death, inflammation, hematologic systems, and ~700 variants related to drug absorption, disposition, metabolism and excretion. Validated, functional polymorphisms including non-synonymous variants with a minor allele frequency (MAF) >5%, and variants within conserved (in humans and mouse) transcriptional regulatory regions were chosen. In the absence of functional variants, intragenic tagging variants were used. Genotyping has been described in detail elsewhere.^(78, 79) Data quality was assessed by negative controls and duplicate samples (3% on Affymetrix, 7% SNPlex, and 1% Sequenom). On the Affymetrix gene chip, duplicate samples from 31 individuals were genotyped with >99% concordance. Variants with concordance <90% and calls <60% were excluded. To verify concordance across platforms, twenty variants were run on multiple platforms and had a concordance rate of >97% and with calls >82%. The Hardy-Weinberg equilibrium assumption was tested by χ^2 analysis in the AA and non-AA subgroups separately, and variants that deviated (p -value < 1×10^{-6}) in either group were removed from the analysis. Variants were also excluded from analysis if the MAF was <5% in both the AA and non-AA subgroups. The final analysis included 2,724 variants: 2,552 from Affymetrix gene chip, 165 from SNPlex and 7 from Sequenom platform (Appendix 1).

Statistical Analysis

The hazard ratio associated with CNI use (cyclosporine vs. tacrolimus) as a time-varying covariate was estimated using Cox proportional hazards regression, stratifying for transplant center. The proportional hazards assumption was tested using the `cox.zph` function in R version 2.10.1 (Package *survival* version 2.36-1).^(80, 81)

Cox proportional hazards regression models investigated the association of each SNP with time to first cyclosporine-related and tacrolimus-related nephrotoxicity. SNPs were coded for the additive genetic model. Individuals were only considered at risk for cyclosporine-related nephrotoxicity while on cyclosporine, and likewise for tacrolimus, starting at the later of 7 days posttransplant or first CNI use. Censoring occurred at the earliest of death, graft failure, permanent CNI discontinuation, or 6 months posttransplant. Participants who temporarily stopped a CNI for reasons other than nephrotoxicity were excluded from the risk set until restarting the CNI. To account for multiple testing, we used an effective number of SNPs $m = 2110$, which was computed based on LD between all SNPs.⁽⁸²⁾

We first performed simple single-SNP analyses, stratifying by transplant center and adjusting for recipient race (AA versus non-AA) due to potential population stratification. Next, multivariate single-SNP analyses were conducted, stratifying by transplant center and adjusting for potential confounding clinical factors that were identified by backward selection with a retention p-value of 0.10. Tested clinical factors were recipient gender, age, weight, prior kidney transplant, primary cause of original kidney failure, deceased or living donor, preemptive transplant, T or B cell cross match, general panel reactive antibodies (PRA) (positive/negative), CMV status of recipient and donor (D+R-, R+, D-R-), number HLA mismatches, posttransplant dialysis, blood type (A, B, AB, O), simultaneous pancreas-kidney transplant, antibody induction, donor age and gender; and time-varying covariates: corticosteroid use, ACE inhibitor use, antiviral use, calcium channel blocker use, and proximal CNI trough concentration. The backward selection procedure retained recipient race at all stages regardless of level of significance.

Finally a multiple-SNP Cox proportional hazards regression model was then developed for time to cyclosporine-related nephrotoxicity using the SNPs that passed an FDR cutoff of 20%, adjusting for the clinical factors used in the multivariate single-SNP analysis and stratifying by transplant center. All statistical analyses were conducted using SAS/Genetics v9.2 (The SAS Institute, Cary, NC, USA, <http://www.sas.com>).

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Abbreviations

FDR	false discovery rate – statistical method to correct for multiple comparisons. For example, an FDR of 5%, we would expect no more than 5% false positives among the variants that are declared as significant. For FDR 20%, we expect no more than 20% false positives
therefore	5% is a more stringent cutoff

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Table 1**Recipient and Donor Demographics and Clinical Factors**

	Cyclosporine (n=323)		Tacrolimus (n=692)	
	Nephrotoxicity (n=73)	No Toxicity (n=250)	Nephrotoxicity (n=137)	No Toxicity (n=555)
Recipient age, median (range)	51 (18–75)	51 (20–79)	51 (22–82)	50 (20–82)
Recipient male gender	48 (65.8%)	155 (62.0%)	92 (67.2%)	346 (62.3%)
Recipient African American	8 (11.0%)	31 (12.4%)	14 (10.2%)	129 (23.2%)
Recipient weight (kg), median (range)	82 (47–137)	80 (44–148)	82 (39–138)	81 (38–152)
Prior kidney transplant	9 (12.3%)	32 (12.8%)	12 (8.8%)	93 (16.8%)
Primary Cause of Kidney Failure^a				
Diabetes Mellitus	25 (35.7%)	75 (31.9%)	42 (31.6%)	184 (34.5%)
Glomerulonephritis	19 (27.1%)	53 (22.6%)	31 (23.3%)	103 (19.3%)
Polycystic kidney disease	5 (7.1%)	28 (11.9%)	21 (15.8%)	65 (12.2%)
Hypertension	7 (10.0%)	24 (10.2%)	13 (9.8%)	86 (16.1%)
Others	14 (20.0%)	55 (23.4%)	26 (19.6%)	95 (17.8%)
Preemptive transplantation	29 (39.7%)	78 (31.2%)	48 (35.0%)	170 (30.6%)
Simultaneous pancreas-kidney transplant	2 (2.7%)	2 (0.8%)	16 (11.7%)	44 (7.9%)
Donor age, median (range)	45 (4–65)	42 (1–69)	42 (10–71)	41 (4–71)
Donor male gender ^b	31 (42.5%)	107 (42.8%)	55 (40.2%)	274 (49.6%)
Living donor	44 (60.3%)	153 (61.2%)	91 (66.4%)	314 (56.6%)
Zero HLA mismatches	11 (15.1%)	42 (16.8%)	14 (10.2%)	51 (9.2%)
Cross T-cell/B-cell match ^c	1 (1.6%)	1 (0.4%)	10 (7.4%)	38 (6.9%)
PRA positive	21 (28.8%)	83 (33.2%)	55 (40.2%)	207 (37.7%)
CMV status of recipient and donor^d				
D–R–	16 (25.0%)	63 (26.1%)	31 (23.7%)	103 (19.3%)
R+	36 (56.3%)	133 (55.2%)	81 (61.8%)	351 (65.9%)
D+R–	12 (18.8%)	45 (18.7%)	19 (14.5%)	79 (14.8%)
Recipient blood types				
A	33 (45.2%)	100 (40.0%)	61 (44.5%)	233 (42.0%)
B	10 (13.7%)	25 (10.0%)	21 (15.3%)	67 (12.1%)
AB	5 (6.9%)	12 (4.8%)	6 (4.4%)	23 (4.1%)
O	25 (34.3%)	113 (45.2%)	49 (35.8%)	232 (41.8%)
Antibody induction at transplantation	73 (100.0%)	249 (99.6%)	134 (97.8%)	548 (98.7%)
Dialysis after transplantation ^e	4 (5.5%)	30 (12.0%)	7 (5.1%)	43 (7.8%)
Corticosteroid ^f	29 (39.7%)	142 (56.8%)	63 (46.0%)	284 (51.2%)
ACE inhibitor ^g	24 (32.9%)	103 (41.2%)	48 (35.0%)	193 (34.8%)
Antiviral prophylaxis ^g	73 (100.0%)	215 (86.0%)	124 (90.5%)	531 (95.7%)
Calcium channel blocker ^g	53 (72.6%)	202 (80.8%)	94 (68.3%)	368 (66.3%)

^a 6% missing in CSA group, 4% missing in TAC group,

^b <1% missing in TAC group,

^c8% missing in CSA group, 1 % missing in TAC group,

^d6% missing in CSA group, 4% missing in TAC group,

^e<1% missing in TAC group,

^fUse of a corticosteroid on day 14 posttransplant,

^gUse of drug at any time while at risk for nephrotoxicity

Table 2

Characteristics of Calcineurin Inhibitor Related Nephrotoxicity

	Cyclosporine (n=73)	Tacrolimus (n=137)
Serum creatinine (mg/dL)		
Baseline ^a , median (inter-quartile range)	1.6 (1.3–1.8)	1.3 (1.1–1.7)
At time of toxicity, median (inter-quartile range)	2.0 (1.7–2.5)	1.7 (1.5–2.2)
Increase from baseline to time of toxicity, median (inter-quartile range)	0.4 (0.3–0.5)	0.4 (0.2–0.5)
Day posttransplant of toxicity onset, median (inter-quartile range)	21 (14–41)	31 (13–62)
Daily dose of CNI at time of toxicity, median (inter-quartile range) (mg)	400 (400–500)	6 (4–8)
Trough concentration at time of toxicity ^b , median (inter-quartile range) (ng/ml)	228 (190–272)	12.6 (10.2–15.9)

^a SCr within 2 weeks prior to the nephrotoxicity onset^b trough concentration obtained prior to and nearest to toxicity onset but no greater than 2 weeks of onset

Table 3

Clinical Factors Associated With Acute CNI-Related Nephrotoxicity and Adjusted For in the SNP Models^a

Clinical Factors	Hazard Ratio (95% CI)	p-value
Tacrolimus Nephrotoxicity		
African American	0.72 (0.37–1.42)	0.34
Proximal tacrolimus trough (per 1 ng/mL) ^b	1.22 (1.18–1.26)	1.0×10 ⁻³¹
Antiviral prophylaxis	0.44 (0.26–0.74)	0.0020
Prior kidney transplant	0.38 (0.21–0.69)	0.0017
Male donor	0.73 (0.51–1.03)	0.075
Cyclosporine Nephrotoxicity		
African American	1.50 (0.68–3.30)	0.31
Proximal cyclosporine trough (per 100 ng/mL)	1.59 (1.32–1.93)	1.8×10 ⁻⁶
Age (per 10 years), linear	0.97 (0.82–1.14)	0.67
Age (per 10 years), quadratic	1.12 (1.02–1.24)	0.024
Weight at baseline (per 10 kg)	1.14 (1.01–1.29)	0.034
Preemptive transplant	1.58 (0.94–2.65)	0.083

^a All analyses were stratified by center.

^b Proximal CNI trough is a time-varying covariate which is defined as the closest trough concentration obtained prior to any nephrotoxicity event.

Table 4

SNPs Associated With Acute Cyclosporine Nephrotoxicity in Single SNP Analyses

SNP no.	rs number	Gene Name	Unadjusted Hazard Ratio (95% CI)	p-value	Adjusted Hazard Ratio (95% CI) ³	p-value	Allele and MAF ^d
1	rs2228001	XPC	0.44 (0.30–0.66)	0.00007 ^e	0.41 (0.27–0.63)	0.00004 ^e	C 0.36
2	rs1057910	CYP2C9 ^f	3.20 (1.74–5.89)	0.00019 ^e	3.55 (1.91–6.61)	0.00006 ^e	C 0.05
3	rs1057911	CYP2C9 ^f	3.20 (1.74–5.89)	0.00019 ^e	3.55 (1.91–6.61)	0.00006 ^e	T 0.05
4	rs9332093	CYP2C9 ^f	3.20 (1.74–5.89)	0.00019 ^e	3.55 (1.91–6.61)	0.00006 ^e	G 0.05
5	rs9332098	CYP2C9 ^f	3.20 (1.74–5.89)	0.00019 ^e	3.55 (1.91–6.61)	0.00006 ^e	A 0.05
6	rs712704	PAX4	1.85 (1.29–2.65)	0.00082	2.09 (1.45–3.01)	0.00007 ^e	C 0.20
7	rs1802059	MTRR ^g	0.41 (0.27–0.62)	0.00003 ^e	0.42 (0.27–0.65)	0.00009 ^e	A 0.34
8	rs2608555	GAN	1.80 (1.25–2.58)	0.00146	2.11 (1.44–3.10)	0.00013 ^e	T 0.24
9	rs1532268	MTRR ^g	0.42 (0.28–0.64)	0.00006 ^e	0.43 (0.28–0.67)	0.00018 ^e	A 0.34
10	rs1126672	ADH4	1.73 (1.25–2.41)	0.00103	1.85 (1.31–2.63)	0.00055 ^h	T 0.24
11	rs3771342	UGT1A1	1.97 (1.30–2.98)	0.00133	2.09 (1.37–3.19)	0.00062 ^h	A 0.15

^a SNPs are adjusted for race to account for possible population stratification.

^b hazard ratio and 95% confidence interval (CI) of developing toxicity for each risk allele.

^c Analyses were adjusted for factors significant at 10% level or less, proximal cyclosporine trough, age, age squared, weight, preemptive transplant but always adjusted for race regardless of p-value.

^d allele associated with the hazard ratio and minor allele frequency (MAF).

^e significant accounting for a FDR of 5%.

^f CYP2C9 SNPs are in LD: $r^2 > 0.66$ among African Americans, $r^2 = 1.0$ among non-African American.

^g MTRR SNPs are in LD: $r^2 = 0.96$ among African Americans, $r^2 = 0.99$ among non-African American.

^h significant accounting for a FDR of 20%.

Table 5
SNPs Associated with Acute Cyclosporine Nephrotoxicity in Multiple SNP Analysis Adjusting For Clinical Factors^a

SNPs	Hazard Ratio (95% CI) ^b	p-value
rs2228001 (XPC)	0.624 (0.412–0.947)	0.027
rs1057910 (CYP2C9)	2.334 (1.217–4.476)	0.011
rs712704 (PAX4)	1.864 (1.252–2.775)	0.0022
rs1802059 (MTRR)	0.428 (0.277–0.662)	0.00013
rs2608555 (GAN)	2.183 (1.446–3.297)	0.00020
rs1126672 (ADH4)	1.874 (1.283–2.736)	0.0012
rs3771342 (UGT1A1)	2.170 (1.408–3.346)	0.00045

^a Analysis adjusted for race, proximal cyclosporine trough, age, age squared, weight, and preemptive transplant and stratified by transplant center.

^b Due to the selection of SNPs through prior multiple comparisons, the hazard ratios may be inflated due to optimism. However, the model shows that the selected SNPs are jointly and independently predictive of cyclosporine nephrotoxicity.