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Genetic Determinants of Mycophenolate Related Anemia and Leukopenia Following Transplantation

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Abstract

Mycophenolate related anemia and leukopenia are well-known toxicities after transplantation. Toxicity leads to dose reduction, addition of colony-stimulating factors or erythropoietin, or discontinuation of immunosuppressive therapy. The causes of and risk factors associated with toxicity are unclear.

Methods—We studied the association between mycophenolate related anemia and leukopenia and 2,724 single nucleotide plymorphisms (SNP) in 978 patients undergoing living or deceased donor kidney transplant. Patients were followed to time of first anemia (hemoglobin <10gm/dL or hematocrit <30%) or first leukopenia (white blood cell [WBC] count <3000 cells/mm^3) which required clinical intervention in the first 6 months posttransplant.

Results—Anemia occurred in 87 (9.5%) subjects and leukopenia in 224 (22.9%). In single SNP analyses, none of the SNPs were associated with time to leukopenia at a false discovery rate (FDR) of 20%. However, SNPs from the IL12A, HUS, CYP2C8 genes were associated with time to anemia allowing for an FDR of 20%. To assess the independence of these SNPs as predictors of anemia, we conducted a multi-SNP analysis including one SNP from each of the three genes. All three SNPs were associated with time to anemia, after adjusting for recipient age, weight, posttransplant dialysis and antiviral drug use and stratifying by clinical center.

Conclusion—While these SNPs require validation in an independent population, our results suggest genetics may play a role in risk of mycophenolate related hematologic toxicity. This may ultimately provide for better management of maintenance immunosuppression and gives insights into potential mechanism(s) by which toxicity occurs.

Keywords

mycophenolate; transplantation; adverse effects; pharmacogenomics; pharmacogenetics; toxicity; anemia; leukopenia; mycophenolic acid

INTRODUCTION

Anemia and leukopenia toxicity is a well recognized problem associated with mycophenolate therapy.(1–2) Mycophenolate-related leukopenia occurs in 11.8–40% of recipients and anemia in 13.5–66% of recipients.(3–8) Mycophenolate has been associated with pure red cell aplasia which prompted package insert warnings.(8–9) Development of mycophenolate toxicity usually results in a dose reduction or discontinuation, and places patients at higher risk of acute rejection and graft failure.(10–12) Mycophenolate dose reductions increase the relative risk of rejection by 4% for every week of reduction.(13) In addition, patients who are anemic posttransplantation have higher mortality and graft failure. (14–16)

Few data are available regarding risk factors for mycophenolate-related toxicity. Studies suggest that high mycophenolic acid (MPA) area under the curve or trough concentrations

are associated with greater risk of anemia or leukopenia; however, others find no association.(3–5, 7, 10, 17–21) We hypothesized that genetic variation may be associated with the development of mycophenolate-related anemia and leukopenia. Our long-term goal is to identify individuals pretransplantation at risk for toxicity and provide alternative immunosuppression strategies for these individuals.

RESULTS

Patients and Mycophenolate-Related Toxicity

Demographics of the 978 subjects are shown in Table 1. Mycophenolate mofetil was the initial mycophenolate in 971 subjects and mycophenolate sodium in 7 subjects. Seven subjects were switched from mycophenolate mofetil to mycophenolate sodium during the study. Nearly all patients received a calcineurin inhibitor with mycophenolate and over half received Thymoglobulin induction (Table 2).

Complete clinical data were available to evaluate time to mycophenolate-related anemia in 918 patients. Anemia occurred in 87 (9.4%) individuals within the first 6 months posttransplant (Table 2). Patients experiencing the event received mycophenolate a median (95%CI) of 45.0 (34–55) days before the development of anemia. The median (range) hemoglobin at time of anemia was 9.3 g/dL (7.1 to 10.0), and hematocrit 28% (11.2 to 29.9). Interventions for those with anemia were mycophenolate dose reduction (n=27), discontinuation (n=1) and erythropoietin with mycophenolate continuation (n=68). Nine patients underwent more than one of the above changes.

Time to mycophenolate-related leukopenia was evaluable in 978 patients. Leukopenia occurred in 224 (22.9%) individuals within the first 6 months posttransplant (Table 2). Those experiencing an anemia event received mycophenolate a median (95% CI) of 81.5 (76–88) days before the development of leukopenia. Median (range) white blood cell count (WBC) at time of leukopenia was 1,900 cells/mm,3 (400 to 2,900). Interventions for leukopenia were mycophenolate dose reduction (n=184), discontinuation (n=12), and colony-stimulating factor with mycophenolate continuation (n=66). Thirty eight patients underwent more than one of the above. Of note, 31 developed both leukopenia and anemia within the 6 month period of followup.

Clinical Factors and SNPs Associated with Mycophenolate-Related Anemia

Clinical factors associated with time to anemia ($p \leq 0.10$) were antiviral use (p=0.025), age at time of transplant (linear, p=0.01), weight at time of transplant (linear, p=0.018) and need for posttransplant dialysis (p=0.002). The effect of each SNP was tested individually in Cox regression models not adjusting and adjusting for the clinical factors above, and stratified by center. Unadjusted and adjusted analysis are shown in Table 3. Hazard ratio, 95% CI and p-value for each SNP was similar between the two analyses. In adjusted analysis, SNPs in the interleukin (IL) 12A, checkpoint homolog protein (HUS), and cytochrome P450 (CYP) 2C8 genes were associated with an increased hazard for time to anemia accounting for a false discovery rate (FDR) of 20%. The presence of one A allele for the IL12A SNP (rs568408, minor allele frequency [MAF] 15%), increased the hazard (95% CI) of anemia by 1.98 (1.39–2.82), and two A alleles increased the hazard by 3.93 (1.95–7.95) relative to noncarriers. The HUS1 SNP (rs2037483, MAF 50%) was associated with a reduced hazard (95% CI), 0.54 (0.39–0.74), of anemia. CYP2C8 was associated with an increased hazard [3.24 (1.7–6.2)] but had a low MAF (3%).

To assess whether these SNPs were independent predictors, a multiple-SNP Cox regression model for time to anemia was developed from three of the four top SNPs from the single SNP analysis and the above associated clinical factors (Table 4). Of the two SNPs from

HUS1, only one SNP (rs1056663) was included due to high LD (r^2=1.0). All three of the SNPs remained associated with time to anemia at the 0.005 level adjusting for the other SNPs and the clinical factors. For every increase in 10 years of age from the mean age the hazard of anemia was increased by 1.28 (95%CI, 1.09–1.50) and need for posttransplant dialysis increased hazard of anemia by 2.44 (95%CI, 1.42–4.20). Antiviral drug use was not significant in the final model at the 5% level.

Clinical Factors and SNPs Associated with Mycophenolate-Related Leukopenia

Clinical factors associated with leukopenia were corticosteroid use (p=0.018), weight at time of transplant (linear, p=0.018), prior kidney transplant (p=0.038), deceased donor (p=0.051) and negative-recipient and positive-donor CMV serostatus (p=0.006). The effect of each SNP was then tested individually in Cox regression models adjusting and not adjusting for the above clinical factors, and stratifying by center. Eighteen SNPs had a p<0.01 and 95% CIs that did not cross one however they were not significant at an FDR of 20% (Table 5).

DISCUSSION

This is the first pharmacogenetic study to evaluate the association between SNPs and mycophenolate-related toxicities using a broad panel of SNPs in a large population (n=978). Limited numbers of candidate SNPs in genes related to mycophenolate metabolism, transport or its target have been evaluated towards toxicity in small analyses.(22–24) Previously no association between SNPs of the inosine monophosphate dehydrogenase-1 (IMPDH) gene and leukopenia were found in 191 kidney transplant recipients; although some of these SNPs increased the risk of acute rejection.(22) A recent analysis of IMPDH2 and ABCC2 SNPs in 59 pediatric heart recipients found an association between the IMPDH2 SNP (rs11706052) and neutropenia which required dose holding but no association with anemia or thrombocytopenia.(23) In our analysis, these and other IMPDH and ABCC2 SNPs were not found to be associated with toxicity.

We identified multiple novel SNPs potentially associated with anemia (Table 3). Potential SNPs for anemia were in the interleukin-12A (IL12A), checkpoint homolog protein (HUS1) and cytochrome P4502C8 (CYP2C8) genes which were each significant in single-SNP analysis after accounting for a 20% FDR, and remained independent predictors in the multiple-SNP analysis. The IL-12 gene is important in the regulation of T-cell response and is involved in innate and adaptive immunity, inflammation and autoimmune disease.(25–26) IL-12 mRNA expression and IL-12 levels have been shown to be elevated patients with aplastic anemia.(27) The HUS1 protein has multiple functions and is involved in cellular response to DNA damage.(28) It is thought to participate in cell cycle arrest, activation of DNA repair pathways, movement of DNA repair proteins to sites of damage, activation of transcription, and apoptosis.(29) It is possible that altered HUS1 function may place a cell at higher risk of cytotoxicity. CYP2C8 is involved in drug metabolism and the 6-O-desmethyl-mycophenolate acid metabolite is produced by CYP3A4/5 and probably CYP2C8.(30–32) Therefore, SNPs of this enzyme may affect mycophenolic acid (MPA) metabolism resulting in higher exposure.

We identified SNPs potentially associated with leukopenia (Table 5). These SNPs were not significant after accounting for a 20% FDR in the single SNP analysis. We consider these SNPs exploratory and will require validation. The most promising SNPs in the leukopenia analysis were from the vascular cell adhesion molecule (VCAM) and solute carrier organic anion transporter (SLCO1B1, rs4149056) genes. VCAM is expressed on vascular endothelial cells after stimulation by cytokines that mediate leukocyte-endothelial cell adhesion and signal transduction and regulates the migration of stem cells and homing of lymphocytes.(33) Mice with deficient VCAM-1 develop leukocytosis.(34) SLCO1B1 is an

influx hepatic transporter expressed at the basolateral membrane of hepatocytes and to a lesser degree in spleen, mammary gland and testis. The SLCO1B1 and SLCO1B3 transporters are substrates for the MPA glucuronide metabolite (MPAG).(35) The SLCO1B1*5 (rs4149056,c.521T>C) SNP, which we identified as possibly important towards leukopenia is also associated with serum bilirubin, statin pharmacokinetics, statin adverse events, irinotecan pharmacokinetics and hematologic toxicity.(36–42) No association between MPA pharmacokinetics and SLCO1B1 SNPs has been observed by others, although trends show that individuals with rs4149056 have higher MPA concentrations.(35, 43) We also evaluated SLCO1B3 SNPs in our study and we found no association with toxicity; however, it is possible that other SLCO1B3 SNPs not on our SNP chip are important. Although conflicting, SLCO1B3 SNPs have been associated with MPA pharmacokinetics but only in patients receiving concomitant tacrolimus or sirolimus.(35, 43) The mechanism by which SLCO1B1 SNPs might increase the risk of leukopenia may be through accumulation of MPA and/or its active metabolites in the blood due to altered hepatic transport or accumulation within white blood cells. There were no overlapping SNPs between leukopenia and anemia suggesting different mechanisms by which these toxicities occur.

The pathogenesis of anemia and leukopenia after transplantation is multifactorial and these factors were evaluated in our study.(44–48) Interestingly, we did not find the use of corticosteroids protective towards leucopenia in the final models. Such therapies have been associated with higher white counts (21) but have not been universally observed.(10, 49) Although controversial, most data suggest that high total MPA concentrations are associated with toxicity.(3–5, 7, 10, 17–18, 20–21, 50) Most centers participating in our study do not monitor MPA concentrations and data are not available; therefore, we cannot exclude the possibility of an association between concentration and toxicity. Renal impairment has also been associated with leukopenia (21) which may be due to accumulation of the active MPA acyl-glucuronide metabolite or high unbound MPA concentrations that may occur during renal impairment.(20) However, in our analysis SCr early posttransplant was not associated with toxicity.

The mechanism by which mycophenolate toxicities occur is unclear. Microarray analysis of liver and gut in rats treated with high-dose mycophenolate showed down regulation of the expression of the major α-hemoglobin, polymeric immunoglobulin receptor, catalase gene and CCAAT/enhancer protein-α suggesting that these genes may be linked to adverse events.(51) Mycophenolate-treated rats had significantly lower erythrocytes and lymphocytes, and hemoglobin concentrations than untreated rats. In another study, significant drops in erythrocyte counts and hemoglobin were observed in rats after only one week of mycophenolate.(52) There was nearly a complete absence of hematopoietic progenitor cells in the bone marrow with reduced expression of major α-hemoglobin, catalase and erythropoietin receptors in the bone marrow suggesting suppression of erythropoiesis by mycophenolate. Interestingly in our analysis, a catalase SNP increased the risk of leukopenia in the single SNP analysis (p=0.0093); however, it was not significant in the multiple-SNP model. This SNP requires further followup.

In summary, we identified multiple SNPs potentially associated with anemia and leukopenia, many of which have a strong biologic basis. Of interest, SNPs associated with anemia differed from those associated with leukopenia. However, these SNPs require independent confirmation in other centers. If these associations are confirmed, these finding may have clinical application and help to assess the toxicity risk prettransplantation. Patients with these risk SNPs may be more safely treated with azathioprine therapy. However, to our knowledge these novel SNPs have yet to be tested towards azathioprine toxicity.

MATERIALS AND METHODS

Study Design and Patients

This is a multi-center prospective study to identify SNPs associated with mycophenolate-related anemia and leukopenia. Subjects who received mycophenolate mofetil or mycophenolate sodium after kidney transplantation, for any period of time, between day of transplant and 6 months posttransplant were studied. Subjects were recruited from the Deterioration of Kidney Allograft Function (DeKAF) study, which is designed to characterize the causes of late allograft failure.(53–55) This study is registered at www.clinicaltrials.gov (NCT00270712). Transplant recipients were enrolled at time of transplant and were eligible if they were undergoing kidney or simultaneous kidney-pancreas transplantation. Written informed consent was obtained from all subjects and was approved by the Institutional Review Boards of the participating institutions.

Mycophenolate-related anemia was defined as the use of mycophenolate at least 14 days prior to a hemoglobin <10gm/dL (U.S. centers) or hematocrit <30% (Canadian centers), that resulted in a clinical intervention. Clinical interventions were a mycophenolate dose reduction lasting ≥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 mycophenolate-related if the patient had an active case of bleeding or antibody administration or a diagnosis of acute rejection within 2 weeks of anemia onset.

Mycophenolate related leukopenia was defined as the use of mycophenolate at least 14 days prior to a WBC count<3000 cells/mm3 that resulted in a clinical intervention. Clinical interventions were a mycophenolate dose reduction lasting ≥2 weeks, discontinuation for ≥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 mycophenolate related if the subject had concurrent sepsis, an active CMV infection, or if the low WBC count was within 2 weeks of antibody administration or acute rejection. The time to anemia or leukopenia was calculated from first mycophenolate use to the date of the first respective low count.

Donor and recipient demographic information and drugs or class of drug prescribed over the 6 month study period were compiled. Drugs included were alemtuzumab, equine or rabbit antithymocyte globulin, basiliximab, daclizumab, azathioprine, corticosteroid, cyclosporine or tacrolimus, sirolimus, fluoxetine, flupenazine, captopril, enalapril, ticlopidine, fondaparinux, aspirin, heparin, clopidogrel, iron, erythropoietin and antivirals. Acute rejection as diagnosed by the treating physician, allograft failure and patient death during the first 6 months posttransplant were also recorded.

Genotyping

DNA was isolated from peripheral blood lymphocytes. Lymphocytes were isolated by centrifugation after RBC lysis and the DNA isolated. DNA was quantified by measuring the absorbance at 260nm. Genotyping of SNPs was done using a customized Affymetrix GeneChip.(56) Additional SNPs were genotyped using the SNPlex (Applied Biosystems Inc, Foster City, California, USA) and Sequenom (Sequenom, Inc, San Diego, CA, USA) platforms. SNPs within genes associated with pathways affecting immunity, cell cycle, signaling, growth, proliferation, differentiation, movement, structure and death, inflammation, hematologic systems, and drug absorption, disposition, metabolism and excretion were initially selected. Approximately 700 SNPs were related to drug absorption, disposition, metabolism and excretion. Validated, functionally relevant polymorphisms including non-synonymous SNPs with a minor allele frequency greater than 5%, and SNPs within conserved (in humans and mouse) transcriptional regulatory regions were chosen for

genotyping. In the absence of functional SNPs, intragenic tagging SNPs were used. For quality control of genotyping data, negative controls (water) and duplicate samples (3% on Affymetrix, 7% on SNPlex, and 1% on Sequenom) were included in the analysis. Duplicate samples from 31 individuals genotyped on the Affymetrix platform exhibited >99% concordance. For all platforms, SNPs with concordance rates <90% and with call rates <60% were excluded. Twenty SNPs were run on multiple platforms and had a concordance rate of >97% and with call rates >82%. The Hardy-Weinberg equilibrium assumption was tested by χ^2 analysis and SNPs that deviated from that assumption (p-value $< 1\times10^{-6}$) were removed from the analysis. SNPs were excluded from further analysis if the MAF was <5% in the African American and non-African American groups; therefore, 2724 SNPs were used in the final analysis (Appendix 1).

Association Testing of Clinical Factors and SNPs with Toxicity

Cox proportional hazards regression models were used to test the association between each SNP and time to anemia or time to leukopenia. Individuals were considered at risk of toxicity beginning on the day of transplant or first mycophenolate use, if mycophenolate was initiated after day of transplant. Censoring occurred at the earliest of death, graft failure, 14 days after permanent mycophenolate discontinuation, last date of follow up, or 6 months posttransplant. Participants who temporarily stopped mycophenolate for reasons other than toxicity for more than 14 days were excluded from the risk set from 14 days after discontinuation until restarting mycophenolate.

Prior to testing for association of any SNPs, confounding clinical factors occurring in ≥5% of individuals were identified by backwards selection with a retention p-value <0.10 for time to anemia and leukopenia separately. Tested clinical factors were fixed covariates; gender, race, thymoglobulin induction, smoker, other tobacco use, age (linear and squared), weight (linear and squared), malignancy at baseline, primary cause of kidney failure, prior kidney transplant (yes/no), prior other transplant (yes/no), deceased or living donor, preemptive transplant (yes/no), T or B cell cross match (yes/no), general panel reactive antibodies (PRA) (positive/negative), CMV status of recipient and donor (D+R−, D+R+, D−R−, D+R+), posttransplant dialysis (yes/no), blood type (A, B, AB, O) and SCr between day 6–8 posttransplant, and time-varying covariates (cyclosporine, tacrolimus, corticosteroids, ACE inhibitors, aspirin, clopidogrel, heparin and antiviral use at time of event) and were tested through regression analysis. Sirolimus was not evaluated since it was used in only 2.7% of recipients in the first 6 months. Age and weight were mean centered at 49.2 years and 81.4 kg, respectively. Unadjusted and adjusted Cox regression SNP models for time to anemia and leukopenia stratified by study center were conducted. Anemia SNP models were adjusted for age (linear), weight (linear and squared), posttransplant dialysis, and antiviral use. Leukopenia SNP models were adjusted for weight (linear), prior kidney transplant, deceased or living donor, CMV serostatus, and corticosteroid use.

Single-SNP Cox proportional hazards regression models were created for time to anemia and leukopenia by adding each SNP into a separate background model. SNPs were coded for the additive genetic model. P-values for SNP association were ordered in increasing order and denoted by $P_{(1)}, \ldots, P_{(m)}$. They were considered significant if below a FDR of 20%, i.e., $P_{(k)} < 0.2k/m$. We used an effective number of SNPs $m = 2110$, which was computed based on linkage disequilibrium (LD) between all SNPs.(57)

A Cox proportional hazards regression model with multiple SNPs was then developed for time to anemia. The top four SNPs from the single-SNP analyses (Tables 3) that were significant accounting for an FDR of 20% and the retained clinical factors were considered for entry into models. Since the two HUS1 SNPs (rs2037483 and rs1056663) are in high

linkage disequilibrium (r^2=1.0), only the SNP with the least missing data, rs1056663, was used.

All statistical analyses were conducted using SAS/Genetics v9.2 (The SAS Institute, Cary, NC, USA, http://www.sas.com).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

false discovery rate (FDR)	statistical method to correct for multiple comparisons. For a FDR of 20%, as used in this paper, we would expect no more than 20% false positives among the SNPs that are declared as significant

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Participating Centers

Participating transplant centers were University of Alberta, Edmonton, CA; University of Manitoba, Winnipeg, CA; University of Minnesota, Minneapolis, MN, USA; Hennepin County Medical Center, Minneapolis, MN, USA; Mayo Clinic, Rochester, MN, USA; University of Iowa, Iowa City, IA, USA; and University of Alabama, Birmingham, AL, USA.

Table 1

Characteristics of Study Subjects (n=978)

Recipient gender (male/female)	610/368
Age at transplant (yr)[1]	49.2±14.1
Weight at time of transplant (kg)[1]	81.3±20.1
Living-donor transplant	574 (59%)
No. with diabetes at time of transplantation	368 (38%)
Race (n)	
Caucasian	747
African American	171
Asian	32
Native American	19
Hawaiian/Pacific Islander	3
Multi-race	3
Not given	3
Primary Cause of Kidney Disease (n)	
Diabetes	300
Glomerular disease	199
Hypertensive nephrosclerosis	121
Polycystic kidney disease	116
Congenital, rare familial or metabolic disease	44
Tubular and interstitial disease	32
Other	166
No. with prior kidney transplant	136 (14%)
No. with preemptive transplant	309 (32%)

[1] mean±SD

Table 2

Mycophenolate Related Anemia and Leukopenia Toxicity

	Subjects with toxicity	Subjects without toxicity
Anemia (n=918)[1]	87	831
Median days to anemia or censoring (95% CI)	45 (34–55)	183 (183–183)
Age (mean±SD)	51.6±14.5	48.8±13.9
Weight (kg) at transplant (mean±SD)	79.2±22.8	81.8±20.0
Male gender	56.3%	62.8%
Prior kidney transplant	17%	14%
Living donor	51%	61%
Thymoglobulin induction	66%	52%
Concomitant medications		
Cyclosporine	57%, (26%)[2]	35%, (24%)[2]
Tacrolimus	39%, (0%)[2]	63%, (5%)[2]
Corticosteroids	44%[3]	50%[3]
Antiviral prophylaxis	91%[4]	95%[4]
Posttransplant dialysis	22%	7%
CMV serostatus pretransplant (R−/D−, R+, R−/D+)[3]	18%, 55%, 21%	21%, 59%, 16%
Leukopenia (n=978)[1]	224	754
Median days to leukopenia or censoring (95% CI)	81.5 (76–88)	183 (183–183)
Age (mean±SD)	47.7±15	49.6±13.7
Weight at transplant (mean±SD)	78.3±20.2	82.2±20.0
Male gender	60%	63%
Prior kidney transplant	10%	15%
Living donor	53%	60%
Thymoglobulin induction	60%	49%
Concomitant medications		
Cyclosporine	37%, (22%)[2]	35%, (25%)[2]
Tacrolimus	61%, (7%)[2]	63%, (4%)[2]
Corticosteroids	65%[3]	41%[3]
Antiviral prophylaxis	98%[4]	92%[4]
Posttransplant dialysis	8.5%	8%
CMV serostatus (R−/D−, R+, R−/D+)[5]	18%, 53%, 23%	22%, 61%, 14%

[1]Number of subjects analyzed.

[2]% of patients receiving concomitant calcineurin inhibitor anytime within the 14 days posttransplant (% switched off the calcineurin inhibitor at anytime in the first 6 months posttransplant),

[3]%of patients who never received corticosteroids or stopped taking corticosteroids within 14 days posttransplant,

[4]% of patients who received antiviral prophylaxis at any time while at risk for toxicity,

[5] Pretransplant CMV serostatus missing in 44 subjects.

Table 3

Adjusted and Unadjusted Single SNP Analysis Towards Mycophenolate Related Anemia

SNP no.	rs number	Gene Name	Unadjusted Hazard Ratio (95% CI)[1]	p-value	Adjusted Hazard Ratio (95% CI)[2]	p-value	MAF[3]	
1	rs568408	IL12A	1.99 (1.40–2.83)	0.00012[4]	1.98 (1.39–2.82)	0.00014[4]	A	0.15
2	rs2037483	HUS1[5]	0.58 (0.42–0.79)	0.00058	0.54 (0.39–0.74)	0.00016[4]	A	0.50
3	rs1056663	HUS1[5]	0.58 (0.43–0.80)	0.00068	0.54 (0.39–0.75)	0.00018[4]	A	0.49
4	rs11572076	CYP2C8[6]	3.44(1.90–6.25)	0.00005[4]	3.24 (1.70–6.20)	0.00037[4]	A	0.03
5	rs11572103	CYP2C8[6]	3.40 (1.92–6.03)	0.00003[4]	3.04 (1.61–5.73)	0.00059	T	0.03
6	rs5005	ADM	3.26 (1.62–6.57)	0.00090	3.52 (1.68–7.63)	0.00085	G	0.01
7	rs3797897	MSH3	1.95 (1.33–2.86)	0.00058	1.89 (1.28–2.79)	0.00137	G	0.09
8	rs12441817	CYP1A1	1.76 (1.24–2.50)	0.00143	1.77 (1.24–2.51)	0.00150	C	0.13
9	rs4135113	TDG	2.27 (1.28–4.01)	0.00479	2.48 (1.38–4.43)	0.00227	A	0.05
10	rs2227551	PLAU[7]	1.61 (1.20–2.17)	0.00166	1.57 (1.17–2.11)	0.00261	G	0.32
11	rs2239393	COMT	1.61 (1.20–2.17)	0.00155	1.57 (1.17–2.11)	0.00292	G	0.37
12	rs3087243	CTLA4	0.57 (0.40–0.79)	0.00093	0.60 (0.43–0.84)	0.00300	A	0.38
13	rs4633	COMT	0.61 (0.45–0.83)	0.00167	0.63 (0.46–0.85)	0.00301	T	0.48
14	rs610899	MRE11A[8]	1.48 (1.11–1.98)	0.00811	1.55 (1.16–2.07)	0.00322	G	0.42
15	rs4303	ACE	4.84 (2.03–11.6)	0.00038	3.74 (1.54–9.08)	0.00357	T	0.02
16	rs2227552	C10orf55[7]	1.55 (1.14–2.11)	0.00504	1.57 (1.16–2.13)	0.00380	C	0.28
17	rs2010963	VEGFA	0.58 (0.40–0.84)	0.00364	0.58 (0.40–0.84)	0.00449	C	0.31
18	rs518276	MRE11A[8]	1.49 (1.13–1.98)	0.00527	1.50 (1.13–1.99)	0.00457	A	0.41
19	rs12922317	RUNDC2A	1.46 (1.09–1.95)	0.01046	1.50 (1.13–1.99)	0.00544	G	0.34
20	rs497763	ANKRD49	1.46 (1.09–1.95)	0.01086	1.51 (1.13–2.02)	0.00566	A	0.44
21	rs373496	TNFRSF17	2.83 (1.47–5.47)	0.00195	2.55 (1.31–4.96)	0.00572	T	0.03
22	rs3020314	ESR1	1.49 (1.11–1.99)	0.00713	1.50 (1.13–2.01)	0.00574	C	0.41
23	rs1272744	MRE11A[8]	1.50 (1.12–1.99)	0.00615	1.49 (1.12–1.99)	0.00675	C	0.40
24	rs1049631	IL4R	0.64 (0.47–0.87)	0.00464	0.65 (0.48–0.89)	0.00691	A	0.48
25	rs1056836	CYP1B1	0.70 (0.52–0.95)	0.02053	0.66 (0.48–0.89)	0.00694	G	0.48
26	rs2193587	DGKG	0.51 (0.33–0.79)	0.00279	0.55 (0.35–0.85)	0.00705	G	0.20

SNP no.	rs number	Gene Name	Unadjusted Hazard Ratio (95% CI)[1]	p-value	Adjusted Hazard Ratio (95% CI)[2]	p-value	MAF[3]	
27	rs2740560	CYP3A7[9]	1.71 (1.21–2.41)	0.00228	1.62 (1.14–2.31)	0.00716	A	0.18
28	rs2687140	CYP3A7[9]	1.72 (1.22–2.42)	0.00206	1.62 (1.14–2.30)	0.00718	A	0.18
29	rs2074086	ABCC1	0.63 (0.45–0.88)	0.00637	0.63 (0.45–0.88)	0.00754	C	0.38
30	rs836802	MSH3	0.58 (0.38–0.88)	0.00988	0.56 (0.37–0.86)	0.00767	G	0.24
31	rs3813867	CYP2E1	2.10 (1.24–3.54)	0.00828	2.03 (1.2–3.42)	0.00552	C	0.04
32	rs1946519	IL18	0.66 (0.49–0.90)	0.00847	0.66 (0.49–0.90)	0.00955	A	0.40
33	rs943975	CYP2E1	1.65 (1.14–2.39)	0.00860	1.64 (1.13–2.36)	0.00764	C	0.16
34	rs20432	PTGS2	1.73 (1.19–2.51)	0.00909	1.54 (1.11–2.13)	0.00420	G	0.23
35	rs2802269	CDC42BPA	1.66 (1.11–2.49)	0.00953	1.74 (1.14–2.64)	0.01489	G	0.09
36	rs2020869	FMO2	1.71 (1.20–2.43)	0.00967	1.62 (1.12–2.33)	0.00317	G	0.15

[1] SNPs are not adjusted for clinical factors,

[2] adjusted for antiviral use, age, weight, posttransplant dialysis, [2] hazard and 95% confidence interval (CI) of developing anemia for each risk allele,

[3] allele associated with the hazard and the minor allele frequency (MAF) in the population,

[4] significant accounting for a FDR of 20%,

[5] HUS1 SNPs are correlated r^2=1.0,

[6] CYP2C8 SNPs are correlated r^2=0.93,

[7] PLAU and C10orf55 SNPs are correlated r^2=0.93,

[8] MRE11A SNPs are correlated r^2=0.89,

[9] CYP3A7 SNPs are correlated r^2 =0.99.

Table 4

Multiple SNP and Clinical Factor Analysis for Association with the Development of Mycophenolate Related Anemia

Clinical Factors and SNPs	Hazard Ratio (95% CI)[1]
Antiviral prophylaxis	1.89 (0.84–4.25)
Decade of age[2]	1.28 (1.09–1.50)
Weight	0.99 (0.98–1.00)
Weight squared[3]	1.00 (1.00–1.001)
Posttransplant dialysis[4]	2.44 (1.41–4.20)
rs1056663 (HUS1)[5]	0.55 (0.40–0.77)
rs568408 (IL12A)[6]	1.84 (1.28–2.66)
rs11572076 (CYP2C8)	2.62 (1.34–5.10)

[1] CI is 95% confidence interval,

[2] 1.28 (28%) increase in hazard of anemia for every decade increase from the mean age,

[3] square term for the mean-centered weight,

[4] receipt of dialysis is a 2.44 greater hazard than not receiving dialysis,

[5] HUS1 was protective against anemia with a hazard of 0.55 (45% reduction) of developing anemia for one allele relative to those recipients without the SNP.

[6] IL12A increases hazard of anemia by 1.84 (84%) for every one allele relative to those recipients without the SNP.

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Table 5

Adjusted and Unadjusted Single SNP Analysis Towards Mycophenolate Related Leukopenia

SNP no.	rs number	Gene Name	Unadjusted Hazard Ratio (95% CI)[1,2]	p-value	Adjusted Hazard Ratio (95% CI)[2,3]	p-value	MAF[4]	
1	rs1041163	VCAM1[5]	0.58 (0.43–0.77)	0.00023	0.60 (0.44–0.81)	0.0008	C	0.17
2	rs2392221	VCAM1[5]	0.55 (0.40–0.77)	0.00038	0.59 (0.42–0.82)	0.0016	T	0.15
3	rs4149056	SLCO1B1	1.46 (1.14–1.87)	0.00283	1.52 (1.17–1.98)	0.0017	C	0.13
4	rs9658655	CHGA	0.52 (0.35–0.77)	0.00089	0.54 (0.36–0.79)	0.0017	C	0.10
5	rs12418	CHST3	1.28 (1.07–1.53)	0.00726	1.34 (1.11–1.62)	0.002	A	0.43
6	rs10759326	IKBKAP[6]	1.33 (1.09–1.64)	0.00583	1.40 (1.13–1.73)	0.002	G	0.26
7	rs4148950	CHST3[7]	1.28 (1.07–1.53)	0.00756	1.32 (1.1–1.6)	0.003	A	0.43
8	rs10979601	IKBKAP[6]	1.31 (1.07–1.61)	0.00932	1.37 (1.11–1.7)	0.004	T	0.25
9	rs2020870	FMO2	0.46 (0.29–0.74)	0.00131	0.50 (0.31–0.81)	0.004	G	0.07
10	rs9282626	CAT	1.67 (1.17–2.40)	0.00489	1.71 (1.18–2.47)	0.004	C	0.05
11	rs5744934	POLE	1.48 (1.15–1.91)	0.00246	1.47 (1.12–1.91)	0.005	G	0.12
12	rs4148947	CHST3[7]	1.26 (1.05–1.52)	0.01225	1.31 (1.08–1.58)	0.005	C	0.43
13	rs2140516	SLC13A1	1.30 (1.08–1.58)	0.00638	1.32 (1.09–1.61)	0.006	C	0.31
14	rs7201683	WWOX	1.68 (1.11–2.55)	0.01413	1.81 (1.19–2.76)	0.006	G	0.03
15	rs6941583	POLH	1.36 (1.03–1.81)	0.03209	1.49 (1.12–1.99)	0.006	T	0.08
16	rs2706338	RAD50	1.33 (1.07–1.65)	0.01020	1.35 1.08–1.68)	0.007	T	0.18
17	rs1058172	CYP2D6	0.69 (0.49–0.96)	0.02809	0.62 (0.44–0.88)	0.008	A	0.13
18	rs769214	CAT	0.72 (0.59–0.88)	0.00168	0.76 (0.62–0.93)	0.009	G	0.36

[1] SNPs are not adjusted for clinical factors,

[2] hazard and 95% confidence interval (CI) of developing leukopenia for each risk allele,

[3] Adjusted for corticosteroid use, weight, prior kidney transplant, donor (living vs deceased), CMV serostatus,

[4] allele associated with the hazard and minor allele frequency (MAF) in the population,

[5] VCAM1 SNPs are correlated r^2=0.78,

[6] IKBKAP SNPs are correlated r^2=0.97,

[7] CHST3 SNPs are correlated r^2=0.997.