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Mycophenolate mofetil-related leukopenia in children and young adults following kidney transplantation: Influence of genes and drugs

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DISCLOSURE

CDV, TF, CLK, LJM, BLW, HPP, GMB, SKV, RGV, DJW, AW, PSV, AAV, JG, and DKH have nothing to disclose. DHC is an employee at and shareholder in Abbvie.

AUTHORS' CONTRIBUTIONS

Charles D. Varnell: Participated in the data analysis and drafting, critical review, and revision of the manuscript; Tsuyoshi Fukuda: Participated in the research design, drafting of the manuscript, and acquisition and analysis of data and contributed new reagents; Cassie L. Kirby, Barry L. Warshaw, Hiren P Patel, Deepa H. Chand, Gina-Marie Barletta, Scott K. Van Why, Rene G. VanDeVoorde, Donald J. Weaver, Amy Wilson, Priya S. Verghese, Larry Greenbaum, and Jens Goebel: Participated in the research design, acquisition of data, and critical review and revision of the manuscript; Lisa J. Martin: Participated in the research design, data analysis and interpretation, and drafting, critical revision, and review of the manuscript; Alexander A. Vinks: Participated in the research design, acquisition of data, and critical review and revision of the manuscript and contributed new reagents; Larry Greenbaum: Participated in the research design; and David K. Hooper: Participated in the research design, acquisition and analysis of data, and drafting, critical review, and revision of the manuscript and contributed new reagents.

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Abstract

MMF is commonly prescribed following kidney transplantation, yet its use is complicated by leukopenia. Understanding the genetics mediating this risk will help clinicians administer MMF safely. We evaluated 284 patients under 21 years of age for incidence and time course of MMF-related leukopenia and performed a candidate gene association study comparing the frequency of 26 SNPs between cases with MMF-related leukopenia and controls. We matched cases by induction, steroid duration, race, center, and age. We also evaluated the impact of induction and SNPs on time to leukopenia in all cases. Sixty-eight (24%) patients had MMF-related leukopenia, of which 59 consented for genotyping and 38 were matched with controls. Among matched pairs, no SNPs were associated with leukopenia. With non-depleting induction, UGT2B7-900A>G (rs7438135) was associated with increased risk of MMF-related leukopenia ($P = .038$). Time to leukopenia did not differ between patients by induction agent, but 2 SNPs (rs2228075, rs2278294) in IMPDH1 were associated with increased time to leukopenia. MMF-related leukopenia is common after transplantation. UGT2B7 may influence leukopenia risk especially in patients without lymphocyte-depleting induction. IMPDH1 may influence time course of leukopenia after transplant.

Keywords

adverse effects; kidney transplantation; leukopenia

1 | INTRODUCTION

MMF is among the most commonly prescribed medications for immunosuppression following kidney transplantation.[1] Yet, its use in children and young adults is complicated by a high incidence of side effects (gastrointestinal, hematologic)[2–4] that may lead to empiric dose reduction, which has been associated with increased risk for rejection.[4] The incidence of MMF-related leukopenia is a function of overall exposure to MPA as estimated by the area under the time-concentration curve in both children and adults.[5–7] Multiple factors influence the risk of leukopenia in MMF-treated transplant recipients in the first year following kidney transplantation including induction immunosuppression with lymphocyte-depleting antibodies, other medications that cause marrow suppression such as valganciclovir and trimethoprim/sulfamethoxazole, and viral infections such as cytomegalovirus.[8,9] In addition, it is now evident that pharmacogenetic factors significantly impact the metabolism, transport, and target enzyme activity of MPA.[10–17] SNPs in genes encoding UGT have been associated with 50% increased or decreased drug exposure in

humans;[12,13] SNPs in the genes encoding multidrug resistance proteins (MDR1 and MRP2) may predict altered transport and distribution of MPA,[14] and SNPs in genes encoding IMPDH may influence the inhibitory activity of MPA.[15–17]

Because of its narrow therapeutic index, a number of studies have examined ways to decrease side effects and increase the efficacy of MMF by relying on pharmacokinetic analysis[18,19] and/or IMPDH activity.[20–23] While these methods have potential to improve the efficacy of therapy, they have not been widely accepted for routine use, perhaps because they require multiple blood samples and can be technically challenging.[24] Knowledge of a patient's genetic risk for developing MMF-related side effects may help identify the subset of patients that would benefit from more intense monitoring of drug levels or IMPDH activity. Likewise, an understanding of the increased risk of leukopenia related to other medications and opportunistic viral infections may further allow clinicians to tailor therapy in a manner that avoids this complication. Thus, knowledge about the gene-environment association in these patients would be beneficial when considering treatment regimens.

We conducted a multicenter study to better understand the incidence and time course of MMF-related leukopenia in children and young adults based on induction therapy and examined the association of 26 SNPs involving genes that encode proteins involved in MPA metabolism, transport, or target enzyme activity. We controlled our analysis for other factors associated with leukopenia including induction with a depleting or non-depleting antibody, other medications (eg, trimethoprim/sulfamethoxazole and ganciclovir/valganciclovir), and viral infections. Finally, we evaluated time to leukopenia in all cases of MMF-related leukopenia according to induction type (depleting or non-depleting) and these 26 SNPs.

2 | MATERIALS AND METHODS

2.1 | Study design

This was a retrospective multicenter study to evaluate the incidence and time course of MMF-related leukopenia within the first year following kidney transplantation and a candidate gene association study comparing frequency of 26 SNPs between cases that experienced MMF-related leukopenia and controls that did not. Participating centers were recruited from the Midwest Pediatric Nephrology Consortium and obtained Institutional Review Board (IRB) approval for participation.

2.2 | Study Population

Ten participating centers conducted systematic chart review of all eligible patients in their post-kidney transplant cohort and all patients who received a kidney transplant during the conduct of the study to identify cases and controls for inclusion. Patients 1–21 years of age who experienced MMF-related leukopenia or neutropenia during the first year following kidney transplantation were approached for enrollment as cases. MMF-related leukopenia was defined as WBC $<3.0 \times 10^9$/L and/or ANC $<1.5 \times 10^9$/L that prompted MMF discontinuation and/or dose reduction and recovered within 6 weeks.

Patients were considered for enrollment as controls if they had been treated for a full year with MMF at full dose (per each center's protocol) without leukopenia or neutropenia.

When a patient meeting the above criteria could not be found to match with a given case, patients who experienced only one mild, transient episode of leukopenia that recovered spontaneously without intervention were also eligible to participate as controls. Each case was matched with one control according to five criteria listed in order of importance: (i) induction regimen: depleting (antithymocyte globulin) vs non-depleting (basiliximab or daclizumab) antibody vs none, (ii) duration of steroid therapy following transplantation, (iii) race (black vs white or Hispanic), (iv) transplant center (to control for population stratification), and (v) age (0–3, 4–12, >12 years of age). Unmatched cases (matching control unavailable) were not included in the case-control analysis, but were included in a post hoc analysis of SNP predictors of time to leukopenia stratified by induction antibody type.

For the gene association study, patients were excluded for the presence of conditions that could affect MMF exposure and for known causes of leukopenia not otherwise accounted for in our model including the following: MMF dose reduction for reasons other than leukopenia (eg, diarrhea, anemia), liver failure or transplant, bone marrow transplant, active nephrotic syndrome, underlying diagnosis of systemic lupus erythematosus, therapy with bile acid sequestrants, history of non-adherence, induction with alemtuzumab, estimated glomerular filtration rate <30 mL/min/1.73 m^2 calculated using the bedside Schwartz formula[25] (0.413*$height_{cm}$/serum creatinine),[26] and evidence of cytomegalovirus viremia within 1 month of the diagnosis of leukopenia.

2.3 | Data collection

The following data were gathered and recorded in a secure online database about each case and control: demographic and anthropometric data; date of current and all previous transplants; induction and maintenance immunosuppression; steroid treatment duration; HLA mismatch; duration of treatment with ganciclovir and/or valganciclovir; date, height, and weight at the time of leukopenia for cases and at 1 year for controls; MMF dose prior to and after dose adjustment for cases and at 1 year for controls; serum creatinine; history of other MMF-related side effects (eg, diarrhea, anemia), date and treatment of biopsy-proven rejection; maintenance immunosuppression drug levels for cyclosporine, tacrolimus, or sirolimus at the time of leukopenia for cases or at one year for controls; concurrent medications; and concurrent viral infections (eg, EBV, CMV, adenovirus).

2.4 | Selection of candidate genes and genotyping

We selected candidate genes based on protein products which are involved in MPA metabolism, transport, or target enzyme activity. Genetic variants in the candidate genes were selected from previous publications with at least one positive association with activity of the protein products. Eleven SNPs were selected from enzymes involved in MPA metabolism [UGT1A8: *3 (rs1786762) and *2 (rs1042597); UGT1A9 -2152C>T (rs17868320), -665C>T (rs10176426), -440C>T (rs2741045), -275T>A (rs6714486); UGT2B7 -900A>G (rs7438135); UGT1A7 -57T>G (rs7586110); CYP3A5*3 (rs776746); CYP3A4*22 (rs35599367); CYP2C8 (rs11572076)], 10 SNPs were selected from enzymes involved in either MPA or MPA metabolite transports [ABCB1/MDR1: 3435C>T (rs1045642), 1236C>T (rs1128503), 2677G>T/A (rs2032582); SLCO1B3: 334T>G

(rs4149117 and rs1104585); SLCO1B1: Val174Ala (rs4149056) and Pro155Thr (rs11045819); ABCC2/MRP2: -24C>T (rs717620 and rs12762549); ABCG2 421C>A (rs2231142)], and five SNPs were selected from MMF target enzymes (IMPDH1: rs2278293, rs2278294, rs2228075; IMPDH2: rs11706052, rs4974081).

All enrolled cases and matched controls were asked to submit a saliva specimen using the Oragene® DNA Self-Collection Kit or a single blood specimen using an EDTA tube for genotyping at the time of enrollment during a regular follow-up visit. Samples were collected locally at participating centers and then shipped directly to a centralized laboratory where DNA isolation and genotyping were performed. Commercially available TaqMan® assays (Applied Biosystems, Foster City, CA, USA) were used to determine these genotypes after cross-validation by direct sequencing.

2.5 | Statistical analysis

The incidence of MMF-related leukopenia was determined from seven of the participating centers (3 of the initial 10 did not have complete data on their entire cohort). Descriptive statistics were performed and compared between cases and controls using frequencies for categorical data and median with interquartile range for continuous data. We used chi-square analysis for categorical data, t tests for normally distributed continuous data, and nonparametric tests for non-normally distributed continuous data. Prior to genetic association analyses, all variants were evaluated for deviations from HWE by race. The threshold for exceeding HWE was set as $P = .0019$ (Bonferroni correction of 26 SNPs). Two variants surpassed this threshold, SLCO1B3 (rs1104585) and UGT1A9-2152C>T (rs17868320), and thus were excluded from further analysis.

To determine whether our candidate variants were associated with the development of leukopenia, we performed a matched case-control analysis using logistic regression. Covariates in the model included induction therapy, concurrent medications, and viral infections. An additive genetic model was used to test whether there was a dose-dependent relationship effect with the number of copies of the minor allele and risk of leukopenia. In addition, because the pathways leading to leukopenia may differ by induction agent, analyses were also stratified by induction agent. Given the small sample size and exploratory nature of this study, multiple testing correction was not performed. Using the sample of 38 matched case-control pairs, we would have 80% power to detect an odds ratio of 3.6 for a SNP with a MAF equaling 0.15 at $\alpha = 0.05$. For variants with a MAF equaling 0.30, we have 80% power to detect an odds ratio of 2.9.

As a secondary analysis, we performed a case-only analysis for the time to leukopenia using linear regression. To maximize our power, we included all recruited cases with a DNA sample even if they did not have a matching control (n = 57, 38 matched cases plus 19 unmatched cases). Time to leukopenia exhibited skewing, so the variable was natural-logarithm-transformed. Covariates included sex, race, ethnicity, induction agent, trimethoprim/sulfamethoxazole use, and ganciclovir/valganciclovir use. As with the case-control analysis, stratified analyses were performed by induction agent. Given the small sample size and exploratory nature of this study, multiple testing correction was not performed.

3 | RESULTS

3.1 | Incidence of MMF-related leukopenia

A chart review was performed for 284 subjects from seven centers. Of these, 68 (24%) met our definition for MMF-related leukopenia. Fifty-eight (21%) patients never experienced a single episode of leukopenia or neutropenia, and another 72 (25%) had only one episode of transient leukopenia (recovered without intervention). Forty-eight (17%) patients experienced other MMF-related side effects, and 38 (13%) were deemed to have other causes of leukopenia (eg, cytomegalovirus infection).

3.2 | Candidate gene association study

Of the 68 identified potential cases, we were unable to enroll 11. An additional 19 did not have an enrolled matched control for the following reasons: steroid duration (n = 10), race (n = 7), induction antibody (n = 1), or site (n = 1). Thus, we included 38 matched pairs in our candidate gene association study. Cases and controls were well matched according to all measured variables, including matching criteria (Table 1). Specifically, they did not differ in induction agent, duration of steroids, age, race and ethnicity, gender, MMF dose (per body surface area), treatment with other leukopenia-causing medications, viral illness, or biopsy-proven rejection. Among cases, the mean WBC and ANC at the time of leukopenia were 1.85×10^9/L (range: 0.8–2.9) and 0.55×10^9/L (range: 0–1.3) respectively. Using all 38 matched pairs, none of the SNPs were associated with MMF-related leukopenia ($P > .07$) (Table 2). However, among patients receiving non-depleting antibodies, the G allele for UGT2B7-900A>G (rs7438135) was associated with increased risk of MMF-related leukopenia [$P = .038$, OR 5.26 (1.1-25.0)].

3.3 | Time to leukopenia analysis

For a secondary analysis of time to leukopenia, we analyzed our entire cohort of enrolled cases, which included 38 matched cases and 19 unmatched cases (n = 57). The 19 cases not included in the case-control analysis did not differ from the 38 patients who were included (Table 1) and were similar in the time to leukopenia ($P = .2$). Median time (interquartile range) to leukopenia for the entire group was 105.8 (69.3-153.3) days and did not differ significantly in those who received depleting antibody compared to those who received non-depleting antibody induction [131.7 (81.0-174.6) days vs 99.7 (64.4-140.0) days ($P = .22$)]. Two SNPs in the IMPDH1 gene were significantly associated with time to leukopenia (Table 3). The G allele in rs2228075 was associated with an increased time to leukopenia ($P = .0046$). When examining the genotypic least squares means, those with GG genotype had a time to leukopenia 92 days longer than individuals with either the GA or AA genotype. The G allele in rs2278294 was also associated with an increased time to leukopenia ($P = .0382$). When examining the genotypic least squares means, the GG genotype had time to leukopenia 90 days longer than individuals with either the GA or AA genotype.

4 | DISCUSSION

In this study, we examined three important aspects of MMF-related leukopenia in a cohort of nearly three hundred children and young adults during their first year following kidney

transplantation including the incidence and time course of MMF-related leukopenia, the influence of induction agents, and the association of 26 candidate SNPs. This is the largest study of MMF-related leukopenia in pediatric and young adult kidney transplant recipients to date, and the findings further the understanding of this important problem in a high-risk population. For the candidate gene association study, patients were well matched according to clinical, demographic, and treatment characteristics to provide the best chance for isolating the impact of genetics.

Of the 284 children in the first year following kidney transplantation, we found that 24% (n = 68) experienced MMF-related leukopenia as evidenced by WBC $<3.0 \times 10^9$/L and/or ANC $<1.5 \times 10^9$/L that recovered within 6 weeks following a dose adjustment or discontinuation of medication. When combined with the 48 patients who were excluded from case-control analysis because they required MMF dose modification caused by other side effects (diarrhea/anemia), 41% (116/284) of all patients required an MMF dose modification in the first year following transplantation due to one or more side effects. Others have looked at MMF-related leukopenia in pediatric cohorts and have found varying rates of leukopenia. Ohmann et al[27] and Siddiqi et al[28] have reported adverse event data in pediatric heart transplant patients, and they found the incidence of MMF-related leukopenia to be 35.6% (21/59 patients) defining leukopenia as WBC <2000 cells/μL and 59% (13/22 patients) defining leukopenia as WBC <3000 cells/μL, respectively, with MMF dosing of 600 mg/m^2. Bunchman et al[29] reported MMF use in pediatric kidney transplant patients and found an incidence of leukopenia of 24% (24/100), which is consistent with our findings. When we looked at the time to leukopenia based on induction antibody type, there was no significant difference based on depleting vs non-depleting antibodies. While it may be assumed that the depleting antibodies would lead to decreased time to leukopenia based on the mechanism of action, in a study of rabbit antithymocyte globulin vs basiliximab in renal transplantation, Brennan et al described that immediately after transplantation, leukopenia and thrombocytopenia were more common among patients who received antithymocyte globulin than among those who received basiliximab ($P < .001$) but that by day 14 there were no significant differences noted.[30]

Given the high rate of MMF-related leukopenia, we sought also to identify the impact of genetic variants on this important treatment-limiting side effect via an exploratory case-control candidate gene association study of 26 SNPs known to affect the metabolism, transport, or target enzyme activity of MMF. Without any stratification of patients, we did not find any of the 26 SNPs that predicted a statistically significant increased risk for leukopenia. We did find that in patients with non-depleting antibody induction, the G allele for UGT2B7-900A>G was associated with increased risk of MMF-related leukopenia. We can only speculate as to why we found an association only in patients with non-depleting antibody induction, but it is possible that the well-described leukopenia-causing effect of lymphocyte-depleting antibody induction[31] washed out the ability to detect any association between this SNP and leukopenia. In the absence of the lymphocyte depletion, the effects of genetic polymorphisms may play a more important role. MPA is mainly metabolized by UGT1A family members (particularly UGT1A9 and UGT1A8) to MPA glucuronide (MPAG) and UGT2B7 to the acyl glucuronide of MPA (AcMPAG).[10,32] It has been reported that the G allele for UGT2B7-900A>G is associated with decreased metabolic activity and

subsequent higher AUCs of MPA, resulting in a higher risk for adverse events in pediatric kidney transplant recipients.[10] While the specific mechanism of leukopenia and MMF metabolites is still a topic of investigation, our finding that the G allele was associated with an increased risk for MMF-related leukopenia is consistent with prior literature suggesting impaired metabolism and overall higher systemic exposure to MPA increases the risk of leukopenia.[5–7,10,32,33]

Others have studied the metabolite AcMPAG as a potential mediator of adverse side effects for patients prescribed MMF both in vitro and in vivo and suggest there may be a relationship between AcMPAG and gastrointestinal side effects;[34,35] however, AcMPAG has not been convincingly shown to be a mediator of leukopenia. In a study by van Agteren et al, they did not find a difference in AcMPAG concentrations in 332 kidney transplant patients that experienced leukopenia compared to those that did not.[36] We did not measure serum MPA or its metabolites (MPAG and AcMPAG) in this study, so we are unable to correlate whether or not the UGT2B7-900G allele had an effect on plasma levels of MPA or its metabolites; however, this could be performed in future studies looking for allelic differences in MMF-related side effects in pediatric kidney transplant recipients.

Compared to adults, it may be that children and adolescents are more susceptible to MMF-related leukopenia and/or the effects of SNPs due to maturation of these enzymes. Indeed, it has been reported that the levels of some key drug-metabolizing enzymes commonly reach adult levels after 3 years of age, while the isoforms of the UGT enzymes (especially UGT1A9, UGT2B7, and UGT1A6) develop more slowly and typically do not reach adult levels until 10 years of age.[37] As such, it is important to recognize that the ability to metabolize various drugs is dynamic throughout childhood[38] and that age-related studies, specifically looking at pharmacokinetics and how SNPs contribute to metabolism, are required. These differences in metabolism between adults and children may modify the effect of SNPs on the incidence of MMF-related side effects.

Of the two SNPs in the IMPDH1 gene, the G alleles in rs2228075 and rs2278294 were associated with increased time to leukopenia. This would suggest a protective effect against the development of leukopenia in individuals homozygous for the G allele compared to the GA or AA phenotype. In pediatric heart transplant patients, Ohmann et al reported that the GG phenotype in rs2278294 was significantly associated with reduced risk of MMF dose-holding or discontinuation, with 55% of GG carriers discontinuing and/or holding MMF compared with 86.7% of patients with an A allele at this locus ($P = .01$; adjusted OR = 0.15, 95% CI: 0.04-0.62, $P = .008$).[27] Similar findings have also be described in adult kidney transplant patients.[39] Of note, rs2278294 and rs2228075 exhibit moderate linkage disequilibrium ($r^2 = .64$) based on 1000 Genomes CEU data. This suggests that the associations we detected may be a marker for another genetic variant in this region. Future studies should examine this further. While our findings do not directly provide a mechanism for increased time to leukopenia, given that these alleles have lower incidence of other adverse events, it is possible that these alleles affect sensitivity of this target enzyme to MPA.

Strengths of our study include that it is the largest cohort of children and young adults to be assessed for MMF-related leukopenia. For the gene association study, cases were well matched to their controls according to five important parameters and a variety of important SNPs with plausible mechanisms for causing increased MMF exposure and related leukopenia were evaluated. However, the results must be interpreted in light of weaknesses common to studies of rare conditions. We were unable to directly assess exposure to MPA or its metabolites to link genetic findings to a mechanism of action. Unfortunately our data cannot determine causality, only establish associations. Finally, because it was exploratory, the candidate gene association study was powered only to detect large effects of genetic polymorphisms (OR of 2.9-3.6 for MAFs ranging from 0.15 to 0.30). Most often, SNPs in complex genetic traits are associated with smaller effects sizes that our study was underpowered to detect. Thus, the lack of association with MMF-related leukopenia in our study does not necessarily mean that no association exists. Larger studies with multiple testing corrections will be required to detect more subtle effects and provide conclusive results. Interestingly, however, as highlighted in the discussion, our findings align with those of other investigators and therefore supplement to the growing body of literature documenting the effects of SNPs on risk of MMF-related side effects.

In summary, we found that a quarter of patients treated with MMF developed leukopenia and 41% developed an MMF-related side effect that required dose modification within the first year post-transplant. The UGT2B7 -900A>G allele is associated with a increased risk for MMF-related leukopenia in patients treated with non-depleting antibodies, and two SNPs in the IMPDH1 gene are associated with delayed time to leukopenia. These findings need to be validated in other studies prior to application in clinical care. Further, we reiterate the need for such studies in children due to the unique implications of enzymatic maturation in this population.

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Abbreviations

ANC	absolute neutrophil count
AUC	area under the curve
CMV	cytomegalovirus
DNA	deoxyribonucleic acid
EBV	Epstein-Barr virus
EDTA	ethylenediaminetetraacetic acid
HLA	human leukocyte antigen

HWE	Hardy-Weinberg equilibrium
IMPDH	inosine monophosphate dehydrogenase
MAF	minor allele frequency
MMF	mycophenolate mofetil
MPA	mycophenolic acid
SNP	single nucleotide polymorphism
UGT	uridinediphosphate glucuronosyltransferase
WBC	white blood cell

References

1. Organ Procurement and Transplantation Network (OPTN) and Scientific Registry of Transplant Recipients (SRTR). OPTN/SRTR 2011 Annual Data Report. Rockville, MD: Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation; 2012.
2. Roberti I, Reisman L. A comparative analysis of the use of mycophenolate mofetil in pediatric vs. adult renal allograft recipients. Pediatr Transplant. 1999; 3:231–235. [PubMed: 10487285]
3. Butani L, Palmer J, Baluarte HJ, Polinsky MS. Adverse effects of mycophenolate mofetil in pediatric renal transplant recipients with presumed chronic rejection. Transplantation. 1999; 68:83–86. [PubMed: 10428272]
4. Knoll GA, MacDonald I, Khan A, Van Walraven C. Mycophenolate mofetil dose reduction and the risk of acute rejection after renal transplantation. J Am Soc Nephrol. 2003; 14:2381–2386. [PubMed: 12937317]
5. Kuypers DRJ, de Jonge H, Naesens M, et al. Current target ranges of mycophenolic acid exposure and drug-related adverse events: a 5-year, open-label, prospective, clinical follow-up study in renal allograft recipients. Clin Ther. 2008; 30:673–683. [PubMed: 18498916]
6. Weber LT, Hoecker B, Armstrong VW, Oellerich M, Tonshoff B. Long-term pharmacokinetics of mycophenolic acid in pediatric renal transplant recipients over 3 years posttransplant. Ther Drug Monit. 2008; 30:570–575. [PubMed: 18758392]
7. Kuypers DRJ, Claes K, Evenepoel P, Maes B, Vanrenterghem Y. Clinical efficacy and toxicity profile of tacrolimus and mycophenolic acid in relation to combined long-term pharmacokinetics in de novo renal allograft recipients. Clinical Pharmacol Ther. 2004; 75:434–447. [PubMed: 15116056]
8. Brum S, Nolasco F, Sousa J, et al. Leukopenia in kidney transplant patients with the association of valganciclovir and mycophenolate mofetil. Transplant Proc. 2008; 40:752–754. [PubMed: 18455007]
9. Mitsides N, Greenan K, Green D, et al. Complications and outcomes of trimethoprim-sulphamethoxazole as chemoprophylaxis for *Pneumocystis* pneumonia in renal transplant recipients. Nephrology. 2014; 19:157–163. [PubMed: 24387294]
10. Fukuda T, Goebel J, Cox S, et al. UGT1A9, UGT2B7, and MRP2 genotypes can predict mycophenolic acid pharmacokinetic variability in pediatric kidney transplant recipients. Ther Drug Monit. 2012; 34:671–679. [PubMed: 23131697]
11. Cattaneo D, Vinks AA. Optimizing immunosuppressive drug dosing in pediatric renal transplantation. Part of a special series on Paediatric Pharmacology, guest edited by Gianvincenzo Zuccotti, Emilio Clementi, and Massimo Molteni. Pharmacol Res. 2012; 65:163–167. [PubMed: 22015636]

12. Baldelli S, Merlini S, Perico N, et al. C-440T/T-331C polymorphisms in the UGT1A9 gene affect the pharmacokinetics of mycophenolic acid in kidney transplantation. Pharmacogenomics. 2007; 8:1127–1141. [PubMed: 17924828]
13. Kuypers DRJ, Naesens M, Vermeire S, Vanrenterghem Y. The impact of uridine diphosphate-glucuronosyltransferase 1A9 (UGT1A9) gene promoter region single-nucleotide polymorphisms T-275A and C-2152T on early mycophenolic acid dose-interval exposure in de novo renal allograft recipients. Clin Pharmacol Ther. 2005; 78:351–361. [PubMed: 16198654]
14. Naesens M, Kuypers DRJ, Verbeke K, Vanrenterghem Y. Multidrug resistance protein 2 genetic polymorphisms influence mycophenolic acid exposure in renal allograft recipients. Transplantation. 2006; 82:1074–1084. [PubMed: 17060857]
15. Wang J, Yang JW, Zeevi A, et al. IMPDH1 gene polymorphisms and association with acute rejection in renal transplant patients. Clin Pharmacol Ther. 2008; 83:711–717. [PubMed: 17851563]
16. Grinyo J, Vanrenterghem Y, Nashan B, et al. Association of four DNA polymorphisms with acute rejection after kidney transplantation. Transpl Int. 2008; 21:879–891. [PubMed: 18444945]
17. Cattaneo D, Baldelli S, Perico N. Pharmacogenetics of immunosuppressants: progress, pitfalls and promises. Am J Transplant. 2008; 8:1374–1383. [PubMed: 18510642]
18. van Gelder T, Silva HT, de Fijter JW, et al. Comparing mycophenolate mofetil regimens for de novo renal transplant recipients: the fixed-dose concentration-controlled trial. Transplantation. 2008; 86:1043–1051. [PubMed: 18946341]
19. Le Meur Y, Buchler M, Thierry A, et al. Individualized mycophenolate mofetil dosing based on drug exposure significantly improves patient outcomes after renal transplantation. Am J Transplant. 2007; 7:2496–2503. [PubMed: 17908276]
20. Sombogaard F, Peeters AM, Baan CC, et al. Inosine monophosphate dehydrogenase messenger RNA expression is correlated to clinical outcomes in mycophenolate mofetil-treated kidney transplant patients, whereas inosine monophosphate dehydrogenase activity is not. Ther Drug Monit. 2009; 31:549–556. [PubMed: 19704402]
21. Kamar N, Glander P, Nolting J, et al. Pharmacodynamic evaluation of the first dose of mycophenolate mofetil before kidney transplantation. Clin J Am Soc Nephrol. 2009; 4:936–942. [PubMed: 19339413]
22. Fukuda T, Goebel J, Thogersen H, et al. Inosine monophosphate dehydrogenase (IMPDH) activity as a pharmacodynamic biomarker of mycophenolic acid effects in pediatric kidney transplant recipients. J Clin Pharmacol. 2011; 51:309–320. [PubMed: 20418509]
23. Chiarelli LR, Molinaro M, Libetta C, et al. Inosine monophosphate dehydrogenase variability in renal transplant patients on long-term mycophenolate mofetil therapy. Br J Clin Pharmacol. 2010; 69:38–50. [PubMed: 20078611]
24. LeMeur Y, Borrows R, Pescovitz MD, et al. Therapeutic drug monitoring of mycophenolates in kidney transplantation: report of The Transplantation Society consensus meeting. Transplant Rev (Orlando, FL). 2011; 25:58–64.
25. Naesens M, de Loor H, Vanrenterghem Y, Kuypers DR. The impact of renal allograft function on exposure and elimination of mycophenolic acid (MPA) and its metabolite MPA 7-O-glucuronide. Transplantation. 2007; 15:362–373.
26. Schwartz GJ, Munoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol. 2009; 20:629–637. [PubMed: 19158356]
27. Ohmann EL, Burckart GJ, Brooks MM, et al. Genetic polymorphisms influence mycophenolate mofetil-related adverse events in pediatric heart transplant patients. J Heart Lung Transplant. 2010; 29:509–516. [PubMed: 20061166]
28. Siddiqi N, Lamour JM, Hsu DT. The effect of MMF dose and trough levels on adverse effects in pediatric heart transplant recipients. Pediatr Transplant. 2015; 19:618–622. [PubMed: 26082342]
29. Bunchman T, Navarro M, Broyer M, et al. The use of mycophenolate mofetil suspension in pediatric renal allograft recipients. Pediatr Nephrol. 2001; 16:978–984. [PubMed: 11793083]
30. Brennan DC, Daller JA, Lake KD, et al. Rabbit antithymocyte globulin versus basiliximab in renal transplantation. N Engl J Med. 2006; 355:1967–1977. [PubMed: 17093248]

31. Brennan DC, Flavin K, Lowell JA, et al. A randomized, double-blinded comparison of thymoglobulin versus atgam for induction immunosuppressive therapy in adult renal transplant recipients. Transplantation. 1999; 67:1011–1018. [PubMed: 10221486]

32. Djebli N, Picard N, Rerolle JP, Le Meur Y, Marquet P. Influence of the UGT2B7 promoter region and exon 2 polymorphisms and comedications on Acyl-MPAG production in vitro and in adult renal transplant patients. Pharmacogenet Genom. 2007; 17:321–330.

33. Prausa SE, Fukuda T, Maseck D, et al. UGT Genotype may contribute to adverse events following medication with mycophenolate mofetil in pediatric kidney transplant recipients. Clin Pharmacol Ther. 2009; 85:495–500. [PubMed: 19225446]

34. Wieland E, Shipkova M, Schellhaas U, et al. Induction of cytokine release by the acyl glucuronide of mycophenolic acid: a link to side effects? Clin Biochem. 2000; 33:107–113. [PubMed: 10751588]

35. Zegarska J, Hryniewiecka E, Zochowska D, et al. Mycophenolic acid metabolites acyl-glucuronide and glucoside affect the occurrence of infectious complications and bone marrow dysfunction in liver transplant recipients. Ann Transplant. 2015; 20:483–492. [PubMed: 26313036]

36. van Agteren M, Armstrong VW, van Schaik RHN, et al. AcylMPAG plasma concentrations and mycophenolic acid-related side effects in patients undergoing renal transplantation are not related to UGT2B7-840G>A gene polymorphism. Ther Drug Monit. 2008; 30:439–444. [PubMed: 18641546]

37. Yoo EC, Alvarez-Elias AC, Todorova EK, Filler G. Developmental changes of MPA exposure in children. Pediatr Nephrol. 2016; 31:975–982. [PubMed: 26743220]

38. Filler G, Alvarez-Elias AC, McIntyre C, Medeiros M. The compelling case for therapeutic drug monitoring of mycophenolate mofetil therapy. Pediatr Nephrol. 2017; 32:21–29. [PubMed: 26921212]

39. Gensburger O, van Schaik RHN, Picard N, et al. Polymorphisms in type I and II inosine monophosphate dehydrogenase genes and association with clinical outcome in patients on mycophenolate mofetil. Pharmacogenet Genom. 2010; 20:537–543.

TABLE 1

Patient characteristics for case-control analysis of genetic variants associated with MMF-related leukopenia

	Matched cases	Controls	*P* value	Unmatched cases
Matching criteria	38	38		19
Induction antibody (%)			1	
Depleting	14 (37)	14 (37)		9 (47)
Non-depleting	23 (61)	23 (61)		10 (53)
None	1 (3)	1 (3)		0 (0)
Weeks of steroid therapy: median (Q1–Q3)	52 (1–52)	52 (1–52)	.89	
Race (%)			.64	
White	31 (81)	28 (74)		11 (58)
Black	6 (16)	7 (18)		5 (26)
American Indian	0	1 (3)		2 (11)
Other	2 (5)	2 (5)		1 (5)
Ethnicity (% Hispanic)	3 (8)	2 (5)	.64	1 (5)
Other patient characteristics				
Age at TX (years, mean ± SD)	11.3 ± 5.1	10.2 ± 5.0	.32	11.13 ± 5.32
Time to leukopenia (years, median, IQR)	0.27 (0.17–0.42)	–	–	0.36 (0.25–0.46)
Sex (%M)	24 (63)	22 (58)	.64	9 (47)
MMF dose (mg/m^2/d)	850.8 ± 236.7	795.9 ± 252.0	.34	1012.9 ± 523.7
Body surface area (m^2)	1.2 ± 0.4	1.2 ± 0.5	.77	1.3 ± 0.5
Primary DX (%)			.47	
0—aplasia/hypoplasia/dysplasia	7 (18)	5 (13)		
1—chronic glomerulosclerosis	4 (11)	1 (3)		
2—focal segmental glomerulosclerosis	4 (11)	4 (11)		
3—obstructive/reflux nephropathy	4 (11)	8 (21)		
4—other	19 (50)	20 (53)		
Viral illness within 1 year post-transplant (%)				
Cytomegalovirus	1 (3)	1 (3)	1.0	
Epstein-Barr virus	3 (8)	3 (8)	1.0	
Adenovirus	0	0		
Anemia	3 (8)	1 (3)	.61	
Diarrhea	4 (11)	4 (11)	1.0	
Biopsy-proven acute rejection (%)	6 (16)	3 (8)	.48	1 (6)
Trimethoprim/sulfamethoxazole use at the time of leukopenia[a]	30 (79)	29 (76)	.78	17 (94)[b]
Valganciclovir use at the time of leukopenia[a]	22 (58)	22 (58)	1.0	10 (56)
Trimethoprim/sulfamethoxazole and valganciclovir at the time of leukopenia[a]	21 (55)	20 (53)	.82	10 (56)

[a]Use for med in controls based on the time of a leukopenia in the cases, and for cases med use within 8 d of leukopenia.

[b] Missing data for one individual.

TABLE 2

Case-control analysis of genetic variants associated with MMF-related leukopenia adjusting for trimethoprim/sulfamethoxazole, valganciclovir, and induction status

					All		Depleting		Non-depleting	
Function	**Gene**	**Change**	**RS#**	**Major allele**	***P* value**	**OR CI**	***P* value**	**OR CI**	***P* value**	**OR CI**
Metabolism	UGT1A9	−2152C>T	rs17868320	C	.235	0.38 (0.08–1.88)	.991		1.000	1.00 (0.14–7.10)
	UGT1A9	−440C>T	rs2741045	C	.171	1.85 (0.77–4.45)	.980		.780	1.17 (0.39–3.54)
	UGT1A9	−665C>T	rs10176426	C	.618	0.67 (0.14–3.21)	.989		.976	
	UGT1A9	−275T>A	rs6714486	T	.225	0.39 (0.09–1.79)	.991		.649	0.65 (0.10–4.09)
	UGT1A8	*2	rs1042597	C	.614	0.80 (0.34–1.90)	1.000	1.00 (0.20–4.95)	1.000	1.00 (0.28–3.51)
	UGT1A8	*3	rs17863762	G	.678	0.68 (0.11–4.20)	.991		.571	0.50 (0.05–5.51)
	UGT2B7	−900A>G	rs7438135	A	.071	0.45 (0.19–1.07)	.303	2.85 (0.39–20.85)	.038*	0.19 (0.04–0.91)
	UGT1A7	−57T>G	rs7586110	T	.398	1.47 (0.60–3.59)	.215	4.00 (0.45–35.79)	1.000	1.00 (0.25–4.00)
	CYP3A5	*3	rs776746	G	.319	1.72 (0.59–4.98)	1.000	1.00 (0.06–15.99)	.101	5.78 (0.71–47.06)
	CYP3A4	*22	rs35599367	C	.201	0.20 (0.02–2.33)	.984			
	CYP2C8		rs11572076	G	.296	0.26 (0.02–3.27)	.994		.978	
Transport	MDR1/ABCB1	1236C>T	rs1128503	C	.411	0.77 (0.40–1.45)	.197	0.29 (0.05–1.89)	.654	0.82 (0.33–1.99)
	MDR1/ABCB1	3435C>T	rs1045642	T	.360	1.36 (0.70–2.62)	.215	4.00 (0.45–35.79)	1.000	1.00 (0.46–2.17)
	MDR1/ABCB1	2677G>T/A	rs2032582	G	.366	1.40 (0.67–2.92)	.491	0.59 (0.13–2.65)	.178	2.08 (0.72–6.05)
	MRP2/ABCC2	−24C>T	rs717620	C	.997	1.00 (0.37–2.73)	.986		.703	0.74 (0.16–3.50)
	MRP2/ABCC2		rs12762549	C	.601	1.21 (0.59–2.49)	.378	1.78 (0.49–6.40)	.780	0.85 (0.28–2.58)
	SLCO1B1	Val 174 Ala	rs4149056	T	.568	1.32 (0.51–3.46)	.571	2.00 (0.18–22.06)	.752	1.22 (0.35–4.25)
	SLCO1B1	Pro 155 Thr	rs11045819	C	.655	1.22 (0.51–2.96)	.740	0.80 (0.21–3.01)	.372	1.75 (0.51–5.98)
	SLCO1B3	334T>G	rs4149117	G	.478	0.75 (0.34–1.66)	.984		.773	1.18 (0.38–3.68)
	SLCO1B3		rs11045585	A	.737	1.17 (0.48–2.86)	.980		.761	1.21 (0.36–4.00)
	BCRP/ABCG2	421C>A	rs2231142	C	.612	1.30 (0.47–3.63)	.571	0.50 (0.05–5.51)	.259	3.17 (0.43–23.44)
Target	IMPDH1		rs2278293	G	.590	1.24 (0.57–2.70)	.740	0.80 (0.21–3.01)	.596	1.34 (0.46–3.93)
	IMPDH1		rs2278294	G	.512	0.77 (0.35–1.69)	.740	1.25 (0.33–4.71)	.198	0.39 (0.10–1.63)
	IMPDH1		rs2228075	G	.648	1.23 (0.50–3.05)	.708	0.75 (0.16–3.43)	.761	1.21 (0.36–4.06)

					All		Depleting		Non-depleting	
Function	**Gene**	**Change**	**RS#**	**Major allele**	***P* value**	**OR CI**	***P* value**	**OR CI**	***P* value**	**OR CI**
	IMPDH2		rs4974081	T	.639	1.23 (0.52–2.87)	.657	1.50 (0.25–8.98)	.596	0.75 (0.25–2.20)
	IMPDH2	3757T>C	rs11706052	A	.297	0.53 (0.16–1.75)	.979		.173	0.16 (0.01–2.21)

*
p value < 0.05

Pediatr Transplant. Author manuscript; available in PMC 2018 November 01.

TABLE 3

Genetic association with time to leukopenia

		All		Depleting		Non-depleting	
Marker	**Major allele**	**Beta ± SE**	***P* value**	**Beta ± SE**	***P* value**	**Beta ± SE**	***P* value**
#01: MDR1-3435 C-T_rs1045642	T	0.02 ± 0.10	.8459	−0.002 ± 0.20	.9932	0.03 ± 0.12	.8022
#02: ABCB1_rs1128503_C___7586662	C	−0.001 ± 0.0	.9899	0.09 ± 0.20	.6474	−0.06 ± 0.12	.6400
#03: SLCO1B3_rs4149117_C__256391	G	0.03 ± 0.12	.7933	0.38 ± 0.24	.1221	−0.15 ± 0.13	.2544
#04: SLCO1B1_rs4149056_C__306339	T	−0.10 ± 0.17	.5580	0.25 ± 0.34	.4592	−0.28 ± 0.19	.1487
#06: MRP2-24_rs717620	C	−0.13 ± 0.18	.4815	0.38 ± 0.36	.2984	−0.37 ± 0.19	.0560
#07: IMPDH2_rs11706052_C___18429	A	−0.21 ± 0.18	.2458	−0.50 ± 0.28	.0866	0.03 ± 0.24	.9092
#08: IMPDH1_rs2228075_C__2694004	G	−0.35 ± 0.12	**.0046** *	−0.46 ± 0.23	.0587	−0.33 ± 0.15	***.0343***
#09: IMPDH1_rs2278293_C__1596518	G	−0.23 ± 0.11	.0458 *	−0.40 ± 0.21	.0705	−0.14 ± 0.14	.3040
#10: SLCO1B1-155_rs11045819_SLCO	C	0.19 ± 0.17	.2551	0.07 ± 0.27	.8077	0.43 ± 0.23	.0743
#11/#12: ABCB1_RS2032582	G	0.05 ± 0.10	.6370	0.26 ± 0.19	.1921	−0.06 ± 0.12	.6301
#13: ABCG2_rs2231142_C__15854163	C	−0.25 ± 0.19	.1799	0.02 ± 0.35	.9647	−0.50 ± 0.22	***.0248***
#14: UGT1A8 *3_rs17863762	G	−0.19 ± 0.34	.5825	–	–	−0.17 ± 0.32	.5987
#15: UGT1A8 *2_rs1042597_C__11742	C	−0.18 ± 0.15	.2370	−0.28 ± 0.28	.3270	−0.13 ± 0.19	.4874
#17: UGT1A9-2152_rs17868320	C	−0.26 ± 0.22	.2416	−0.33 ± 0.34	.3400	−0.17 ± 0.32	.5987
#18: UGT1A9-275_rs6714486	T	−0.15 ± 0.19	.4149	−0.33 ± 0.34	.3400	−0.01 ± 0.26	.9823
#20: UGT1A9-440_rs2741045	C	0.08 ± 0.12	.5034	−0.41 ± 0.32	.2216	0.23 ± 0.12	.0664
#21: UGT1A9_rs10176426_C__303416	C	0.18 ± 0.21	.4015	0.11 ± 0.37	.7694	0.27 ± 0.28	.3455
#22: UGT2B7-900_rs7438135	A	−0.09 ± 0.11	.4404	−0.31 ± 0.22	.1880	0.03 ± 0.13	.7752
#23: UGT1A7_rs7586110_C____28726	T	0.02 ± 0.11	.8603	−0.45 ± 0.24	.0807	0.23 ± 0.12	.0648
#24: ABCC2_rs12762549_C__1121491	C	−0.02 ± 0.12	.8493	0.34 ± 0.22	.1406	−0.21 ± 0.13	.1147
#25: SLCO1B3_rs11045585_C__31106	A	0.20 ± 0.14	.1552	0.38 ± 0.30	.2286	0.11 ± 0.15	.4782
#27: CYP3A5 *3_rs776746	G	−0.17 ± 0.14	.2439	0.03 ± 0.25	.8942	−0.34 ± 0.17	.0553
#36: CYP3A4 *22_rs35599367_C__590134	C	−0.53 ± 0.29	.0718	−0.43 ± 0.40	.2937	−0.86 ± 0.50	.0977
#49: IMPDH1_rs2278294_C___283083	G	−0.23 ± 0.11	**.0382** *	−0.42 ± 0.21	.0592	−0.13 ± 0.12	.3241

Marker	Major allele	All		Depleting		Non-depleting	
		Beta ± SE	*P* value	Beta ± SE	*P* value	Beta ± SE	*P* value
#50: IMPDH2_rs4974081_C__2576349	T	−0.02 ± 0.13	.8901	0.13 ± 0.21	.5303	−0.14 ± 0.16	.3809
#56: CYP2C8_rs11572076_C__3165811	G	−0.18 ± 0.28	.5273	0.07 ± 0.51	.8907	−0.35 ± 0.33	.2898

Beta values are for natural log transformation of days.

* p value < 0.05