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Variants in mycophenolate and CMV antiviral drug pharmacokinetic and pharmacodynamic genes and leukopenia in heart transplant recipients

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BACKGROUND: The objective was to assess the relationship between single nucleotide polymorphisms in mycophenolate and cytomegalovirus antiviral drug pharmacokinetic and pharmacodynamic genes and drug-induced leukopenia in adult heart transplant recipients.

METHODS: This retrospective analysis included $n = 148$ patients receiving mycophenolate and a cytomegalovirus antiviral drug. In total, 81 single nucleotide polymorphisms in 21 pharmacokinetic and 23 pharmacodynamic genes were selected for investigation. The primary and secondary outcomes were mycophenolate and/or cytomegalovirus antiviral drug-induced leukopenia, defined as a white blood cell count $<3.0 \times 10^9$/L, in the first six and 12 months post-heart transplant, respectively.

RESULTS: Mycophenolate and/or cytomegalovirus antiviral drug-induced leukopenia occurred in 20.3% of patients. *HNF1A* rs1169288 A>C (p.I27L) was associated with drug-induced leukopenia (unadjusted $p = 0.002$; false discovery rate <20%) in the first six months post-transplant. After adjusting for covariates, *HNF1A* rs1169288 variant C allele carriers had significantly higher odds of leukopenia compared to A/A homozygotes (odds ratio 6.19; 95% CI 1.97-19.43; $p = 0.002$). Single nucleotide polymorphisms in *HNF1A, SLC13A1*, and *MBOAT1* were suggestively associated ($p < 0.05$) with the secondary outcome but were not significant after adjusting for multiple comparisons.

CONCLUSION: Our data suggest genetic variation may play a role in the development of leukopenia in patients receiving mycophenolate and cytomegalovirus antiviral drugs after heart transplantation. Following replication, pharmacogenetic markers, such as *HNF1A* rs1169288, could help identify patients

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at higher risk of drug-induced leukopenia, allowing for more personalized immunosuppressant therapy and cytomegalovirus prophylaxis following heart transplantation.
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Leukopenia is a major cause of morbidity following heart transplantation, occurring in approximately 30% of patients.[1,2] In the heart transplant setting, leukopenia is often caused by administration of mycophenolate and cytomegalovirus (CMV) antiviral drugs (i.e., ganciclovir and valganciclovir). The occurrence of leukopenia often results in a dose reduction or temporary discontinuation of one or both of these medications.[3] When the mycophenolate dose is reduced or held, it places patients at higher risk of cardiac allograft rejection, whereas lowering the dose or discontinuing CMV antiviral drugs increases the risk of CMV infection and associated adverse outcomes.[4,5] Variability in the pharmacokinetics and pharmacodynamics of these medications may contribute to the development of leukopenia in heart transplant recipients.

Mycophenolate, available as mycophenolate mofetil and mycophenolate sodium, is rapidly converted in the intestine and blood to its active metabolite, mycophenolic acid (MPA).[6] MPA is further metabolized into 7-O-MPA-glucuronide (MPAG) and an acyl glucuronide (Ac-MPAG) by UDP glucuronosyltransferases (UGTs) in the liver, gastrointestinal tract, and kidneys.[7] MPAG is excreted into the bile and deconjugated to MPA before undergoing enterohepatic recirculation, which contributes to approximately 40% of the MPA area under the plasma concentration-time curve.[8] MPA inhibits inosine 5'-monophosphate dehydrogenase (IMPDH), which is the rate limiting step in the de novo synthesis of guanosine nucleotides, a pathway required for T-cell proliferation.[9] Previous studies suggest that increased MPA exposure is associated with the development of adverse effects, including leukopenia, after heart transplantation.[10,11]

Valganciclovir is a prodrug that is converted into ganciclovir by esterases in the liver and intestine.[12] Although ganciclovir does not undergo extensive metabolism, it is a substrate for multiple transporters, including organic cation transporter 1 (OCT1) and organic anion transporter 2 (OAT2).[13,14] Ganciclovir is a potent inhibitor of viral DNA polymerase in CMV infected cells and non-specifically inhibits cellular DNA synthesis in uninfected cells.[15] Previous studies indicate that increased systemic exposure to ganciclovir is associated with neutropenia post-transplant.[16,17]

The field of pharmacogenetics seeks to understand how single nucleotide polymorphisms (SNPs) affect drug disposition, response, and toxicity.[18] Identifying genetic variants that influence the pharmacokinetic and pharmacodynamic properties of mycophenolate and CMV antiviral drugs could help clinicians better assess the risk of drug-induced leukopenia in heart transplant recipients. However, previous studies assessing mycophenolate or valganciclovir-induced leukopenia were mostly narrow in scope, analyzing only a few SNPs in select genes involved in the clinical pharmacology of these medications.[19-24] Given the complexity of drug-induced leukopenia and the limited number of genetic variants assessed to date, a larger panel of SNPs in additional genes may help identify patients at increased risk for this adverse effect. Additionally, previous studies have generally applied a narrow definition of drug-induced leukopenia post-transplant, focusing primarily on either mycophenolate- or valganciclovir-induced leukopenia. As mycophenolate and CMV antiviral drugs are routinely administered together and both reduce white blood cell counts, a broader definition of drug-induced leukopenia, that incorporates both medications, is more clinically relevant in the heart transplant population. Thus, we utilized a candidate gene approach to comprehensively evaluate the association between SNPs in mycophenolate and CMV antiviral drug pharmacokinetic and pharmacodynamic genes and mycophenolate and/or CMV antiviral drug-induced leukopenia in adult heart transplant recipients.

Methods

Study population

Patients recruited from a previous pharmacogenetic study (ClinicalTrials.gov, NCT01686191), hereafter referred to as the parent study, were considered for inclusion in this retrospective analysis. The parent study enrolled 253 adult heart transplant recipients followed at the University of Colorado Advanced Heart Failure and Transplant Program who were transplanted between March 1985 and August 2016. Participants were included in the parent study if they received a heart-only transplant, were at least 18 years old at the time of transplant, and were treated with a calcineurin inhibitor post-transplant. Patients were excluded from the parent study if they underwent multi-organ transplant or were unwilling to provide written informed consent. Patients were recruited at routine post-transplant clinic visits and a mouthwash or blood sample was collected for genetic analysis. Patients' medical records were retrospectively reviewed to collect demographic and clinical data at the following time points: pre-transplant, transplant discharge, 6 months post-transplant, and annually from one-year to five-years post-transplant.

The retrospective analysis described hereafter used parent study clinical data obtained from pre-transplant to one-year post-transplant. Participants were included in this analysis if they were ≥18 years old at the time of transplant, received a heart-only transplant at the University of Colorado, were at least one-year post-transplant, and received mycophenolate and a CMV antiviral drug at any point during the first six months following their primary heart transplant. Patients were excluded if they received a multi-organ transplant, did not receive mycophenolate and/or a CMV antiviral drug in the first six months post-transplant, were transplanted outside of the University of Colorado, or if they did not provide consent for their samples and clinical data to be used for future research. The parent study and this retrospective analysis were approved by the Colorado Multiple Institutional Review Board.

Immunosuppression and biopsy grading

All patients received triple-immunosuppressive therapy in the form of corticosteroids, a calcineurin inhibitor, and an antiproliferative agent. The immunosuppressive regimen in the 1990's consisted of corticosteroids, cyclosporine, and azathioprine. Mycophenolate replaced azathioprine as the primary antiproliferative agent in 2000 and tacrolimus became the preferred calcineurin inhibitor in 2010. Induction therapy with thymoglobulin was used on a case-by-case basis for transplant recipients with renal dysfunction, panel reactive antibody (PRA) >15%, and/or a positive crossmatch. Biopsies were graded according to International Society for Heart and Lung Transplantation (ISHLT) standards. Biopsies graded prior to 2004 were categorized using the ISHLT 1990 grading scale, while biopsies thereafter were assessed using the ISHLT 2004 revised classification system.[25,26]

Outcomes

The primary outcome was the occurrence of mycophenolate and/or CMV antiviral drug-induced leukopenia (yes/no) at any point in the first six months following heart transplantation. Leukopenia was defined as a white blood cell count $<3.0 \times 10^9$/L within 14 days of the administration of mycophenolate and/or CMV antiviral drugs (i.e., ganciclovir or valganciclovir) that resulted in a decrease in dose or temporary discontinuation of either medication.[27,28] Leukopenia was not considered to be mycophenolate and/or CMV antiviral drug-induced if it occurred within 14 days of grade 2R cellular rejection or higher according to the 2004 ISHLT classification system, infection that required hospital admission, or administration of other potent myelotoxic medications (i.e., thymoglobulin, cyclophosphamide, or methotrexate).[26] The secondary outcome was the occurrence of mycophenolate and/or CMV antiviral drug-induced leukopenia (yes/no), as defined above, in the first 12 months post-transplant.

Genotyping, imputation, and candidate SNP selection

Genotyping, quality control, and imputation methods are described in the Supplementary Data. SNPs for this study were selected in three steps. First, following an extensive literature search, we selected variants previously associated with common mycophenolate and/or CMV antiviral drug adverse effects (i.e., leukopenia, neutropenia, anemia, and gastrointestinal symptoms). Second, we included SNPs reported to impact mycophenolate or CMV antiviral drug pharmacokinetics and pharmacodynamics. Lastly, to supplement the SNPs identified in the first two steps, we chose select variants located within genes involved in the clinical pharmacology of mycophenolate and/or CMV antiviral drugs based on functional or clinical significance, annotations in pharmacogenetic databases, and/or HapMap tagging variants. In total, 81 SNPs, including 37 SNPs in 21 pharmacokinetic genes and 44 SNPs in 23 pharmacodynamic genes, were selected for analysis (Supplementary Table 1).

Statistics

Data are presented as mean ± standard deviation (SD) unless otherwise indicated. Normality was assessed using the Shapiro-Wilk test, with non-normal data log transformed prior to analysis and relevant statistics back-transformed for presentation. Relationships between demographic and clinical covariates (i.e., age at transplant, sex, reason for transplant (ischemic cardiomyopathy vs other), pre-transplant weight, pre-transplant white blood cell count, receiving >2000 mg/day of mycophenolate mofetil or >1440 mg/day of mycophenolate sodium at any time in the first six months post-transplant (yes/no), primary calcineurin inhibitor (tacrolimus vs cyclosporine), primary maintenance CMV antiviral drug (valganciclovir vs ganciclovir), days post-transplant on date of mycophenolate initiation, azathioprine use prior to mycophenolate initiation (yes/no), recipient CMV status (positive/negative), and thymoglobulin induction (yes/no)] and the primary and secondary outcomes were evaluated using Pearson's correlation, with significant ($p < 0.05$) clinical covariates included in the respective multivariate models. The top six principal components (PCs) were included in all multivariate models to account for potential population stratification. All analyses were performed as wild type homozygotes versus variant carriers under a dominant model.

For the primary and secondary outcomes, univariate analyses were performed to assess the relationship between each SNP and mycophenolate and/or CMV antiviral drug-induced leukopenia (yes/no) using Fisher's exact tests. Suggestive SNPs in the univariate approach ($p < 0.05$) were analyzed using logistic regression in multivariate models, adjusting for the relevant demographic and clinical covariates for each outcome and the top six PCs. Multivariate logistic regression models adjusting for pertinent demographic and clinical covariates were also performed in non-Hispanic Americans of European ancestry to assess whether suggestive SNPs from the entire cohort remained associated with each outcome in the largest subpopulation of participants. A false discovery rate (FDR) threshold of 20% was used to account for multiple testing.[27] Statistical analyses were performed using R version 3.5.2 (R Foundation for Statistical Computing, Vienna Austria).

Results

Patient characteristics

Demographic and clinical characteristics of the 148 participants that met the inclusion criteria for this analysis are listed in Table 1. The cohort consisted primarily of men (76.4%) and individuals of European ancestry (77.7%), with the mean ± SD age at transplant of 49 ± 13 years. Patients were transplanted between October 1996 and August 2016. All patients received mycophenolate mofetil, although four patients (2.7%) were transitioned to mycophenolate sodium due to gastrointestinal adverse effects. Approximately one-fifth (22.2%) of participants were initiated on azathioprine and switched to mycophenolate [median (range) days on azathioprine = 7 (1-131)]. Valganciclovir (56.1%) was the primary CMV antiviral drug prescribed in this cohort. All patients were prescribed a calcineurin inhibitor, primarily tacrolimus (56.8%), in the first six months post-transplant.

Mycophenolate and CMV antiviral drug dose adjustments

In total, 103 patients (69.6%) had either the mycophenolate and/or CMV antiviral drug dose reduced or temporarily discontinued in the first six months post-transplant (Table 2). Doses were reduced or temporarily discontinued 149 times

Table 1 Patient Demographic and Clinical Characteristics

Characteristic	Entire cohort (n = 148)	No drug-induced leukopenia (n = 118)	Drug-induced leukopenia[a] (n = 30)	p-value[b]
Men	113 (76.4%)	91 (77.1%)	22 (73.3%)	0.638
Age at transplant (years)	49 ± 13	49 ± 12	48 ± 16	0.682
Race				0.133
European American	115 (77.7%)	95 (80.5%)	20 (66.7%)	
African American	16 (10.8%)	13 (11.0%)	3 (10.0%)	
Asian	6 (4.1%)	3 (2.5%)	3 (10.0%)	
Other[c]	11 (7.4%)	7 (5.9%)	4 (13.3%)	
Ethnicity				0.484
Non-Hispanic	134 (90.5%)	108 (91.5%)	26 (86.7%)	
Hispanic	14 (9.5%)	10 (8.5%)	4 (13.3%)	
Reason for transplant				0.011
Nonischemic CM	75 (50.7%)	58 (49.2%)	17 (56.7%)	
Ischemic CM	41 (27.7%)	38 (32.2%)	3 (10.0%)	
Valvular CM	7 (4.7%)	5 (4.2%)	2 (6.7%)	
Mixed etiology	7 (4.7%)	4 (3.4%)	3 (10.0%)	
Congenital CM	7 (4.7%)	3 (2.5%)	4 (13.3%)	
Other	11 (7.4%)	10 (8.5%)	1 (3.3%)	
Primary CMV antiviral drug[d]				0.221
Valganciclovir	83 (56.1%)	63 (53.4%)	20 (66.7%)	
Ganciclovir	65 (43.9%)	55 (46.6%)	10 (33.3%)	
Recipient CMV status				0.688
Positive	79 (53.4%)	64 (54.2%)	15 (50.0%)	
Negative	69 (46.6%)	54 (45.8%)	15 (50.0%)	
Median days post-transplant to initiation of mycophenolate (range)[e]	0.5 (0-131)	1 (0-131)	0 (0-38)	0.294
MMF >2000 mg/day or MPS >1440 mg/day at any point in the first six months post-transplant	99 (66.9%)	78 (66.1%)	21 (70.0%)	0.829
Primary calcineurin inhibitor[f]				0.104
Tacrolimus	84 (56.8%)	71 (60.2%)	13 (43.3%)	
Cyclosporine	64 (43.2%)	47 (39.8%)	17 (56.7%)	
Thymoglobulin induction	25 (16.9%)	18 (15.3%)	7 (23.3%)	0.287
Pre-transplant weight (kg)	78.7 ± 16.9	80.7 ± 17.3	71.2 ± 13.4	0.006
Pre-transplant white blood cell count (x10^9/L)	8.35 ± 3.20	8.49 ± 3.26	7.81 ± 2.96	0.203

CM,cardiomyopathy; CMV, cytomegalovirus; MMF, mycophenolate mofetil; MPS, mycophenolate sodium.
Data are presented as mean ± standard deviation or N (%) unless otherwise specified.
[a]As defined by a white blood cell count <3.0 × 10^9/L while receiving mycophenolate and/or CMV antiviral drugs in the first six months;
[b]Groups were compared using t-tests or Fisher's exact tests, where appropriate;
[c]Includes n = 10 Hispanics and n = 1 American Indian;
[d]CMV antiviral drug prescribed for the most total days in the first six months post-transplant
[e]n = 33 patients initiated on azathioprine and switched to mycophenolate;
[f]Calcineurin inhibitor prescribed for the most total days in the first six months post-transplant.

in 56.8% of patients (mycophenolate) and 99 times in 45.9% of patients (CMV antiviral drugs) during this time frame. The primary reason for mycophenolate and CMV antiviral drug dose adjustments was a decline in white blood cell count (n = 113 and n = 43 dose adjustments, respectively).

Mycophenolate and/or CMV antiviral drug-induced leukopenia in the first six months post-transplant

Mycophenolate and/or CMV antiviral drug-induced leukopenia occurred in 30 participants (20.3%) during the first six months post-transplant. All patients were prescribed mycophenolate and a CMV antiviral drug on the date of leukopenia. Mycophenolate alone (n = 15) was the primary drug reduced or temporarily discontinued as a result of leukopenia, followed by both mycophenolate and CMV antiviral drugs (n = 12), and CMV antiviral drugs alone (n = 3). Demographic and clinical variables that were correlated with mycophenolate and/or CMV antiviral drug-induced leukopenia and included in the multivariate model were lower pre-transplant weight (p = 0.006) and reason for transplant other than ischemic cardiomyopathy (p = 0.015). Receipt of > 2000 mg/day of mycophenolate mofetil or > 1440 mg/day of mycophenolate sodium, azathioprine use

Table 2 Indications for 149 Mycophenolate and 99 CMV Antiviral Drug Dose Adjustments in the First Six Months Post-Transplant

Mycophenolate dose adjustment (n = 84 patients)[a]	
Reason for dose adjustment	Number of dose adjustments (% of dose adjustments)
Decline in white blood cell count	113 (75.8%)
Gastrointestinal symptoms	23 (15.4%)
Infection	5 (3.4%)
Unintentional dose reduction by patient	3 (2.0%)
Other[b]	5 (3.4%)
CMV antiviral drug dose adjustment (n = 68 patients)[a]	
Reason for dose adjustment	Number of dose adjustments (% of dose adjustments)
Decline in white blood cell count	43 (43.4%)
Renal dysfunction	38 (38.4%)
Unknown	9 (9.1%)
Unintentional dose reduction by patient	3 (3.0%)
Other[c]	6 (6.1%)

[a]Multiple dose adjustments could occur in each patient;
[b]Includes skin cancer (n = 2), concern for potential infection (n = 1), thrombocytopenia (n = 1), and unknown (n = 1);
[c]Includes thrombocytopenia (n = 1), nausea (n = 1), elevated liver function tests (n = 1), tremor (n = 1), ulcer (n = 1), and gastrointestinal bleed (n = 1).

prior to mycophenolate initiation, and the primary maintenance CMV antiviral drug prescribed, i.e., valganciclovir vs ganciclovir, were not significantly correlated with leukopenia.

The nonsynonymous SNP, rs1169288 A>C (p.I27L), in the hepatocyte nuclear factor 1 homeobox A (*HNF1A*) gene was significantly associated with mycophenolate and/or CMV antiviral drug-induced leukopenia in the first six months post-transplant in the univariate analysis (p = 0.002; Table 3). This SNP remained significant after accounting for an FDR of 20%. After adjusting for pre-transplant weight, reason for transplant, and the top six PCs, *HNF1A* rs1169288 variant C allele carriers had higher odds of developing mycophenolate and/or CMV antiviral drug-induced leukopenia than A/A homozygotes [odds ratio (OR) 6.19; 95% CI 1.97-19.43; p = 0.002]. The primary outcome occurred in 30% of variant C allele carriers compared to 10% of patients with the A/A genotype. The frequency of the *HNF1A* rs1169288 variant C allele was 32%. Similar findings were observed when the analysis was limited to non-Hispanic Americans of European ancestry only (OR 5.42; 95% CI 1.54-19.11; p = 0.009). In the whole cohort, higher odds of drug-induced leukopenia were observed in *HNF1A* rs1169288 heterozygotes (n = 58; OR 6.84; 95% CI 2.11-22.12; p = 0.001) and C/C homozygotes (n = 18; OR 4.27; 95% CI 0.90-20.34; p = 0.069) when compared to A/A patients (n = 72). No other SNPs were significantly associated with the primary outcome in the entire study cohort.

Mycophenolate and/or CMV antiviral drug-induced leukopenia in the first 12 months post-transplant

Mycophenolate and/or CMV antiviral drug-induced leukopenia developed in 46 patients (31.1%) in the first 12 months post-transplant. Mycophenolate alone (n = 29) was the most common medication reduced or temporarily discontinued as a result of leukopenia, followed by both mycophenolate and CMV antiviral drugs (n = 12), and CMV antiviral drugs alone (n = 5). Demographic and clinical characteristics that were correlated with the secondary outcome included lower pre-transplant weight (p = 0.002), lower pre-transplant white blood cell count (p < 0.001), and reason for transplant other than ischemic cardiomyopathy (p = 0.023).

Six SNPs were suggestively associated (p < 0.05) with mycophenolate and/or CMV antiviral drug-induced leukopenia in the first 12 months post-transplant in univariate analyses (Table 3); however, none of these SNPs remained significant after accounting for multiple comparisons. As an exploratory analysis, these six SNPs were evaluated in single-SNP multivariate models, adjusting for pre-transplant weight, pre-transplant white blood cell count, reason for transplant, and the top six PCs. Four SNPs [*HNF1A* rs1169288 A>C, solute carrier family 13 member 1 (*SLC13A1*) rs2140516 T>C, *HNF1A* rs2393791 T>C, and membrane bound O-acyltransferase domain containing 1 (*MBOAT1*) rs13218743 G>A] remained suggestively associated (p < 0.05) with the secondary outcome, with variant carriers having higher odds of mycophenolate and/or CMV antiviral drug-induced leukopenia when compared to wild-type homozygotes (Table 3). When restricting the analysis to non-Hispanic Americans of European ancestry, only *HNF1A* rs1169288 and *SLC13A1* rs2140516 were suggestively associated with the outcome in single-SNP multivariate models (Supplementary Table 2).

Discussion

This is the first pharmacogenetic study to comprehensively assess the relationship between SNPs in a broad range of mycophenolate and CMV antiviral drug pharmacokinetic and pharmacodynamic genes and drug-induced leukopenia

Table 3 Associations Between Single Nucleotide Polymorphisms and Mycophenolate and/or CMV Antiviral Drug-Induced Leukopenia[a]

Drug-induced leukopenia in the first six months post-transplant

Gene name	rs number	Chromosome	Alleles[b]	MAF	Reference group	Unadjusted odds ratio (95% CI)	Unadjusted *p*-value	Adjusted[c] odds ratio (95% CI)	Adjusted *p*-value
HNF1A	rs1169288	12	A>C	32%	AA	4.03 (1.61-10.12)	0.002	6.19 (1.97-19.43)	0.002

Drug-induced leukopenia in the first 12 months post-transplant

Gene name	rs number	Chromosome	Alleles[b]	MAF	Reference group	Unadjusted odds ratio (95% CI)	Unadjusted *p*-value	Adjusted[d] odds ratio (95% CI)	Adjusted *p*-value
HNF1A[e]	rs1169288	12	A>C	32%	AA	2.28 (1.11-4.70)	0.033	4.14 (1.62-10.55)	0.003
SLC13A1	rs2140516	7	T>C	32%	TT	2.19 (1.07-4.51)	0.034	3.08 (1.29-7.35)	0.011
HNF1A[e]	rs2393791	12	T>C	39%	TT	2.32 (1.06-5.08)	0.042	2.77 (1.07-7.17)	0.035
MBOAT1	rs13218743	6	G>A	6%	GG	3.77 (1.33-10.66)	0.013	3.77 (1.08-13.18)	0.038
UGT1A9	rs6731242	2	T>G	19%	TT	0.43 (0.19-0.96)	0.041	0.44 (0.18-1.10)	0.081
IMPDH1	rs11761662	7	A>G	9%	AA	0.23 (0.06-0.80)	0.012	0.31 (0.08-1.18)	0.087

HNF1A, hepatocyte nuclear factor 1 homeobox A; *IMPDH1*, inosine monophosphate dehydrogenase 1; MAF, minor allele frequency; *MBOAT1*, membrane bound O-acyltransferase domain containing 1; *SLC13A1*, solute carrier family 13 member 1; *UGT1A9*, UDP glucuronosyltransferase family 1 member A9.

[a]Defined as a white blood cell count $<3.0 \times 10^9$/L while receiving mycophenolate and/or cytomegalovirus antiviral drugs;

[b]Major>minor alleles, respectively;

[c]Adjusted for pre-transplant weight, reason for transplant (ischemic cardiomyopathy vs other), and the top six principal components;

[d]Adjusted for pre-transplant white blood cell count, pre-transplant weight, reason for transplant (ischemic cardiomyopathy vs other), and the top six principal components;

[e]*HNF1A* SNVs are correlated ($r^2 = 0.69$).

in adult heart transplant recipients. In addition to lower pre-transplant weight and reason for transplant other than ischemic cardiomyopathy, the *HNF1A* rs1169288 variant C allele was associated with a higher odds of mycophenolate and/or CMV antiviral drug-induced leukopenia in the first six months post-transplant after accounting for an FDR of 20%. Additionally, SNPs in *HNF1A*, the sulfate transporter *SLC13A1*, and acyltransferase *MBOAT1* were suggestively associated with drug-induced leukopenia in the first 12 months post-transplant.

HNF1A encodes a transcription factor that increases the expression of numerous genes in the liver, kidneys, and intestines, including the *UGT1As, UGT2B*s, *SLC22A7*, and *SLC22A1*.[29-31] Multiple UGT1A enzymes and UGT2B7 are involved in the metabolism of MPA, while OAT2 and OCT1, encoded by *SLC22A7* and *SLC22A1*, respectively, transport ganciclovir in the kidneys.[7,13,14] Functionally, the substitution of isoleucine for leucine in rs1169288 results in reduced transcriptional activity of *HNF1A*, possibly due to a change in the structure of the protein.[32,33] Specifically, the *HNF1A* rs1169288 variant C allele results in a change from a loop to an alpha helix at that site.[33] As HNF1A is biologically active as a dimer, and rs1169288 is located in the dimerization domain of HNF1A, a leucine at this location may reduce dimer formation, thereby inhibiting HNF1A function.[34] This decline in function may decrease expression of genes that play a role in the metabolism of MPA (e.g., *UGT1A*s, *UGT2B7*) and transport of ganciclovir (e.g., *SLC22A7, SLC22A1*).[32] Thus, *HNF1A* rs1169288 variant C allele carriers may have decreased ability to metabolize and excrete MPA and/or ganciclovir from the body, leading to increased blood levels, which could explain the higher odds of drug-induced leukopenia in this group.

To date, two studies have assessed the relationship between *HNF1A* rs1169288 and mycophenolate adverse effects following transplantation. Jacobson et al. reported no association between rs1169288 and mycophenolate-induced leukopenia in kidney transplant patients.[27] Discordant genotypes between kidney donor and recipient may explain, in part, the discrepant results, as HNF1A modulates the expression of multiple MPA transporters in the kidney (e.g., MRP2, P-glycoprotein).[31] Since the heart is not significantly involved in the excretion of MPA, differences in donor and recipient *HNF1A* genotypes are not likely to impact this process in our population. Additionally, in another cohort of kidney transplant recipients receiving mycophenolate, *HNF1A* rs1169288 variant C allele carriers had less gastrointestinal adverse effects compared to A/A homozygotes.[35] The authors hypothesized that better gastrointestinal tolerability in variant C allele carriers was due to decreased production of the reactive MPA metabolite, Ac-MPAG. Lower levels of Ac-MPAG may be a result of decreased MPA metabolism, which correspondingly would lead to an increase in systemic MPA exposure. In light of the conflicting studies in the literature, validation of our findings in a larger heart transplant cohort, such as the International Genetics and Translational Research in Transplantation Network (iGeneTRAIN) or Swiss Transplant Cohort Study, is needed to confirm the role of this variant in mycophenolate and/or a CMV antiviral drug-induced leukopenia.[36,37]

Previous pharmacogenetic studies with other drugs support the hypothesis that genetic variation in *HNF1A*

contributes to pharmacokinetic variability and concentration-dependent clinical outcomes. For example, in a study of colorectal patients receiving irinotecan, Labriet et al. found carriers of the *HNF1A* rs2244608 variant G allele, which is in strong linkage disequilibrium with the rs1169288 variant C allele in patients of European ancestry (r^2 = 0.98, 1000 Genomes CEU Phase 3 population), had higher area under the plasma concentration-time curve of irinotecan's active metabolite, SN-38, compared to wild-type homozygotes.[38] Improved progression free survival was also reported in rs2244608 variant G carriers, which the authors attributed to increased SN-38 exposure. SN-38 and MPA are substrates for many of the same metabolizing enzymes and transporters (e.g., UGT1As, MRP2),[39,40] and HNF1A regulates the expression of the genes that encode these proteins (e.g., *UGTA1s, ABCC2*).[29,41] Given the overlap in metabolism and excretion pathways of SN-38 and MPA, in addition to the linkage between *HNF1A* rs2244608 and rs1169288, it is possible that the associations with rs1169288 observed in our study are due, in part, to alterations in MPA pharmacokinetics.

If validated in other cohorts, *HNF1A* rs1169288 could serve as a potential pharmacogenetic marker for drug-induced leukopenia, allowing clinicians to personalize mycophenolate and CMV antiviral drug therapy post-transplant. For example, monitoring of MPA blood levels in patients with the rs1169288 variant C allele may be warranted, allowing for more precise tailoring of mycophenolate doses, thereby lowering the risk of leukopenia and associated comorbidities. Alternatively, CMV antiviral drug dosing in transplant recipients could be modified to reduce the likelihood of a decline in white blood cell count. For instance, rs1169288 variant C allele carriers that have a low (i.e., donor and recipient CMV seronegative; D-/R-) or intermediate (i.e., CMV D+/R+ and D-/R+) risk of CMV infection may be prescribed lower maintenance doses of valganciclovir or have this medication discontinued earlier post-transplant.[42] In this way, CMV antiviral drug therapy could be personalized to the individual heart transplant patient.

In addition to *HNF1A* rs1169288, the clinical factors of pre-transplant weight and reason for transplant other than ischemic cardiomyopathy were associated with leukopenia in the first six months post-transplant. Lower body weight has previously been associated with leukopenia in heart transplant patients receiving valganciclovir and lung transplant recipients prescribed mycophenolate and valganciclovir.[43,44] As mycophenolate and maintenance CMV antiviral medications are often initially prescribed at fixed doses independent of weight following heart transplantation, patients with lower weights may experience greater drug exposure, thereby increasing the risk of leukopenia. With respect to reason for transplant, this association is likely driven by racial differences between groups, as 85% (n=35) of patients with ischemic cardiomyopathy were of European ancestry and pre-transplant WBC counts were generally higher in patients of European descent in our cohort.

SNPs located in genes beyond *HNF1A* were also suggestively associated with the secondary outcome in our cohort. Variant carriers of the nonsynonymous, loss-of-function *SLC13A1* rs2140516 T>C (p.N174S) and the intronic *MBOAT1* rs13218743 G>A had higher odds of mycophenolate and/or CMV antiviral drug-induced leukopenia in the first 12 months after heart transplantation when compared to wild-type homozygotes. *SLC13A1* and *MBOAT1* encode for the Na^+ sulfate cotransporter 1 (NAS1) and MBOAT1 proteins, respectively. NAS1 is involved in sulfate reabsorption in the kidneys, whereas MBOAT1 is a lysophosphatidylserine acyltransferase expressed in neutrophils.[45,46] The *SLC13A1* rs2140516 variant C allele and *MBOAT1* rs13218743 variant A allele have previously been associated with a higher hazard of mycophenolate-related leukopenia in kidney transplant recipients.[27,28] Our results, in combination with these findings, suggest *SLC13A1* rs2140516 and *MBOAT1* rs13218743 may play a role in the occurrence of drug-induced leukopenia after transplantation. However, additional work is necessary to determine the role of *SLC13A1* rs2140516 and *MBOAT1* rs13218743 in mycophenolate and CMV antiviral drug clinical pharmacology and to identify the mechanisms by which these SNPs contribute to the development of mycophenolate and CMV antiviral drug-induced leukopenia in the setting of heart transplantation.

We were unable to replicate SNPs in genes (e.g., *ABCC2, IMPDH2*) previously associated with mycophenolate-induced leukopenia in heart and other transplant populations.[19,20,22-24,27] Many of these studies were performed in kidney transplant recipients, where non-genetic factors (e.g., allograft function, rejection) may contribute to the pharmacokinetics of mycophenolate and/or CMV antiviral drugs, as both medications are primarily excreted in the urine. Furthermore, varying definitions of leukopenia between studies may explain, in part, differences observed in the literature. For example, Ting et al. found an association between two variants (*ABCC2* rs17222723 T>A and *UGT1A1* rs10929303 C>T) and leukopenia in thoracic transplant recipients receiving mycophenolate; however, a white blood cell count $<4.0 \times 10^3/mm^3$ was used to define leukopenia in that study.[19] Additionally, Ohmann et al. reported *IMPDH2* rs11706052 variant G carriers had a higher risk of mycophenolate dose-holding due to bone marrow toxicity, defined as a white blood cell count $<2.0 \times 10^3/mm^3$ or absolute neutrophil count $<1.0 \times 10^3/mm^3$, in pediatric heart transplant patients.[20] We also included both mycophenolate and CMV antiviral drug dose reductions in our definition of leukopenia, whereas previous work focused almost exclusively on leukopenia that resulted in mycophenolate dose reductions.

Our study has limitations that deserve to be acknowledged. First, while our definition of mycophenolate and/or CMV antiviral drug-induced leukopenia is broader than previously published literature, it is still possible that we missed potential cases.[27,28] Second, despite excluding select medications known to cause leukopenia (e.g., thymoglobulin) from our outcome definition, it is possible that patients were taking other drugs that contributed to the development of leukopenia (e.g., sulfamethoxazole-trimethoprim, dapsone, valacyclovir). Third, we did not assess the

relationship between MPA or ganciclovir blood levels and mycophenolate and/or CMV antiviral drug-induced leukopenia in this study, as our institution does not routinely measure these levels in clinical practice. Fourth, SNPs were selected for this analysis primarily based on a prior association with mycophenolate and/or CMV antiviral drug pharmacokinetics and/or pharmacodynamics. As such, these variants likely did not provide complete coverage of the 21 pharmacokinetic and 23 pharmacodynamic genes investigated in this study. Fifth, in our exploratory analysis, some variants did not remain suggestively associated with the secondary outcome when the analysis was limited to non-Hispanic Americans of European ancestry, perhaps due to the decreased sample size or elements of population stratification. Lastly, our cohort was primarily of European ancestry and male. While our demographics are comparable to the racial and gender statistics of heart transplant recipients in the United States, our results may not be applicable to other heart transplant recipients (e.g., African Americans, women).[47]

In conclusion, our study is the first to report an association between *HNF1A* rs1169288 and mycophenolate and/or CMV antiviral drug-induced leukopenia in adult heart transplant recipients. We found *HNF1A* rs1169288 variant C carriers had higher odds of developing mycophenolate and/or CMV antiviral drug-induced leukopenia following heart transplant. Furthermore, variants in *HNF1A*, the sulfate cotransporter gene *SLC13A1,* and acyltransferase *MBOAT1* were suggestively associated with drug-induced leukopenia in the first 12 months post-transplant. If replicated, these SNPs might serve as novel pharmacogenetic markers of mycophenolate and/or CMV antiviral drug-induced leukopenia after heart transplantation. Ultimately, following replication of our findings, clinicians may be able to identify patients at higher risk for mycophenolate and/or CMV antiviral drug-induced leukopenia prior to initiation of these medications, allowing for more personalized and precise care in this patient population.

Disclosure statement

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Supplementary materials

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