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ORIGINAL ARTICLE

Pharmacogenomic associations in *ABCB1* and *CYP3A5* with acute kidney injury and chronic kidney disease after myeloablative hematopoietic cell transplantation

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Renal disease is a major complication in patients following myeloablative allogeneic hematopoietic cell transplantation (HCT). Post-HCT patients receive immunosuppressive regimens containing calcineurin inhibitor (CNIs), cyclosporine or tacrolimus, for graft-versus-host disease prophylaxis. In this retrospective trial, we investigated pharmacogenomic associations in the multidrug resistance (*ABCB1*) and cytochrome *P*450 3A5 (*CYP3A5*) genes and acute kidney injury (AKI) and chronic kidney disease (CKD) in a cohort of 121 patients. *ABCB1* and *CYP3A5* are responsible for the renal disposition of CNIs, which are known to be nephrotoxic. AKI was defined as doubling of baseline serum creatinine during the first 100 days post-HCT, and CKD as at least one glomerular filtration rate $<60\,ml/min/m^2$ between 6 and 18 months post-HCT. Patients were genotyped for *CYP3A5*1>*3* and *ABCB1* single nucleotide polymorphisms (SNPs) (1199G>A, 1236C>T, 2677G>T/A and 3435C>T). Odds ratios were calculated using logistic regression. Haplotype estimation and univariate association analyses were performed because of strong *ABCB1* linkage disequilibrium (LD). AKI occurred in 48 of 121 patients (39.7%) and CKD in 16 of 66 patients (24.2%). No pharmacogenomic associations were found between *ABCB1* and *CYP3A5* SNPs and the incidences of AKI or CKD. The degree of LD(r^2) between *ABCB1* SNPs was estimated as follows: 2677G>T/3435C>T (0.44), 1236C>T/3435C>T (0.42) and 1236C>T/2677G>T (0.72). *ABCB1* 1199G>A showed no LD to other SNPs (<0.05). No associations were found between the most common *ABCB1* haplotypes and AKI or CKD. Since no significant pharmacogenomic associations were observed, tailoring CNIs dosing based on these genotypes is unlikely to lower significantly the risk of renal injury following myeloablative HCT.
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Introduction

Allogeneic hematopoietic cell transplantation (HCT) following myeloablative conditioning regimens is a mainstay in the treatment of hematologic

malignancies. However, patient survival is limited by regimen-related toxicities.[1] Renal disease is a major complication post-HCT with acute kidney injury (AKI) and chronic kidney disease (CKD) significantly contributing to non-relapse mortality in these patients.[2,3] The genesis of renal disease after myeloablative allogeneic HCT is multifactorial and the identification of risk factors for both AKI and CKD is critical to allow for preventive measures. We have identified previously several risk factors including sinusoidal obstruction syndrome (SOS), amphotericin use and baseline serum creatinine.[2]

Allogeneic HCT recipients routinely receive immunosuppressive regimens containing calcineurin inhibitors (CNIs), cyclosporine or tacrolimus, to prevent graft-versus-host disease (GVHD). Cyclosporine trough concentrations >400 ng/ml are associated with a higher incidence of renal injury.[4] It is important to note that cyclosporine-induced renal injury can occur despite low or normal concentrations of cyclosporine and may be complicated by patients' genetic susceptibility, concurrent use of other nephrotoxic drugs or disease-related factors.

We recently reported that plasma cyclosporine levels were not found to be associated with AKI in a cohort of patients undergoing myeloablative HCT.[2] Therefore, we hypothesized that the intracellular concentrations of CNIs within kidney tubular cells may be more predictive of the incidence of renal injury in these patients. CNI exposure in the kidney tubular cells is primarily mediated by the drug-metabolizing enzyme cytochrome *P*450 3A5 (CYP3A5) and the drug transporter P-glycoprotein (P-gp), which is encoded by the mutidrug resistance gene (*ABCB1*, also known as *MDR1*).[5–7] Genetic variability in the *CYP3A5* and *ABCB1* genes may alter the metabolism and transport of CNIs in renal tubular cells and may be an important risk factor for AKI and CKD post-HCT.[8,9] Our goal was to evaluate the pharmacogenomic associations between the *CYP3A5* and *ABCB1* genotypes and the risk of CNI-induced renal injury in a retrospective study in our cohort of HCT patients. In *CYP3A5*, we focused on the *CYP3A5*3* splice variant that leads to a truncated protein and loss of expression.[8] In *ABCB1*, we focused on four important single nucleotide polymorphisms (SNPs) that have been shown to be associated with changes in expression and activity of P-gp.[9] We investigated two synonymous *ABCB1* SNPs that do not change an amino acid in the protein (1236C>T and 3435C>T). We also evaluated two non-synonymous SNPs; the tri-allelic non-synonymous SNP at nucleotide position 2677, which results in a 2677G>T (Ala893Ser) or a 2677G>A (Ala893Thr) transition, and the 1199G>A SNP (Ser400Asn) that we have shown to cause a functional alteration in P-gp activity.[10,11] Because significant linkage disequilibrium (LD) exists in *ABCB1*, we also evaluated the associated risk of *ABCB1* haplotypes.[12–14] Understanding the influence of genetic variability in the intrarenal concentrations of CNIs in post-HCT patients may allow for the *a priori* identification of patients at high risk of CNI-induced renal dysfunction who would be candidates for non-CNI containing regimens.

Results

Patient characteristics

The demographic characteristics of the study cohort have been described previously.[1] The demographics of the 121 patients for whom DNA was available did not differ significantly from those of the cohort as a whole.

Genotype frequencies

Frequencies of *CYP3A5* and *ABCB1* genotypes observed in this cohort are displayed in Table 1. All genotypes were in Hardy–Weinberg equilibrium. The *ABCB1* 1199G>A SNP was observed with low frequency, but it was comparable to what has been observed in the literature.[13,15,16] We observed all three alleles at the 2677 locus; however, the 2677A allele was found with low prevalence and was only observed in heterozygous patients 2677TA or 2677GA.

SNP associations with acute kidney injury

Since trough blood concentrations of CNIs were targeted according to standard clinical guidelines, we decided to focus on the clinical end point of renal injury to evaluate pharmacogenomic associations. AKI was observed in 48 of

Table 1 ***CYP3A5* and *ABCB1* genotype frequencies (*n* = 121)**

SNP	*Genotype*	*No. of patients*	*Expected frequency (%)*[a]	*Observed frequency (%)*
*CYP3A5*1/*3*				
	*1/*1	4	1.3	3.3
	*1/*3	19	19.8	15.7
	*3/*3	98	78.9	81.0
ABCB1 1199G>A				
	GG	110	89.5	90.9
	GA	9	10.2	7.4
	AA	2	0.3	1.7
ABCB1 1236C>T				
	CC	47	37.9	38.8
	CT	55	47.3	45.5
	TT	19	14.8	15.7
ABCB1 2677G>T/A				
	GG	51	37.4	42.2
	GT	43	44.0	35.5
	TT	20	12.9	16.5
	TA	4	2.1	3.3
	GA	3	3.5	2.5
	AA	0	0.1	0
ABCB1 3435C>T				
	CC	37	30.7	30.6
	CT	60	49.4	49.6
	TT	24	19.9	19.8

[a]Estimated based on the Hardy–Weinberg equilibrium.
Abbreviations: *ABCB1*, multidrug resistance gene; *CYP3A5*, cytochrome *P*450 3A5 gene; SNP, single nucleotide polymorphism.

121 patients (39.7%). The median day of AKI incidence was day 33 post-HCT (range: day 1–97). We analyzed the associations between the development of AKI and the SNPs in *CYP3A5* and *ABCB1* in this study population. Table 2 shows the univariate odds ratios (OR) for the risk of AKI for the single genetic predictors. None of the genotypes was statistically associated with AKI in this population. Figure 1 shows the cumulative frequency of AKI by genotype in the first 100 days post-HCT for the 1236C>T, 2677G>T and 3435C>T (patients with 2677A allele were omitted since the number of patients was low). While the OR do not indicate a significant association between *ABCB1* genotypes and incidence of AKI, there appears to be non-significant trends toward increased risk of AKI in patients homozygous for

Table 2 Pharmacogenomics associations with AKI ($n = 121$ patients)

SNP	*No. of patients with AKI (% cumulative incidence)*[a]	*OR (95% CI)*	P-*value*
*CYP3A5*1/*3*			
*1/*1	1 (25)		
*1/*3	9 (47)		
*3/*3	38 (39)		
*3/*3 vs (*1/*3 and *1/*1)		0.8 (0.3–2.1)	0.68
ABCB1 1199G>A			
GG	46 (42)		
GA	2 (22)		
AA	0		
GG vs (AA and GA)		3.2 (0.7–15.7)	0.14
ABCB1 1236C>T			
CC	17 (36)		
CT	21 (38)		
TT	10 (53)		
TT vs (CC and CT)		1.9 (0.7–5.0)	0.21
ABCB1 2677G>T/A			
GG	19 (37)		
GT	17 (40)		
TT	10 (50)		
TA	1 (25)		
GA	1 (33)		
TT vs (GG and GT)		1.6 (0.6–4.3)	0.34
TT vs (TA and GA)		2.5 (0.4–16.0)	0.33
ABCB1 3435C>T			
CC	14 (38)		
CT	24 (40)		
TT	10 (42)		
TT vs (CC and CT)		1.1 (0.4–2.8)	0.82

[a]Cumulative incidence estimated as the probability of AKI over the first 100 days post-HCT within each genotype.
Abbreviations: *ABCB1*, multidrug resistance gene; AKI, acute kidney injury; CI, confidence interval; *CYP3A5*, cytochrome *P*450 3A5; HCT, hematopoietic cell transplantation; OR, odds ratio; SNP, single nucleotide polymorphism.

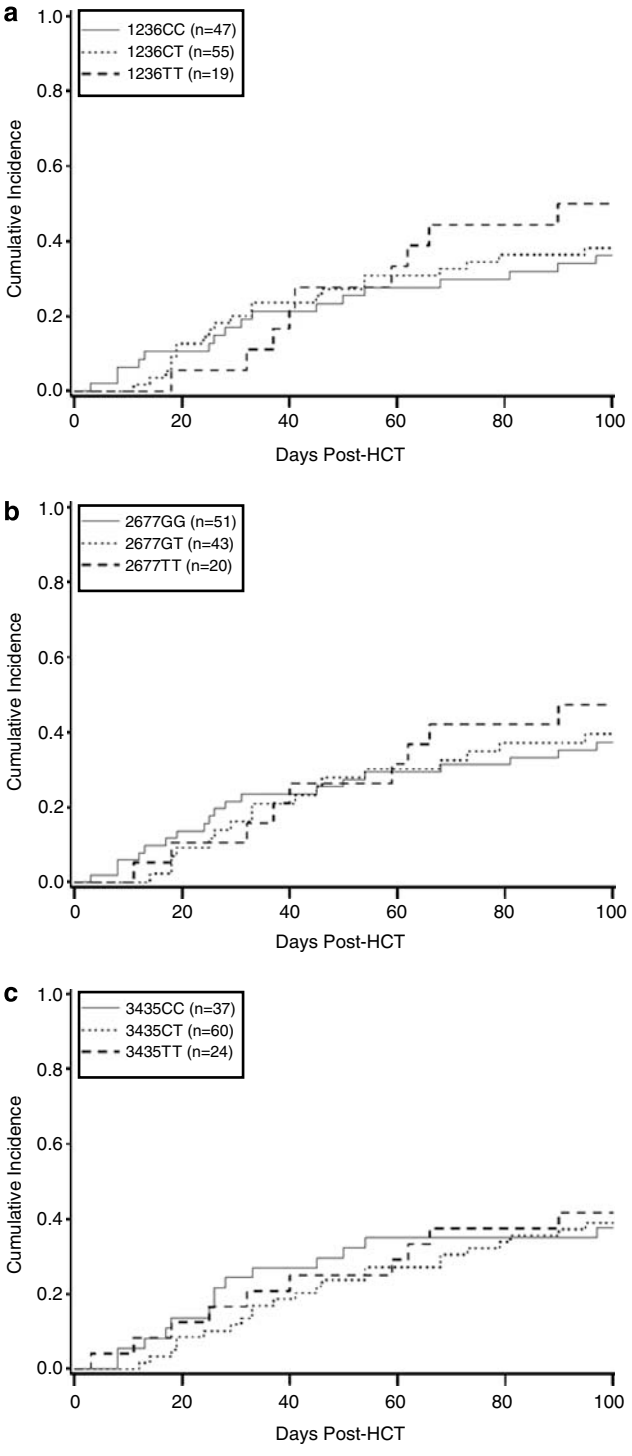

Figure 1 Association of *ABCB1* genotypes with the cumulative incidence of AKI after myeloablative HCT. The cumulative incidence of AKI is plotted over 100 days post-HCT for three *ABCB1* SNPs: 1236C>T (**a**), 2677G>T (**b**) and 3435C>T (**c**). The homozygous wild-type (solid line), heterozygous (dotted line) and homozygous variant (dashed line) genotypes for *ABCB1* are each presented. AKI, acute kidney injury; HCT, hematopoietic cell transplantation; SNP, single nucleotide polymorphism.

1236TT and 2677TT, especially after day 60 post-HCT. An approximate 17% increase in the incidence of AKI was observed in homozygous 1236TT patients compared to 1236CT and 1236CC patients (Figure 1a). A similar non-significant trend was also observed with the non-synonymous *ABCB1* 2677G>T SNP; an approximate 12% increase in the incidence of AKI was observed in homozygous 2677TT patients compared to 2677GT and 2677GG patients (Figure 1b). However, an associated risk of AKI was not apparent with the *ABCB1* 3435C>T SNP (Figure 1c). Although the number of patients with the *ABCB1* 1199A allele is low, it appeared that heterozygous 1199GA patients have an approximate 20% decrease in the incidence of AKI. On the basis of power analyses of the differences in risk between genotype groups, we were underpowered to detect a significant association in this retrospective trial.

SNP associations with chronic kidney disease

Among the 66 patients with relevant data in the time period, there were 16 cases of CKD (24.2%); therefore, we were limited in patient numbers for analysis. Table 3 shows the univariate OR of CKD for the single genetic predictors. None of the genotypes was statistically significantly associated with CKD in this population. The OR could not be estimated for the *ABCB1* 1199G>A SNP since there were no CKD cases in individuals possessing the 1199A allele.

ABCB1 *haplotype inference and association with renal disease*

Significant LD exists between *ABCB1* SNPs;[12–14] therefore, we also performed haplotype association analyses. The degree of LD (calculated as r^2) between *ABCB1* SNPs was estimated in this population. The 1236C>T and 2677G>T/A SNPs were in tight LD (0.72). The 3435C>T SNP was in significant LD with other SNPs, but to a lesser degree; 3435C>T and 2677G>T/A (0.44), and 3435C>T and 1236C>T (0.42). *ABCB1* 1199G>A showed no LD to other SNPs (<0.05). Next, the haplotype structure in this population was inferred and haplotype frequencies, with their corresponding standard error (±s.e.), were estimated. The three most common haplotypes among the *ABCB1* SNPs are 1199G/1236C/2677G/3435C (40.9±0.9%), 1199G/1236T/2677T/3435T (29.9±0.7%) and 1199G/1236C/2677G/3435T (11.8±0.8%). All other haplotypes inferred in this cohort had a frequency less than 5% and were grouped together for subsequent analyses. Table 4 shows the relationship between *ABCB1* haplotypes and the incidence of AKI and CKD post-HCT. There were no associations between the *ABCB1* haplotypes and the incidence of AKI or CKD.

Table 3 Pharmacogenomics associations with CKD (*n*=66 patients)

SNP	*No. of patients with CKD (% cumulative incidence)*[a]	*OR (95% CI)*	P-*value*
*CYP3A5*1/*3*			
*1/*1	0		
*1/*3	2 (18)		
*3/*3	14 (26)		
*3/*3 vs (*1/*3 and *1/*1)		2.0 (0.4–10.0)	0.41
ABCB1 1199G>A			
GG	16 (27)		
GA	0		
AA	0		
GG vs (AA and GA)		—	
ABCB1 1236C>T			
CC	7 (28)		
CT	7 (23)		
TT	2 (20)		
TT vs (CC and CT)		0.8 (0.1–4.0)	0.73
ABCB1 2677G>T/A			
GG	6 (21)		
GT	5 (22)		
TT	3 (30)		
TA	0		
GA	2 (67)		
TT vs (GG and GT)		1.6 (0.3–7.0)	0.56
TT vs (TA and GA)		0.6 (0.1–6.1)	0.70
ABCB1 3435C>T			
CC	4 (17)		
CT	9 (31)		
TT	3 (23)		
TT vs (CC and CT)		0.9 (0.2–3.9)	0.91

[a]Cumulative incidence estimated as the probability of CKD between 6 and 18 months post-HCT within each genotype.

Abbreviations: *ABCB1*, multidrug resistance gene; CI, confidence interval; CKD, chronic kidney disease; *CYP3A5*, cytochrome *P*450 3A5; HCT, hematopoietic cell transplantation; OR, odds ratio; SNP, single nucleotide polymorphism.

Discussion

In this analysis, we did not observe significant pharmacogenomic associations between AKI and CKD and the *ABCB1* and *CYP3A5* SNPs that regulate protein expression and activity of enzymes and transporters influencing CNI disposition in the kidney. In our cohort of HCT patients, genomic DNA was available for 121 patients who received a myeloablative conditioning regimen before HCT. While the data are suggestive of a relationship between *ABCB1* 1236C>T, 2677G>T and 1199G>A SNPs and the development of AKI in a myeloablative allogeneic HCT setting, the declining use of myeloablative HCT conditioning regimens makes it unlikely that studies in a larger patient population are feasible.

Renal injury remains a common problem after allogeneic HCT and exposure to CNI-containing immunosuppressive regimens may play a role in the development of disease. We recently showed that systemic cyclosporine levels are not associated with AKI.[2] Therefore, intracellular CNI exposure in kidney tubule cells, mediated by CYP3A5 and P-gp, may

Table 4 ***ABCB1*** **haplotypes and association with AKI and CKD**

Haplotype[a]	*Estimated haplotype frequency (%) ± s.e.*		*OR (95% CI)*	P-*value*
Incidence of AKI	No ($n=73$)	Yes ($n=48$)		
GCGC	39.6±3.1	44.1±10.4	1.0[b]	—
GTTT	30.7±9.1	32.5±2.9	1.0 (0.6–1.8)	0.99
GCGT	11.5±9.3	9.0±6.8	0.8 (0.4–2.0)	0.70
Remainder[c]	18.1 (–)[d]	14.4 (–)[d]	0.7 (0.4–1.5)	0.37
Incidence of CKD	No ($n=50$)	Yes ($n=16$)		
GCGC	39.7 (6.0)	49.5 (6.0)	1.0[b]	—
GTTT	30.8 (4.7)	31.3 (9.7)	0.9 (0.4–2.1)	0.77
GCGT	9.2 (3.1)	6.7 (6.0)	0.9 (0.3–3.2)	0.91
Remainder[c]	20.3 (–)[d]	12.5 (–)[d]	0.6 (0.3–1.5)	0.28

[a]Haplotype definitions: GCGC (1199G/1236C/2677G/3435C); GTTT (1199G/1236T/2677T/3435T); and GCGT (1199G/1236C/2677G/3435T).
[b]An odds ratio of 1.0 signifies the reference group.
[c]Represents the total frequency of the remaining haplotypes.
[d]s.e. not calculated since remaining haplotypes were grouped for analysis.
Abbreviations: AKI, acute kidney injury; CI, confidence interval; CKD, chronic kidney disease; OR, odds ratio.

be more predictive of renal injury. Since CYP3A5 and P-gp are highly expressed in the kidney, genetic variability in their expression and activity may be critical determinants of intracellular concentrations of CNIs in the kidney tubules; and therefore, may influence CNI-induced renal injury.

The primary drug-metabolizing enzymes involved in CNI metabolism are CYP3A4 and CYP3A5 in humans. Tissue expression of CYP3A5 is widespread and it is the major CYP3A isozyme in the kidneys.[17–19] The major cause of polymorphic *CYP3A5* expression is due to the *CYP3A5*3* polymorphism. *CYP3A5*3* results from a single base substitution within intron-3 (A6986G), which creates an alternative splice site and results in the production of aberrant mRNA and a truncated, non-functional protein.[8,20] CYP3A5 protein expression in the kidney and CYP3A5 catalytic activity in kidney microsomes are substantially reduced in samples with the *CYP3A5*3/*3* genotype.[8,17]

P-gp is expressed in many tissues important for CNI drug disposition including the kidneys.[21] P-gp limits cellular exposure to CNIs by actively effluxing the drugs out of cells. *ABCB1* is highly polymorphic and displays considerable LD and several SNPs have been associated with changes in the expression and activity of P-gp.[9] The first *ABCB1* polymorphism found to be associated with alterations in P-gp was the synonymous SNP 3435C>T.[16] Subsequently, it was reported that 3435C>T is in LD with two other *ABCB1* SNPs; a tri-allelic non-synonymous SNP at nucleotide position 2677, which results in a 2677G>T (Ala893Ser) and a 2677G>A (Ala893Thr) transition, as well as 1236C>T, another synonymous SNP.[13] In addition, we have recently observed a functional alteration in P-gp activity due to another non-synonymous *ABCB1* polymorphism, 1199G>A (Ser400Asn).[10,11] The *in vivo* significance of *ABCB1* polymorphisms has been conflicting as to their functional effect on renal P-gp expression.[22–24]

Since significant linkage exists in the *ABCB1* gene, it is important to understand the role of *ABCB1* haplotypes in pharmacogenomic association studies. We investigated the LD in our cohort of patients, and while strong LD was observed between SNPs, the degree of LD varied. We found that the 1199G>A SNP was not in LD with other *ABCB1* SNPs. The strongest LD was observed between the 1236C>T and 2677G>T/A SNPs. The 3435C>T SNP was also in significant LD with 1236C>T and 2677G>T/A, but to a lesser degree. Because there is a high degree of LD between these three *ABCB1* SNPs, and that the degree varies between SNPs, it may be important to evaluate the significance not only of individual SNPs, but also the significance of *ABCB1* haplotypes. The three most common haplotypes inferred in this cohort of patients were not associated with a risk of AKI or CKD.

Our study is the first to evaluate the pharmacogenomic association of *ABCB1* SNPs with AKI and CKD in HCT patients. While statistical significance was not achieved, several non-significant trends were observed between AKI and three of the *ABCB1* SNPs (1199G>A, 1236C>T and 2677G>T). While the number of patients with the 1199A allele is low, there was an observed decreased risk for the development of AKI in patients heterozygous for 1199GA, suggesting that patients with this genotype have a lower exposure to CNIs in kidney tubule cells due to increased P-gp-mediated efflux. This is consistent with previous *in vitro* studies in which we showed that recombinant cells expressing the 1199A variant showed increased P-gp-mediated efflux of anticancer agents and HIV protease inhibitors.[10,11] Analysis of the impact of the 1199G>A SNP is not complicated by linkage to other *ABCB1* SNPs. An increased risk of AKI was observed in patients homozygous for either 1236TT or 2677TT. However, we did not observe a trend in incidence of AKI for the 3435C>T SNP. The discordance between these observed results may be due to the stronger degree of LD observed between 1236C>T and 2677G>T compared to the LD of either SNP with 3435C>T in this cohort of patients. A synonymous SNP would not be

expected to alter protein function; however, a recent study has shown that the silent 3435C>T SNP alters substrate specificity.[25] While we did not observe a trend with 3435C>T, the other 1236C>T synonymous SNP in our study may influence the incidence of AKI. The observed trend towards an increased risk of AKI with the 1236TT and 2677TT genotypes suggests that patients expressing these genotypes have a higher exposure to CNIs in the kidney tubule cells due to decreased P-gp-mediated efflux.

In the solid organ transplant setting, studies evaluating the effect of *ABCB1* polymorphisms on CNI dose requirements, blood concentrations and CNI-induced renal injury have produced variable results.[26,27] Liver transplant patients receiving CNI-containing immunosuppressive regimens who were homozygous for 2677TT had a reduced risk of renal injury, but no association was observed with the 3435C>T SNP.[28] In contrast, a study in renal transplant patients receiving CNIs found that 3435TT donor genotypes were associated with an increased risk of renal injury, while no association was observed with the 2677G>T SNP.[29] Therefore, the influence of *ABCB1* polymorphisms on the risk of CNI-induced renal injury in solid organ transplant patients is conflicting, and our data in HCT patients also are not consistent with these results. Several confounding factors may play a role in these discordant results such as disease states, concomitant medication (particularly amphotericin, used in 38 patients in our cohort), environmental factors and differences in CNI regimens. In addition, other genetic factors may play a role in CNI-induced kidney disease. We have shown that several genes in the immune and inflammatory pathways are associated with the development of AKI post-HCT.[30]

In conclusion, there do not appear to be pharmacogenomic associations between polymorphisms and haplotypes in the *CYP3A5* and *ABCB1* genes and the development of AKI or CKD in patients following myeloablative HCT. Therefore, tailoring the CNI dose based on these genotypes is unlikely to lower significantly the risk of AKI or CKD. Alternative strategies for identifying risk factors for kidney injury in patients undergoing myeloablative HCT should be pursued given the necessity of CNI as GVHD prophylaxis and the frequent onset of renal dysfunction post-HCT.

Materials and methods

Study population

One hundred forty-seven patients with hematologic malignancies who were undergoing allogeneic transplantation following a conditioning regimen of cyclophosphamide plus total body irradiation were enrolled between April 1997 and January 2000 at the Fred Hutchinson Cancer Research Center (Seattle, WA, USA). All signed informed consent was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center. The clinical risk factors for SOS and AKI in this cohort have been reported previously.[1,2]

Seven days before the infusion of stem cells, cyclophosphamide was infused through a central venous access catheter for over 1–2 h at a dose of 60 mg/kg body weight. On the following day, a second infusion of the same dose of cyclophosphamide was given. The pharmacokinetics and pharmacodynamics of cyclophosphamide and metabolites in this cohort of patients has been published.[1] After a day of rest, total body irradiation (9–14.2 Gy) was given daily for three or four subsequent days in hyperfractionated doses from opposing cobalt sources. The kidneys were not shielded. Prophylaxis regimens against GVHD included a CNI, cyclosporine or tacrolimus and methotrexate in the majority of patients, or variations on the above, including prednisolone and BC3; BC3 is a murine antibody specific for human CD3.[1,2] CNIs were targeted to trough concentrations according to standard practice guidelines or individual protocol specifications; cyclosporine trough levels are generally targeted to between 150 and 450 ng/ml and tacrolimus trough levels are generally targeted to between 5 and 15 ng/ml. Donor hematopoietic cells were infused on day 'zero'; by convention, all subsequent days are numbered from this day.

Preparation of DNA

B-lymphoblastoid cells were collected from patients before HCT and were used to establish permanent growing lymphoblastoid cell lines using an Epstein-Barr virus transformation method.[31] Genomic DNA was extracted using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) from immortalized B-lymphoblastoid cell lines as described previously.[32] Genomic DNA was available for 121 of the 147 patients enrolled in the study. The quality of DNA samples was tested by spectrophotometric analysis at 260 and 280 nm.

Determination of CYP3A5 *and* ABCB1 *genotypes*

Genotyping was performed at the DNA Sequencing and Gene Analysis Center (Department of Pharmaceutics, University of Washington, Seattle, WA, USA). Patients were genotyped for SNPs in the *CYP3A5* and *ABCB1* genes using allelic-discrimination TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA). The reference SNP ID number for these assays were *CYP3A5*1>*3* (rs776746) and *ABCB1* 1199G>A (rs2229109), 1236C>T (rs1128503) and 3435C>T (rs1045642) SNPs. The tri-allelic *ABCB1* 2677G>T/A SNP was determined based on a custom-made assay that has been described previously: forward primer (5′-GTAAGCAGTAGGGAGTAACAAAATAACACT-3′), reverse primer (5′-GACAAGCACTGAAAGATAAGAAAGAA CT-3′), 2677G allele probe (5′-CCTTCCCAGCACCT-3′), 2677T allele probe: (5′-CTTCCCAGAACCTT-3′) and 2677A allele probe (5′-CTTCCCAGTACCTTC-3′).[33] The cycling conditions for PCR amplification were one cycle at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 1 min in a reaction volume of 10 μl containing 1 μl of genomic DNA ($\sim$25–100 ng), and 1$\times$ final concentrations of TaqMan Universal PCR Master Mix and TaqMan SNP Genotyping Assay primers and probes (Applied Biosystems). The allelic discrimination was determined in a post-PCR

analysis on a 7900HT Fast Real-Time PCR System (Applied Biosystems) with allele-specific probes for the common and variant SNPs (FAM and VIC, respectively) by Sequence Detection Systems version 2.2.1 (Applied Biosystems). Internal controls of sequence-verified genotypes were included for each assay.

Definitions of kidney injury

AKI was defined as a doubling of baseline serum creatinine within the first 100 days after transplant.[2,34] Baseline serum creatinine was the value obtained before the start of myeloablative conditioning therapy. AKI was assessed in all 121 patients.

CKD was defined as at least one value of glomerular filtration rate (GFR) $<60\,ml/min/m^2$ between 6 and 18 months post-HCT (days 180–540).[35] GFR was calculated using the modification of diet in renal disease equation for adults and the Schwartz formula for children.[36,37] Because of difficulties in acquiring patient samples on later days post-HCT, only 66 of the 121 patients in this cohort had GFR measurements in the time period of interest. Therefore, CKD was assessed only in these 66 patients.

Statistical analysis

We analyzed the associations between the incidences of AKI and CKD with genetic polymorphisms in *CYP3A5* and *ABCB1* in this study population, as well as the inferred haplotypes of *ABCB1*. OR for the development of AKI and CKD were calculated using logistic regression models with 95% confidence intervals. Analyses were performed using SAS statistical software (Cary, NC, USA). The following factors were considered as potential confounders: age at transplant, sex, gain in weight from baseline to the end of exposure period, baseline serum creatinine, amphotericin use and the incidence of moderate to severe SOS based on our previous analysis.[2] Cumulative incidence plots of the probability of AKI over the first 100 days post-HCT by genotype was estimated.[38] Death without AKI was treated as a competing risk.

LD between *ABCB1* SNPs was estimated based on the r^2 LD statistics using a display format, visual genotypes 2 (SeattleSNPs Variation Discovery Resource, Seattle, WA, USA). Haplotype inference was generated using HPlus software (qge.fhcrc.org/hplus/; Fred Hutchinson Cancer Research Center) for simultaneous haplotype estimation and univariate association analysis.

Abbreviations

ABCB1 or MDR1	*multidrug resistance gene*
AKI	*acute kidney injury*
CKD	*chronic kidney disease*
CNIs	*calcineurin inhibitors*
CYP3A5	*cytochrome P450 3A5*
GVHD	*graft-versus-host disease*
HCT	*hematopoietic cell transplantation*
LD	*linkage disequilibrium*
OR	*odds ratio*
P-gp	*P-glycoprotein*
SNP	*single nucleotide polymorphism*
SOS	*sinusoidal obstruction syndrome*

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Duality of interest

No duality of interest.

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