Pharmacogenetic determinants of calcineurin inhibitor associated nephrotoxicity in liver transplant patients

Catherine K. Yeung

A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Public Health

University of Washington

2013

Committee:

Karen Edwards, Ph.D.

Kenneth E. Thummel, Ph.D.

Program Authorized to Offer Degree:

Public Health - Epidemiology

ABSTRACT

The use of calcineurin inhibitors (tacrolimus and cyclosporine) has become standard of care in post-transplant immunosuppression. While these medications have greatly reduced organ rejection, they can cause nephrotoxicity and renal failure in some patients. A possible risk factor for this toxicity may be pharmacogenetic variability in the enzymes involved in the clearance of the calcineurin inhibitors. We conducted a prospective study in liver transplant patients at the University of Washington to evaluate whether or not variation in the genes encoding CYP3A5 (*1 vs *3) and P-glycoprotein (MDR2677, MDR3435, or MDR1236) were associated with a decline in kidney function (assessed by the estimated glomerular filtration rate) in liver transplant patients using continuous calcineurin inhibitor therapy. This study did not find a significant association between the inheritance of the CYP3A5 *1 or *3 alleles, or MDR2677, MDR3435, or MDR1236 alleles and the rate of decline of estimated glomerular filtration rate in post-liver transplant patients.

INTRODUCTION

The use of calcineurin inhibitors (CNIs), tacrolimus and cyclosporine, as primary immunosuppressive therapy in liver transplant patients has resulted in decreased incidence of organ rejection and an increased lifespan. The majority of liver transplant patients live at least 5 years, with many living more than 15 years. CNIs are narrow therapeutic index drugs, with sub therapeutic levels resulting in graft rejection and elevated levels associated with nephrotoxicity and other morbidities. Unfortunately, even therapeutic levels of CNIs have been shown to be nephrotoxic, and a small study conducted at the University of Washington concluded that, at 3 years post-transplant, 44% of liver transplant patients receiving CNI therapy exhibited significant renal dysfunction [2].

The CNIs undergo extensive biotransformation, principally by cytochrome P450s (CYP) 3A4 and 3A5, which are abundant in the liver and intestinal tract. They are also subject to transport by intestinal and hepatic P-glycoprotein (encoded by the ABCB1 gene), an efflux transporter which transports drugs out of the enterocyte and into the intestinal lumen or from the hepatocyte into the bile canaliculi (**Figure 1**).

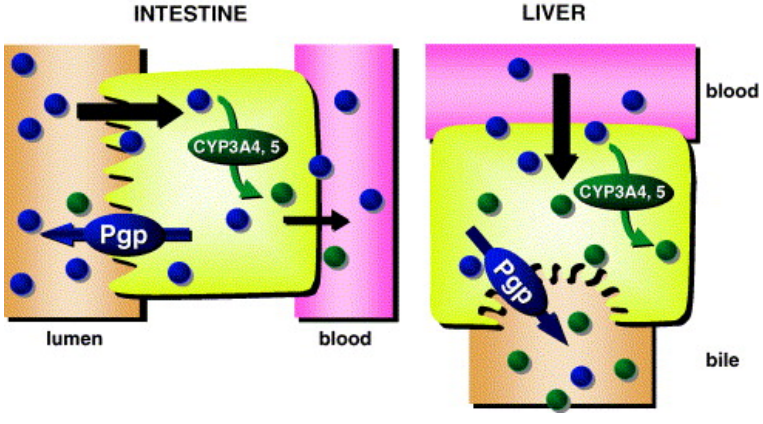

Figure 1. Role of P-glycoprotein (ABCB1) and CYP3A4/5 on the enterohepatic handling of tacrolimus and cyclosporine. Blue and green symbols represent the unchanged form and metabolite, respectively (from [1]).

CYP3A5 and Pgp are highly polymorphic in the population. CYP3A5 has two primary variants, designated CYP3A5 *1 and CYP3A5 *3, with the *1 variant producing a catalytically active protein and the *3 variant resulting in an inactive product. In the Caucasian population, the prevalence of *3 variant approaches 96%, with >70% prevalence in Chinese, Japanese,

Ethiopian, Zimbabwean, Middle Eastern/Arab, and Central American populations. However, in some African populations, specifically those localized to Gabon and Zanzibar, the prevalence is less than 20%. Pgp, encoded by the ABCB1 gene, has three major variants at positions 1236, 2677, and 3435 with the frequencies shown in the **Table 1** (all variant data extracted from: http://www.pharmacogeneticsinfo.org). CYP3A5 variation has been shown to alter drug exposure of tacrolimus and cyclosporine [2-5] and altered transporter function may also affect exposure although the data are more equivocal.

Table 1. Allele Frequencies for major ABCB1 (Pgp) variants in different populations

	Allele Frequency (%)						
	ABCB1 c.1236		**ABCB1 c.2677**			**ABCB1 c.3435**	
Population	**C**	**T**	**G**	**T**	**A**	**C**	**T**
Asian American	31.5	68.5	48.3	45	6.7	60	40
African American	79.1	20.9	89.5	10	0.5	84	16
European American	54.1	45.9	50	46.4	3.6	43.9	56.1
Mexican American	55	45	60	40		50	50

CYP3A5 and Pgp are also present in the kidney, along with many other drug metabolizing and transport enzymes (**Figure 2**). The presence of gene variants of CYP3A5 and Pgp can contribute to increased or decreased renal exposure of parent drug and/or metabolites, even when circulating parent drug levels are kept within a narrow range by therapeutic blood level monitoring. Recently published work [7] has shown that, despite similar oral clearance values, CYP3A5 expressors had a 30% higher $AUC_{metabolite}/AUC_{parent}$ ratio for cyclosporine (AM19 and AM1c metabolites) and 20.4% lower urinary clearance (48-hour collection) compared with non-expressors, indicating that cyclosporine exhibits CYP3A5 dependent intrarenal metabolism. Similar results were seen for tacrolimus and its metabolites [8], where CYP3A5 expressors had 2.0-2.7 fold higher metabolite/parent ratios in blood and a 36% lower renal tacrolimus clearance than non-expressors. With regard to CNI-induced nephrotoxicity, CYP3A5 non-expressing

patients receiving continuous immunosuppression therapy are expected to accumulate higher concentrations of unchanged CNI drug and lower metabolite concentrations in the renal epithelium than that of CYP3A5-expressing patients. These differences may influence the risk of drug-induced nephrotoxicity.

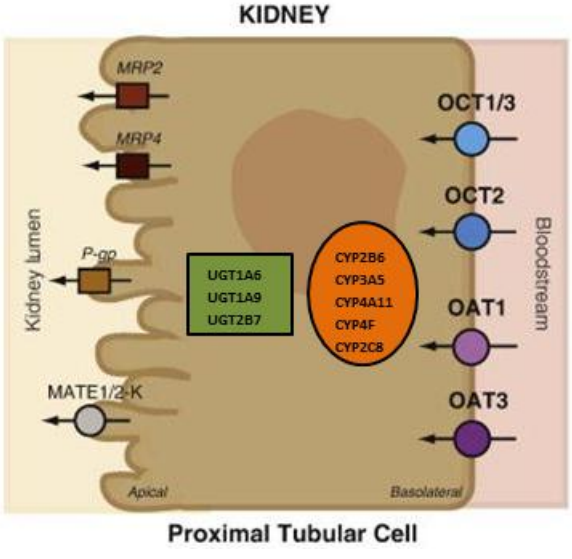

Figure 2. Expression of human drug uptake transporters (from the *SLC* superfamily) and efflux transporters (from the *ABC* superfamily) in kidney (proximal tubular cell). ABC transporters are depicted with a square and are shown in italics (e.g. MRP2). For SLC transporters, abbreviated names, gene family and colours are the following: OCT, for organic cation transporter, and OAT, organic anion transporters (SLC22 gene family; light blue, dark blue and violet, respectively); MATE, multidrug and toxin extrusion transporter (SLC47 gene family; light grey); For ABC transporters (all coloured in brown or black): P-gp, P-glycoprotein (ABCB1); MRP, multidrug resistance protein (ABCC gene family). Cytochrome P450s and UGTs shown in orange and dark green. Modified from [6]

Based on the assumption that Pgp/CYP3A5 function can influence intra-renal exposure to unchanged CNI drugs and their metabolites, and that variation in the genes encoding these proteins results in reduced function, *we hypothesized that genetic variants that result in increased drug exposure will be associated with increased risk of nephrotoxicity among liver transplant patients* . A clearer understanding of the pharmacogenetic risk factors that contribute to calcineurin-associated nephrotoxicity can result in altered drug selection or dosing regimen in patients who are at escalated risk. Prospective genetic testing may prove to be a valuable tool to optimize therapeutic effect and reduce adverse events in transplant patients.

METHODS

Study design: We conducted a prospective study to evaluate whether or not variation in the genes encoding CYP3A5 and P-glycoprotein (Pgp; *ABCB1*) were associated with a decline or loss of kidney function in liver transplant patients using continuous CNI therapy.

Study setting: Subjects were recruited from the liver transplant follow-up clinic at the University of Washington Medical Center (UWMC).

Study subjects: Male and non-pregnant female patients >18 years old who had received a single-organ liver transplant at UWMC and were receiving a calcineurin inhibitor as part of their post-transplant immunosuppressive therapy, with unimpaired kidney function, were eligible for the study. Patients with abnormal lab values or physical exam, history of irreversible kidney damage at the time of transplant or who received a multi-organ transplant were excluded from the study. Patients with pre-existing kidney disease were not eligible for the study because of the difficulty in isolating drug effects from pre-existing conditions. Eligible participants received either tacrolimus or cyclosporine according to clinical protocol; at UWMC, the current standard of care was to titrate the dose of CNIs to specific trough concentration levels (5-7ng/mL tacrolimus and 150ng/mL cyclosporine). Consent for using this population for this study was approved by the Institutional Review Board at the University of Washington (current HSRC approval number 04-1322-D04).

Sample and data collection: During the screening exam, buccal swabs of cheek cells were collected and then genotyped for CYP3A5 and ABCB1 allelic variants. All pertinent clinical data was collected from the electronic medical records for each patient at the appropriate post-transplantation time-points. Immediately prior (within 24 hours) to transplant surgery, lab values for serum creatinine (SCr) and bilirubin, as well as height and weight were collected. For the 6-month and one-year post-transplant time-points, clinical measures collected included SCr, bilirubin, height, weight, CNI dose, CNI serum level, GFR, and creatinine clearance. When

patients did not have timely 6-month or 1-year follow-up exams, the lab values obtained closest to the scheduled timepoints were recorded. When available, a liver biopsy sample from the implanted liver was obtained from the Transplant Services biorepository for DNA extraction and genotyping.

Genotyping: Buccal swabs and liver biopsy samples were genotyped for CYP3A5*3 and Pgp variants 2677, 3534, and 1236 (rs776746, rs2032582, rs1045642 and rs 2032582, respectively) as previously described[5]. In brief, DNA was purified using Qiagen QIAamp DNA mini kit and samples were genotyped using 5'-nuclease assays with specific primers and fluorogenic probes designed for each SNP using the assay-by-design service from Applied Biosystems (Foster City, California, USA). All SNP detection assays were performed in the Functional Genomics Laboratory, Center for Ecogenetics and Environmental Health at the University of Washington.

Clinical Endpoints: Kidney function was determined by estimated Glomerular Filtration Rate (eGFR), which was calculated from SCr using the Cockraft Gault equation:

$$\text{eGFR} = [(140\text{-age}) \times (\text{weight in kg}) \times (0.85 \text{ if female})] / (0.72 \times \text{SCr})$$

When glomerular filtration in the kidney is functioning normally, eGFR levels are greater than 90 ml/min/1.73 m^2, with stage 1 and 2 kidney disease (mildly impaired) defined as an eGFR = 60-90 ml/min/1.73 m^2, stage 3 (moderate impairment) as eGFR = 45-59, and stage 4 (severe impairment) as eGFR = 15-30 ml/min/1.73 m^2. When eGFR is below 15 ml/min/1.73 m^2, patients require dialysis therapy.

Data analysis: Because we had data for these patients at multiple time points (baseline, 6 months post-transplant, 1-year post-transplant), we evaluated the longitudinal change in renal function post-transplant. Time trends for different genotypes were compared by the method of generalized estimating equations. This analysis included a characterization of the differences in time trends by testing genotype groups x time interaction. We adjusted for patient gender, age, race (white vs. nonwhite) in the rate of change in renal function. Subsequent adjustment for pre-

transplant hypertension and diabetes, and donor 3A5 genotype did not alter the associations between genotype and decline in renal function and therefore these coefficients have not been presented.

Results

Subject Demographics

A total of 215 participants who were consented for this study and were at least one year post-liver transplant at the time of this analysis were evaluated. The characteristics of the study participants are listed in Table 1. The average age of participants at baseline was 53.9 years, with a mean eGFR=104 mL/min. Men made up a majority of the subjects (73%). Most of the participants were Caucasian, with only 15% of the study population identifying as non-Caucasian. There were no significant differences between study subjects that completed the study (0, 6, 12 month time points) compared with subjects that did not have available data at the 12 month time point (Table 2). The frequency of variants of CYP3A5 and MDR were consistent with previously published values.

Statistical Analysis

For CYP3A5*3 polymorphism, each additional copy of the *1 allele was associated with a 2% faster decline in eGFR per year compared with the wild type allele (GG), however this change was not statistically significant (p=0.72, Table 4). Similarly, no significant differences in the association between eGFR decline and MDR2677, MDR3435, or MDR1236 variants (p=0.91, p=0.41, and p=0.29 respectively). MDR1236 showed a tendency towards an association (p=0.29) between the slope of log eGFR vs. time, but the association was not significant, possibly due to small sample size. Adjustment for age, race (Caucasian vs. non-caucasian), and gender did not alter the lack of association (Table 5). Wald tests for the change in slope for CYP3A5*3, MDR2677, MDR3435, and MDR1236 were not significant (p=0.81, p=0.91, p=0.41, and p=0.29 respectively). No difference in decline of renal function was observed between subjects with CYP3A5*3 and CYP3A5*1/*3 donor genotype (Table 5).

Discussion

This study did not find a significant association between the inheritance of the *MDR2677, MDR3435, or MDR1236* alleles and the rate of decline of eGFR in post-liver transplant patients. This finding is surprising, as it is in contrast to an earlier study[2] that showed that frequency of renal dysfunction was reduced among liver transplant patients treated with calcineurin inhibitors and who carried an ABCB1 2677TT genotype, as compared to those with a 2677GG genotype; subjects with a heterozygote genotype behaved phenotypically like the 2677GG group. The discrepancy may be due to differences in treatment protocols, namely the shift from predominantly cyclosporin –based CNI therapy to almost exclusively tacrolimus based therapy, along with a decrease in targeted trough levels (tacrolimus target trough = 10-14 mg/dL in 2003 vs tacrolimus target trough = 4-7 mg/dL after 2010). The previous study also followed subjects for 3 years, whereas we studied subjects only for 1 year, leading to the possibility that nephrotoxicity occurs after cumulative exposure of several years. Our results are consistent with a more recent study[9] in heart transplant patients receiving tacrolimus and cyclosporine (mean tacrolimus trough = 5.89-6.26 mg/dL) for more than 8 years that showed no association between genetic polymorphism of either MDR1 or CYP3A5 with the development of nephrotoxicity.

Similarly, this study also did not find an association between the inheritance of the CYP3A5*1 allele and the rate of decline in this cohort of liver transplant patients. The CYP3A5*1 has been purported to have a protective effect on renal function due to more rapid metabolism and decreased exposure to the CNIs and is most common in individuals of African descent. A recent study by Shi and colleagues[10] showed that subjects carrying a CYP3A5*1 allele experienced decreased risk of early renal glomerular injury, compared to the subjects that were homozygous for the CYP3A5*3 allele (P=0.01), when observed for a mean duration of 4.3 years. MDR-1 polymorphisms were not associated with early decline in renal function. We were unable to replicate the CYP3A5 results in this study, possibly due to our shorter period of observation (1

year) and that nephrotoxic effects of the CNI require extended exposure to manifest. Additionally, our cohort contained few African American subjects (n=7), and only 3 subjects that were homozygous for the *1 allele, and as such, we were unable to detect any significant difference between CYP3A5*3 and CYP3A5*1 populations.

Zheng and colleagues[8] recently predicted that tacrolimus exposure in the renal epithelium of CYP3A5 expressors (*1) is 53% of that for CYP3A5 nonexpressors (*3) when normalized to blood AUC and suggested that intrarenal accumulation of tacrolimus and its primary metabolites depends on the CYP3A5 genotype of the liver and kidneys. Therefore, we expected to observe a change in decline in renal function between the donor CYP3A5 *3 and CYP3A5 *1/*3 groups. However, no such difference was observed, possibly due to the multifactorial nature of the renal disposition of the CNIs, involving not only oxidative enzymes such as CYP3A5 but also drug transporters. A larger study, including subjects with the CYP3A5 *1/*1 donor genotype would be required to confirm the predictions of Zheng and colleagues.

The strength of this study is its prospective design that enrolled 214 subjects between July 2004 and Jan 2009. During this time period, 407 patients were transplanted, with an overall trend that patients in poorer health with multiple comorbidities (and therefore ineligible for consent), were transplanted towards the end of the study. In general, during the study period, the number of liver transplants performed by the UWMC declined (from 126 in 2004 to 82 in 2009, a decline of 35%), which further complicated subject recruitment. While we were able to consent 53% of transplanted patients, we cannot rule out that the statistical power of this study was insufficient to detect a significant effect of a single gene variant on renal function. However, even if we had been able to enroll more subjects and detected a statistically significant difference in genotype groups, it is unlikely that the small differences observed would be sufficient to revise current clinical protocols.

Our results do not support routine genotyping of the SNPs investigated in this study to assess the risk of nephrotoxicity in patients undergoing liver transplant and the clinical relevance of these SNPs remains unclear. In the 30 years since the introduction of cyclosporine, the CNIs have been a major contributor to the success of graft survival and the prevention of organ rejection, but CNIs are also responsible for serious and harmful side effects. As the field of liver transplantation continues to progress and personalized medicine becomes standard of practice, success will be measured by long term outcomes. The ability to identify patients that are susceptible to drug-based adverse events will be critical to ensure long term survival for transplant recipients.

REFERENCES

1. Masuda S, Inui K: An up-date review on individualized dosage adjustment of calcineurin inhibitors in organ transplant patients. *Pharmacol Ther,* 112**:** 184-198, 2006

2. Hebert MF, Dowling AL, Gierwatowski C, Lin YS, Edwards KL, Davis CL, Marsh CL, Schuetz EG, Thummel KE: Association between ABCB1 (multidrug resistance transporter) genotype and post-liver transplantation renal dysfunction in patients receiving calcineurin inhibitors. *Pharmacogenetics,* 13**:** 661-674, 2003

3. Anglicheau D, Legendre C, Beaune P, Thervet E: Cytochrome P450 3A polymorphisms and immunosuppressive drugs: an update. *Pharmacogenomics,* 8**:** 835-849, 2007

4. Kuypers DR, de Jonge H, Naesens M, Vanrenterghem Y: A prospective, open-label, observational clinical cohort study of the association between delayed renal allograft function, tacrolimus exposure, and CYP3A5 genotype in adult recipients. *Clin Ther,* 32**:** 2012-2023, 2010

5. Smith HE, Jones JP, 3rd, Kalhorn TF, Farin FM, Stapleton PL, Davis CL, Perkins JD, Blough DK, Hebert MF, Thummel KE, Totah RA: Role of cytochrome P450 2C8 and 2J2 genotypes in calcineurin inhibitor-induced chronic kidney disease. *Pharmacogenet Genomics,* 18**:** 943-953, 2008

6. Minuesa G, Huber-Ruano I, Pastor-Anglada M, Koepsell H, Clotet B, Martinez-Picado J: Drug uptake transporters in antiretroviral therapy. *Pharmacol Ther,* 132**:** 268-279, 2011

7. Zheng S, Tasnif Y, Hebert MF, Davis CL, Shitara Y, Calamia JC, Lin YS, Shen DD, Thummel KE: CYP3A5 Gene Variation Influences Cyclosporine A Metabolite Formation and Renal Cyclosporine Disposition. *Transplantation*, 2013

8. Zheng S, Tasnif Y, Hebert MF, Davis CL, Shitara Y, Calamia JC, Lin YS, Shen DD, Thummel KE: Measurement and compartmental modeling of the effect of CYP3A5 gene variation on systemic and intrarenal tacrolimus disposition. *Clin Pharmacol Ther,* 92**:** 737-745, 2012

9. Klauke B, Wirth A, Zittermann A, Bohms B, Tenderich G, Korfer R, Milting H: No association between single nucleotide polymorphisms and the development of nephrotoxicity after orthotopic heart transplantation. *J Heart Lung Transplant,* 27**:** 741-745, 2008

10. Shi Y, Li Y, Tang J, Zhang J, Zou Y, Cai B, Wang L: Influence of CYP3A4, CYP3A5 and MDR-1 polymorphisms on tacrolimus pharmacokinetics and early renal dysfunction in liver transplant recipients. *Gene,* 512**:** 226-231, 2013

Table 1. Participant characteristics prior to transplant

	% (n) *total n=215*
Female	27 (61)
White	85.1 (183)
African American	3.3 (7)
American Indian/Alaska Native	1.4 (3)
Asian	6.5 (14)
Hispanic	0.5 (1)
Other or unknown	3.5 (7)
Hypertensive	24 (50)
Diabetic	24 (50)
	Mean (sd)
Age	53.9 (8.2) years
Serum creatinine	1.2 (0.6) mg/dL
eGFR*	104 (48) mL/min

**eGFR calculated with Cockraft Gault equation*

Table 2. Participant characteristics by number of study visits

# study visits	2	3
n	58	152
age (mean, sd)	51.6, 9.99	54.6, 7.37
baseline eGFR (mean, sd)	108.2, 50.2	103.4, 47.7
female (%, n)	24.6, 14	30.5, 46
baseline HTN (%, n)	26.4, 14	23.7, 35
baseline DM (%, n)	24.5, 13	23.7, 35

Table 3. Unadjusted associations between CYP3A5 or P-gp genotypes and loss of GFR*

Gene	Genotype	n	slope (logGFR)	95% CI		Δ slope	95% CI		p value
CYP3A5*3	GG	163	0.84	0.78	0.89	1.02	0.89	1.16	0.81
	AG	39	0.85	0.76	0.95				
	AA	3	1.03	0.79	1.34				
	missing	9							
MDR2677	TT	32	0.85	0.75	0.95	0.99	0.91	1.08	0.88
	TA/TG	100	0.84	0.79	0.89				
	GG/GA	73	0.99	0.83	1.17				
	missing	9							
MDR3435	CC	46	0.87	0.78	0.97	0.96	0.88	1.05	0.41
	CT	113	0.84	0.79	0.89				
	TT	46	0.93	0.77	1.11				
	missing	9							
MDR1236	CC	76	0.81	0.74	0.88	1.04	0.96	1.13	0.29
	CT	95	0.85	0.79	0.90				
	TT	34	1.09	0.92	1.29				
	missing	9							

Table 4. Adjusted associations between CYP3A5 or P-gp genotypes and loss of GFR*

Gene	Genotype	n	slope (logGFR)	95% CI		Δ slope	95% CI		p value
CYP3A5*3	GG	163	0.84	0.78	0.89	1.02	0.90	1.17	0.72
	AG	39	0.86	0.76	0.96				
	AA	3	1.05	0.81	1.36				
	missing	9							
MDR2677	TT	32	0.84	0.75	0.95	1.00	0.92	1.09	0.99
	TA/TG	100	0.84	0.79	0.89				
	GG/GA	73	1.00	0.84	1.19				
	missing	9							
MDR3435	CC	46	0.87	0.78	0.97	0.97	0.88	1.06	0.48
	CT	113	0.84	0.79	0.89				
	TT	46	0.94	0.78	1.12				
	missing	9							
MDR1236	CC	76	0.82	0.75	0.89	1.04	0.96	1.13	0.37
	CT	95	0.85	0.80	0.90				
	TT	34	1.08	0.91	1.28				
	missing	9							

**adjusted for age, gender, and race with race classified as white or non-white*

Table 5: Unadjusted and adjusted* associations between donor CYP3A5 genotype and loss of eGFR

Donor Genotype	n	Unadjusted slope (logGFR)	95% CI		Adjusted slope (logGFR)	95% CI	
GG	33	0.70	0.48	1.01	0.70	0.48	1.02
AG	13	0.79	0.66	0.95	0.79	0.66	0.95
missing	168						

**adjusted for age, gender, and race with race classified as white or non-white*