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ASSOCIATIONS OF *ABCB1* 3435C>T AND *IL-10* -1082G>A POLYMORPHISMS WITH LONG-TERM SIROLIMUS DOSE REQUIREMENTS IN RENAL TRANSPLANT PATIENTS

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Abstract

Backgrounds—SRL absorption and metabolism are affected by Pgp-mediated transport and CYP3A enzyme activity, which are further under the influences of cytokine concentrations. This retrospective study determined the associations of *ABCB1* 1236C>T, 2677 G>T/A, and 3435C>T, *CYP3A4* -392A>G, *CYP3A5* 6986A>G and 14690G>A, *IL-10* -1082G>A, and *TNF* -308G>A polymorphisms with SRL dose-adjusted, weight-normalized trough concentrations (C/D) at 7 days, and at 1, 3, 6, and 12 months post initiation of SRL.

Methods—Genotypes for 86 renal transplant patients who received SRL-based maintenance immunosuppressive therapy were determined using polymerase chain reaction followed by chip-based mass spectrometry. The changes of log-transformed C/D over the days post transplantation were analyzed using a linear mixed-effects model, with adjustments for body mass index and weight-normalized doses of tacrolimus, prednisone, clotrimazole, and statins.

Results—*ABCB1* 3435C>T and *IL-10* -1082G>A were significantly associated with log C/D ($p=0.0016$ and 0.0394 , respectively). Mean SRL C/D was 48% higher in patients with *ABCB1* 3435CT/TT genotype than those with 3435CC genotype, and was 24% higher in *IL-10* -1082GG compared to -1082AG/AA.

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Footnotes

The authors declare no conflict of interest.

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Conclusions—*ABCB1* 3435C>T and *IL-10* -1082G>A were significantly associated with long-term SRL dose requirements. Genetics can play a significant role in SRL dosing and may be useful in therapeutic monitoring of SRL in renal transplantation. Future replication studies are needed to confirm these associations.

Keywords

Sirolimus; *ABCB1*; *CYP3A5*; pharmacogenetics; pharmacokinetics

Introduction

SRL (Rapamune®, Wyeth Pharmaceuticals Inc., Philadelphia, PA) is a potent immunosuppressant that is used for the prevention of renal transplant rejection. SRL pharmacokinetics exhibits wide inter- and intra-subject variability and dosages used in kidney transplantation range from 2–15 mg/day. Steady state SRL trough concentrations <5 ng/mL and >15 ng/mL were associated with the occurrence of acute rejection episodes and adverse reactions such as leukopenia and thrombocytopenia, respectively (1). TDM of SRL is therefore suggested to be a useful tool to optimize the outcomes of patients with renal transplantation (2). The systemic availability of oral SRL is low, likely due to the fact that SRL is a substrate for the drug efflux pump P-gp, as well as the hepatic and intestinal CYP3A4 and 3A5 enzymes (3,4). The genes encoding P-gp (i.e. *ABCB1*), CYP3A4, and CYP3A5 could therefore be important determinants of SRL bioavailability and metabolism.

Some of the most commonly studied *ABCB1*, *CYP3A4*, and *CYP3A5* variants include *ABCB1* 1236C>T, 2677G>T/A, and 3435C>T, *CYP3A4* -392A>G, and *CYP3A5* 6986A>G and 14690G>A. The *ABCB1* 1236C>T and 3435C>T are synonymous SNPs; the latter was correlated with *ABCB1* function (5) and resulted in substrate specificity changes (6). The *ABCB1* 2677G>T/A causes an amino acid change in exon 21, which led to increased vincristine transport rates in the variants (7). *CYP3A5* 6986A>G and 14690G>A represent two splice variants that cause alternative splicing and protein truncation result in the absence of CYP3A5 protein (8). Allelic frequency for 6986A>G is highest in Whites (93%), whereas 14690G>A occurs almost exclusively in Blacks (12%) (9). Although *CYP3A4* -392A>G represents a mutation in the nifedipine-specific element in the 5'-flanking region of the gene, its associations with higher prostate cancer tumor stage (10) and reduced hepatic CYP3A activity (11) were likely attributed to its strong linkage equilibrium with *CYP3A5* 6986A>G (8).

Cytokines such as IL-10 and TNF- α have been shown to decrease CYP3A activity (12,13) and affect P-gp expression (14). Therefore, SRL pharmacokinetics may be influenced by *IL-10* and *TNF* SNPs as well. It has been shown that IL-10 basal protein expression levels were higher in healthy individuals with *IL-10* -1082GG genotype (15), and *TNF* -308A variant allele was associated with much powerful transcriptional activation of *TNF* in human B cells (16).

The effects of genetic polymorphisms on SRL dose requirements and pharmacokinetics in renal transplantation have been studied previously (17–21). However, all these studies were cross-sectional in nature. Since physiological factors affecting drug absorption and disposition could be different during early versus late post transplantation, it is important to examine the influence of genetics on long-term SRL usage. This retrospective study was designed to explore the associations of *ABCB1*, *CYP3A4*, *CYP3A5*, *IL-10*, and *TNF* genetic polymorphisms with longitudinal dose requirements of SRL in kidney transplant recipients. Information obtained from this study can potentially help develop an optimal dosing strategy for long-term therapeutic monitoring of SRL.

Results

Ninety-three renal transplant patients were enrolled in this study; a complete genotype set was obtained in 87 subjects. The variant allele frequencies for the *ABCB1* 1236C>T, 2677G>T/A, and 3435C>T, *CYP3A4* -392A>G, *CYP3A5* 6986A>G and 14690G>A, *IL-10* -1082G>A, and *TNF* -308G>A polymorphisms were 0.359, 0.352/0.033, 0.413, 0.167, 0.806, 0.269, 0.544, and 0.161, respectively (Supplemental Digital Content 1). With the exception of *CYP3A4* -392A>G and *CYP3A5* 6986A>G, all SNPs were in Hardy-Weinberg equilibrium (i.e. no significant differences between observed and expected genotype frequencies).

Among the 93 subjects enrolled, 86 recipients were included in data analysis. Six subjects were excluded because of incomplete genetic information and one is known to be noncompliant with medications. Patient demographics and immunosuppressive therapies are summarized in Table 1. Using a linear mixed-effects model, BMI and weight-normalized doses of tacrolimus, prednisone, clotrimazole, and statins were found to have significant effects on log-transformed, dose adjusted, weight-normalized trough concentrations of SRL (ng/mL per mg/kg body weight) (expressed as C/D ratio) and were included as covariates in subsequent modeling.

Table 2 summarizes the univariate effects of each SNP under the dominant, co-dominant, and recessive genetic models on SRL log (C/D), with adjustments for the significant covariates as described above. The *ABCB1* 1236C>T and 3435C>T were significantly associated with log (C/D) under both the co-dominant and dominant models, while *ABCB1* 2677G>T/A was significant under all three genetic models. For all these *ABCB1* SNPs, the dominant model had the lowest AIC value and was used in the multivariate analysis. The *IL-10* -1082G>A was significant under both the co-dominant and recessive models, with the recessive model having the lower AIC and was used in the multivariate analysis.

The *ABCB1* 3435C>T under the dominant model and *IL-10* -1082G>A under the recessive model remained significant in the multivariate analysis, with p values of 0.0016 and 0.0394, respectively. The coefficients (standard errors) for *ABCB1* 3435C>T and *IL-10* -1082G>A were 0.171 (0.0521) and 0.0930 (0.0444), respectively. These represent a 48% higher mean SRL C/D in patients with at least one *ABCB1* 3435T allele than those with 3435CC genotype, and a 24% higher mean SRL C/D in *IL-10* -1082GG homozygotes compared to -1082A heterozygotes and homozygotes. There were no significant interactions between *ABCB1* 3435C>T and *IL-10* -1082G>A as well as between these SNPs with the covariates in the final model.

Discussion

In this study, SRL trough concentrations were obtained longitudinally from the start to one year after the initiation of SRL therapy, with post-operative days ranging from immediate to two years post transplantation. These data, obtained from TDM, reflected the actual use of SRL in clinical practice but were subjected to the influences of various confounding factors such as demographics, time post transplantation, and concomitant medications. We minimized the effects of these confounding factors by selecting and adjusting for those factors that have significant impact on the mixed-effects model. Among all significant covariates, BMI correlated positively with SRL log (C/D), similar to a previous finding showing higher initial tacrolimus blood levels in overweight renal transplant recipients (22). Additionally, SRL concentration/dose ratios were increased during clotrimazole or statins co-administration and were decreased during tacrolimus or prednisone co-administration, suggesting CYP3A enzyme competition between substrates (statins and tacrolimus) as well

as CYP3A induction and inhibition by corticosteroids (23,24) and clotrimazole, respectively (25,26). The effect of steroids may influence the observation that prednisone sparing sirolimus protocols have high rates of SRL related adverse effects such as mouth ulcers (27,28).

Various studies examining drug-metabolizing enzyme and transporter polymorphisms reported conflicting results on the associations of *CYP3A5* genotypes with SRL dose requirements and pharmacokinetics, while no associations were observed with *ABCB1* SNPs to date. SRL concentration/dose ratios were significantly lower in patients who are homozygous and heterozygous for the wild type *CYP3A5* 6986A allele (expressors) than in *CYP3A5* 6986GG homozygotes (non-expressors) in a subgroup of 69 renal transplant recipients receiving SRL as rescue therapy without calcineurin inhibitors (17). However, no association was observed in the same study in the entire studied population ($n = 149$), in which patients receiving *de novo* SRL and SRL-calcineurin inhibitor combination were included (17). Two other studies showed significant influences of *CYP3A5* status on SRL pharmacokinetics in renal transplant recipients. SRL concentration/dose ratios and dose-adjusted C_0 , C_{max} , and AUC_{0-9} at steady state were lower in 47 *CYP3A5* Chinese (21) and Caucasian expressors, respectively (18). On the contrary, no differences were observed between *CYP3A5* expressors and nonexpressors in dose-adjusted SRL trough in 85 renal transplant patients (19), as well as in SRL dose/trough ratio and dose-adjusted AUC_{0-24} in a small patient population ($n=20$) (20). The lack of associations between SRL log (C/D) with *CYP3A5* genotypes in our study suggest that *CYP3A5* 6986A>G and 14690G>A do not contribute significantly to the variability in SRL dose requirements. This is consistent with findings from an *in vitro* study showing that CYP3A4 was a more efficient catalyst of SRL metabolism than CYP3A5 (29). It is noted that *CYP3A4* -392A>G and *CYP3A5* 6986A>G were not in Hardy-Weinberg equilibrium, and typically, they should be excluded from data analysis in genetic association study such as ours. However, inclusion of these SNPs did not affect our final results. *CYP3A4* -392A>G and *CYP3A5* 6986A>G were not found to be significant factors.

While our results on *CYP3A5* polymorphisms are not totally unexpected, it is surprising to find a significant association between *ABCB1* 3435C>T and SRL log (C/D). As a matter of fact, this study is the first to report such an association despite all negative findings previously. The reason for the discrepancy in results may be related to the differences in patient population, type of concentration data, study duration, and the methodology used in statistical analysis. The patient population in the current study was relatively heterogeneous when compared to those of the others; 16% of our patients were Blacks versus almost all Whites or Chinese in the other studies (17–21). Our data were longitudinal with an average of 4.4 data points/patient lasting up to one year post SRL initiation, and were analyzed by a mixed-effects model that took into account the intra-subject variability as well as various confounding factors. It is calculated that the post-hoc powers to detect the observed mean differences in SRL log (C/D) at day 7, assuming the observed effects are true effects, were 67% and 6.6% for *ABCB1* 3435C>T and *IL-10* -1082G>A, respectively. These powers were increased to 97% and 18%, respectively, when the observed average means across all time points and the “ball park” sample sizes ($n = 190$) for longitudinal studies were used. The “ball park” sample sizes represent the numbers of study subjects as if each subject only contributes one observation, taking into consideration the correlation between repeated observations ($\rho = 0.291$ in this study) (30). Finally, we did not make assumptions on the genetic model for the loci being examined; the co-dominant, dominant, and recessive models were all tested for each SNP. This testing allowed us to select the model that best fitted our data for the *ABCB1* 3435C>T, which was found to be the dominant model and was significantly associated with SRL log (C/D). The co-dominant model, in which the mean of the heterozygotes falls between the two homozygote means, may not be the best model for

our data because SRL concentration/dose ratios were higher in the CT heterozygotes than in the TT homozygotes (Table 2). Notably, concentration data from all previous studies were also inconsistent with a co-dominant model (17–21,21). Presumably, the lack of associations in some of these studies may be attributed to analyzing the data with respect to three instead of two genotype groups, as well as the cross-sectional nature of the studies. The “true” genetic model of *ABCB1* 3435C>T and its association with long-term SRL dose requirements remain to be confirmed in future studies.

The higher SRL log (C/D) in *ABCB1* 3435CT heterozygotes compared to the TT homozygotes is perplexing. It may be related to the complex interplay between *ABCB1* genotype/P-gp phenotype, CYP3A4 expression, as well as physiological and environmental influences. As discussed earlier, CYP3A4 was a more efficient catalyst of SRL metabolism than CYP3A5 *in vitro* (29). It was also found to be the major factor, rather than P-gp, in limiting SRL absorption in CYP3A4-transfected Caco-2 cells (31). Therefore, CYP3A4 phenotype may play a more significant role in SRL trough concentrations compared to other drug metabolizing enzymes and transporters. Previous studies have shown that baseline hepatic CYP3A4 expression was increased in *Abcb1* knockout mice (32) as well as *ABCB1* 2677T allele carriers (33), and higher jejunal CYP3A4 mRNA levels were observed in women with *ABCB1* 2677TT-3435TT haplotype than those with 2677GT-3435CT haplotype (34). Presumably, CYP3A4 is increased in *ABCB1* variants due to the up-regulation of the major CYP enzyme regulator PXR, an effect that could be caused by higher concentrations of PXR ligands such as endogenous steroids, which are also P-gp substrates (35). This increase could in turn compensate for the effect of reduced P-gp activity as a result of *ABCB1* 3435C>T. It can be hypothesized that in 3435TT homozygotes, the enhanced CYP3A4 activity overrode the effect of reduced P-gp transport causing a smaller overall increase in SRL log (C/D). It remains to be determined the relative contribution of *ABCB1* 3435CT heterozygotes and TT homozygotes to SRL disposition and pharmacokinetics.

In addition to *ABCB1* 34354C>T, our results demonstrated that *IL-10* -1082GG homozygotes had higher SRL concentration/dose ratios, consistent with enhanced *IL-10* expression (15) leading to lowered CYP3A activity (13) and reduced SRL metabolism in patients with this genotype. This result appears to agree with previous finding showing *IL-10* -1082A carriers as a risk factor for steroid dependency at one year after transplantation (36). However, because of multiple testing in this study and the borderline significance of this SNP in the final model, the association of *IL-10* -1082G>A with SRL dose requirements cannot be certain. Regardless of this *IL-10* genotype, simulation of our final model showed that SRL trough concentrations would be in the range of 8 – 10 ng/mL for a 70-kg patient with *ABCB1* 3435CC genotype receiving 5-mg SRL without concomitant medications (Supplemental Digital Content 2). These levels are lower than the 12 – 20 ng/mL concentration range that is recommended in the package insert for cyclosporine withdrawal in patients receiving SRL-cyclosporine combination, and suggest that patients with *ABCB1* 3435CC genotype may be at a higher risk for rejection and may require more intensive immunosuppression as well as slow steroid weaning. While it is important to perform future studies to validate our results and evaluate the effects of *ABCB1* SNPs on outcomes and adverse effects, the current study indicates that genetics can play a significant role in SRL dosing and may be useful in TDM of SRL in renal transplantation.

Materials and Methods

Patients and Data Collection

The study population consisted of renal transplant recipients who participated in one of the treatment protocols at the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Transplantation Branch between 1999 and 2006.

Patients were enrolled if they received or switched to SRL as the maintenance immunosuppressive therapy. Informed consent was obtained from all patients in the screening protocol for genetic analysis. This study, as well as the screening and treatment protocols, was approved by the NIDDK Institutional Review Board. SRL, and all other prescribed immunosuppressive medications, was provided for all patients free of charge to lessen the variability associated with limited access to drug.

Clinical data including patient demographics, type and date of transplantation, induction and maintenance immunosuppression therapies, hematocrit, and concomitant medications were collected for all patients from the NIDDK transplant database. Most of the patients were started on oral SRL loading dose followed by daily maintenance therapy. Whole blood SRL trough concentrations were measured as clinically indicated. SRL dosage was adjusted to maintain levels between 15–20 ng/mL when given alone or between 10–12 ng/mL when administered concurrently with tacrolimus (Prograf®, Astellas, Deerfield, IL) as specified in the parent protocols. Dose adjustments were also performed if patients experience thrombocytopenia, severe arthralgias or other side effects known to be associated with this agent. SRL daily dosages and pre-dose concentrations (C) at 7 days and at 1, 3, 6, and 12 months post initiation of SRL were collected for each patient and SRL log (C/D) were calculated. SRL whole blood concentrations were measured by a validated high-performance liquid chromatography tandem mass spectrometric assay with a quantification limit of 2 ng/mL.

Genotyping

Genomic DNA was isolated from whole blood using the Gentra® DNA purification kit (Gentra Systems, Minneapolis, MN) according to the manufacturer's instructions. Genotyping of *ABCB1* 1236C>T (rs1128503), 2677G>T/A (rs2032582), and 3435C>T (rs1045642), *CYP3A4* -392A>G (rs2740574), *CYP3A5* 6986 A>G (rs776746) and 14690G>A (rs10264272), *IL-10* -1082G>A (rs1800896), and *TNF* -308G>A (rs1800629) were determined using the Sequenom™ MassArray iPLEX™ platform (37) by Bioserve™ (Beltsville, MD). This platform had an average error rate of < 0.2% and provided call rates of ≥ 95% on our samples. Quality control was achieved by typing internal positive control samples of known genotypes with no template controls. In initial assay development, DNAs from 20 individuals from Coriell's Polymorphism Discovery Resource were used.

Statistical analyses

Deviation from the Hardy-Weinberg equilibrium was assessed using the Pearson's Chi-squared test, using the observed genotype frequencies obtained from the data and the expected frequencies obtained from the Hardy-Weinberg principle. The longitudinal changes in SRL log C/D were analyzed using a linear mixed-effects model, which incorporates correlation inherent within observations from the same individual. The outcome variable, C/D, and the primary independent time variable, number of post-operative day was log-transformed prior to analysis to achieve a normal distribution.

Initially, various covariates were examined using a mixed-effects model and backward elimination to select the confounding factors that had significant impact on SRL log (C/D). Univariate analysis was then performed to investigate the effect of each SNP on log (C/D) under the co-dominant, dominant, or the recessive genetic model, with adjustments for the selected covariates. Under the co-dominant model, genotypes were coded into three separate groups i.e. major allele homozygote, heterozygote, and minor allele homozygote. Under the dominant model, genotypes were coded into two groups, which consisted of the major allele homozygote as one group and heterozygote and the minor allele homozygote as the other group. Under the recessive model, the minor allele homozygote was coded as one group and

the heterozygote and the major allele homozygote as the other group. The major allele homozygote was used as the reference group for the co-dominant and dominant models. The group containing at least one major allele was designated as the reference group for the recessive model. The major allele is the more common allele and the minor allele is the less common allele. The genetic model that best fitted the concentration data for each SNP was chosen based on the AIC. The model with the lowest AIC value was the best model.

Finally, a multivariate analysis was performed using the genetic models that showed significant associations in the univariate analysis, with adjustments for the same covariates as previously determined. Statistical analysis was performed using the open source statistical software* R (version 2.5.0). P-values <0.05 were considered statistically significant. Adjustments for multiple testing were not performed. As such, our results are considered exploratory and hypothesis generating.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ABCB1	Adenosine triphosphate-binding cassette, subfamily B, member 1
AIC	Akaike's information criterion
C/D	dose-adjusted, weighted-normalized trough concentration
CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4
CYP3A5	cytochrome P450, family 3, subfamily A, polypeptide 5
IL-10	interleukin 10
P-gp	p-glycoprotein
PXR	pregnane X receptor
SNP	single nucleotide polymorphism
SRL	sirolimus
TDM	therapeutic drug monitoring
TNF	tumor necrosis factor

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Table 1

Demographics at the time of transplantation and the induction and maintenance immunosuppressive therapies of the 86 patients included in the analysis

Gender (M/F)	52 / 34
Age (yr) ^a	44.2 (20.6 – 68.3)
Weight (kg) ^b	77.4 ± 17.5
Body mass index (kg/m ²) ^b	26.8 ± 6.65
Race	
White	69
Black	14
Asian	3
Type of transplantation	
Kidney/Kidney-pancreas	83/3
First/Second transplant	82/4
Induction therapy	
Depletional (rabbit anti-thymocyte globulin or alemtuzumab) with intraoperative steroids	76
Daclizumab with intraoperative steroids	6
Daclizumab alone	2
Steroids alone	2
Maintenance immunosuppressive therapy	
SRL/TAC ± MMF	29
SRL/TAC/PRED ± MMF	27
SRL/PRED ± MMF	18
SRL ± MMF	12
Time between transplantation and SRL initiation (days) ^a	10(6–432)
SRL daily dose (mg/kg) ^b	0.0584 ± 0.0337
SRL trough concentration (ng/mL) ^b	11.0 ± 6.09

Abbreviations: MMF, mycophenolate mofetil; TAC, tacrolimus; PRED prednisone

^aData expressed as median (range);

^bdata expressed as mean ± standard deviation

Table 2

Univariate analysis of ABCB1, CYP3A4, CYP3A5, IL-10, and TNF genetic polymorphisms with SRL log (C/D) in renal transplant patients

Gene	Nucleotide	Model	Coefficient	Standard Error	P Value	AIC Value
<i>ABCB1</i>	1236C>T	Co-dominant	0.0770	0.0442	0.085	-7.05
		Dominant	0.139	0.0578	0.0182	
		Recessive	0.0955	0.0408	0.0193	-7.89
			0.102	0.0544	0.0591	-5.98
<i>ABCB1</i>	2677G>T/A	Co-dominant	0.0880	0.0458	0.0582	-9.16
		Dominant	0.145	0.0516	0.0063	
		Recessive	0.111	0.0400	0.0056	-10.1
			0.110	0.0490	0.0251	-7.43
<i>ABCB1</i>	3435C>T	Co-dominant	0.183 *	0.0421	<0.0001	-18.6
		Dominant	0.135 **	0.0510	0.01	
		Recessive	0.167	0.0387	<0.0001	-19.7
			0.0445	0.0512	0.381	-3.19
<i>CYP3A4</i>	-392A>G	Co-dominant	-0.0211	0.0534	0.693	-0.744
		Dominant	-0.0348	0.0767	0.651	
		Recessive	-0.0252	0.0465	0.584	-2.72
			-0.0306	0.0760	0.684	-2.58
<i>CYP3A5</i>	6986A>G	Co-dominant	-0.0848	0.0552	0.128	-2.88
		Dominant	-0.0378	0.0682	0.581	
		Recessive	-0.0672	0.0464	0.146	-4.53
			-0.200	0.0682	0.767	-2.51
<i>CYP3A5</i>	14690G>A	Dominant	0.208	0.108	0.0551	-6.10
<i>IL-10</i>	-1082G>A	Co-dominant	0.0488	0.0456	0.288	-6.76
		Dominant	0.140	0.0553	0.0130	
		Recessive	0.0773	0.0435	0.0763	-5.56
			0.111	0.0484	0.0228	-7.60

Gene	Nucleotide	Model	Coefficient	Standard Error	P Value	AIC Value
<i>TNF</i>	-308G>A	Co-dominant	0.0513	0.0451	0.259	-2.30
			0.179	0.218	0.415	
		Dominant	0.0550	0.0446	0.215	-3.96
		Recessive	0.163	0.219	0.452	-2.99

* *ABCB1* 3435CT;

** *ABCB1* 3435TT