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Melding Pharmacogenomic Effect of *MDR1* and CYP3A5 Gene Polymorphism on Tacrolimus Dosing in Renal Transplant Recipients in Northern India

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Introduction: Tacrolimus (TAC) is the mainstay immunosuppressant for renal transplantation. A narrow therapeutic index, multiple drug interactions, and interindividual variability in pharmacokinetics make it obligatory to monitor therapeutic drug levels. The *Multidrug resistance gene 1* (*MDR1*) and CYP3A5 gene polymorphism may blend to achieve the optimal level. The optimal dose as per body weight is difficult to single out in the early posttransplantation period. In this study, we aimed to analyze the melding effect of both gene polymorphisms and to elicit the dose depending on the combination of genetic single nucleotide polymorphisms (SNPs) in northern Indian transplant recipients, for whom data are limited.

Methods: The daily TAC dose, weight-adjusted doses (mg/kg per day), TAC trough blood concentration (average of at least 3 levels), dose normalized with a corresponding dose using TAC concentration/weight-adjusted dose ratio (ng/ml per mg/kg per day) of 248 patients were recorded. All recipients were genotyped for the SNPs of CYP3A5 at intron 3 A6986G (the *3 or *1 allele), *MDR1* at exons 12 (C1236T), 21 (G2677A/T), and 26 (C3435T). We analyzed the blending effect of mutant SNPs of the *MDR* gene and CYP3A5 for optimized TAC levels.

Results: Among CYP3A5 genotypic variants, the dose-adjusted TAC level was significantly lower, and the TAC dose required to achieve the target level was significantly higher, in CYP3A5*1*1 (expressor) than that of CYP3A5*1*3 and CYP3A5*3*3. Of the *MDR1* gene SNPs, only the G2677T/A homozygous mutant was significantly associated with TAC level, and it was strongly correlated with P-gp expression.The daily TAC dose requirement was highest with a combination of CYP3A5*1*1 and homozygous mutant TT+AA genotype of G2677T/A, and was lowest with CYP3A5*3*3 and wild-type GG of the G2677T/A genotype.

Conclusion: Both CYP gene and *MDR1* gene polymorphism affect TAC dose requirements, and there is a need to look for both in an individual to achieve the target trough concentration.

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The calcineurin inhibitor TAC is the cornerstone immunosuppressant for any solid organ transplantation. It has reduced the rate of acute and chronic rejection.[1] However, due to the narrow therapeutic index, multiple drug interactions, and greater interindividual variability in pharmacokinetics, therapeutic drug monitoring is essential to achieve optimal immunosuppression while avoiding undue adverse effects.[1,2]

The pharmacokinetics of TAC are regulated predominantly by cytochrome P450 enzymes and gene (CYP3A4 and CYP3A5); moreover, TAC is also affected by P-glycoprotein (P-gp) and *MDR1* gene. P-glycoprotein is a product of the multidrug-resistance gene *MDR1*, commonly expressed in a variety of tissues, such as those of the intestines, renal tubular cells, hepatocytes, and peripheral blood T-lymphocytes.[3–5] It works as a transmembrane efflux pump that exports

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xenobiotics from inside to outside the cell in order to prevent cytotoxicity, and limits luminal absorptions as well.[6] On the other hand, systemic clearance of TAC is performed mainly via CYP3A5 isoenzymes and, to a lesser extent, via CYP3A4, which belong to cytochromes P450 superfamily expressed in the gut and liver.[7] There is a possibility that polymorphism of these genes can cause significant phenotypic differences in their expression. Polymorphism of the *MDR1* gene will affect drug absorption, and that of CYP3A5 will affect systemic clearance.[8] This may correlate with interindividual variation in the pharmacokinetics of TAC and hence affect the therapeutic drug level. Racial differences in polymorphisms of this gene have been reported.[9,10] Despite the use of TAC in clinical practice for a long time, its optimal use and role in pharmacogenetics in the individualization of the therapy is still in infancy, and singling out a starting dose to achieve a therapeutic level is cumbersome in day-to-day clinical practice. Moreover, all polymorphisms may not be clinically relevant to all populations. There is a paucity of data from northern Indian patients, particularly studies combining the *MDR1* gene and CYP3A5 in the same cohort of patients and their effect on trough levels of TAC in the early posttransplantation period. This study was conducted to evaluate the influence of *MDR1* and CYP3A5 gene polymorphisms on daily TAC dosage in northern Indian renal transplant patients so that pharmacogenetics could be used to individualize the therapy.

METHODS

Patients

In this prospective observational study, a total of 255 living donor renal transplant patients were recruited at our institute between October 2015 and September 2018. We recruited only those kidney transplant patients who met the following inclusion criteria: (i) TAC-based immunosuppressive regimens; (ii) no delayed graft function; and (iii) no clinical history of taking medications known to interact with calcineurin inhibitors, such as antimycotics (fluconazole and ketoconazole), calcium channel blockers (diltiazem, nicardipine, and verapamil), macrolide antibiotics (erythromycin and clarithromycin, and antiepileptics (phenytoin and carbamazepine). The study was approved by the Institutional Ethics Committee and conducted as per ethics standards laid down by the Declaration of Istanbul. Informed written consent was obtained from each patient. Patients who developed delayed graft function (n = 4) and acute rejection (n = 3) were excluded. Finally, data for 248 patients including 41 female individuals with a mean age of 34.40 ± 10.77 years (range 18–60 years), and a mean weight of 55 ± 9.63 kg were analyzed.

All recipients were on triple immunosuppressive TAC, mycophenolate mofetil, and steroid. The initial dose of tacrolimus (Tacromus, Zydus, India) was 0.15 mg/kg per day in 2 divided doses from day minus 2 of transplantation, and the dose was then adjusted to achieve trough blood TAC concentration (Co) of 8 to 12 ng/ml at 12 hours, which is advocated for first 3 months of transplantation at our institute.

Tacrolimus Trough Level Monitoring and C/D Ratio Assessment

Blood trough TAC concentration (Co) was measured by chemiluminescent microparticle enzyme immunoassay (Abbott Co., Ltd., Plano, TX) with 200 µl blood in ethylenediamine tetraacetic acid (EDTA) tubes after 12 hours at the previous dose. The daily TAC dose was recorded, and weight-adjusted doses (daily TAC requirement) were calculated by the ratio of daily TAC dose/weight (mg/kg per day). TAC trough blood concentration was measured and then dose normalized with a corresponding dose using a TAC Co/weight-adjusted dose ratio (ng/ml per mg/kg per day).

Genotype Analysis

DNA Isolation and Genotyping

All recipients were also genotyped for the SNP of CYP3A5 at intron 3 A6986G (the *3 or *1 allele, rs776746), *MDR1* at exons 12 (C1236T,rs128503), 21 (G2677A/T,rs2032582), and 26 (C3435T,rs1045642). In brief, peripheral blood samples (1.0 ml) were collected in EDTA-treated tubes and genomic DNA was extracted with a QIA amp Blood kit (Qiagen, Hilden, Germany) and stored at −20 °C. The SNPs were identified using the polymerase chain reaction–restriction fragment length polymorphism method. The primers, restriction enzymes, and polymerase chain reaction conditions are shown in Table 1 as used in the previous study.[11]

P-gp Expression Assay

The P-gp expression on lymphocytes was analyzed from whole blood. A 50-µl quantity of heparinized blood was incubated with 20 µl phycoerythrin-conjugated human anti−P-gp mAb (BD Pharmingen, San Diego, CA) and 20 µl phycoerythrin-conjugated matched-isotype control antibody for 30 minutes at room temperature. Then, red blood cells were lysed with BD FACS lysing solution and washed twice in phosphate-buffered saline solution. Subsequently, peripheral blood mononuclear cells were fixed with 0.4% paraformaldehyde and analyzed on a FACS Canto (Becton Dickinson, San Diego, CA). At least 10,000 cells were counted and analyzed and separated according to

Table 1. Primers, restriction enzymes, and polymerase chain reaction conditions used for identifying single nucleotide polymorphisms

Marker	Primer sequence	Primer annealing conditions	Enzyme	Allele	Restriction digestion product size
CYP3A5*1/*3	F 5′-CATCAGTTAGTAGACAGATGA-3′ R 5′-GGTCCAAACAGGGAAGAAATA-3′	55 °C for 1 min	*SspI*	*1 *3	148, 125, 20 (W) 168, 125 (M)
MDR C3435T	F 5′- TCTTTTCAGCTGCTTGATGG-3′ R 5′-AAGGCATCTATGTTGGCCTC-3′′	61 °C for 30 s	*SauIIIAI*	C T	39, 158 (W) 197 (M)
MDR C1236T	F 5′-TATCCTGTGTCTCTGAATTGCC-3′ R 5′ CCTGACTCACCACACCAATG-3′	63.7 °C for 30 s	*HaeIII*	C T	269, 97 (W) 269, 62, 35 (M)
MDR G2677T	F 5′-TGCAGGCTATAGGTTCCAGG-3′ R 5′-TTTAGTTTGACTCACCTTCCCG-3′	64.3 °C for 30 s	*BanI*	G T	224 (W) 198, 26 (M)
			RsaI	G A	224 (W) 198, 26(M)

M, mutant; W, wild-type.

their forward and side scatter characteristics. Results are expressed as the percentage of positive cells for P-gp and relative fluorescence intensity as performed in our previous study.[12]

Statistical Analyses

Data were analyzed using IBM SPSS software version 20 (IBM Corp., Armonk, NY). The categorical values were presented as frequencies and percentages, and the continuous variables as mean values with standard deviations. The Student *t* test was used to compare the mean values between the 2 groups when appropriate. One-way analysis of variance using Bonferroni (Dun) methods for multiple comparisons was used to find significant differences in mean values between the groups.

Table 2. Demographic and clinical characteristics of the renal transplant recipients

Characteristics	Values
Sex	
Male	207 (83.5%)
Female	41 (16.5%)
Mean age (yr)	34.40 ± 10.77 (18–60)
Height (cm)	162.05 ± 11.27
Weight (kg)	55 ± 9.63
Body mass index (kg/m^2)	20.85 ± 2.46
Serum creatinine at discharge (mg/dl)	1.117 ± 0.287 (0.49–2.29)
Native kidney disease	
CGN	110 (44%)
CIN	65 (26%)
Diabetic kidney disease	21 (9%)
CKDu	8 (3%)
ADPKD	7 (3%)
DGGS	7 (3%)
Alport syndrome	3 (1%)
Others	27 (11%)
Tacrolimus dose and adjusted level	
TAC dose (mg/day)	6.19 ± 1.91
Weight-adjusted dose (mg/kg per day)	0.118 ± 0.038
TAC level (Co) (ng/ml)	11.91 ± 4.47
Dose-adjusted TAC (ng/ml per mg/kg per day)	119.53 ± 79.80

ADPKD, autosomal dominant polycystic kidney disease; CGN, chronic glomerulonephritis; CIN, chronic interstitial nephritis; CKDu, chronic kidney disease of unknown etiology; DGGS, diffuse glomerulosclerosis; TAC, tacrolimus.

RESULTS

Demographic characteristics of the patients are summarized in Table 2. A total of 248 living donor kidney transplant recipients (207 male and 41 female, mean age 34.40 ± 10.77 years), were enrolled. The native kidney diseases of the recipients included 110 cases of chronic glomerulonephritis, 65 of interstitial nephropathy, 21 of diabetic nephropathy, 8 of chronic kidney disease of unknown etiology, 7 of autosomal-dominant polycystic kidney disease, 7 of diffuse global glomerulosclerosis, 3 of Alport syndrome, and 27 cases of other, undefined causes.

Adjusted Tacrolimus Doses and Levels of Transplant Recipients

The details of the TAC doses, weight-adjusted doses, trough levels, and dose-adjusted TAC are shown in Table 2. The daily TAC dose and level were recorded a minimum of 3 times (day 3, day 7, and day 11) in each patient for dose stabilization to achieve a target trough level of 8 to 12 ng/ml.

The mean daily doses and levels were included in the final analysis. The mean TAC dose was 6.19 ± 1.91 mg/d, and the daily TAC dose requirement was 0.118 ± 0.038 mg/kg per day. The TAC level (Co) was 11.91 ± 4.47 ng/ml, and the dose-adjusted TAC level was 119.53 ± 79.80 ng/ml per mg/kg per day.

CYP3A5 and *MDR1* Genotypic Distribution in Recipients

CYP3A5 and *MDR1* genotypic distribution in recipients are shown in Table 3. On analyzing CYP3A5 A6986G. the polymorphisms CYP3A5*1/*1 (AA), CYP3A5*1/*3 (AG), and CYP3A5*3/*3 (GG) were observed in 31 (12.5%), 94 (37.9%), and 123 (49.59%) cases, respectively, whereas A and G alleles were

Table 3. Genotype and allele distribution of CYP3A5 and *MDR1* gene in renal transplant recipients

CYP3A5 A6986G genotype	Genotypes	Number (%)
AA	*1/*1 (%)	31 (12.5%)
AG	*1/*3 (%)	94 (37.90%)
GG	*3/*3 (%)	123 (49.59%)
	A allele	156 (31.45%)
	G allele	340 (68.55%)
MDR1 genotype		
C1236T	CC (%)	109 (43.95%)
	CT (%)	111 (44.75%)
	TT (%)	28 (11.29%)
	C allele	329 (66.33%)
	T allele	167 (33.66%)
G2677T/A	GG (%)	99 (39.91%)
	GT (%)	89 (35.88%)
	GA (%)	15 (6.04%)
	TT (%)	40 (16.12%)
	AA (%)	5 (2.01%)
	G allele	302 (60.88%)
	T allele	169 (34.07%)
	A allele	25 (5.04%)
C3435T	CC (%)	29 (11.69%)
	CT (%)	137 (55.24%)
	TT (%)	82 (33.06%)
	C allele	195 (39.31%)
	T allele	301 (60.68%)

observed in 156 (31.45%) and 340 (68.55%) recipients, respectively.

For *MDR1* C1236T, the genotypes CC, CT, and TT were observed in 109 cases (43.95%), 111 cases (44.75%), and 28 cases (11.29%), respectively; and C and T alleles were found in 329 cases (66.33%) and 167 cases (33.66%), respectively. The *MDR1* G2677T/A genotypes wild-type GG, heterozygous mutant GT and GA, homozygous mutant TT and AA, and alleles G, T, and A frequencies were 99 recipients (39.91%), 89 (35.88%), 15 (6.04%), 40 (16.12%), 5 (2.01%), 302 (60.88%), 169 (34.07%), and 25 recipients (5.04%), respectively. Moreover, in *MDR1* C3435T mutation, the frequencies of CC, CT, and TT genotypes and C and T alleles were 29 (11.69%), 137 (55.24%), 82 (33.06%), 195 (39.31%), and 301 (60.68%), respectively, in our cohort (Table 3).

Effect of CYP3A5 A6986G Genotype on TAC Level and Their Disposition

According to CYP3A5 genotypic variations, recipients were mainly of 2 types: expressor (*1*1 homozygous and *1*3 heterozygous), and nonexpressor (*3*3 homozygous). The TAC dose requirement to achieve target level was significantly higher in CYP3A5*1*1 (0.138 ± 0.023 mg/kg per day) than that of CYP3A5*3*3 (0.11 ± 0.04 mg/kg per day; $P = 0.015$) and comparable to CYP3A5*1*3 (0.122 ± 0.036 mg/kg per day; $P = 0.381$). Daily TAC dose was significantly lower in CYP3A5*3*3 (5.63 ± 1.65 mg/d) than in CYP3A5*1*1 (7.01 ± 1.64 mg/d; $P = 0.014$) and CYP3A5*1*3 (6.68 ± 2.09 mg/d; $P = 0.003$) (Figure 1a and b).

The TAC level was significantly lower in CYP3A5*1*1 (7.39 ± 3.36 ng/ml) than that of both the genotypes CYP3A5*1*3 (11.42 ± 4.05 ng/ml; $P = 0.001$) and CYP3A5*3*3 (13.33 ± 4.26 ng/ml; $P < 0.001$). Moreover, the TAC level was also significantly lower in CYP3A5*1*3 than in CYP3A5*3*3 ($P = 0.022$). CYP3A5*1*1 patients had a lower level of dose-adjusted TAC (54.73 ± 27.33 ng/ml per mg/kg per day) to achieve target blood concentration than that of CYP3A5*1*3 (109.73 ± 70.84 ng/ml per mg/kg per day; $P = 0.022$) and CYP3A5*3/*3 patients (141.90 ± 85.02 ng/ml per mg/kg per day; $P < .001$), and the dose-adjusted TAC level was also significantly lower in CYP3A5*1*3 than in CYP3A5*3/*3 ($P = 0.043$) patients (Figure 1c and d)

Effect of *MDR1* Genotype in Recipient on TAC Dosing and Their Disposition

Considering the possible influence of *MDR1* SNPs on TAC pharmacokinetics in recipients, we finally assessed the effects of SNPs of *MDR1* on TAC dosing. As shown in Figures 2 and 3, we did not find any significant difference in TAC dose requirement, TAC Co, and dose-adjusted TAC level among the recipients with *MDR1* at position C1236T CC, CT, and TT genotype (Figure 2a–d) and among those with C3435T CC, CT, and TT genotype (Figure 3a–d).

However, patients with G2677T/A homozygous mutant TT+AA (0.14 ± 0.034 mg/kg per day) had significantly higher daily TAC dose per weight than wild-type GG (0.10 ± 0.033 mg/kg per day; $P < 0.001$) and heterozygous mutant GT+GA (0.11 ± 0.039 mg/kg per day; $P = 0.027$) genotype to achieve target trough levels (Figure 4a–d).

Significant differences in TAC blood trough levels and dose-normalized trough levels were observed between the groups. Homozygous mutant TT+AA (8.21 ± 2.10 ng/ml; 64.19 ± 35.09 ng/ml per mg/kg per day) displayed significantly lower TAC blood trough levels and dose-normalized trough levels than wild-type GG (12.48 ± 4.51 ng/ml, $P < 0.001$; 134.94 ± 73.41 ng/ml per mg/kg per day, $P < 0.001$) and heterozygous mutant GT+GA (12.97 ± 4.41 ng/ml, $P < 0.001$; 128.58 ± 89.73 ng/ml per mg/kg per day, $P = 0.001$) genotype (Figure 4). There were no significant differences in daily TAC dose requirements, TAC trough levels, and dose-normalized trough levels between wild-type GG and heterozygous mutant GT+GA variants.

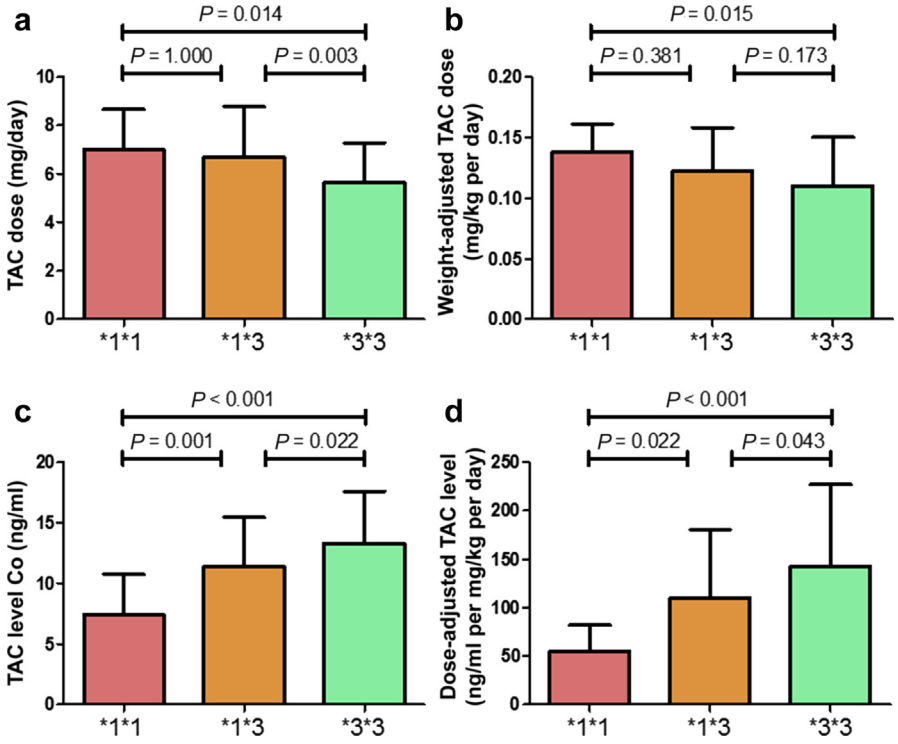

Figure 1. Daily (a) tacrolimus (TAC) dose, (b) TAC dose requirement, (c) TAC concentration (Co), and (d) dose-adjusted TAC level compared between recipients with CYP3A5 expressor (*1*1) and those with nonexpressor (*1*3 and *3*3).

MDR1 Genotypes, P-gp Expression, and TAC Concentration and Dosing

There was no significant difference in expression of P-gp among SNPs CC, CT, and TT of the recipients with *MDR1* gene C1236T and CC, CT, and TT genotypes of *MDR* gene C3435T. However, P-gp expression was significantly higher in recipients with homozygous mutant (TT+AA; 14.47 ± 3.24) compared to the

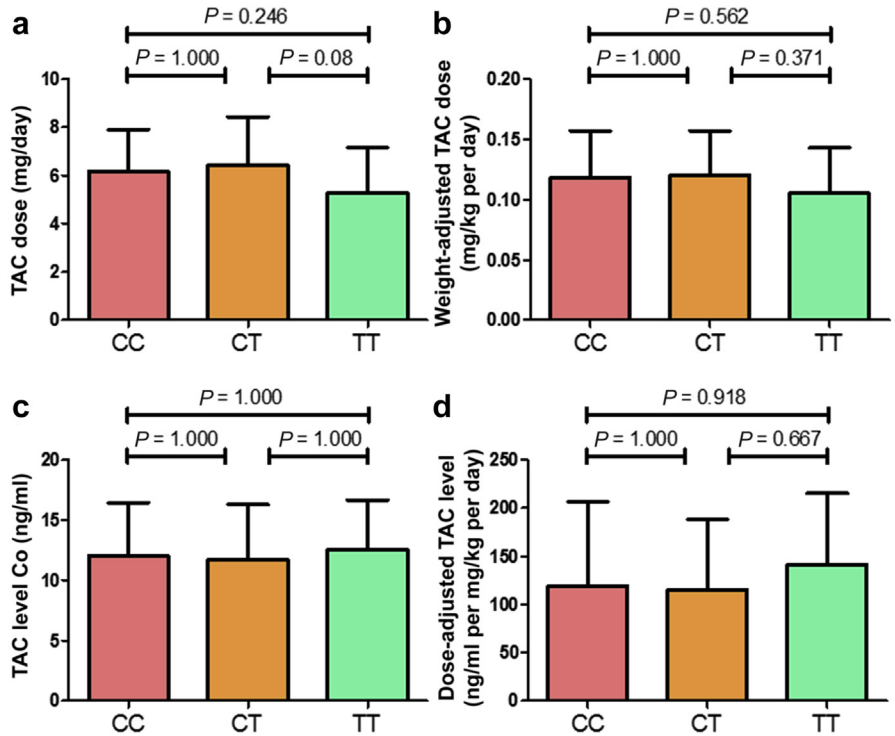

Figure 2. Daily (a) tacrolimus (TAC) dose, (b) TAC dose requirement, (c) TAC concentration (Co), and (d) dose-adjusted TAC level compared among recipients with wild-type homozygous CC, heterozygous CT, and mutant homozygous TT genotype of *MDR1* C1236T gene.

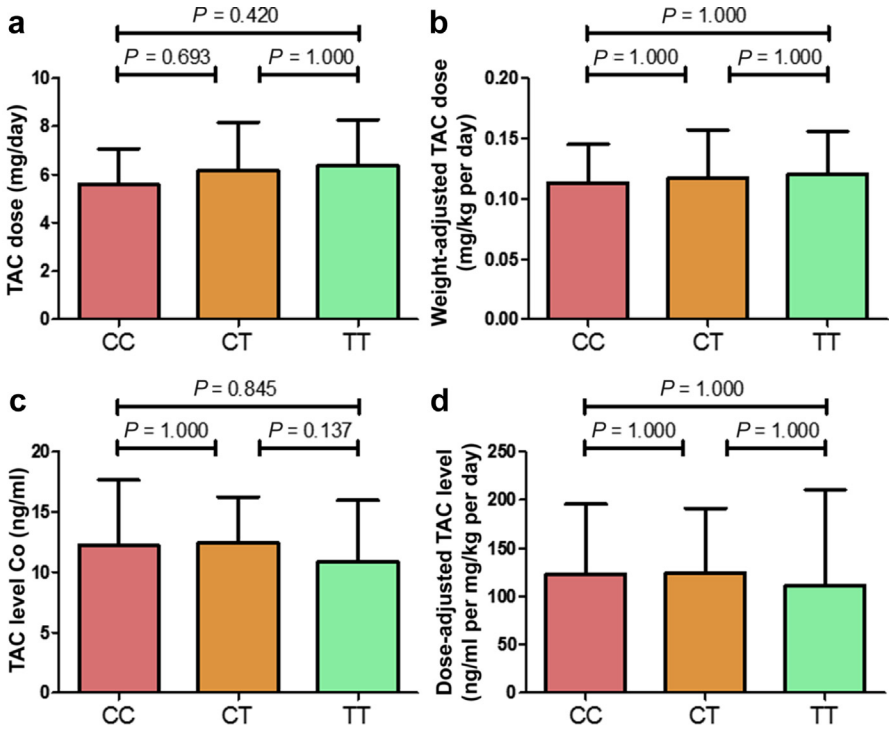

Figure 3. Daily (a) tacrolimus (TAC) dose, (b) TAC dose requirement, (c) TAC concentration (Co), and (d) dose-adjusted TAC level compared among recipients with wild-type homozygous CC, heterozygous CT, and mutant homozygous TT genotype of *MDR1* C3435T gene.

wild-type (GG; 6.08 ± 3.42, $P < 0.001$) and heterozygous mutant (GT+GA; 6.47 ± 3.58, $P < 0.001$) of *MDR* gene G2677T/A. However, there was no significant difference between wild-type and heterozygous mutants (Figure 5).

On analyzing the correlation of the P-gp expression, with TAC concentration and dosing, we found a significant positive correlation with daily TAC dose ($r = 0.519$, $P < 0.001$) and daily dose requirement ($r = 0.534$, $P < 0.001$) (Figure 6a and b) and

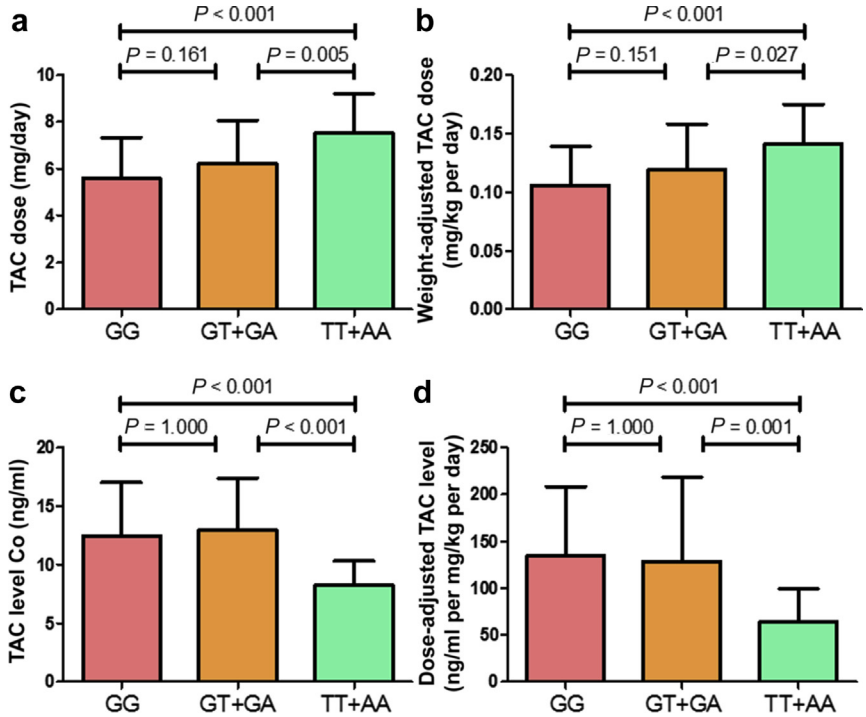

Figure 4. Daily (a) tacrolimus (TAC) dose, (b) TAC dose requirement, (c) TAC concentration (Co), and (d) dose-adjusted TAC level compared among recipients with wild-type homozygous GG, heterozygous GT+GA, and mutant homozygous TT+AA genotype of *MDR1* G2677T/A gene.

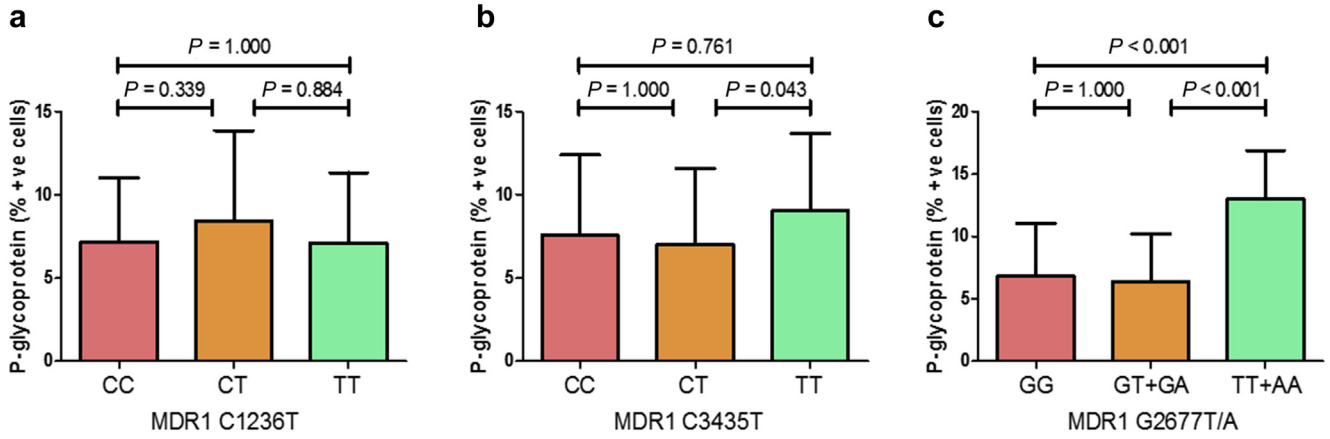

Figure 5. P-glycoprotein (P-gp) expression in different variants at positions C1236T, C3435T, and G2677T/A of *MDR1* gene.

negative correlation between P-gp and TAC blood trough levels ($r = -0.702$, $P < 0.001$) and dose-adjusted TAC level ($r = -0.735$, $P < 0.001$) (Figure 6c and d).

Synergistic Effect of CYP3A5 and *MDR1* Genotype

On analyzing the combined effect of CYP3A5 and *MDR1* G2677T/A genotype (Table 4), we observed that patients with a combination of expressor CYP3A5*1*1 and homozygous mutant TT+AA of G2677T/A required the highest daily TAC dose to meet the target trough level of TAC. Moreover, the lowest daily TAC dose requirement was found while melding patients with nonexpressor CYP3A5*3*3 and wild-type GG of G2677T/A genotype.

On multiple comparison test using analysis of variance and the Bonferroni method to see the combined effect of CYP3A5 *1*1, *1*3, and *3*3 genotypes and *MDR1* G2677T/A genotypes, there were significant differences in dose requirement with *MDR* G2677T GG ($P = 0.003$) and TT/AA ($P = 0.024$) genotypes. With *MDR* G2677T GG, the daily TAC dose requirement to achieve target level was significantly higher in GG*1*1 (0.135 ± 0.012 mg/kg per day) than that of GG*1*3 (0.106 ± 0.032 mg/kg per day; $P = 0.017$) and GG*3*3 (0.101 ± 0.027 mg/kg per day; $P = 0.002$) genotype. In *MDR* G2677T TT/AA variant, the daily TAC dose requirement to achieve target concentration was significantly lower in TT/AA*3*3 (0.116 ± 0.021 mg/kg per day) than that of both TT/AA*1*1 (0.147 ± 0.032 mg/kg per day; $P = 0.035$) and

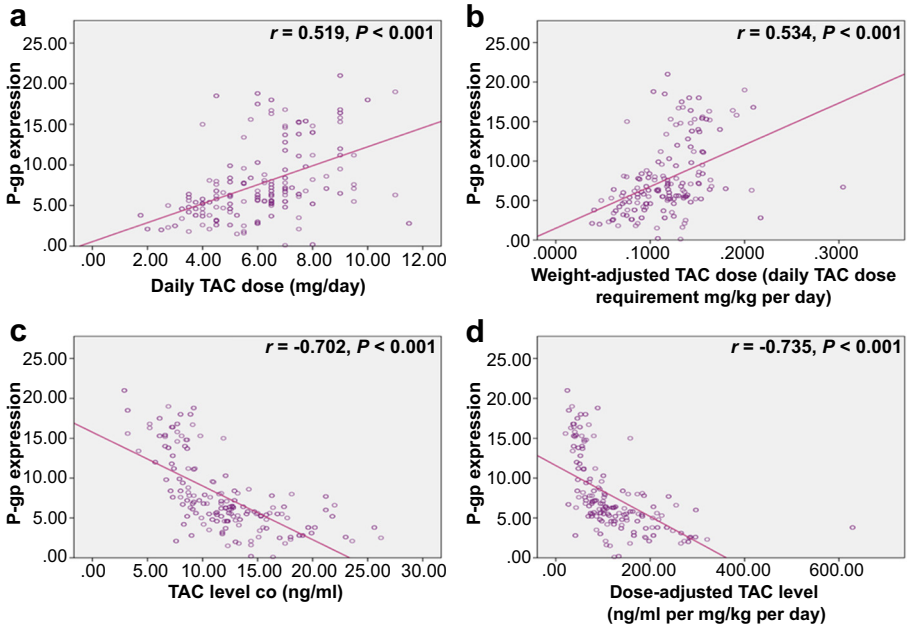

Figure 6. Correlation of P-glycoprotein (P-gp) expression with tacrolimus (TAC) dose, daily TAC dose requirement, TAC level, and dose-adjusted TAC level. There was (a,b) a positive correlation with TAC dose and daily TAC dose requirement and (c,d) a negative correlation with TAC level and dose-adjusted TAC level.

Table 4. Daily tacrolimus (TAC) dose requirement in different combinations of *MDR* G2677T/A and CYP3A5 genotype to achieve target TAC trough concentration

Genotype combinations	Recipients (n)	Dose requirement (mg/kg per d)
GG*3*3	56	0.101
GG*1*1	10	0.135
GG*1*3	35	0.106
GA/GT*1*1	8	0.128
GA/GT*3*3	57	0.114
TT/AA*3*3	10	0.116
GA/GT*1*3	37	0.125
TT/AA*1*3	22	0.143
TT/AA*1*1	13	0.147

TT/AA*1*3 (0.143 ± 0.027 mg/kg per day; $P = 0.049$) genotype (Table 5).

Table 5. Multiple comparison testing with analysis of variance and Bonferroni method for daily dose requirement between different groups

Daily tacrolimus dose requirement (mg/kg per d) in different genotype combinations					
Genotype	*1*1	*1*3	*3*3	*P* value	Multiple comparisons ($P < 0.05$)
GG	0.135 ± 0.012 (n = 10)	0.106 ± 0.032 (n = 35)	0.101 ± 0.027 (n = 56)	0.003	GG*1*1 vs. GG*1*3; $P = 0.017$ GG*1*1 vs. GG*3*3; $P = 0.002$
GA/GT	0.128 ± 0.007 (n = 8)	0.125 ± 0.037 (n = 37)	0.114 ± 0.04 (n = 57)	0.293	—
TT/AA	0.147 ± 0.032 (n = 13)	0.143 ± 0.027 (n = 22)	0.116 ± 0.021 (n = 10)	0.024	TT/AA*1*1 vs. TT/AA*3*3; $P = 0.035$ TT/AA*1*3 vs. TT/AA*3*3; $P = 0.049$

DISCUSSION

In this study, we observed that the expressor (CYP3A5*1*1) of CYP3A5 required a higher TAC dose than the nonexpressor to achieve the target trough concentration, and the mean dose difference between the 2 was significant. We also found the melding effect of the combination of expressor and homozygous mutant SNPs of *MDR1* gene G2677T/A required the highest dose per kilogram of weight, whereas the combination of nonexpressor and wild-type GG of G2677T/A genotype required the lowest dose per kilogram to meet the required trough concentration of TAC.

Variability in the pharmacokinetics of TAC depends on polymorphisms of the genes involved in transport and metabolism of the drug.[13,14] P-gp, a product of the *MDR1* gene, is a membrane transporter glycoprotein that acts as an efflux pump, which effluxes drugs out of cells from inside, preventing the absorption of drugs, resulting in limited availability of the drug at the site of action within the cells. P-gp is expressed on luminal cells of the intestine and affects the absorption of the drug and thus the drug level indirectly.[3–5,14] In the present study, we estimated P-gp on peripheral blood lymphocytes rather than luminal tissue because it is a relatively easy and noninvasive way to assay, and the translatability into day-to-day clinical practice is relatively more than a luminal biopsy and assessing P-pg on immunohistochemistry. Systemic clearance of TAC happens via CYP3A5 isoenzymes, a member of the cytochrome P450 superfamily.[7,13] The activity of these 2 proteins, which is dependent on genetic polymorphism of corresponding genes, determines the ultimate drug level.[13–15]

In our study, we examined gene polymorphism of membrane transporter (*MDR1* C1236T, *MDR1* C3435T, and *MDR1* G2677T/A) and metabolizer (CYP3A5 A6986G) proteins. Among them, SNPs of *MDR1* G2677T/A and CYP3A5 A6986G genes were significantly associated with drug level. Gene polymorphism of rest (*MDR1* C1236T, and *MDR1* C3435T) did not affect drug level. A nonsignificant association of *MDR1* C1236T and *MDR1* C3435T gene polymorphisms and ponderously significant association of *MDR1* G2677T/A in steroid-resistant nephrotic syndrome in a large cohort of northern Indian individuals from the general population and nephrotic syndrome patients was observed in our previous study.[11] In the present study, the dose-adjusted TAC level was significantly lower in TT+AA, followed by heterozygous mutant GT+GA and wild-type GG, and there were no significant differences in dose-adjusted TAC and TAC dose requirement among *MDR1* C3435T and C1236T SNPs. The study further affirms the applicability of *MDR1* G2677T/A gene in the assessment of drug dosing and resistance in the population of northern India. Analysis of *MDR1* G2677T/A gene polymorphism, wild-type GG, heterozygous mutant GT and GA, and homozygous mutant TT and AA, were prevalent in 39.91%, 41.92%, and 18.13% in this study cohort.

The association data of TAC metabolism with *MDR1* gene is not consistent and varied with the patient population. Shi *et al.*,[16] Jun *et al.*,[17] and Kurzawaski *et al.*[18] have not shown an association of TAC with ABCB1 gene polymorphisms. However, several studies reported that *MDR1* gene SNPs are associated with daily TAC requirement.[19–24] Anglicheau *et al.*[14] found that TAC dose requirement was generally lower in patients with exon 21 and 26 SNPs.[14] The most important relationship was reported for the exon 21 2677G>T/A SNPs, and the dose requirement was 40% higher in homozygous than in the wild-type carriers.[14] Hoffmeyer *et al.* reported that SNP in exon 26 3435C>T was associated with variations in intestinal expression and function of P-gp.[23] Li *et al.* also showed interactive

effects of CYP3A4, CYP3A5, and *MDR1* polymorphisms on TAC trough concentrations.[25] It is possible that P-gp acts in synergy with the CYP3A subfamily in limiting intestinal absorption of various drugs.[26] One study showed that genetic polymorphisms in exon26 (3435C>T) were correlated with the cellular expression level of P-gp in relation to ABCB1 mRNA stability and/or the protein's timing of cotranslational folding.[27]

In a recent study from southern India, Fernando *et al*.[28] also did not show an association of TAC with the ABCB1 gene; however, they studied only C3435T. Other genes G2677T/A, C1236T, and P-gp expression have not been studied. We studied all 3 genes including P-gp expression and found an association with G 2677T/A, which is consistent with our previous other study findings as well.[11,12] In order to analyze the functional part of *MDR1* SNPs, we observed that P-gp expression was significantly higher in recipients with homozygous mutant (TT+AA) compared to the wild-type (GG) and heterozygous mutant (GT+GA) at position G2677T/A. There was a significant negative correlation between P-gp expression and dose-adjusted TAC level.

The CYP3A5 enzyme is responsible mainly for hepatic elimination of TAC. It may potentially affect TAC pharmacokinetics, particularly in patients with CYP3A5/4 gene mutations.[24,29] The CYP3A5 gene polymorphism accounts for a key element of the interindividual variability observed with TAC bioavailability.[30]

Analysis of the CYP3A5 A6986G gene polymorphism shows the almost equal prevalence of expressor (CYP3A5*1*1/*1*3, 50.41%) and nonexpressor (CYP3A5*3*3, 49.59 %) in our study population. Among expressors, the prevalence of CYP3A5*1*3 polymorphism (37.9%) is higher than that of CYP3A5*1*1 (12.5%). CYP3A5 SNPs are distributed differently among races. The CYP3A5*1 allele was found in 5% to 15% of Caucasians, 15% to 35% of Asians, 25% of Mexicans, and 45% to 73% of African Americans, which is similar to our study.[13,31] In another study from northern India, Singh *et al*.[32] showed the distribution of CYP3A5 SNPs as expressor in 54.54% and nonexpressor 45.45%. Loh *et al*.,[15] in a study of Asian renal transplant populations, also showed expressor in 51% and nonexpressor in 49%. These prevalence findings of expressor and nonexpressor are similar to our findings of expressor in 50.41% and nonexpressor in 49.59%. However, a small Indian transplant population study of 100 patients by Ashivaid *et al*. reported that the distribution of CYP3A5*1*1, CYP3A5*1*3, and CYP3A5*3*3 SNPs among were 3%, 62%, and 35%, respectively.[33] The prevalence of homozygous expressor CYP3A5*1*1 (3%) was less than 12% in our study.-This variation is probably due to the small sample size in the study by Ashivaid *et al*.[33]

We have clearly observed that among CYP3A5 genotypic variants, dose-adjusted TAC level was significantly lower, and the TAC dose required to achieve the target level was significantly higher, in CYP3A5*1*1 than those of CYP3A5*1*3 and CYP3A5*3*3. A similar observation was made in other studies.[15,20,21,32,34]

In this study, we tried to observe the melding effect of CYP3A5 and *MDR1* genotype together. The study evidently showed that the daily TAC dose requirement was highest with CYP3A5*1*1 and homozygous mutant TT+AA of G2677T/A genotype, and lowest with CYP3A5*3*3 and wild-type GG of G2677T/A genotype. To achieve the therapeutic TAC level of 8 to 12 ng/ml, we need to start the TAC dose at 0.147 mg/kg body weight in cases with a combination of mutant of G2677T/A and expressor (TT/AA*1*1), and at 0.101 mg/kg BW in cases of combination of wild-type G2677T/A and nonexpressor (GG*3*3) genotype.

The study adds to the value on the existing literature in the sense that we used both *MDR1* gene and CYP p450 SNPs, both having an independent effect on TAC level, in the same cohort of patients. The study has potential utility in terms of providing a numerical figure to determine the dose of tacrolimus in terms of milligrams per kilogram per day to achieve initial target level in early posttransplantation, depending on the SNP combination.

CONCLUSION

To conclude, the CYP gene and *MDR1* gene polymorphisms affect the TAC dose required to achieve the target trough concentration. Expressors of CYP require a higher dose than nonexpressors. The homozygous mutant SNP of G2677A/T requires a higher dose than the heterozygous and wild-type genotypes. The combinations of expression (CYP) and mutant (*MDR1*) required the highest dose, and the nonexpressor and wild-type (GG of G2677T/A) genotype the lowest dose, to achieve the target trough level.

DISCLOSURE

All the authors declared no competing interests.

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