Influence of *CYP3A5* and *ABCB1* gene polymorphisms and other factors on tacrolimus dosing in Caucasian liver and kidney transplant patients

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Received July 13, 2011; Accepted August 29, 2011

DOI: 10.3892/ijmm.2011.794

Abstract. Tacrolimus is a substrate of cytochrome P4503A (CYP3A) enzymes as well as of the drug transporter ABCB1. We have investigated the possible influence of *CYP3A5* and *ABCB1* single nucleotide polymorphisms (SNPs) and other factors (e.g. albumin, hematocrit and steroids) on tacrolimus blood levels achieved in a population of Caucasian liver (n=51) and kidney (n=50) transplant recipients. At 1, 3 and 6 months after transplantation, tacrolimus doses (mg/kg/day) and trough blood levels (C_0) were recorded and the weight-adjusted tacrolimus dosage (mg/kg/day) was calculated. Polymerase chain reaction followed by restriction fragment length polymorphism analysis was used for genotyping *CYP3A5*1* and **3* [6986A>G] as well as *ABCB1* at exons 21 [2677G>T/A] and 26 [3435C>T] in both liver transplant donors and recipients and in kidney transplant recipients. Of the 152 subjects studied, 84.9% showed a *CYP3A5*3/*3* genotype. The total frequency of the allelic variant *3 was 93%. For the *G2677T/A* and *C3435T* polymorphisms the total frequencies of the allelic variants T/A and T were 44.7 and 46.7%, respectively. At 1, 3 and 6 months after transplantation the dose-adjusted C_0 levels were significantly lower in patients with one copy of the *1 allele compared to those homozygous for the *3 allele. In the case of liver transplant patients the tacrolimus dose requirements were dominantly influenced by the polymorphisms of the *CYP3A5* gene in the donors. With regard to the *ABCB1* SNPs, in general they did not show any appreciable influence on tacrolimus dosing requirements; however, kidney transplant recipients carrying the 2677T/A allele required significantly higher daily tacrolimus doses than subjects homozygous for the wild-type allele. Identification of *CYP3A5* single nucleotide polymorphisms prior to transplantation could contribute to evaluate the appropriate initial dosage of tacrolimus in the patients.

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Key words: tacrolimus, single nucleotide polymorphisms, CYP3A5, ABCB1, liver and kidney transplant patients

Introduction

Tacrolimus is an immunosuppressive drug widely used and of great benefit in transplant patients. However, its narrow therapeutic index and elevated inter- and intra-individual pharmacokinetic variability require the close monitoring of its administration regimens (1). Achieving the desired target blood concentrations as soon as possible after transplantation is crucial to avoid rejection or excessive immunosuppression and to limit significant dose-related adverse reactions such as nephrotoxicity, neurotoxicity and hyperglycemia (1). To date, trough whole blood concentration (C_0) represents the most common parameter used for the therapeutic monitoring of tacrolimus; it is measured 12 h after dose administration and correlates well with the area under the concentration-time curve (2,3).

Tacrolimus has poor bioavailability after oral administration (~25%; range, 4-93%) and is extensively metabolized by the cytochrome P4503A (CYP3A) oxidative enzymes CYP3A4 and CYP3A5 in the liver and small intestine (1,4). Furthermore, tacrolimus is also a substrate of P-glycoprotein (P-gp), a membrane drug efflux transporter encoded by the multi-drug resistance (*MDR1*) gene, also known as *ABCB1* (5). P-gp is present in several human tissues and organs and affects the disposition of several xenobiotics by limiting their absorption from the gut lumen and increasing their biliary and urinary excretion (6,7).

Several studies have suggested a link between the variability in the pharmacokinetics of tacrolimus and the polymorphisms of the *CYP3A5* and *ABCB1* genes (8,9). It is known that a single nucleotide polymorphism (SNP) *CYP3A5*3* in the homozygous state, correlates with reduced CYP3A5 activity because of alternative splicing and formation of a truncated protein that is

non-functional (10,11). Several studies have indeed indicated that this *CYP3A5* SNP can be associated with changes in tacrolimus dose requirements (9,12).

ABCB1 also has a polymorphic expression, with at least 50 SNPs identified to date (13). It has been suggested that some of these SNPs, like 2677G>T/A at exon 21 and 3435C>T at exon 26, may alter the expression and function of P-gp (13). Although 3435C>T is a synonymous polymorphism, it may be in linkage disequilibrium with some non-synonymous SNP of *ABCB1*, including 2677G>T/A, which causes a serine-alanine substitution and results in a low expression of intestinal P-gp (14). In addition, recent data have suggested that 3435C>T may reduce ABCB1 mRNA stability in the liver (15) or affect the insertion and folding of P-gp into the membrane resulting in an altered substrate specificity of the transporter (16).

Accordingly, at equivalent dose levels, subjects with the exon 21 and 26 polymorphisms in the *ABCB1* gene would experience greater tacrolimus bioavailability and level/dose ratios than those carrying the wild-type genotypes (13). However, the conclusions drawn so far on the actual influence of *ABCB1* SNPs on tacrolimus or also cyclosporine pharmacokinetics are largely controversial (12,14,17,18).

Importantly, the allele frequencies of the afore-mentioned *CYP3A5* and *ABCB1* SNPs can widely differ depending on ethnicities (19-21). Further, interactions have been described between tacrolimus and other drugs commonly used in transplantation, such as steroids. Steroids can induce both CYP3A and P-gp activity through pathways which involve different nuclear receptors and it is controversial whether their concomitant use with tacrolimus may change the pharmacokinetics of the latter agent with important clinical consequences (22). It has also been suggested that the *CYP3A5*1* non-carriers may be more susceptible to the inductive effects of steroids owing to their lower basal activity of CYP3A (23).

The principal objective of this study was to analyze the possible effect of the *CYP3A5* and *ABCB1* SNPs, also in conjunction with steroids use, on tacrolimus dose requirements in a Caucasian, mainly Sicilian, population of liver and kidney transplant patients. We examined also the possible influences of age, gender and plasma protein and hematocrit values on tacrolimus clearance.

Materials and methods

Subjects. A total of 101 subjects who underwent liver (n=39 male, 12 female) and kidney (26 male, 24 female) transplantation at the Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT) were consecutively included in this study. For the liver transplant patients we took into account also their co-respective donors (n=51). The average age of the liver transplant recipients was 54±12.30 (range, 15-68) years; all were Caucasians and 48 (94%) were Sicilians. The average age of the transplant donors was 42.80±20.30 (range, 15-82) years; 25 were males and 26 were females and all were Caucasians (70.6% Sicilians). Regarding the kidney transplant patients, the average age was 42.94±13.55 (range, 17-62) years; all were Caucasians and 46 (92%) were Sicilians.

After transplantation, the recipients were treated with tacrolimus, alone or in combination with steroids (mostly during the first month) and/or mycophenolate mofetil. The patients were checked to make sure that they were not taking any drug known to interact with tacrolimus. The average initial tacrolimus dose for liver and kidney transplant recipients was 0.017 and 0.05 mg/kg every 12 h, respectively. The dose was subsequently adjusted according to the whole blood trough (C_0) levels. The target C_0 level was set between 5 and 12 ng/ml for liver transplant patients and between 8 and 12 ng/ml for kidney transplant patients.

Body weight, laboratory data (albumin, serum creatinine and liver function tests), tacrolimus dosage (mg/kg/day) and C_0 levels were recorded at 1, 3 and 6 months after transplantation. Blood samples for tacrolimus C_0 levels determinations were drawn just prior to the morning dose. Whole blood tacrolimus concentrations were measured by EMIT 2000 immunoassay (DADE Behring, Hilden, Germany). The dose-adjusted C_0 level (L/D) was calculated by dividing the tacrolimus C_0 level by the corresponding 24 h dose. The information thus obtained was the tacrolimus dose needed to obtain a given C_0 level. The protocol was approved by the Institutional Review Board and Ethic Committee of ISMETT and informed consent was obtained from all patients.

Genotype identification. Recipient and donor genotypes were determined by analyzing 200 μl of EDTA-anticoagulated blood. Genomic DNA was extracted using the QIAmp DNA mini kit (Qiagen, Crawley, UK). The PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) assay was then applied to identify the *CYP3A5*3*, 2677G>T/A and 3435C>T genetic polymorphisms. We used 150 ng of genomic DNA for PCR amplification. The forward primer for *CYP3A5*3* was 5'-CAT CAG TTA GTA GAC AGA TGA-3' and the reverse primer was 5'-GGT CCA AAC AGG GAA GAA ATA-3'; the forward primer for 2677G>T/A was 5'-TAC CCA TCA TTG CAA TAG CAG-3' and the reverse primer was 5'-TTT AGT TTG ACT CAC CTT TCT AG-3'; the forward primer for 3435C>T was 5'-CAT GCT CCC AGG CTG TTT AT-3' and the reverse primer was 5'-GTA ACT TGG CAG TTT CAG TG-3'. PCR was performed in a total volume of 50 μl with 40 pmoles of each primer, 0.2 mM dNTP, 1X High Fidelity PCR buffer (600 mM Tris-SO_4 pH 8.9 and 180 mM $NHSO_4$), 2 mM $MgSO_4$ and 1.5 U of TaqDNA polymerase (Platinum Taq High Fidelity, Invitrogen, Carlsbad, CA, USA). PCR process included initial denaturation at 94˚C for 7 min, followed by 35 cycles of denaturation for 1 min at 94˚C, 35 cycles of annealing for 1 min at 55˚C, and 35 cycles of synthesis for 1 min at 72˚C. The final extension was carried out for 7 min at 72˚C. The PCR products (10 μl for each samples) were analyzed by electrophoresis on 1% agarose gel. The enzymatic digestion was performed for 2 h at 37˚C using specific restriction enzymes. For *CYP3A5*3* we used 15 μl of PCR product and 10 units of *Ssp*I plus 1X buffer (50 mM NaCl, 100 mM Tris-HCl, 10 mM $MgCl_2$ and 0.025% Triton X-100) in a total volume of 20 μl; for 2677G>T/A, we used 15 μl of PCR product and 20 units of *Xba*I plus 1X buffer (50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$ and 1 mM DTT) in a total volume of 20 μl; and for 3435C>T we used 10 μl of PCR product and 10 units of *Dpn*II plus 1X buffer (100 mM NaCl, 50 mM Bis Tris-HCl, 10 mM $MgCl_2$ and 1 mM dithiothreitol) in a total volume of 15 μl. The digestion products were then

Table I. Distribution of *CYP3A5* and *ABCB1* genotypes in liver transplant donors and recipients and in kidney transplant recipients.

Genotype	Allelic status	Liver transplant patients		Kidney transplant recipients (n=50) n (%)	Total expression % (n/152)
		Donors (n=51) n (%)	Recipients (n=51) n (%)		
*CYP3A5*3*	*3/*3	40 (78.4)	44 (86.3)	45 (90)	84.9 (129/152)
	*1/*3	10 (19.6)	6 (11.7)	5 (10)	13.8 (21/152)
	*1/*1	1 (2)	1 (2)	-	1.3 (2/152)
ABCB1 2677G>T/A (Exon 21)					
	G/G	15 (29.4)	18 (35.3)	15 (30)	31.6 (48/152)
	G/T	27 (52.9)	23 (45.1)	18 (36)	44.7 (68/152)
	G/A	1 (2)	-	3 (6)	2.6 (4/152)
	T/T	6 (11.7)	8 (15.7)	13 (26)	17.8 (27/152)
	T/A	2 (4)	2 (4)	1 (2)	3.3 (5/152)
ABCB1 3435C>T (Exon 26)					
	C/C	12 (23.5)	9 (17.6)	11 (22)	21 (32/152)
	C/T	26 (51)	26 (51)	26 (52)	51.3 (78/152)
	T/T	13 (25.5)	16 (31.4)	13 (26)	27.7 (42/152)

subjected to 3% agarose gel electrophoresis and detected by staining with ethidium bromide.

Genotype identification by PCR-RFLP was confirmed by random evaluation of at least 10 samples per gene SNP through sequencing. The PCR products were purified using the Wizard PCR Preps DNA Purification System kit (Promega, Madison, WI).

Direct sequencing of the amplified and purified amplicons was performed using a Cycle Sequencing Termination kit (ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kits, version 1.1) in an ABI PRISM 310 apparatus (Applied Biosystems, Foster City, CA).

Statistical analysis. All values were expressed as means ± SD and the two-tailed Mann-Whitney U test was employed to determine the difference in continuous values among groups. The χ^2 test was used to analyze differences in the frequencies between Sicilian and non-Sicilian subjects. The allele and genotype frequencies of the *CYP3A5* and *ABCB1* polymorphisms were assessed for deviation from the Hardy-Weinberg equilibrium using the χ^2 test. P-values <0.05 were considered to indicate statistically significant differences.

Results

Frequency of CYP3A5 and ABCB1 variants in liver transplant recipients and donors and in kidney recipients. As shown in Table I, among the 51 liver transplant recipients involved in the study, the *CYP3A5*3/*3* genotype was observed in 44 (86.3%) recipient cases, *CYP3A5*1/*3* in 6 (11.7%) cases and *CYP3A5*1/*1* in 1 (2%) case. For the corresponding donors, *CYP3A5*3/*3* was present in 40 (78.4%) cases, *CYP3A5*1/*3* in 10 (19.6%) cases and *CYP3A5*1/*1* in 1 (2%) case (Table I). Among the 50 kidney transplant recipients, the *CYP3A5*3/*3* genotype was observed in 45 cases and *CYP3A5*1/*3* in 5 cases. Overall, the frequency of *CYP3A5*3/*3* was 84.9% (129/152 subjects), that of **1/*3* 13.8% (21/152 subjects) and that of **1/*1* 1.3% (2/152 subjects). The total allelic frequency was 91.7% for *CYP3A5*3* and 8.3% for *CYP3A5*1*.

As for the *ABCB1* SNP at exon 21 (2677G>T/A), the G/G, G/T, T/T and T/A genotypes were found in 18 (35.3%), 23 (45.1%), 8 (15.7%) and 2 (4%) of the liver transplant recipients, respectively. The G/G, G/T, G/A, T/T and T/A genotypes were found in 15 (29.4%), 27 (52.9%), 1 (2%), 6 (11.7%) and 2 (4%) of the donors, respectively. For the kidney recipients, the G/G, G/T, G/A, T/T and T/A genotypes were observed in 15 (30%), 18 (36%), 3 (6%), 13 (26%) and 1 (2%) of the patients (Table I). The overall percentages of expression of G/G, G/T, G/A, T/T and T/A were 31.6 (48/152 subjects), 44.7 (68/152), 2.6 (4/152), 17.8 (27/152) and 3.3% (5/152), respectively (Table I). The total allelic frequencies of the G, T and A variants were 55.3, 41.7 and 3%, respectively.

For the SNP of *ABCB1* at exon 26 (3435C>T), among the liver transplant recipients, the C/C, C/T and T/T genotypes were observed in 9 (17.7%), 26 (51%) and 16 (31.3%) cases, respectively. Among the donors, the C/C, C/T and T/T genotypes were observed in 12 (23.5%), 26 (51%) and 13 (25.5%) cases, respectively. For the kidney recipients, the C/C, C/T and T/T genotypes were identified in 11 (22%), 26 (52%) and 13 (26%) cases, respectively (Table I).

The overall percentages of expression were 21 (32/152), 51.3 (78/152) and 27.7% (42/152) for the C/C, C/T and T/T genotypes, respectively (Table I). The total allelic frequency observed for the variant T was 46.7%.

The data showed strong linkage (49.3%) between the two *ABCB1* polymorphisms. The *CYP3A5* and *ABCB1* (exons 21

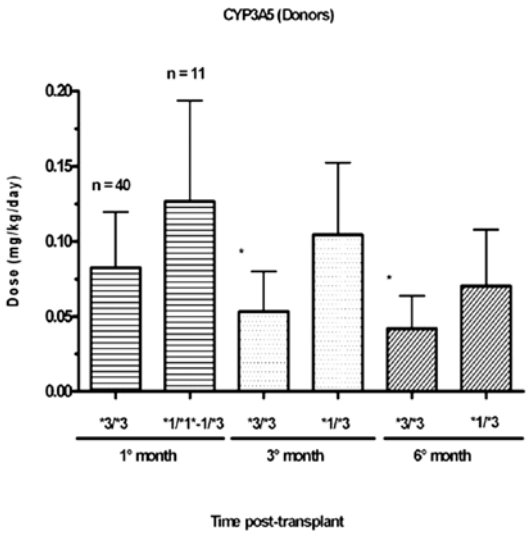

Figure 1. The tacrolimus doses (mg/kg/day) according to donors CYP3A5 genotypes at 1, 3 and 6 months after liver transplantation. *Designates that the differences between the groups were statistically significant (P<0.05).

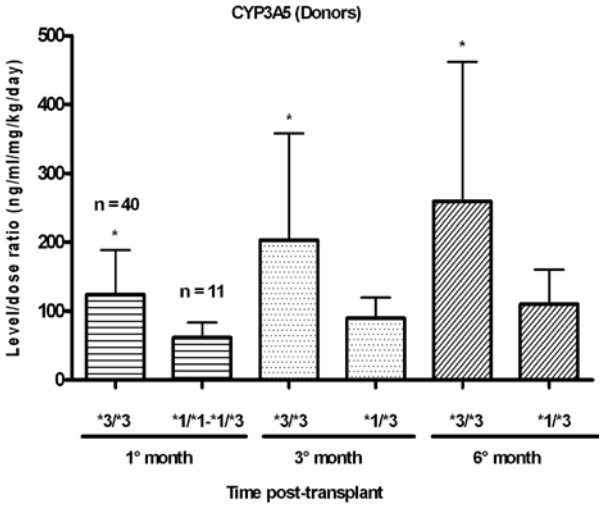

Figure 2. The tacrolimus blood level/dose ratios (ng/ml/mg/kg/day) according to donors CYP3A5 genotypes at 1, 3 and 6 months after liver transplantation. *Designates that the differences between the groups were statistically significant (P<0.05).

Table II. Tacrolimus blood L/D ratio (ng/ml per mg/kg/day) in different groups of combined *CYP3A5* genotypes in both donors and recipients at 1, 3 and 6 months after liver transplantation.

		L/D ratio (ng/ml/mg/kg/day)		
Group comparison	n	1 month	3 months	6 months
Non-expressor donors - Expressor vs. non-expressor	3	117.23±27.05	144.49±81.84	229.50±120.31
recipients	37	124.73±66.63	207.53±159.25	261.41±208.97
Non-expressor recipients - Expressor vs.	11	61.42±21.93[a]	89.91±29.75[a]	110.03±50.07[a]
non-expressor donors	37	124.73±66.63	207.53±159.25	261.41±208.97

[a]The differences between the groups were statistically significant (P<0.05).

and 26) genotype frequencies were not significantly different from those predicted by the Hardy-Weinberg equilibrium.

Effect of CYP3A5 and ABCB1 SNPs on tacrolimus dosage and level-dose ratio (L/D) in the liver transplant recipients. At 1, 3 and 6 months after liver transplantation, the average tacrolimus doses (mg/kg/day) were 0.092 (0.025-0.257), 0.064 (0.014-0.21) and 0.048 (0.006-0.177), respectively.

The tacrolimus doses and blood L/D ratios of liver recipients in relation to donors genotype are shown in Figs. 1 and 2. After 3 and 6 months of transplantation, at equivalent tacrolimus blood levels, the doses of the immunosuppressive agent required to reach the desired trough concentrations were significantly higher (P<0.05) in the group of subjects receiving a liver with at least one copy of allele *1 compared to the subjects receiving a liver homozygous for allele *3 (Fig. 1).

A similar, though of borderline statistical significance (P=0.0503), trend was observed one month after transplantation (Fig. 1); in addition, at that time the tacrolimus C_0 levels were substantially lower in the patients receiving livers with *1/*3 or *1/*1 alleles rather than with the *3/*3 allele.

A statistically significant difference was also evidenced by relating L/D ratios with the *CYP3A5* genotypes of the liver donors at 1, 3 and 6 months after transplantation (Fig. 2).

Also for the recipient genotypes, the presence of at least one *1 copy tended to increase tacrolimus doses and to lower dose-adjusted C_0 levels, though the differences with respect to the *3/*3 genotypes were statistically significant only at the third month (data not shown).

For further investigation we analyzed the effects of combining the *CYP3A5* genotypes of the donor and of the recipient (Table II). At 1, 3 and 6 months after liver transplantation, only the presence of the *CYP3A5*1* allele in donors, but not in recipients, had a statistically significant effect (P<0.05) on the tacrolimus L/D ratio.

Table III shows that, at equivalent tacrolimus trough blood levels, the presence, either in the liver donors or in the liver recipients, of the two examined *ABCB1* polymorphisms, did not have any significant effect on the tacrolimus doses or L/D ratios during the 6 months of observation.

Furthermore, we evaluated the influence of the *ABCB1* genotypes (according to both the donors and recipients

Table III. Relationship of *ABCB1* SNPs to tacrolimus C_0 blood levels, dose requirements and L/D ratio after liver transplantation.

	1 month			3 months			6 months		
	Level (ng/ml)	Dose (mg/kg/day)	L/D (ng/ml/mg/kg/day)	Level (ng/ml)	Dose (mg/kg/day)	L/D (ng/ml/mg/kg/day)	Level (ng/ml)	Dose (mg/kg/day)	L/D (ng/ml/mg/kg/day)
Donor genotype									
ABCB1 Ex-21									
G/G (n=41)	8.83±3.53	0.096±0.05	112.45±68.24	8.20±2.89	0.063±0.04	190.42±159.43	7.89±2.60	0.046±0.03	244.95±207.77
G/T (n=7)	7.23±3.33	0.069±0.02	109.28±44.2	8.31±2.41	0.068±0.02	127.12±29.21	7.31± 1.40	0.050±0.01	156.73±57.93
T/T (n=3)	6.81±1.90	0.076±0.01	88.93±18.24	8.83±1.80	0.069±0.01	134.61±46.00	8.20 ±0.75	0.062±0.02	143.58±48.96
G/T+T/T (n=10)	7.10±2.87	0.071±0.02	103.17±38.38	8.46±2.16	0.068±0.02	123.37±32.43	7.57±1.27	0.053±0.01	152.78±53.01
Recipient genotype									
ABCB1 Ex-21									
G/G (n=43)	8.67±3.59	0.092±0.04	109.32±57.22	8.17±2.80	0.062±0.03	184.53±154.55	7.86±2.48	0.046±0.02	236.62±202.38
G/T (n=6)	7.33±2.96	0.103±0.06	85.60±54.80	8.84±3.00	0.087±0.03	116.60±58.47	7.79±2.36	0.062±0.03	137.72±73.78
T/T (n=2)	8.10±0.14	0.050±0.03	213.94±120.20	8.30±0.84	0.038±0.01	233.40±73.22	7.40±0.00	0.028±0.01	285±120.20
G/T+T/T (n=8)	7.52±2.53	0.090±0.06	117.69±94.27	8.70±2.57	0.075±0.04	145.80±78.30	7.69±2.00	0.054±0.03	174.54±102.95
Donor genotype									
ABCB1 Ex-26									
C/C (n=12)	8.12±3.25	0.087±0.05	106.71±43.78	7.76±1.86	0.068±0.03	167.97±174.78	8.29±2.01	0.054±0.02	226.29±267.65
C/T (n=26)	8.19±3.31	0.089±0.048	115.97±76.11	8.37±2.22	0.066±0.043	167.45±100.13	8.17±2.53	0.048±0.033	222.50±116.48
T/T (n=13)	9.10±3.50	0.102±0.047	96.31±37.00	8.50±4.15	0.061±0.026	176.03±181.08	6.73±2.26	0.041±0.017	224.60±230.63
C/T+T/T (n=39)	8.49±3.38	0.093±0.047	109.42±65.82	8.41±2.99	0.064±0.038	170.31±130.26	7.69±2.51	0.046±0.028	223.20±160.39
Recipient genotype									
ABCB1 Ex-26									
C/C (n=9)	7.50±2.37	0.089±0.05	114.50±88.70	8.29±2.70	0.073±0.03	139.35±75.76	7.69±1.87	0.054±0.02	170.99±96.89
C/T (n=26)	9.00±3.64	0.086±0.035	121.28±64.05	8.44±3.03	0.061±0.040	208.54±188.34	8.31±2.53	0.048±0.033	270.92±247.58
T/T (n=16)	8.24±3.67	0.102±0.060	91.15±41.26	7.93±2.42	0.063±0.036	151.56±70.54	7.13±2.38	0.044±0.017	186.76±85.06
C/T+T/T (n=42)	8.71±3.63	0.092±0.046	109.80±57.82	8.24±2.79	0.062±0.038	186.83±155.67	7.86±2.51	0.046±0.028	238.86±204.29

Table IV. Relationship of *CYP3A5* and *ABCB1* SNPs to tacrolimus C_0 blood levels, dose requirements and L/D ratio after kidney transplantation.

	1 month			3 months			6 months		
Kidney transplant recipients	Level (ng/ml)	Dose (mg/kg/day)	L/D (ng/ml/mg/kg/day)	Level (ng/ml)	Dose (mg/kg/day)	L/D (ng/ml/mg/kg/day)	Level (ng/ml)	Dose (mg/kg/day)	L/D (ng/ml/mg/kg/day)
*CYP3A5*3/*3* (n=45)	12.81±3.55	0.119±0.098	160.41±114.19[a]	11.51±3.16	0.087±0.066[a]	187.13±98.17[a]	9.34±2.57	0.070±0.051	188.70±114.87[a]
*CYP3A5*1/*3* (n=5)	8.04±3.15	0.131±0.088	76.94±35.06	9.46±2.04	0.119±0.027	79.92±9.03	6.25±2.01	0.091±0.040	72.75±20.69
ABCB1 Ex-21									
G/G (n=15)	12.24±3.26	0.082±0.03	171.26±71.06	11.01±3.03	0.058±0.03	239.58±109.99	8.69±2.21	0.047±0.02	235.38±126.53
G/T (n=18)	12.21±4.27	0.106±0.07	148.54±85.56	11.09±2.66	0.098±0.06[a]	161.23±98.80[a]	9.20±3.41	0.087±0.06[a]	159.06±120.80[a]
G/A (n=3)	16.30±6.78	0.327±0.27	74.42±50.29[a]	16.45±6.27	0.212±0.11[a]	90.74±38.49[a]	10.36±2.05	0.086±0.04	139.54±72.84
T/A (n=1)	10.64	0.014	714.09	8.35	0.052	159.96	8.4	0.058	142.86
T/T (n=13)	11.82±2.75	0.142±0.05[a]	92.83±34.13[a]	10.98±2.13	0.089±0.04	146.34±59.82[a]	8.94±2.35	0.078±0.03[a]	146.16±84.41
G/T+G/A+T/T+T/A (n=35)	12.37±4.01	0.136±0.10[a]	137.65±123.19[a]	11.43±3.18	0.103±0.06[a]	149.62±81.34[a]	9.18±2.86	0.083±0.05[a]	152.13±100.89[a]
ABCB1 Ex-26									
C/C (n=11)	11.32±2.04	0.107±0.052	132.45±67.78	10.78±2.41	0.081±0.044	167.47±87.69	9.24±1.92	0.069±0.036	162.22±69.95
C/T (n=26)	12.53±4.15	0.115±0.07	157.11±137.27	10.74±2.42	0.090±0.05	162.82±89.97	9.21±3.16	0.078±0.05	168.37±111.30
T/T (n=13)	12.80±4.16	0.140±0.15	151.77±91.63	12.87±4.35	0.097±0.08	211.85±121.68	8.49±2.19	0.062±0.04	207.25±146.42
C/T+T/T (n=39)	12.62±4.10	0.123±0.106	157.59±121.25	11.45±3.29	0.092±0.068	178.93±102.35	8.97±2.87	0.073±0.054	181.30±124.68

[a]The differences between the groups were statistically significant ($P<0.05$).

Table V. Tacrolimus levels, doses and level/dose ratios in liver and kidney transplant patients carrying the *CYP3A5*3/*3* genotype at 1 and 3 months after transplantation treated or not treated with steroids.

	Treated with steroids				Not treated with steroids			
	n	Level	Dose	L/D	n	Level	Dose	L/D
Liver transplant	23				14			
1 month		8.59±4.23	0.082±0.042[a]	115.53±51.37[a]		9.13±2.34	0.079±0.029	139.83±86.21
3 months		8.57±3.29	0.050±0.025	222.50±184.29		8.26±2.22	0.059±0.030	182.94±108.21
Kidney transplant	37				8			
1 month		12.89±3.70	0.125±0.104[a]	145.48±114.54[a]		12.41±2.92[a]	0.087±0.062	202.24±101.71
3 months		11.97±3.26	0.092±0.066	174.79±88.93		9.36±1.20	0.063±0.060	245.47±125.00

Tacrolimus levels (L) (ng/ml), doses (D) (mg/kg/day) and level/dose (L/D) ratios (ng/ml/mg/kg/day) in liver and kidney transplant patients carrying the *CYP3A5*3/*3* genotype at 1 and 3 months after transplantation treated or not treated with steroids. [a]Within the patient groups the differences in tacrolimus levels, doses or L/D ratios between the first and third month after transplantation were statistically significant (P<0.05).

genetic profiles) by analyzing their role in association with the *CYP3A5* status. Therefore, all donors and recipients with at least one copy of the allele *1 were excluded from the analysis. Also in this case, as regards the 37 subjects examined, the two *ABCB1* polymorphisms had no statistically significant effect on tacrolimus pharmacokinetics (data not shown).

In kidney recipients, at 1, 3 and 6 months after transplantation, the mean dose of tacrolimus was equal to 0.120 (0.014-0.316), 0.090 (0.020-0.288) and 0.074 (0.013-0.157) mg/kg/day, respectively.

Again, the *CYP3A5* genotype appeared to have a determining effect: at 1, 3 and 6 months after transplant, the L/D ratio was statistically lower (P<0.05) in the 5 patients with one copy of allele *1 compared to patients homozygous for allele *3 (Table IV). Also, three months after the transplant, the dose required to maintain the drug trough blood levels was statistically lower (P<0.05) in the patients homozygous for allele *3 compared to the heterozygous patients (Table IV).

Interestingly, despite the higher doses administered to heterozygous patients, at 1 and 6 months after transplantation an equivalency of the trough blood levels was not achieved in comparison with the patients homozygous for allele *3.

In reference to the ABCB1 behavior in kidney recipients, during the six months of observation the C3435>T polymorphism did not affect the dosage or level/dose ratio, when the polymorphisms were evaluated among the subjects (n=45) homozygous for allele *3 (data not shown). However, as shown in Table III, subjects carrying the 2677T/A allele required a significantly higher daily tacrolimus dose than subjects homozygous for the wild-type allele.

We also found that after liver or kidney transplant there were increases in plasma albumin concentrations and in hematocrit values and statistical analysis showed that they were significantly associated with a reduction in the tacrolimus doses needed to reach the target dose levels (data not shown).

For steroids, in the present study these agents were used only in a part of the patients; they were administered in moderate doses and in general suspended one month after transplant. The results did not show any difference in the tacrolimus doses at 1 month and further between patients treated with steroids and not, also considering the different *CYP3A5* and *ABCB1* genotypes (data not shown). However, in the group of patients treated with steroids, the reduction of the doses between the first and third month (usually required to maintain the target blood levels) was significantly lower than in the patients without steroids. This was observed only in patients without CYP3A5 expression (Table V).

Discussion

The findings on the *CYP3A5* gene in our population are in agreement with literature data, according to which up to 90% of Caucasian subjects are homozygous for the non-functional variant *CYP3A5*3* (10,11,24,25): in our series the percentage of *CYP3A5*3/*3* subjects was 84.9 and the total frequency of the variant *3 was 91.7%. The percentage of *CYP3A5*3/*3* subjects is lower (~20-30%) in other ethnic groups, in particular among Africans and African Americans (10,11,25-27). Regarding the *ABCB1* gene polymorphisms, in the case of G2677T/A at exon 21, the total frequency of the variant T in our patients (41%) was similar to that of other Caucasian populations (40-50%) (19,20,28-30) and different from that recorded in Africans and African Americans (0.9-13%) (30-33). The allelic frequency of 2677A was 3%. In Caucasians the frequency of this allele is low (1-4%) and different from that reported for Asian populations like the Chinese (~6%) and Japanese (~20%) ones (20). As regards C3435T at exon 26, the total frequency of the variant T was 46.7%, also in agreement with data of other authors who have indicated that it is 33-65% among Caucasian populations (28-30,34-36).

Our data confirmed strong linkage between the two *ABCB1* polymorphisms, (12,14,30,37). The *ABCB1* 2677T-3435T (T-T) haplotype is present in ~32% of Caucasians, 62% of European Americans, 27% of Asian Americans and 35% of Mexican Americans (14,38). In other ethnic groups, like the African and African American populations, a lower T/T frequency and even the absence of a linkage disequilibrium between the two *ABCB1* polymorphisms have been reported (14,31,38,39).

Considering the relationship between genotype and tacrolimus dosage and L/D ratios, our data in both the liver and kidney transplant situations have confirmed the significant influence of CYP3A5 on tacrolimus pharmacokinetics. Subjects homozygous for the variant *3, likely owing to the lower metabolic drug clearance, require a lower tacrolimus dose to reach the desired therapeutic levels compared to the subjects with at least one *1 copy who are considered to express the functional enzyme (21,40,41). Similar results have been previously reported for liver (8,41-43), kidney (12,44-47), heart (48), and lung recipients (49). In particular, as regards to liver transplantation, our data support the primary importance of the donor with respect to the *CYP3A5* genotype (8,43,50-53); however, they suggest a possible influence of the recipient genotype also (52), which might be corroborated by the known metabolic role of CYP3A5 in extra-hepatic tissues such as the intestine (51,53).

Data from literature concerning ABCB1 suggest that subjects homozygous for the exon 26 3435T variant present a 2-fold reduction of intestinal P-gp, (54-56) which might influence the bioavailability of P-gp substrates such as tacrolimus and cyclosporine (14,50,56,57). Nonetheless, other studies focusing on this polymorphism in the context of kidney, liver and lung transplants, have generally excluded this role of T/T or at most indicated a minor influence of such recipient genotype in the disposition of tacrolimus and cyclosporine (14,17,18,43,49,52). Our data support these findings, confirming that the presence of the T allele in liver and kidney recipients does not affect the dose required to achieve the target levels of tacrolimus. On the other hand, the exon 21 G2677T/A polymorphisms, did not affect tacrolimus dose in our liver transplant patients, but increased it in kidney transplant patients, in contrast with other studies reporting that carriers of the G/G wild-type genotype require higher daily tacrolimus doses (13). We have no explanation for the last result; overall, however, as other authors have shown (58), the effect of *ABCB1* polymorphisms on tacrolimus dosage was not as evident as it was for the *CYP3A5*3* polymorphism.

Since the CYP3A activity may influence the contribution of P-gp on tacrolimus disposition, we analyzed the effect of the *ABCB1* polymorphisms only in the subjects with the *CYP3A5*3/*3* genotype. Also in this instance, both in liver and kidney recipients, the two *ABCB1* polymorphisms, with the exception of the G2677T/A polymorphism in kidney transplanted patients, did not show any statistically significant effect on the tacrolimus dose.

It has been proposed that in liver transplant patients, regardless of the status of the donors with respect to CYP3A5, the doses needed to reach the target trough blood levels may increase along time. This may be due to the fact that, early after transplantation, the liver cannot fully perform its metabolic function owing to the damage associated with ischemia and hepatic reperfusion (51).

However, our results did not show such time-related dose increases, but rather progressive reductions. This behavior was especially evident in the liver transplanted patients. Indeed, another multi-center study on liver transplant recipients suggested that, as time elapses, lower doses of tacrolimus are necessary to reach the same target concentrations in whole blood (59). The data of this study revealed that the low concentrations of plasma albumin and total proteins recorded soon after transplantation underwent subsequent constant increases and resumed normal levels within 4-8 weeks. The restore of plasma albumin and total protein concentrations could lead to a higher rate of bound tacrolimus in plasma and therefore to a reduction in tacrolimus clearance. Our data support the afore-mentioned findings; in particular, we found that after transplantation there were increases in plasma albumin concentrations and in hematocrit values. Statistical analysis showed that they were significantly associated with a reduction in the tacrolimus doses needed to reach the target dose levels (data not shown). A previous study also provided evidence of the influence of plasma albumin concentrations and hematocrit on the clearance of tacrolimus (60).

As regards to the influence of steroids, which were administered in not intensive doses, there was no difference in the tacrolimus doses between patients treated with these agents and not, also considering the different *CYP3A5* and *ABCB1* genotypes. However, the reduction in the doses between the first and third month in the *CYP3A5*3/*3* subjects treated with steroids was significantly lower than that in the patients with the same genotype but not treated with steroids. This result may perhaps support that the subjects lacking *CYP3A5*1* can undergo some inductive effects of these drugs on tacrolimus metabolism (23).

Some studies have evidenced that the CYP3A enzyme activity is low immediately after birth and that then it progressively increases reaching a peak during youth and adulthood and eventually decreasing in old age (61). Other studies have demonstrated that the P-gp activity in peripheral blood lymphocytes is greater in the umbilical cord, and that it steadily decreases with age (62). On the contrary, other authors have reported that the P-gp activity does not decline in old age (63). However, our data did not show any influence of age on the tacrolimus doses used during the six-month observation period (data not shown). Furthermore, it has been proposed, that drugs that are a substrate of both CYP3A and P-gp, such as tacrolimus, may undergo a greater clearance in women than in men following i.v. administration. Nevertheless our data did not suggest any statistically significant difference in the dosage between male and female patients, also taking into account the genotypes of both donors and recipients (data not shown).

Neurotoxicity is an important dose-limiting effect of calcineurin inhibitors and corticosteroids. On the other hand, P-gp is highly expressed in the hematoencephalic barrier, where its function is to limit drug access into the central nervous system. In a study carried out on 17 liver recipients treated with tacrolimus, the presence of the mutant T allele in exon 21 was a predictor of tacrolimus-related neurotoxicity (64). In our population only 1 patient developed neurotoxicity ascribable to the administration of tacrolimus. Interestingly, this patient exhibited a mutant T homozygous genotype at exon 21.

In conclusion, our study has demonstrated the high variability of the tacrolimus doses required to maintain the desired drug levels, even in subjects expressing the same genotype (with particular reference to the expression of functional CYP3A5), and the noticeable overlap of such doses in subjects with the wild-type and mutant genotypes.

This shows that genetic polymorphism is only one of the factors influencing tacrolimus pharmacokinetics, which, in

fact, can be modified by many other variables (51,65,66). Thus, genotyping cannot substitute but only support the therapeutic monitoring of the immunosuppressor blood concentrations. Nonetheless, *CYP3A5* genotyping may represent a tool especially useful to evaluate the appropriate initial dose of tacrolimus for transplant recipients, even in populations like the Caucasians which are fairly homogenous with respect to the lack of *CYP3A5*1*.

Acknowledgements

This study was supported by ISMETT and by the University of Palermo.

References

1. Venkataraman R, Swaminatan A, Prasad T, *et al*: Clinical pharmacokinetics of tacrolimus. Clin Pharmacokinet 29: 404-430, 1995.
2. Ihara H, Shinkuma D, Ichikawa Y, Nojima M, Nagano S and Ikoma F: Intra- and interindividual variation in the pharmacokinetics of tacrolimus (FK506) in kidney transplant recipients-importance of trough level as a practical indicator. Int J Urol 2: 151-155, 1995.
3. Jusko WJ: Analysis of tacrolimus (FK506) in relation to therapeutic drug monitoring. Ther Drug Monit 17: 596-601, 1995.
4. Sattler M, Guengerich FP, Yun CH, Christians U and Sewing KF: Cytochrome P-450 3A enzymes are responsible for biotransformation of FK506 and rapamycin in man and rat. Drug Metab Dispos 20: 753-761, 1992.
5. Saeki T, Ueda K, Tanigawara Y, Hori R and Komano T: Human P-glycoprotein transports cyclosporin A and FK506. J Biol Chem 268: 6077-6080, 1993.
6. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I and Willingham MC: Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissue: Proc Natl Acad Sci USA 84: 7735-7738, 1987.
7. Hall SD, Thumel KE, Watkins PB, *et al*: Molecular and physical mechanisms of first-pass extraction. Drug Metab Dispos 27: 161-166, 1999.
8. Provenzani A, Notarbartolo M, Labbozzetta M, *et al*: The effect of CYP3A5 and ABCB1 single nucleotide polymorphisms on tacrolimus dose requirements in Caucasian liver transplant patients. Ann Transplant 14: 23-31, 2009.
9. Macphee I, Fredericks S, Tai T, *et al*: Tacrolimus pharmacogenetics: polymorphisms associated with expression of cytochrome P4503A5 and P-glycoprotein correlate with dose requirement. Transplantation 74: 1486-1489, 2002.
10. Kuehl P, Zhang J, Lin Y, *et al*: Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet 27: 383-391, 2001.
11. Hustert E, Haberl M, Burk O, *et al*: The genetic determinants of the CYP3A5 polymorphism. Pharmacogenetics 11: 773-779, 2001.
12. Tsuchiya N, Satoh S, Tada H, *et al*: Influence of CYP3A5 and MDR1 (ABCB1) polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. Transplantation 78: 1182-1187, 2004.
13. Staatz CE, Goodman LK and Tett SE: Effect of CYP3A and ABCB1 single bucleotide polymorphisms on the pharmacokinetics and pharmacodynamics inhibitors: part I. Clin Pharmacokinet 49: 141-175, 2010.
14. Anglicheau D, Verstuyft C, Laurent-Puig P, *et al*: Association of the multidrug resistance-1 gene single-nucleotide polymorphisms with the tacrolimus dose requirements in renal transplant recipients: J Am Soc Nephrol 14: 1889-1896, 2003.
15. Wang D, Johnson AD, Papp AC, Kroetz DL and Sadée W: Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C>T affects mRNA stability. Pharmacogenet Genomics 15: 693-704, 2005.
16. Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV and Gottesman MM: A 'silent' polymorphism in the MDR1 gene changes substrate specificity. Science 315: 525-528, 2007.
17. Fredericks S, Moreton M, Reboux S, Carter ND, Goldberg L, Holt DW and MacPhee IA: Multi-drug resistance gene-1 (MDR-1) haplotypes have a minor influence on tacrolimus dose requirements. Transplantation 82: 705-708, 2006.
18. von Ahsen N, Richter M, Grupp C, Ringe B, Oellerich M and Armstrong VW: No influence of the MDR-1 C3435T polymorphisms or a CYP3A4 promoter polymorphism (CYP3A4-V allele) on dose-adjusted cyclosporine A trough concentrations or rejection incidence in stable renal transplant recipients. Clin Chem 47: 1048-1052, 2001.
19. Jeannesson E, Albertini L, Siest G, *et al*: Determination of ABCB1 polymorphisms and haplotypes frequencies in a French population. Fundam Clin Pharmacol 21: 411-418, 2007.
20. Cascorbi I, Gerloff T, Johne A, *et al*: Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. Clin Pharmacol Ther 69: 169-174, 2001.
21. Utecht KN, Hiles JJ and Kolesar J: Effects of genetic polymorphisms on the pharmacokinetics of calcineurin inhibitors. Am J Health Syst Pharm 63: 2340-2348, 2006.
22. Anglicheau D, Flamant M, Schlageter MH, *et al*: Pharmacokinetic interaction between corticosteroids and tacrolimus after renal transplantation. Nephrol Dial Transplant 18: 2409-2414, 2003.
23. Roberts PJ, Rollins KD, Kashuba AD, *et al*: The influence of CYP3A5 genotype on dexamethasone induction of CYP3A activity in African Americans. Drug Metab Dispos 36: 1465-1469, 2008.
24. van Schaik RHN, van der Heiden IP, van den Anker JN and Lindemans J: CYP3A5 variant allele frequencies in Dutch Caucasians. Clin Chem 48: 1668-1671, 2002.
25. Lamba JK, Lin YS, Shuetz EG and Thummel KE: Genetic contribution to variable human CYP3A-mediated metabolism. Adv Drug Deliv Rev 54: 1271-1294, 2002.
26. Macphee IA and Holt DW: A pharmacogenetic strategy for immunosuppression based on the CYP3A5 genotype. Transplantation 85: 163-165, 2008.
27. Dirks NL, Huth B, Yates CR and Meibohm B: Pharmacokinetics of immunosuppressants: a perspective on ethnic differences. Int J Clin Pharmacol Ther 42: 701-718, 2004.
28. Ifergan I, Bernard NF, Bruneau J, Alary M, Tsoukas CM and Roger M: Allele frequency of three functionally active polymorphisms of the MDR-1 gene in high-risk HIV-negative and HIV-positive Caucasians. AIDS 16: 2340-2342, 2002.
29. Cavaco I, Gil JP, Gil-Berglund E and Ribeiro V: CYP3A4 and MDR1 alleles in a Portuguese population. Clin Chem Lab 41: 1345-1350, 2003.
30. Kim RB, Leake BF, Choo EF, *et al*: Identification of functionally variant MDR1 alleles among Europeans Americans and Africans Americans. Clin Pharmacol Ther 70: 189-199, 2001.
31. Allabi AC, Horsmans Y, Bouchra I and Gala JL: Single nucleotide polymorphisms of *ABCB1* (*MDR1*) gene and distinct haplotype in a West Black African population. Eur J Clin Pharmacol 61: 97-102, 2005.
32. Tang K, Wong LP, Lee EJ, Chong SS and Lee CG: Genomic evidence for recent positive selection at the human MDR1 gene locus. Hum Mol Genet 13: 783-797, 2004.
33. Tang K, Ngoi SM, Gwee PC, Chua JM, Lee EJ, Chong SS and Lee CG: Distinct haplotype profiles and strong linkage disequilibrium at the MDR1 multidrug transporter gene locus in three ethnic Asian populations. Pharmacogenetics 12: 437-450, 2007.
34. Fromm MF: The influence of MDR1 polymorphisms on P-glycoprotein expression and function in humans. Adv Drug Deliv Rev 54: 1295-1310, 2002.
35. Pauli-Magnus C and Kroetz DL: Functional implications of genetic polymoprhisms in the multidrug resistance gene *MDR1* (*ABCB1*). Pharm Res 21: 904-913, 2004.
36. Ameyaw MM, Regateiro F, Li T, *et al*: MDR1 pharmacogenetics of the C3435T mutation in exon 26 is significantly influenced by ethnicity. Pharmacogenetics 11: 217-221, 2001.
37. Wang J, Zeevi A, McCurry K, *et al*: Impact of ABCB1 (MDR1) haplotypes on tacrolimus dosing in adult lung transplant patients who are CYP3A5*3/*3 non-expressors. Transpl Immunol 15: 235-240, 2006.
38. Kroetz DL, Pauli-Magnus C, Hodges LM, *et al*: Sequence diversity and haplotype structure in the human ABCB1 (MDR1, multidrug resistance transporter) gene. Pharmacogenetics 13: 481-494, 2003.
39. Wang H, Ding K, Zhang Y, Jin L, Kullo IJ and He F: Comparative and evolutionary pharmacogenetics of ABCB1: complex signatures of positive selection on coding and regulatory regions. Pharmacogenet Genomics 17: 667-678, 2007.

40. Day Y, Hebert MF, Isoherranen N, Davis CL, Marsh C, Shen DD and Thummel KE: Effects of CYP3A5 polymorphism on tacrolimus metabolic clearance in vitro. Drug Metab Dispos 34: 836-847, 2007.
41. Li D, Lu W, Zhu JY, Gao J, Lou YQ and Zhang GL: Population pharmacokinetic of tacrolimus and CYP3A5, MDR1 and IL-10 polymorphisms in adult liver transplant patients. J Clin Pharm Ther 32: 505-515, 2007.
42. Kalow W, Tang BK and Endrenyi L: Hypothesis: comparisons of inter- and intra-individual variations can substitute for twin studies in drug research. Pharmacogenetics 8: 283-289, 1998.
43. Goto M, Masuda S, Kiuchi T, *et al*: CYP3A5*1-carrying graft liver reduces the concentration/oral dose ratio of tacrolimus in recipients of living-donor liver transplantation. Pharmacogenetics 14: 471-478, 2004.
44. MacPhee IA, Fredericks S, Tai T, *et al*: The influence of pharmacogenetics on the time to achieve target tacrolimus concentration after kidney transplantation. Am J Transplant 4: 914-919, 2004.
45. Thervet E, Anglicheau D, King B, *et al*: Impact of cytochrome P450 3A5 genetic polymorphism on tacrolimus doses and concentration-to dose ratio in renal transplant recipients. Transplantation 76: 1233-1235, 2003.
46. Haufroid V, Mourad M, Van Kerckhove V, *et al*: The effect of CYP3A5 and MDR1 (ABCB1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. Pharmacogenetics 14: 147-154, 2004.
47. Roy JN, Barama A, Poirier C, Vinet B and Roger M: CYP3A4, CYP3A5, and MDR-1 genetic influences on tacrolimus pharmacokinetics in renal transplant recipients. Pharmacogenet Genomics 16: 659-665, 2006.
48. Zheng H, Webber S, Zeevi A, *et al*: Tacrolimus dosing in pediatric heart transplant patients is related to CYP3A5 and MDR1 gene polymorphisms. Am J Transplant 3: 477-483, 2003.
49. Zheng H, Zeevi A, Schuetz E, *et al*: Tacrolimus dosing in adult lung transplant patients is related to cytochrome P4503A5 gene polymorphism. J Clin Pharmacol 44: 135-140, 2004.
50. Wei-lin W, Jing J, Shu-sen Z, Li-hua W, Ting-bo L, Song-feng Y and Sheng Y: Tacrolimus dose requirements in relation to donor and recipients ABCB1 and CYP3A5 gene polymorphisms in Chinese liver transplant patients. Liver Transpl 12: 775-780, 2006.
51. Uesugi M, Masuda S, Katsura T, Oike F, Takada Y and Inui K: Effect of intestinal CYP3A5 on postoperative tacrolimus trough levels in living-donor liver transplant recipients. Pharmacogenet Genomics 16: 119-127, 2006.
52. Li D, Zhu JY, Gao J, Wang X, Lou YQ and Zhang GL: Polymorphisms of tumor necrosis factor-α, interleukin-10, cytochrome P450 3A5 and ABCB1 in Chinese liver transplant patients treated with immunosuppressant tacrolimus. Clin Chim Acta 383: 133-139, 2007.
53. Fukudo M, Yano I, Yoshimura A, *et al*: Impact of MDR1 and CYP3A5 on the oral clearance of tacrolimus and tacrolimus-related renal dysfunction in adult living-donor liver transplant patients. Pharmacogenet Genomics 18: 413-423, 2008.
54. Hitzl M, Drescher S, van der Kuip H, *et al*: The C3435T mutation in the human MDR1 gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from $CD56^+$ natural killer cells. Pharmacogenetics 11: 293-298, 2001.
55. Goto M, Masuda S and Saito H: C3435T polymorphism in the MDR1 gene affects the enterocyte expression level of CYP3A4 rather than Pgp in recipients of living-donor liver transplantation. Pharmacogenetics 12: 451-457, 2002.
56. Sakaeda T, Nakamura T and Okumura K: Pharmacogenetics of MDR1 and its impact on the pharmacokinetics and pharmacodinamics of drugs. Pharmacogenetics 4: 397-410, 2003.
57. Hoffmeyer S, Burk O, von Richter O, *et al*: Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci USA 97: 3473-3478, 2000.
58. Cheung CY, Op den Buijsch RA, Wong KM, *et al*: Influence of different allelic variants of the CYP3A and ABCB1 genes on the tacrolimus pharmacokinetic profile of Chinese renal transplant recipients. Pharmacogenomics 7: 563-574, 2006.
59. Undre NA and Möller A: Pharmacokinetic interpretation of FK 506 levels in blood and in plasma during a European randomised study in primary liver transplant patients. The FK 506 European Study Group. Transpl Int 7 (Suppl 1): S15-S21, 1994.
60. Hu RH, Lee PH and Tsai MK: Clinical influencing factors for daily dose, trough level, and relative clearance of tacrolimus in renal transplant recipients. Transplant Proc 32: 1689-1692, 2000.
61. May DG, Porter J, Wilkinson GR and Branch RA: Frequency distribution of dapsone-N-hydroxylase, a putative probe for P4503A4 activity, in a white population. Clin Pharm Ther 55: 492-500, 1994.
62. Machado CG, Calado RT, Garcia AB and Falcão RP: Age-related changes of the multidrug resistance P-glycoprotein function in normal human peripheral blood T-lymphocytes. Braz J Med Biol Res 36: 1653-1657, 2003.
63. Brenner SS and Klotz U: P-glycoprotein function in the elderly. Eur J Clin Pharmacol 60: 97-102, 2004.
64. Yamauchi A, Ieiri I, Kataoka Y, *et al*: Neurotoxicity induced by tacrolimus after liver transplantation: relation to genetic polymorphisms of the ABCB1 (MDR1) gene. Transplantation 74: 571-572, 2002.
65. Zahir H, McLachlan AJ, Nelson A, McCaughan G, Gleeson M and Akhlaghi F: Population pharmacokinetic estimation of tacrolimus apparent clearance in adult liver transplant recipients. Ther Drug Monit 27: 422-430, 2005.
66. Lee JY, Hahn HJ, Son IJ, Suh KS, Yi NJ, Oh JM and Shin WG: Factors affecting the apparent clearance of tacrolimus in Korean adult liver transplant recipients. Pharmacotherapy 26: 1069-1077, 2006.