Association of the Multidrug Resistance-1 Gene Single-Nucleotide Polymorphisms with the Tacrolimus Dose Requirements in Renal Transplant Recipients

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Abstract. The immunosuppressive drug tacrolimus, whose pharmacokinetic characteristics display large interindividual variations, is a substrate for P-glycoprotein (P-gp), the product of the multidrug resistance-1 (*MDR1*) gene. Some of the single nucleotide polymorphisms (SNP) of *MDR1* reported correlated with the *in vivo* activity of P-gp. Because P-gp is known to control tacrolimus intestinal absorption, it was postulated that these polymorphisms are associated with tacrolimus pharmacokinetic variations in renal transplant recipients. The objective of this study was to evaluate in a retrospective study of 81 renal transplant recipients the effect on tacrolimus dosages and concentration/dose ratio of four frequent *MDR1* SNP possibly associated with P-gp function (T-129C in exon 1b, 1236C>T in exon 12, 2677G>T,A in exon 21, and 3435C>T in exon 26). As in the general population, the SNP in exons 12, 21, and 26 were frequent (16, 17.3, and 22.2% for the variant homozygous genotype, respectively) and exhibited incomplete linkage disequilibrium. One month after tacrolimus introduction, exon 21 SNP correlated significantly with the daily tacrolimus dose ($P \leq 0.05$) and the concentration/dose ratio ($P \leq 0.02$). Tacrolimus dose requirements were 40% higher in homozygous than wild-type patients for this SNP. The concentration/dose ratio was 36% lower in the wild-type patients, suggesting that, for a given dose, their tacrolimus blood concentration is lower. Haplotype analysis substantiated these results and suggested that exons 26 and 21 SNP may be associated with tacrolimus dose requirements. Genotype monitoring of the *MDR1* gene reliably predicts the optimal dose of tacrolimus in renal transplant recipients and may predict the initial daily dose needed by individual patients to obtain adequate immunosuppression.

Tacrolimus, like cyclosporine, is a member of the calcineurin inhibitor family. It is used as an alternative to cyclosporine to prevent allograft rejection in solid organ transplantation and therefore is a basic component of immunosuppressive regimens for renal transplant recipients. Tacrolimus is a critical-dose drug with a narrow therapeutic index. Moreover, its pharmacokinetic characteristics may vary greatly among individuals, and daily doses must be adjusted according to the whole-blood trough tacrolimus concentrations measured 12 h post-dose, just before the next dose. Achieving therapeutic trough levels is of critical importance during the initial period after transplantation, when the risk of rejection is greatest. Identification of the parameters that are predictive of the optimal tacrolimus dosage might be clinically important for rapid determination of the adequate therapeutic concentration.

Tacrolimus is a substrate for P-glycoprotein (P-gp), the product of the multidrug resistance (*MDR1*) gene (1). P-gp acts as a transmembrane efflux pump involved in energy-dependent export of xenobiotics from inside the cells. It is present in intestinal epithelial cells, in biliary canalicular cells, in the blood-brain barrier, in lymphocytes, and on the luminal surface of proximal tubule kidney cells (2–4). P-gp affects the absorption of drugs from the gut and their distribution among the body's compartments and also their metabolism and excretion (5). In the gut, strong expression and/or function of P-gp lowers the substrate's absorption. Conversely, alteration in expression and/or function raises this drug absorption.

Previous data for liver transplant recipients who were treated with tacrolimus have demonstrated that intestinal mRNA expression of the *MDR1* gene is inversely correlated to the concentration/dose ratio, which expresses the tacrolimus dose needed to obtain a given blood concentration (6). In this study, patients with strongly expressed *MDR1* required higher tacrolimus doses to achieve trough concentrations similar to those of patients with weak expression. These results suggested that

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P-gp expression plays a critical role in the ability of the gut to absorb tacrolimus.

Significant interindividual variations in the expression and function of P-gp may be due to genetic factors. Various single nucleotide polymorphisms (SNP) have been identified within the *MDR1* gene in the past 3 yr (7–17). In particular, one SNP, located in exon 26 (exon 26 3435C>T), has been studied extensively. When first described, this SNP was associated with variations in intestinal expression and function of P-gp (7). This expression was stronger for the 3435C/C allele than for the 3435T/T allele, and the area under the plasma concentration curve for orally administered digoxin was larger for the 3435T/T allele than for the 3435C/C allele (7). Several groups have reported that this SNP is associated with decreased *MDR1* expression in CD56+ natural-killer cells (15) and placental tissues (12). However, other reports have led to contradictory results regarding the effect on this SNP in phenotypic consequences or P-gp protein expression. Von Ahsen *et al.* (16) found no association between this SNP and the dose and efficacy of cyclosporin A, another P-gp substrate, and Kim *et al.* (17) reported a decrease in fexofenadine with the T allele of the SNP. Because SNP C3435T is a silent polymorphism that does not result in any amino acid changes, the associations and discrepancies described suggest that it may be in linkage disequilibrium with other functional polymorphisms within the *MDR1* gene. In white individuals, Kim *et al.* (17) indeed reported the co-segregation of exon 26 3435T with the T allele of the nonsynonymous exon 21 G2677T SNP (exon 21 2677G>T), resulting in an A893S amino acid change, and with the T allele of the synonymous exon 12 SNP C1236T (exon 12 1236C>T). A noncoding SNP located in the promoter of *MDR1* (T-129C) has also been associated with weaker expression of P-gp in human placenta (12). Taken together, the association of several SNP led to haplotype analysis of the *MDR1* gene to identify the links between the genomic variations, represented by each haplotype on the one hand and by altered *MDR1* function on the other (11,13,14).

Because these polymorphisms may partly explain the large interindividual variations in the pharmacokinetic characteristics of tacrolimus and may control the extent of its uptake after ingestion, we investigated the effect of the four *MDR1* SNP (exon 1b T-129C, exon 12 1236C>T, exon 21 2677G>T,A, and exon 26 3435C>T) most frequently suggested to be associated with P-gp function on tacrolimus doses and trough levels in renal transplant recipients (Figure 1).

Materials and Methods

Study Population

All renal transplant recipients who received a transplant in our center between 1998 and 2001 and were treated with tacrolimus were invited to participate in this retrospective study. All of those included gave written informed consent to participate. In all, 81 kidney transplant recipients (41 men and 40 women), who were given tacrolimus-based immunosuppressive treatment, were recruited for the investigation. Mean recipient age was 40.9 ± 11.3 yr. Mean recipient body weight was 62.7 ± 13.7 kg. Seventy-three patients were white, 7 were black, and 1 was Indian. For all patients, the initial dosage of tacrolimus was 0.2 mg/kg per d. The daily dose was then adapted to blood tacrolimus concentrations. During the first 3 mo, the target blood trough concentration was 10 to 15 ng/ml. In addition to tacrolimus, most patients received a purine inhibitor that was either mycophenolate mofetil ($n = 50$) or azathioprine ($n = 26$) and steroid given at a standard dosage regimen of 500 mg of intravenous methylprednisolone at the time of surgery, 125 mg intravenously the following day, and then 20 mg of prednisone daily. Oral prednisone was then progressively tapered to 5 mg daily at 3 mo posttransplantation. Apart from steroids, the absence of any medication known to interfere with P-gp function (inhibitors or inducers) was checked for all patients. However, no patient was excluded from the study for taking such medication. Particularly, steroid dosage was never significantly different in the different genotype groups.

Data Collection

All patients had a clinical evaluation and laboratory tests 1 mo after tacrolimus initiation. The clinical evaluation included body weight and the screening of all concomitant medications with possible

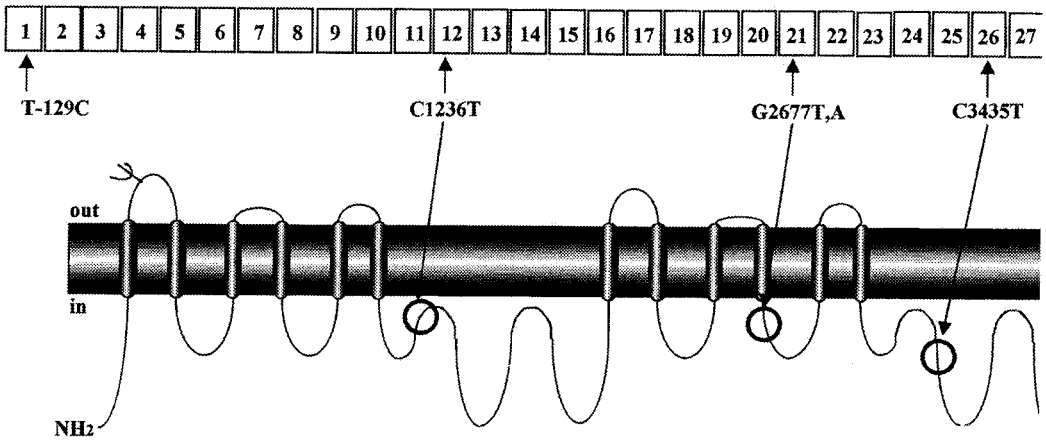

Figure 1. Schematic representation of the multidrug resistance-1 (*MDR1*) gene and putative protein secondary structure. Boxes represent exons. The location of the different single nucleotide polymorphisms (SNP) studied is indicated (arrows) in relation to the exon structure of the *MDR1* gene and the predicted topology of the P-glycoprotein (P-gp). (Adapted from Tanabe *et al.* (9).)

CYP3A and/or P-gp interaction. The daily dose of tacrolimus (mg) was recorded, and the weight-adjusted tacrolimus dosage (mg/kg per d) was calculated. Laboratory evaluations included the tacrolimus blood trough concentrations (ng/ml). Blood tacrolimus levels were assayed 12 h after the previous dose, using the semiautomated microparticle enzyme immunoassay (Abbott, Rungis, France). For reducing intraindividual variability, the tacrolimus blood trough concentration was calculated as the mean of three consecutive measurements. The concentration measured was dose-normalized using the concentration/dose ratio, obtained by dividing the tacrolimus trough concentration by the corresponding 24-h dose, on an mg/kg basis. The information thus obtained was the tacrolimus dose needed to obtain a given trough level.

Identification of Genotypes

For genotype determination, genomic DNA was extracted from EDTA-treated blood using the Qiagen Kit (Courtabeauf, France).

MDR1 exon 26 (C3435T) single nucleotide polymorphism was detected by 5' nuclease allelic discrimination assay (ABI PRISM 7700 Sequence Detection System; Applied Biosystems, Foster City, CA) using the forward 5'-ATGTATGTTGGCCTCCTTTGCT-3' and reverse 5'-AACAGCCGGGTGGTGTCA-3' primers for amplification. Specific probes for each allele (5'-CCTCACGATCTCT-3' and 5'-CCTCACAATCTCTT-3') were respectively labeled with the fluorescence reporter dyes FAM and VIC at their 5' extremities.

MDR1 exon 1b, 12, and 21 SNP were detected by direct sequencing. The following primers were used for PCR amplification: exon 1b forward, 5'-TGATTGGCTGGGCaGGAACAG-3'; exon 1b reverse, 5'-AATCTTGGAAGAAGATACTCC-3'; exon 12 forward, 5'-TCCTGTGTCTGTGAATTGCCTTG-3'; exon 12 reverse, 5'-GCTGATCACCGCAGTCTAGCTCGC-3'; exon 21 forward, 5'-GCAGGCTATAGGTTCCAGGCT-3'; and exon 21 reverse, 5'-TGAGGA ATGGTTATAAACACAT-3'. PCR were performed in a total volume of 25 μl using 100 ng of genomic DNA with 0.2 μM of each primer (0.4 μM for exon 1b), 0.2 mM dNTP, 1× PCR buffer, 2.5 mM $MgCl_2$ for exon 1b or 2 mM $MgCl_2$ for exon 12 and 21, and 0.625 units of AmpliTaq Gold DNA-polymerase (Perkin-Elmer, France). PCR products were sequenced with the BigDye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer) on an ABI PRISM 3100 sequencer (Applied Biosystems).

Statistical Analyses

For analysis of continuous pharmacologic variables, patient genotypes were used as categorical independent variables. For the analyses of daily tacrolimus doses, blood tacrolimus levels, and tacrolimus concentration/dose ratios, groups were compared using nonparametric tests. We used the Mann-Whitney *U* test for comparisons between two groups and the Kruskal-Wallis test for comparisons among several groups. Allele and genotype frequencies for the various SNP were assessed for deviation from Hardy-Weinberg equilibrium using Fisher exact test. $P < 0.05$ was considered significant.

The different genotypes for the G2677T,A SNP were classified as follows: wild type (G/G), heterozygous (G/T or G/A), and homozygous for the variant (T/T, T/A, or A/A). Haplotype frequencies were estimated on the basis of the Expectation-Maximization algorithm (18) using the population genetics data analysis program Arlequin (www.lgb.unige.ch/arlequin/).

Results

Frequency of MDR1 Variants in Renal Transplant Patients

Table 1 shows the frequency of the four *MDR1* SNP for each allele in the 81 renal transplant recipients. The frequency expected for each genotype was evaluated on the basis of Hardy-Weinberg equilibrium proportions. None of the observed frequencies was significantly different from the expected frequencies. In our population, the presence of the homozygous variant in the promoter region (exon 1b) was not observed, but nine patients were heterozygous for the mutant allele. A high frequency was observed for variants leading to an amino acid modification in exon 21 G2677T,A (Ala ^{893}Ser or Ala ^{893}Thr). More than 50% of the patients exhibited a mutated nucleotide at position 2677, and 17.3% were homozygous for the mutation. The other exonic variants, C1236T in exon 12 and C3435T in exon 26, which do not affect the amino acid sequence, were also frequently observed. The mutations were present homozygously in 16 and 22.2% of patients for exons 12 and 26, respectively.

Linkage Disequilibrium Study

We investigated the four SNP for any linkage disequilibrium to determine whether they were randomly associated in the same patient. Pairwise linkages between SNP were not randomly distributed, and only a few major linkages were observed (defined as >10% occurrence; Figure 2). It is interest-

Table 1. Positions, effects, sequences, and frequencies of the four SNP evaluated in 81 renal transplant recipients

Mutation	Effect	Nucleotide Sequence		Genotype[a] (n = 81)		
		Wild Type (wt)	Mutation (m)	wt/wt	wt/m	m/m
T-129C	Noncoding	cgag**T**agcg	cgag**C**agcg	72 (88.9%)	9 (11.1%)	0
C1236T	wobble	aggg**C**ctga	aggg**T**ctga	29 (35.8%)	39 (48.1%)	13 (16%)
G2677T,A						
G2677T	Ala893Ser	aggt**G**ctgg	aggt**T**ctgg	26 (32.1%)	38 (46.9%)	T/T 12 (14.8%)
G2677A	Ala893Thr	aggt**G**ctgg	aggt**A**ctgg		3 (3.7%)	A/A: 0
						A/T: 2 (2.5%)
C3435T	wobble	agat**C**gtga	agat**T**gtga	29 (35.8%)	34 (42%)	18 (22.2%)

[a] The frequency expected for each genotype was evaluated on the basis of Hardy-Weinberg equilibrium proportions. None of the observed frequencies was significantly different from the expected frequencies.

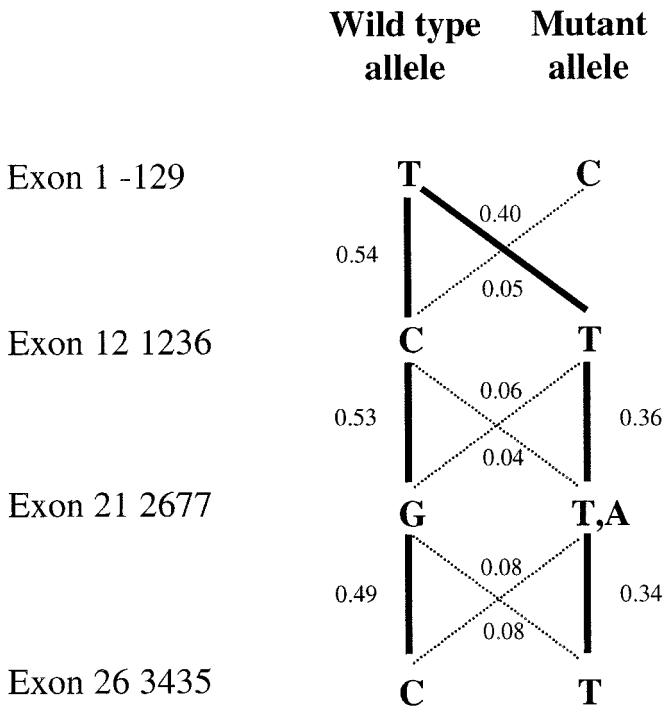

Figure 2. Pairwise linkage profiles of the four SNP of the *MDR1* gene. The frequency of the different pairwise linkages was calculated on the basis of Expectation-Maximization (24). Dotted lines represent weak linkages (<10%), and solid lines represent strong linkages (frequency >10%). The number beside each line indicates the percentage of the specific linkage.

ing that the association of exon 1-129C with exon 12 1236T was never observed. All nine patients who harbored the C allele in exon 1 SNP also had the C allele in exon 12 SNP. Significant linkage disequilibrium was also observed between the exon 12, 21, and 26 SNP. Most of the wild-type alleles at any one position were associated with the wild-type allele at the following position. Conversely, a mutant allele in the exon 12 SNP was usually associated with mutant alleles in the exon 21 and 26 SNP. Comparison of the linkages between the four SNP with the correlation coefficient Δ (equivalent to coefficient correlation, r) showed that the T-129C SNP was not significantly associated with the genotypes at any of the positions of the three other SNP. The C1236T SNP correlated with the G2677T,A and C3435T SNP ($\Delta = 0.79$ and 0.66, respectively), and the G2677T,A SNP correlated with the C3435T SNP ($\Delta = 0.66$).

Tacrolimus Dose Requirements

The tacrolimus doses required 1 mo after tacrolimus initiation varied greatly (Figure 3). They ranged from 0.029 to 0.364 mg/kg per d (median, 0.169), thus confirming the large interindividual variations in tacrolimus pharmacokinetics. The mean tacrolimus trough blood concentrations were 11.8 ± 2.8 ng/ml. More than 80% of the tacrolimus trough concentrations were within the desired target 1 mo after tacrolimus initiation.

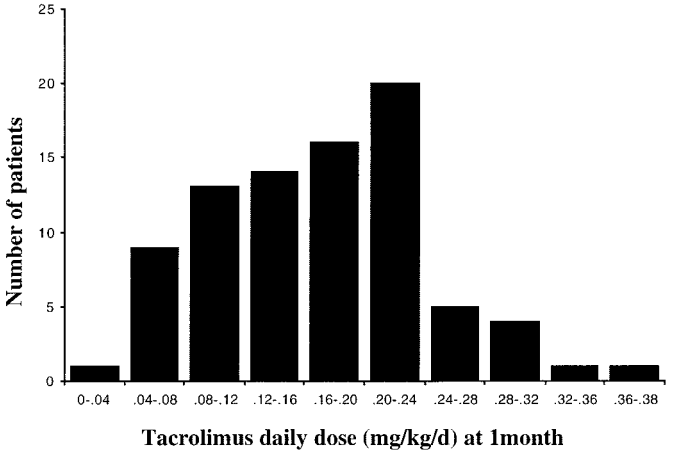

Figure 3. Distribution histograms of tacrolimus daily doses required to obtain the target trough level 1 mo after tacrolimus initiation in 81 renal transplant patients.

Effects of MDR1 SNP on Tacrolimus Dose Requirement

We examined the relationship between each SNP of the *MDR1* gene and the tacrolimus daily dose and concentration/dose ratio (Table 2). One month after tacrolimus introduction, there was no difference between the blood trough levels of the different *MDR1* genotypes, confirming that the daily dose was adjusted to reach target tacrolimus blood level. As shown in Figure 4, 1 mo after tacrolimus introduction, the exon 21 SNP of 2677G>T,A SNP was significantly associated with the tacrolimus daily dose ($P \leq 0.05$, Kruskal-Wallis test). The mean dose required to obtain the target trough concentration was significantly higher in patients with the wild-type genotype than in those with one or two mutant alleles (0.20 ± 0.07 *versus* 0.16 ± 0.07, and 0.14 ± 0.07 mg/kg per d; $P = 0.05$). Tacrolimus dose requirements were not significantly different for exons 1b, 12, and 26.

The average concentration/dose ratio correlated with the exon 21 2677G>T,A SNP. The concentration/dose ratios were respectively 65, 94, and 101 (ng/ml)/(mg/kg) for the wild-type, heterozygous, and homozygous variants ($P = 0.01$). The average concentration/dose ratio also correlated with the C1236T SNP ($P \leq 0.05$). Note that a similar nonsignificant trend was observed for the C3435T SNP (Table 2). The mean daily dose and concentration/dose ratio were also related to the number of mutant copies for each SNP.

Haplotype Analysis

There is increasing evidence that gene-based haplotype approaches that take into account the combination of SNP present in an allele make it easier to predict changes in response to drugs than SNP-based approaches (15,16). The *in vivo* effects of isolated mutations are difficult to evaluate because of the high frequency of the SNP and the close linkage disequilibrium detected between the exon 12, 21, and 26 SNP. This prompted us to perform a multipoint haplotype determination of the *MDR1* gene to identify the associations between the genomic

Table 2. Relationship between the four SNP tested and tacrolimus daily doses and concentration/dose ratios after 1 mo of tacrolimus treatment in 81 renal transplant recipients

Mutation	Genotype	*n*	Tacrolimus Requirements at 1 Mo	
			Daily Dose (mg/kg/d)	Concentration/Dose Ratio (ng/ml)/(mg/kg)
T-129C	T/T	72	0.16 ± 0.07	89 ± 45
	T/C	9	0.19 ± 0.07	65 ± 33
C1236T	C/C	29	0.19 ± 0.08	71 ± 38[a]
	C/T	39	0.16 ± 0.07	91 ± 46[a]
	T/T	13	0.14 ± 0.07	103 ± 48[a]
G2677T, A	G/G	26	0.20 ± 0.07[a]	65 ± 30[b]
	G/mutant	41	0.16 ± 0.07[a]	94 ± 47[b]
	mutant/mutant	14	0.14 ± 0.07[a]	101 ± 50[b]
C3435T	C/C	29	0.19 ± 0.07	76 ± 46
	C/T	34	0.16 ± 0.07	88 ± 41
	T/T	18	0.15 ± 0.07	98 ± 48

The three genotype groups were compared using the Kruskal-Wallis test.
[a] $P \leq 0.05$.
[b] $P \leq 0.02$.

variations represented by each haplotype and tacrolimus dose requirements.

The exon 1-129T>C SNP was excluded from this determination because the combination of sample size and low frequency of the minor C allele would have resulted in nonsignificance of the predicted haplotypes. Haplotype analysis therefore was restricted to the coding SNP of exon 12, 21, and 26 (for the exon 21 SNP, the mutated alleles T and A were considered as a single group). For individuals who were homozygous at both variants or who were heterozygous at only one variant, the haplotypes could be assigned unambiguously. The haplotype remained uncertain for five patients. Of eight possible haplotypes, six were present in our patients but only two occurred with high frequency, suggesting tight linkage among the loci of exons 12, 21, and 26. These two haplotypes, C-G-C (haplotype 1) and T-T,A-T (haplotype 2), accounted for 45.4 and 36.2% of the haplotypes, respectively. Each of the six remaining haplotypes constituted <9% of the patient's haplotypes. Individuals with haplotype 1 required significantly higher daily doses of tacrolimus than those with haplotype 2 (0.18 ± 0.07 *versus* 0.15 ± 0.07 mg/kg per d; $P < 0.02$).

Because the two predominant haplotypes were determined, it was possible to define the genotypes 1/1, 1/2, and 2/2, which respectively occurred in 20, 22, and 8 patients. We then analyzed the treatment characteristics for each of these three genotypes (Table 3). The daily tacrolimus dose was significantly higher in patients with the wild-type genotype for the three SNP than in those with one and two mutated haplotypes (0.21 ± 0.07, 0.15 ± 0.06, and 0.13 ± 0.07 mg/kg respectively; $P = 0.03$). The average concentration/dose ratio also correlated with the *MDR1* haplotype-derived genotypes (63, 93, and 102 [ng/ml]/[mg/kg], respectively; $P = 0.01$).

Discussion

The pharmacogenetic evaluation of several genes implicated in drug pharmacokinetics should provide efficient tools for individualizing drug therapy by optimizing drug dosage. This would both improve drug efficacy and prevent adverse effects (19). Such evaluation may be particularly useful for drugs characterized by a narrow therapeutic index and/or significant toxicity, such as the calcineurin inhibitors tacrolimus and cyclosporine. In this study, we demonstrated that several polymorphisms of the *MDR1* gene partly explain the variability of tacrolimus dose requirement in renal transplant recipients.

Abundantly expressed in the enterocytes, P-gp, the *MDR1* gene product, may play an important role in the variability of tacrolimus absorption (7,20–23). Thus, interindividual variations in P-gp expression and/or function may explain the interindividual variability of tacrolimus bioavailability among individuals. Genetic polymorphisms related to the intestinal expression of P-gp therefore may affect the uptake of tacrolimus in transplant patients. The correlations between *MDR1* SNP and tacrolimus requirements may be interpreted as an enhanced uptake by the intestine of tacrolimus in the mutant carriers. Patients with no mutation in the *MDR1* gene are more likely to extrude tacrolimus from intestinal cells and therefore need a higher daily dose to achieve adequate blood tacrolimus levels. Conversely, low expression of intestinal P-gp may reduce the P-gp–mediated drug efflux that directs intestinal secretion of drug into the gut lumen, resulting in greater tacrolimus bioavailability. Although the gut is the likely source, we cannot exclude that the SNP action could be occurring elsewhere. Because P-gp is located mainly in the apical membrane of excretory cells in the liver, kidney, and intestine (2,3), its expression is important for the absorption, distribution, and elimination of xenobiotics. Functional polymor-

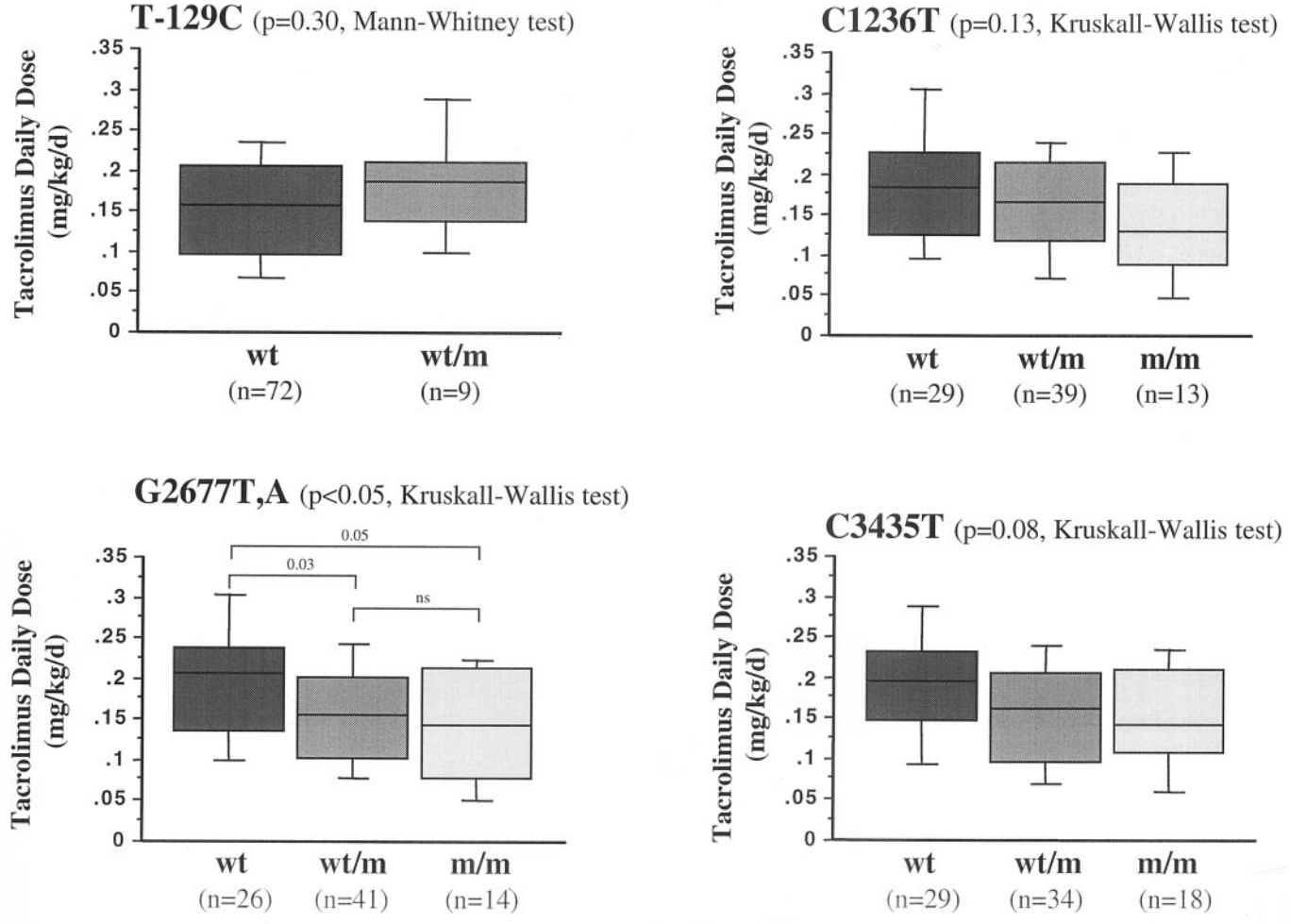

Figure 4. Correlation of the SNPs of the *MDR1* gene in exons 1b, 12, 21, and 26 with the tacrolimus dose requirements (mg/kg per d) recorded in 81 kidney transplant recipients 1 mo after tacrolimus introduction. The box plot shows the tacrolimus dose distribution, clustered according to the allelic variations in *MDR1*. The *P* value for the pairwise comparison of each genotype is indicated in the figure. Wt, wild type genotype; m, mutated genotype.

phisms in *MDR1* gene may then alter these pharmacologic parameters. However, a recent study showing an association between haplotypes of the *MDR1* gene and the steady-state kinetics of the P-gp substrate digoxin showed that *MDR1* polymorphisms were significantly associated with the absorption but not with the elimination of the drug, suggesting that the SNP action occurred mainly in the gut (24).

Our patients exhibited genotype frequencies of the different SNP in accordance with those already described in white populations. For example, Cascorbi *et al.* (25) in a genetic analysis of 461 German volunteers reported frequencies for the exon 12 1236C>T polymorphism of 34.4, 49.2, and 16.4 for the C/C, C/T, and T/T genotypes, respectively. For the exon 21 SNP, 30.9% of their subjects were homozygous for the wild-type allele, 51.2% were heterozygous, and 17.9% exhibited two copies of mutant alleles. For the exon 26 polymorphism, the reported frequencies reveal that almost half of the individuals were heterozygous and 25 to 30% were homozygous carriers for the variant allele. These three polymorphisms therefore are found frequently in the general population, and their putative role in drug disposition may have a relevant impact in clinical practice.

The linkage that we found between the SNP in exons 12, 21, and 26 has already been reported in other populations (12,17). In European Americans, Kim *et al.* (17) reported co-segregation of the exon 26 mutant allele with the T allele of the nonsynonymous exon 21 SNP G2677T and with the T allele of the synonymous exon 12 SNP C1236T. These three SNP in exon 12, 21, and 26 are closely linked at high frequency and occurred in 62% of European Americans and 13% of African Americans. This high level of incomplete disequilibrium is at the origin of the haplotype approach. Haplotypes generally contain more information than did individual SNP (26,27).

Our results confirmed the large interindividual variations in tacrolimus pharmacokinetics. After 1 mo of tacrolimus treatment, a 1- to 12-fold range of doses is needed to achieve therapeutic concentrations. Our results suggest that genetic polymorphisms may explain a significant part of this variation

Table 3. Daily doses and concentration/dose ratios of tacrolimus according to the genotypes derived from the two predominant haplotypes in 81 renal transplant recipients after 1 mo of tacrolimus treatment

Genotype	*n*	Tacrolimus Requirements at 1 Mo	
		Daily Dose (mg/kg/d)	Concentration/Dose Ratio (ng/ml)/(mg/kg)
1/1	20	0.21 ± 0.07[a]	63 ± 31[a]
1/2	22	0.15 ± 0.06[a]	93 ± 44[a]
2/2	8	0.13 ± 0.07[a]	102 ± 49[a]

Haplotypes 1 and 2 are derived from the exon 12, 21, and 26 SNP: haplotype 1, C-G-C; haplotype 2, T-T, A-T.

The three genotype groups were compared using the Kruskal-Wallis test.

[a] $p \leq 0.05$.

in tacrolimus bioavailability after oral administration. Exon 1-129T>C mutations have been associated with P-gp expression (12). However, in accordance with previous authors (14), we found no phenotypic correlation with tacrolimus pharmacokinetics for this mutation. However, we found that tacrolimus dose requirements were generally lower in patients with one or two mutant alleles in their exon 12, 21, and 26 SNP, who therefore exhibited a gene-dose effect. The most important relation was noted for the exon 21 2677G>T,A SNP, as the tacrolimus dose requirement was 40% higher in homozygous than in wild-type carriers. Similarly, the concentration/dose ratio was 36% lower for this mutation in wild-type patients, suggesting that for a given dose, the tacrolimus blood concentration is lower in the wild-type patients. A similar nonsignificant trend was also observed with SNP in exon 12 and 26. The association of the *MDR1* gene SNP with the tacrolimus dose requirements was evaluated in two recent studies. Goto *et al.* (14) found no association between 10 SNP of *MDR1* gene and the tacrolimus concentration/dose ratio during the first postoperative days after liver transplantation. Conversely, MacPhee *et al.* (28) recently reported a weak association between the exon 26 3435C>T SNP and the dose requirement of tacrolimus 3 mo after renal transplantation. In this study, they tested only the synonymous C3435T SNP of *MDR1*. This SNP is a silent polymorphism that does not result in any amino acid changes. The conflicting results of these two studies and our own may be partly due to the high level of disequilibrium between the exon 12, 21, and 26 SNP, as the exon 12 and 26 SNP are strongly associated with the exon 21 2677G>T,A mutation. The analysis that we performed with the two most frequent haplotypes showed a 61% increase in tacrolimus dose requirements in patients who were homozygous for the wild-type allele in the SNP off all three exons. Comparison of the different haplotype profiles showed that the exon 12 mutation alone does not contribute to tacrolimus dose requirements (data not shown). However, the impact of the exon 21 SNP was substantiated by our haplotype-analysis, which included the exon 26 SNP, suggesting that these two SNP may have separate impacts on P-gp function. These results are in accordance with the impact of the association between the SNP in exons 21 and 26 on P-gp functional changes *in vivo*. In a recent study, a significant relationship was indeed found between the exons 21 and 26 genotypes and the pharmacokinetics of digoxin (10). In this study, the bioavailability of digoxin was lowest in the wild-type/wild-type subjects, intermediate in double-heterozygous subjects, and highest in homozygous mutant subjects. The possibility cannot be excluded that other mutations and haplotypes may be associated with the changes in P-gp function.

Of note, tacrolimus doses may be modified and blood levels may be altered by other possible mechanisms from diet to timing of blood draws to other enzymes that regulate tacrolimus levels. The retrospective design of this study does not permit avoidance of such confounding factors. Only a prospective study may limit these confounding factors.

In summary, this study demonstrates that *MDR1* gene SNP are associated with the tacrolimus requirements. Pharmacogenetic testing therefore could contribute to the individualization of drug treatment and enhance drug safety and efficacy. Genotype characterization can easily be performed before transplantation and may help to determine the initial daily dose needed after transplantation by individual patients to obtain adequate immunosuppression without increasing the risk of toxicity. It should be highlighted that these results have been obtained from a relatively small number of patients. The confirmation of the association of the *MDR1* SNP with tacrolimus requirements is needed in a larger and more diverse population. Large-scale genotype-phenotype correlation trials are required to increase our knowledge of the effects of SNP on clinical outcome.

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