TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Impact of genetic polymorphisms on tacrolimus pharmacokinetics and the clinical outcome of renal transplantation

Guillermo Gervasini,[1] Montserrat Garcia,[1] Rosa María Macias,[2] Juan Jose Cubero,[2] Francisco Caravaca[2] and Julio Benitez[1]

1 Department of Medical and Surgical Therapeutics, Medical School, University of Extremadura, Badajoz, Spain
2 Service of Nephrology, Infanta Cristina Hospital, Badajoz, Spain

Keywords
ABCB1, *CYP3A*, polymorphisms, renal transplant, tacrolimus.

Correspondence
Guillermo Gervasini PhD, Department of Medical and Surgical Therapeutics, Medical School, University of Extremadura, Av. Elvas s/n 06071, Badajoz, Spain.
Tel.: +34 927 257 120;
fax: +34 927 257 106;
e-mail: ggervasi@unex.es

Conflicts of Interest
The authors have declared no conflicts of interest.

Received: 14 October 2011
Revision requested: 7 November 2011
Accepted: 23 January 2012
Published online: 28 February 2012

doi:10.1111/j.1432-2277.2012.01446.x

Summary

We retrospectively examined the association of polymorphisms in the *CYP3A*, *CYP2J2*, *CYP2C8*, and *ABCB1* genes with pharmacokinetic (PKs) and pharmacodynamic (PDs) parameters of tacrolimus in 103 renal transplant recipients for a period of 1 year. CYP3A5 expressers had lower predose concentrations (C_0)/dose and higher dose requirements than nonexpressers throughout the study. Among *CYP3A5*1* carriers, those also carrying the *CYP3A4*1B* allele showed the lowest C_0/dose, as compared with *CYP3A4*1/CYP3A5*3* carriers (54.28 ± 26.45, 59.12 ± 24.00, 62.43 ± 41.12, and 57.01 ± 17.34 vs. 112.37 ± 76.60, 123.21 ± 59.57, 163.34 ± 76.23, and 183.07 ± 107.82 at 1 week, 1 month, 5 months, and 1 year after transplantation). In addition, *CYP3A4*1B/CYP3A5*1* carriers showed significantly lower dose-corrected exposure than *CYP3A4*1/CYP3A5*1* carriers 1 year after transplantation (57.01 ± 17.34 vs. 100.09 ± 24.78; $P = 0.016$). Only the *ABCB1* TGC (3435-2677-1236) haplotype showed a consistent association with PDs (nephrotoxicity; OR = 4.73; CI: 1.3–16.7; $P = 0.02$). Our findings indicate that the *CYP3A4*1B-CYP3A5*1* haplotype may have a more profound impact in tacrolimus PKs than the *CYP3A5*1* allele. This study does not support a critical role of the *CYP450* or *ABCB1* single nucleotide polymorphisms in the occurrence of toxicity or acute rejection in renal transplant recipients treated with tacrolimus.

Introduction

The clinical use of tacrolimus is hampered by a narrow therapeutic window and its large interindividual variability in its pharmacokinetics (PKs) [1]. In the last years, a number of studies have shown that pharmacogenetics could be helpful in tailoring immunosuppressant therapy to the needs of transplant recipients [2,3]. CYP3A4 and CYP3A5 are the major enzymes responsible for tacrolimus biotransformation [4] and hence have received much attention regarding the presence of functional polymorphisms that could affect their activity. Amongst the most frequently studied polymorphisms, *CYP3A5*3* is the most common *CYP3A5* allele in Caucasians and produces a nonfunctional protein [5]. It has been shown that transplant recipients with at least one *CYP3A5*1* allele (expressing CYP3A5 protein) show lower tacrolimus concentration to dose ratios and higher dose requirements than nonexpressers (carriers of the *CYP3A5*3/*3* genotype) [6–8]. More controversy exists, however, regarding the clinical significance of the major polymorphism in the *CYP3A4* gene (*CYP3A4*1B*) [9–11].

Tacrolimus is also a substrate for P-glycoprotein (P-gp, ABCB1, MDR1) [12], an efflux transporter that limits the oral absorption of its substrates among other functions. Despite large research efforts, reported results on the influence of the major *ABCB1* polymorphisms (C1236T, G2177T/A and C1236T) on tacrolimus PKs and pharmacodynamics (PDs) are still inconclusive [13,14].

In addition, there are other CYP enzymes that could affect the outcome of renal transplantation. CYP2C8 and CYP2J2 are polymorphically expressed in the kidney and

are involved in the synthesis of epoxyeicosatrienoic acids (EETs) [15–18], which play a protective role by vasodilator mechanisms against processes that could lead to acute rejection or toxicity.

In the present work, our goal was to identify relevant single nucleotide polymorphisms (SNPs) and haplotypes in the aforementioned enzymes and transporters in a population of renal transplant recipients treated with tacrolimus. These genetic variants were retrospectively associated with PK parameters collected during 1 year after the transplant, as well as with kidney function and the occurrence of tacrolimus-related adverse effects and acute rejection.

Patients and methods

Study population

The study included 103 consecutive adult renal transplant recipients who received a single kidney at the Infanta Cristina Hospital (Badajoz, Spain) between June 2000 and February 2003 and were administered a tacrolimus-based immunosuppressive therapy. The Infanta Cristina Hospital is a 890-bed hospital situated in a rural area of Spain where people of mostly Caucasian ethnicity live. The transplant program started in 1990 and as a result, most patients presented here were nonimmunized and received a first kidney allograft (Table 1). All patients reported herein received a kidney from a deceased donor, as no transplants from living donors are performed in our institution.

Initial dosage of tacrolimus was 0.1 mg/kg twice a day for all patients and was subsequently adjusted according to blood concentrations. Tacrolimus target levels were 7–15 ng/ml between June 2000 and September 2001, and were then adjusted to 10–15 ng/ml (months 0–3) and 5–10 ng/ml (months 4–12) between October 2001 and February 2003.

Tacrolimus was given in combination with mycophenolate mofetil (2 g/day) and a tapering schedule of steroids (500 mg IV methylprednisolone at the time of surgery, 125 mg IV the following day, and then 20 mg of oral prednisone daily, which was progressively tapered to 5 mg daily at 2 months after transplantation). Thirty-one patients (30.1%) received antibodies against the interleukin 2 receptor. Induction therapy with antithymocyte globulin was not implemented. Subjects with a chronic use of substances known to interfere with tacrolimus absorption, distribution, metabolism, and excretion (e.g. macrolides, rifampin, phenytoin, carbamezipine, etc.) were excluded from the study.

All participants were of Caucasian origin and gave oral and written consent for their participation. The study was approved by the Ethics Committee of the Infanta Cristina University Hospital and was conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

Table 1. PK parameters and clinical characteristics of renal transplant recipients (n = 103) at the four time points considered in the study.

	1 Week	1 Month	5 Months	1 Year
C_0 (ng/ml)*	12.1 ± 5.3	11.8 ± 3.9	9.8 ± 3.6	8.3 ± 3.3
Dose (mg/kg)	0.13 ± 0.06	0.12 ± 0.05	0.08 ± 0.05	0.06 ± 0.04
Weight (kg)	69.0 ± 14.2	68.9 ± 13.8	72.0 ± 13.9	72.6 ± 15.0
C_0/dose (ng/ml per mg/day per kg body weight)	107.2 ± 74.9	117.5 ± 59.8	155.6 ± 77.8	172.4 ± 107.3
Serum creatinine (mg/dl)	2.4 ± 1.8	1.7 ± 1.0	1.5 ± 0.7	1.4 ± 0.6
Creatinine clearance (ml/min)	57.1 ± 28.0	61.5 ± 28.8	71.2 ± 32.5	80.9 ± 38.3
% Recipients below target concentration range†	35.4	32.4	3.0	7.1
% Recipients within target concentration range†	44.4	49.0	57.4	72.4
% Recipients above target concentration range†	20.2	18.6	39.6	20.4
Duration of dialysis before transplantation (years)				3.7 ± 3.1
Number of transplants (first/second)				97/6
Cold ischemia (hours)				16.8 ± 5.4
Mismatch HLA-A				1.2 ± 0.7
Mismatch HLA-B				1.3 ± 0.6
Mismatch HLA-DR				0.6 ± 0.6
PRA activity max				9.7 ± 17.9
PRA activity max. >50%				6 (5.8)
DGF				26 (25.2)

Data are shown as number (percentage) or mean ± standard deviation.

*C_0, predose concentrations.

†Target concentrations range was 10–15 ng/ml during the first month and 5–10 ng/ml for the third month onwards.

Data collection

Tacrolimus predose concentrations (C_0; ng/ml), daily doses (mg/kg), and dose-normalized predose concentrations (C_0/dose; ng/ml per mg/day per kg body weight) were retrieved from medical records at 1 week, 1 month, 5 months, and 1 year after transplantation. Tacrolimus blood concentrations were routinely measured using an immunoassay performed using a Cobas Mira Plus analyzer (Roche Diagnostics, Basel, Switzerland).

Renal function was assessed by estimating the glomerular filtration rate (GFR) from serum creatinine, using the 4-variable modification of diet in renal disease formula [19], GFR = $186(\mathrm{Cr}^{-1.154} \times \mathrm{age}^{-0.203}) \times (1.212 \text{ if black}) \times (0.742 \text{ if female})$. Delayed graft function (DGF) was defined according to standard criteria, creatinine clearance <10 ml/min during the first 3 days post-transplantation and/or the need for dialysis treatment during the first postoperative week [20].

CNIT was diagnosed by experienced nephrologists based on (i) impairment of renal function defined by elevated serum creatinine values (25% increase from basal figures) coinciding with high tacrolimus plasma levels with subsequent decrease following dose reduction and/or (ii) exclusion of renal impairment as a result of acute rejection either proved by biopsy and/or judged by clinical evaluation when patients presented with a rise ≥0.30 mg/dl in serum creatinine not coincidental with supratherapeutic tacrolimus predose concentrations, fever without signs of infection, graft swelling, or tenderness, oliguria, increased resistive index on Doppler ultrasonography or clinical response to steroid treatment consistent with rejection. Acute allograft rejection was established by histological findings in renal biopsies according to the 1997 Banff classification and/or by clinical evaluation (see above). Diagnosis was confirmed by biopsy in all corticoid-resistant patients.

Symptoms defining neurotoxicity included tremor, headache, insomnia, hyperesthesia, itching, and seizures. As part of the protocol followed in our center, patients were specifically questioned about the presence of these neurologic symptoms.

Genotyping analyses

Blood samples were drawn from each subject and immediately stored at −80 °C until genotype analysis. Genomic DNA was isolated from peripheral blood leukocytes in each subject's whole-blood sample using a QIAamp DNA Blood Kit method (Qiagen Inc., Chatsworth, CA, USA).

Polymerase chain reaction and restriction fragment length polymorphism analysis were used for the determination of the *CYP3A4*1B*, *CYP3A5*3*, *CYP2C8*3*, and *CYP2J2*7* variants following previously described protocols [21–24]. The analysis of index samples was duplicated and confirmed by direct sequencing. The three major *ABCB1* polymorphisms, namely C1236T, G2677T/A, and C3435T were detected by direct sequencing (ABI3700 Genetic Analyzer; Applied Biosystems, Weiterstadt, Germany) using primers and PCR conditions previously described [25].

Statistical analyses

The distribution of the outcome variables was tested by the Kolgomorov–Smirnov test for normality. The statistical significance of the differences of quantitative variables (dose-adjusted predose concentrations, dose requirements, creatinine values, etc.) among individuals with different genotypes was calculated by using the ANOVA-Bonferroni or Kruskall–Wallis tests, as appropriate. When only two different genotypes were considered, T-student or Mann–Whitney tests were applied. Fisher's exact test or Pearson's X^2 was used for the univariate analysis of the associations between categorical data (incidence of acute rejection or adverse effects) in the different genotype groups.

The statistical power of the sample size was evaluated with a genetic model analyzing the frequency for carriers of the variant alleles with arbitrarily established effect size at 2.5 ($\alpha = 0.05$). With the available sample size, the power for unilateral associations with efficacy and safety parameters was 0.831. Power calculations were made using the program G*Power version 3.1.3.

The effect of haplotypes on quantitative variables was calculated through linear regression modeling assuming an additive mode of inheritance using SNPstats [26] and Hapstat software version 3.0. For binary traits, the logistic regression model was employed, and the regression parameters pertained to the log odds ratios. Differences were considered significant when P values were under 0.05.

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

A total of 103 renal transplant recipients (41 women) with a mean age of 48.7 ± 16.9 years at the time of transplantation were included in the study. Underlying diseases for renal failure were classified as followed: glomerulonephritis (33.0%), chronic interstitial nephritis (11.7%), polycystic kidney disease (9.7%), diabetic nephropathy (7.8%), and uncertain (28.2%). Other causes (9.7%) included Alport's syndrome, hemolytic uremic syndrome,

nephroangiosclerosis, nephronophtisis, and ischemic nephropathy. Additional clinical characteristics of the patients at the four time points considered in the study are shown in Table 1.

Table 2 shows the frequency of the seven studied polymorphisms (*CYP3A4*1B*, *CYP3A5*3*, *CYP2C8*3*, *CYP2J2*7*, and *ABCB1* C3435T, G2677T/A, and C1236T). Genotype frequencies did not show statistically significant differences from those expected by the Hardy-Weinberg principle. One *CYP3A5*1/*1* subject was identified and included in the *CYP3A5*1/*3* group for statistical purposes, as it is assumed that only one *CYP3A5*1* allele is enough to express the protein. There was a moderately high degree of linkage disequilibrium between the *CYP3A4*1B* and *CYP3A5*1* SNPs ($D' = 0.99$, $r^2 = 0.44$) that resulted in all the subjects carrying the *CYP3A4*1B* variant also having the *CYP3A5*1* allele. Linkage disequilibrium was also observed between the *ABCB1* G2677T and C3435T SNPs ($D' = 0.90$, $r^2 = 0.57$), the *ABCB1* G2677T, and C1236T SNPs ($D' = 0.90$, $r^2 = 0.67$) and between the *ABCB1* C3435T and C1236T SNPs ($D' = 0.71$, $r^2 = 0.43$). No further significant linkage was found between other combinations of SNPs ($r^2 < 0.1$).

Effect of CYP3A4 and CYP3A5 genotypes on tacrolimus predose concentrations and dose requirements

The presence of the *CYP3A5*1* allele was strongly associated with lower dose-corrected tacrolimus trough blood levels (C_0) at all time points considered (Table 3). Furthermore, carriers of the *CYP3A4*1B* variant allele displayed tacrolimus predose concentrations that were on average 59% lower than those of subjects with the *CYP3A4*1/*1* genotype (Table 3). A gene-dose effect was observed when the combination of both the *CYP3A4*1B* and *CYP3A5*3* SNPs was analyzed. Mean tacrolimus dose-adjusted C_0 values throughout the study were 145.59, 86.89 and 58.21 ng/ml per mg/kg/day for the *3A4*1-3A5*3*, *3A4*1-3A5*1*, and *3A4*1B-3A5*1* haplotypes, respectively (Table 3). *P* values for the significance of the difference between the three haplotypes were 0.120, 0.012, 0.006, and 0.010 at 1 week, 1 month, 5 months, and 1 year, respectively. The difference in dose-corrected exposure between CYP3A5 expressers carrying or not carrying the *CYP3A4*1B* allele reached statistical significance 1 year after grafting ($P = 0.016$).

Table 4 depicts dose requirements for carriers of the different genotypes analyzed. *CYP3A5*1* carriers showed significantly higher values at 1, 5, and 12 months after transplantation. Dose requirements for the *3A4*1-3A5*1* and *3A4*1B-3A5*1* haplotypes were approximately 2-fold higher than those showed by the most common *3A4*1-3A5*3* combination. *P* values for the statistical significance of the difference between the three combined genotypes were 0.892, <0.01, <0.001, and <0.01 at 1 week, 1 month, 5 months, and 1 year, respectively.

Remarkably, none of the CYP3A5 expressers who were also harboring the *CYP3A4*1B* variant reached target predose concentrations during the first month post-transplantation. However, because of the two different criteria followed to define predose concentration target range (see Methods section), a formal statistical analysis could not be performed and hence this particular observation should be taken with caution.

Table 2. Distribution of allelic and genotypic frequencies in 103 renal transplant recipients.

Polymorphism	wt/wt, *n* (%)	wt/m, *n* (%)	m/m, *n* (%)	MAF
*CYP3A4 *1/*1B*	98 (95)	5 (5)	0 (0)	0.02
*CYP3A5 *1/*3*	1 (1)	9 (9)	93 (90)	0.05
*CYP2C8 *1/*3*	74 (72)	27 (26)	2 (2)	0.15
*CYP2J2 *1/*7*	99 (96)	4 (4)	0 (0)	0.02
ABCB1 C3435T	31 (30)	50 (49)	22 (21)	0.46
ABCB1 G2677T/A	40 (39)	49 (47)*	14 (14)	0.37
ABCB1 C1236T	34 (33)	52 (50)	17 (0.17)	0.42

n, Number of subjects; MAF, minor allele frequency; m, mutant; wt, wild type.

*One subject was carrier of one *ABCB1* 2677A allele.

Effect of ABCB1 genotypes/haplotypes on tacrolimus predose concentrations and dose requirements

None of the *ABCB1* polymorphisms studied (C3435T, G2677T/A or C1236T) was individually associated with C_0/dose values or dose requirements (data not shown). In the same manner, there were no significant differences between the major wild type (CGC, frequency = 0.48) and mutant (TTT, frequency = 0.34) *ABCB1* haplotypes.

However, when the analysis was confined only to *CYP3A5* nonexpressers (*CYP3A5*3/*3* carriers), a statistical trend was observed towards lower dose-corrected exposure and higher dose requirements in those patients carrying 4 to 6 *ABCB1* variants ($n = 69$) compared with patients with 0 to 3 variants ($n = 22$) (Table 5).

Associations with clinical outcomes

Renal function measured as creatinine clearance was not statistically significantly different between the *CYP3A* or *ABCB1* genotypes or haplotypes studied. DGF was observed in 26 patients (25.2%) and was found to be moderately associated with the presence of the *CYP2C8*3/*3* genotype [OR = 2.4, CI (0.9–6.1), $P = 0.05$, vs. *CYP2C8*1* carriers].

Out of the 103 kidney transplant recipients included in the study, 19 (18.4%) developed CNIT during the

Table 3. Effect of *CYP3A4* and *CYP3A5* genotypes on tacrolimus predose concentrations at the four different time points considered.

		Predose concentrations (ng/ml per mg/day per kg body weight)			
Genotype	*n*	1 Week	1 Month	5 Months	12 Months
All	103	107.18 ± 74.93	117.48 ± 59.82	155.62 ± 77.76	172.40 ± 107.35
*CYP3A4*1/*1*	98	110.03 ± 75.70	120.48 ± 59.61	160.47 ± 76.25	178.61 ± 106.64
*CYP3A4*1/*1B*	5	54.28 ± 26.45	59.12 ± 24.00†*	62.43 ± 41.12†**	57.01 ± 17.34†**
*CYP3A5*3/*3*	93	112.37 ± 76.60	123.21 ± 59.57	163.34 ± 76.23	183.07 ± 107.82
CYP3A5 expressers	10	61.53 ± 34.99‡**	64.74 ± 29.17‡**	85.38 ± 54.74‡**	78.54 ± 30.37‡***
*3A4*1/3A5*3*	93	112.37 ± 76.60	123.21 ± 59.57	163.34 ± 76.23	183.07 ± 107.82
*3A4*1/3A5*1*	5	68.77 ± 43.86	70.36 ± 35.49§*	108.32 ± 61.11	100.09 ± 24.78§*
*3A4*1B/3A5*1*	5	54.28 ± 26.45§*	59.12 ± 24.00§*	62.43 ± 41.12§**	57.01 ± 17.34§***

Values shown are mean ± standard deviation. Because of the existing linkage disequilibrium, values for CYP3A5 nonexpressers are the same as those shown by carriers of the *CYP3A4*1/CYP3A5*3* haplotype. In the same manner, all the patients who carried the *CYP3A4*1B* variant were also carriers of the *CYP3A*1B-CYP3A5*1* haplotype and hence displayed values are identical.
**P* < 0.05; ***P* < 0.01; ****P* < 0.001.
†*P* value vs. *CYP3A4*1/*1* carriers.
‡*P* value vs. CYP3A5*3/*3 carriers.
§*P* value vs. *CYP3A4*1/CYP3A5*3* carriers.

Table 4. Effect of *CYP3A4* and *CYP3A5* genotypes on tacrolimus dose requirements at the four different time points considered.

		Dose requirements (mg/kg)			
Genotype	*n*	1 Week	1 Month	5 Months	12 Months
All	103	0.13 ± 0.06	0.12 ± 0.05	0.08 ± 0.05	0.06 ± 0.04
*CYP3A4*1/*1*	98	0.13 ± 0.06	0.12 ± 0.05	0.08 ± 0.04	0.06 ± 0.04
*CYP3A4*1/*1B*	5	0.14 ± 0.03	0.15 ± 0.03	0.14 ± 0.05†**	0.12 ± 0.04†***
*CYP3A5*3/*3*	93	0.13 ± 0.06	0.11 ± 0.05	0.07 ± 0.04	0.06 ± 0.04
CYP3A5 expressers	10	0.14 ± 0.05	0.16 ± 0.04‡**	0.13 ± 0.05‡∫	0.12 ± 0.05‡***
*3A4*1/3A5*3*	93	0.13 ± 0.06	0.11 ± 0.05	0.07 ± 0.04	0.06 ± 0.04
*3A4*1/3A5*1*	5	0.14 ± 0.06	0.17 ± 0.04§**	0.12 ± 0.06§**	0.11 ± 0.05§*
*3A4*1B/3A5*1*	5	0.14 ± 0.03	0.15 ± 0.03	0.14 ± 0.05§***	0.12 ± 0.04§**

Values shown are mean ± standard deviation. Because of the existing linkage disequilibrium, values for CYP3A5 nonexpressers are the same as those shown by carriers of the *CYP3A4*1/CYP3A5*3* haplotype. In the same manner, all the patients who carried the *CYP3A4*1B* variant were also carriers of the *CYP3A*1B-CYP3A5*1* haplotype and hence displayed values are identical.
**P* < 0.05; ***P* < 0.01, ****P* < 0.001, ∫*P* < 0.0001.
†*P* value vs. *CYP3A4*1/*1* carriers.
‡*P* value vs. *CYP3A5*3/*3* carriers.
§*P* value vs. *CYP3A4*1/CYP3A5*3* carriers.

Table 5. Effect of the number of *ABCB1* variant alleles on dose-adjusted predose concentrations and dose requirements in CYP3A5 nonexpressers (*n* = 93). Mean ± standard deviation values are shown.

ABCB1 variants	*n*	1 Week	*P*	1 Month	*P*	5 Months	*P*	12 Months	*P*
Dose-corrected predose concentrations (ng/ml per mg/day per kg body weight)									
0–3	70	110.04 ± 55.28	0.64	127.82 ± 62.05	0.13	168.51 ± 75.84	0.25	191.13 ± 114.44	0.21
4–6	23	118.87 ± 122.19		105.59 ± 47.80		146.74 ± 78.55		156.40 ± 82.18	
Dose requirements (mg/kg)									
0–3	70	0.12 ± 0.06	0.06	0.11 ± 0.05	0.01	0.07 ± 0.04	0.09	0.05 ± 0.04	0.14
4–6	23	0.15 ± 0.06		0.14 ± 0.05		0.09 ± 0.04		0.07 ± 0.04	

period of study. Diagnosis was confirmed by biopsy in six patients. No protocol biopsies were performed in individuals with stable graft function. Sixteen patients (15.5%) underwent acute rejection according to diagnostic criteria described in the Methods section (diagnosis was confirmed by biopsy in three of those

subjects). Fourteen (13.6%) individuals showed neurotoxicity.

No *CYP3A4/CYP3A5* SNPs or haplotypes showed a significant effect on tacrolimus-induced toxicity (Table 6). In contrast, both the *ABCB1* 2677GG and 1236CC wild type genotypes increased the risk for neurotoxic events [OR (CI) = 3.12 (1.0–10.7), $P = 0.04$ and OR (CI) = 3.19 (1.1–10.4), $P = 0.04$; respectively] in analyses carried out considering a dominant model of inheritance (2677GG vs. GT+TT carriers or 1236CC vs. CT + TT carriers, respectively). No further associations were found for the rest of the *ABCB1* SNPs (data not shown).

Four major *ABCB1* haplotypes (3435-2677-1236), namely CGC, TTT, TGC, and CGT, were identified in the population of study and their associations with the occurrence of adverse effects are depicted in Table 6. Four other rare haplotypes, namely TGT, TTC, CTT, and CTC, were also identified with frequencies equal to or lower than 0.01, and hence their associations with toxicity were not tested. Most notably, and in comparison with the wildtype CGC haplotype, the TGC haplotype significantly increased the risk for CNIT [OR (CI) = 4.73 (1.3–16.7), $P = 0.02$]. In addition, the mutant TTT haplotype was observed to moderately reduce the risk for neurotoxicity [OR (CI) = 0.34 (0.1–1.0), $P = 0.05$] (Table 6). The analyzed SNPs in the EETs-synthesizing genes *CYP2C8*3* and *CYP2J2*7* were not associated with tacrolimus toxicity.

Finally, no associations were found between SNPs affecting tacrolimus blood levels or dose requirements and the occurrence of acute rejection. In the same manner, we did not observe any significant association between acute rejection and polymorphisms in the *ABCB1*, *CYP2C8,* or *CYP2J2* genes (Table 6).

Discussion

Our findings confirm in our population previous data indicating that renal transplant recipients who are carriers of the *CYP3A5*1* allele display lower dose-corrected exposure and higher dose requirements of tacrolimus. Overall, mean C_0/dose in CYP3A5 expressers was 50.5% lower than that of nonexpressers, which is also consistent with previous studies reporting an approximate halving of the tacrolimus C_0/dose [11,27,28]. Less and more controversial data are available on the effect of the *CYP3A4*1B* SNP [10,29]. We observed that the *CYP3A4*1B* variant was associated with lower C_0/dose levels and higher dose requirements. However, linkage disequilibrium between *CYP3A4* and *CYP3A5* SNPs resulted in all *CYP3A4*1B* carriers also harboring the *CYP3A5*1* allele, and therefore the possibility exists that the observed clinical impact could be a consequence of the *CYP3A5*1* allele [11]. Still, our results suggest otherwise. PK data were different depending on the *CYP3A4/CYP3A5* haplotype. *CYP3A4*1/CYP3A5*3* carriers showed the highest predose concentrations followed by *3A4*1/3A5*1* patients and then by *3A4*1B/3A5*1* carriers, which would indicate a significant role of the *CYP3A4*1B*. Furthermore, we observed that differences between the two groups of CYP3A5 expressers (carrying or not carrying the *CYP3A4*1B* variant) increased significantly over time. In this regard, Kuypers *et al.* [30] found a similar trend for the same three *CYP3A4-CYP3A5* haplotypes in 95 renal transplant recipients for an extended period of 5 years. However, in the study by Kuypers *et al.*, C_0/dose values were not corrected by weight and statistical differences between the two groups of CYP3A5 expressers were not

Table 6. Effect of *CYP450* polymorphisms and *ABCB1* haplotypes (3435-2677-1236) on the clinical outcome of renal transplantation. Percentages of patients or estimated haplotype frequencies in the different groups are shown.

	Nephrotoxicity			Neurotoxicity			Acute rejection		
Genotype/haplotype	No	Yes	OR (CI)	No	Yes	OR (CI)	No	Yes	OR (CI)
*3A4*1-3A5*3*	89.3	94.7	Ref.	88.8	100	Ref.	89.7	93.8	Ref.
*3A4*1-3A5*1*	6.0	0.0	NC	5.6	0.0	NC	5.7	0.0	NC
*3A4*1B-3A5*1*	4.8	15.3	1.04 (0.1–9.9)	5.6	0.0	NC	4.6	6.3	1.3 (0.1–12.4)
ABCB1									
CGC	48.2	44.7	Ref.	45.5	60.7	Ref.	48.0	43.5	Ref.
TTT	32.9	36.8	1.13 (0.5–2.6)	36.2	17.9	0.34 (0.1–1.0)*	33.1	37.2	1.41 (0.6–3.4)
TGC	6.2	18.4	4.73 (1.3–16.7)**	7.6	14.3	2.00 (0.5–8.2)	9.2	6.6	1.1 (0.2–6.0)
CGT	5.8	0.0	NC	4.3	7.1	1.27 (0.2–7.8)	5.9	0.0	NC
*CYP2C8*1/*1*	70.2	78.9	Ref.	71.9	71.4	Ref.	73.6	62.5	Ref.
*CYP2C8*1/*3 + *3/*3*	29.8	21.1	0.63 (0.2–2.1)	28.1	28.6	1.02 (0.3–3.6)	26.4	37.5	1.58 (0.5–4.8)
*CYP2J2*1/*1*	96.4	94.7	Ref.	95.5	100.0	Ref.	95.4	100	Ref.
*CYP2J2*1/*7*	3.6	5.3	1.5 (0.1–15.2)	4.5	0.0	NC	4.6	0 (0)	NC

*$P = 0.05$; **$P = 0.02$.

Ref., reference; NC, noncalculable; OR, Odds ratio with 95% confidence intervals (CI).

tested, which makes comparisons with our results difficult. One of the putative explanations for the differences observed between the different *CYP3A4-CYP3A5* haplotypes could be the tapering of glucocorticoids. These drugs are known CYP3A4 inducers [31], and hence lower doses would lead to a decrease induction and subsequent higher drug exposure. This would be particularly noticeable in carriers of the wild type allele, as it has been shown that the *CYP3A4*1B* variant is less responsive to drug inducibility [32].

In accordance with the majority of studies (reviewed by Staatz *et al.* [13]), we found no evidence of a significant role of *ABCB1* SNPs or haplotypes on tacrolimus PKs. It is possible that those studies reporting a PK impact of *ABCB1* SNPs may in fact be observing the effect of *CYP3A* variants. Most studies have observed that the impact of *ABCB1* SNPs is lost after eliminating the confounder *CYP3A5* genotype [33–35], which is consistent with our results. A reason for the observed trend in the present study toward higher dose requirements in carriers of 4–6 variants after controlling for the *CYP3A5* genotype may be the different distribution of some *ABCB1* SNPs between CYP3A5 expressers and nonexpressers in our population (e.g. 3435 TT subjects accounted for 23.7% among nonexpressers while being absent in the CYP3A5 expresser group).

The results of this study do not support a critical role of *CYP3A* or *ABCB1* SNPs in the development of toxicity induced by tacrolimus. Despite the strong association of *CYP3A5*1* with tacrolimus PKs, there is no consensus regarding its role in CNIT [36]. In agreement with our results, most studies have failed to find an association between this SNP and renal dysfunction [2,9,37–43]. Conversely, Kuypers *et al.* found that CNIT was related to the presence of the *CYP3A5*1* allele in renal recipients [30,44]. However, the authors observed that the proportion of *CYP3A5*3* carriers who also had higher dose requirements were as well more prone to develop CNIT, which probably diminishes the significance of the reported association with the *CYP3A5*1* allele [44].

We found that the *ABCB1* TGC haplotype was consistently associated with CNIT. As the precise impact of *ABCB1* haplotypes on P-glycoprotein function is yet to be established, we can only speculate on the explanation for this finding. It might indicate an accumulation of the drug due to reduced efflux because of the presence of the 3435T variant. The donor's genotype (influencing P-glycoprotein status in the allograft) would most likely be more revealing on this matter. It needs to be pointed out that the protocol followed in our center does not include performing biopsies in all patients with signs of renal dysfunction and therefore potential underreporting of the true incidence of CNIT might have occurred.

To our knowledge, there are no previous genetic association studies on tacrolimus-induced neurotoxicity in renal transplant recipients. Two studies in different settings have found a positive association for the 2677T [45] and 1236T [46] mutant alleles. However, the first study only included 17 patients (six with neurotoxicity) and none of them carried out haplotype analyses. Our study shows opposite results in the sense that both the wild type 2677GG and 1236CC genotypes were associated with increased risk of neurotoxicity compared with T-allele carriers. Notwithstanding, we acknowledge that the clinical significance of these findings is probably limited, as further analyses using a codominant model of inheritance did not find significant associations. A statistical trend toward lower risk of neurotoxicity in carriers of the mutant *ABCB1* TTT haplotype was also observed. These results would argue against the general conception that a mutated P-glycoprotein in the blood–brain barrier would cause an accumulation of drug in the brain and hence higher risk of neurotoxicity. However, it is still not clear how exactly these *ABCB1* haplotypes affect the transporting capacity of the protein. Moreover, the precise mechanism for tacrolimus neurotoxicity remains unknown, and studies other than case reports and including determinations of tacrolimus in the cerebrospinal fluid are warranted. Finally, there is also a potential risk that the relatively low sample size of the study, along with the low frequency of some polymorphisms, may have resulted in putative spurious associations.

In agreement with our results, and despite the observed underexposure to tacrolimus, most association studies have failed to find a correlation between the presence of the *CYP3A5*1* allele and the rate of acute rejection in renal transplant recipients [2,9,29,30,37,47,48]. The effect of *ABCB1* genetic variants has been less studied. A large study has reported a moderately increased risk for acute rejection in carriers of the *ABCB1* TGC (3435-2677-1236) haplotype, although their analysis included both tacrolimus- and cyclosporine-treated patients [47]. We and others [29,37] could not confirm the significance of *ABCB1* in acute rejection. However, the fact that an association of the very same TGC haplotype with CNIT was observed in this study could be an indication of a relevant clinical role for this combination of SNPs in the *ABCB1* gene.

To date, the influence of *CYP2C8*3* and *CYP2J2*7* SNPs on the outcome of renal transplantation had not been tested. We have shown that the incidence of DGF was higher in *CYP2C8*3/*3* carriers, a genotype associated with reduced arachidonic acid biotransformation to EETs [22]. It is tempting to speculate that lower amounts of these protective compounds could have played a role in the development of DGF. Consistent with these results,

Smith *et al.* [49] have reported a higher risk of tacrolimus nephrotoxicity in liver recipients who were carriers of the *CYP2C8*3* allele.

Recently, a prospective study in renal transplant recipients analyzed the effect of administering either a standard tacrolimus dose or a dose according to the patient's *CYP3A5* genotype. The authors concluded that the dose-adapted group reached target concentrations earlier and with less dose changes [2]. The results of this study support that view, but also suggest that the additional genotyping of the *CYP3A4*1B* SNP could probably make dose adjustments more accurate. On the other hand, both our results and the general perception in the literature indicate that those PK differences hardly translate into different clinical outcomes. There are several reasons that can explain this. Renal recipients are usually subjected to intensive concentration-controlled dose adaptations in the first days after grafting, which, along with other nongenetic factors (e.g. steroids dosing, drugs interactions, etc.), might overshadow the effect of genetic variants. Second, new data have revealed other significant SNPs besides *CYP3A5*1* that may be important in a pharmacogenetic approach to dose adaptations of tacrolimus [50–52]. Third, the precise effect of *ABCB1* SNPs and haplotypes on the P-glycoprotein transporting capacity is as yet not fully understood and hence results from association studies do not have a straightforward interpretation. Genetic analyses of donors together with gene expression studies in the allograft will probably help elucidate the link between genetic variations and tacrolimus PDs in the near future.

Authorship

GG: designed study and wrote the paper. MG, RMM: performed the genetic analyses and collected clinical data. JJC, FC: designed study and collected data. JB: designed study.

Funding

This work has been supported in part by grant GR10022 from Junta de Extremadura, Consejeria de Economia, Comercio e Innovacion, Merida (Spain), and grant PRiS113 from Fundesalud, Merida (Spain).

References

1. Venkataramanan R, Swaminathan A, Prasad T, *et al.* Clinical pharmacokinetics of tacrolimus. *Clin Pharmacokinet* 1995; **29**: 404.
2. Thervet E, Loriot MA, Barbier S, *et al.* Optimization of initial tacrolimus dose using pharmacogenetic testing. *Clin Pharmacol Therapeut* 2010; **87**: 721.
3. Chandel N, Aggarwal PK, Minz M, Sakhuja V, Kohli KK, Jha V. CYP3A5*1/*3 genotype influences the blood concentration of tacrolimus in response to metabolic inhibition by ketoconazole. *Pharmacogenet Genom* 2009; **19**: 458.
4. Sattler M, Guengerich FP, Yun CH, Christians U, Sewing KF. Cytochrome P-450 3A enzymes are responsible for biotransformation of FK506 and rapamycin in man and rat. *Drug Metab Dispos* 1992; **20**: 753.
5. 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* 2001; **27**: 383.
6. Macphee IA, Fredericks S, Tai T, *et al.* Tacrolimus pharmacogenetics: polymorphisms associated with expression of cytochrome p4503A5 and P-glycoprotein correlate with dose requirement. *Transplantation* 2002; **74**: 1486.
7. 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* 2004; **14**: 147.
8. 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* 2003; **76**: 1233.
9. Hesselink DA, van Schaik RH, van Agteren M, *et al.* CYP3A5 genotype is not associated with a higher risk of acute rejection in tacrolimus-treated renal transplant recipients. *Pharmacogenet Genom* 2008; **18**: 339.
10. Hesselink DA, van Schaik RH, van der Heiden IP, *et al.* Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Therapeut* 2003; **74**: 245.
11. Op den Buijsch RA, Christiaans MH, Stolk LM, *et al.* Tacrolimus pharmacokinetics and pharmacogenetics: influence of adenosine triphosphate-binding cassette B1 (ABCB1) and cytochrome (CYP) 3A polymorphisms. *Fundamental Clin Pharm* 2007; **21**: 427.
12. Saeki T, Ueda K, Tanigawara Y, Hori R, Komano T. Human P-glycoprotein transports cyclosporin A and FK506. *J Biol Chem* 1993; **268**: 6077.
13. Staatz CE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: Part I. *Clin Pharmacokinet* 2010; **49**: 141.
14. Staatz CE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: Part II. *Clinical Pharmacokinet* 2010; **49**: 207.
15. Zeldin DC, Moomaw CR, Jesse N, *et al.* Biochemical characterization of the human liver cytochrome P450 arachidonic acid epoxygenase pathway. *Arch Biochem Biophys* 1996; **330**: 87.

16. Zeldin DC, DuBois RN, Falck JR, Capdevila JH. Molecular cloning, expression and characterization of an endogenous human cytochrome P450 arachidonic acid epoxygenase isoform. *Arch Biochem Biophys* 1995; **322**: 76.
17. Node K, Huo Y, Ruan X, *et al.* Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* 1999; **285**: 1276.
18. Chen G, Wang P, Zhao G, *et al.* Cytochrome P450 epoxygenase CYP2J2 attenuates nephropathy in streptozotocin-induced diabetic mice. *Prostaglandins Other Lipid Mediat* 2011; **96**: 63–71.
19. Levey AS, Greene T, Kuske J, Beck GJ, Group MS. A simplified equation to predict glomerular filtration rate from serum creatinine. *J Am Soc Nephrol* 2000; **11**: 155A.
20. Geddes CC, Woo YM, Jardine AG. The impact of delayed graft function on the long-term outcome of renal transplantation. *J Nephrol* 2002; **15**: 17.
21. Cavalli SA, Hirata MH, Hirata RD. Detection of MboII polymorphism at the 5' promoter region of CYP3A4. *Clin Chem* 2001; **47**: 348.
22. Dai D, Zeldin DC, Blaisdell JA, *et al.* Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid. *Pharmacogenetics* 2001; **11**: 597.
23. van Schaik RH, van der Heiden IP, van den Anker JN, Lindemans J. CYP3A5 variant allele frequencies in Dutch Caucasians. *Clin Chem* 2002; **48**: 1668.
24. King LM, Ma J, Srettabunjong S, *et al.* Cloning of CYP2J2 gene and identification of functional polymorphisms. *Mol Pharmacol* 2002; **61**: 840.
25. 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* 2003; **14**: 1889.
26. Sole X, Guino E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. *Bioinformatics (Oxford, England)* 2006; **22**: 1928.
27. Macphee IA, Fredericks S, Mohamed M, *et al.* Tacrolimus pharmacogenetics: the CYP3A5*1 allele predicts low dose-normalized tacrolimus blood concentrations in whites and South Asians. *Transplantation* 2005; **79**: 499.
28. Zhang X, Liu ZH, Zheng JM, *et al.* Influence of CYP3A5 and MDR1 polymorphisms on tacrolimus concentration in the early stage after renal transplantation. *Clin Transplant* 2005; **19**: 638.
29. Roy JN, Barama A, Poirier C, Vinet B, Roger M. Cyp3A4, Cyp3A5, and MDR-1 genetic influences on tacrolimus pharmacokinetics in renal transplant recipients. *Pharmacogenet Genom* 2006; **16**: 659.
30. Kuypers DR, de Jonge H, Naesens M, Lerut E, Verbeke K, Vanrenterghem Y. CYP3A5 and CYP3A4 but not MDR1 single-nucleotide polymorphisms determine long-term tacrolimus disposition and drug-related nephrotoxicity in renal recipients. *Clin Pharmacol Therapeut* 2007; **82**: 711.
31. Sewing KF. Pharmacokinetics, dosing principles, and blood level monitoring of FK506. *Transplant Proc* 1994; **26**: 3267.
32. Floyd MD, Gervasini G, Masica AL, *et al.* Genotype-phenotype associations for common CYP3A4 and CYP3A5 variants in the basal and induced metabolism of midazolam in European- and African-American men and women. *Pharmacogenetics* 2003; **13**: 595.
33. Fredericks S, Moreton M, Reboux S, *et al.* Multidrug resistance gene-1 (MDR-1) haplotypes have a minor influence on tacrolimus dose requirements. *Transplantation* 2006; **82**: 705.
34. 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* 2006; **7**: 563.
35. Loh PT, Lou HX, Zhao Y, Chin YM, Vathsala A. Significant impact of gene polymorphisms on tacrolimus but not cyclosporine dosing in Asian renal transplant recipients. *Transplant Proc* 2008; **40**: 1690.
36. Hesselink DA, Bouamar R, van Gelder T. The pharmacogenetics of calcineurin inhibitor-related nephrotoxicity. *Therapeut Drug Monitoring* 2010; **32**: 387.
37. Glowacki F, Lionet A, Buob D, *et al.* CYP3A5 and ABCB1 polymorphisms in donor and recipient: impact on Tacrolimus dose requirements and clinical outcome after renal transplantation. *Nephrol Dial Transplant* 2011; **26**: 3046.
38. Ferraresso M, Tirelli A, Ghio L, *et al.* Influence of the CYP3A5 genotype on tacrolimus pharmacokinetics and pharmacodynamics in young kidney transplant recipients. *Pediatric Transplant* 2007; **11**: 296.
39. Renders L, Frisman M, Ufer M, *et al.* CYP3A5 genotype markedly influences the pharmacokinetics of tacrolimus and sirolimus in kidney transplant recipients. *Clin Pharmacol Therapeut* 2007; **81**: 228.
40. Zheng HX, Zeevi A, McCurry K, *et al.* The impact of pharmacogenomic factors on acute persistent rejection in adult lung transplant patients. *Transplant Immunol* 2005; **14**: 37.
41. Grenda R, Prokurat S, Ciechanowicz A, Piatosa B, Kalicinski P. Evaluation of the genetic background of standard-immunosuppressant-related toxicity in a cohort of 200 paediatric renal allograft recipients–a retrospective study. *Ann Transplant* 2009; **14**: 18.
42. Quteineh L, Verstuyft C, Furlan V, *et al.* Influence of CYP3A5 genetic polymorphism on tacrolimus daily dose requirements and acute rejection in renal graft recipients. *Basic Clin Pharmacol Toxicol* 2008; **103**: 546.
43. Metalidis C, Lerut E, Naesens M, Kuypers DR. Expression of CYP3A5 and P-glycoprotein in renal allografts with histological signs of calcineurin inhibitor nephrotoxicity. *Transplantation* 2011; **91**: 1098.
44. Kuypers DR, Naesens M, de Jonge H, Lerut E, Verbeke K, Vanrenterghem Y. Tacrolimus dose requirements and CYP3A5 genotype and the development of calcineurin

inhibitor-associated nephrotoxicity in renal allograft recipients. *Therapeut Drug Monitoring* 2010; **32**: 394–404.
45. 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* 2002; **74**: 571.
46. Yanagimachi M, Naruto T, Tanoshima R, *et al.* Influence of CYP3A5 and ABCB1 gene polymorphisms on calcineurin inhibitor-related neurotoxicity after hematopoietic stem cell transplantation. *Clin Transplant* 2010; **24**: 855.
47. Bandur S, Petrasek J, Hribova P, Novotna E, Brabcova I, Viklicky O. Haplotypic structure of ABCB1/MDR1 gene modifies the risk of the acute allograft rejection in renal transplant recipients. *Transplantation* 2008; **86**: 1206.
48. MacPhee IA, Fredericks S, Tai T, *et al.* The influence of pharmacogenetics on the time to achieve target tacrolimus concentrations after kidney transplantation. *Am J Transplant* 2004; **4**: 914.
49. Smith HE, Jones JP 3rd, Kalhorn TF, *et al.* Role of cytochrome P450 2C8 and 2J2 genotypes in calcineurin inhibitor-induced chronic kidney disease. *Pharmacogenet Genom* 2008; **18**: 943.
50. de Jonge H, Metalidis C, Naesens M, Lambrechts D, Kuypers DR. The P450 oxidoreductase *28 SNP is associated with low initial tacrolimus exposure and increased dose requirements in CYP3A5-expressing renal recipients. *Pharmacogenomics* 2011; **12**: 1281.
51. Elens L, Bouamar R, Hesselink DA, *et al.* A new functional CYP3A4 intron 6 polymorphism significantly affects tacrolimus pharmacokinetics in kidney transplant recipients. *Clin Chem* 2011; **57**: 1574.
52. Jacobson PA, Oetting WS, Brearley AM, *et al.* Novel polymorphisms associated with tacrolimus trough concentrations: results from a multicenter kidney transplant consortium. *Transplantation* 2011; **91**: 300.