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Increased Hospital Stay and Allograft Disfunction in Renal Transplant Recipients with Cyp2c19 AA Variant in SNP rs4244285

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ABSTRACT

Pharmacogenetics correlates certain genetic variants, such as single nucleotide polymorphisms (SNPs), with blood drug levels, efficacy, and adverse effects of the treatment. Tacrolimus is mainly metabolized via *CYP3A4/5*, whereas *CYP2C19* and *CYP3A4/5* are responsible for omeprazole metabolism. Omeprazole inhibits tacrolimus metabolism via CYP3A5 in patients carrying variant alleles of *CYP2C19*, increasing tacrolimus blood concentrations. Seventy-five renal transplant recipients treated with tacrolimus and concomitant omeprazole were genotyped in a panel of 37 SNPs with use of Sequenom MassArray. The patients with *CYP2C19*2/*2* genotype (n = 4) showed a median posttransplantation hospital stay of 27.5 days (95% confidence interval [CI], 23–39 days), compared with 12 days (95% CI, 10–15 days) in patients with *CYP2C19*1/*1* or *CYP2C19*1/*2* (n = 71; P = 0.016, Kruskal-Wallis test).The difference in hospital stay was directly correlated with an increase in tacrolimus levels (C_{min}/[dose/weight]) during the first week after trasplantation (in 59 patients with data on levels; P = 0.021, Kruskal-Wallis), excluding the patients with atypical metabolisms due to *CYP3A5*1/*3* or *CYP3A5*1/*1* genotype. Recipients with *CYP2C19*2/*2* genotype also showed allograft delayed function (acute tubular necrosis in 3 patients). Genotyping of *CYP3A5* and *CYP2C19* in renal transplantation should be considered to be of interest when treating with tacrolimus and omeprazole, because *CYP2C19*2/*2* variant indirectly elicits an increase of tacrolimus blood levels and, in our study population, the adverse effects described.

Introduction

Optimizing balance between therapeutic efficacy and the occurrence of adverse events is the main goal of individualized medicine. This takes even more importance in narrow therapeutic index drugs, such as immunosuppressants. Calcineurin inhibitors are central in the pharmacological treatment after solid organ transplantation. These drugs are highly effective in preventing acute graft rejection, but both tacrolimus and cyclosporine show highly variable pharmacokinetics and pharmacodynamics (Hesselink et al., 2003). Therefore, the fragile equilibrium between the risks and benefits of immunosuppression makes the management of immunosuppressive pharmacotherapy a challenge.

Therapeutic drug monitoring (TDM) is an essential and indispensable instrument for calcineurin inhibitor dosing and reduces the pharmacokinetic component of variability by controlling drug blood concentrations. However, TDM is only possible after the drug is administered and steady state and patient's compliance are achieved; thus, complementary strategies are needed (Cattaneo et al., 2004). Moreover, despite correct TDM, it may take several days or even weeks to reach target blood concentrations. For many patients, this period is not appropriate to achieve sufficiently high concentrations to prevent graft rejection or adverse reactions without exposing the patient to excessive toxicity (Ware and MacPhee, 2010). In this sense, pharmacogenetics is an interesting approach, helpful to manage immunosuppressant drugs. Pharmacogenetics is defined as the study of variations in DNA sequence as related to drug response (definitions for genomic biomarkers, pharmacogenomics, pharmacogenetics, genomic data, and sample coding categories; www.ich.org, 2007). Changes in expression or function of proteins and enzymes involved in drug transportation, metabolism, or mechanism of action will cause changes in drug's absorption, metabolism, and distribution and, therefore, can lead to changes in the response and toxicity of the treatment.

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ABBREVIATIONS: C_0, trough level; C_0/D_c, trough concentration/dose corrected by weight; CI, confidence interval; PPI, proton pump inhibitors; SNP, single nucleotide polymorphism; TDM, therapeutic drug monitoring.

Characterization of these genetic variants can help to establish effective doses and to minimize adverse reactions (Staatz et al., 2010). The pharmacogenetic strategy that is closest to translation into clinical practice is the use of *CYP3A5* genotype to predict the optimal initial dose for tacrolimus (Ware and MacPhee, 2010). A polymorphism in intron 3 of *CYP3A5*, the *CYP3A5*3* allele, results in a premature stop codon, whereas individuals carrying the *CYP3A5*1* allele express this enzyme normally (Kim et al., 2012). Several studies have associated the presence of *CYP3A5*1* allele with lower dose-adjusted tacrolimus blood concentrations and higher tacrolimus dose requirements (Glowacki et al., 2011; Cho et al., 2012). On the other hand, the influence of *ABCB1* polymorphisms (gene coding for P glycoprotein) on tacrolimus pharmacokinetics and clinical outcomes is still controversial (Kim et al., 2012; Glowacki et al., 2011).

In addition, because calcineurin inhibitors are substrates of cytocrome P450, especially the CYP3A5 isoform, they may have interactions with many other widely prescribed drugs also metabolized by this enzyme that can lead to altered blood concentrations. These interactions might be very frequent, because transplant recipients take several other drugs for the treatment or prevention of complications. Most of the time, the relevance of the interaction is also determined by genetic polymorphisms, which modulate the expression or function of the metabolizing enzyme. In this sense, the interaction between proton pump inhibitors (PPI) and tacrolimus has been described previously in liver transplant recipients (Hosohata et al., 2008; Hosohata et al., 2009a; Hosohata et al., 2009b) with *CYP2C19* variant genotype in the polymorphism rs4244285 (*CYP2C19*2*) leading to elevated blood concentrations.

Given these facts, we designed a platform with 37 single nucleotide polymorphisms (SNPs) in 14 genes coding for metabolizing enzymes, transporters, and molecular targets of tacrolimus and other immunosuppressive agents, including some polymorphisms related to other concomitant drugs. We proceeded to its evaluation as a potential tool to support decision making in the complex context of pharmacotherapy for transplant recipients.

Materials and Methods

Study Design and Patients. From March 2008 through December 2009, all de novo adult renal transplant recipients (n = 75) and their respective donors (n = 54) who underwent surgery in our hospital and provided their written informed consent were included in the study. Patients who underwent simultaneous pancreatic transplantation were excluded. Immunosuppression regimen after surgery was based in tacrolimus.

Patients were followed up for the first two weeks after transplantation for the pharmacokinetic data and for 18 months for clinical data. Analytical and clinical data and the length of hospital stay after organ transplantation (days from surgery to discharge after transplantation) were obtained retrospectively from medical records.

Genetic data and any other relevant information were retrieved from the study population without interfering with patient treatment and were handled according to standard regulations for data registration, use, and preservation of patient anonymity and privacy.

The study was approved by the local Clinical Research Ethics Committee (registry number 2008/0263) and was conducted in accordance with the Declaration of Helsinki and Istanbul and its amendments.

Baseline Immunosuppression and Measurement of Tacrolimus Concentrations. The immunosuppression regimen consisted of tacrolimus, corticosteroids, and mycophenolate mofetil or sodium. Oral treatment was started within 24 hours after surgery unless there were complications. All the patients received tacrolimus orally as the primary immunosuppression drug at an initial dose of 0.1–0.2 mg/kg/day divided into two doses. The dose of tacrolimus was individualized by TDM to maintain the target blood trough concentration of 10–15 ng/ml.

Tacrolimus blood concentrations were measured in whole-blood samples collected immediately before tacrolimus morning dose administration (C_0, trough level, in nanograms per milliliter) using a clinical chemistry system (Dimension; Siemens Healthcare, Deerfield, IL). We obtained a minimum of four samples per patient: two during the first week after transplantation (days 4 and 7 approximately) and two during the second week (days 9 and 14 approximately).

The mean of dose-normalized blood concentration of tacrolimus during the first and second week after transplantation was assessed separately for each recipient and expressed as the ratio trough concentration/dose corrected by weight (C_0/D_c) [(ng/ml)/(mg/kg/24h)].

Genotyping. Genomic DNA was collected from EDTA-anticoagulated whole blood samples from transplant recipients and donors. The DNA was extracted from 200 μl of blood with use of a commercially available kit based on centrifugation in microcolumns (UltraCleanBloodSpin DNA Isolation Kit; MoBio Laboratories Inc., Carlsbad, CA). After quantification using a spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE) to determine the concentration and purity, DNA was stored at −20°C until use.

A genetic analysis platform (MassARRAY; Sequenom, Inc, San Diego, CA) was used to genotype each sample. Thirty-seven SNPs in 14 different genes were analyzed: *ABCB1 (rs1045642, rs2032582, rs1128503, rs2229109, rs2235013, rs2235033, rs3213619, rs9282564), ABCC2/MRP2 (rs2273697, rs3740066, rs717620), ABCG2/BCRP (rs2231137, rs2231142), CYP2B6 (rs2279343, rs3745274), CYP2C19 (rs4244285), CYP2C9 (rs1057910, rs1799853), CYP3A4 (rs2740574), CYP3A5 (rs776746, rs10264272, rs41303343), MTHFR (rs1801131, rs1801133), NOD2/CARD15 (rs2066844, rs2066845), SLCO1A2 (rs11568563, rs72559749, rs11568564), SLCO1B1 (rs2306283, rs4149056), TPMT (rs1142345, rs1800460, rs1800462), UGT1A9 (rs6714486, rs72551330, rs17868320).*

Statistical Analyses. Statistical analysis and calculations were performed using SPSS software (version 19; SPSS, IBM, Armonk, NY) and Prism 4 (GraphPad Software, Inc., La Jolla, CA). Categorical variables were expressed as percentage (95% confidence interval [CI]). Continuous variables were expressed as mean or median (with 95% CI or interquartile range, with outlayers for the graphs), depending on the result of Kolmogorov-Smirnoff or Shapiro-Wilk normality tests, performed depending on the sample size. The association between categorical variables and genotypes was assessed using a χ^2 test. For continuous variables, a Mann-Whitney U test to compare two groups and Kruskal-Wallis test to compare several groups was used. For all the analyses, $P < 0.05$ was considered to be statistically significant.

Results

Genomic DNA from renal transplant recipients and donors (all white) was genotyped. The allele frequencies were consistent with those described in SNP PubMed Database for the white population. The characteristics of the study population are shown in Table 1. After

TABLE 1

Recipients' characteristics

Characteristic	Value
Age, years, mean (95% CI)	49.7 (46.6–52.9)
White race, no. (%)	75 (100.0)
Sex, no. (%)	
Male	47 (62.7)
Female	28 (37.3)
Immunosuppressant treatment, no (%)	
Tacrolimus + mycophenolate mofetil + corticoids	50 (66.7)
Tacrolimus + sodium mycophenolate + corticoids	25 (33.3)
Primary disease, no (%)	
Glomerulonephritis	21 (28.0)
Idiopathic chronic renal disease	20 (26.7)
Polycystic kidney disease	12 (16.0)
Diabetic nephropathy	4 (5.3)
Pyelonephritis	3 (4.0)
Nephroangiosclerosis	3 (4.0)
Other	12 (16.0)

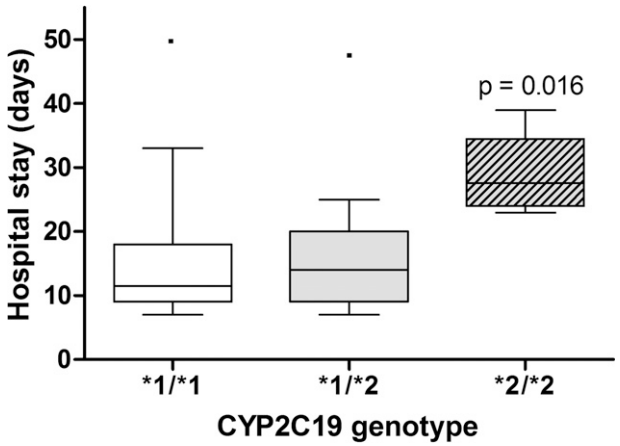

Fig. 1. Hospital stay (days) by rs4244285 CYP2C19 genotype (*1/*1, $n = 51$; *1/*2, $n = 20$; *2/*2, $n = 4$). The box plots represent the median and interquartile range, marking with little dots the outlying values. Kruskal-Wallis test showed a statistically significant difference in group *2/*2 with $P = 0.016$.

applying the corresponding statistical tests, no associations between patients' characteristics and the SNPs studied were found.

A statistically significant difference ($P = 0.016$, Kruskal-Wallis test) in the length of hospital stay was found for *CYP2C19 rs4244285* and *NOD2/CARD15 rs2066844*. Figure 1 represents the median hospital stay in days (with interquartile range) regarding *CYP2C19* genotype. Median hospital stay was double for renal transplant recipients with *CYP2C19*2/*2* genotype, compared with the other two genotype groups: median hospital stay in recipients with *CYP2C19*2/*2* genotype was 27.5 days (95% CI, 23.0–39.0 days), compared with 12.0 days (95% CI, 10.0–13.0 days) for patients with *CYP2C19*1/*1* and 14.5 days (95% CI, 10.0–20.0 days) for patients with *CYP2C19*1/*2*. When data analysis was repeated grouping patients with *CYP2C19*1/*1* plus *CYP2C19*1/*2* genotype, the median hospital stay was 12.0 days (95% CI, 10.0–15.0 days), and when comparing with *CYP2C19*2/*2* group, the difference was even stronger ($P = 0.006$).

A statistically significant difference in hospital stay was also found by *NOD2/CARD15 rs2066844* genotype. Median hospital stay for patients with *CC* in *NOD2/CARD15 rs2066844* was 13.5 days (95% CI, 11.0–19.0 days), compared with 9.5 days (95% CI, 9.0–12.0 days) for patients with *CT/TT* ($P = 0.013$). This difference in median hospital stay was in lesser magnitude than the one found for *CYP2C19 rs4244285*.

No other associations were found between hospital stay and the rest of the SNPs studied. Kruskal-Wallis test *P* values for all the SNPs studied are shown in Table 2.

To explain these findings, because CYP2C19 is not a metabolizing enzyme related to tacrolimus and that drug was our first hypothesis for clinical correlations, all concomitant medication was reviewed and pharmacokinetic analysis was performed. Complete pharmacokinetic data during the first two weeks after transplantation were available for 61 patients, 59 of whom were treated concomitantly with tacrolimus and omeprazole and were included in the analysis. Pharmacokinetic data were assessed for tacrolimus main metabolizing enzyme's genotype, *CYP3A5*, and *CYP2C19* genotype. CYP3A5 is the main metabolizing enzyme of tacrolimus, and lower tacrolimus blood levels are expected for recipients carrying *CYP3A5*1/*3* or *CYP3A5*1/*1*. To avoid this influence, the study of the association between *CYP2C19* genotype and tacrolimus C_0/D_c was performed only with the subset of patients carrying *CYP3A5*3/*3* genotype,

TABLE 2

P values from Kruskal-Wallis test for length of hospital stay (days) in all the studied SNPs

Statistically significant results are shown in bold. NA, not applicable (all patients with the same genotype).

Gene	SNP	Classic nomenclature	*P* value
ABCB1	rs1045642	3435 C>T	0.652
	rs2032582	2677 G>T/A	0.300
	rs1128503		0.609
	rs2229109	1199 G>A	0.449
	rs2235013	1725+38 G>A	0.615
	rs2235033	1554+24T>C	0.615
	rs3213619		0.506
	rs9282564		0.636
ABCC2/MRP2	rs2273697	1249 G>A	0.167
	rs3740066	3972 C>T	0.101
	rs717620	−24 C>T	0.309
ABCG2/BCRP	rs2231137		0.402
	rs2231142	421 C>A	0.104
CYP2B6	rs2279343	CYP2B6*4	0.897
	rs3745274	CYP2B6*6	0.304
CYP2C19	rs4244285	CYP2C19*2	**0.016**
CYP2C9	rs1057910	CYP2C9*3	0.734
	rs1799853	CYP2C9*2	0.544
CYP3A4	rs2740574	CYP3A4*1B	0.428
CYP3A5	rs776746	CYP3A5*3	0.822
	rs10264272	CYP3A5*6	0.331
	rs41303343	CYP3A5*7	NA
MTHFR	rs1801131	1298 A>C	0.375
	rs1801133	677 C>T	0.252
NOD2/CARD15	rs2066844	R702W	**0.028**
	rs2066845		NA
SLCO1A2	rs11568563		0.935
	rs72559749		NA
	rs11568564		NA
SLCO1B1	rs2306283	SLCO1B1*1B	0.518
	rs4149056	SLCO1B1*5	0.839
TPMT	rs1142345	TPMT*3C	0.302
	rs1800460	TPMT*3B	0.284
	rs1800462	TPMT*2	0.095
UGT1A9	rs6714486		0.806
	rs72551330		0.225
	rs17868320		NA

the main variant in the white population ($n = 45$), which is associated with higher tacrolimus levels. Parameters of liver function were also checked and remained within normal range during the first and second week after transplantation (medium bilirubin level, 0.56 mg/dl [95% CI, 0.50–0.62 mg/dl]; aspartate aminotransferase level, 19.4 UI/ml [95% CI, 17.4–21.4 UI/ml]; alanine aminotransferase level, 24.8 UI/ml [95% CI, 21.4–28.2 UI/ml]). No differences were found in *CYP3A5* genotype or *CYP2C19* genotype, during both the first and the second week after transplantation (unpublished data).

To investigate the influence of the genotype of recipients and donors, patients were divided into groups according to recipient/donor genotype. For *CYP3A5* analysis, patients were allocated in two groups: *CYP3A5*1/*1* or *CYP3A5*1/*3* (expressers) and *CYP3A5*3/*3* (nonexpressers). In accordance with *CYP2C19* genotype, patients were classified as *CYP2C19*1/*1* or *CYP2C19*1/*2* (extensive plus intermediate metabolizers) and *CYP2C19*2/*2* (poor metabolizers). Tacrolimus C_0/D_c during the first and second week after transplantation according to *CYP3A5* and *CYP2C19* recipient/donor genotype are shown in Fig. 2. Figure 2, A and C, shows the association between *CYP3A5* recipient/donor genotype and tacrolimus C_0/D_c during the first and second week after transplantation. Tacrolimus C_0/D_c was higher when recipients were *CYP3A5*3/*3*. Donor's influence could not be demonstrated, although slightdifferences in tacrolimus C_0/D_c

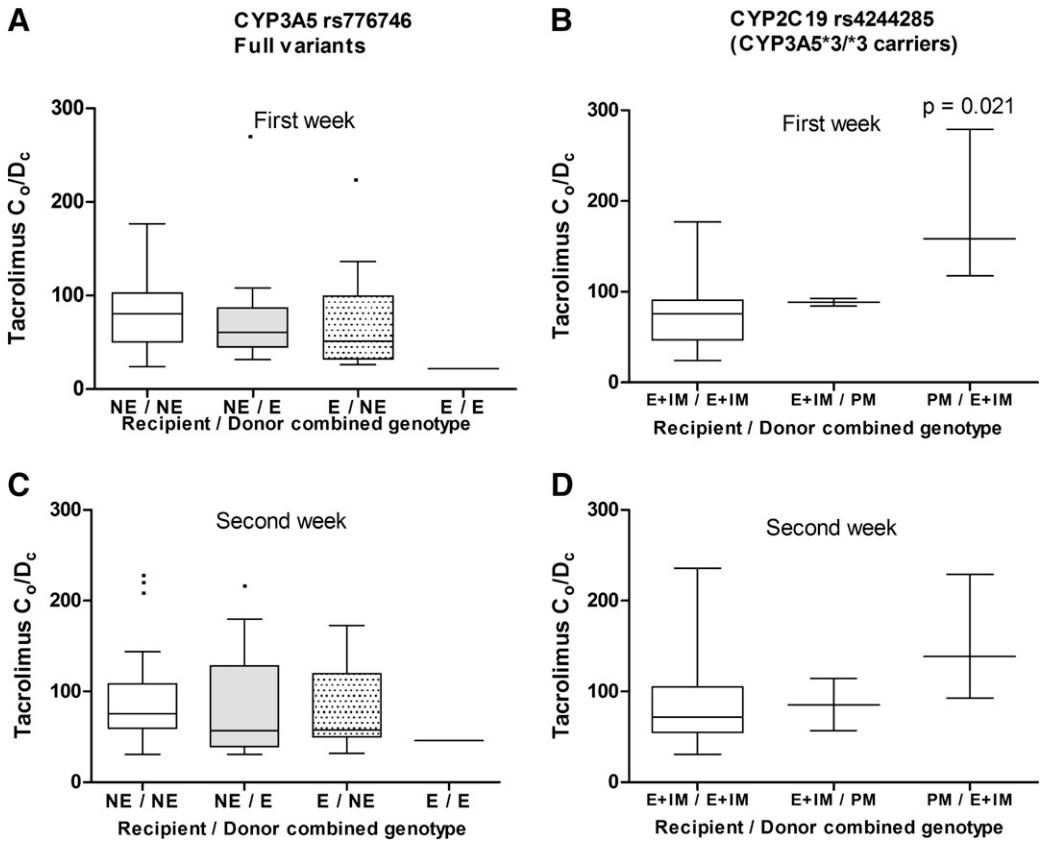

Fig. 2. Tacrolimus dose-normalized blood concentrations by combined recipients/donors genotype. Box plots (median, interquartile range and outlayers) of tacrolimus dose-normalized trough levels (C_0 /D_c [ng/ml]/[mg/kg/24h]) in renal transplant recipients grouped by combined recipient/own donor genotypes. (A and C) Results by CYP3A5 rs776746 where nonexpresser (NE) is *3/*3 (GG) and expresser (E) is *1/*3 or *1/*1 (AG or AA). The data from the first week after transplantation are shown in (A) (NE/NE [n = 32] versus NE/E [n = 12] versus E/NE [n = 7] versus E/E [n = 1]), and the data from the second week after transplantation in (C) (NE/NE [n = 35] versus NE/E [n = 12] versus E/NE [n = 8] versus E/E [n = 1]). No statistically significant differences were found among the groups, applying Kruskal-Wallis tests. (B and D) Results only for those patients carrying CYP3A5*3/*3 in (A) and (C), respectively, but they have been grouped by CYP2C19 rs4244285, whereextensive plus intermediate metabolizer (E+IM) is *1/*1 (GG) or *1/*2 (GA) andpoor metabolizer (PM) is *2/*2 (AA). The results from the first week after transplantation are shown in (B) (E+IM/E+IM [n = 40] versus E+IM/PM [n = 2] versus PM/E+IM [n = 3]), whereas those from the second week are shown in (D) (E+IM/E+IM [n = 42] versus E+IM/PM [n = 2] versus PM/E+IM [n = 3]). Kruskal-Wallis tests showed statistically significant difference in (B) at PM/E+IM group, with P = 0.021.

between groups were observed in some cases. No statistically significant association was found in any case. Figure 2, B and D, shows the association between *CYP2C19* recipient/donor genotype and tacrolimus C_0/D_c during the first and second week after transplantation, respectively, considering only the subset of *CYP3A5*3/*3* carriers from Fig. 2, A and C. In this case, during the first week after transplantation, there was a statistically significant association between *CYP2C19* genotype and tacrolimus C_0/D_c (P = 0.021) that was doubled when the recipient's genotype was *CYP2C19*2/*2*. This tendency was maintained during the second week after transplantation, although no statistically significant association was found.

To determine from these results which genotype is the most relevant, that from the recipient or the one from the donor, tacrolimus C_0/D_c was evaluated separately according to recipients' or donors' genotypes. Figure 3 shows tacrolimus C_0/D_c during the first week after transplantation. Median tacrolimus C_0/D_c was higher for *CYP3A5*3/*3* recipients, but this difference was not statistically significant. No *CYP3A5*1/*1* recipients were found (Fig. 3A). A small difference in C_0/D_c by donor's *CYP3A5 rs776746* genotype was also found (Fig. 3C), comparing *CYP3A5**3/*3 with *CYP3A5**1/*3, whereas the difference with *CYP3A5**1/*1 was higher, although there were only two patients in that group. Nevertheless, no statistically significant differences were found in any case.

With regard to *CYP2C19* genotype, during the first week after transplantation, median tacrolimus C_0/D_c was 158.0 (ng/ml)/(mg/kg) (95% CI, 117.2–279.0) for renal transplantation recipients with *CYP2C19*2/*2* genotype, 78.2 (ng/ml)/(mg/kg) (95% CI, 48.5–92.4) for patients with *CYP2C19*1/*2,* and 75.1 (ng/ml)/(mg/kg) (95% CI, 50.3–87.9) for patients with *CYP2C19*1/*1*. This was found to be statistically significant (P = 0.028) by Kruskal-Wallis test (Fig. 3B). In contrast, very small differences were observed between groups in tacrolimus C_0/D_c regarding *CYP2C19* donors' genotype, and these were not statistically significant (Fig. 3D). Similar results were found during the second week after transplantation (Fig. 4), although no statistically significant results were found.

Moreover, our main goal after these findings was trying to elucidate whether those four patients with *CYP2C19*2/*2* genotype (and *CYP3A5*3/*3*) had experienced any important clinical complications after transplantation that could be consistent with their increase of tacrolimus C_0/D_c and could be the cause for their longer hospital stay. The results of our findings in this search are shown in Table 3. All four recipients showed allograft delayed function, which led to the need of dialysis after transplantation in three of the four cases, ranging from 7 to 25 days of dialysis. In addition, in three recipients, premature biopsy of the allograft was required, confirming acute tubular necrosis and even signs of focal segmental sclerosis in one of the recipients (patient 4), who finally lost the allograft.

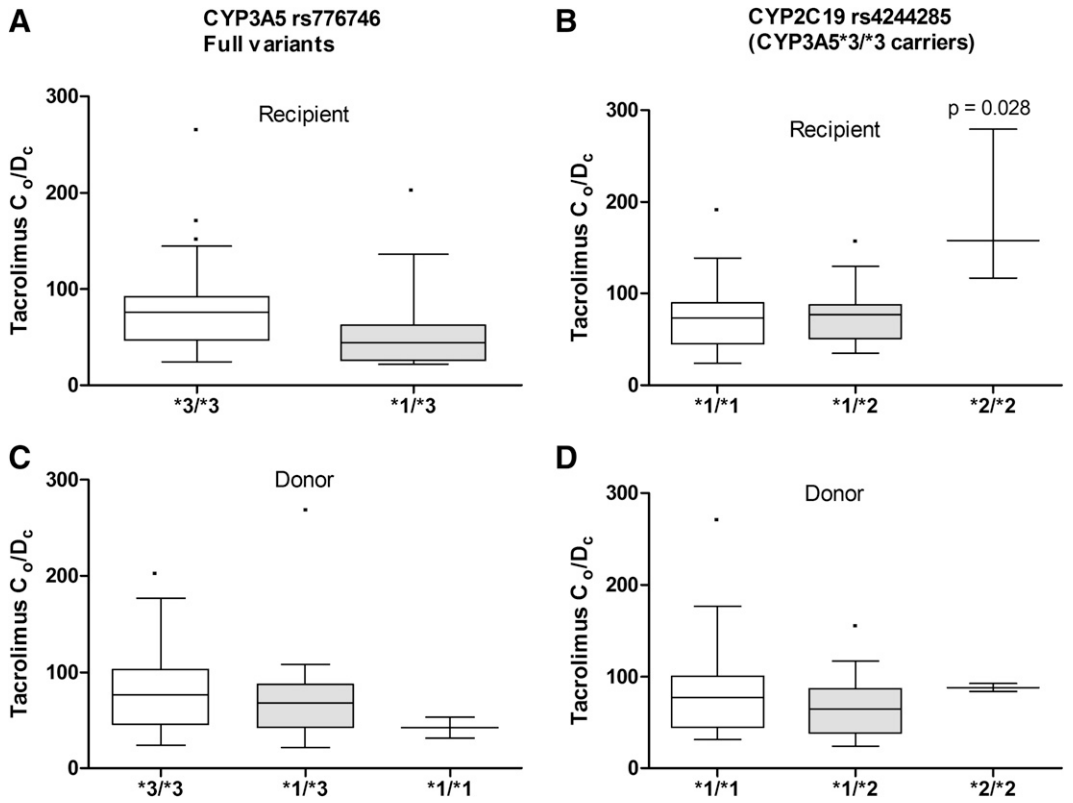

Fig. 3. First week tacrolimus dose-normalized blood concentrations. Box plots (median, interquartile range. and outlyers) of tacrolimus levels (C_0 /D_c [ng/ml]/[mg/kg/24h]) in renal transplant recipients by CYP3A5 rs776746 genotype are shown according to the recipient's genotype in (A) (*3/*3 [*n* = 45] versus *1/*3 [*n* = 8]) or grouping the recipients by their donor's genotype in (C) (*3/*3 [*n* = 39] versus *1/*3 [*n* = 11] versus *1/*1 [*n* = 2]). No statistically significant differences were found after applying Kruskal-Wallis and Mann-Whitney *U* tests among these groups. (B and D) Results of tacrolimus levels only for those patients carrying CYP3A5*3/*3, subdivided by CYP2C19 rs4244285 genotype of the recipients in (B) (*1/*1 [*n* = 29] versus *1/*2 [*n* = 13] versus *2/*2 [*n* = 3]) or the genotypes of their donors in (D) (*1/*1 [*n* = 32] versus*1/*2 [*n* = 11] versus *2/*2 [*n* = 2]).

Discussion

In this study, we present data on renal transplant recipients undergoing routine clinical follow-up after surgery, to evaluate the potential usefulness of pharmacogenetics in daily clinical practice. A significant correlation was obtained for hospital stay immediately after transplantation with two SNPs: *rs4244285* in *CYP2C19* and *rs2066844* in *NOD2/CARD15*. The implication of *NOD2/CARD15* in a worse outcome after transplantation could be explained by its role in the infection and inflammation processes. There is evidence of polymorphisms in this gene associated with all-cause, cardiovascular mortality and graft survival after renal transplantation (Krüger et al., 2007; Landfried et al., 2010). In our results, the difference in hospital stay was only 4 days between the different genotype groups; thus, we do not grant a high clinical impact without further studies.

With regard to the association found with CYP2C19, this enzyme has not been described to be associated with the immunosuppressive regimen (McDonagh et al., 2011); thus, there was a need for taking a deeper look into the concomitant drugs administered to our patients, to find any that could be related to *CYP2C19*. In this respect, the elevation of tacrolimus levels when it is administered concomitantly with omeprazole in patients with *CYP2C19*2/*2* had previously been described in liver recipients (Hosohata et al., 2008; Hosohata et al., 2009a; Hosohata et al., 2009b) and in healthy volunteers and renal recipients (Itagaki et al., 2002; Takahashi et al., 2007; Maguire et al., 2012). However, no clinical relevance of this elevation has been reported to our knowledge. Therefore, the importance of our work is that, for the first time, there are clinical implications for this patient that resulted in an increase in hospital stay, allograft delayed function, and acute tubular necrosis.

To evaluate whether there was an association between this described interaction and our patients with longer hospital stay, tacrolimus blood levels (C_0 /D_c) during the first two weeks after transplantation were plotted according to the patients' genotype. To avoid the effect of the most known SNP affecting tacrolimus C_0 /D_c, *CYP3A5 rs776746*, we always plotted the patients according to this SNP and confirmed the expected effects: *CYP3A5*3/*3* genotype (nonexpresser) showed higher tacrolimus C_0 /D_c than did *CYP3A5*1/*1* or *CYP3A5*1/*3* genotypes (expressers). The decreasing effects of *CYP3A5* expressers could hide the hypothetical increasing effect of *CYP2C19*2/*2* genotype because of the described omeprazole-tacrolimus interaction. This is the reason why we decided to plot tacrolimus levels with regard to *CYP2C19* only in the subset of patients with *CYP3A5*3/*3*s, as other authors have previously done (Hosohata et al., 2009a; Hosohata et al., 2009b). There is a work (Katsakiori et al., 2010) in which no elevation of tacrolimus levels was found in patients cotreated with omeprazole, but this was probably because the authors only considered *CYP3A5* genotype and not *CYP2C19*; thus, they could not see the interaction.

With regard to *CYP3A5 rs776746*, we failed to find statistically significant differences between groups, probably because of the reduced number of patients included. However, a clear tendency is observed, confirming the expected behavior. This was especially observed according to recipients' genotype, but seems to be also observed according to the donor's, although in a less significant way.

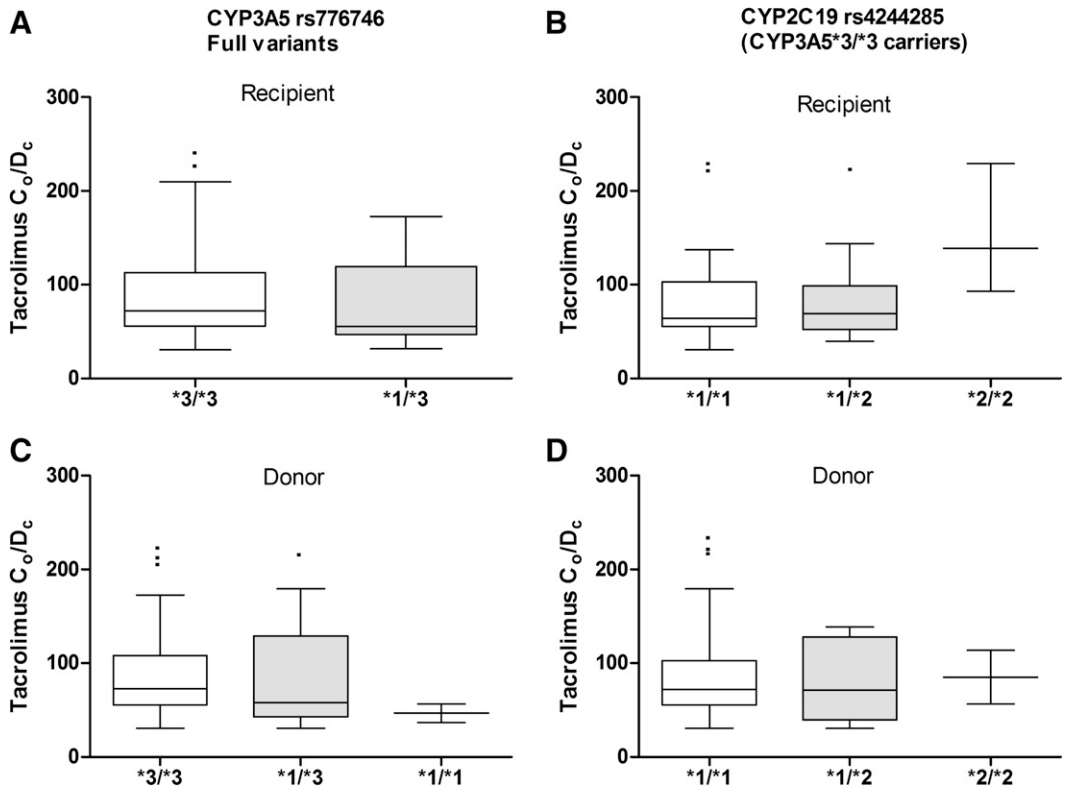

Fig. 4. Second week tacrolimus dose-normalized blood concentrations. Box plots (median, interquartile range, and outlyers) of tacrolimus levels (C_0 /D_c [ng/ml]/[mg/kg/24h]) in renal transplant recipients by CYP3A5 rs776746 genotype are shown according to the recipient's genotype in (A) (*3/*3 [n = 48] versus *1/*3 [n = 9]) or grouping the recipients by their donor's genotype in (C) (*3/*3 [n = 43] versus *1/*3 [n = 11] versus *1/*1 [n = 2]). (B and D) Results of tacrolimus levels only for those patients carrying CYP3A5*3/*3, subdivided by CYP2C19 rs4244285 genotype of the recipients in (B) (*1/*1 [n = 29] versus *1/*2 [n = 15] versus *2/*2 [n = 3]) or the genotypes of their donors in (D) (*1/*1 [n = 34] versus *1/*2 [n = 11] versus *2/*2 [n = 2]). No statistically significant differences were found after applying Kruskal-Wallis and Mann-Whitney U tests among these groups.

Many publications, including our own, have found statistically significant correlations between this SNP and tacrolimus and/or cyclosporine C_0 /D_c (Hesselink et al., 2003; Jun et al., 2009; Mendes et al., 2009; Herrero et al., 2010a; Herrero et al., 2010b; López-Montenegro et al., 2010; Jacobson et al., 2011; Jordán de Luna et al., 2011; Galiana et al., 2012). Even some researchers, working groups, and consortia recommend guidelines for initial dose adjustment with this SNP (Haufroid et al., 2006; Provenzani et al., 2009; Kuypers et al., 2010; Thervet et al., 2010; Becquemont et al., 2011; Jacobson et al., 2011; Passey et al., 2011; Singh et al., 2011; Tang et al., 2011; Tavira et al., 2011; Zhu et al., 2011), but always followed by TDM.

Several studies have highlighted the relevance of CYP3A4 in tacrolimus metabolism. Wild-type (*CYP3A4*1/*1*) is associated with higher tacrolimus dose-adjusted C_0 than are variant genotypes (*CYP3A4*1B, *1G*) (Hesselink et al., 2003; Miura et al., 2011). Although *CYP3A4* contribution to tacrolimus C_0 /D_c is less than *CYP3A5*, it is not worthless and might explain our failure to find statistically significant differences among *CYP3A5* genotype groups (Kuypers et al., 2007).

To analyze tacrolimus C_0 /D_c in *CYP2C19 rs4244285*, we considered the complete recipient/donor combined genotype. Recipients carrying *CYP3A5*3/*3* alleles were subplotted according to the variants in *CYP2C19 rs4244285*, showing that the highest tacrolimus C_0 /D_c is reached in *CYP2C19*2/*2* recipients, especially during the first week after transplantation. To clarify the contribution to this effect of the recipient and the donor genotypes separately, the study was divided. The consideration of the donor's genotype regarding these two SNPs has not been previously reported in renal transplantation, to our knowledge. Here, the results showed that the increase of tacrolimus C_0 /D_c was only clear when the recipient had *CYP2C19*2/*2*, reaching statistical significance only in the first week after transplantation. We cannot discard a possible little effect of the

TABLE 3

Complications after transplantation for patients with CYP2C19*2/*2 genotype

Patient	Hospital stay, days	Allograft delayed function	Need of dialysis after transplantation	Days of dialysis	Need of premature biopsy	Biopsy results	Other complications
1	31	Yes	Yes	7	Yes, 1	Acute Tubular Necrosis	Seroma, Acute urine retention
2	23	Yes	No	0	No	—	Posttransplantation hyperglucemia, proteinuria
3	25	Yes	Yes	10	Yes, 1	Acute Tubular Necrosis	
4	39	Yes	Yes	25	Yes, 2	1st:Acute Tubular Necrosis; 2nd:Signs of Focal Segmental Sclerosis	Recidive of Focal Segmental Glomerulosclerosis, Allograft Loss

donor's genotype, but in our hands, this effect seems not to be as important as it has been described in liver transplantation, where the allograft genotype (donor's genotype) is logically relevant regarding metabolic enzymes (Hosohata et al., 2009a). Further studies will be required in the renal transplantation context to clearly define the donor's genotype contribution.

On the other hand, omeprazole is also a known inducer of *CYP3A4/5* in addition to an inducer of *CYP1A*. Therefore, omeprazole can affect tacrolimus concentrations via *CYP3A4/5* inhibition (first stage) and induction (later stage, usually 7 days or longer because of latent effect). These properties of omeprazole may explain the finding that significantly higher tacrolimus concentrations were observed in the *CYP2C19* poor metabolizers only during the first week but not during the second week.

Other polymorphisms in *CYP2C19* have been associated with increased enzymatic activity, as *CYP2C19*17* allele, that is expected to show opposite effects to those seen with *CYP2C19*2* or *CYP2C19*3*, which are associated with loss of function. However, as it has been reported (Li-Wan-Po et al., 2010), the magnitude of effects for *CYP2C19*17* is considerably smaller than has been reported for *CYP2C19*2*. Even in homozygotes, any observed ultrafast metabolic profiles have been within the range seen in the wild-type homozygotes. In addition, other authors have reported (Kearns et al., 2010) that, although for pantoprazole, a statistically significant relationship was observed between *CYP2C19*17* and both dose-corrected areas under the curve and the apparent elimination rate constant, no significant genotype-phenotype relationships were observed for omeprazole. Unfortunately, *CYP2C19*17* was not initially included in the study, and we cannot contribute to clarify how it influences the observed effects.

Because tacrolimus is a highly nephrotoxic drug, a simple explanation to the renal damage in our four patients could be the higher drug exposure. Kuypers et al. (Kuypers et al., 2010) reported that delayed allograft function was associated with higher initial mean tacrolimus C_0 values predominantly in *CYP3A5* nonexpressers. Other authors have reported (Metalidis et al., 2011) that expression and localization of *CYP3A5* in renal allografts is associated with histologic signs of calcineurin inhibitor nephrotoxicity. In our patients, the dose-normalized blood concentrations of tacrolimus doubled for patients with *CYP2C19*2/*2* genotype (inside the group of *CYP3A5* nonexpressers). A priori, *CYP2C19* polymorphisms should not affect tacrolimus pharmacokinetics, and because data during concomitant use of azoles, potent inhibitors of CYP3A, have been excluded and no other medication with potential interaction was administered during this period, C_0/D_c elevation in patients with *CYP2C19*2/*2* in a uniform group for *CYP3A5* genotype was attributed to a drug interaction with omeprazole. The inclusion of a control group not treated with PPI would have given more evidence to the occurrence of the interaction, but unfortunately, because this is a retrospective study, we did not have this control group.

Although important data are reported, it has to be emphasized that, because of the small number of patients in the *CYP2C19*2/*2* group, our study lacks potency and evidence in demonstrating both the interaction and the supposed medical complications derived from it, but should serve as an alarm and starting point for controlled studies with larger numbers of renal transplant recipients, because gastric protection is essential in these patients and potential consequences of this interaction can be serious. Several works (Itagaki et al., 2002; Takahashi et al., 2007; Itagaki et al., 2004; Homma et al., 2002) propose that other PPIs, such as rabeprazole or pantoprazole, instead of omeprazole, do not present this interaction with tacrolimus via CYP3A5 and, thus, would be more suitable for these patients to avoid potential complications.

To summarize, our increase in hospital stay is directly related to a bad evolution of the transplantation that could be the consequence of high tacrolimus levels as a result of an interaction with omeprazole in patients with *CYP2C19*2/*2*. Although an economic study of the costs of these complications has not been performed, it is obvious that all these events imply a very high cost, economically and related to patient health and life quality.

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Authorship Contributions

Participated in research design: Herrero, Aliño, Sánchez-Plumed.
Conducted experiments: Bosó, Herrero, Galiana, Marrero, Marqués, Bea.
Contributed new reagents or analytic tools: Herrero, Aliño, Bosó.
Performed data analysis: Bosó, Herrero, Aliño.
Wrote or contributed to the writing of the manuscript: Bosó, Herrero, Hernández, Sánchez-Plumed, Poveda, Aliño.

References

Becquemont L, Alfirevic A, and Amstutz U, et al. (2011) Practical recommendations for pharmacogenomics-based prescription: 2010 ESF-UB Conference on Pharmacogenetics and Pharmacogenomics. *Pharmacogenomics* **12**:113–124.

Cattaneo D, Perico N, and Remuzzi G (2004) From pharmacokinetics to pharmacogenomics: a new approach to tailor immunosuppressive therapy. *Am J Transplant* **4**:299–310.

Cho JH, Yoon YD, Park JY, Song EJ, Choi JY, Yoon SH, Park SH, Kim YL, and Kim CD (2012) Impact of cytochrome P450 3A and ATP-binding cassette subfamily B member 1 polymorphisms on tacrolimus dose-adjusted trough concentrations among Korean renal transplant recipients. *Transplant Proc* **44**:109–114.

Galiana M, Herrero MJ, Bosó V, Bea S, Ros E, Sánchez-Plumed J, Poveda JL, and Aliño SF (2012) Pharmacogenetics of immunosuppressive drugs in renal transplantation, in *Renal Transplantation - Updates and Advances* (Long L, ed, ed) pp 143–162, InTech, Rijeka, Croatia.

Glowacki F, Lionet A, Buob D, Labalette M, Allorge D, Provôt F, Hazzan M, Noël C, Broly F, and Cauffiez C (2011) CYP3A5 and ABCB1 polymorphisms in donor and recipient: impact on Tacrolimus dose requirements and clinical outcome after renal transplantation. *Nephrol Dial Transplant* **26**:3046–3050.

Haufroid V, Wallemacq P, VanKerckhove V, Elens L, De Meyer M, Eddour DC, Malaise J, Lison D, and Mourad M (2006) CYP3A5 and ABCB1 polymorphisms and tacrolimus pharmacokinetics in renal transplant candidates: guidelines from an experimental study. *Am J Transplant* **6**:2706–2713.

Herrero MJ, Almenar L, Jordán C, Sánchez I, Poveda JL, and Aliño SF (2010a) Clinical interest of pharmacogenetic polymorphisms in the immunosuppressive treatment after heart transplantation. *Transplant Proc* **42**:3181–3182.

Herrero MJ, Sánchez-Plumed J, Galiana M, Bea S, Marqués MR, and Aliño SF (2010b) Influence of pharmacogenetic polymorphisms in routine immunosuppression therapy after renal transplantation. *Transplant Proc* **42**:3134–3136.

Hesselink DA, van Schaik RH, van der Heiden IP, van der Werf M, Gregoor PJ, Lindemans J, Weimar W, and van Gelder T (2003) Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther* **74**:245–254.

Homma M, Itagaki F, Yuzawa K, Fukao K, and Kohda Y (2002) Effects of lansoprazole and rabeprazole on tacrolimus blood concentration: case of a renal transplant recipient with CYP2C19 gene mutation. *Transplantation* **73**:303–304.

Hosohata K, Masuda S, Ogura Y, Oike F, Takada Y, Katsura T, Uemoto S, and Inui K (2008) Interaction between tacrolimus and lansoprazole, but not rabeprazole in living-donor liver transplant patients with defects of CYP2C19 and CYP3A5. *Drug Metab Pharmacokinet* **23**: 134–138.

Hosohata K, Masuda S, Katsura T, Takada Y, Kaido T, Ogura Y, Oike F, Egawa H, Uemoto S, and Inui K (2009a) Impact of intestinal CYP2C19 genotypes on the interaction between tacrolimus and omeprazole, but not lansoprazole, in adult living-donor liver transplant patients. *Drug Metab Dispos* **37**:821–826.

Hosohata K, Masuda S, Yonezawa A, Sugimoto M, Takada Y, Kaido T, Ogura Y, Oike F, Uemoto S, and Inui K (2009b) Absence of influence of concomitant administration of rabeprazole on the pharmacokinetics of tacrolimus in adult living-donor liver transplant patients: a case-control study. *Drug Metab Pharmacokinet* **24**:458–463.

Itagaki F, Homma M, Yuzawa K, Fukao K, and Kohda Y (2002) Drug interaction of tacrolimus and proton pump inhibitors in renal transplant recipients with CYP2C19 gene mutation. *Transplant Proc* **34**:2777–2778.

Itagaki F, Homma M, Yuzawa K, Nishimura M, Naito S, Ueda N, Ohkohchi N, and Kohda Y (2004) Effect of lansoprazole and rabeprazole on tacrolimus pharmacokinetics in healthy volunteers with CYP2C19 mutations. *J Pharm Pharmacol* **56**:1055–1059.

Jacobson PA, Oetting WS, and Brearley AM, et al.; DeKAF Investigators (2011) Novel polymorphisms associated with tacrolimus trough concentrations: results from a multicenter kidney transplant consortium. *Transplantation* **91**:300–308.

Jordán de Luna C, Herrero Cervera MJ, Sánchez Lázaro I, Almenar Bonet L, Poveda Andrés JL, and Aliño Pellicer SF (2011) Pharmacogenetic study of ABCB1 and CYP3A5 genes during the

first year following heart transplantation regarding tacrolimus or cyclosporine levels. *Transplant Proc* **43**:2241–2243.
Jun KR, Lee W, and Jang MS, et al. (2009) Tacrolimus concentrations in relation to CYP3A and ABCB1 polymorphisms among solid organ transplant recipients in Korea. *Transplantation* **87**: 1225–1231.
Katsakiori PF, Papapetrou EP, Goumenos DS, Nikiforidis GC, and Flordellis CS (2010) Investigation of clinical interaction between omeprazole and tacrolimus in CYP3A5 non-expressors, renal transplant recipients. *Ther Clin Risk Manag* **6**:265–269.
Kearns GL, Leeder JS, and Gaedigk A (2010) Impact of the CYP2C19*17 allele on the pharmacokinetics of omeprazole and pantoprazole in children: evidence for a differential effect. *Drug Metab Dispos* **38**:894–897.
Kim IW, Moon YJ, and Ji E, et al. (2012) Clinical and genetic factors affecting tacrolimus trough levels and drug-related outcomes in Korean kidney transplant recipients. *Eur J Clin Pharmacol* **68**:657–669.
Krüger B, Böger CA, Schröppel B, Obed A, Hoffmann U, Murphy BT, Fischereder M, Holler E, Banas B, and Krämer BK (2007) Impact of NOD2/CARD15 haplotypes on the outcome after kidney transplantation. *Transpl Int* **20**:600–607.
Kuypers DR, de Jonge H, Naesens M, Lerut E, Verbeke K, and Vanrenterghem Y (2007) CYP3A5 and CYP3A4 but not MDR1 single-nucleotide polymorphisms determine long-term tacrolimus disposition and drug-related nephrotoxicity in renal recipients. *Clin Pharmacol Ther* **82**:711–725.
Kuypers DR, de Jonge H, Naesens M, and Vanrenterghem Y (2010) A prospective, open-label, observational clinical cohort study of the association between delayed renal allograft function, tacrolimus exposure, and CYP3A5 genotype in adult recipients. *Clin Ther* **32**:2012–2023.
Landfried K, Bataille F, and Rogler G, et al. (2010) Recipient NOD2/CARD15 status affects cellular infiltrates in human intestinal graft-versus-host disease. *Clin Exp Immunol* **159**:87–92.
Li-Wan-Po A, Girard T, Farndon P, Cooley C, and Lithgow J (2010) Pharmacogenetics of CYP2C19: functional and clinical implications of a new variant CYP2C19*17. *Br J Clin Pharmacol* **69**:222–230.
López-Montenegro Soria MA, Kanter Berga J, Beltrán Catalán S, Milara Payá J, Pallardó Mateu LM, and Jiménez Torres NV (2010) Genetic polymorphisms and individualized tacrolimus dosing. *Transplant Proc* **42**:3031–3033.
Maguire M, Franz T, and Hains DS (2012) A clinically significant interaction between tacrolimus and multiple proton pump inhibitors in a kidney transplant recipient. *Pediatr Transplant* **16**: E217–E220
McDonagh EM, Whirl-Carrillo M, Garten Y, Altman RB, and Klein TE(2011) From pharmacogenomic knowledge acquisition to clinical applications: the PharmGKB as a clinical pharmacogenomic biomarker resource. *Biomarkers in Medicine* **5**: 795-806 (www.pharmgkb.org, accession date: November 2011).
Mendes J, Martinho A, Simoes O, Mota A, Breitenfeld L, and Pais L (2009) Genetic polymorphisms in CYP3A5 and MDR1 genes and their correlations with plasma levels of tacrolimus and cyclosporine in renal transplant recipients. *Transplant Proc* **41**:840–842.
Metalidis C, Lerut E, Naesens M, and Kuypers DR (2011) Expression of CYP3A5 and P-glycoprotein in renal allografts with histological signs of calcineurin inhibitor nephrotoxicity. *Transplantation* **91**:1098–1102.
Miura M, Satoh S, Kagaya H, Saito M, Numakura K, Tsuchiya N, and Habuchi T (2011) Impact of the CYP3A4*1G polymorphism and its combination with CYP3A5 genotypes on tacrolimus pharmacokinetics in renal transplant patients. *Pharmacogenomics* **12**:977–984.
Passey C, Birnbaum AK, Brundage RC, Oetting WS, Israni AK, and Jacobson PA (2011) Dosing equation for tacrolimus using genetic variants and clinical factors. *Br J Clin Pharmacol* **72**: 948–957.
Provenzani A, Notarbartolo M, and Labbozzetta M, et al. (2009) The effect of CYP3A5 and ABCB1 single nucleotide polymorphisms on tacrolimus dose requirements in Caucasian liver transplant patients. *Ann Transplant* **14**:23–31.
Singh R, Srivastava A, Kapoor R, and Mittal RD (2011) Do drug transporter (ABCB1) SNPs influence cyclosporine and tacrolimus dose requirements and renal allograft outcome in the posttransplantation period? *J Clin Pharmacol* **51**:603–615.
Staatz CE, Goodman LK, and Tett SE (2010) Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: Part I. *Clin Pharmacokinet* **49**:141–175.
Takahashi K, Yano I, Fukuhara Y, Katsura T, Takahashi T, Ito N, Yamamoto S, Ogawa O, and Inui K (2007) Distinct effects of omeprazole and rabeprazole on the tacrolimus blood concentration in a kidney transplant recipient. *Drug Metab Pharmacokinet* **22**:441–444.
Tang HL, Xie HG, Yao Y, and Hu YF (2011) Lower tacrolimus daily dose requirements and acute rejection rates in the CYP3A5 nonexpressers than expressers. *Pharmacogenet Genomics* **21**:713–720.
Tavira B, Coto E, and Díaz-Corte C, et al.; REDINREN Pharmacogenetics group (2011) Pharmacogenetics of tacrolimus after renal transplantation: analysis of polymorphisms in genes encoding 16 drug metabolizing enzymes. *Clin Chem Lab Med* **49**:825–833 Erratum in: Clin Chem Lab Med 2011; 49:1087.
Thervet E, Loriot MA, and Barbier S, et al. (2010) Optimization of initial tacrolimus dose using pharmacogenetic testing. *Clin Pharmacol Ther* **87**:721–726.
Ware N and MacPhee IA (2010) Current progress in pharmacogenetics and individualized immunosuppressive drug dosing in organ transplantation. *Curr Opin Mol Ther* **12**:270–283.
Zhu HJ, Yuan SH, Fang Y, Sun XZ, Kong H, and Ge WH (2011) The effect of CYP3A5 polymorphism on dose-adjusted cyclosporine concentration in renal transplant recipients: a meta-analysis. *Pharmacogenomics J* **11**:237–246.

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