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SHORT COMMUNICATION

The donor *ABCB1* (MDR-1) C3435T polymorphism is a determinant of the graft glomerular filtration rate among tacrolimus treated kidney transplanted patients

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The *ABCB1* gene encodes the P-glycoprotein (P-gp) that drives the transmembrane efflux of many drugs. The donor *ABCB1* polymorphisms have been related with chronic histological damage and long-term renal function among kidney transplanted patients who received cyclosporine A and tacrolimus (Tac). The aim of our study was to determine whether the donor *ABCB1* 3435 C/T genotype was related with renal function among Tac-treated renal transplanted patients. Kidney donors ($n=65$) and recipients ($n=90$) were genotyped for the *ABCB1* rs1045642 (c.3435 C/T) and *CYP3A5* rs776746 single-nucleotide polymorphisms. The estimated glomerular filtration rate (eGFR) was calculated with the modification of diet in renal disease formulae at five post-transplant times (2 weeks and 1, 3, 6 and 12 months). The recipient *ABCB1* and *CYP3A5* genotypes had no significant effect on the eGFR at all the post-transplant times. We found significantly lower eGFR values among patients who received a kidney from *ABCB1* 3435 T carriers ($P<0.01$). In conclusion, our study confirmed the potential impact of the donor *ABCB1* 3435 genotype on the post-transplant renal function among patients treated with Tac.
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Tacrolimus (Tac, FK-506) is a calcineurin inhibitor (CNI) widely used to avoid organ rejection among kidney transplanted patients. Tac is characterized by a narrow therapeutic index and large interindividual dose requirement variability, which makes necessary the monitoring of blood concentration to adjust the dose in order to prevent rejection and toxicity. The *CYP3A5*3* allele (SNP rs776746) is the main genetic determinant of Tac dose requirements: homozygotes for the null *CYP3A5*3* allele showed an almost complete absence of enzyme activity and required significantly lower Tac compared with *CYP3A5*1* (wild-type allele) carriers.[1–5] The *ABCB1* gene (also known as multi-drug response-1, *MDR1*) encodes the P-glycoprotein (P-gp) that drives the transmembrane efflux of many drugs. The effect of *ABCB1* variants on Tac dose is controversial: the common c.C3435T SNP (rs1045642) has been related to Tac bioavailability by some authors, but others failed to confirm the association.[6–9]

Recent studies suggested that the donor (instead of the recipient) *ABCB1* genotype was related with chronic histological damage and low glomerular filtration rate(GFR) in response to CNIs, either cyclosporine A (CsA) or Tac.[10–13] In a large cohort from UK, the donor *ABCB1* genotype was associated with long-term graft survival.[13] The donor genotype was also linked to differences in the estimated GFRs (eGFRs) at 1 year post transplant (PT).[12] These effects on graft function might be explained by a heterogeneous expression of the of P-gp in the renal tubular epithelial cells that could in turn result in differences in Tac or CsA absorption by these cells.[11] However, the reported positive effects should be taken with caution because at least one study based on a cohort followed for >2 years did not find differences between the donor *ABCB1* genotypes.[14]

The aim of our study was to determine whether the donor *CYP3A5* and *ABCB1* genotypes were related with kidney function among Tac-treated renal transplanted patients. The study was approved by the Ethical Committee of Hospital Universitario Central Asturias (HUCA) and involved a total of 65 deceased donors and 90 patients who received a first graft from these donors in the years 2010–2013. Patients aged >18 years, who had not received a previous organ, who were treated with Tac as primary immunosuppressor and had been followed for at least 1 year PT were included in the study. All the donors and recipients were Caucasians from Spain. The 90 kidney transplanted patients were treated with Tac, micophenolate mofetil (MMF) and prednisone as reported.[5,15] The eGFR was calculated with the Modification of Diet in Renal Disease (MDRD) formula: eGFR ($\mathrm{ml\,min^{-1}\,1.73\,m^{-2}}$) $= 186 \times [\mathrm{plasma\ creatinin\ (mg\,dl^{-1})}] - 1.154 \times (\mathrm{age}) - 0.203 \times (0.742$ if female).[16] Some studies reported that among renal transplanted patients the MDRD values showed a better

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correlation with the true filtration rate (compared with other renal function estimates, such as the raw serum creatinine value or the Cockcroft–Gault formula).[17] The diagnosis of acute rejection was based on laboratory and clinical findings, such as decreased urine production and a rise in plasma creatinine that lowered in response to antirejection treatment.[18]

The DNA from the donors and recipients was obtained and the genotypes for *ABCB1* rs1045642 was determined through real-time PCR Taqman (assay C_7586657_20; www.appliedbiosystems.com). We also determined the *CYP3A5*3* allele (rs776746, Taqman assay id. C_25201809_30) in recipients and donors. There were no significant effect of the recipient *CYP3A5* and *ABCB1* genotypes on the eGFR at the five PT times (Table 1). As expected, recipients who were *CYP3A5*3* homozygotes required lower Tac dose and showed a significantly higher normalized value (the ratio of Tac blood concentration/Tac dose; Table 1). In reference to the donor genotypes, there were no differences in the recipient Tac dose and Tac blood concentrations (data not shown). The donor *ABCB1* 3435 T allele was linked to significantly lower eGFR values ($P<0.01$) at 1, 3, 6 and 12 months PT (Table 2). The eGFR value decreased with the number of T-allele copies (CC > CT > TT; Figure 1).

The P-gp is expressed in renal tubular epithelial cells and would contribute to exporting Tac out of the cells. The *ABCB1* 3435 C/T is a silent polymorphism (p.I1145I) and therefore it was not expected to change the function of the protein. This SNP was in linkage disequilibrium with other polymorphisms, including missense changes that might have functional consequences on P-gp activity and the ability of the protein to drive the efflux of Tac.[19] Studies that determined the P-gp expression and function in the duodenum by western blots and quantitative immunohistology found that the homozygous T-allele was associated with more than twofold lower protein expression levels compared with homozygous CC samples. Heterozygous individuals display an intermediate phenotype.[20] Moreover, after receiving an oral dose of digoxin (an indicator of Pg-P activity) individuals homozygous for the T allele showed significantly highest digoxin plasma levels.[20]

Interestingly, the *ABCB1* 3435 C > T was associated with differences in Tac concentrations in peripheral blood cells (PBCs) from renal transplanted patients, and the T allele was also linked to increased

Table 1 *CYP3A5* and *ABCB1* recipient ($n=90$) genotypes on Tac pharmacokinetics and renal function values

	*CYP3A5 *3/*3* (n = *74*) *Mean ± s.d. (range)*	*CYP3A5 *1/*3* (n = *16*) *Mean ± s.d. (range)*	P-*values*
Age (years)	57.40 ± 9 (46–68)	52.53 ± 13 (24–78)	0.41
eGFR			
2 weeks	33.59 ± 15 (8–60)	37.25 ± 11 (27–53)	0.57
1 month	41.07 ± 11 (14–61)	33 ± 8 (27–39)	0.39
3 months	43.10 ± 10 (22–61)	49.33 ± 10 (38–58)	0.40
6 months	43.30 ± 9 (23–61)	49.75 ± 4 (46–56)	0.11
1 year	43.70 ± 9 (25–65)	49.35 ± 6 (41–58)	0.21
	Median (range)	*Median (range)*	*Median (range)*
Dose-normalized Tac blood concentration (ng ml^{-1} per mg kg^{-1})			
2 weeks	89.27 (14–700)	61.75 (15–450)	<0.001
6 months	125.31 (14–1300)	87.01 (24–370)	<0.001
1 year	147.97 (12–1490)	102.03 (29–634)	<0.001

	ABCB1 CC (n = *17*) *Mean ± s.d. (range)*	*ABCB1 CT* (n = *49*) *Mean ± s.d. (range)*	*ABCB1 TT* (n = *24*) *Mean ± s.d. (range)*	P-*values*
Age (years)	55.29 ± 15 (27–76)	53.41 ± 12 (24–75)	50.70 ± 4 (27–78)	0.43
eGFR				
2 weeks	39.06 ± 14 (12–60)	39.08 ± 10 (16–59)	39.64 ± 11 (12–43)	0.98
1 month	43.24 ± 9 (24–59)	41.18 ± 10 (21–61)	40 ± 12 (14–59)	0.74
3 months	44.01 ± 7 (33–59)	42.98 ± 8 (22–61)	47.18 ± 10 (22–60)	0.26
6 months	44.35 ± 7 (31–60)	43.04 ± 8 (23–61)	43.07 ± 9 (29–36)	0.74
1 year	47.24 ± 9 (29–60)	44.53 ± 8 (25–61)	44.52 ± 6 (26–65)	0.42
	Median (range)	*Median (range)*	*Median (range)*	
Dose-normalized Tac blood concentration (ng ml^{-1} per mg kg^{-1})				
2 weeks	90.02 (27–614)	85.52 (14–700)	93.35 (21–432)	0.39
6 months	137.64 (34–770)	134.72 (14–1300)	146.47 (34–399)	0.19
1 year	147.02 (36–990)	142.63 (12–1490)	159.09 (41–832)	0.23

Abbreviations: eGFR, estimated glomerular filtration rate; Tac, tacromilus.
Data are presented as mean ± s.d. or median and range. We used the Student's *t*-test and ANOVA to compare mean values between the groups. A $P<0.05$ was considered as statistically significant.

Table 2 Allograft clinical outcome in the 90 recipients according to the donor *CYP3A5* and *ABCB1* polymorphisms

	Donor CYP3A5		
	**3/*3* (n = *71*) *Mean ± s.d. (range)*	**1/*3* (n = *19*) *Mean ± s.d. (range)*	P-*values*
Donor age (years)	55.65 ± 11.62 (22–76)	58.50 ± 10.86 (25–75)	0.09
Recipient age (years)	55.11 ± 12.52 (24–76)	58.11 ± 12.14 (25–78)	0.26
eGFR			
2 weeks	33.41 ± 15.16 (8–60)	39.61 ± 12.28 (21–58)	0.17
1 month	41.17 ± 11.43 (14–61)	41.50 ± 7.26 (31–59)	0.92
3 months	43.67 ± 9.89 (22–61)	42.58 ± 10.27 (26–60)	0.73
6 months	42.96 ± 9.63 (23–61)	41.18 ± 8.34 (29–60)	0.57
1 year	45.75 ± 9.08 (25–65)	44.91 ± 7.31 (27–62)	0.72

	Donor ABCB1			
	CC (n = *23*) *Mean ± s.d. (range)*	*CT* (n = *43*) *Mean ± s.d. (range)*	*TT* (n = *24*) *Mean ± s.d. (range)*	P-*values*
Donor age (years)	55.61 ± 11 (35–76)	53.02 ± 13 (22–73)	57.22 ± 9 (27–75)	0.12
Recipient age (years)	50.29 ± 13 (26–71)	51.65 ± 13 (27–76)	57.58 ± 13 (24–78)	0.12
eGFR				
2 weeks	40.46 ± 10 (12–57)	40.05 ± 10 (18–59)	36.54 ± 13 (12–60)	0.35
1 month	46.54 ± 9 (28–61)	42.62 ± 8 (23–59)	34.57 ± 11 (14–59)	0.001
3 months	46.92 ± 6 (35–61)	44.91 ± 7 (26–59)	39.77 ± 10 (22–60)	0.012
6 months	46.75 ± 5 (36–60)	42.95 ± 7 (29–61)	38.83 ± 10 (23–56)	0.011
1 year	49.46 ± 5 (37–60)	44.47 ± 7 (26–61)	41.59 ± 7 (25–59)	0.001

Data were presented as mean ± s.d. or median and range (*P*-values for the Student's *t*-test or ANOVA).

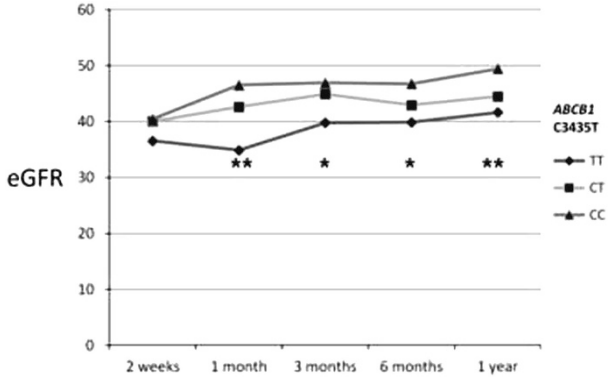

Figure 1 Mean eGFR at five post-transplant times according to the three *ABCB1* donor genotypes. We show the mean reduced eGFR among the patients who received a kidney from 3435 T-donors. **P*<0.05, ***P*<0.01, significantly different from allele-CC donors. eGFR, estimated glomerular filteration rate.

intracellular levels of CsA.[21,22] The reported differences between the genotypes in Pg-P level and activity might also result in heterogeneous intrarenal accumulation, and the increased accumulation of CNI in *ABCB1* 3435 T kidneys could result in an increased apoptotic stress in tubular cells and the subsequent reduction of graft function. This would explain the reported higher risk of CsA-related nephrotoxicity and Tac-chronic damage among patients receiving a kidney bearing the low P-gp expression genotype.[11,23]

In conclusion, our study showed a potential impact of *ABCB1* 3435 donor genotype on the post-transplant renal function among patients treated with Tac. Although our results confirm previous studies, it should be taken with caution due to the limited sample size.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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