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ORIGINAL ARTICLE

Weight of *ABCB1* and *POR* genes on oral tacrolimus exposure in *CYP3A5* nonexpressor pediatric patients with stable kidney transplant

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Tacrolimus (TAC) is highly effective for the prevention of acute organ rejection. However, its clinical use may be challenging due to its large interindividual pharmacokinetic variability, which can be partially explained by genetic variations in TAC-metabolizing enzymes and transporters. The aim of this study was to evaluate the influence of genetic and clinical factors on TAC pharmacokinetic variability in 21 stable pediatric renal transplant patients. This study was nested in a previous Prograf to Advagraf conversion clinical trial. *CYP3A5*, *ABCB1* and two *POR* genotypes were assessed by real-time PCR. The impact on TAC pharmacokinetics of individual genetic variants on *CYP3A5* nonexpressors was evaluated by genetic score. Explicative models for TAC AUC_{0-24h}, C_{max} and C_{min} after Advagraf were developed by linear regression. The built genetic scores explain 13.7 and 26.5% of the total AUC_{0-24h} and C_{min} total variability, respectively. Patients genetic information should be considered to monitorizate and predict TAC exposure.

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INTRODUCTION

Tacrolimus (TAC) is a calcineurin inhibitor highly effective in preventing acute organ rejection after transplantation.[1,2] However, TAC administration is complicated with side effects directly related to its drug blood concentrations such as nephrotoxicity, hypertension, hypercholesterolemia and diabetes mellitus.[3,4] This drug has a high grade of complexity on its use mainly due to its narrow therapeutic index and its high inter and intra subject pharmacokinetic (PK) variability, requiring therapeutic drug monitoring to optimize treatment[1,5,6] and avoid graft loss and toxicity.[7]

However, despite therapeutic drug monitoring some transplant recipients experience TAC concentrations above or below the therapeutic range and therefore are either at a greater risk for toxicity (those with higher concentrations) or acute rejection (those with lower concentrations). TAC interindividual PK variability depends on many clinical co-variants such as serum levels of albumin, hematocrit and hemoglobin, concomitant use of drugs, post-operative time, as well as genetic factors.[8–11] TAC presents an extensive hepatic metabolism by cytochrome P450, and its bioavailability is also influenced by the multidrug resistance genotypes (*MDR1* or *ABCB1*).[12,13] Single-nucleotide polymorphisms (SNPs) in the *CYP3A5* gene explain 40–50% of TAC dose variability.[14] In particular the rs776746 SNP (c.219-237A > G), also referred to as *CYP3A5**3 allele, is a consistent predictor of TAC-dosing requirements. Homozygous carriers of the G allele of this SNP (*CYP3A5* *3/*3) are referred to CYP3A5 nonexpressers in contrast to CYP3A5*1 carriers (known as CYP3A5 expressers) who show normal protein function and therefore lower trough concentrations related to higher metabolic rates.[10,15–22] The *CYP3A5**3 allele causes an abnormal spliced messenger RNA that results in protein truncation, a decrease of functional CYP3A5 enzyme and reduced TAC-dosing requirements.[23–25]

ABCB1 is thought to be responsible for the low oral bioavailability of TAC and is also involved in the distribution of TAC throughout the body and its excretion.[14,26,27] However, associations between *ABCB1* genotype and TAC PKs remain unclear. Some authors describe that there is no association between *ABCB1* variations and TAC trough concentrations.[28–30] However other groups found significant differences between patients with different *ABCB1* genotypes.[27,31] Up to now, the most studied polymorphism affecting P-glycoprotein expression in human tissue is the silent mutation at position in exon 26 of the gene (c.3435 T > C rs1045642 SNP).[32] The CC genotype has been related to a higher expression of P-glycoprotein in the small intestine compared with the TT genotype.[33] Polymorphisms in the P450 oxireductase (*POR*) have been recently demonstrated to modulate the activity of P450 enzymes such as *CYP1A2*, *CYP2C19* and CYP3A.[34,35] POR seems to be essential for CYP-mediated drug oxidation through electron donation. *POR* is highly polymorphic and more than 100 SNPs have been identified and linked to differential CYP activities. The rs1057868 (c.1508 C > T, POR*28) is the most common variant of the gene, and has been associated to a reduced protein activity *in vitro*.[35] Homozygous *POR**28 carriers (TT-expressers) require higher doses of TAC to maintain similar exposure to the drug when compared with wild-type patients (*POR**1/*1).[36] An association between *POR* rs2868177 and *CYP* activity has also been described as it is strongly related to warfarin maintenance dose variations.[37]

Our main objective was to identify and report the influence of *CYP3A5, ABCB1* and two different *POR* polymorphisms on the PKs

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of TAC in order to describe a simple method for TAC AUC, C_{max} and C_{min} prediction in our study population.

MATERIALS AND METHODS

This study was nested in a previous Prograf to Advagraf conversion clinical trial in pediatric patients (EudraCT: 2009-017600-89).[38]

Twenty-one pediatric patients who underwent kidney transplantation and with stable TAC-based immunosuppressive treatment were included in this study (Table 1). All patients were transplanted at La Paz University Hospital in Madrid, which is a reference hospital for pediatric kidney transplantation. Written informed consent was obtained from all subjects or from their legal tutors. All of them continued with its regular TAC doses and were converted from Prograf to Advagraf following a 1:1 (mg: mg) daily dose relation. Twenty-four hours PK profiles at steady state were obtained for Prograf and Advagraf after 7 days administration. For the first 7 days patients received Prograf and then switched to the same dose of Advagraf.

All patients included in the study had stable renal function, followed stable TAC doses over the past 30 days and none of them had changes in co-medications that could modify TAC PKs.

Table 1. Patients characterization

Gender (male/female %)	57/43
Race	
Caucasian (*n*)	17
Hispanic (*n*)	1
Asian (*n*)	2
Arabian (*n*)	1
Age (years ± s.d.)	12.29 ± 4.17
Weight (kg ± s.d.)	42.85 ± 15.42
Height (cm ± s.d.)	143.4 ± 18.16
BMI (kg m^{-2} ± s.d.)	19.87 ± 3.28
BSA (m^2 ± s.d.)	1.30 ± 0.33
Total Advagraf dose (mg ± s.d.)	4.8 ± 1.70
Time since transplant until conversion (years ± s.d.)	5.390 ± 3.25
Creatinine (mg dl^{-1} ± s.d.)	0.90 ± 0.29
Albumin (g dl^{-1} ± s.d.)	3.83 ± 0.23
Hemoglobin (g dl^{-1} ± s.d.)	12.23 ± 1.23
Cystatin C (mg l^{-1} ± s.d.)	1.23 ± 0.27
Bilirubin (mg dl^{-1} ± s.d.)	0.39 ± 0.12
Hematocrit (% ± s.d.)	37.57 ± 3.54
eGFR (ml min^{-1} ± s.d.)	76.57 ± 23.01

Genetic information

Variant	*Genotype*	*No.*	*Study frequency*	*Population frequency (1000G)*
CYP3A5 rs776746[a]	TT (*1/*1)	1	5%	22,7%
	CT (*1/*3)	3	14%	30,4%
	CC (*3/*3)	17	81%	47%
ABCB1 rs1045642[a]	GG	7	33%	39,6%
	AG	9	43%	41,7%
	AA	5	24%	18,7%
*POR*28 rs1057868	CC (*1/*1)	11	52%	51,1%
	CT (*1/*28)	9	43%	40,5%
	TT (*28/*28)	1	5%	8,3%
POR rs2868177	AA	4	14%	35,8%
	AG	12	57%	48,4%
	GG	5	24%	15,8%

Abbreviations: 1000G, 1000 Genomes Database; BMI, body mass index; BSA, body surface area; eGFR, estimated glomerular filtration rate. [a]*CYP3A5* and *ABCB1* genes are located in the reverse strand and therefore variants are reported in reverse orientation of the genome.

Drug concentration measurement and AUC estimation

Advagraf blood samples were gathered before the beginning of the treatment and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 15 and 24 h after intake. Whole-blood concentrations were analyzed by enzyme immunoassay method made on the DIMENSION RXL platform (Siemens Healthcare, Erlangen, Germany). The lower and upper limits of quantification were 2 and 30 ng ml^{-1}, respectively. The PK data analysis was performed following a non-compartmental model using WinNonlin Pro 2.0 software (Pharsight Corporation, Cary, NC, USA). AUC_{0-24} was calculated by the trapezoidal rule.

Genotyping assays

Blood samples were collected from each patient and DNA was extracted using a commercial extraction kit QuickGene DNA Whole Blood Kit S on a QuickGene-810 semiautomatic extractor (Fijifilm Corporation, Tokyo, Japan). All subjects were genotyped for *CYP3A5**3 c.219-237A>G (rs776746), *ABCB1* c.3435C>T (rs1045642) and two SNPs: c.1508C>T (*POR*28*, rs1057868) and c.188+6405A>G (rs2868177) in the *POR* gene using commercial RT-PCR Taqman assays following the manufacturer recommendations (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics 19.0 (SPSS: an IBM company, IBM Corporation, Armonk, NY, USA). The differences between genotype groups for each genetic variant were assessed by the nonparametric Jonckheere–Terpstra trend test. The results were considered significant when *P*-values were lower than 0.05. No adjustments for multiple comparisons were performed.

Multivariate linear regression was used to assess the impact of clinical, demographic, concomitant medication and genetic covariates on subject variability of dose- and body weight-adjusted Advagraf PK parameters AUC, C_{max} and C_{min}.

Genetic score

Aiming to increase the sensibility to detect the influence of genetic on PK disposition factors in our population a genetic score was built. In order to evaluate the impact of *CYP3A5* SNPs on TAC PK parameters the study sample was divided in two groups: CYP3A5 expressers (*1/*1 or *1/*3) and CYP3A5 nonexpressers (*3/*3). Due to the reduced sample size and the physiological plausibility, the contribution of the genetic variants in *ABCB1* and *POR* on TAC PKs was only evaluated in *CYP3A5* nonexpressors. An additive genetic score was built using the analysed genetic variants in *ABCB1* (*rs1045642*) and *POR* (*rs1057868* and *rs2868177*) genes. Three genetic groups were delimited to build the score: one with no variant alleles, another with one or two and a third group with three variant alleles. The differences between *CYP3A5* expressors and nonexpressors, as well as between the three genetic score groups were assessed by the nonparametric Jonckheere–Terpstra trend test.

Model building

Two different linear regression models were built. One model including all subjects and a second model for *CYP3A5* nonexpressors. Genetic and nongenetic (race, body weight, body height, age, sex, body mass index, body surface area (BSA), hemoglobin, hematocrit, albumin and concomitant treatments) covariates were tested in a step-wise manner. Covariates removal was also performed in a step by step way. To evaluate the bias and precision of our model we calculated dose-/weight-adjusted AUC_{0-24h} and dose-/weight-adjusted C_{min} (predicted values) in our patients and we compared them with the real ones, determining mean error, mean absolute error and mean absolute error as a percentage of the real value.

All models were built using dose/weight AUC_{0-24h}, C_{max} and C_{min} with logarithmic transformation in order to assure a normal distribution of dependant variables.

Same procedures were followed for Prograf before the conversion to Advagraf.

RESULTS

Study population characteristics

Twenty-one stable kidney-transplanted children aged between 4 and 17 years were included in the study. Most of them were

Table 2. Comparison of the main pharmacokinetic parameters adjusted by dose and weight according to the different genotypes analysed

Pharmacokinetic parameter	*Variant*	*Genotype*	*Mean ± s.d.*	P-*value*
AUC_{0-24}	*CYP3A5* (rs776746)[a]	TT	879.6	0.009
		CT	1232.24 ± 578.64	
		CC	2507.88 ± 967.676	
	ABCB1 (rs1045642)[a]	GG	1967.69 ± 2301.42	0.255
		AG	2301.42 ± 1253.14	
		AA	2544.74 ± 950.02	
	*POR**28 (rs1057868)	CC	1884.88 ± 808.24	0.178
		CT	2812.42 ± 1103.67	
		TT	1164.86	
	POR (rs2868177)	AA	1886.27 ± 985.47	0.946
		AG	2456.11 ± 1198.81	
		GG	2038.38 ± 667.65	
C_{max}	*CYP3A5* (rs776746)[a]	TT	71.28	0.036
		CT	112.16 ± 34.92	
		CC	175.59 ± 71.96	
	ABCB1 (rs1045642)[a]	GG	144.47 ± 48.40	0.922
		AG	182.65 ± 94.00	
		AA	147.51 ± 55.49	
	*POR**28 (rs1057868)	CC	141.59 ± 59.24	0.512
		CT	194.54 ± 78.17	
		TT	84.37	
	POR (rs2868177)	AA	123.04 ± 54.54	0.413
		AG	178.25 ± 82.09	
		GG	152.32 ± 53.27	
C_{min}	*CYP3A5* (rs776746)[a]	TT	33.17	0.004
		CT	29.96 ± 11.70	
		CC	95.38 ± 33.79	
	ABCB1 (rs1045642)[a]	GG	60.87 ± 24.28	0.055
		AG	87.14 ± 46.99	
		AA	106.85 ± 33.31	
	*POR**28	CC	71.06 ± 34.40	0.388
		CT	99.19 ± 44.93	
		TT	70.15	
	POR (rs2868177)	AA	70.74 ± 21.61	0.682
		AG	87.42 ± 48.30	
		GG	82.53 ± 32.27	

[a]*CYP3A5* and *ABCB1* genes are located in the reverse strand and therefore variants are reported in reverse orientation of the genome.

Caucasian males. Study population characteristics are shown in Table 1. Allelic frequencies for *CYP3A5, ABCB1* and *POR* SNPs in our study population are also shown in Table 1. All genetic variants are in Hardy–Weinberg equilibrium.

Pharmacokinetic and pharmacogenetic results

For this analysis we selected AUC_{0-24h}, C_{max} and C_{min} as the main PK parameters adjusted by daily dose administered and body weight.

As shown in Table 2 and Figure 1 the univariate analysis found significant differences in AUC_{0-24h}, C_{max} and C_{min} between different genotypes of *CYP3A5 (CYP3A5*3, rs776746)*. *CYP3A5* nonexpressors (*CYP3A5*3/*3*, $n = 17$) presented a C_{max}, AUC_{0-24h} and C_{min} 72, 119 and 210% higher than expressors (*CYP3A5*1/*1 or *1/*3)*. No other significant differences were observed.

In order to evaluate the contribution of the genetic variants in *ABCB1* and *POR* genes we selected the 17 *CYP3A5* nonexpressors (*CYP3A5*3/*3*) and built a three-group additive genetic score. The genetic score groups includes subjects with 0 (group 1, reference), 1 or 2 (group 2) SNPs in *POR*, and 3 (group 3) variants in *ABCB1* and *POR* genes. Mean values for TAC PK parameters for these three groups are shown in Table 3. Group 2 (carriers of 1 or 2 *POR* genes variant alleles) have a C_{max}, AUC_{0-24h} and C_{min} 43, 27 and 11% higher than subjects in group 1, respectively (Table 3 and Figure 2). The C_{max}, AUC_{0-24h} and C_{min} observed in group 3 are 94, 82 and 68% higher than subjects without variant alleles (group 1), respectively (Table 3). Comparing the AUC_{0-24h}, C_{max} and C_{min} values of each group we can find significant differences between them ($P = 0.018$, $P = 0.037$ and $P = 0.018$, respectively) (Table 4).

Explicative models

A multivariate linear regression was used to evaluate the contribution of genetic and nongenetic factors to daily dose and body weight-adjusted C_{max}, AUC_{0-24h} and C_{min} variability. In

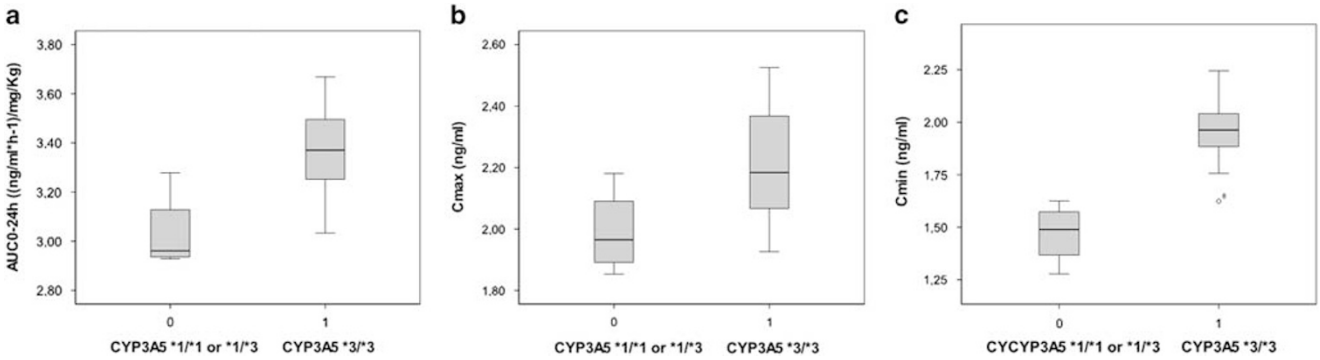

Figure 1. Pharmacokinetic parameters in *CYP3A5* (*CYP3A5*1*1 or*1/*3 and CYP3A5*3/*3)* genotypes ($n = 21$). (**a**) Dose-/weight-adjusted AUC_{0-24h}. (**b**) Dose-/weight-adjusted C_{max}. (**c**) Dose-/weight-adjusted C_{min}.

Table 3. Pharmacokinetic parameters in *CYP3A5* expressors and nonexpressors, and by genetic score in *CYP3A5* nonexpressors

	Variants	*CYP3A5 *1/*1 or *1/*3* n = *4*	*CYP3A5 *3/*3* n = *17*	*Genetic score of the CYP3A5 nonexpressors*		
				0 n = *2*	*1–2* n = *10*	*3* n = *5*
AUC_{0-24}/dose/weight	Mean ± s.d.	1144.09 ± 504.28	2507.86 ± 967.68	1805.65 ± 530.37	2308.53 ± 824.63	3295.26 ± 915.10
C_{max}/ dose/weight	Mean ± s.d.	101.94 ± 35.08	175.58 ± 71.96	115.63 ± 23.80	165.72 ± 61.00	224.76 ± 79.75
C_{min}/dose/weight	Mean ± s.d.	30.76 ± 9.69	95.38 ± 33.79	76.01 ± 29.40	84.48 ± 19.97	128.11 ± 36.36

Genetic score: 0, any variant allele in *ABCB1* or *POR;* 1–2, one or two genes with at least one variant allele in *ABCB1* or *POR;* 3, all three genes with at least one variant.

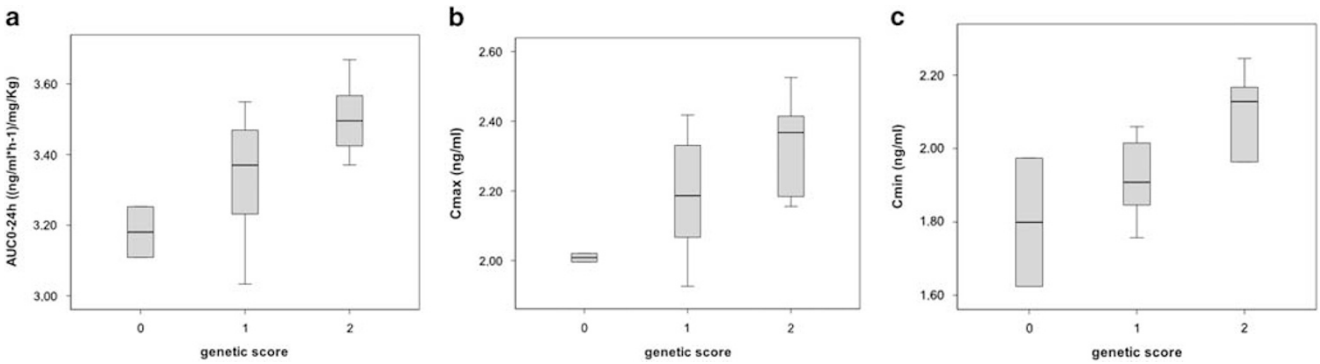

Figure 2. Pharmacokinetic parameters by genetic score ($n = 17$). (**a**) Dose-/weight-adjusted AUC_{0-24h}. (**b**) Dose-/weight-adjusted C_{max}. (**c**) Dose-/weight-adjusted C_{min}.

Table 4. Jonckheere–Terpstra trend test performed for the 17 patients, *CYP3A5* nonexpressors for all the remaining three genetic variants and for the built score

Variant	*Pharmacokinetic parameter*	*Jonckheere–Terpstra trend test (p)*
ABCB1 (rs1045642)	AUC_{0-24h}	0.507
	C_{max}	0.965
	C_{min}	0.102
*POR**28 (rs1057868)	AUC_{0-24h}	0.262
	C_{max}	0.575
	C_{min}	0.852
POR (rs2868177)	AUC_{0-24h}	0.715
	C_{max}	0.235
	C_{min}	0.273
Genetic score	AUC_{0-24h}	0.018
	C_{max}	0.037
	C_{min}	0.018

the model including the whole-study population ($n = 21$) the three retained covariates were: *CYP3A5* rs776746 SNP and Deflazacort and methylprednisolone co-medications. *CYP3A5* nonexpressors (*CYP3A5*3/*3*) showed increased TAC PK parameters and *CYP3A5* genotype explained 39.6, 20.6 and 59.5% of AUC_{0-24h}, C_{max} and C_{min} variability, respectively (Table 5). Deflazacort and methylprednisolone co-medications were related to decreased TAC PK parameters. Deflazacort explained 20.9 and 19.5% of AUC_{0-24h} and C_{max} variability, respectively, and methylprednisolone explained 14.3% of the AUC_{0-24h} variability (Table 5).

A second model was built for the subgroup of *CYP3A5* nonexpressors (*CYP3A5 *3/*3*, $n = 17$) in order to assess the effect of the genetic variants in *ABCB1* and *POR* genes through a genetic score. Genetic variants explained 13.7% of total AUC_{0-24h} variability. On the other hand, Deflazacort and methylprednisolone co-medications explained 32 and 11.1% of total AUC_{0-24h} variability. The genetic score was the only significant covariate in case of C_{min} explaining 26.5% of its variability. None of the genetic and nongenetic covariates included in this study were found to explain a significant part of C_{max} variability.

Table 5. Developed models with respective MAE%

Model	*Non-standardized coefficients*	*Standardized coefficients*	P-value	*Explained variability (%)*	*MAE% mean (IQR)*
	B ± s.e.	*Beta*			
All participants (n = 21)					
AUC_{0-24} r_c^2 = 0.571					
(Constant)	3.179 ± 0.083		0.000		25.78 (7.13–34.88)
CYP3A5	0.371 ± 0.089	0.519	0.001	39.6%	
Methylprednisolone	−0.212 ± 0.080	−0.436	0.017	14.3%	
Deflazacort	−0.376 ± 0.121	−0.513	0.006	20.9%	
C_{max} r_c^2 = 0.365					
(Constant)	2.115 ± 0.088		0.000		25.63 (9.43–40.57)
CYP3A5	0.246 ± 0.094	0.519	0.018	20.6%	
Methylprednisolone	−0.172 ± 0.085	−0.436	0.059	–	
Deflazacort	−0.325 ± 0.127	−0.513	0.021	19.5%	
C_{min} r_c^2 = 0.604					
(Constant)	1.527 ± 0.090		0.000		27.66 (6.91–40.22)
CYP3A5	0.504 ± 0.095	0.827	0.000	59.6%	
Methylprednisolone	−0.093 ± 0.086	−0.184	0.295	–	
Deflazacort	−0.143 ± 0.130	−0.175	0.285	–	
CYP3A5 nonexpressors (n = 17)					
AUC_{0-24} r_c^2 = 0.573					
(Constant)	3.390 ± 0.090		0.000		18.07 (7.34–26.91)
Genetic score	0.117 ± 0.050	0.413	0.035	13.7%	
Methylprednisolone	−0.174 ± 0.081	−0.424	0.050	11.1%	
Deflazacort	−0.474 ± 0.140	−0.639	0.005	32.0%	
C_{max} r_c^2 = 0.367					
(Constant)	2.209 ± 0.129		0.000		22.36 (5.96–32.53)
Genetic score	0.115 ± 0.060	0.410	0.078	–	
Methylprednisolone	−0.148 ± 0.098	−0.363	0.153	–	
Deflazacort	−0.352 ± 0.169	−0.478	0.058	–	
C_{min} r_c^2 = 0.403					
(Constant)	1.860 ± 0.109		0.000		21.11 (6.90–28.98)
Genetic score	0.136 ± 0.050	0.558	0.019	26.5%	
Methylprednisolone	−0.067 ± 0.082	−0.189	0.429	–	
Deflazacort	−0.239 ± 0.142	−0.375	0.116	–	

Abbreviations: AUC, area under the curve; IQR, interquartile range; MAE, mean predicted absolute error.

Model evaluation

Using the developed models for daily dose and body weight-adjusted C_{max}, AUC_{0-24h} and C_{min} the mean predicted absolute error for each model was calculated. The mean predicted absolute error expressed as percentage for C_{max}, AUC_{0-24h} and C_{min} is shown in Table 5. Similar results were found for the Prograf formulation and are presented in Supplementary Material.

DISCUSSION

Optimization of therapeutic strategies through individual genetic information can maximize therapeutic efficacy and reduce adverse drug reactions,[39,40] thus contributing to the development of personalized medicine.

TAC shows a large interindividual variation in oral bioavailability ranging from 4 to 89%.[7] A significant amount of this variability is explained by genetic variants affecting the drug metabolizing enzymes CYP3A4/5, genes regulating their activity as well as those genetic variants in cellular transporters as *ABCB1*.[10,11,13,23,41]

The aim of this project was to explore the impact of genetic and clinical factors in the exposure to TAC in kidney-transplanted children. As far as we know this is the first study assessing the joint influence of *CYP3A5 (rs776746), POR* (rs1057868 POR*28 and rs2868177) and *ABCB1 (rs1045642)* in the disposition of TAC after the administration of both Prograf and Advagraf. Advagraf results are reported in the main body of the article. Information about the Prograf study is reported in the Supplementary Material.

In a first univariate analysis with all 21 patients (Table 2) we found that the only genetic variant significantly related to TAC disposition is the rs776746 SNP (CYP3A5*3 allele) affecting all three PK parameters analysed (dose weight-adjusted AUC_{0-24}, C_{max} and C_{min}) and showing a clear gene–dose response. This confirms the major impact of this polymorphism in the disposition of TAC. This fact has been well described in the literature.[10,11,13,15–17,19,20]

We therefore performed a multivariate analysis including all the patients ($n = 21$) and found that *CYP3A5*3* genotype explains 40, 21 and 60% of variability in daily dose and body weight-adjusted AUC_{0-24h}, C_{max} and C_{min}, respectively, in stable renal transplant children receiving one-daily TAC formulation (Advagraf) (Table 5). Similar results were found for Prograf (see Supplementary Table 1S to Supplementary Table 3S presented in Supplementary Material). These findings are in agreement with previous published results by de Jonge *et al.*[42] in adult stable Prograf-treated renal allograft recipients, where *CYP3A5*3 (rs776746)* explains more than 29% of the TAC dose requirements. The well-known *CYP3A* activity

inducers Deflazacort and methylprednisolone were the only nongenetic covariates retained in the model. Deflazacort and methylprednisolone explain 21 and 14% of variability in adjusted AUC_{0-24h}, respectively (Table 5). Only Deflazacort was retained in the case of C_{max}, explaining 20% of its variability. C_{min} variability was not influenced by these co-medications. No other clinical, biochemical or demographic variables explain a significant part of TAC exposure variability. Some authors have described that hematocrit could explain up to 14 and 11% of TAC clearance and dose-adjusted AUC_{0-12h}, respectively.[42] The discrepancy found in our study may be due to the reduced sample size and low variability in the hematocrit values among our patients (CV % = 9.4%).

Recently *CYP3A4*22* has been described as a determinant genetic factor influencing TAC total exposure, as this variant increases the formation of the non-functional CYP3A4 splice variant.[3,14,42,43] We did not evaluate these variants as we considered that its low frequency (minor allele frequency = 0.025) made it very unlikely to be found in our cohort due to the number of patients included (we would require at least twice the patients to find more than 1 carrier). We therefore assumed that all patients were wildtype for *CYP3A4* (CYP3A4*1/*1) for the interpretation and evaluation of our results.

The influence of *ABCB1* polymorphisms on TAC PKs has been extensively investigated; however the results are still controversial.[27,30] Rong *et al.*[44] describe that *ABCB1* variants have no effect on TAC exposure, whereas some others found that patients homozygous for allele C (rs1045642) would require higher daily doses of TAC to obtain levels into the therapeutic range when compared with the T allele carriers.[26] In our determinations we haven't found any significant difference between the different *ABCB1 (rs1045642)* genotypes (Table 2).

POR is a membrane-bound coenzyme that functions as an electron donor for the CYP enzymes, therefore genetic variability in this gene may be related to CYP3A enzymatic activity variations. De Jongue *et al.*[36] and Gijsen *et al.*[45] found that *POR*28* T allele carriers had significantly higher TAC dose requirements in CYP3A5 expressors (CYP3A5*1 carriers) but not in CYP3A5 nonexpressers *(CYP3A5*3/*3)* However, Elens *et al.*[41] found that *POR*28* homozygosity (CC) was related to a significant higher *CYP3A4* activity in *CYP3A5* nonexpressors for TAC metabolism. We found no significant differences in dose weight-adjusted AUC_{0-24}, C_{max} or C_{min} in patients with different *POR*28* genotypes ($P = 0.178$, $P = 0.512$ and $P = 0.388$, respectively) but like Elens *et al.*[41] we did find an increasing trend in these parameters in *POR*28* carriers (Table 2). Recently, an intronic polymorphism (rs2868177) in *POR* gene was related to higher warfarin maintenance dose needs (mutated patients require lower doses)[37] but in a univariate analysis we found no significant relation between the TAC PK parameters and the different *POR rs2868177* genotypes (Table 2).

Figure 1 shows there is a great variability in the TAC PK parameters dose-adjusted AUC_{0-24} and C_{max} mainly in *CYP3A5* nonexpressors (CYP3A5*3/*3). In addition, due to the majority of *CYP3A5* nonexpressors in Caucasian population we decided to study this variability only in this group of patients ($n = 17$).

To increase the sensibility of our statistical analysis we developed an additive genetic score using the analysed genetic variants in *ABCB1* (rs1045642) and *POR* (rs1057868 and rs2868177) and we found significant differences in TAC PK parameters between different *ABCB1* and *POR* genotypes (Table 4 and Figure 2).

By applying the built genetic score in a multivariate analysis we found that this variable explains 14 and 27% of the daily dose and body weight-adjusted AUC_{0-24h} and C_{min}, variability, respectively (Table 5). The concomitant administration of Deflazacort and methylprednisolone explained 32 and 11% of daily dose and body weight-adjusted AUC_{0-24h} variability (Table 5).

To our knowledge, this is the first multivariate model developed, for pediatric population with kidney transplant, for daily dose and body weight-adjusted TAC PK parameters. The mean absolute error of all models is lower than 28% indicating that if these results are replicated in another independent pediatric population the models could be used in clinics to improve TAC exposure prediction.

Similar results have been found for the Prograf formulation (Supplementary Material). This study confirmed that *CYP3A5* genetic variants have a major effect in TAC-dosing requirements, with *CYP3A5* genotype explaining ~21% of C_{max} and 60% of C_{min} variability. Nonetheless, our results show that some previously reported SNPs in *ABCB1 (rs*1045642) and *POR* (rs1057868 and 2868177) may explain residual variability in response to TAC in CYP3A5 nonexpressors and shouldn´t be underestimated when evaluating TAC exposure.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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