

Original Article

# Prediction of tacrolimus metabolism and dosage requirements based on CYP3A4 phenotype and CYP3A5\*3 genotype in Chinese renal transplant recipients

Xi LUO<sup>1,2,3</sup>, Li-jun ZHU<sup>4</sup>, Ning-fang CAI<sup>5</sup>, Li-yun ZHENG<sup>2</sup>, Ze-neng CHENG<sup>2,\*</sup>

<sup>1</sup>School of Life Sciences, Central South University, Changsha 410083, China; <sup>2</sup>School of Pharmaceutical Sciences, Central South University, Changsha 410013, China; <sup>3</sup>The State Key Laboratory of Medical Genetics, Central South University, Changsha 410078, China; <sup>4</sup>Research Center of National Health Ministry on Transplantation Medicine Engineering and Technology, the 3rd Affiliated Hospital of Xiangya Medical Institute, Central South University, Changsha 410013, China; <sup>5</sup>Department of Pharmacy, Zhangzhou Municipal Hospital of Fujian Province, Zhangzhou 363000, China

**Aim:** To examine how the endogenous CYP3A4 phenotype and CYP3A5\*3 genotype of Chinese renal transplant recipients influenced the dose-corrected trough concentration ( $C_0/D$ ) and weight-corrected daily dose ( $D/W$ ) of tacrolimus.

**Methods:** A total of 101 medically stable kidney transplant recipients were enrolled, and their blood and urine samples were gathered. The endogenous CYP3A4 phenotype was assessed by the ratio of 6 $\beta$ -hydroxycortisol and 6 $\beta$ -hydroxycortisone to cortisol and cortisone in urine. CYP3A5\*3 genotype was determined using PCR-RELP.

**Results:** In overall renal transplant recipients, a multiple regression analysis including the endogenous CYP3A4 phenotype, CYP3A5\*3 genotype and post-operative period accounted for 60.1% of the variability in  $C_0/D$  ratio; a regression equation consisting of the endogenous CYP3A4 phenotype, post-operative period, body mass index, CYP3A5\*3 genotype, gender, total bilirubin and age explained 61.0% of the variability in  $D/W$  ratio. In CYP3A5\*3/\*3 subjects, a combination of the endogenous CYP3A4 phenotype, post-operative period and age was responsible for 65.3% of the variability in  $C_0/D$  ratio; a predictive equation including the endogenous CYP3A4 phenotype, post-operative period, body mass index, gender and age explained 61.2% of the variability in the  $D/W$  ratio. Based on desired target range of tacrolimus trough concentrations, individual daily dosage regimen was calculated, and all the observed daily doses were within the predicted range.

**Conclusion:** This study provides the equations to predict tacrolimus metabolism and dosage requirements based on the endogenous CYP3A4 phenotype, CYP3A5\*3 genotype and other non-genetic variables.

**Keywords:** tacrolimus; CYP3A phenotype; CYP3A5\*3 genotype; Chinese renal transplant recipients

Acta Pharmacologica Sinica (2016) 37: 555–560; doi: 10.1038/aps.2015.163; published online 29 Feb 2016

## Introduction

Tacrolimus (Tac) is a potent immunosuppressive agent that has been widely used to improve the outcome of organ transplantation. The need for frequent and specific monitoring of drug concentrations remains essential because the therapeutic dosing and pharmacokinetics of Tac show great variability among recipients<sup>[1]</sup>. Tac is known to be extensively metabolized by cytochrome P450 (CYP) 3A4 and CYP3A5. Intestinal

P-glycoprotein (P-gp) is a product of the multidrug resistant 1 (*MDR1*) gene in humans and limits the bioavailability of Tac via active efflux<sup>[2,3]</sup>. Importantly, most single nucleotide polymorphisms (SNPs) for CYP3A4 are unable to fully explain the inter-individual variability in CYP3A enzymatic activity<sup>[4]</sup>. In contrast, the CYP3A5\*3 SNP (A6896G) in exon 3 is strongly associated with CYP3A5 expression<sup>[5]</sup>, and the effect of the CYP3A5\*3 allele on the pharmacokinetics of oral Tac has been confirmed by consistently positive results<sup>[1,6]</sup>. Other reports have indicated that the most common SNPs for *MDR1*, including the synonymous SNPs C1236T in exon 12, C3435T in exon 26 and the nonsynonymous SNP G2677T/A in exon 21<sup>[7]</sup>, play

\* To whom correspondence should be addressed.

E-mail chengzn@csu.edu.cn

Received 2015-07-11 Accepted 2015-11-03

a minor role in the metabolism of Tac<sup>[1]</sup>. Hence, *CYP3A5\*3* polymorphisms may be the only useful SNP for Tac dosage adjustment<sup>[6]</sup>. However, such genetic information is unlikely to be responsible for the residual inter-individual variability in the pharmacokinetics of Tac among *CYP3A5* expressers or *CYP3A5* non-expressers. Therefore, accurately formulating an individual's dosing regimen remains limited<sup>[6]</sup>. As a complementary or alternative strategy, determination of a patient's *CYP3A4* phenotype may help to overcome the limitations of genetic assays and optimize Tac dosage regimens.

Currently, the ratio of 6 $\beta$ -hydroxycortisol to free cortisol (6 $\beta$ -OHF/F) in urine is thought to be a useful marker of both the induction and the inhibition of hepatic *CYP3A4* activity<sup>[8]</sup>. The interconversion of cortisol (F) to cortisone (E) and 6 $\beta$ -hydroxycortisol (6 $\beta$ -OHF) to 6 $\beta$ -hydroxycortisone (6 $\beta$ -OHE) by 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD) plays an important role in cortisol metabolism; thus, using cortisol as a *CYP3A4* phenotyping probe may be confounded due to the regulation of cortisol feedback<sup>[9,10]</sup>. Our previous experiments confirmed that the combined ratio of 6 $\beta$ -hydroxycortisol and 6 $\beta$ -hydroxycortisone to cortisol and cortisone in urinary MR reflects *CYP3A4* catalytic ability and not the total activity of *CYP3A4* and *CYP3A5* isoforms (unpublished data). Another previous study completed by our laboratory showed that the MR was significantly related with the dose-corrected trough concentrations ( $C_0/D$ ) of Tac in renal transplant recipients ( $r = -0.824$ ,  $P < 0.05$ )<sup>[11]</sup>.

The major aim of our current study was to investigate the relationship between the endogenous *CYP3A4* phenotype (assessed by urinary MR) and Tac metabolism and dosage requirements in Chinese renal transplant recipients. We also investigated the influence of *CYP3A5\*3* genotype and other potential determinants of Tac disposition.

## Materials and methods

### Ethics statement

This study was performed in accordance with the declaration of Helsinki and its amendments. The experimental protocol was approved by the Ethical Committee of the School of Pharmaceutical Sciences at Central South University. The study is a part of registered clinical trial in ClinicalTrials.gov and the identifier is NCT01699360 (<http://clinicaltrials.gov/show/NCT01699360>). Written informed consents were obtained from all subjects before commencing the study.

### Materials

The 6 $\beta$ -OHF, 6 $\beta$ -OHE, F, E and dexamethasone were purchased from Sigma-Aldrich (St Louis, MO, USA). They were of at least 98% purity. Acetonitrile, methanol and formic acid were of LC grade and purchased from Tedia (Tedia Company Inc, Fairfield, CT, USA). All other chemicals were of AR grade and obtained from commercial sources (Sinopharm Chemical Reagent Co Ltd, Shanghai, China).

### Human subject study

The study was comprised of 101 medically stable kidney trans-

plant recipients between 1 and 2 years after transplantation. A total of 231 specimens were collected at the 3rd Affiliated Hospital of Xiangya Medical Institute at Central South University. Patients who met the following criteria were included: receiving an immunosuppressive regimen containing Tac (tacrolimus capsules, Prograf<sup>®</sup>, Astellas Ireland Co Ltd, Ireland), mycophenolate mofetil (CellCept<sup>®</sup>, Roche, Shanghai, China) and prednisolone; oral administration of Tac at twice the daily dose at least 5 d prior to the study; undergone no more than one renal transplant; normal liver and renal function; and older than 18 years of age. The following exclusion criteria were used for this study: an acute rejection episode or infection; multiple organ transplantation; use of any other medications known to induce or inhibit the *CYP3A* enzyme or interact with immunosuppressive agents, with the exception of amlodipine; or any abnormal findings on physical examinations or laboratory tests.

At the time of investigation, the dose of Tac ranged between 1.0 and 8.0 mg/day. The doses of mycophenolate mofetil and prednisolone were 0.36–1.5 mg/day and 2.0–30.0 mg/day, respectively. On the morning of the study, urine specimens were collected between 8:00 AM and 10:00 AM. Blood samples were obtained before Tac administration. Blood samples were immediately sent to the clinical laboratory for Tac analysis and *CYP3A5\*3* genotyping, and urine samples were stored at -20 °C for subsequent analysis of 6 $\beta$ -OHF, 6 $\beta$ -OHE, F and E. All subjects underwent routine laboratory tests, including hematology, blood chemistries and urinalysis. The demographics of each subject and their co-medications were also recorded (Table 1). Each patient was instructed to eat his or her usual breakfast, excluding caffeine- and grapefruit-containing foods, and to take any other morning medication except Tac.

**Table 1.** The data of renal transplant recipients. Mean $\pm$ SD.

Parameter	Value
Age (year)	37.4 $\pm$ 9.9
Gender (male/female)	77/24
Body weight (kg)	60.2 $\pm$ 10.5
Height (cm)	165.7 $\pm$ 6.4
Body mass index (kg/m <sup>2</sup> )	21.8 $\pm$ 3.2
Postoperative periods (h)	11 887.6 $\pm$ 2032.6
Glutamic-pyruvic transaminase (U/L)	25.2 $\pm$ 16.8
Glutamic oxalacetic transaminase (U/L)	21.2 $\pm$ 12.3
Total bilirubin ( $\mu$ mol/L)	13.2 $\pm$ 4.3
Blood urea nitrogen (mmol/L)	6.1 $\pm$ 2.0
Serum creatinine ( $\mu$ mol/L)	97.3 $\pm$ 47.6
Serum uric acid ( $\mu$ mol/L)	337.4 $\pm$ 70.1
Comedication of amlodipine (with/without)	50/51

### *CYP3A5\*3* genotyping

Genomic DNA was extracted from peripheral lymphocytes according to the instructions provided with the Genomic DNA

Purification Kit (Promega, Madison, WI, USA). Identification of *CYP3A5*\*3 genotypes were performed using the previously reported polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay<sup>[12]</sup>. The forward primer was 5'-CATGACTTAGTAGACAGATGAC-3' and the reverse primer was 5'-GGTCCAAACAGGGAAGAAATA-3'. The amplified DNA fragment was 293 bp and was digested with *Ssp* I (Fermentas, Vilnius, Lithuania) at 37 °C for 4 h. The genotype for each individual was further validated by a sequencing assay (data not shown).

#### Analytical methods

##### 6β-OHF, 6β-OHE, F and E in urine

The 6β-OHF, 6β-OHE, F and E in urine were measured using the HPLC-UV method developed in our laboratory<sup>[11]</sup>. The lower limit of quantification was 5 µg/L for all of the compounds in the urine. The accuracy was determined at three concentrations and ranged between 98.2% and 115.5%.

##### Trough concentrations of tacrolimus in whole blood

Trough concentrations of Tac in whole blood were assayed by the Microparticle Enzyme Immunoassay (MEIA, Abbott, Princeton, NJ, USA) using blood samples taken on the morning of the study. The assay was performed according to the instructions supplied with the Tacrolimus II monoclonal antibody kit (Abbott, Princeton, NJ, USA).

#### Data and statistical analysis

The normality distribution of parameters was assessed using the Kolmogorov-Smirnov test. The  $C_0/D$  ratio and weight-corrected daily dosage (D/W) of Tac between *CYP3A5*\*3 genotypes was compared using a one-way ANOVA followed by *post-hoc* Bonferroni corrected *t*-tests. The relationship between the  $C_0/D$  and D/W ratios of Tac and urinary MR were analyzed by linear regression. Correlations of the  $C_0/D$  and D/W ratios of Tac with each factor (as shown in Table 1) were analyzed with an  $\alpha=0.05$ . Factors that were not statistically significant were rejected. A stepwise multiple regression analysis with statistically significant factors was used to obtain the coefficient of determination ( $R^2$ ). The models for the pre-

diction of the  $C_0/D$  and D/W ratios of Tac were constructed based on the multiple regression equation. All statistical analysis was performed using SPSS 17.0 statistics software, and a *P* value <0.05 was considered significant.

#### Results

##### Effect of *CYP3A5*\*3 genotype on the $C_0/D$ and D/W ratios of Tac

The Kolmogorov-Smirnov test illustrated that the logarithm of  $C_0/D$  and D/W ratios of Tac and the logarithm of urinary MR, postoperative periods, body mass index and total bilirubin were normally distributed. The values of all other continuous variables (as shown in Table 1) showed a normal distribution in Chinese renal transplant recipients.

The  $C_0/D$  ratios of Tac in the *CYP3A5*\*3/\*3 group were significantly higher when compared with those in the *CYP3A5*\*1/\*1 and *CYP3A5*\*1/\*3 groups (Supplementary Table S1). Consistent with the relationship between *CYP3A5* genotype and phenotype, the D/W ratios of Tac in *CYP3A5*\*3/\*3 subjects were markedly lower when compared with *CYP3A5*\*1 carriers (*CYP3A5*\*1/\*1 and *CYP3A5*\*1/\*3 genotypes) (Supplementary Table S1).

##### The relationship between the endogenous *CYP3A4* phenotype and tacrolimus metabolism

As shown in Figure 1, the endogenous *CYP3A4* phenotype (assessed by urinary MR) significantly correlated with the  $C_0/D$  and D/W ratios of Tac. Within *CYP3A5*\*3/\*3 subjects, urinary MR was related to the  $C_0/D$  and D/W ratios; however, only weak correlations were observed in *CYP3A5*\*1 carriers (*CYP3A5*\*1/\*1 and *CYP3A5*\*1/\*3 genotypes).

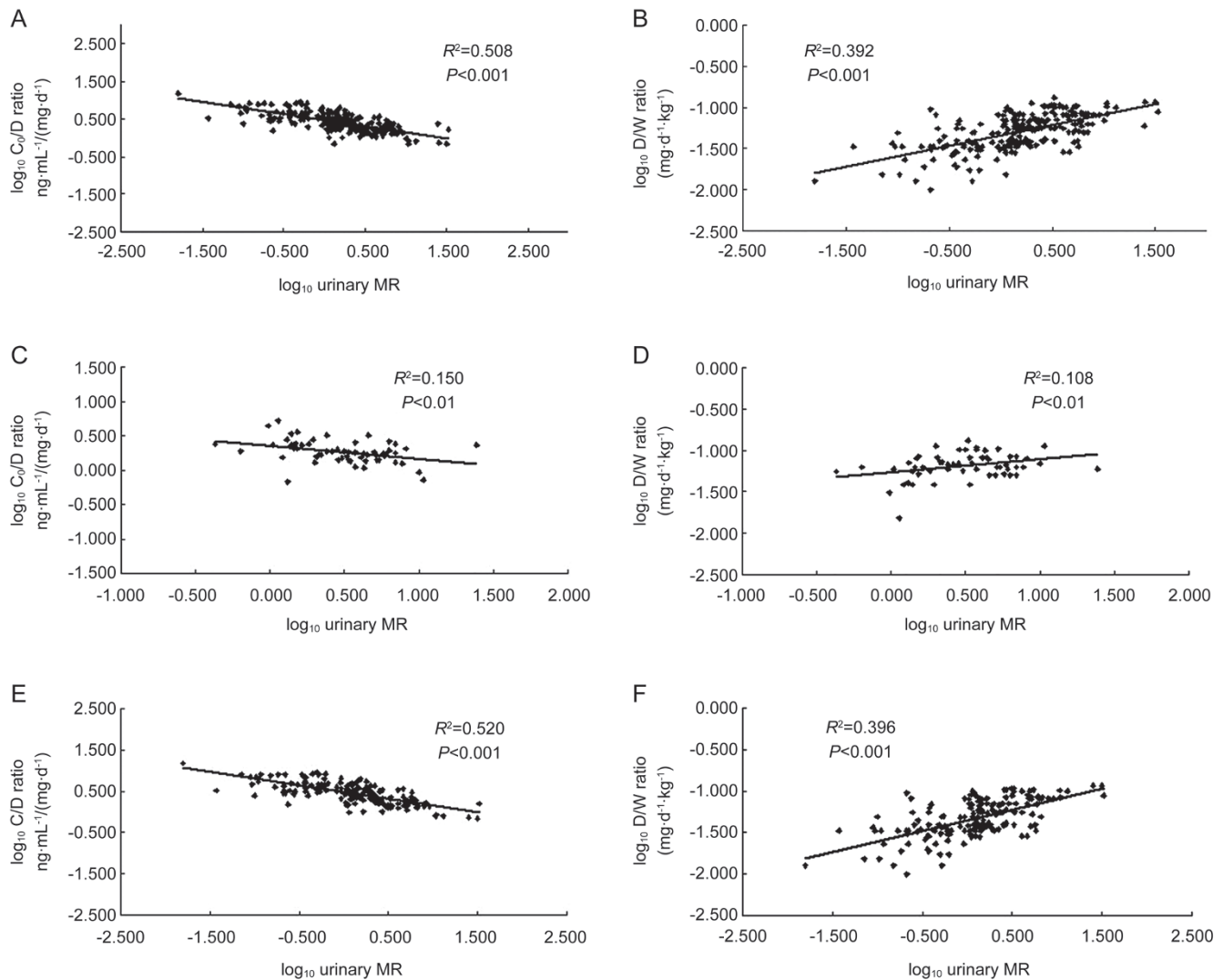
##### Multiple regression analysis for the prediction of tacrolimus metabolism and dosage requirements

Final regression models were established to predict the  $C_0/D$  and D/W ratios of Tac (Table 2). The coefficients of determination ( $R^2$ ) were 0.601 and 0.610 for the  $C_0/D$  and D/W ratios, respectively. Within the *CYP3A5*\*3/\*3 genotype, the values of  $R^2$  were 0.653 and 0.612 for the  $C_0/D$  and D/W ratios, respectively (Table 3). According to equations (1) and (3), the daily dosage regimen was determined:

**Table 2.** The regression analysis for the prediction of the  $C_0/D$  and D/W ratios of Tac in overall renal transplant recipients ( $n=231$ ).

No	Predicted value	Regression equation	$R^2$	<i>P</i>
Equation (1)	$C_0/D$ ratio $\text{ng}\cdot\text{mL}^{-1}/(\text{mg}\cdot\text{d}^{-1})$	$\text{Log}_{10} C_0/D = 0.331 - 0.338 * \text{Log}_{10} \text{MR} - 0.083 * \text{CYP3A5} + 0.046 * \text{Log}_{10} \text{POT}$	0.601	<0.001
Equation (2)	D/W ratio $(\text{mg}\cdot\text{d}^{-1}\cdot\text{kg}^{-1})$	$\text{Log}_{10} D/W = 0.303 + 0.230 * \text{Log}_{10} \text{MR} - 0.133 * \text{Log}_{10} \text{POT} - 0.750 * \text{Log}_{10} \text{BMI} + 0.081 * \text{CYP3A5} + 0.066 * \text{gender} - 0.133 * \text{Log}_{10} \text{TBIL} - 0.002 * \text{age}$	0.610	<0.001

$C_0/D$ , dose-corrected trough concentration of Tac; D/W, weight-corrected stable dose of Tac;  $\text{Log}_{10} \text{MR}$ , the logarithm of the ratio of 6β-hydroxycortisol and 6β-hydroxycortisone to cortisol and cortisone in urine; *CYP3A5*\*1/\*1 and *CYP3A5*\*1/\*3 genotypes were set at the value of "1"; *CYP3A5*\*3/\*3 genotype was set at the value of "0";  $\text{Log}_{10} \text{POT}$ , the logarithm of post-operative period;  $\text{Log}_{10} \text{BMI}$ , the logarithm of body mass index; gender, male was defined as the value of "0"; female was defined as the value of "1";  $\text{Log}_{10} \text{TBIL}$ , the logarithm of total bilirubin;  $R^2$ , coefficient of determination; *P*, probability.



**Figure 1.** Linear regression relationships between urinary MR and the  $C_0/D$  and  $D/W$  ratios of tacrolimus across the different  $CYP3A5^*3$  genotypes. (A) with the  $C_0/D$  ratio in overall subjects ( $n=231$ ); (B) with the  $D/W$  ratio in overall subjects ( $n=231$ ); (C) with the  $C_0/D$  ratio in  $CYP3A5^*1$  carriers ( $n=58$ ); (D) with the  $D/W$  ratio in  $CYP3A5^*1$  carriers ( $n=58$ ); (E) with the  $C_0/D$  ratio in  $CYP3A5^*3/*3$  genotype ( $n=173$ ); (F) with the  $D/W$  ratio in  $CYP3A5^*3/*3$  genotype ( $n=173$ ).

Daily dosage regimen for all subjects= $C_0/\text{Power}$  [10, (0.331-0.338\* $\text{Log}_{10}\text{MR}$ -0.083\* $CYP3A5$ +0.046\* $\text{Log}_{10}\text{POT}$ )]

Equation (5)

$C_0$ , trough concentration of Tac;  $\text{Log}_{10}\text{MR}$ , the logarithm of the ratio of 6 $\beta$ -hydroxycortisol and 6 $\beta$ -hydroxycortisone to cortisol and cortisone in urine;  $CYP3A5^*1/*1$  and  $CYP3A5^*1/*3$  genotypes were set at "1"; the  $CYP3A5^*3/*3$  genotype was set at "0";  $\text{Log}_{10}\text{POT}$ , the logarithm of post-operative period.

Daily dosage regimen for  $CYP3A5^*3/*3$  subjects= $C_0/\text{Power}$  [10, (0.132-0.357\* $\text{Log}_{10}\text{MR}$ +0.074\* $\text{Log}_{10}\text{POT}$ +0.003\*age)]

Equation (6)

$C_0$ , trough concentration of Tac;  $\text{Log}_{10}\text{MR}$ , the logarithm of the ratio of 6 $\beta$ -hydroxycortisol and 6 $\beta$ -hydroxycortisone to cortisol and cortisone in urine;  $\text{Log}_{10}\text{POT}$ , the logarithm of post-operative period.

Based on the desired target range of Tac  $C_0$  levels, an individual's daily dosage regimen was directly calculated by equation (5) and (6). The results illustrate that all of the observed doses were within the predicted dosage range (Table 4).

## Discussion

Therapeutic drug monitoring (TDM) and  $CYP3A5^*3$  genotyping are common tools for the adjustment of an individual's Tac dosage in the clinic. However, TDM can be labor intensive because this method requires the frequent monitoring of drug concentrations in blood. Notably, TDM cannot predict an individual's dosage; thus, many patients can have adverse reactions before drug monitoring begins<sup>[1]</sup>.  $CYP3A5^*3$  genotyping can help to optimize the Tac dose regimen between  $CYP3A5$  expressers and  $CYP3A5$  non-expressers. Our results confirm previous reports showing that  $CYP3A5^*3$  polymor-

**Table 3.** The regression analysis for the prediction of the  $C_0/D$  and  $D/W$  ratios of Tac in renal transplant recipients with  $CYP3A5^*3/*3$  genotype ( $n=173$ ).

No	Predicted value	Regression equation	$R^2$	$P$
Equation (3)	$C_0/D$ ratio $\text{ng}\cdot\text{mL}^{-1}/(\text{mg}\cdot\text{d}^{-1})$	$\text{Log}_{10} C_0/D=0.132-0.357*\text{Log}_{10} \text{MR}+0.074*\text{Log}_{10} \text{POT}+0.003*\text{age}$	0.653	<0.001
Equation (4)	$D/W$ ratio $(\text{mg}\cdot\text{d}^{-1}\cdot\text{kg}^{-1})$	$\text{Log}_{10} D/W=0.333+0.251*\text{Log}_{10} \text{MR}-0.151*\text{Log}_{10} \text{POT}-0.820*\text{Log}_{10} \text{BMI}+0.061*\text{gender}-0.002*\text{age}$	0.612	<0.001

$C_0/D$ , dose-corrected trough concentration of Tac;  $D/W$ , weight-corrected stable dose of Tac;  $\text{Log}_{10} \text{MR}$ , the logarithm of the ratio of  $6\beta$ -hydroxycortisol and  $6\beta$ -hydroxycortisone to cortisol and cortisone in urine;  $\text{Log}_{10} \text{POT}$ , the logarithm of post-operative period;  $\text{Log}_{10} \text{BMI}$ , the logarithm of body mass index; gender, male was defined as the value of "0"; female was defined as the value of "1";  $R^2$ , coefficient of determination;  $P$ , probability.

**Table 4.** The desired target range of Tac concentration, the predicted daily dosage range and the observed daily dose of Tac in overall ( $n=231$ ) and  $CYP3A5^*3/*3$  subjects ( $n=173$ ). Mean $\pm$ SD.

Subjects	Desired target range of concentration ( $\text{ng}\cdot\text{mL}^{-1}$ ) <sup>a</sup>	Predicted daily dosage range ( $\text{mg}\cdot\text{d}^{-1}$ )		Observed daily dose ( $\text{mg}\cdot\text{d}^{-1}$ )
		Lower bound	Upper bound	
Overall	3–7	1.0 $\pm$ 0.4	3.7 $\pm$ 1.5	3.4 $\pm$ 1.5
$CYP3A5^*3/*3$ genotype	3–7	0.9 $\pm$ 0.4	3.2 $\pm$ 1.6	3.1 $\pm$ 1.5

<sup>a</sup>The desired target range of concentration was set according to the clinical practice in the 3rd Affiliated Hospital of Xiangya Medical Institute, Central South University;

phisms play an important role in Tac metabolism<sup>[1,6]</sup>.  $CYP3A5$  non-expressers ( $CYP3A5^*3/*3$ ) displayed higher  $C_0/D$  ratios and lower  $D/W$  ratios when compared with  $CYP3A5$  expressers ( $CYP3A5^*1/*1$  and  $CYP3A5^*1/*3$ ) (Supplementary Table S1). Within  $CYP3A5$  expressers or  $CYP3A5$  non-expressers, there was a residual inter-individual variability in the dosage requirements and pharmacokinetics of Tac. Therefore, we hypothesized that *in vivo*  $CYP3A4$  phenotyping was an independent predictor of Tac disposition.

A previous study suggested that 56%–59% of the variability in Tac dose requirements and clearance could be explained by *in vivo*  $CYP3A4$  activity assessed by midazolam clearance and  $CYP3A5$  genotype<sup>[6]</sup>. Administration of midazolam is not convenient because of its pharmacological effects as a central nervous system depressant; thus, taking advantage of endogenous substances, such as markers of the  $CYP3A4$  phenotype, can avoid unnecessary administration of probe drugs to humans. In our study, a non-invasive  $CYP3A4$  phenotype was used by determining the metabolic ratio of endogenous  $6\beta$ -hydroxymetabolites to cortisol and cortisone. No serial blood sampling for the  $CYP3A4$  phenotype was needed. The findings showed that this endogenous  $CYP3A4$  probe explained 50.8% and 39.2% of variability in the  $C_0/D$  and  $D/W$  ratios, respectively, of all subjects (Figure 1). The multiple regression analysis, including  $CYP3A4$  phenotype assessed by urinary MR,  $CYP3A5^*3$  genotype and post-operative period, accounted for 60.1% of the variability in the  $C_0/D$  ratio (Table 2). Combination of the *in vivo*  $CYP3A4$  phenotype, post-operative period, body mass index,  $CYP3A5^*3$  genotype, gender, total bilirubin and age explained 61.0% of variability in the  $D/W$  ratio (Table 2). These results are similar to a previ-

ous report on midazolam clearance and  $CYP3A5^*3$  genotype<sup>[6]</sup>.

Within  $CYP3A5$  non-expressers ( $CYP3A5^*3/*3$ ), urinary MR was responsible for 52.0% and 39.6% of the variability in the  $C_0/D$  and  $D/W$  ratios of Tac, respectively; however, only a 15.0% and 10.8% contribution was found among  $CYP3A5$  expressers ( $CYP3A5^*1/*1$  and  $CYP3A5^*1/*3$ ) (Figure 1). Furthermore, 65.3% and 61.2% of the variance in the  $C_0/D$  and  $D/W$  ratios, respectively, was explained by the multiple regression equations presented in Table 3. Our previous study suggested that urinary MR reflects  $CYP3A4$  activity (unpublished data), and the metabolism of Tac depends on a total of  $CYP3A4$  and  $CYP3A5$  activities<sup>[13]</sup>. Among  $CYP3A5^*3/*3$  subjects, we assumed that all compounds were mainly catalyzed by the  $CYP3A4$  isoform, and the absence of the  $CYP3A5$  enzyme may contribute to the marked correlation between urinary MR and Tac disposition.

The recommended blood concentration of Tac is 3–7  $\text{ng}/\text{mL}$  at least 3 months after transplantation. The dosage requirements should be carefully controlled in order to achieve the desired concentration of Tac. Using equations (5) and (6), the desired daily dosage was predicted, and all of the observed doses were within the predicted range (Table 4). Our findings suggest that equations (5) and (6) can provide additional valuable information for the use of Tac in the clinic.

In our study, all patients received an immunosuppressive regimen containing Tac, mycophenolate mofetil and prednisolone. Prednisolone can be converted to prednisone, which is a weak inducer of  $CYP3A4$ <sup>[14]</sup>. However, we found that a varying dosage of prednisolone had no significant effect on Tac disposition and  $CYP3A4$  phenotype (data not shown). Furthermore, calcium-channel blockers were more commonly

used after transplantation. Amlodipine does not interact with the CYP3A enzyme at therapeutic doses<sup>[14]</sup> and was included in the study population. Our results suggest that co-medication with amlodipine did not affect the  $C_0/D$  and  $D/W$  ratios of Tac in Chinese renal transplant recipients (data not shown).

Tac is metabolized by the CYP3A enzyme, but it can also be influenced by the patient's physical condition after transplantation. Thus, only patients with a similar postoperative period were selected. In our study, patients at a stable stage (between 1–2 years after transplantation) were enrolled. As a result, only stable doses were investigated, and further studies should focus on the prediction of initial dosage requirements in the early post-operative period.

In summary, we developed equations that describe the association between the endogenous CYP3A4 phenotype and Tac metabolism and dosage requirements. These equations take into account the CYP3A5\*3 genotype and other potential variables. Within CYP3A5\*3/\*3 genotypes, 65.3% and 61.2% of the inter-individual variability in Tac metabolism and dosage requirements were explained by the endogenous CYP3A4 phenotype and other non-genetic variables. These findings provide a potential basis for estimating a Tac dosing regimen using a non-invasive CYP3A4 probe, rather than the administration of probe drugs or serial blood sampling. Furthermore, our predictive algorithm based on phenotype and genotype can be used prior to treatment.

### Acknowledgements

The research was funded by the National Natural Science Foundation of China (No 81072700/H3110). The project was funded by the China Postdoctoral Science Foundation (No 2014M562140), Research Fund for Young Scholars of Fujian Province Health and Family Planning Commission (No 2014-1-79) and Scientific Research Foundation for Postdoctoral of Central South University (No 31000-160320068). We gratefully thank Wen-zhao XIE and Ya GONG for gathering the biological samples. We also thank Qing LIU for her kind help with the data analysis.

### Author contribution

Xi LUO and Ze-neng CHENG designed the research; Xi LUO, Li-jun ZHU and Li-yun ZHENG performed the research; Li-jun ZHU and Ze-neng CHENG contributed analytic tools; Xi LUO, Ning-fang CAI and Li-yun ZHENG analyzed the data; and Xi LUO and Ze-neng CHENG wrote the paper.

### Supplementary information

Supplementary Table S1 is available at *Acta Pharmacologica*

*Sinica's* website.

### References

- 1 Masuda S, Inui K. An up-date review on individualized dosage adjustment of calcineurin inhibitors in organ transplant patients. *Pharmacol Ther* 2006; 112: 184–98.
- 2 Hebert MF. Contributions of hepatic and intestinal metabolism and P-glycoprotein to cyclosporine and tacrolimus oral drug delivery. *Adv Drug Deliv Rev* 1997; 27: 201–14.
- 3 Feng Y, Zhang S, Poloyac S, Strom S, Venkataramanan R. Determination of 13-O-demethyl tacrolimus in human liver microsomal incubates using liquid chromatography–mass spectrometric assay (LC–MS). *J Chromatogr B Analyt Technol Biomed Life Sci* 2005; 821: 31–7.
- 4 Oleson L, von Moltke LL, Greenblatt DJ, Court MH. Identification of polymorphisms in the 3'-untranslated region of the human pregnane X receptor (PXR) gene associated with variability in cytochrome P450 3A (CYP3A) metabolism. *Xenobiotica* 2010; 40: 146–62.
- 5 Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, *et al*. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* 2001; 27: 383–91.
- 6 de Jonge H, de Looor H, Verbeke K, Vanrenterghem Y, Kuypers DR. *In vivo* CYP3A4 activity, CYP3A5 genotype, and hematocrit predict tacrolimus dose requirements and clearance in renal transplant patients. *Clin Pharmacol Ther* 2012; 92: 366–75.
- 7 Cascorbi I, Gerloff T, Johne A, Meisel C, Hoffmeyer S, Schwab M, *et al*. Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. *Clin Pharmacol Ther* 2001; 69: 169–74.
- 8 Galteau MM, Shamsa F. Urinary 6 $\beta$ -hydroxycortisol: a validated test for evaluating drug induction or drug inhibition mediated through CYP3A in humans and in animals. *Eur J Clin Pharmacol* 2003; 59: 713–33.
- 9 Peng CC, Templeton I, Thummel KE, Davis C, Kunze KL, Isoherranen N. Evaluation of 6 $\beta$ -hydroxycortisol, 6 $\beta$ -hydroxycortisone and their combination as endogenous probes for inhibition of CYP3A4 *in vivo*. *Clin Pharmacol Ther* 2011; 89: 888–95.
- 10 Luo X, Li XM, Hu ZY, Cheng ZN. Evaluation of CYP3A activity in humans using three different parameters based on endogenous cortisol metabolism. *Acta Pharmacol Sin* 2009; 30: 1323–9.
- 11 Zheng L, Luo X, Zhu L, Xie W, Liu S, Cheng Z. Simultaneous determination of cortisol, cortisone, 6 $\beta$ -hydroxycortisol and 6 $\beta$ -hydroxycortisone by HPLC. *J Chromatogr Sci* 2015; 53: 451–5.
- 12 Katz DA, Grimm DR, Cassar SC, Gentile MC, Ye X, Rieser MJ, *et al*. CYP3A5 genotype has a dose-dependent effect on ABT-773 plasma levels. *Clin Pharmacol Ther* 2004; 75: 516–28.
- 13 Renders L, Frisman M, Ufer M, Mosyagin I, Haenisch S, Ott U, *et al*. CYP3A5 genotype markedly influences the pharmacokinetics of tacrolimus and sirolimus in kidney transplant recipients. *Clin Pharmacol Ther* 2007; 81: 228–34.
- 14 Anglicheau D, Flamant M, Schlageter MH, Martinez F, Cassinat B, Beaune P, *et al*. Pharmacokinetic interaction between corticosteroids and tacrolimus after renal transplantation. *Nephrol Dial Transplant* 2003; 18: 2409–14.