



# Effect of pregnane X receptor polymorphisms on tacrolimus blood concentrations and the resulting adverse reactions in kidney transplantation recipients

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**ABSTRACT.** We investigated the effect of pregnane X receptor (PXR) polymorphisms on tacrolimus (FK506) blood trough concentrations and the associated adverse reactions in kidney transplantation recipients (KTRs). Polymerase chain reaction (PCR)-restriction fragment length polymorphism was used to detect the genotypes of single nucleotide polymorphism loci in 336 KTRs. The PXR six-base deletion mutation was classified using specific allele PCR, and the FK506 blood trough concentration in the KTRs was measured by chemiluminescent microparticle immunoassay. There were significant differences in adverse reactions resulting from FK506 in age, weight, body mass index (BMI) and treatment course ( $P < 0.05$ ). Logistical regression

revealed that the FK506 treatment course and BMI were risk factors for hyperlipidemia, and the risk of hyperlipidemia increased 27.534 times when the BMI was less than 18.5. Moreover, age was also a risk factor leading to hyperglycemia. FK506 blood trough concentration and  $C_0/D$  value had an impact on adverse reactions induced by hyperglycemia. The KTRs' PXR rs3842689, rs6785049, and rs1523127 mutation frequencies were 26.07, 11.79, and 16.07%, respectively. There was no statistically significant difference in the mutation frequency of each locus between the control group and the adverse reaction groups. Therefore, rs3842689, 7635G>A (rs6785049), and 24381C>A (rs1523127) PXR polymorphisms have no obvious impact on FK506; furthermore, the PXR rs3842689 wild-type homozygous WW genotype is a risk factor of FK506 and results in gastrointestinal reactions.

**Key words:** Pregnane X receptor; Tacrolimus; Kidney transplantation; Adverse reaction; Gene polymorphism

## INTRODUCTION

Tacrolimus (FK506) metabolizes via the liver and intestinal tract, and is a substrate of P-glycoprotein. To date, research on FK506 pharmacogenetics has mainly focused on CYP3A4, CYP3A5, and MDR1 gene polymorphisms (Hesselink et al., 2003; Wang et al., 2015a,b). Pregnane X receptor (PXR), a new-found orphan nuclear receptor, expresses in the liver and partially expresses in the colon and small intestine; it participates in the regulation of substance absorption and metabolism. Miura et al. (2008) reported that the prednisolone area under the curve in PXR 7635 G allele carriers decreases significantly; the PXR A7635G gene polymorphism can also affect the FK506 clearance rate, and the clearance rates in A allele carrier and GG genotype patients are  $3.9 \pm 0.3$  and  $5.4 \pm 0.6$  L/h, respectively (Press et al., 2009). PXR C25385T is effective in affecting FK506 apparent clearance (Cl/F) (Benkali et al., 2009). However, Elens et al. (2007) did not discover pharmacokinetic correlations between A11156C and T11193C and FK506 in liver transplantation recipients.

Therefore, the PXR gene polymorphism may play an important role in FK506 metabolism. However, race has an effect on individual differences in drug metabolism, curative effects, and adverse reactions; consequently, whether the unknown single nucleotide polymorphism affects FK506 pharmacokinetics in the Chinese population requires further investigation (Fei et al., 2006; Qi et al., 2012; Yuan et al., 2012). In this study, we examined the correlation between PXR gene polymorphisms and FK506 blood trough concentration, and investigated susceptible genetic factors that lead to adverse reactions after kidney transplantation.

## MATERIAL AND METHODS

### Research objects

We selected a total of 336 kidney transplantation recipients (KTRs) (aged 18 to 78 years) who had received transplants in the Zhengzhou People's Hospital between 1998 and 2014, and who were devoid of other serious diseases. The KTRs were willing to take part in

the experiment and signed written informed consent. Patients who had failed to follow-up, had received multi-organ transplantation, had suffered from liver or kidney injury before medication, or had been diagnosed with tuberculosis or hepatitis B/C were excluded from this study. This experiment was approved by the Ethics Committee of Zhengzhou People's Hospital.

### **Dosage regimen**

A trigeminy immunosuppression regimen (FK506 + mycophenolate mofetil (MMF) + prednisone) was administered. Patients were first given 0.15-0.3 mg/kg immunosuppression therapy and then MMF by oral administration on an empty stomach (1.5-2.0 g/day). The dosage was then gradually decreased to 0.5-1 g/day. During the operation, the patients were treated with methylprednisolone by intravenous drip (300 mg/day). Three days after the operation, the patients took prednisone orally (40 mg/day), and the dosage was reduced to 30 mg/day on the 7th day, followed by 5-10 mg/day to maintain the treatment.

### **Observational indices**

The subjects were asked to provide data on gender, age, stature, weight, body mass index, transplantation time, time of dialysis, cadaveric kidney donation or related renal transplantation, and history of diabetes, hypertension, or hepatitis B/C infection. Daily oral dosages of FK506 at different time points, blood trough concentrations, prednisone and MMF dosages, drug combination data, hepatic and renal function, fasting plasma glucose, blood routine, blood lipid, blood pressure, and other inspection results were also recorded.

### **Measurement of FK506 blood concentration**

#### ***Sample preparation and measurement***

Venous blood (2 mL) was taken from transplant recipients during the morning following medication administration. After ethylenediaminetetraacetic acid (EDTA) anticoagulation, 200  $\mu$ L blood was mixed for 5 s and moved into a centrifuge tube with a pipette. The precipitant (200  $\mu$ L) was added, fully mixed for 30 s, and centrifuged for 5 min at 13,000 rpm. The supernatant was added to an EDTA glass tube and mixed for 2 s, then subjected to fully automated immunochemistry analysis to measure FK506 trough concentration ( $C_0$ ) by chemiluminescence microparticle immunoassay.

#### ***Quality control***

Three standard substances (3, 6, and 12 ng/mL) were measured once per week in high, medium, and low order, over a 3-month cycle for quality control. All quality control values were found to be in an effective range, suggesting that the FK506 concentration values of samples were reliable.

### **DNA extraction and measurement**

DNA was extracted from peripheral venous blood from the KTRs per the manufacturer

instructions (WIZARD Genomic DNA purification kit, Beijing Solarbio Bioscience & Technology Co., Ltd., Beijing, China) for polymerase chain reaction (PCR). Allele-specific PCR was applied to classify the PXR six-base deletion (rs3842689) mutation and the direct sequencing method was used to investigate the genotypes of PXR 7635G>A (rs6785049) and PXR 24381C>A (rs1523127).

### Measurement methods

The PXR six-base deletion gene was detected using the allelic-special touchdown PCR method. Each DNA sample was configured with two tubes: one with a normal upstream primer (F1) and the other with a mutant upstream primer (F2) (Yang et al., 2010). They were then reacted in a PCR system (25  $\mu$ L) containing 2X PCR Master Mix (12.5  $\mu$ L) comprising 50 U/mL Taq DNA polymerase, 400  $\mu$ M deoxynucleoside triphosphates, 3 mM MgCl<sub>2</sub>, 1  $\mu$ L upstream and downstream primers (10  $\mu$ M), 2  $\mu$ L DNA template, and 8.5  $\mu$ L nuclease-free water. The PCR regimen was as follows: pre-denaturation at 94°C for 5 min; 20 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 30 s, in which the annealing temperature decreased by 0.5°C per cycle; 20 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 30 s; and a final extension at 72°C for 5 min. After the paired PCR amplification products had been electrophoresed on 2.5% agarose gel, the results were analyzed using an ultraviolet gel imaging system.

The PXR 24381C>A mutation gene was reacted using the same PCR system (25  $\mu$ L), and the PCR products were treated in the same way for direct sequencing (de Jonge et al., 2008).

### Statistical analysis

All data were processed using the SPSS19.0 software and are reported as means  $\pm$  standard deviation (SD). Differences were considered to be statistically significant if  $P < 0.05$ . The *t*-test was used to compare the differences in clinical data between each group, and non-normal distribution variables were transformed logarithmically. Enumeration data were subjected to the chi-square test and are reported as N (%). Logistical regression was applied to analyze risk factors of adverse reactions resulting from FK506.

## RESULTS

### PCR amplification primer sequences

The PCR primer sequences and the lengths of the amplification products are shown in Table 1.

### Comparison of general clinical data from patients with different PXR six-base deletion (rs3842689) genotypes

The general clinical data for the 336 KTRs were compared (Table 2), and no statistical differences were found ( $P > 0.05$ ).

**Table 1.** Polymerase chain reaction (PCR) amplification primer sequences and lengths of the amplification products.

	Name of primer	Primer sequence	Length of amplification products
rs3842689	6-base deletion-F1	5'-AATCACCACAGGAGAAGC-3'	163 bp
	6-base deletion-F2	5'-AAACACCACAGCCTTAAC-3'	169 bp
	6-base deletion-R	5'-AAAAGGAATCAGGACTGAGT-3'	
rs6785049	7635G>A	5'-TAGGTCAGGATTGTATAGGAGC-3'	
	7635G>A	5'-CCATCCCAGAACCTGCATATC-3'	
rs1523127	24381C>A	5'-CCCCTTTTCCTGTGTTTTTG-3'	
	24381C>A	5'-CAACATTAAGTGATTGTTTCATGC-3'	

**Table 2.** Comparison of clinical data for patients with different PXR rs3842689 genotypes.

	WW (N = 212)	MM (N = 52)	WM (N = 72)
Cadaveric kidney (%)	161 (75.94%)	43 (82.69%)	58 (80.56%)
Male/female	137/75	40/12	48/24
Age at transplant (years)	40.35 ± 10.09	40.58 ± 12.13	38.89 ± 10.34
Time of dialysis (months)	10.84 ± 12.07	11.60 ± 10.86	10.01 ± 8.55
Transplant weight (kg)	57.24 ± 9.76	56.52 ± 9.16	57.87 ± 12.30
BMI (kg/m <sup>2</sup> )	20.58 ± 2.78	20.47 ± 2.88	21.03 ± 2.96
Treatment course (months)	38.82 ± 25.41	36.04 ± 22.21	33.17 ± 21.22

BMI = body mass index.

### Influence of PXR six-base deletion (rs3842689) gene polymorphism on FK506 blood trough concentration and $C_0/D$ value

Table 3 and Figures 1 and 2 show the influence of the PXR rs3842689 gene polymorphism on FK506 blood trough concentration and the  $C_0/D$  value. At different time points after drug administration, homozygous mutation MM gene carriers had a higher blood trough concentration than wild-type homozygous WW gene carriers, but the difference was not statistically significant ( $P > 0.05$ ).

### Results of PXR 7635G>A (rs6785049) and PXR 24381C>A (rs1523127) genotyping

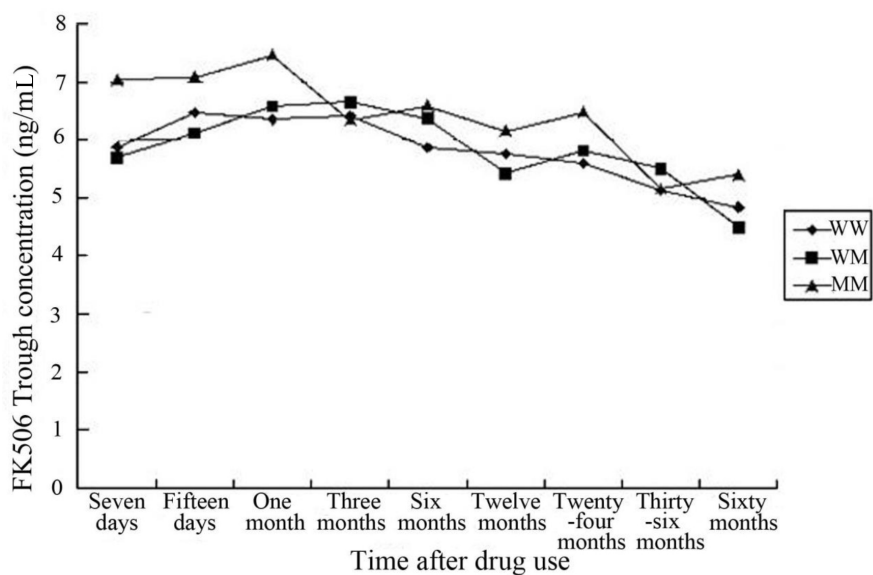
The included KTRs were subjected to PXR rs6785049 and rs1523127 genotype detection and the results are shown in Table 4. Of the 336 included patients, 39 had the rs6785049 mutation, with a mutation frequency of 11.61%, and 55 had the rs1523127 mutation, with a mutation frequency of 16.37%.

### Influence of the PXR 7635G>A (rs6785049) gene polymorphism on FK506 blood trough concentration and $C_0/D$ value

Table 5 shows the influence of the PXR 7635G>A (rs6785049) gene polymorphism on FK506 blood trough concentration and  $C_0/D$  value. The PXR 7635G>A (rs6785049) gene polymorphism did not have any obvious influence on FK506 blood trough concentration or  $C_0/D$  value at the different time points after drug administration ( $P > 0.05$ ).

**Table 3.** Influence of the PXR six-base deletion gene polymorphism on FK506 blood trough concentration and  $C_0/D$  (means  $\pm$  SD).

Time after drug use	PXR rs3842689 Genotype	Daily dosage (mg/kg)	Blood trough concentration (ng/mL)	$C_0/D$ (ng·kg·mL <sup>-1</sup> ·mg <sup>-1</sup> )
Seven days	WW	0.06 $\pm$ 0.02	5.88 $\pm$ 3.45	103.26 $\pm$ 75.81
	MM	0.06 $\pm$ 0.02	7.04 $\pm$ 3.71	123.72 $\pm$ 69.12
	WM	0.08 $\pm$ 0.04	5.70 $\pm$ 3.41	129.76 $\pm$ 74.18
Fifteen days	WW	0.07 $\pm$ 0.04	6.47 $\pm$ 3.29	113.28 $\pm$ 64.27
	MM	0.08 $\pm$ 0.02	7.08 $\pm$ 2.75	104.84 $\pm$ 71.40
	WM	0.07 $\pm$ 0.04	6.12 $\pm$ 2.52	87.49 $\pm$ 67.56
One month	WW	0.07 $\pm$ 0.04	6.35 $\pm$ 2.74	110.47 $\pm$ 73.43
	MM	0.07 $\pm$ 0.04	7.47 $\pm$ 2.87	118.45 $\pm$ 81.76
	WM	0.09 $\pm$ 0.04	6.58 $\pm$ 3.09	96.39 $\pm$ 75.48
Three months	WW	0.06 $\pm$ 0.03	6.42 $\pm$ 2.64	135.07 $\pm$ 85.31
	MM	0.06 $\pm$ 0.03	6.34 $\pm$ 2.60	110.96 $\pm$ 57.23
	WM	0.07 $\pm$ 0.04	6.65 $\pm$ 2.63	117.60 $\pm$ 83.85
Six months	WW	0.05 $\pm$ 0.03	5.87 $\pm$ 2.81	144.88 $\pm$ 103.34
	MM	0.06 $\pm$ 0.03	6.59 $\pm$ 2.35	124.74 $\pm$ 76.38
	WM	0.06 $\pm$ 0.03	6.37 $\pm$ 2.69	125.17 $\pm$ 76.50
Twelve months	WW	0.06 $\pm$ 0.03	5.76 $\pm$ 2.30	147.37 $\pm$ 86.91
	MM	0.06 $\pm$ 0.03	6.15 $\pm$ 2.16	111.03 $\pm$ 51.24
	WM	0.06 $\pm$ 0.03	5.42 $\pm$ 1.89	100.99 $\pm$ 51.52
Twenty-four months	WW	0.05 $\pm$ 0.03	5.60 $\pm$ 2.31	147.86 $\pm$ 97.78
	MM	0.05 $\pm$ 0.03	6.48 $\pm$ 4.11	130.31 $\pm$ 85.65
	WM	0.05 $\pm$ 0.03	5.82 $\pm$ 4.05	129.67 $\pm$ 44.26
Thirty-six months	WW	0.05 $\pm$ 0.03	5.13 $\pm$ 1.87	154.22 $\pm$ 102.13
	MM	0.06 $\pm$ 0.02	5.16 $\pm$ 1.86	87.44 $\pm$ 28.83
	WM	0.04 $\pm$ 0.03	5.51 $\pm$ 3.04	107.20 $\pm$ 46.89
Sixty months	WW	0.04 $\pm$ 0.03	4.84 $\pm$ 1.71	193.66 $\pm$ 187.00
	MM	0.04 $\pm$ 0.03	5.40 $\pm$ 2.11	134.27 $\pm$ 50.43
	WM	0.04 $\pm$ 0.03	4.49 $\pm$ 1.47	123.71 $\pm$ 23.94

**Figure 1.** Influence of medication time on FK506 blood trough concentration.

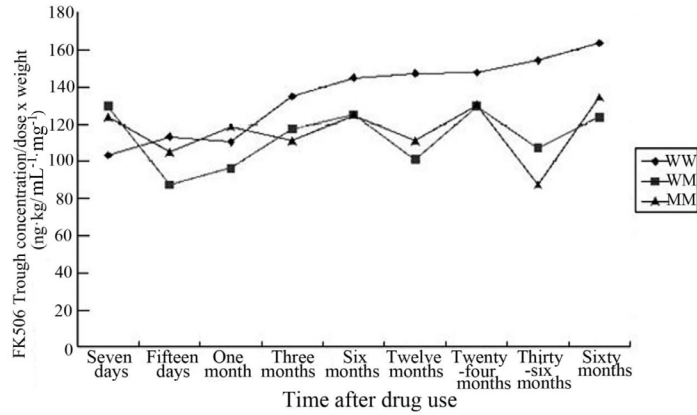


Figure 2. Influence of medication time on C<sub>0</sub>/D.

Table 4. PXR rs6785049 and rs1523127 mutation frequencies.

Genotypes	Number of cases with mutation	Mutation frequency
PXR 7635G>A (rs6785049)	39	11.61%
PXR 24381C>A (rs1523127)	55	16.37%

Table 5. Influence of the PXR 7635G>A (rs6785049) gene polymorphism on FK506 blood trough concentration and C<sub>0</sub>/D value (means ± SD).

Time after drug use	7635G>A genotype	Daily dosage (mg/kg)	Blood trough concentration (ng/mL)	C <sub>0</sub> /D (ng kg <sup>-1</sup> mL <sup>-1</sup> mg <sup>-1</sup> )
Seven days	Mutant type	0.07 ± 0.03	5.84 ± 3.98	103.67 ± 43.51
	Primitive type	0.06 ± 0.03	6.02 ± 3.42	110.29 ± 77.80
Fifteen days	Mutant type	0.07 ± 0.03	5.93 ± 3.11	102.79 ± 69.17
	Primitive type	0.07 ± 0.03	6.50 ± 3.06	107.45 ± 71.59
One month	Mutant type	0.08 ± 0.03	6.27 ± 2.57	88.34 ± 62.26
	Primitive type	0.07 ± 0.03	6.62 ± 2.89	113.42 ± 76.48
Three months	Mutant type	0.06 ± 0.03	6.91 ± 3.05	140.57 ± 80.39
	Primitive type	0.06 ± 0.03	6.37 ± 2.54	126.63 ± 82.53
Six months	Mutant type	0.06 ± 0.03	6.58 ± 2.48	159.80 ± 108.37
	Primitive type	0.05 ± 0.03	6.10 ± 2.73	134.07 ± 92.18
Twelve months	Mutant type	0.06 ± 0.03	5.54 ± 2.20	122.79 ± 58.96
	Primitive type	0.05 ± 0.03	5.80 ± 2.22	135.77 ± 82.81
Twenty-four months	Mutant type	0.05 ± 0.03	5.85 ± 3.29	154.00 ± 92.03
	Primitive type	0.05 ± 0.03	5.77 ± 2.96	140.27 ± 91.07
Thirty-six months	Mutant type	0.05 ± 0.04	4.64 ± 1.62	135.29 ± 81.25
	Primitive type	0.04 ± 0.02	5.32 ± 2.21	157.21 ± 100.27
Sixty months	Mutant type	0.05 ± 0.05	5.06 ± 1.44	187.64 ± 133.86
	Primitive type	0.04 ± 0.02	4.87 ± 1.81	212.04 ± 210.73

### Influence of the PXR 24381C>A (rs1523127) gene polymorphism on FK506 blood trough concentration and C<sub>0</sub>/D value

Table 6 shows the influence of the PXR 24381C>A (rs1523127) gene polymorphism on FK506 blood trough concentration and C<sub>0</sub>/D value. After 3 months of medication, the blood

trough concentration was higher in the mutant individuals than in the wild-type individuals ( $P = 0.030$ ), and the mutant individuals had a markedly higher  $C_0/D$  value than the wild-type individuals did after 6 months of medication ( $P = 0.009$ ). There were no significant differences in blood trough concentration or  $C_0/D$  value at the other time points ( $P > 0.05$ ).

**Table 6.** Influence of the PXR rs1523127 gene polymorphism on FK506 blood trough concentration and  $C_0/D$  (means  $\pm$  SD).

Time after drug use	24381C>A genotype	Daily dosage (mg/kg)	Blood trough concentration (ng/mL)	$C_0/D$ (ng·kg·mL <sup>-1</sup> ·mg <sup>-1</sup> )
Seven days	Mutant type	0.07 $\pm$ 0.03	6.37 $\pm$ 3.48	127.42 $\pm$ 84.06
	Primitive type	0.06 $\pm$ 0.03	5.95 $\pm$ 3.49	107.24 $\pm$ 73.85
Fifteen days	Mutant type	0.07 $\pm$ 0.03	6.41 $\pm$ 2.96	107.37 $\pm$ 81.99
	Primitive type	0.07 $\pm$ 0.03	6.50 $\pm$ 3.11	106.55 $\pm$ 69.32
One month	Mutant type	0.07 $\pm$ 0.03	6.48 $\pm$ 2.93	104.28 $\pm$ 73.90
	Primitive type	0.07 $\pm$ 0.03	6.57 $\pm$ 2.84	109.28 $\pm$ 75.06
Three months	Mutant type	0.06 $\pm$ 0.03	6.77 $\pm$ 3.34	146.71 $\pm$ 89.42
	Primitive type	0.06 $\pm$ 0.03	6.40 $\pm$ 2.41	125.70 $\pm$ 80.52
Six months	Mutant type	0.06 $\pm$ 0.03	6.75 $\pm$ 2.71	184.20 $\pm$ 142.69
	Primitive type	0.05 $\pm$ 0.03	6.00 $\pm$ 2.73	129.83 $\pm$ 81.95
Twelve months	Mutant type	0.05 $\pm$ 0.03	6.18 $\pm$ 2.32	155.87 $\pm$ 85.56
	Primitive type	0.05 $\pm$ 0.03	5.63 $\pm$ 2.19	129.10 $\pm$ 77.88
Twenty-four months	Mutant type	0.04 $\pm$ 0.03	5.62 $\pm$ 4.23	152.74 $\pm$ 64.61
	Primitive type	0.05 $\pm$ 0.03	5.18 $\pm$ 2.20	141.61 $\pm$ 94.17
Thirty-six months	Mutant type	0.03 $\pm$ 0.03	5.22 $\pm$ 1.51	193.75 $\pm$ 88.97
	Primitive type	0.05 $\pm$ 0.03	5.23 $\pm$ 2.26	148.22 $\pm$ 97.14
Sixty months	Mutant type	0.02 $\pm$ 0.01	4.35 $\pm$ 1.62	212.90 $\pm$ 95.66
	Primitive type	0.05 $\pm$ 0.03	4.83 $\pm$ 1.77	202.83 $\pm$ 217.40

### Distribution of the PXR gene polymorphism in the control group and the groups with different adverse reactions

The data for the PXR gene polymorphism are shown in Table 7. The PXR rs3842689 and rs6785049 genotypes were distributed in each group without statistical significance ( $\chi^2 = 8.364$ ,  $P = 0.756$ ;  $\chi^2 = 6.273$ ,  $P = 0.393$ ). There were statistically significant differences in PXR rs1523127 genotype distribution between the various groups ( $\chi^2 = 13.126$ ,  $P = 0.041$ ) and the PXR rs1523127 mutation increased in the hyperlipidemia group in comparison with the control group.

**Table 7.** Relationship between the PXR genotype and adverse reactions resulting from FK506.

	Control group (203 cases)	Liver injury group (8 cases)	Hyperglycemia group (34 cases)	Hematotoxicity group (32 cases)	Hyperlipidemia group (30 cases)	Hypertension group (14 cases)	Gastrointestinal reaction group (15 cases)	Total adverse reaction (133 cases)
rs3842689 Primitive type	126 (62.07%)	8 (100%)	21 (61.76%)	20 (62.50%)	21 (70%)	9 (64.29%)	8 (53.33%)	87 (65.41%)
Mutation homozygote	32 (15.76%)	0 (0%)	6 (17.65%)	6 (18.75%)	3 (10%)	0 (0%)	4 (26.67%)	19 (14.28%)
Mutation heterozygote	45 (22.17%)	0 (0%)	7 (20.59%)	6 (18.75%)	6 (20%)	5 (35.71%)	3 (20%)	27 (20.31%)
rs6785049 Primitive type	175 (86.21%)	8 (100%)	32 (94.12%)	28 (87.50%)	29 (96.67%)	10 (71.43%)	14 (93.33%)	121 (90.98%)
Mutant type	28 (13.79%)	0 (0%)	2 (5.88%)	4 (12.50%)	1 (3.33%)	4 (28.57%)	1 (6.67%)	12 (9.02%)
rs1523127 Primitive type	171 (84.24%)	7 (87.50%)	29 (85.29%)	31 (96.88%)	20 (66.67%)	10 (71.43%)	15 (100%)	111 (83.46)
Mutant type	32 (15.76%)	1 (12.50%)	5 (14.71%)	1 (3.12%)	10 (33.33%)	4 (28.57%)	0 (0%)	22 (16.54)



## Logistical regression

According to the regression results, the rs6785049 and rs1523127 genotypes had no obvious correlation with adverse reactions resulting from FK506 ( $P > 0.05$ ). Logistical regression revealed that PXR rs3842689 patients with the wild-type homozygote WW genotype were 15.8 times more likely to suffer from gastrointestinal reaction after medication compared with mutation homozygote MM genotype individuals.

## DISCUSSION

Because it is a key transcriptional regulation factor, PXR participates in the inducible expression of a variety of drug metabolic enzymes and transport proteins, there by indirectly affecting FK506 drug metabolism and transport. At present, international research on NR1I2 gene polymorphisms focuses only on African Americans and Caucasians, instead of Asians or Chinese. Moreover, NR1I2 mutations differ greatly between the races (Tsuchiya et al., 2004; Wang et al., 2007; Swart and Dandara, 2014).

Uno et al. (2003) first discovered the NR1I2 promoter region six-base pair deletion mutation (rs3842689, -/GAGAAG), which occurs in a potential liver nuclear factor (HNF-1) binding site. Research indicated that the Hpar-2 promoter activity of HepG2 cells was essentially lost with the six-base deletion mutation, and the PXR six-base deletion mutation caused a decrease in PXR mRNA expression, there by significantly reducing the expression of its target genes *CYP3A4* and *MDR1* (Liu et al., 2009). Lamba et al. (2008) also discovered an obvious decrease in *CYP3A4* expression resulting from six-base deletions under base-induced conditions. Of the included 336 KTRs, the frequency of the PXR six-base deletion mutation was 26.07%. Homozygous MM gene carriers had a higher blood trough concentration than native homozygous WW gene carriers at the different time points after medication, but the differences were not statistically significant. Compared with homozygous MM genotype individuals, the risk of native homozygous WW genotype patients developing gastrointestinal reactions increased 15.8 times after medication (95%CI = 10.07-24.80;  $P = 0.000$ ). PXR G7635A is mainly associated with *CYP3A4* in the intestinal tract (Zhang et al., 2001). Press et al. (2009) found that gene polymorphism in the locus affected the clearance rate of FK506 and the clearance rates of A allele gene carriers and GG genotype patients were  $3.9 \pm 0.3$  and  $5.4 \pm 0.6$  L/h, respectively. Applying the direct sequencing PCR method, Wang et al. (2013) carried out PXR-C24381A genotyping on 60 patients who were given an oral administration of FK506 after renal transplantation, and the results suggested that the  $C_0/D$  value in the C/A and C/C genotype patients was higher than in the A/A genotype patients, which suggested that the A/A genotype patients required a higher dosage of FK506 than the C/A and C/C genotype patients to obtain a similar blood trough concentration. Moreover, the PXR six-base deletion (rs3842689), and the PXR 7635G>A (rs6785049) and PXR 24381C>A (rs1523127) gene polymorphisms had no influence on FK506 blood trough concentration or  $C_0/D$  value. Logistical regression revealed that the PXR rs3842689 native homozygous WW genotype was a risk factor that resulted in gastrointestinal reactions but the other genotypes had no significant effects on adverse reactions resulting from FK506.

There are some caveats in this study, which could not be addressed owing to certain limitations with respect to experimental conditions; currently, we are in the process of making the necessary improvements to rectify these limitations.

This study aimed to investigate the effect of PXR polymorphisms on tacrolimus blood concentrations and the resulting adverse reactions in KTRs to provide a reliable basis for further studies.

### Conflicts of interest

The authors declare no conflict of interest.

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