# Chapter 11

# Pregnane X receptor (PXR) polymorphisms involved in the tacrolimus pharmacokinetics

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## Abstract

Background and aim

Cytochrome P450 (CYP) 3A4/5 are responsible for metabolizing tacrolimus while the P-glycoprotein pump (ABCB1) modulates its bioavailability. Differences in gene expression can not be fully explained by CYP3A4/5 and/or ABCB1 polymorphisms. The pregnane X receptor (PXR) is a transcriptional regulator of the CYP450 and the ABCB1 expression.

Materials and methods

Real-time PCR FRET assays determine the genotypes of four PXR polymorphisms in 103 Chinese renal transplant recipients to examine its influence on dose-normalized $AUC_{0\text{-}12}$ ($dnAUC_{0\text{-}12}$).

Results

Individuals carrying a variant allele for PXR polymorphisms A7635G, C8055T, A11156C and T11193C, associated with increased expression of CYP3A4 or ABCB1, had lower $dnAUC_{0\text{-}12}$ for tacrolimus compared to individuals carrying the wild type variant for these PXR polymorphisms (respectively, Mann-Whitney; $P = 0.038$, $P = 0.088$, $P = 0.038$ and $P = 0.038$).

Conclusion

These PXR polymorphisms are associated with a trend towards a lower $dnAUC_{0\text{-}12}$ of tacrolimus which confirms a role of PXR in the CYP3A and ABCB1 expression and thereby on the tacrolimus metabolism.

## Introduction

Tacrolimus has a narrow therapeutic index which requires close monitoring of the drug concentration to achieve an optimal efficiency and minimizing the risks of acute rejection and drug overdose. The polymorphisms present in the cytochrome P450 3A5 (CYP3A5) iso-enzyme and adenosine triphosphate-binding cassette gene B1 (ABCB1) can partly explain their variability in expression and activity and thus clarify to a certain extend the interindividual differences in the tacrolimus pharmacokinetics. However, cytochrome P450 3A4 (CYP3A4) expression shows a large inter-individual variation that can not be explained solely by polymorphisms identified in this gene[1]. The pregnane X receptor (PXR)[2] also known as SXR[3], hPAR[4] or NR1I2 has been identified as a transcriptional regulator of CYP3A4 [2,4] and ABCB1 [5,6]. PXR binds as a heterodimer with the 9-*cis* retinoic acid receptor (RXR; NR2B) to previously characterised xenobiotic response elements in CYP3A gene promoters, and importantly, PXR is activated by the spectrum of chemicals that are known to induce CYP3A gene expression[7,8]. Single nucleotide polymorphisms (SNPs) in the PXR gene can influence the PXR activity and thereby the CYP3A4 and ABCB1 expression. Zhang *et al.*[9] identified 38 different polymorphisms in the PXR gene and determined their allelic frequency in a Caucasian (n = 75) and African American (n = 11) population. Two other studies also screened the PXR gene for polymorphisms and determined their allele frequency in a Caucasian population[10,11]. The polymorphisms A7635G and C8055T located in correspondingly intron 5 and intron 6 of the PXR gene have a high allelic frequency in two different Caucasian populations. Bosch *et al.*[10] reported that 32% and 15% of the Caucasians were carrier of, respectively, PXR 7635G and 8055T variant alleles while Zhang *et al.*[9] found that 35% and 16% of the Caucasians was carrier of correspondingly PXR 7635G and 8055T variant alleles. In addition, Zhang *et al.*[9] observed that 16% of the Caucasians was carrier of the 11156C or 11193C variant allele which is located in the 3'-untranslated region of the PXR gene. The PXR polymorphisms A7635G and C8055T have been shown to have a higher magnitude of induction of the intestinal CYP3A4 by rifampicin while the homozygous carriers of the PXR 11156C or 11193C variant alleles had lower P-glycoprotein levels in gut biopsies compared to those with homozygous 11156A and 11193T alleles[9]. In the present study, a 12 hour area under the time concentration curve ($AUC_{0-12}$) calculated with a two time point sample strategy is correlated with the corresponding PXR genotypes for 103 Chinese renal transplant patients to elucidate whether these PXR polymorphisms can contribute to a decrease within the inter-individual variability of the tacrolimus pharmacokinetics.

# Patients and methods

## Study population

A total of 103 Chinese renal transplant recipients who received tacrolimus as part of the immunosuppressive therapy and had regular follow up in the Queen Elizabeth Hospital or Tuen Mun Hospital in Hong Kong were included in this retrospective study. There was no change in the daily tacrolimus dose for at least two weeks. Patients who were taking medication known to have interaction with tacrolimus, such as calcium channel blockers, anti-epileptics, anti-mycotics and macrolide antibiotics were excluded from this study. Additionally, patients who suffered from gastrointestinal tract disease, liver disease or other disorders that may alter the absorption of tacrolimus were also excluded. Apart from tacrolimus and steroids, the patients were normally put on azathioprine, however some patients preferred to use mycophenolic acid on advice of their private physician. The dosage of azathioprine was 1.5 mg/kg/day, while the dosage of mycophenolic acid was 0.5 gram twice daily. The initial tacrolimus dosage, administrated twice daily, was 0.3 mg/kg per day for all patients. The daily tacrolimus dose was then adjusted according to the $AUC_{0\text{-}12}$ value, which was kept at around 100-150 ng $\times$ hr/ml during the first three months. After three months the target $AUC_{0\text{-}12}$ value was decreased at around 80-100 ng $\times$ hr/ml for long term maintenance. These $AUC_{0\text{-}12}$ values were based on our previous pilot study[12] and the experiences obtained in our transplantation centers. The steroid regimen for the first month was 30 mg/day of oral prednisolone, progressively tapered by 2.5 mg every two weeks until a daily maintenance dose of 5 mg. Demographic as well as clinical data were collected at the time of the tacrolimus blood sample collection.

## Determination whole blood tacrolimus concentrations

Blood samples were collected 2 (C2) and 4 (C4) hours after the morning tacrolimus administration. Tacrolimus blood concentrations were determined in ethylene diamine tetra-acetic acid (EDTA) whole blood using a semi-automated microparticle enzyme immunoassay (MEIA) on an IMx II clinical analyser (Abbott Laboratories, Abbott Park, IL, USA). The two tacrolimus blood concentrations determined were used to calculate the $AUC_{0\text{-}12}$ according to the equation based strategy as described earlier[12]: $AUC_{0\text{-}12}$ = 16.2 + 2.4 $\times$ C2 + 5.9 $\times$ C4. Dose-normalised AUC ($dnAUC_{0\text{-}12}$) were calculated by dividing the $AUC_{0\text{-}12}$ by the corresponding 24 hour dose on a milligrams per kilogram basis.

## DNA isolation

Genomic DNA was extracted from 103 Chinese renal transplant recipients by using 200 μl EDTA anticoagulated blood for isolation with a QIAamp blood mini kit (Qiagen, Leusden, the Netherlands) according to the manufacturers' instructions.

## Primers and probes for the PXR A11156C and T11193C polymorphisms

The real-time PCR fluorescence resonance energy transfer (FRET) assays were designed and optimised in our laboratory. Genotyping for the PXR A11156C and T11193C polymorphisms was performed using the primers F22: 5'-CAA TCA gTT AAA CAC ACC ggA g-'3 (sense; 81370-391) and R22: 5'-TTT TAT gTT CTT ACg CCg gAg T-'3 (anti-sense; 81766-745) to amplify a 397 bp part from the PXR gene which covers both A11156C and T11193C polymorphisms in the 3'-untranslated region (3' UTR) of the PXR gene (Genbank acces no: AF364606). Detection of the PXR A11156C polymorphism was carried out using the anchor probe 56A22: 5'-TTT Tgg gAA ATg TAg CCC Tgg gT-'3 (sense; 81553-575) which was labelled at the 5'-end with LCRed640 and phosphorylated at the 3'-end to block extension from the sensor probe 56P22AL: 5'-CAT TCC ACA CCT AXA gAA CTA-'3 (sense; 81532-551) which is complementary to the PXR 11156A polymorphism. This 3'-fluorescence labelled sensor probe, in which the polymorphic locked nucleic acid (LNA) nucleotide is underlined binds with a distance of one base 5' to the detection probe. A locked nucleic acid (indicated as XA in the sensor probe 56P22AL ) was necessary to discriminate both melting peaks[13]. Detection of the PXR T11193C polymorphism was carried out using the anchor probe 93A22: 5'-ACC CAg ggC TAC ATT TCC CAA AA-'3 (anti-sense; 81575-553) which was labelled at the 5'-end with LCRed640 and phosphorylated at the 3' end to block extension from the sensor probe 93P22: 5'-CTT TTg CCT TgA TTT ggC ATT A-'3 (anti-sense; 81598-577) which is complementary to the PXR 11193C polymorphism. This 3'-fluorescence labelled sensor probe, in which the polymorphic nucleotide is underlined binds with a distance of one base 5' to the detection probe. The PCR mixture for both real-time PCR FRET assays contained: 3.2 μl sterile water; 3.0 mM $MgCl_2$; 1.0 μl LC Faststart DNA Master Hybridization Probes (Roche Diagnostics GmbH, Mannheim, Germany); 0.5 μM of each primer and 0.2 μM of both sensor and anchor probe (TIB MOLBIOL, Berlin, Germany). After adding 1.0 μl containing 50-100 ng genomic DNA to the PCR mixture, the total volume was 10 μl. The PCR protocol included the following steps: denaturation for ten minutes at 95°C; 45 cycles at 95°C for ten seconds, 55°C for ten seconds and 72°C for 16 seconds. After amplification was completed, a melting curve was recorded by cooling to 45°C (20°C/second) holding at 45°C for one minute and then heating slowly to 70°C at 0.2°C/second.

## Primers and probes for the PXR A7635G and C8055T polymorphism

Additionally, a real-time PCR FRET assay was designed and optimised for the PXR A7635G polymorphism. Genotyping for the PXR A7635G polymorphism was performed using the primers F23: 5'-gAg CTg TCT gCT ggg TTg Tg-'3 (sense; 77928-974) and R23: 5'-ggT CCT CgA Tgg gCA AgT C-'3 (anti-sense; 78136-118) to amplify a 209 bp part from the PXR gene which covers the A7635G polymorphisms in intron 5 (Genbank

access no: AF364606). Detection of the PXR A7635G polymorphism was carried out using the anchor probe A23: 5'-ATT ATg ggA Tgg CTg CTg gTg CCg gC-'3 (sense; 78039-064) which was labelled at the 5'- end with LCRed640 and phosphorylated at the 3'- end to block extension from the sensor probe, P23: 5'-CCT CTC ACC CCC AAC TTC T-'3 which is complementary to the PXR 7635A polymorphism. This 3'-fluorescence labelled sensor probe, in which the polymorphic nucleotide is underlined binds with a distance of one base 5' to the detection probe. Finally, a real-time PCR FRET assay was designed, optimised and validated for the PXR C8055T polymorphism. Genotyping for the PXR C8055T polymorphism was performed using the primers F24: 5'-gCT ggA TTA AAg CAT gTA CTT CA-'3 (sense; 78312-330) and R24: 5'-ggC CTg CAT CAg CAC ATA CT-'3 (anti-sense; 78558-539) to amplify a 246 bp part from the PXR gene which covers the C8055T polymorphisms in intron 6 (Genbank access no: AF364606). Detection of the PXR C8055T polymorphism was carried out using the anchor probe A24: 5'-TCC ACA ggT ggC TTC CAg CAA CTT CTA CTg-'3 (sense; 78454-483) which was labelled at the 5'- end with LCRed705 and phosphorylated at the 3'- end to block extension from the sensor probe. P24: 5'-CTg CCC CTC CAT CCT gTT ACC-'3 which is complementary to the PXR 8055C polymorphism. This 3'-fluorescence labelled sensor probe, in which the polymorphic nucleotide is underlined binds with a distance of one base 5' to the detection probe. The PCR mixture for the real-time PCR FRET assays for the PXR A7635G and C8055T polymorphisms contained: 5.2 μl sterile water; 2.0 mM $MgCl_2$; 1.0 μl LC Faststart DNA Master Hybridization Probes (Roche Diagnostics GmbH, Mannheim, Germany); 0.6 μM of each primer and 0.2 μM of both sensor and anchor probe (TIB MOLBIOL, Berlin, Germany). After adding 1.0 μl containing 50-100 ng genomic DNA to the PCR mixture the total volume was 10 μl. The PCR protocol for the two PXR polymorphisms A7635G and C8055T included the following steps: denaturation for ten minutes at 95°C; 40 cycles at 95°C for ten seconds, 55°C for ten seconds and 72°C for ten seconds. After amplification was completed, a melting curve was recorded by heating to 95°C (20°C/second) holding at 95°C for 30 seconds; subsequently cooling to 40°C (20°C/second) holding at 40°C for one minute and then heating slowly to 75°C at 0.1°C/second. All real-time PCR and melting curve analyses were performed on a LightCycler (Roche Diagnostics, Almere, the Netherlands). Additionally, sequencing is performed on a capillary sequencer ABI Prism 3100 using the Bridge version 1.1 sequence kit (Both products from Applied Biosystems, Fostercity, USA) and is used for identifying the presence of the expected PXR polymorphisms. The sequenced samples are used as controls in the different real-time PCR FRET assays.

## Statistical analysis

The statistical analysis of the data was performed with use of the statistical software SPSS 11.0 for windows (Chicago, IL, USA). Patients' genotypes were used as categorical independent variables for analysis of continuous clinical and

pharmacological variables. To examine the population homogeneity of the patients, the genotype frequencies of the PXR polymorphisms were tested against Hardy-Weinberg equilibrium by the Pearson's goodness-of-fit test. For analysis of the daily dose of tacrolimus (mg/kg/day) and the $dnAUC_{0\text{-}12}$ (ng × hr/ml per mg/kg), groups were compared using non parametric statistical tests. To compare two groups we used the Mann-Whitney test, and to compare several groups, the Kruskal Wallis test. *P* values less than 0.05 were considered statistically significant. All values are expressed as median and range unless stated otherwise.

# Results

## Real-time PCR FRET assays

After analysing 103 DNA samples obtained from Chinese renal transplant recipients the melting point of the sensor probe for the PXR A11156C polymorphism was 58.7 ± 0.08°C (n = 113) when hybridised to the PXR 11156A allele and 51.9 ± 0.11°C (n = 93) when hybridised to the PXR 11156C allele, whereas for the PXR T11193C polymorphism, the melting point of the sensor probe was 56.4 ± 0.16°C (n = 113) when hybridised to the PXR 11193T allele and 61.3 ± 0.14°C (n = 93) when hybridised to the PXR 11193C allele. In addition, for the PXR A7635G polymorphism, the melting point of the sensor probe was 61.2 ± 0.19°C (n = 95) when hybridised to the PXR 7635A allele and 55.0 ± 0.11°C (n = 111) for the PXR 7635G allele, whereas for the PXR C8055T polymorphism, the melting point of the sensor probe was 64.7 ± 0.32°C (n = 116) when hybridised to the PXR 8055C allele and 58.1 ± 0.33°C (n = 90) when hybridised to the PXR 8055T allele. Heterozygote samples for all studied polymorphisms showed both melting peaks. The presence of the expected polymorphisms was confirmed by sequencing the different allelic variants of the PXR gene according to a direct sequence procedure on a capillary sequencer ABI Prism 3100 using the Bridge version 1.1 sequence kit (Both products from Applied Biosystems, Fostercity, USA).

## Distribution of allele frequencies

Table 11.1 illustrates the demographic characteristics of 103 Chinese renal transplant recipients included in the present study while Table 11.2 shows the distribution of the allele frequencies and genotypes of the PXR polymorphisms among Chinese transplant recipients examined in the present study. Moreover, Table 11.2 summarised the allele frequencies from Caucasians and African American individuals.

Table 11.1 Demographic characteristics of the renal transplant recipients

| Demographic characteristics | |
|---|---|
| Gender (male/female) | 58/45 |
| Age (years, mean ± SD, (range)) | 43.1 ± 11.1 (21-77) |
| Weight (kg, mean ± SD, (range)) | 62.5 ± 11.4 (37.4-87.8) |
| Primary kidney disease | |
| Glomerulonephritis | 57 |
| Chronic pyelonephritis | 1 |
| Diabetic nephropathy | 10 |
| Hypertensive nephropathy | 9 |
| Polycystic kidney disease | 2 |
| Obstructive Uropathy | 3 |
| Unknown | 18 |
| Other | 3 |
| Transplantation number | |
| First | 97 |
| Second | 6 |
| Tacrolimus dose (mg/kg body weight/day, mean ± SD, (range)) | 0.067 ± 0.032 (0.01-0.18) |
| Tacrolimus conc. 2 hour post dose (ng/ml, mean ± SD, (range)) | 13.5 ± 4.30 (5.7-29.4) |
| Tacrolimus conc. 4 hour post dose (ng/ml, mean ± SD, (range)) | 8.7 ± 2.60 (2.3-16.4) |
| $AUC_{0\text{-}12}$ (ng × hr/ml, mean, (range)) | 100.3 (47.8-183.5) |
| $DnAUC_{0\text{-}12}$ (ng × hr/ml per mg/kg body weight, mean, (range)) | 1940 (300-11125) |
| Use of azathiopurine/mycophenolate mofetil | 78/25 |
| Current steroid dose (mg, mean ± SD, (range)) | 6.98 ± 2.37 (4-25) |
| Time since transplantation (days, mean, (range)) | 977 (133-4982) |
| Haemoglobin (mmol/l, mean ± SD, (range) ref. ♂ 8.2-11.0, ♀ 7.3-9.7) | 12.8 ± 2.0 (8.5-19) |
| Haematocrit fraction (mean ± SD, (range) ref. ♂ 0.41-0.52, ♀ 0.36-0.48) | 0.38 ± 0.058 (0.25-0.57) |
| ALAT (U/l, mean ± SD, (range) ref. ♂ < 45, ♀ < 35) | 22 ± 18 (8-147) |
| Serum albumin (g/l, mean ± SD, (range) ref. 34-45) | 43 ± 3.2 (34-50) |
| Serum creatinine (µmol/l, mean ± SD, (range) ref. ♂ 71-110, ♀ 53-97) | 156 ± 76 (62-462) |

Ref. are the reference values applied in the clinical chemistry and haematology laboratories of the University Hospital in Maastricht. ♂ male, ♀ female.

In total, 79.6% (n = 82) of the Chinese renal transplant recipients have a similar haplotype for these four PXR polymorphisms. The allele frequencies of the Chinese renal transplant patients examined were found to be 45.1% (11156C and 11193C), 53.9% (7635G) and 43.7% (8055T) which is not in line with those published in previous studies[9,10]. However, these studies determined the allele frequencies in Caucasians or African Americans which may explain the differences. According to the Pearson's goodness-of-fit test the genotype frequencies of these PXR polymorphisms differed not significantly from the Hardy-Weinberg equilibrium ($P > 0.05$).

Table 11.2 Distribution allele frequencies/genotypes PXR polymorphisms.

| PXR Polymorphisms | Genotype n (%) | Allele frequency n (%) | Allele frequencies observed in other studies[9,10] |
|---|---|---|---|
| A7635G | AA: 20 (19.4%) | AA: 95 (46.1%) | AA: 65%[a], 23%[a], 32%[b] |
| | AG: 55 (53.4%) | | |
| | GG: 28 (27.2%) | GG: 111 (53.9%) | GG: 35%[a], 77%[a], 68%[b] |
| C8055T | CC: 33 (32.0%) | CC: 116 (56.3%) | CC: 85%[a], 82%[a], 84% |
| | CT: 50 (48.5%) | | |
| | TT: 20 (19.4%) | TT: 90 (43.7%) | TT: 15%[a], 18%[a], 16% |
| A11156C | AA: 31 (30.1%) | AA: 113 (54.9%) | AA: 84%[a], 67%[a] |
| | AC: 51 (49.5%) | | |
| | CC: 21 (20.4%) | CC: 93 (45.1%) | CC: 16%[a], 33%[a] |
| T11193C | TT: 31 (30.1%) | TT: 113 (54.9%) | TT: 84%[a], 67%[a] |
| | TC: 51 (49.5%) | | |
| | CC: 21 (20.4%) | CC: 93 (45.1%) | CC: 16%[a], 33%[a] |

[a] Zhang *et al.*[9] determined for 150 alleles originated from Caucasians and 22 alleles originated from African Americans the allele frequencies of these PXR polymorphisms. [b] Bosch *et al.*[10] determined for 200 alleles obtained from healthy Caucasian volunteers the allele frequencies of these PXR polymorphisms.

## Influence of the different PXR allelic variants on the pharmacokinetic tacrolimus profiles

Since identical genotype distributions were found among the Chinese renal transplant recipients for the PXR polymorphisms A11156C and T11193C, the difference observed between the $dnAUC_{0\text{-}12}$ was also the same. A significant higher $dnAUC_{0\text{-}12}$ (1864 *versus* 1334 ng × hr/ml per mg/kg; Mann-Whitney, $P = 0.038$ and 1918 *versus* 1342 ng × hr/ml per mg/kg; Mann-Whitney, $P = 0.038$) was observed for carriers of respectively the PXR 7635AA and 11156AA/11193TT genotypes compared to the PXR 7635GG and 11156CC/11193CC genotypes while a strong trend towards a lower $dnAUC_{0\text{-}12}$ (1882 *versus* 1390 ng × hr/ml per mg/kg; Mann-Whitney, $P = 0.088$) was observed between carriers of the PXR 8055CC genotype compared to carriers of the PXR 8055TT genotype. Additionally, similar results were obtained for the daily tacrolimus dose, a significantly higher daily tacrolimus dose (0.052 *versus* 0.070 mg/kg/day; Mann-Whitney, $P = 0.042$ and 0.051 *versus* 0.070 mg/kg/day; Mann-Whitney, $P = 0.061$) was observed for carriers of respectively the PXR 7635AA and 11156AA/11193TT genotypes compared to the PXR 7635GG and 11156CC/11193CC genotypes while a strong trend towards a higher daily tacrolimus dose (0.052 *versus* 0.070 mg/kg/day; Mann-Whitney, $P = 0.108$) was observed between carriers of the PXR 8055CC genotype compared to carriers of the PXR 8055TT genotype. Figure 11.1A-D illustrate the effect of the different PXR polymorphisms on the $dnAUC_{0\text{-}12}$ recorded in 103 Chinese renal transplant recipients. Furthermore, the PXR haplotypes consisting of the four polymorphisms are associated with the $dnAUC_{0\text{-}12}$, as is shown in Figure 11.1E.

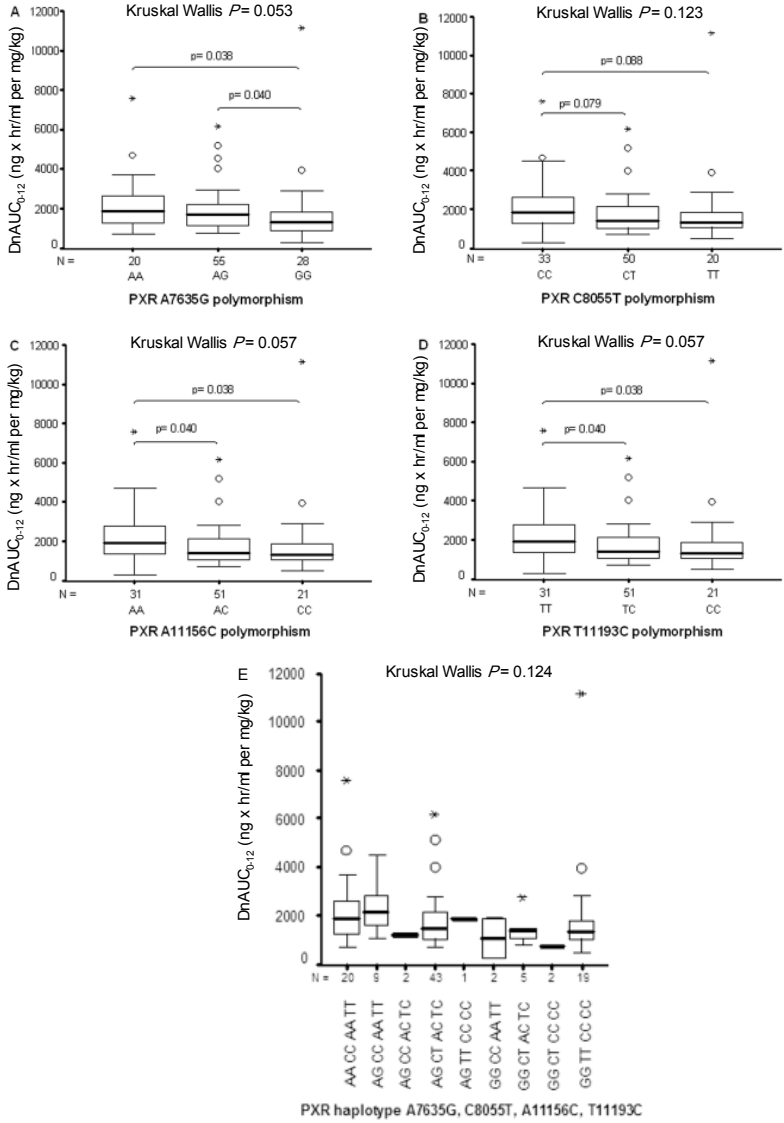


Figure 11.1 Influence of the PXR A7635G, C8055T, A11156C and T11193C genotypes on the area under the time tacrolimus concentration curve ($AUC_{0-12}$) that were recorded in 103 stable Chinese renal transplant recipients. Figure 11.1 illustrates the boxplots of the distribution of the dose normalized $AUC_{0-12}$ (ng × hr/ml per mg/kg) clustered according to the (A) PXR A7635G genotype, (B) PXR C8055T genotype, (C) PXR A11156C genotype, (D) PXR T11193C genotype and (E) the PXR haplotypes consisting of the PXR polymorphisms A7635G, C8055T, A11156C and T11193C. The open circles indicate an outlier value at more than 1.5 box lengths above the box, while the asterices indicate an extreme value at more than 3 box lengths above the box.

However, previously we[14] demonstrated in the same renal transplant patient group an important impact of the CYP3A5 A6986G polymorphism on the tacrolimus concentrations whereas a minor association was reported between both ABCB1 polymorphisms G2677T/A and C3435T and the tacrolimus concentration. To exclude influence of one of these polymorphisms, the renal transplant recipients were categorized on their CYP3A5 and ABCB1 G2677T/A, C3435T genotype in order to examine solely the effect of the four PXR polymorphisms on the dnAUC$_{0\text{-}12}$ and the daily tacrolimus dose. After categorizing the patients for respectively the CYP3A5 A6986G, ABCB1 G2677T/A and ABCB1 C3435T genotype there is, as is illustrated in Figure 11.2A-C, a tendency towards a lower dnAUC$_{0\text{-}12}$ within the groups of the combination ABCB1 polymorphisms and the PXR A7635G polymorphism. Although these data are not shown, similar results were observed for the PXR polymorphisms C8055T, A11156C and T11193C. Additionally, comparable results were achieved for the daily tacrolimus dose requirement and the four PXR polymorphisms.

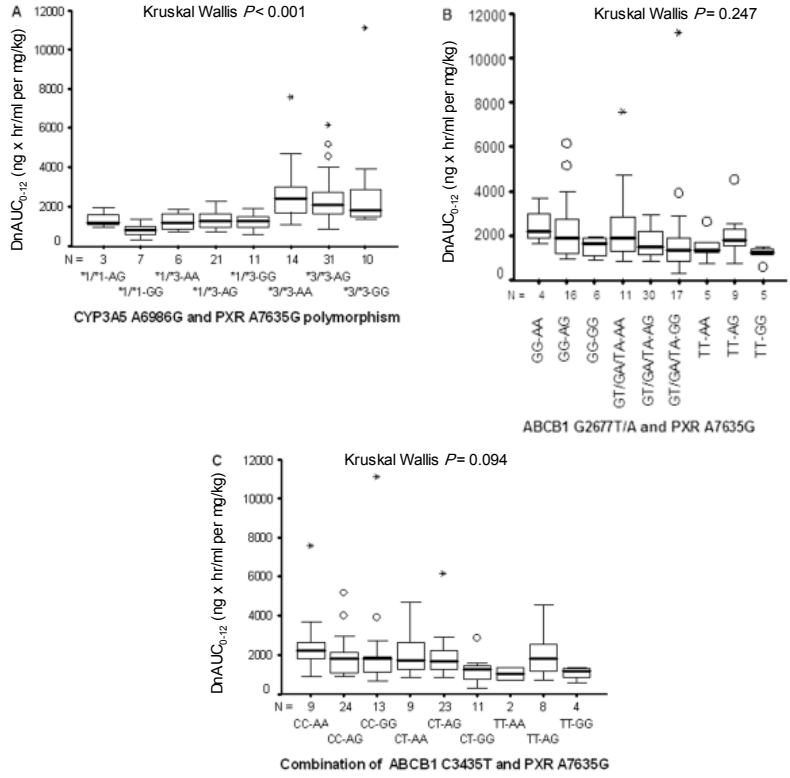


Figure 11.2 Boxplots of the distribution of the dose normalized AUC$_{0\text{-}12}$ (ng×hr/ml per mg/kg) clustered according to (A) the combination of respectively CYP3A5 A6986G and PXR A7635G, (B) ABCB1 G2677T/A and PXR A7635G genotypes and (C) ABCB1 C3435T and PXR A7635G genotypes. The open circles indicate an outlier value at more than 1.5 box lengths above the box, while the asterices indicate an extreme value at more than 3 box lengths above the box.

## Discussion

Tacrolimus is predominantely metabolised by CYP3A iso-enzymes into 13-O-demethyltacrolimus in the liver and the intestine[15-19]. Although CYP3A4 and CYP3A5 are the most important members of the CYP3A family, the CYP3A5 A6986G polymorphism is the only relevant polymorphism in the CYP3A4 and CYP3A5 genes which has a relative high allele frequency in different populations and a significant impact on the tacrolimus pharmacokinetics. Although CYP3A4 expression shows a large inter-individual variation, this can not only be explained by the polymorphisms in the CYP3A4 gene[1]. Elucidation of the mechanisms underlying CYP3A gene expression may be important for the role of the inter-individual variation in the tacrolimus pharmacokinetics. Moreover, there is also conflicting evidence regarding the role of the individual ABCB1 polymorphisms and ABCB1 haplotypes in the tacrolimus pharmacokinetics. Several studies reported a significant impact of the ABCB1 polymorphisms or haplotypes on the tacrolimus trough ($C_0$) concentrations[20-26] while other studies found no association between the different genotypes of the ABCB1 polymorphisms or ABCB1 haplotypes and the tacrolimus $C_0$ concentrations[27-32] or pharmacokinetics[14,33,34]. Since PXR is emerged as a transcriptional regulator of the expression of different members of the CYP450 family, polymorphisms in the PXR gene may influence the PXR activity and thereby the expression of these CYP450s. PXR binds as a heterodimer with RXR to the functional direct repeat-3 (DR-3) response element and to the functional everted repeat-6 (ER-6) response element in the proximal promoter region of the CYP3A4 gene while the CYP3A5 gene only contains the functional ER-6 response element in the promoter. In addition, the PXR/RXR heterodimer also binds to the functional direct repeat-4 (DR-4) response element in the ABCB1 promoter[5,6]. Zhang *et al.*[9] found that the 7635A to G transition in intron 5 of the PXR gene was associated with higher magnitude of induction of intestinal CYP3A by rifampicin. Individuals homozygous for the 7635G allele had a twofold higher induction of CYP3A compared to those homozygous for 7635A. An 8055C to T substitution was also associated with intestinal CYP3A phenotype that was more inducible. Individuals carrying at least one T-allele had twofold higher levels of intestinal CYP3A following rifampicin treatment compared to individuals homozygous for the 8055C allele. The 3'-untranslated region (3'-UTR) polymorphisms 11156A to C and 11193T to C showed that those individuals heterozygous with at least one 11156C or one 11193C allele had 1.45-fold lower P-glycoprotein levels in gut biopsies compared to those with homozygous 11156A and 11193T alleles. Since no CYP3A4*1B variant allele is found in this Chinese renal transplant patient group[14], there is no influence of the CYP3A4*1B variant allele on the CYP3A4 expression and thereby on the $dnAUC_{0-12}$ of tacrolimus. However, to exclude the effect of the CYP3A5*3 variant allele and the individual ABCB1 polymorphisms G2677T/A and C3435T or ABCB1 haplotypes, the renal transplant population is categorized according to the CYP3A5 A6986G, ABCB1 G2677T/A and C3435T genotype. In the present study a significant difference was observed regarding

the PXR polymorphisms A7635G, A11156C and T11193C when the dnAUC$_{0\text{-}12}$ was plotted *versus* the corresponding PXR polymorphisms of the 103 Chinese renal transplant patient recipients while a trend was observed for the PXR C8055T polymorphism. These results underline the *in vitro* findings demonstrated by Zhang *et al.*[9]. However, after excluding the effect of the CYP3A5 A6986G polymorphism and the ABCB1 polymorphisms G2677T/A and C3435T, only a trend is observed within the patients that were carrier of a similar genotype for the CYP3A5 A6986G or ABCB1 G2677T/A, ABCB1 C3435T polymorphisms. Probably due to the presence of at least eight different groups with other genotype combinations it is likely that the small number of patients included in one group has a relevant impact on these findings. Additional studies with larger patient numbers included in each group could possibly confirm our preliminary results.

## Conclusion

We described real-time PCR FRET assays for four PXR polymorphisms and determined the allele frequency of these four PXR polymorphisms in a Chinese renal transplant recipient group. Moreover, a strong trend towards a lower dnAUC$_{0\text{-}12}$ is found for individuals carrying a variant PXR allele suggesting that these PXR polymorphisms may play a role in the tacrolimus metabolism.

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