Background. Tacrolimus, a drug for prevention of rejection after kidney transplantation, has a narrow therapeutic window and is metabolized by the cytochrome P540 3A (CYP3A) system. Tacrolimus exposure increases after steroid tapering in many patients. The pregnane X receptor (PXR) — a mediator for CYP3A — has a steroid receptor and might regulate CYP3A5 activity depending on single nucleotide polymorphisms (SNPs) of CYP3A5 or PXR. This may contribute to differences in tacrolimus exposure after steroid tapering.

Methods. In a cohort of renal transplant recipients, the influence of CYP3A5 and PXR SNPs (A7635G, C8055T and C25385T) on the dose-normalized Tacrolimus trough concentration (DnC0) and their potential interaction with each other after steroid taper were analysed by linear regression. Eligible were all 83 outpatient renal transplant patients on tacrolimus treatment (DnC0) and their potential interaction with each other after steroid taper were analysed by linear regression. Eligible were all 83 outpatient renal transplant patients on tacrolimus after kidney transplantation, has a narrow therapeutic window and is metabolized by the cytochrome P540 3A (CYP3A) system. Tacrolimus exposure increases after steroid tapering in many patients. The pregnane X receptor (PXR) — a mediator for CYP3A — has a steroid receptor and might regulate CYP3A5 activity depending on single nucleotide polymorphisms (SNPs) of CYP3A5 or PXR. This may contribute to differences in tacrolimus exposure after steroid tapering.

Methods. In a cohort of renal transplant recipients, the influence of CYP3A5 and PXR SNPs (A7635G, C8055T and C25385T) on the dose-normalized Tacrolimus trough concentration (DnC0) and their potential interaction with each other after steroid taper were analysed by linear regression. Eligible were all 83 outpatient renal transplant patients on tacrolimus
INTRODUCTION

Tacrolimus is a frequently used immunosuppressive drug after renal transplantation. Because of its narrow therapeutic window between underexposure (with risk of rejection) and overexposure (with risk of side effects), therapeutic drug monitoring is advised. In clinical practice, dosing is adapted on the trough concentration (as surrogate for the exposure). With increasing time post-transplant, a decrease in the dosage of tacrolimus required to maintain similar trough concentrations has been reported [1], the reason being a decrease in tacrolimus clearance with time [2].

However, tacrolimus dose requirements also diminish after steroid withdrawal [1, 3–5]. Clinically relevant increases (>20% rise of trough concentration) have been reported in 43% of patients after withdrawal of 5 mg and in 61% of patients after withdrawal of 10 mg prednisolone [4]. This increase in trough concentration after steroid tapering has been demonstrated to be present also when tapering takes place >6 months after transplantation, when mechanisms like improving graft function, liver function and an increase in haemoglobin or albumin concentration are less likely to occur [1, 4].

The reason for this phenomenon has not yet been clearly elucidated. Relevant pharmacokinetic interaction between corticosteroids and tacrolimus has been linked to cytochrome P450 3A (CYP3A) and P-glycoprotein (P-gp), a drug efflux pump produced by the multidrug resistance 1 (MDR-1) gene [3]. Since steroids are reported to induce the activity of CYP3A enzymes [6], it has been postulated that steroid tapering may result in diminished CYP3A5 activity [4]. CYP3A enzymes in the liver and small intestine are majorly responsible for tacrolimus metabolism leading to a substantial first-pass effect [7, 8]. Tacrolimus exposure is also related to single-nucleotide polymorphisms (SNPs) of CYP3A5. To our knowledge, it is not known whether the effect of tapering steroids on tacrolimus exposure is different between these SNPs.

The induction of CYP3A, on the other hand, is mediated by the pregnane X receptor (PXR, also called NR1I2) [9]. It is a regulatory factor of the nuclear receptor family with a steroid receptor and is involved in the upregulation of many drug-metabolizing enzymes and drug transporters [10, 11]. Induction of the CYP3A gene in response to treatment with a variety of compounds, such as the glucocorticoid dexamethasone, the antibiotic rifampicin and the antymycotic clotrimazole [9, 12], could then be linked to PXR.

Several SNPs in the PXR gene have been found to increase CYP3A induction [6]. However, it is unknown whether systemic exposure to tacrolimus is then (in-)directly related to the inducing capacity mediated by SNPs in the PXR gene. If so, carriers of these inducing SNPs should have higher tacrolimus requirements while on steroids and thus have a more pronounced increase in dose-corrected trough concentrations after steroid withdrawal compared with the wild-type allele carriers.

In this study, we hypothesize that the steroid-mediated induction of PXR upregulates metabolic clearance through CYP3A5 and therefore contributes to the increase in tacrolimus exposure after steroid tapering or withdrawal. The aim of the current study is to determine whether the observed increase in tacrolimus exposure after steroid tapering is associated with certain SNPs of the CYP3A5 or PXR gene and whether there is an interaction between SNPs of the CYP3A5 and PXR genes. To test this hypothesis we retrospectively analysed data in a population of renal transplant patients for whom we obtained relevant biochemical, pharmaceutical and genetic data.

MATERIALS AND METHODS

Patients, samples and clinical data

Between December 2002 and February 2005, leftover ethylene-diaminetetraacetic acid (EDTA) blood samples of renal transplant patients routinely visiting our outpatient clinic were collected. Patient data and tacrolimus trough concentrations were retrieved from the patient files and hospital information system. Collection, storage and use of blood and patient data were performed in agreement with the Federation of Dutch University Medical Centers (FEDERA) code of conduct (www.nfu.nl). Secondary usage of this leftover material was approved by the Medical Ethical Committee of the Maastricht University Medical Centre (MEC 04-188).

All clinically stable renal transplant patients at least 6 weeks post-transplant on tacrolimus-based immunosuppression and whose leftover samples for DNA analysis were available were included. In addition, pharmacokinetic steady-state tacrolimus trough concentrations had to be present at a prednisolone dose of 10 mg/day and/or 5 mg/day and after complete steroid withdrawal. According to our centre protocol at that time, the prednisolone dose was 10 mg during the first 6 weeks and 7.5 mg for 4 weeks and 5 mg for 4 weeks during the ensuing 2 months and complete withdrawal thereafter, provided there were no signs of rejection or a higher degree of immunization.

The tacrolimus trough concentration in the pharmacokinetic steady state was defined as at least 7 days after the last prednisolone dose reduction and/or the last tacrolimus dose change. Excluded from analysis were patients who were treated for acute rejection within 1 month before measurements and...
who were suffering from any condition influencing absorption or elimination of tacrolimus, like gastrointestinal disorders, hepatic dysfunction or medication interfering with tacrolimus pharmacokinetics.

**Parameters**

We collected the following biochemical, pharmaceutical and genetic parameters from hospital clinical and laboratory files: age, time since transplantation, body weight, dose-normalized Tacrolimus trough concentration (DnC₀) [calculated from the tacrolimus trough level (C₀) and tacrolimus total daily dose], haemoglobin and serum albumin. The CYP3A5*1 and *3 alleles as well as three different SNPs of the PXR gene (A7635G, C8055T and C25385T) were determined. The choice of the PXR SNPs was based on previous reports: A7635G and C8055T are associated with a higher magnitude of (intestinal) CYP3A inducibility [6], while C25385T has been reported to be related to differences in tacrolimus apparent clearance [13].

**DNA analysis**

Genomic DNA from a venous blood sample was extracted according to the manufacturers’ instructions (Qiagen, Leusden, The Netherlands). Real-time polymerase chain reaction (PCR) fluorescence resonance transfer (FRET) assays were used for genotyping with the LightCycler (Roche Diagnostics, Almere, The Netherlands), as described previously (R. Op Den Buijsch, unpublished work).

**Statistical analysis**

The baseline characteristics of the patients are presented as mean and standard deviation (SD) or absolute value and percentage of all three prednisolone dosages. DnC₀ values were compared using an independent sample t-test. The unadjusted association of both CYP3A5 and PXR SNPs and the DnC₀ value for 10 mg steroid use were first computed using linear regression. In addition, we computed the adjusted associations after correction for all other SNPs and potential confounding factors [i.e. gender, age, weight at 10 mg steroid use (baseline), elapsed time since transplantation, haemoglobin concentration, serum albumin concentration, DnC₀ at 10 mg steroid use, time interval between 10 and 0 mg steroid use]. These potential confounders were tested for significance using backward stepwise elimination. To assess interactions between CYP3A5 and PXR SNPs for the individual slopes of the DnC₀ value depending on steroid use, we computed interaction terms and tested using linear regression.

Next, we determined DnC₀ changes over the three measures by performing a linear regression analysis per patient to obtain each patient’s individual DnC₀ slope. These slopes were used for subsequent analyses using the same methods as for the associations with DnC₀ at 10 mg steroid use. Finally, we used Fisher’s exact test to assess whether there were any associations between CYP3A5 and PXR SNPs. All analyses were performed using SPSS, version 23 (IBM, Armonk, NY, USA). P-values ≤0.05 were considered statistically significant.

**RESULTS**

Patients screened for this study received a renal transplant between March 1993 and January 2003. After this time our centre protocol changed to early steroid withdrawal, therefore patients transplanted after 2003 could not be included. By collecting leftover EDTA blood samples for DNA analyses between December 2002 and February 2005, we were able to collect material for all patients who were under regular control in our centre. This way we had a database consisting of 325 patients. When retrospectively applying the criteria for eligibility (at least 6 weeks post-transplant on tacrolimus-based immunosuppression, pharmacokinetic steady-state tacrolimus trough concentration at a prednisolone dose of 10 mg/day and/or 5 mg/day and after complete steroid withdrawal), 105 patients were eligible for further analyses. After application of the exclusion criteria we had tacrolimus trough concentration measurements for 83 patients after complete steroid withdrawal. Of these, 66 patients also had a measurement at 5 mg prednisolone dose and 81 at 10 mg. Tacrolimus trough concentrations were available at all three prednisolone doses in 64 patients. Of these, 17 patients did not have a stable tacrolimus trough concentration at prednisolone 5 mg and 2 patients at 10 mg. The baseline characteristics of the entire cohort for the respective prednisolone doses are shown in Table 1.

**Correlation of CYP3A5 and PXR SNPs for DnC₀ at 10 mg steroid dose**

The unadjusted (univariable) and adjusted (multivariable) associations between the tested CYP3A5 and PXR SNPs and DnC₀ for 10 mg steroid dose (baseline measurement) are shown in Table 2. In the multivariable model, all SNPs were entered simultaneously together with all potential relevant confounders (i.e. except DnC₀ at 10 mg steroid use and time interval between 10 and 0 mg steroid use). None of the potential confounders were statistically significant and they were therefore left out of the final multivariable model. Both in the univariable and multivariable analysis, the CYP3A5 carrier state was the only significant factor correlated with DnC₀: *3/*3 SNP individuals had a 42% higher DnC₀ compared with *1/*3 SNP individuals (1.4 versus 0.8 μg/L/mg). At baseline, i.e. <10 mg prednisolone dose, none of the PXR SNPs showed a statistically significant difference in DnC₀.

**Influence of steroid withdrawal on DnC₀**

DnC₀ increased by 58% (from a mean of 1.2 to 1.9 μg/L/mg) after withdrawal of prednisolone from 10 to 0 mg/day (P = 0.001; Table 1). As illustrated in Figure 1, DnC₀ increased with every step of prednisolone tapering, with a large interindividual variability. The individual changes in DnC₀ after steroid withdrawal are demonstrated in Figure 2. By considering (arbitrarily) a 20% change to be clinically relevant, ~75% of the individuals (n = 62) had a clinically relevant increase up to 260% (with a single outlier >400%), while 18 remained stable and 3 individuals had a clinically relevant decline in DnC₀. Since we observed one patient with an extreme increase in DnC₀ after steroid withdrawal of >400% (Figure 2), we performed a sensitivity analysis in which the extreme outlier was
omitted from the analyses. The sensitivity analysis did not result in different conclusions, nor did it result in different effect sizes or the signs of effects (results not shown), as described in the following sections.

**Relationship of CYP3A5 and PXR SNPs with the change in DnC0 for different steroid dosages**

In accordance with the literature, CYP3A5*3/*3 homozygotes have a higher DnC0 compared with CYP3A5*1/*3 carriers. This finding is valid at all prednisolone dosages (Figure 3 and Table 3). As shown in Table 3, not only the DnC0 but also its increase by steroid tapering is significantly higher in CYP3A5*3/*3 individuals (+64%) compared with CYP3A5*1/*3 individuals (+37%) (P < 0.001).

The univariable and multivariable associations between CYP3A5 and PXR SNPs and the change in DnC0 with every 5-mg decline in prednisolone dose are depicted in Table 4. In the multivariable model, all SNPs were entered together with all potential confounders. As none of the latter were statistically significant related to the outcome parameter, the final multivariable model contains only the CYP3A5 and PXR SNPs. For CYP3A5, both the univariable and the multivariable models show that, compared with CYP3A5*1/*3 heterozygotes, CYP3A5*3/*3 homozygotes had a clinically significant

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**Table 1. Baseline characteristics of the study cohort at three different prednisolone dosages**

<table>
<thead>
<tr>
<th></th>
<th>Prednisolone 10 mg (n = 81)</th>
<th>Prednisolone 5 mg (n = 66)</th>
<th>Prednisolone 0 mg (n = 83)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.1 (12.5)</td>
<td>48.4 (11.9)</td>
<td>50.2 (12.7)</td>
</tr>
<tr>
<td>Gender (male), n (%)</td>
<td>50 (61.0)</td>
<td>39 (59)</td>
<td>51 (61.4)</td>
</tr>
<tr>
<td>Time since transplant (days)</td>
<td>88.5 (276.8)</td>
<td>151 (218.9)</td>
<td>580 (759.5)</td>
</tr>
<tr>
<td>Days on stable prednisolone dose</td>
<td>41.5 (132.8)</td>
<td>48.8 (115.1)</td>
<td>51.3 (139.9)</td>
</tr>
<tr>
<td>Kreatinin (µmol/L)</td>
<td>222.5 (168.6)</td>
<td>168.9 (67.7)</td>
<td>146.1 (50.8)</td>
</tr>
<tr>
<td>Haemoglobin (mmol/L)</td>
<td>6.8 (5.6)</td>
<td>7.4 (1.2)</td>
<td>8.1 (1.1)</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>33.8 (5.1)</td>
<td>39.5 (4.3)</td>
<td>38.8 (4.1)</td>
</tr>
<tr>
<td>ALAT (µmol/L)</td>
<td>28.1 (20.4)</td>
<td>21.3 (9.8)</td>
<td>24.7 (14.1)</td>
</tr>
<tr>
<td>Daily tacrolimus dose (mg)</td>
<td>15.5 (9.9)</td>
<td>10.2 (6.1)</td>
<td>6.7 (4.4)</td>
</tr>
<tr>
<td>Tacrolimus trough concentration (µg/L)</td>
<td>13.9 (4.1)</td>
<td>11.9 (3.7)</td>
<td>9.6 (3.2)</td>
</tr>
<tr>
<td>Dn trough concentration (µg/L/mg)</td>
<td>1.2 (0.7)</td>
<td>1.6 (1.1)</td>
<td>1.9 (1.1)</td>
</tr>
<tr>
<td>CYP3A5, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*3/*3</td>
<td>52 (64.2)</td>
<td>43 (65.1)</td>
<td>54 (65)</td>
</tr>
<tr>
<td>*1/*3</td>
<td>29 (35.8)</td>
<td>23 (34.9)</td>
<td>29 (34.9)</td>
</tr>
<tr>
<td>PXR C25385T, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>38 (46.9)</td>
<td>36 (54.5)</td>
<td>39 (47.0)</td>
</tr>
<tr>
<td>CT</td>
<td>35 (43.2)</td>
<td>25 (37.9)</td>
<td>35 (43.4)</td>
</tr>
<tr>
<td>TT</td>
<td>8 (9.8)</td>
<td>5 (7.6)</td>
<td>8 (9.6)</td>
</tr>
<tr>
<td>PXR A7635G, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>33 (40.7)</td>
<td>24 (36.4)</td>
<td>33 (39.7)</td>
</tr>
<tr>
<td>AG</td>
<td>29 (35.8)</td>
<td>25 (37.9)</td>
<td>30 (36.1)</td>
</tr>
<tr>
<td>GG</td>
<td>19 (23.5)</td>
<td>16 (25.7)</td>
<td>19 (24.0)</td>
</tr>
<tr>
<td>PXR C8055T, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>56 (69.1)</td>
<td>48 (72.3)</td>
<td>59 (71.1)</td>
</tr>
<tr>
<td>CT</td>
<td>22 (27.2)</td>
<td>15 (22.7)</td>
<td>22 (26.3)</td>
</tr>
<tr>
<td>TT</td>
<td>3 (3.7)</td>
<td>3 (4.5)</td>
<td>3 (3.6)</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD) unless stated otherwise.

ALAT, Alanine Aminotransferase; Dn, dose-normalized.

---

**Table 2. Association of PXR and cytochrome gene SNPs with DnC0 for 10 mg of steroid use**

<table>
<thead>
<tr>
<th></th>
<th>Univariable</th>
<th>Multivariable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>SE</td>
</tr>
<tr>
<td>PXR C25385T (CC is reference)</td>
<td>-0.02</td>
<td>0.15</td>
</tr>
<tr>
<td>CT</td>
<td>-0.11</td>
<td>0.25</td>
</tr>
<tr>
<td>PXR A7635G (AA is reference)</td>
<td>-0.05</td>
<td>0.15</td>
</tr>
<tr>
<td>AG</td>
<td>0.24</td>
<td>0.17</td>
</tr>
<tr>
<td>PXR C8055T (CC is reference)</td>
<td>0.01</td>
<td>0.17</td>
</tr>
<tr>
<td>CT</td>
<td>0.56</td>
<td>0.38</td>
</tr>
<tr>
<td>CYP3A5 (*3/*3 is reference)</td>
<td>-0.66</td>
<td>0.13</td>
</tr>
<tr>
<td>*1/*3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Tacrolimus exposure after steroid tapering in renal transplant patients 1671

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additional ±0.30 μg/L/mg increase in DnC₀ for every 5 mg ste-
roid taper (P = 0.002).

Compared with the homozygote PXR A7635A genotype, the
A7635G heterozygotes had a trend towards a greater increase in
DnC₀ (+0.17 μg/L/mg for every 5 mg prednisolone tapering),
while for the G7635G homozygotes this increase was statisti-
cally and clinically significant and nearly twice as high
(+0.31 μg/L/mg; P = 0.02; Figure 4). In contrast, the homozy-
gote PXR T8055T genotype had a statistically and clinically re-
levant lower DnC₀ compared with the homozygote PXR
C8055C genotype in both univariable and multivariable analy-
ses (−0.50 and −0.72, respectively, for every 5 mg prednisolone
tapering). However, interpretation of this has to be cautious
because of the small number of individuals with PXR T8055T
(n = 3; Table 1) and the fact that there was no indication for a
dose–effect relationship (no decline in the C8055T genotype).

There were no statistically significant interactions between
CYP3A5 and the two statistically significant PXR SNPs (interaction
with PXR A7635G GG: B = 0.14, SE = 0.23, P = 0.559;
interaction with PXR C8055T TT: B = −0.70, SE = 0.48,
P = 0.149). Fisher’s exact test revealed no associations between
the presence of PXR SNPs and CYP3A5 SNPs. P-values derived
from these tests were 0.840, 0.131 and 0.433, respectively, for
PXR C25385T, A7635G and C8055T.

DISCUSSION

This study was designed to determine whether the observed
increase in tacrolimus exposure after steroid tapering correlates
with certain SNPs of the CYP3A5 or PXR gene and whether
these SNPs showed any interaction. First, we confirmed the for-
mer finding of increasing DnC₀ with steroid tapering (Figure 1)
with a magnitude consistent with an earlier report from our
group [4]. Second, in line with CYP3A5 activity, carriers of the
CYP3A5*1 genotype had a lower DnC₀ compared with the
CYP3A5*3 SNP homozygotes (Figure 3 and Tables 2 and 3).
Third, not only do CYP3A5*1 carriers have a lower DnC₀, but a
new finding was that they also exhibit a significant ~30% lower
increase after steroid taper compared with the CYP3A5*3/*3
SNP. Finally, we identified a correlation between the PXR 7635
carrier state and the change in DnC₀ and that this correlation is
independent from the CYP3A5 carrier state.

The mechanism for the smaller increase in DnC₀ in
CYP3A5*1 carriers is not known. Although the increased drug
clearance in stable long-term post-transplant patients in
CYP3A5*1 carriers has been extensively described and reviewed
[14–17], the majority of this increase was already within the
diminished increase in DnC₀ over time and has only been
described by Kuypers et al. [18], who found a 39% increase in
dose-corrected tacrolimus exposure only in the CYP3A5*3/*3
group during the first 5 years after renal transplant. The major-
ity of this increase was within the first year post-transplant. As
most of the steroid reduction takes place during this first year
and we found a 25% increase in drug exposure per 5 mg steroid
reduction, we conclude that this increase in tacrolimus expos-
ure can be primarily attributed to the steroid taper. This is
strengthened by the fact that the time since transplantation of the steroid tapering was not related to the change in tacrolimus exposure in our analysis.

More recently, it has been suggested that the phenomenon of maturation of tacrolimus exposure in the first year after renal transplantation observed in CYP3A5*3/*3 homozygous patients can partly be explained by a (steroid tapering–related) decline in CYP3A4 activity (measured by diminished apparent oral clearance of midazolam) and a progressive increase in haematocrit [19]. We measured a progressive increase of the haemoglobin concentration and assume that this is equivalent to an increase in haematocrit. However, we did not establish a significant relationship of the increase in haemoglobin concentration and the increasing dose-corrected tacrolimus concentration during the total time of observation, which was longer than the reported 1-year period in the study of de Jonge et al. [20].

Since PXR is a transcriptional regulator of CYP3A5 [21–23] and this regulation is mediated by steroids [24, 25], carriers of inducing SNPs could then have higher tacrolimus requirements while on steroids and thus have a more pronounced increase in DnC0 after steroid withdrawal compared with the wild-type allele carriers. Our analysis revealed two PXR SNPs to be related to the change in DnC0 with steroid tapering (Table 4). First, compared with PXR A7635A patients, patients with the homozygote G7635G allele had a 0.31 μg/L/mg higher increase in DnC0 after complete steroid withdrawal, and this was nearly double the increase of heterozygote PXR A7635G carriers (P = 0.02). These results are in line with the results of Zhang et al. [25], who found a 2-fold higher CYP3A4 mRNA content after 2 days of rifampicine exposure in homozygous G7635G carriers compared with homozygous A7635A carriers. This should translate into higher CYP3A activity. Reducing the inducing influence of steroids through dose reduction would then result in diminished CYP3A activity, leading to a greater increase in DnC0. Notably, at a stable steroid dose we did not find differences in DnC0 between heterozygote PXR A7635G carriers and homozygote PXR G7635G carriers. However, PXR

Table 3. Main outcomes stratified by CYP3A5 genotype

<table>
<thead>
<tr>
<th>CYP3A5*3/*3</th>
<th>Prednisolone 10 mg (n = 52)</th>
<th>Prednisolone 5 mg (n = 43)</th>
<th>Prednisolone 0 mg (n = 54)</th>
<th>CYP3A5*1/*3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily tacrolimus dose (mg)</td>
<td>11.5 (6.1)</td>
<td>7.3 (3.8)</td>
<td>4.6 (2.2)</td>
<td>23.0 (9.3)</td>
</tr>
<tr>
<td>Tacrolimus trough concentration (μg/L)</td>
<td>13.1 (3.5)</td>
<td>11.8 (3.9)</td>
<td>9.2 (3.0)</td>
<td>15.3 (4.7)</td>
</tr>
<tr>
<td>Dn trough concentration (μg/L/mg)</td>
<td>1.4 (0.7)</td>
<td>2.0 (1.2)</td>
<td>2.3 (1.1)</td>
<td>0.8 (0.3)</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD). Dn, dose-normalized.

Table 4. Association of PXR and cytochrome gene SNPs with steroid decrease–induced DnC0 increase

| | Univariable | Multivariable |
| | Coefficient | SE | P-value | Coefficient | SE | P-value |
| PXR C25385T (CC is reference) | | | | | | |
| CT | 0.07 | 0.09 | 0.489 | 0.04 | 0.09 | 0.644 |
| TT | −0.16 | 0.16 | 0.308 | −0.11 | 0.15 | 0.486 |
| PXR A7635G (AA is reference) | | | | | | |
| AG | −0.02 | 0.10 | 0.837 | 0.17 | 0.11 | 0.138 |
| GG | 0.11 | 0.11 | 0.316 | 0.31 | 0.13 | 0.020 |
| PXR C8055T (CC is reference) | | | | | | |
| CT | −0.08 | 0.11 | 0.478 | −0.18 | 0.11 | 0.110 |
| TT | −0.50 | 0.24 | 0.044 | −0.72 | 0.25 | 0.006 |
| CYP3A5 (*3/*3 is reference) | *1/*3 | | | | | |
| −0.30 | 0.09 | 0.001 | −0.29 | 0.09 | 0.002 |

FIGURE 3: Tacrolimus DnC0 stratified by CYP3A5 genotype.
G7635G homozygotes display a significantly greater increase after steroid withdrawal, which is compatible with our hypothesis that carriers of inducing SNPs have higher tacrolimus requirements while on steroids and thus have a more pronounced increase in DnC₀ after steroid withdrawal. Our analysis reveals that this greater increase in DnC₀ is independent of the CYP3A5 genotype.

One has to be aware that the PXR 7635 genotype was the only tested genotype not in Hardy–Weinberg (HW) equilibrium: our study cohort had a relative abundance of homozygous 7635G patients. We were unable to identify any plausible explanation for this finding in this population that was not pre-selected by any other parameter than the availability of steady-state tacrolimus trough concentration while on prednisone (10 mg and/or 5 mg) and after complete tapering of prednisone. Also, the entire pharmacogenetic renal transplant database (n = 325; without differences in patient characteristics compared with the studied cohort) was in HW equilibrium. We therefore think that the HW disequilibrium of the A7635G genotype is due to coincidence.

The second SNP statistically related to the change in DnC₀ with steroid tapering was the PXR T8055T genotype. It showed a significantly lower DnC₀ increase compared with the heterozygote PXR C8055T or the PXR C8055C reference (multivariable coefficient = −0.72; P = 0.06; Table 4), which means there is less effect of steroid withdrawal. This is not consistent with what would be expected given the intestinal CYP3A inducibility as shown by Zhang et al. [6]. One should consider that in our study only three patients carried this PXR T8055T genotype. So no firm conclusions can be drawn from these findings concerning the C8055T SNP unless they are confirmed in an independent larger cohort.

An advantage of our study was that we had longitudinal data of pharmacokinetically stable patients at different phases of steroid taper during the first 2 years of transplantation instead of studying cross-sectional data. Since we adapted our immunosuppressive protocol towards early steroid withdrawal in our centre later on, we are unable to extend the number of patients in this study. As could be expected in a Caucasian population of this size, we lack in this study CYP3A5*1/*1 homozygotes and therefore have to refrain from making any conclusions about them. Given the low genotype frequency of the PXR T8055T SNP, we could not elucidate in detail the potential influence of this apparently rare SNP.

In conclusion, we confirmed the clinically relevant increase in tacrolimus exposure due to steroid tapering in renal transplantation. This phenomenon is not time dependent and probably largely explains the reported increase in drug exposure in the first year post-transplant. Given the large interpatient variability, the fact that tacrolimus exposure increases in all CYP3A5 groups after steroid tapering and the dose–response relation with every 5 mg prednisone taper, our advice is to monitor the tacrolimus trough concentration after every steroid dose change. Above all, we found that the increase in DnC₀ after steroid tapering will be larger in CYP3A5*3 homozygotes compared with CYP3A5*1 carriers (and probably CYP3A5*1/*1 homozygotes). In addition, we also demonstrated that some SNPs of the PXR gene (especially G7635G, but also possibly T8055T) were related to a clinically relevant change in tacrolimus exposure due to steroid tapering and that this was independent of the CYP3A5 SNP. Therefore this study is the first clinical study showing that the steroid receptor PXR might be of clinical relevance for tacrolimus metabolism.

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AUTHORS’ CONTRIBUTIONS

F.S. was responsible for performance of the research, data analysis and writing and revision of the manuscript. S.M.J.v.K. performed the statistical analysis. O.B. was responsible for the contribution of analytical tools and revision of the manuscript. M.H.L.C. was the principal investigator, designed the research, provided data analysis and wrote and revised the manuscript.

CONFLICTS OF INTEREST STATEMENT

F.S., O.B. and S.M.J.v.K. have no conflicts of interest that are relevant to the content of this research. M.H.L.C. has been an investigator in company-driven studies by Novartis and Astellas and his institute has received consulting and lecture fees from Astellas. The results presented in this article have not been published previously in whole or part.

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