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Increase in tacrolimus exposure after steroid tapering is influenced by *CYP3A5* and pregnane X receptor genetic polymorphisms in renal transplant recipients

Frank Stifft[1], Sander M.J. van Kuijk[2], Otto Bekers[3] and Maarten H.L. Christiaans[1]

[1]Department of Internal Medicine, Division of Nephrology, Maastricht University Medical Centre, Maastricht, The Netherlands, [2]Department of Clinical Epidemiology and Medical Technology Assessment, Maastricht University Medical Centre, Maastricht, The Netherlands and [3]Central Diagnostic Laboratory, Department of Clinical Chemistry, Maastricht University Medical Centre, Maastricht, The Netherlands

Correspondence and offprint requests to: Frank Stifft; E-mail: F.Stifft@zuyderland.nl; Twitter handle: @FrankStifft

ABSTRACT

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 (DnC_0) 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

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and steroids in a pharmacokinetic steady state at least 6 weeks after transplantation and whose blood was available for genetic analysis.

Results. Compared with the *CYP3A5*1/*3* genotype, the *CYP3A5*3/*3* SNP showed a significantly stronger increase in DnC_0 after steroid taper (+0.29 μg/L/mg; P = 0.002). Of the tested *PXR* SNPs, *PXR* G7635G individuals had a significantly stronger increase in DnC_0 (compared with A7635A, +0.31 μg/L/mg; P = 0.02), with a weaker increase in A7635G heterozygotes (+0.17 μg/L/mg; P = 0.124). There was neither interaction nor association between *CYP3A5* and *PXR* SNPs.

Conclusions. The magnitude of the DnC_0 increase due to steroid taper after renal transplantation is related to *CYP3A5* SNPs. Independently, the *PXR* G7635G SNP is related to this increase, proving the role of *PXR* in tacrolimus metabolism.

Keywords: pharmacokinetics, pharmacogenetics, pregnane X receptor (*PXR*), tacrolimus, therapeutic drug monitoring

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* (nuclear receptor 1I2)] [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 antimycotic 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 obatined relevant biochemical, pharmaceutical and genetic data.

MATERIALS AND METHODS

Patients, samples and clinical data

Between December 2002 and February 2005, leftover ethylenediaminetetraacetic 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

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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 (DnC_0) [calculated from the tacrolimus trough level (C_0)] 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. DnC_0 values were compared using an independent sample t-test. The unadjusted association of both *CYP3A5* and *PXR* SNPs and the DnC_0 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, DnC_0 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 DnC_0 value depending on steroid use, we computed interaction terms and tested using linear regression.

Next, we determined DnC_0 changes over the three measures by performing a linear regression analysis per patient to obtain each patient's individual DnC_0 slope. These slopes were used for subsequent analyses using the same methods as for the associations with DnC_0 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 dosages are shown in Table 1.

Correlation of *CYP3A5* and *PXR* SNPs for DnC_0 at 10 mg steroid dose

The unadjusted (univariable) and adjusted (multivariable) associations between the tested *CYP3A5* and *PXR* SNPs and DnC_0 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 DnC_0 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 DnC_0: **3/*3* SNP individuals had a 42% higher DnC_0 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 DnC_0.

Influence of steroid withdrawal on DnC_0

DnC_0 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, DnC_0 increased with every step of prednisolone tapering, with a large interindividual variability. The individual changes in DnC_0 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 DnC_0. Since we observed one patient with an extreme increase in DnC_0 after steroid withdrawal of >400% (Figure 2), we performed a sensitivity analysis in which the extreme outlier was

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

	Prednisolone 10 mg ($n = 81$)	Prednisolone 5 mg ($n = 66$)	Prednisolone 0 mg ($n = 83$)
Age (years)	49.1 (12.5)	48.4 (11.9)	50.2 (12.7)
Gender (male), *n* (%)	50 (61.0)	39 (59)	51 (61.4)
Time since transplant (days)	88.5 (276.8)	151 (218.9)	580 (759.5)
Days on stable prednidolone dose	41.5 (132.8)	48.8 (115.1)	95.1 (293.9)
Kreatinin (μmol/L)	222.5 (168.6)	168.9 (67.7)	146.1 (50.8)
Haemoglobin (mmol/L)	6.8 (5.6)	7.4 (1.2)	8.1 (1.1)
Albumin (g/L)	33.8 (5.1)	39.5 (4.3)	38.8 (4.1)
ALAT (mmol/L)	28.1 (20.4)	21.3 (9.8)	24.7 (14.1)
Daily tacrolimus dose (mg)	15.5 (9)	10.2 (6.1)	6.7 (4.4)
Tacrolimus trough concentration (μg/L)	13.9 (4.1)	11.9 (3.7)	9.6 (3.2)
Dn trough concentration (μg/L/mg)	1.2 (0.7)	1.6 (1.1)	1.9 (1.1)
CYP3A5, *n* (%)			
**3/*3*	52 (64.2)	43 (65.1)	54 (65)
**1/*3*	29 (35.8)	23 (34.9)	29 (34.9)
PXR C25385T, *n* (%)			
CC	38 (46.9)	36 (54.5)	39 (47.0)
CT	35 (43.2)	25 (37.9)	35 (43.4)
TT	8 (9.8)	5 (7.6)	8 (9.6)
PXR A7635G, *n* (%)			
AA	33 (40.7)	24 (36.4)	33 (39.7)
AG	29 (35.8)	25 (37.9)	30 (36.1)
GG	19 (23.5)	16 (25.7)	19 (24.0)
PXR C8055T, *n* (%)			
CC	56 (69.1)	48 (72.3)	59 (71.1)
CT	22 (27.2)	15 (22.7)	22 (26.5)
TT	3 (3.7)	3 (4.5)	3 (3.6)

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 DnC_0 for 10 mg of steroid use

	Univariable			Multivariable		
	Coefficient	SE	P-value	Coefficient	SE	P-value
PXR C25385T (CC is reference)						
CT	−0.02	0.15	0.916	−0.10	0.14	0.497
TT	−0.11	0.25	0.650	−0.27	0.24	0.257
PXR A7635G (AA is reference)						
AG	−0.05	0.15	0.740	0.13	0.17	0.446
GG	0.24	0.17	0.170	0.15	0.21	0.477
PXR C8055T (CC is reference)						
CT	0.01	0.17	0.977	0.04	0.18	0.832
TT	0.56	0.38	0.151	0.58	0.38	0.143
CYP3A5 (**3/*3* is reference)						
**1/*3*	−0.66	0.13	<0.001	−0.71	0.14	<0.001

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 DnC_0 for different steroid dosages

In accordance with the literature, *CYP3A5*3/*3* homozygotes have a higher DnC_0 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 DnC_0 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 DnC_0 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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additional ±0.30 µg/L/mg increase in DnC_0 for every 5 mg steroid taper (P = 0.002).

Compared with the homozygote *PXR* A7635A genotype, the A7635G heterozygotes had a trend towards a greater increase in DnC_0 (+0.17 µg/L/mg for every 5 mg prednisolone tapering), while for the G7635G homozygotes this increase was statistically and clinically significant and nearly twice as high (+0.31 µg/L/mg; P = 0.02; Figure 4). In contrast, the homozygote *PXR* T8055T genotype had a statistically and clinically relevant lower DnC_0 compared with the homozygote *PXR* C8055C genotype in both univariable and multivariable analyses (−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).

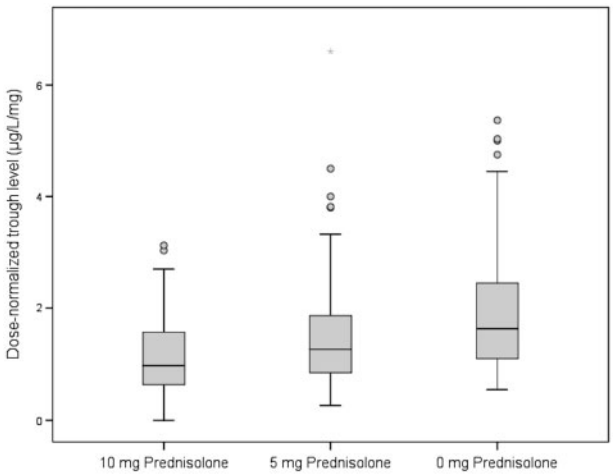

FIGURE 1: Box plots of the tacrolimus DnC_0 for three different prednisolone dosages. The band inside the box shows the median value, whereas the bottom and top of the box represent the first and third quartiles, respectively. The small vertical lines at the end of the whiskers denote the lowest and highest values that are still within 1.5 times the interquartile range. The individual points are the outliers that fall outside of this boundary.

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 former finding of increasing DnC_0 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 DnC_0 compared with the *CYP3A5*3* SNP homozygotes (Figure 3 and Tables 2 and 3). Third, not only do *CYP3A5*1* carriers have a lower DnC_0, 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 DnC_0 and that this correlation is independent from the *CYP3A5* carrier state.

The mechanism for the smaller increase in DnC_0 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 DnC_0 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 majority 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 exposure can be primarily attributed to the steroid taper. This is

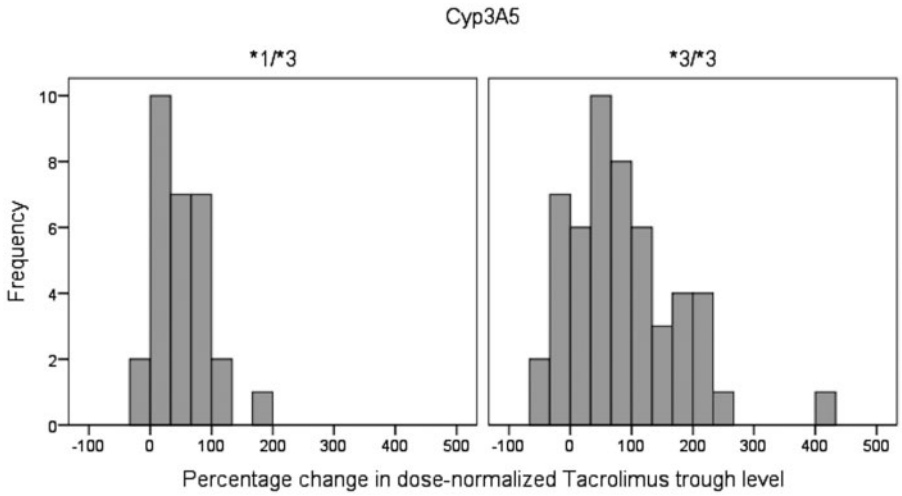

FIGURE 2: Distribution of frequencies of percentage change in tacrolimus DnC_0 after prednisone dose reduction from 10 to 0 mg/day.

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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].

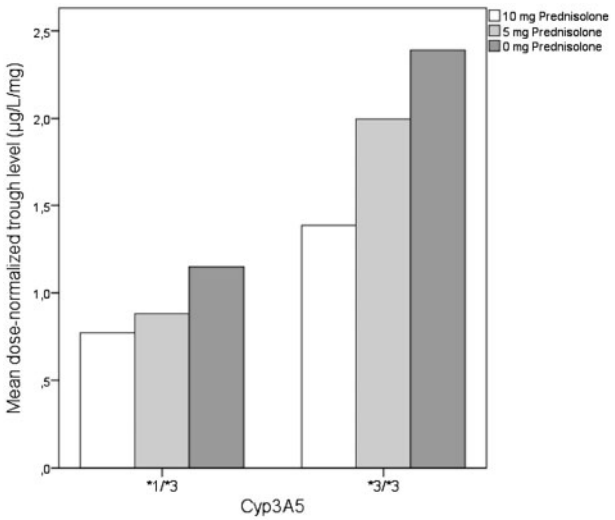

FIGURE 3: Tacrolimus DnC_0 stratified by *CYP3A5* genotype.

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 DnC_0 after steroid withdrawal compared with the wild-type allele carriers. Our analysis revealed two *PXR* SNPs to be related to the change in DnC_0 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 DnC_0 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 DnC_0. Notably, at a stable steroid dose we did not find differences in DnC_0 between heterozygote *PXR* A7635G carriers and homozygote *PXR* G7635G carriers. However, *PXR*

Table 3. Main outcomes stratified by *CYP3A5* genotype

	*CYP3A5*3/*3*			*CYP3A5*1/*3*		
	Prednisolone 10 mg ($n = 52$)	Prednisolone 5 mg ($n = 43$)	Prednisolone 0 mg ($n = 54$)	Prednisolone 10 mg ($n = 29$)	Prednisolone 5 mg ($n = 23$)	Prednisolone 0 mg ($n = 29$)
Daily tacrolimus dose (mg)	11.5 (6.1)	7.3 (3.8)	4.6 (2.2)	23.0 (9.3)	15.5 (6.0)	10.8 (5.2)
Tacrolimus trough concentration (µg/L)	13.1 (3.5)	11.8 (3.9)	9.2 (3.0)	15.3 (4.7)	12.0 (3.2)	10.3 (3.3)
Dn trough concentration (µg/L/mg)	1.4 (0.7)	2.0 (1.2)	2.3 (1.1)	0.8 (0.3)	0.9 (0.4)	1.1 (0.5)

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

Table 4. Association of *PXR* and cytochrome gene SNPs with steroid decrease–induced DnC_0 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

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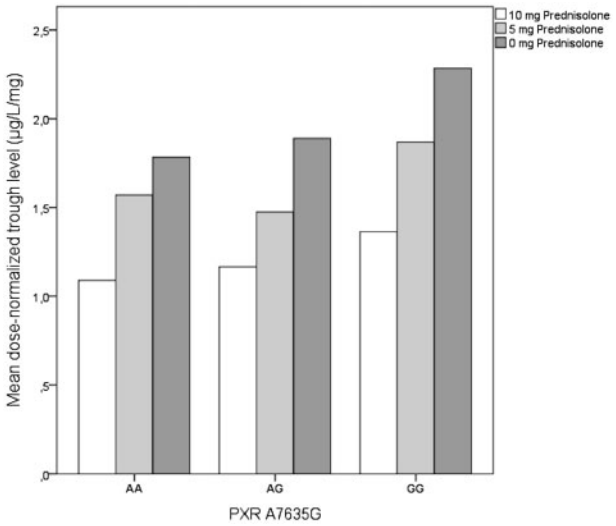

FIGURE 4: Tacrolimus DnC_0 stratified by *PXR* 7635 genotype at different prednisolone doses per day.

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 DnC_0 after steroid withdrawal. Our analysis reveals that this greater increase in DnC_0 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 preselected 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 DnC_0 with steroid tapering was the *PXR* T8055T genotype. It showed a significantly lower DnC_0 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 we 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 DnC_0 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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