Chapter 9

Cytochrome P450 (CYP3A7) *1C allelic variant is associated with the tacrolimus pharmacokinetics in renal transplant recipients

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Submitted

Abstract

Background and aim
Cytochrome P450 3A4/5 (CYP3A4/5) are mainly responsible for the tacrolimus metabolisation although little information is available regarding the contribution of CYP3A7. The CYP3A7*1C allele contains the proximal everted repeat 6 motif of CYP3A4 which implicates that the pregnane x receptor and the constitutively activated receptor bind with a higher affinity to the CYP3A7*1C allele than to the CYP3A7*1 allele, which results in a higher expression of CYP3A7 for individuals carrying a CYP3A7*1C allele.

Materials and methods
Three different renal transplant recipient groups are used to examme the influence of CYP3A7 in the tacrolimus exposure. In total 70 Caucasian and 103 Chinese renal transplant recipient were genotyped for CYP3A7*1C variantalled. The pharmacokinetic parameters of tacrolimus were determined for all renal transplant recipients and correlated with their corresponding genotypes.

Results
An allele frequency of respectively 2.8% and 0.0% was found after genotyping 70 Caucasian and 103 Chinese renal transplant recipients for the CYP3A7*1C allele using a real-time polymerase chain reaction (PCR) fluorescence resonance energy transfer (FRET) assay. Heterozygous carriers of the CYP3A7*1C allele showed no significant lower trough levels, area under the time tacrolimus concentration curve or maximum concentrations for tacrolimus compared to the carriers of the CYP3A7*1 allele.

Conclusion
Based on these findings we conclude that the CYP3A7*1C allele is not associated with lower pharmacokinetic tacrolimus parameters.

Introduction

Tacrolimus, a calcineurin inhibitor used in the transplantation therapy, is characterised by its variable pharmacokinetic characteristics and narrow therapeutic window. Moreover, tacrolimus is predominately metabolised into 13-O-demethyltacrolimus in the liver and the intestines by cytochrome P450 (CYP) 3A[1-4]. The CYP3A subfamily composed of four genes, CYP3A4, CYP3A5, CYP3A7 and CYP3A43 and three pseudogenes CYP3AP1, CYP3AP2 and CYP3AP3, is located on chromosome 7q21-q22.1[5]. The CYP3A concentrations and activities display inter-individual variability, which can at least partly explain the variation in the trough (C_0) levels[6-15] and the other pharmacokinetic parameters of tacrolimus[16-18]. Although more than 30 CYP3A4 allelic variants have been reported[5], most CYP3A4 variants are single nucleotide polymorphisms (SNPs) with low allelic frequencies and many of these SNPs are population specific[19]. Due to their low allelic frequency, the contribution of these CYP3A4 SNPs to the inter-individual variability of CYP3A4 expression is limited. In contrast to CYP3A4, a few SNPs in CYP3A5 have a major impact on the CYP3A5 expression[20,21]. However, in Caucasians CYP3A5 expression appears to be mainly regulated by the CYP3A5*3 allelic variant[20-22]. Individuals carrying a CYP3A5*3 (low expressers) variant allele express a significantly lower amount CYP3A5 compared to individuals carrying a CYP3A5*1 allele (high expressers). Transplant patients categorized as low expressers achieve significantly higher pharmacokinetic parameters for tacrolimus (dnC_0, $dnAUC_{0\text{-}12}$ and dnC_{max}) and therefore require a lower daily tacrolimus dose compared to transplant patients included in the high expresser group[16,17]. Although Kamden *et al.*[23] previously demonstrated with *in vitro* experiments that CYP3A7 compared to CYP3A4 and CYP3A5 plays a minor role in the tacrolimus metabolism, the role of higher CYP3A7 protein levels on the tacrolimus metabolisation *in vivo* is not yet been elucidated. CYP3A7 is the most abundant CYP in human liver during fetal development and the first months of postnatal age[24]. Moreover, CYP3A7 plays an important role in the metabolism of key steroids in the adrenals and gonads of potentially toxic and teratogenic endogenous substrates[24-27] and of many drugs of abuse that reach the fetus[28-31]. Sim *et al.*[32] found that the CYP3A7 protein expression is high in a fraction of the human adult livers and is associated partially with the CYP3A7*1C allele. Additionally, Smit *et al.*[33] found that the CYP3A7*1C variant allele is associated with a nearly 50% reduction of the serum dehydro-epi-androsterone sulfate (DHEAS) levels. Considering the relevance of this CYP3A7*1C allelic variant, we developed a real-time PCR fluorescence resonance energy transfer (FRET) assay for this CYP3A7 polymorphism. Moreover, since clinical studies regarding the influence of CYP3A7*1C polymorphism on the pharmacokinetic parameters of tacrolimus are still lacking, two Caucasian and a Chinese renal transplant recipient group are genotyped for the CYP3A7*1C variant allele. Furthermore, the genotypes of the CYP3A7*1C variant allele found in the renal transplant recipient groups are correlated with their corresponding pharmacokinetic tacrolimus parameters.

Materials and methods

Study populations

In total 70 Caucasian and 103 Chinese renal transplant recipients are included in the present study. The Caucasian renal transplant recipients are divided over two different groups of whom in the past for clinical reasons (group I) or for a clinical trial (group II) a 12 hour time tacrolimus concentration curve was performed. A two time point sampling strategy[34] was used to calculate the abbreviated $AUC_{0\text{-}12}$ in the Chinese renal transplant recipients (group III). Group I included early Caucasian posttransplant patients with a short median time after transplantation and large variability in the tacrolimus $AUC_{0\text{-}12}$ compared to the late Caucasian posttransplant recipients of group II. Most pharmacokinetic profiles of the patients included in group I were recorded within six weeks after transplantation. All renal transplant recipients included in group II and III underwent a renal transplantation at least one year (group II) or four months (group III) ago. Moreover, eight patients in group I used a calcium channel blocker that showed interference with tacrolimus. Group II and III included no patients taking medication known to interact with tacrolimus such as calcium channel blockers, anti-epileptics, anti-mycotics and macrolide antibiotics. In addition, patients that suffer from gastrointestinal, liver disease, or other disorders that may alter the absorption of tacrolimus were also disqualified for inclusion. The initial tacrolimus dosage, administrated twice daily, was 0.2 mg/kg per day for patients included in group I and II and 0.3 mg/kg per day for patients included in group III . For patients in group I and II the dose requirement for tacrolimus was assessed three months after transplantation, when the target C_0 concentration was between 10 to 15 ng/ml while the daily tacrolimus dose for patients in group III was adjusted according to the $AUC_{0\text{-}12}$ value, which was kept at around 100-150 ng $\times$ hr/ml in the first three months. After three months the target $AUC_{0\text{-}12}$ value was decreased to around 80-100 ng $\times$ hr/ml for long term maintenance. Prior to the blood sample collection, there had been no tacrolimus dose change for at least three days in groups I and II and for at least two weeks in group III. After overnight fasting the blood samples were collected immediately pre (C_0) and 0.5 ($C_{0.5}$), 1 (C_1), 2 (C_2), 3 (C_3), 4 (C_4), 5 (C_5), 7.5 ($C_{7.5}$) and 12 (C_{12}) hours after the morning tacrolimus administration for group I and II while blood samples were collected at two time points (C_2 and C_4) to calculate the abbreviated $AUC_{0\text{-}12}$ for the patients included in group III. All patients were not allowed to take food until one hour after ingesting the tacrolimus dose and were advised to avoid grapefruit juice after transplantation to prevent alterations in the tacrolimus metabolism. Demographic as well as clinical data were determined at the time of recording the $AUC_{0\text{-}12}$ or the abbreviated $AUC_{0\text{-}12}$ (Table 9.1). The renal transplant recipients included in group I and II underwent a renal transplantation and a regular follow up in the University Hospital of Maastricht, the Netherlands whereas the renal transplant recipients included in group III had regular follow up in Queen Elizabeth Hospital or Tuen Mun Hospital in Hong Kong. The study was performed in accordance

to the Declaration of Helsinki and its amendments. The protocol was approved by the local Medical Ethics Committees in the Netherlands and Hong Kong and written informed consent for participation in this study was obtained from all patients.

Table 9.1 Demographic characteristics of the three renal transplant recipients groups.

Demographic characteristics	Group I	Group II	Group III
Ethnicity	Caucasians	Caucasians	Chinese
Gender (male/female)	22/11	24/13	58/45
Age (years, mean ± SD)	42.0 ± 12.2	51.3 ± 10.9	43.1 ± 11.1
Weight (kg, mean ± SD)	71.7 ± 16.4	77.4 ± 13.5	62.5 ± 11.4
Primary kidney disease			
Glomerulonephritis	5	1	57
Chronic pyelonephritis	2	2	1
IgA nephropathy	3	4	0
Hypertensive nephropathy	4	7	9
Diabetes Mellitus nephropathy	5	0	10
Polycystic kidney disease	2	8	2
Obstructive Uropathy	0	0	3
Unknown	3	4	18
Other	9	11	3
Transplantation number			
First	26	30	97
Second	6	6	6
Third or more	1	1	0
Tacrolimus mono therapy	2	29	0
Tacrolimus dose (mg/kg/day, mean ± SD)	0.34 ± 0.233	0.054 ± 0.029	0.067 ± 0.032
C_0 (ng/ml, mean ± SD)	14.8 ± 6.60	6.59 ± 1.39	NA
$AUC_{0\text{-}12}$ (ng × hr/ml, mean ± SD)	269.7 ± 110.9	122.5 ± 31.1	NA
Abbreviated $AUC_{0\text{-}12}$ (ng × hr/ml, mean ± SD)	ND	ND	100.3 ± 24.2
C_{max} (ng/ml, mean ± SD)	50.2 ± 22.8	20.9 ± 6.5	NA
T_{max} (hr, mean ± SD)	1.46 ± 1.21	1.24 ± 0.43	NA
Use of AZA[a], MMF[b], rapamycine, steroids	10/3/4/28	3/4/0/0	78/25/0/103
Current steroid dose (mg, dose, no. patients)			
0 mg/day	5	37	0
4 mg/day	0	0	1
5 mg/day	7	0	33
7.5 mg/day	0	0	64
8 mg/day	2	0	0
10 mg/day	10	0	3
15 mg/day	4	0	1
20 mg/day	3	0	0
> 20 mg/day	2	0	1
Time since Tx (days, median, (range))	20 (3-5359)	1465 (453-4128)	977 (133-4982)
Haemoglobin (mmol/l, mean ± SD)	6.03 ± 1.56	8.52 ± 0.83	12.8 ± 2.0
Haematocrit fraction (mean ± SD)	0.27 ± 0.09	0.41 ± 0.04	0.38 ± 0.058
ALAT (U/l, mean ± SD)	32 ± 31	24 ± 13	22 ± 18
Serum albumin (g/l, mean ± SD,)	32.5 ± 5.03	37.0 ± 3.84	43 ± 3.2
Serum creatinine (μmol/l, median ± SD)	189 ± 268	125 ± 29	156 ± 76

[a] azathiopurine; [b] mycophenolate mofetil; ND not determined, NA not available; Abbreviated $AUC_{0\text{-}12}$ is the $AUC_{0\text{-}12}$ calculated with the following formula: $AUC_{0\text{-}12} = 16.2 + 2.4 \times C_2 + 5.9 \times C_4$.

Tacrolimus concentration determination

The tacrolimus measurements for the three groups were performed in different laboratories. For all groups the tacrolimus blood concentrations were determined in ethylene diamine tetra-acetic acid (EDTA) whole blood, using a microparticle enzyme immunoassay (MEIA) with a monoclonal antibody (IMx II assay; Abbott Laboratories, Abbott Park, IL, USA) for group I and III and a method based on high pressure liquid chromatography (LC) tandem mass spectrometry (MS/MS) for group II. All laboratories participate in the International Tacrolimus Proficiency Testing Scheme. The tacrolimus C_0 concentration, the peak blood concentration (C_{max}) during the assessed time interval and the time at which the highest blood concentration was observed (T_{max}) were determined directly from the time *versus* tacrolimus blood concentration data. Additionally, the area under the time tacrolimus concentration curve (AUC_{0-12}) was calculated from the time *versus* tacrolimus concentration plot using the linear trapezoidal rule in MWPharm 3.50 (Mediware, Groningen, the Netherlands). The two tacrolimus blood concentrations determined for the patients included in group III were used to calculate the abbreviated AUC_{0-12} according to the equation based strategy as described by our group[34]: $AUC_{0-12} = 16.2 + 2.4 \times C2 + 5.9 \times C4$. DnC_0, $dnAUC_{0-12}$ and dnC_{max} were calculated by dividing the C_0, AUC_{0-12} and C_{max} respectively by the corresponding morning dose on a milligram per kilogram basis.

Real-time PCR FRET assay for CYP3A7*1C allelic variant

Genomic DNA was isolated from 70 Caucasian and 103 Chinese renal transplant recipients by using 200 μl EDTA anticoagulated blood for isolation with a Qiagen blood mini kit (Qiagen, Leusden, the Netherlands) according to the manufacturers' instructions. Genotyping for the CYP3A7*1C allelic variant was performed using a real-time PCR FRET assay on the LightCycler (Roche Diagnostics, Almere, the Netherlands). Since the different members of the CYP3A subfamily show a high sequence homology (> 90%), the position of the primers is carefully selected. Moreover, the CYP3A7*1C variant allele consists of seven different polymorphisms within a 60 base pair (bp) range in the CYP3A7 promoter region, which narrows the position options of the probe that covers one of these polymorphisms. Specific primers for CYP3A7 and a sensor probe covering the T-188G polymorphism in the CYP3A7 promoter region are used in the real-time PCR FRET assay developed for the CYP3A7*1C variant allele. The primers F41: 5'-CCA TAg AgA CAA gAg gAg AgT TAA T-'3 (sense; 110611-635) and R41: 5'-gAg gCT TCT CCA CCT Cg-'3 (anti-sense; 110790-806) were used to amplify a 196 bp part from the CYP3A7 gene which covers the T110718G polymorphism (corresponding to position –188 of the CYP3A7 promoter; Genbank acces no: AF280107)[35]. Detection was carried out using the anchor probe CYP3A7A41: 5'-ggg AgT CCA Agg gTT CTg gg-'3 (anti-sense; 110677-696) which was labelled at the 5' end with LCRed640 and phosphorylated at the 3'-end to block extension and the sensor probe CYP3A7P41: 5'-ATC ATA CAC AAC TCA ATC AAT gTT ACT-'3 (anti-

sense; 110698-724) which is complementary to the CYP3A7*1 allele. This 3'-fluorescein 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 contained 3.2 μl sterile water; 3.0 mmol/l $MgCl_2$; 1.0 μl LC Faststart DNA Master Hybridization Probes (Roche Diagnostics GmbH, Mannheim, Germany); 0.50 μmol/l of each primer and 0.20 μmol/l of both anchor and sensor probe (TIB MOLBIOL, Berlin, Germany). After adding 1.0 μl genomic DNA to the PCR mixture, the total volume is 10 μl. The PCR protocol included the following steps: a) denaturation for ten minutes at 95°C b) 45 cycles at 95°C for ten seconds; 55°C for ten seconds and 72°C for five 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 then cooling to 40°C (20°C/second), holding at 40°C for one minute and then heating slowly to 85°C at 0.2°C/second. During the slow heating procedure of the melting curves, fluorescence was measured continuously to monitor the dissociation of the fluorophore-labelled detection probe. The fluorescence signals were then converted to melting peaks by plotting the negative derivative of the fluorescence with respect to temperature *versus* temperature (-dF2/dT *versus* T). The melting point of the sensor probe was 61°C when hybridised to the wild type CYP3A7*1 allele and 56.5°C when hybridised to the CYP3A7*1C variant allele. A heterozygous carrier of the CYP3A7*1C variant allele shows both melting points (Figure 9.1). The real-time PCR FRET assay for the CYP3A7*1C variant allele was validated by sequencing a heterozygote sample and a wild type sample of the CYP3A7*1C variant allele according to a direct sequence procedure performed with the capillary sequencer ABI 3100 (Applied Biosystems, Fostercity, USA) using the Bridge version 1.1 sequence kit (Applied Biosystems, Fostercity, USA).

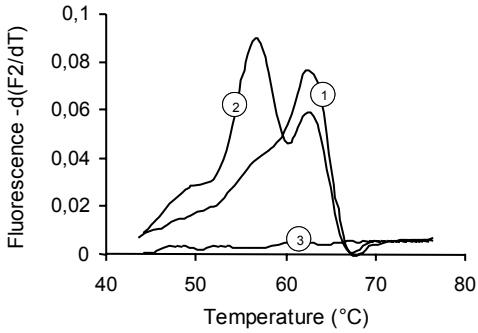

Figure 9.1 Genotyping of the CYP3A7 T-188G polymorphism with allele specific fluorescent probes by derivative melting curve plots. The derivative melting curves are read in channel 2 for the detection of the CYP3A7 fragments. The derivative melting curve is plotted for a sample homozygous for the –188G allele (1; Tm 61.0°C); and a heterozygous sample (2; Tm 56.5°C and 61.0°C). Melting analysis of a no template control was also performed (3).

Statistical analysis

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 CYP3A7*1C polymorphism were tested against Hardy-Weinberg equilibrium by the Pearson's goodness-of-fit test. For analysis of the daily tacrolimus dose (mg/kg/day), dose-normalised (dn)C_0 (ng/ml per mg/kg), dose-normalised (dn)$AUC_{0\text{-}12}$ (ng × hr/ml per mg/kg), dose-normalised (dn)C_{max} (ng/ml per mg/kg) and T_{max} (hr), groups were compared using non parametric statistical tests. To compare the two groups we used the Mann-Whitney test. *P* values less than 0.05 were considered statistically significant. All values are expressed as median and range unless stated otherwise.

Results

In total 70 Caucasian renal transplant recipients divided over 33 early (group I) and 37 late (group II) posttransplant recipients and 103 Chinese renal transplant recipients (group III) were genotyped for the CYP3A7*1C variant allele. In group I and II, respectively one and three heterozygous carriers were found for the CYP3A7*1C variant allele while no CYP3A7*1C variant allele was found in the Chinese renal transplant recipients of group III. The previous findings result in an allele frequency for the CYP3A7*1C variant allele of 1.5%, 4.1% and 0.0% in group I, II and III, respectively. The allele frequencies found in the Caucasian renal transplant recipients are in line with previous studies[20,35]. Since no CYP3A7*1C variant allele was found in the 103 Chinese renal transplant recipients examined, it is likely that the frequency of this CYP3A7 polymorphism in the Chinese population is very low. Renal transplant recipient groups I and II showed no significantly different distribution of the CYP3A7*1C variant allele from that predicted by the Hardy-Weinberg equilibrium ($P > 0.05$). Moreover, no significant difference in the allele frequency for the CYP3A7*1C variant allele was found between the two Caucasian renal transplant recipient groups examined. All heterozygous carriers for the CYP3A7*1C variant allele were homozygous carrier of the CYP3A4*1A and CYP3A5*3 allele. Since one renal transplant recipient in group I was carrier of the CYP3A7*1C variant allele, a statistical analysis was only performed with the 37 Caucasian renal transplant recipients that were included in group II. Renal transplant recipients carrying a CYP3A7*1C variant allele showed no significant lower dnC_0 levels (404 *versus* 264 ng/ml per mg/kg; Mann-Whitney $P = 0.290$), dn$AUC_{0\text{-}12}$ (6376 *versus* 4539 ng × hr/ml per mg/kg; Mann-Whitney $P = 0.504$), dnC_{max} (1179 *versus* 743 ng/ml per mg/kg; Mann-Whitney $P = 0.469$) or T_{max} (1.00 *versus* 1.00 hr; Mann-Whitney $P = 0.312$), compared to the renal transplant recipients carrying a wild type CYP3A7*1 allele.

Additionally, Figure 9.2 illustrates that heterozygote carriers of the CYP3A7*1C variant allele showed no difference in the 12 hour pharmacokinetic tacrolimus profile.

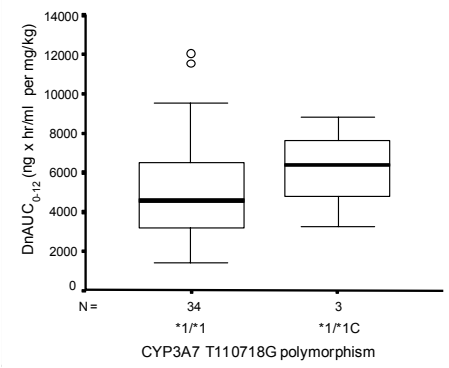

Figure 9.2 The influence of the CYP3A7 T-188G polymorphism on the 12 hour area under the time tacrolimus concentration curve (AUC_{0-12}) in 37 late Caucasian posttransplant patients. The boxplot of the dose-normalized AUC_{0-12} is clustered according to the CYP3A7 genotype. *P* values for the pairwise comparisons of each genotype are given. The open circles indicate an outlier value at more than 1.5 box lengths above the box.

Highlights

- Tacrolimus is predominantly metabolised into 13-O-demethyltacrolimus by cytochrome P450 3A (CYP3A) iso-enzymes. Recently, a study described that the catalytic efficiency for CYP3A5 with tacrolimus is 60% higher than that for CYP3A4. The contribution of the CYP3A7*1C allele, responsible for a higher CYP3A7 expression, to the tacrolimus metabolism in transplant patients is not elucidated.
- The CYP3A7*1C variant allele consists of seven different polymorphisms located in a 60 basepair promoter region of CYP3A7. The proximal everted repeat 6 motif present in the CYP3A7*1C variant allele binds with a higher affinity the pregnane X receptor and the constitutively activated receptor than the CYP3A7*1 allele, which results in a higher CYP3A7 expression for individuals carrying a CYP3A7*1C allele.
- A novel real-time PCR FRET assay on the LightCycler is described for the CYP3A7*1C allelic variant. This real-time PCR FRET assay is validated by sequencing a heterozygous sample and a homozygous wild type DNA sample.
- In total 70 Caucasian and 103 Chinese renal transplant recipients are genotyped for the CYP3A7*1C variant allele. The Caucasian renal transplant recipients were divided in 33 early and 37 late post transplant patients. The allele frequency for the CYP3A7*1C variant allele in the Caucasian and the Chinese renal transplant recipients is 2.8% and 0.0%, respectively.

- The CYP3A7*1C variant allele is not associated with any dose-normalized pharmacokinetic parameter (C_0, AUC_{0-12}, C_{max}) recorded in the late posttransplant recipient group. Our findings indicate that a higher CYP3A7 expression has no influence on the tacrolimus metabolism.

Discussion

The initial and maintenance dosage regime of tacrolimus in transplant patients is difficult to assess due to its highly variable pharmacokinetic characteristics and narrow therapeutic range. Although several clinical studies[16,17] demonstrated that the variation in the pharmacokinetic characteristics of tacrolimus may be explained by the CYP3A5*3 variant allele, there is still a large variation within the patient groups carrying a different CYP3A5 genotype for this CYP3A5*3 variant allele. Sattler *et al.* [1] suggested that other members of the CYP3A subfamily might be involved than CYP3A4. Bader *et al.*[36] later confirmed that CYP3A5 plays an important role in the 13-O-demethylation of tacrolimus. More recently, Kamden *et al.*[23] demonstrated with *in vitro* experiments that the demethylation of tacrolimus into 13-O-demethyltacrolimus in hepatocytes is mainly performed by CYP3A4 and CYP3A5. Although Kamden *et al.*[23] suggested that CYP3A7 played no role in the tacrolimus metabolism *in vivo*, clinical studies confirming these results are still lacking. Sim *et al.*[32] found that the CYP3A7 protein expression contributes 9 to 36% of the total CYP3A levels in 10% of 59 adult livers. Additionally, they demonstrated that the CYP3A7*1C variant allele is partly responsible for these higher CYP3A7 expression levels. The CYP3A7*1C allele contains the proximal everted repeat 6 (ER6) motif of CYP3A4 which implicates that the pregnane X receptor (PXR) and the constitutively actived receptor (CAR) bind with a higher affinity to the CYP3A7*1C allele than to the CYP3A7*1 allele. Consequently only the promoter constructs of CYP3A7 containing this CYP3A4-ER6 motif, CYP3A7*1C, will be transactivated by PXR and CAR. Previously, Burk *et al.*[35] reported that the presence of the ER6 motif of CYP3A4 mediates the high expression of CYP3A7 in individuals carrying a CYP3A7*1C allele. Within the late posttransplant recipient group, no significant effect on both the pharmacokinetic tacrolimus parameters and the daily tacrolimus dose was observed between carriers of the wild type CYP3A7*1 allele or the CYP3A7*1C variant allele. Previously, Kamden *et al.*[23] demonstrated with *in vitro* experiments that CYP3A7 played a minor role in the tacrolimus metabolism. Our findings indicate that a higher CYP3A7 protein expression *in vivo* caused by the CYP3A7*1C variant allele has no significant effect on the tacrolimus metabolism. In conclusion, in the present study a reliable and reproducible real-time PCR FRET assay is described for the CYP3A7*1C variant allele. Additionally, we found that the CYP3A7*1C variant allele, responsible for higher CYP3A7 protein levels, has a (very) low frequency in a Caucasian and a Chinese renal transplant population and is not associated with the pharmacokinetic parameters of tacrolimus.

Outlook

In the last decade pharmacogenomics has gained considerable interest among several medical disciplines. Especially, the immunosuppressive drugs cyclosporin and tacrolimus are of special importance due to their highly variable pharmacokinetic characteristics and narrow therapeutic window. Cyclosporin and tacrolimus are substrates of the cytochrome P450 3A (CYP3A) family. Although CYP3A4 and CYP3A5 both show a great affinity for cyclosporin and tacrolimus, several clinical studies demonstrated that a part of the variation in the pharmacokinetic tacrolimus parameters can be explained by a single nucleotide polymorphism (CYP3A5 A6986G) present in the CYP3A5 gene. However, even within a group transplant patients carrying the same genotype for this CYP3A5 polymorphism, there is still a large variation in the pharmacokinetic tacrolimus parameters. To achieve a better individualized immunosuppressive therapy with a lower occurrence of subtherapeutic or toxic side effects of these immunosuppressive drugs, minimizing the variation in the pharmacokinetic tacrolimus parameters is certainly a prerequisite. Therefore, future studies are necessary to examine the role of other enzymes, transporters or transcriptional regulators involved in the tacrolimus metabolism and whether polymorphisms in these genes have an important impact on the pharmacokinetic parameters and thus on the daily dose requirement.

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