Chapter 10

Influence of allelic variants of uridine
5'-diphosphateglucuronosyltransferase (UGT)
2B7 on the tacrolimus exposure

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Abstract

Background and aim

Tacrolimus is predominantly demethylated into 13-O-demethyltacrolimus although identification of tacrolimus glucuronides demonstrates that conjugation also plays a role in the tacrolimus metabolism. Uridine 5'-diphosphateglucuronosyltransferase (UGT) 2B7 is identified as the most relevant member of the UGT family responsible for tacrolimus glucuronidation. Single nucleotide polymorphisms (SNPs) in the UGT2B7 gene may have a contribution on the interindividual variation of tacrolimus blood concentrations.

Materials and methods

A two time point sampling strategy is used to calculate the AUC_{0-12} for 103 Chinese renal transplant recipients. In the present study real-time polymerase chain reaction (PCR) fluorescence resonance energy transfer (FRET) assays were developed and used to determine the genotype of three UGT2B7 polymorphisms in Chinese renal transplant recipients. The effect of these UGT2B7 polymorphisms on both the dose-normalized area under the time tacrolimus concentration curve ($dnAUC_{0-12}$) and the daily tacrolimus dose is examined.

Results

The variant alleles of UGT2B7 polymorphisms G-79A, T-66C and C816T with an allele frequency of respectively, 0.0%, 5.8% and 29.6% showed no significant differences on both the $dnAUC_{0-12}$ and the daily tacrolimus dose compared to carriers of the wild type UGT2B7 alleles when the patients were categorized based on their CYP3A5 A6986G, ABCB1 G2677T/A and ABCB1 C3435T genotype.

Conclusion

Therefore, genotyping transplant patients for these UGT2B7 polymorphisms before using tacrolimus is irrelevant.

Introduction

Tacrolimus is usually administered orally and has a rather unpredictable and variable absorption rate. Therefore close monitoring of the tacrolimus blood levels is required to prevent both subtherapeutic and toxic blood concentrations. Although cytochrome (CYP) 3A enzyme polymorphisms and possibly also adenosine triphosphate-binding cassette B1 gene (ABCB1) polymorphisms clarify a considerable part of the variation in tacrolimus trough (C_0) levels[1-13], there is still a large inter-individual variation in the tacrolimus C_0 levels. The analysis of tacrolimus metabolites in humans has provided evidence not only for CYP3A catalysed metabolism but also for the formation of glucuronides[14]. Strassburg *et al.*[15] examined the glucuronidation activities of different uridine 5'-diphosphate glucuronosyltransferase (UGT) (sub)families for both tacrolimus and cyclosporin and found that UGT2B7 showed the highest glucuronidation activity in the human gastrointestinal tract. The UGT family, a group of proteins responsible for the glucuronidation of several endogene and exogene compounds in humans, is mainly present in the liver. However, UGTs are also found throughout the gastrointestinal tract, where they are an integral part of prehepatic first-pass metabolism. In addition, UGTs also work in the kidneys, brain, placenta and in several other locations in the human body[16-18]. The major function of glucuronidation is to increase the polarity of the target compound, a process which facilitates their detoxification and excretion. However, glucuronidation can also result in compounds which are biologically active or demonstrate increased toxicity. After glucuronidation, intestinal bacterial β-glucuronidases break down glucuronidation products and release the unconjugated drugs via enterohepatic recirculation. This "recycling" system slowly clears conjugated compounds and releases glucuronides for re-use[19-23]. Although Strassburg *et al.*[15] reported that hepatic and extra-hepatic glucuronidation may influence the therapeutic efficacy of immunosuppressants, the genetic influence of known UGT2B7 polymorphisms on the pharmacokinetic parameters of immunosuppressants has never been examined. In the present study, we have developed real-time polymerase chain reaction (PCR) fluorescence resonance energy transfer (FRET) assays for three UGT2B7 polymorphisms and examined whether the inter-individual variation in the tacrolimus blood levels can be explained more exactly by genotyping the transplant recipients for these UGT2B7 polymorphisms.

Materials and methods

Study populations

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_{0-12} value, which was kept at around 100 - 150 ng $\times$ hr/ml during the first 3 months. After three months the target AUC_{0-12} value was decreased at around 80 - 100 ng $\times$ hr/ml for long term maintenance. These AUC_{0-12} values were based on our previous study[24] 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_{0-12} according to the equation based strategy as described earlier: AUC_{0-12} = 16.2 + 2.4 $\times$ C2 + 5.9 $\times$ C4[24]. Dose-normalized AUC_{0-12} ($dnAUC_{0-12}$) were calculated by dividing the AUC_{0-12} 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.

UGT2B7 G-79A, T-66C and C816T primers and probes

The real-time PCR fluorescence resonance energy transfer (FRET) assays were designed and optimised in our laboratory. Genotyping for the UGT2B7 G-79A and T-66C polymorphisms was performed using the primers F30: 5'-TTg CAT gTC CAT ACA AgA TCC T-'3 (sense; 1150–171) and R30: 5'-ATT TCA CAg ACA TCC Tgg TgC-

'3 (anti-sense;1432–412) to amplify a 273 bp part from the UGT2B7 gene which covers both G-79A and T-66C polymorphisms in the promoter region of the UGT2B7 gene (Genbank acces no: AF282881). Detection of the UGT2B7 G-79A polymorphism was carried out using the anchor probe 79A30: 5'-Tgg CAA AgA gAC AAA TgA Agg TAA ATg ATC TgT TC-3' (anti-sense; 1265 - 231) which was labelled at the 5'-end with LCRed640 and phosphorylated at the 3'- end to block extension from the sensor probe 79P30: 5' CAT TAA ATC AA<u>C</u> AgT CTg AgC ATg Tgg 3' (anti-sense; 1293-267) which is complementary to the UGT2B7 –79G 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. Detection of the UGT2B7 T-66C polymorphism was carried out using the anchor probe 66A30: 5'-Agg gTT ACA TTT TAA CTT CTT ggC TAA TTT ATC TT- 3' (sense; 1283-316) which was labelled at the 5'-end with LCRed640 and phosphorylated at the 3'- end to block extension from the sensor probe 66P30: TTg ATT TAA TgA <u>C</u>AT TgT ATg TAC TTT gAC TTA T (sense; 1283-316) which is complementary to the UGT2B7 -66C 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 11 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 45°C (20°C/second) holding at 45°C for one minute and then heating slowly to 75°C at 0.2°C/second. Genotyping for the UGT2B7 C816T polymorphism was performed using the primers F31: 5'-ACC TTT TTT TTT TCT ATT CCT gT-'3 (sense; 1-23) and R31: 5'-CAA AAT AAA ACC AAC AAA AgT ATg-'3 (anti-sense; 204-181) to amplify a 204 bp part from the UGT2B7 gene which covers the T815C and C816T polymorphisms in exon 1 (Genbank acces no: AF282881). Detection of the UGT2B7 C816T polymorphism was carried out using the anchor probe A31: 5'-CCA AAT gTT gAT TTT gTT ggA ggA CTC C-3' (sense; 119-146) which was labelled at the 5'-end with LCRed640 and phosphorylated at the 3' end to block extension from the sensor probe, P31: 5'-gAA TTT TCA gTT TCC <u>AT</u>A TCC ACT CTT-'3 which is complementary to the UGT2B7 816T 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 assay 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 eight 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 62°C (0.5°C/second) holding at 62°C for one minute and finally cooling to 45°C for 0 seconds (20°C/second) and then heating slowly to 80°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 UGT2B7 polymorphisms. The control samples that are sequenced are also 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 UGT2B7 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$_{0-12}$ (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 for the UGT2B7 G-79A, T-66C, C816T polymorphisms

The melting point of the sensor probe for the UGT2B7 G-79A polymorphism was 59.5°C when hybridised to the UGT2B7 -79G allele and 57.5°C when hybridised to the UGT2B7 -79A allele, whereas for the UGT2B7 T-66C polymorphism, the melting point of the sensor probe was 52°C when hybridised to the UGT2B7 -66T allele was and 59°C when hybridised to the UGT-66C allele. A heterozygote sample for the UGT2B7 G-79A and T-66C polymorphisms showed both melting peaks (Figure 10.1A and 10.1B). In addition, for the UGT2B7 C816T polymorphism, the melting point of the sensor probe was 62.5°C for the UGT2B7 816T allele and 57.5°C when hybridised to the UGT2B7 816C allele. A heterozygote sample for the UGT2B7 C816T polymorphism showed both

melting peaks (Figure 10.1C). The presence of the expected polymorphisms was confirmed by sequencing the allelic variants of UGT2B7 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). A heterozygote DNA sample was found for the UGT2B7 G-79A polymorphism among anonymized healthy Caucasian volunteers using a real-time PCR FRET assay for this UGT2B7 G-79A polymorphism. Additionally, this control sample was sequenced to confirm that this sample is heterozygous for the UGT2B7 G-79A polymorphism.
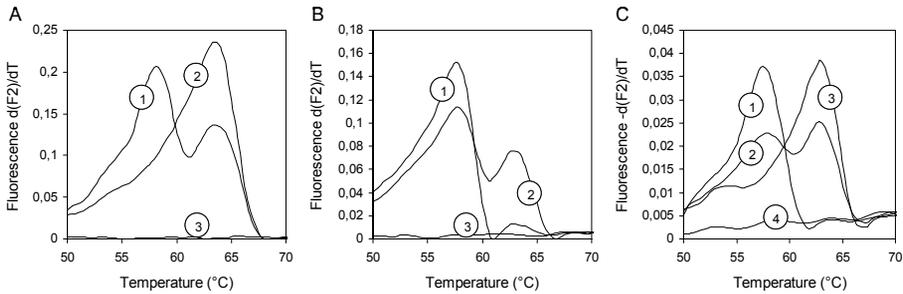

Figure 10.1 Genotyping of UGT2B7 G-79A, T-66C and C816T with allele specific fluorescent probes by derivative melting curve plots. The derivative melting curves are read in channel 2 for the detection of the two UGT2B7 fragments. A. The derivative melting curve is plotted for a sample homozygous for the UGT2B7 -79G allele (1; Tm 59.5°C) and a heterozygous sample (2; Tm 57.5°C and 59.5°C). B. The derivative melting curve is plotted for a sample homozygous for the UGT2B7 -66T allele (1; Tm 52°C) and a heterozygous sample (2; Tm 52°C and 59°C). C. The derivative melting curve is plotted for a sample homozygous for the UGT2B7 816C allele (1; Tm 57.5°C); a sample homozygous for the UGT2B7 816T allele (2; Tm 62.5°C) and a heterozygous sample (3; Tm 57.5°C and 62.5°C) Melting analysis of a no template control was also performed for all assays (3 or 4).

Influence of the different UGT2B7 allelic variants on the pharmacokinetic tacrolimus profiles

Table 10.1 illustrates the demographic characteristics of 103 Chinese renal transplant recipients included in the present study. After genotyping 103 renal transplant patients, no variant allele was found for the UGT2B7 G-79A polymorphism whereas the frequency of the UGT2B7 -66TT genotype was found to be 88% (n = 91) and for the UGT2B7 -66TC the frequency was 12% (n = 12). In addition, 49% (n = 51) of the patients were carrier of the UGT2B7 816CC genotype while 42% (n = 43) was heterozygous carrier of the UGT2B7 816CT genotype and 9% (n = 9) homozygous for the UGT2B7 816TT variant allele. The allele frequencies of the Chinese renal transplant patients examined were found to be 0.0% (-79A), 5.8% (-66C) and 29.6% (816T), which is not in line with those published in previous studies[25,26]. According to the Pearson's

goodness-of-fit test the genotype frequencies of these UGT2B7 polymorphisms differed not significantly from the Hardy-Weinberg equilibrium ($P > 0.05$).

Table 10.1   Demographic characteristics of the renal transplant recipients.

Demographic characteristics	
Gender (male/female)	58/45
Age (years, mean $\pm$ SD, (range))	43.1 $\pm$ 11.1 (21-77)
Weight (kg, mean $\pm$ SD, (range))	62.5 $\pm$ 11.4 (37.4-87.8)
Tacrolimus dose (mg/kg/day, mean $\pm$ SD, (range))	0.067 $\pm$ 0.032 (0.01-0.18)
Tacrolimus conc. 2 hour post dose (ng/ml, mean $\pm$ SD, (range))	13.5 $\pm$ 4.30 (5.7-29.4)
Tacrolimus conc. 4 hour post dose (ng/ml, mean $\pm$ SD, (range))	8.7 $\pm$ 2.60 (2.3-16.4)
AUC_{0-12} (ng $\times$ hr/ml, mean, (range))	100.3 (47.8-183.5)
$DnAUC_{0-12}$ (ng $\times$ hr/ml per mg/kg, mean, (range))	1940 (300-11125)
Use of azathiopurine/mycophenolate mofetil	78/25
Current steroid dose (mg, mean $\pm$ SD, (range))	6.98 $\pm$ 2.37 (4-25)
Time since transplantation (days, mean, (range))	977 (133-4982)
Serum creatinine (µmol/l, mean $\pm$ SD, (range))	156 $\pm$ 76 (62-462)

A significant higher $dnAUC_{0-12}$ (1685 *versus* 1276 ng $\times$ hr/ml per mg/kg; Mann-Whitney, $P = 0.026$) was observed for carriers of the UGT2B7 -66TT genotype compared to carriers of the UGT2B7 -66TC genotype while no significant difference (Kruskal Wallis, $P = 0.817$) was observed between carriers of a different UGT2B7 C816T genotypes. This is illustrated in Figure 10.2A and 10.3A. However, previously we demonstrated in the same renal transplant patient group an important impact of the CYP3A5 A6986G polymorphism whereas a minor association was reported between both ABCB1 polymorphisms G2677T/A and C3435T and the tacrolimus blood concentrations[13]. To exclude influence of one of these polymorphisms, the renal transplant recipients were based on their CYP3A5 and ABCB1 G2677T/A, C3435T genotype categorized in order to examine solely the effect of the two UGT2B7 T-66C and C816T polymorphisms on the $dnAUC_{0-12}$ and the daily tacrolimus dose. As is illustrated in Figure 10.2B - 10.2D, there is no significant influence of UGT2B7 T-66C polymorphism on the $dnAUC_{0-12}$ of tacrolimus after categorizing the patients for respectively CYP3A5 A6986G, ABCB1 G2677T/A and ABCB1 C3435T genotype. Additionally, after categorizing the patients, Figures 10.3B - 10.3D illustrate that there is, as expected, also no significant effect of the UGT2B7 C816T polymorphism on the $dnAUC_{0-12}$ of tacrolimus. Regarding the daily tacrolimus dose requirement similar results were obtained for both UGT2B7 polymorphisms.
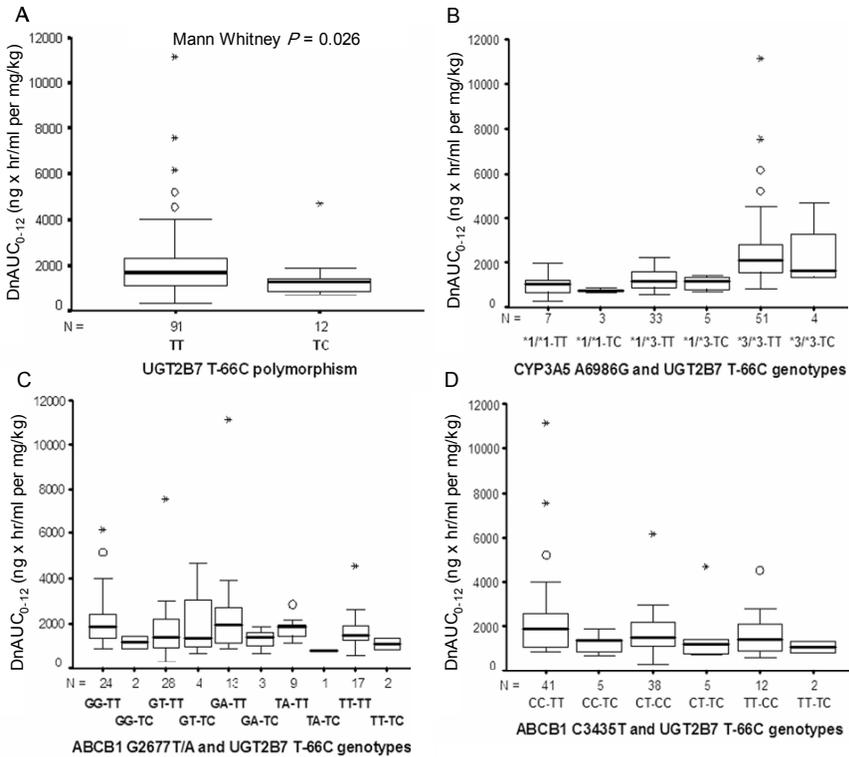
Figure 10.2 Influence of the UGT2B7 T-66C and C816T genotypes on the area under the time – tacrolimus concentration curve (AUC_{0-12}) that were recorded in 103 stable Chinese renal transplant recipients. Figure 10.2 illustrates the boxplots of the distribution of the dose normalized AUC_{0-12} (ng × hr/ml per mg/kg) clustered according to the (A) UGT2B7 T-66C genotype, (B) the combination of respectively CYP3A5 A6986G and UGT2B7 T-66C genotypes, (C) ABCB1 G2677T/A and UGT2B7 T-66C genotypes and (D) ABCB1 C3435T and UGT2B7 T-66C genotypes. Open circles indicate an outlier value at more than 1.5 box lengths above the box, while asterics indicate an extreme value at more than 3 box lengths above the box.
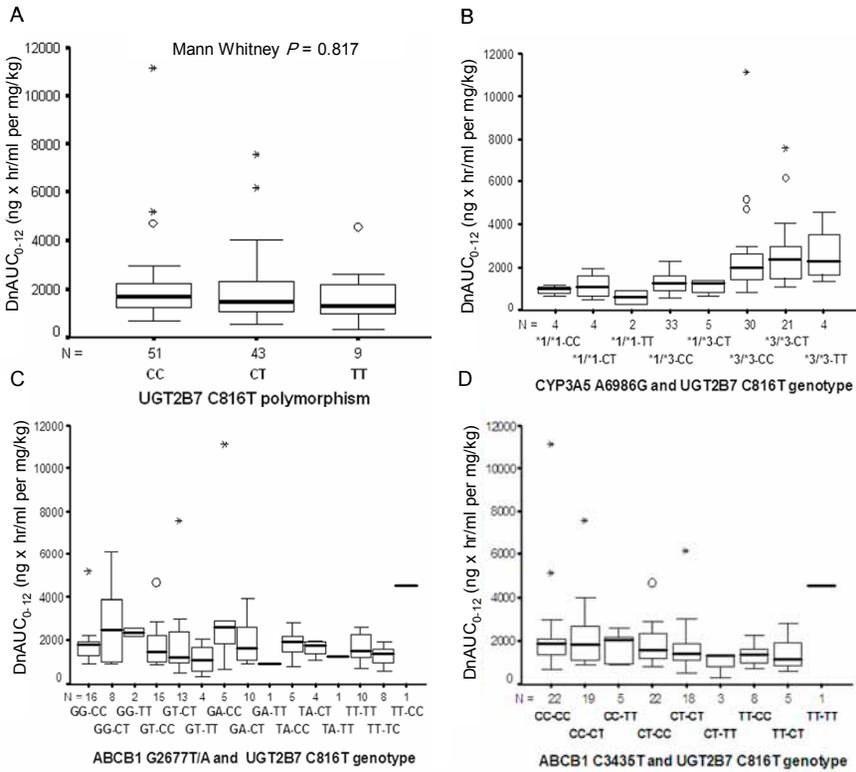
Figure 10.3 This figure illustrates the boxplots of the distributions of the AUC_{0-12} (ng × hr/ml per mg/kg) clustered according to the (A) UGT2B7 C816T genotype, (B) the combination of respectively CYP3A5 A6986G and UGT2B7 C816T genotypes, (C) ABCB1 G2677T/A and UGT2B7 C816T genotypes and (D) ABCB1 C3435T and UGT2B7 C816T genotypes. Open circles indicate an outlier value at more than 1.5 box lengths above the box, while asterics indicate an extreme value at more than 3 box lengths above the box.

Highlights

- Tacrolimus is predominately demethylated into 13-O-demethyltacrolimus by CYP3A iso-enzymes. However, identification of tacrolimus glucuronides demonstrate that besides demethylation also conjugation influences the tacrolimus metabolism.
- Uridine 5'-diphosphate glucuronosyltransferase (UGT) 2B7 mainly present in the liver and intestines is the major UGT isoform responsible for the glucuronidation of tacrolimus.
- Several single nucleotide polymorphisms (SNPs) identified in the UGT2B7 gene may besides the cytochrome 3A (CYP3A) and adenosine diphosphate-binding

cassette B1 gene (ABCB1) polymorphisms also explain the inter-individual differences in the tacrolimus pharmacokinetics.

- A two time point sampling strategy is used to calculate the area under the time tacrolimus concentration curve (AUC_{0-12}) and the dose-normalised (dn) AUC_{0-12} are correlated with the corresponding UGT2B7 genotypes in 103 Chinese renal transplant recipients.

- After eliminating the influence of the CYP3A5 and ABCB1 polymorphisms, no significant differences were found in the $dnAUC_{0-12}$ and daily tacrolimus dose between carriers of different UGT2B7 genotypes.

- Our findings indicate that SNPs in the UGT2B7 gene have no significant impact on the tacrolimus blood concentration and tacrolimus dosage. Therefore, we conclude that polymorphisms in the UGT2B7 gene have no important contribution on the interindividual variability of the tacrolimus pharmacokinetics.

Discussion

Previously, we reported a strong allele-dependent effect between the CYP3A5 A6986G polymorphism and both the $dnAUC_{0-12}$ and the daily tacrolimus dose, while a less pronounced effect was found for the ABCB1 G2677T/A and C3435T polymorphisms in this Chinese renal transplant recipient population[13]. However, there is still variation in the tacrolimus blood concentrations among the renal transplant patients carrying the same CYP3A5 and ABCB1 genotypes. Although identification of tacrolimus[14] glucuronides demonstrates that conjugation also plays a role in the metabolisation of this immunosuppressant, less information is available regarding the UDP-glucuronosyltransferases (UGT) involved in the tacrolimus metabolism. Strassburg *et al.*[15] found that UGT2B7 is the member of the UGT family that is responsible for the tacrolimus glucuronidation. Large inter-individual differences in the mRNA expression levels of UGT2B7[27], which could be caused to a degree by polymorphisms, may possibly explain a part of the inter-individual pharmacokinetic variation of tacrolimus. A common polymorphism in the UGT2B7 gene (UGT2B7 C816T) has been found in Caucasians and Asians[26]. Although one study[28] found no remarkable function difference between the different carriers of the UGT2B7 C816T polymorphism, another study[29] recently reported that UGT2B7 816T variant allele showed significantly higher morphine-6-O-glucuronide/morphine ratios than that with the wild type UGT2B7 allele. More recently, Duguay *et al.*[30] discovered the functional polymorphism –79A in the UGT2B7 promoter with significant impact on the promoter activity while the variation at position -66 resulted in a non significant alteration of promoter activity compared to the wild type promoter. However, no significant difference in the ratios of morphine-6-glucuronide/morphine and morphine-3-glucuronide/morphine were found between 6 heterozygous carriers of the UGT2B7 –79A variant allele and 175 wild type carriers among cancer patients receiving long term oral morphine. Although Strassburg *et al.*[15]

demonstrated that tacrolimus glucuronidation is performed exclusively by UGT2B7, the present study demonstrated that the UGT2B7 polymorphisms T-66C and C816T have no influence on the $dnAUC_{0-12}$ of tacrolimus and the daily tacrolimus dose when categorizing patients in different groups for the CYP3A5 A6986G, ABCB1 G2677T/A and C3435T genotype. Since tacrolimus is predominately demethylated into 13-O-demethyltacrolimus it seems that tacrolimus glucuronidation plays a neglectable role in the tacrolimus metabolisation process.

Outlook

The calcineurin inhibitor tacrolimus is generally used as an immunosuppressive drug in solid organ transplantations. However, it is difficult to maintain an appropriate blood concentration of tacrolimus due to its narrow therapeutic index and significant intra- and inter-individual variety. Tacrolimus is predominantly metabolized into 13-O-demethyltacrolimus by CYP3A4 and CYP3A5 in the liver and intestines, and subsequently pumped out by P-glycoprotein, an ATP dependent efflux pump, encoded by the adenosine triphosphate-binding cassette B1 (ABCB1) gene. Therefore, these proteins are considered to have a major influence on the first-pass effect in the liver and the intestines and on the variation in tacrolimus pharmacokinetics. Several studies already reported the impact of some CYP3A and ABCB1 polymorphisms on the tacrolimus trough (C_0) levels, pharmacokinetic tacrolimus parameters and the daily tacrolimus dose in transplant patients. However, identification of tacrolimus glucuronides demonstrates that conjugation also plays a role in the tacrolimus metabolism. Uridine 5'-diphosphate glucuronosyltransferase (UGT) 2B7 is the most important UGT isoform responsible for the glucuronidation of tacrolimus. Single nucleotide polymorphisms (SNPs) in the UGT2B7 gene may have an effect on the inter-individual variation in the tacrolimus pharmacokinetics. In the present study we calculated an area under the time tacrolimus concentration curve (AUC_{0-12}) based on a two time point sample strategy for 103 Chinese renal transplant recipients and demonstrated that the UGT2B7 polymorphisms T-66C and C816T have no significant role on the variation of the dose-normalised AUC_{0-12} and the daily tacrolimus dose. Therefore, genotyping renal transplant patients for these UGT2B7 polymorphisms will not contribute to less variation in the tacrolimus blood concentrations and a better individualized immunosuppressive therapy.

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