Influence of ABCB1 polymorphisms and haplotypes on tacrolimus nephrotoxicity and dosage requirements in children with liver transplant

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Currently tacrolimus is the mainstay of immunosuppression for most children undergoing liver transplantation (LT).
- The clinical use of this agent, however, is complicated by its various adverse effects (mainly nephrotoxicity), its narrow therapeutic-index and considerable pharmacokinetic variability.
- The low and variable oral bioavailability of tacrolimus is thought to result from the action of the multidrug efflux-pump P-glycoprotein, encoded by the *ABCB1* gene.

WHAT THIS STUDY ADDS

- A significant association between *ABCB1* genetic polymorphisms and tacrolimus-associated nephrotoxicity in paediatric patients following LT is reported for the first time. Genotyping such polymorphisms may have the potential to individualize better initial tacrolimus therapy and enhance drug safety.
- The long-term effect of *ABCB1* polymorphisms on tacrolimus trough concentrations were investigated up to 5 years post-transplantation. A significant effect of intestinal P-glycoprotein genotypes on tacrolimus pharmacokinetics was found at 3 and 4 years post-transplantation suggesting that the effect is maintained long term.

AIMS

The aim of this study was to investigate the influence of genetic polymorphisms in *ABCB1* on the incidence of nephrotoxicity and tacrolimus dosage-requirements in paediatric patients following liver transplantation.

METHODS

Fifty-one paediatric liver transplant recipients receiving tacrolimus were genotyped for *ABCB1* C1236>T, G2677>T and C3435>T polymorphisms. Dose-adjusted tacrolimus trough concentrations and estimated glomerular filtration rates (EGFR) indicative of renal toxicity were determined and correlated with the corresponding genotypes.

RESULTS

The present study revealed a higher incidence of the *ABCB1* variant-alleles examined among patients with renal dysfunction ($\geq 30\%$ reduction in EGFR) at 6 months post-transplantation (1236T allele: 63.3% vs 37.5% in controls, $P = 0.019$; 2677T allele: 63.3% vs. 35.9%, $p = 0.012$; 3435T allele: 60% vs. 39.1%, $P = 0.057$). Carriers of the G2677->T variant allele also had a significant reduction (%) in EGFR at 12 months post-transplant (mean difference = 22.6%; $P = 0.031$). Haplotype analysis showed a significant association between T-T-T haplotypes and an increased incidence of nephrotoxicity at 6 months post-transplantation (haplotype-frequency = 52.9% in nephrotoxic patients vs 29.4% in controls; $P = 0.029$). Furthermore, G2677->T and C3435->T polymorphisms and T-T-T haplotypes were significantly correlated with higher tacrolimus dose-adjusted pre-dose concentrations at various time points examined long after drug initiation.

CONCLUSIONS

These findings suggest that *ABCB1* polymorphisms in the native intestine significantly influence tacrolimus dosage-requirement in the stable phase after transplantation. In addition, *ABCB1* polymorphisms in paediatric liver transplant recipients may predispose them to nephrotoxicity over the first year post-transplantation. Genotyping future transplant recipients for *ABCB1* polymorphisms, therefore, could have the potential to individualize better tacrolimus immunosuppressive therapy and enhance drug safety.

Introduction

Liver transplantation (LT) in children is now a highly successful treatment for liver disease with expected 1- and 5-year survival rates of >85% [1]. This can be attributed to refinements in patient selection, surgical techniques and intensive care management, combined with the evolution of more effective immunosuppressive regimens [2]. Tacrolimus was developed in the mid-1990s as a potent immunosuppressive agent which prevented acute rejection following solid-organ transplantation [3]. Currently, tacrolimus is the mainstay of immunosuppression for most children undergoing LT [4]. The clinical use of this agent, however, is complicated by its highly variable pharmacokinetics [5], a narrow therapeutic index [6] and many side effects; the commonest of these, nephrotoxicity, occurs in as many as half the patients treated with this drug [5].

The variability in the pharmacokinetics of tacrolimus is determined to a large extent by differences in bioavailability. The oral bioavailability is poor and varies widely between patients from 4% to 89% (with a mean value of about 25%) [7]. In recent years much research has focused on the possible causes of these interindividual differences in the pharmacokinetics of tacrolimus. It has become apparent that the biological activity of the permeability-glycoprotein (P-gp) plays an important role in this respect [8].

P-gp is one of the ATP-binding cassette transporters that actively transports its substrates out of cells by the driving force of ATP [9]. P-gp is encoded by the human *ABCB1* gene (ATP binding cassette, subfamily B), also called *MDR1* (multidrug resistance transporter) gene [10]. Physiologically, P-gp is present in the small intestine, liver, kidney, adrenal gland and pancreas [8]. Efflux activity associated with P-gp, therefore, reduces the intestinal absorption of orally administered drugs while enhancing their biliary excretion through the liver and tubular excretion in the kidney [10]. Therefore, it is expected that variation in the intestinal expression of P-gp affects tacrolimus bioavailability and its resultant side effects, particularly renal dysfunction.

P-gp polymorphisms have been extensively studied, following the initial work of Hoffmeyer *et al.* [11] and several detailed reviews are available [10, 12–14]. A synonymous single nucleotide polymorphism (SNP) in exon 26, 3435C>T, has been recognized to play a role in intestinal drug transport [11]. However, the mechanism has not yet been identified. Homozygous individuals for this frequent variation had a lower intestinal P-gp expression. The prevalence of this polymorphism is high, with allele frequency of about 45–50% in Caucasians, but is much less common in Africans and African-Americans [15]. This polymorphism was shown to be associated by linkage disequilibrium with two other SNPs, 2677G>T (exon 21) and 1236C>T (exon 12) [16], the first of which is responsible for a substitution in the amino acid sequence (Ala893Ser).

When the possible genotypic combinations of *ABCB1* polymorphisms in exons 12, 21, 26 were examined, more than 50% of the studied individuals appeared to have strong linkage disequilibrium at the three loci. Of the eight possible haplotypes, only two occurred with high frequency (C-G-C and T-T-T) [17].

No published studies have investigated the impact of these polymorphisms on the incidence of renal toxicity in paediatric patients following LT. Furthermore, studies which assessed the influence of these three polymorphisms in the *ABCB1* gene on tacrolimus pharmacokinetics (mainly tacrolimus blood concentration) have led to conflicting results [6]. One major limitation of these studies, was the use of single polymorphisms rather than haplotypes, which generally provide more information than do individual SNPs [17].

The aim of the present study was to investigate the association of the different haplotypes (and individual SNPs) in the *ABCB1* gene with the incidence of nephrotoxicity in children undergoing LT. In addition, the influence of the most frequent haplotypes of *ABCB1* on tacrolimus trough concentrations and dosage requirements were investigated.

Methods

Study subjects

A total of 51 paediatric patients who underwent LT at the Liver Unit, Children's Hospital NHS Trust, Birmingham, UK were enrolled in this study between December 2006 and June 2007. Children were eligible for the study if they had been maintained on tacrolimus-based immunosuppressive therapy for at least 6 months. Data were collected retrospectively from the Liver Unit database, patient case notes and clinical chemistry records. The demographic details and clinical characteristics of the study subjects are shown in Table 1. The racial breakdown was 38 Caucasian, 10 Asian and 3 Black Caribbean.

The basic immunosuppressant regimen consisted of tacrolimus with low dose steroids. Tacrolimus was administered orally twice daily at an initial dose of 0.075 mg kg⁻¹ body weight. The dose of tacrolimus was adjusted to achieve target concentrations on the basis of whole blood trough concentrations measured about 12 h after the evening dose using enzyme immunoassay (Abbot IMx assay, Abbot Laboratories Ltd., Maidenhead, Berkshire, UK). Target trough concentrations were adjusted according to an established protocol, aiming for higher concentrations in the early post-transplant period.

Study design

One blood sample (1 ml) per patient was obtained for genotyping the *ABCB1* gene. These samples were collected in EDTA tubes and frozen at –20°C until genotyped.

Data were also collected regarding age, gender, weight and height over the period since transplantation. The daily dose (mg) of tacrolimus was recorded and the weight-adjusted tacrolimus dosage ($\text{mg kg}^{-1} \text{ day}^{-1}$) was calculated for each data point. Tacrolimus trough blood concentrations (ng ml^{-1}), collected approximately 12 h after a tacrolimus dose, were recorded retrospectively for samples taken at 0.5, 1, 2, 3, 4 and 5 years post-transplantation. For multiple samples taken within the same period, the mean tacrolimus trough concentration was calculated. Dose-adjusted trough concentrations were calculated for each patient and expressed as concentrations : dose (C : D) ratio.

Serum creatinine concentrations ($\mu\text{mol l}^{-1}$) recorded for each patient were used to estimate renal glomerular filtration rates pre-transplant and at 3, 6 and 12 months post-transplant using the modified Counahan-Barrat formula [Estimated glomerular filtration rate (EGFR; expressed as $\text{ml min}^{-1} 1.73 \text{ m}^{-2}$) = height (cm) $\times$ 40/creatinine ($\mu\text{mol l}^{-1}$)].

Table 1

The demographic and clinical characteristics of the study population

Parameter	
Demographics (n = 51)*	
Gender (male : female)	27 : 24 (53% : 47%)
Age [years, median (range)]	2 (0.6–16)
Weight [kg, median (range)]	11.9 (5.3–81)
Height [cm, median (range)]	82 (60–180)
Serum creatinine [$\mu\text{mol l}^{-1}$, median (range)]	31 (13–92)
EGFR [$\text{ml min}^{-1} 1.73 \text{ m}^{-2}$, median (range)]	109 (39–217)
Pharmacokinetic data of tacrolimus (n = 49)†	
Number of samples	397
Daily dose [mg day^{-1} , median (range)]	2.0 (0.4–12)
Weight-adjusted daily dose [$\text{mg kg}^{-1} \text{ day}^{-1}$, median (range)]	0.1 (0.02–0.98)
Trough concentration [ng ml^{-1} , median (range)]	5.3 (1.2–13.8)
C : D ratio [$\text{ng ml}^{-1} \text{ mg}^{-1} \text{ kg}^{-1}$, median (range)]	49.6 (5.7–155.9)

*Demographic parameters are based on data from all patients at time of liver transplantation. †Pharmacokinetic parameters are based on data collected during 5 years post-transplant.

EGFR, estimated glomerular filtration rate (using Counahan-Barrat formula).

[18]. Tacrolimus-related nephrotoxicity was defined as a 30% reduction in the EGFR compared with the pre-transplant baseline.

Ethical considerations

The study was approved by the National Health Services Office for Research Ethics Committees in England (NHS RECs). Children were included in the study only after their parents or guardians had been fully informed and had signed the study consent form. In addition, verbal assent was obtained from older children (>10 years) before enrolment in the study.

Genotyping of ABCB1 gene polymorphisms

All patients were screened for three different polymorphisms in the ABCB1 gene. Two synonymous SNPs, 1236C>T (exon 12) and 3435C>T (exon 26), together with the non-synonymous SNP, 2677G>T (exon 21), which is responsible for a substitution in the amino acid sequence (ala893ser) were investigated. In addition, haplotype analysis based on these three SNPs was performed.

Genomic DNA was extracted from peripheral blood (1 ml sample per patient) using QIAmp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). The concentration of the extracted DNA was quantified using the Quanti-iT™ PicoGreen dsDNA Quantitation kit (Invitrogen, Oregon, USA) according to the manufacturer's instructions. Detection of the various single nucleotide polymorphisms (SNPs) in the enzymes' genetic loci was carried out using validated TaqMan® genotyping assays obtained from Applied Biosystems (ABI, Foster City, USA). The TaqMan® primer/probe set designed for each SNP allele was included in the kits; their sequences are provided in Table 2.

Polymerase-chain reactions (PCR) were performed in a 96-well microplate format in a 10 μl reaction mixture containing TaqMan® universal PCR master mix and using an amplification protocol of 95°C for 15 min, followed by 50 cycles of 95°C for 15 s, then 60°C for 1.5 min. An allelic discrimination analysis was carried out using the DNA Engine Opticon® 2 System (MJ Research Inc, Alameda, USA).

Table 2

ABCB1 SNPs characteristics and sequences of TaqMan® probes designed for allele detection as supplied by the manufacturer

SNP	Location	NCBI reference	Probe sequence	Reporter 1 Dye	Reporter 2 Dye
ABCB1 C(1236)>T	Chr7	rs1128503	GCCCCACTCTGCACCTTCAGGTTTCAG[AVG]CCCTTCAAGATCTACCAGGACGAGT	VIC	FAM
ABCB1 C(3435)>T	Chr7	rs1045642	TGTTGGCTCTTCTGCTGCCCTCAC[AVG]ATCTCTTCTGTGACACCAACCCGGC	VIC	FAM
			Primer sequence	Probe sequence	
ABCB1 G(2677)>T	Chr7	rs2032582	F: 5'-GTCTGGACAAGCACTGAAAGATAAGA-3' R: 5'-CTTAGAGCATAGTAAGCAGTAGGGAGT-3'	TTCCAG[C/A]ACCTTC	VIC FAM

ABCB1, ATP-binding cassette sub-family B member 1, Chr, chromosome. NCBI, National Centre for Biotechnology Information. VIC and FAM are two different fluorescent reporter dyes available commercially from Applied Biosystems® (P/N 4316033).

Statistical analysis

Statistical analysis was performed using SPSS® computer software (version 15, SPSS Inc, USA). Results were expressed as the mean and 95% confidence intervals or as frequencies (in the case of allele or haplotype carriers in patient groups).

The statistical significance of differences in tacrolimus doses, trough concentrations, C : D ratios and percentage reduction in EGFR between groups of patients with vs those without ABCB1 polymorphisms was assessed at the sequential time points using the Mann-Whitney *U*-test. *P* values for multiple pair-wise comparisons between different ABCB1 haplotypes were adjusted as per the Bonferroni justification for multiple testing.

The differences in allele frequencies and genotype distribution of each polymorphism between patients who developed renal dysfunction vs those who did not were analyzed using the Chi squared (χ^2) test with 1 degree of

freedom. Haplotype frequencies and tests of haplotype associations with nephrotoxicity were determined using Haploview® software version 4.1 (<http://www.broad.mit.edu/mpg/haploview/>). Two-tailed *P* values of less than 0.05 were considered statistically significant.

Results

ABCB1 genotype and haplotype frequencies

Table 3 shows allele and genotype frequencies for the three ABCB1 polymorphisms investigated and their haplotypes in the 51 paediatric liver-transplant recipients studied. The allelic frequencies were not different from those reported in the literature and in all cases, genotype frequencies were not significantly different from those predicted by the Hardy-Weinberg equation.

Effect of ABCB1 genotypes and haplotypes on tacrolimus dosage requirement

At 6 months after LT, the required tacrolimus doses ranged from 0.05 to 0.85 mg kg⁻¹ day⁻¹ (median, 0.165 mg kg⁻¹ day⁻¹). This confirmed the large inter-individual variability in tacrolimus pharmacokinetics. The median tacrolimus trough concentration was 7.13 ng ml⁻¹ ranging from 3.9 to 11.5 ng ml⁻¹. Both the required doses and trough tacrolimus concentrations reduced with time over the 5 years post-transplant (Figure 1).

No statistically significant association was observed between tacrolimus daily doses, trough concentrations or C : D ratios and ABCB1 polymorphisms (C1236->T, G2677->T and C3435->T) at 6, 12 or 24 months after drug initiation. However, C : D ratios were significantly higher at 3 years post-transplantation among carriers of G2677->T (mean = 62 vs 39 ng ml⁻¹ mg⁻¹ kg; *P* = 0.022) and C3435->T variant alleles (mean = 60 vs 33 ng ml⁻¹ mg⁻¹ kg; *P* = 0.021)

Table 3

ABCB1 genotyping data for the study population (*n* = 102 alleles)

Variants	Variant allele frequency, <i>n</i> (%)	Heterozygotes, <i>n</i> (%)	Mutated homozygotes, <i>n</i> (%)
ABCB1 variant alleles			
C1236->T	45 (44.1%)	21 (41.2%)	12 (23.5%)
G2677->T	43 (42.2%)	19 (37.3%)	12 (23.5%)
C3435->T	45 (44.1%)	23 (45.1%)	11 (21.6%)
ABCB1 haplotypes			
C-G-C	47.3%	—	—
T-T-T	34.9%	—	—
C-G-T	7.6%	—	—
T-T-C	6.3%	—	—
T-G-C	2.4%	—	—
C-T-T	1.0%	—	—
T-G-T	0.6%	—	—

Figure 1

Time-dependent changes in tacrolimus trough concentrations (A) and doses (B) over 5 years post-transplantation. Error bars indicate mean ± 95%CI

Figure 2

The sequential changes in mean tacrolimus C : D ratios among carriers of C1236->T (A, ■), G2677->T (B, ■) and C3435->T (C, ■) variant alleles and carriers of T-T-T haplotype (D, ■) in comparison with the rest of the population group (●), over 5 years post-transplantation. Error bars indicate mean $\pm$ 95%CI

and at 4 years post-transplantation for carriers of G2677->T variant allele (mean = 65 vs 41 ng mL⁻¹ mg⁻¹ kg; $P = 0.039$). In addition, carriers of C1236->T showed a trend towards significant association with higher C : D ratios at 3 years post-transplant (mean = 59 vs 41 ng mL⁻¹ mg⁻¹ kg; $P = 0.084$). The sequential changes in mean tacrolimus C : D ratios among carriers of C1236->T, G2677->T and C3435->T variant alleles over 5 years post-transplantation in comparison with the rest of the population group are shown in Figure 2. Separate statistical analysis of the Caucasian subgroup showed significantly higher C : D ratios at 3, 4 and 5 years post-transplantation among carriers of C1236->T (3 years: mean = 69 vs 37 ng mL⁻¹ mg⁻¹ kg, $P < 0.01$; 4 years: mean = 68 vs 48 ng mL⁻¹ mg⁻¹ kg, $P = 0.035$; 5 years: mean =

69 vs 46 ng mL⁻¹ mg⁻¹ kg, $P = 0.036$) and G2677->T variant alleles (3 years: mean = 67 vs 40 ng mL⁻¹ mg⁻¹ kg, $P = 0.018$; 4 years: mean = 70 vs 38 ng mL⁻¹ mg⁻¹ kg, $P < 0.01$; 5 years: mean = 68 vs 47 ng mL⁻¹ mg⁻¹ kg, $P = 0.046$).

The different allelic combinations of C1236->T, G2677->T and C3435->T variants can theoretically lead to eight different haplotypes. Only seven haplotypes, however, were found in this study population (Table 3). The most frequent haplotypes were C-G-C and T-T-T haplotypes which occurred in 47.3% and 34.9% of the study population, respectively.

Pair-wise comparisons between the different haplotypes demonstrated no significant difference in tacrolimus doses, trough concentrations or C : D ratios at the

Figure 3

The influence of ABCB1 polymorphisms and haplotypes on percentage reduction of EGFR throughout the first year post-transplant as compared with pre-transplant baseline. Carriers of ABCB1 C1236->T (A, ■), G2677->T (B, ■) and C3435->T (C, ■) variant alleles and carriers of T-T-T haplotype (D, ■) were compared with the rest of the group (●). * $P < 0.05$, significant difference between two groups. EGFR, estimated glomerular filtration rate. Error bars indicate mean $\pm$ 95% CI

various time points examined except at 3 years post-transplantation where a significant difference was observed in tacrolimus C:D ratios between T-T-T and non-T-T-T haplotype carriers (mean = 61 vs 43 ng ml⁻¹ mg⁻¹ kg; $P = 0.048$; Figure 2).

Effect of ABCB1 genotypes and haplotypes on tacrolimus-related nephrotoxicity

An interesting finding in the present study was that the percentage reduction in EGFR was significantly higher at 6 months post-transplant among carriers of C1236->T (mean = 26.0 vs 8.2%; $P = 0.047$), G2677->T (mean = 26.6 vs 8.1%; $P = 0.045$) and C3435->T variant alleles (mean = 26.3 vs 6.5%; $P = 0.037$). In addition, carriers of G2677->T variant alleles showed a significant cumulative reduction in EGFR at 12 months post-transplantation (mean = 19.7 vs -2.9%; $P = 0.031$) and border-line significance in association with EGFR reduction at 3 months post-transplant (mean = 26.2 vs 7.8%; $P = 0.055$; Figure 3). In contrast, there was only a trend towards significant reduction in EGFR at 12 months post-transplant among carriers of C3435->T variant alleles

(mean = 17.0 vs -1.6%; $P = 0.091$). When separate analysis was performed in the Caucasian sub-group, both carriers of G2677->T and C3435->T variant alleles had greater percentage reduction in EGFR at 6 months (G2677->T: mean = 28.9 vs 2.9%, $P = 0.041$; C3435->T: mean = 28.5 vs -1.7%, $P = 0.025$) and 12 months post-transplant (G2677->T: mean = 22.8 vs -12.7%, $P < 0.01$; C3435->T: mean = 18.4 vs -11.8%, $P = 0.042$).

When compared with the rest of the study group, carriers of T-T-T haplotypes were found to have a trend towards significant reduction in EGFR at 3, 6 and 12 months post-transplant (3 months: mean = 26.8 vs 0.1%, $P = 0.077$; 6 months: mean = 27.0 vs 11.2%, $P = 0.08$; 12 months: mean = 19.4 vs 1.4%, $P = 0.082$; Figure 3). In Caucasians, there was a significant reduction in EGFR at 12 months post-transplant (mean = 22.8 vs -7.9%; $P = 0.021$).

Considering the incidence of renal dysfunction (defined as 30% reduction in EGFR) within the first year after transplantation, single marker analysis showed significant association between ABCB1 1236T, 2677T and 3435T variant alleles and an increased incidence of

Table 4

Influence of ABCB1 allelic variants and haplotypes on tacrolimus-associated renal toxicity at 3, 6 and 12 months post-transplant

	3 months Case, control* frequencies	P value	6 months Case, control frequencies	P value	12 months Case, control frequencies	P value
ABCB1 variant allele						
C3435->T	55.9%, 36.4%	0.062	60%, 39.1%	0.057	50%, 41.4%	0.440
G2677->T	52.9%, 36.4%	0.112	63.3%, 35.9%	0.012	53.6%, 37.1%	0.136
C1236->T	52.9%, 39.4%	0.196	63.3%, 37.5%	0.019	53.6%, 40%	0.221
ABCB1 haplotype						
C-G-C	37.7%, 53.6%	0.132	29.3%, 52.8%	0.032	38.5%, 50.6%	0.281
T-T-T	46.5%, 28.4%	0.071	52.6%, 29.4%	0.029	42.1%, 31.1%	0.301
C-G-T	9.3%, 5.5%	0.469	7.3%, 8.1%	0.899	7.8%, 8%	0.983
T-T-C	6.4%, 6.4%	0.997	10.7%, 5%	0.305	11.4%, 4.6%	0.215
T-G-C	0%, 3.6%	0.265	0%, 3.1%	0.333	0%, 3.4%	0.328
C-T-T	0%, 1.5%	0.480	0%, 1.6%	0.503	0%, 1.4%	0.537

*Case, control patients are those with EGFR changes >30% or <30%, respectively.

nephrotoxicity at 6 months post-transplantation (1236T allele: frequency = 63.3% in nephrotoxic patients vs 37.5% in controls, $P = 0.019$; 2677T allele: frequency = 63.3% vs 35.9%, $P = 0.012$; 3435T allele: frequency = 60% vs 39.1%, $P = 0.057$), the latter of which showed border-line significance only (Table 4). Furthermore, haplotype analysis showed a significant association between T-T-T haplotypes and an increased incidence of tacrolimus-related nephrotoxicity at 6 months post-transplant (haplotype frequency = 52.9% in nephrotoxic patients vs 29.4% in controls; $P = 0.029$). C-G-C haplotypes, on the other hand, were associated with a decreased incidence of nephrotoxicity at 6 months post-transplantation (haplotype frequency = 29.3% in nephrotoxic patients vs 52.8% in controls; $P = 0.032$). None of ABCB1 alleles or haplotypes was associated with renal toxicity after 1 year of transplantation (Table 4).

Discussion

P-gp transport protein has received much attention recently as an important determinant of drug absorption since its expression and function are modified by genetic polymorphisms of the *ABCB1* gene [11]. Of the 28 SNPs, which have been identified in the *ABCB1* gene so far, three (C1236->T, G2677->T and C3435->T) are of particular interest [12]. Several studies have examined the influence of these three polymorphisms on tacrolimus pharmacokinetics and dosage requirements. Conflicting results, however, have been obtained. In renal transplant patients, Macphee *et al.* found that possession of the ABCB1 C3435->T variant allele resulted in 1.44 times higher tacrolimus blood concentrations and lower tacrolimus dose requirements when compared with homozygous patients with the 3435CC genotype [19]. In addition, Anglicheau *et al.* have shown that the tacrolimus dose requirement was higher in the 2677GG group when compared with carriers of the variant allele [20]. However, several other studies did not find an

influence of the three SNPs on tacrolimus trough concentrations or pharmacokinetic parameters [8, 21, 22].

In the setting of LT, various studies which examined the recipient genotype did not find an association between tacrolimus pharmacokinetics and these three SNPs [23, 24]. In contrast to these studies, however, Wei-line *et al.* have shown in a recent study that tacrolimus dose requirements were lower for carriers of the ABCB1 C3435->T variant allele [25]. This finding was observed only during the first month after LT. Information related to the stability of this effect over time is lacking in the literature.

In the present study, the long-term effect of ABCB1 polymorphisms on tacrolimus trough concentrations and dose requirements was investigated for up to 5 years post-transplantation. In addition to individual SNPs, the potential influence of ABCB1 haplotypes was analyzed. Haplotype analysis is superior to that of individual SNPs in revealing genotype-phenotype associations. Knowledge of the haplotype structure of SNPs in strong linkage disequilibrium provides more information than do individual SNPs on the association of candidate-genes with drug responses [26] and might be of higher value in predicting P-gp activity [27].

The present study demonstrated a significant difference in tacrolimus C:D ratios among different ABCB1 genotypes and haplotypes at various time points examined after drug initiation. These results suggest a long-term effect of ABCB1 polymorphisms and haplotypes on tacrolimus pharmacokinetics over time post-transplant.

In addition to its pharmacokinetic variability, it has been shown that tacrolimus has nephrotoxic effects and monitoring of GFR is considered an important part of post-transplant care. Following LT in children a sharp decline (35%) is seen in the EGFR in the first 3 months post-transplant, which persists until 6 months [28]. Coinciding with the reduction in tacrolimus dose, EGFR improves gradually over the next 6 months [28, 29]. As renal function reaches its nadir at 6 months post-transplant, it is at this

time point that the impact of any factors predisposing to nephrotoxicity will be easiest to detect.

Despite the fact that differing ABCB1 polymorphisms were not correlated with tacrolimus pharmacokinetics at 6 months post-transplant, they were significantly related to the incidence of renal dysfunction at that time. To our knowledge, this is the first report in the literature correlating ABCB1 genetic polymorphisms with nephrotoxicity in paediatric patients with LT. Of the three SNPs in linkage disequilibrium, our results suggest that G2677->T seems to have the most pronounced effect on nephrotoxicity since carriers of this variant allele showed a significant reduction in EGFR at 12 months post-transplant. In addition, haplotype analysis showed a significant association between T-T-T haplotypes and an increased incidence of nephrotoxicity ($\geq 30\%$ EGFR reduction) at 6 months post-transplantation.

These results indicate that P-gp protects against the development of renal dysfunction independent of blood tacrolimus concentrations, presumably by reducing the exposure of renal cells to tacrolimus. An alternative explanation is that some P-gp genotypes were associated with higher tacrolimus concentrations during the early phase (first 1–3 months) post-transplantation which led to the subsequent development of nephrotoxicity in these patients. Tacrolimus concentrations at 1–3 months post-transplant, however, were not available in the present study.

Tacrolimus cannot account for all of the nephrotoxicity seen following paediatric liver transplantation but it is accepted as the major modifiable cause. The post-transplant protocol does include other potential nephrotoxic drugs such as low dose Cotrimoxazole and oral amphotericin and acyclovir for short defined periods. These may make small but known contributions to nephrotoxicity. Moreover the improvement of renal function following withdrawal of calcineurin inhibitors suggests that these drugs are the major modifiable factor contributing to renal dysfunction [30].

EGFR is a relatively inaccurate measurement of glomerular filtration. However serial investigations comparing EGFR with more accurate measurements such as Cr EDTA or inulin clearance have consistently shown the same trends in renal function post-transplant in groups of patients [28]. EGFR has the distinct advantage that serial measurements are easy and non-invasive.

In conclusion, the present study demonstrated that ABCB1 polymorphisms in the native intestine do have a significant influence on tacrolimus dose requirement in the stable phase after transplantation. In addition, ABCB1 polymorphisms in paediatric LT recipients may predispose them to nephrotoxicity over the first year post-transplantation. Determination of these SNPs in future transplant recipients may, therefore, have the potential to identify individuals who are at risk of developing tacrolimus-related nephrotoxicity. They may be candidates

for use of tacrolimus sparing regimens. Ultimately, genotyping for ABCB1 may lead to further individualization of tacrolimus therapy in paediatric patients with LT. We await prospective trials to address this issue.

Competing interests

None declared.

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REFERENCES

- Seaberg EC, Belle SH, Beringer KC, Schivins JL, Detre KM. Liver transplantation in the United States from 1987–1998: Updated results from the Pitt-UNOS liver transplant registry. In: Cecka JM, Terasaki PI, Clinical Transplants 1999; 1998: 17–37. Los Angeles, USA: UCLA Tissue Typing Laboratory
- Carter BA, Kilic M, Karpen S. 2006; History of Pediatric Liver Transplantation <http://www.emedicine.com/PED/topic2840>. Last accessed on March 20 2009.
- Masuda S, Inui K. An up-date review on individualized dosage adjustment of calcineurin inhibitors in organ transplant patients. *Pharmacol Ther* 2006; 112: 184–98.
- Fukudo M, Yano I, Masuda S, Goto M, Uesugi M, Katsura T, Ogura Y, Oike F, Takada Y, Egawa H, Uemoto S, Inui K. Population pharmacokinetic and pharmacogenomic analysis of tacrolimus in pediatric living-donor liver transplant recipients. *Clin Pharmacol Ther* 2006; 80: 331–45.
- Staat CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. *Clin Pharmacokinet* 2004; 43: 623–53.
- Hesselink DA, van Gelder T, van Schaik RH. The pharmacogenetics of calcineurin inhibitors: one step closer toward individualized immunosuppression? *Pharmacogenomics* 2005; 6: 323–37.
- Masuda S, Goto M, Fukatsu S, Uesugi M, Ogura Y, Oike F, Kiuchi T, Takada Y, Tanaka K, Inui K. Intestinal MDR1/ABCB1 level at surgery as a risk factor of acute cellular rejection in living-donor liver transplant patients. *Clin Pharmacol Ther* 2006; 79: 90–102.
- Hesselink DA, van Schaik RH, van der Heiden IP, van der Werf M, Gregoor PJ, Lindemans J, Weimar W, van Gelder T. Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther* 2003; 74: 245–54.
- Saito K, Miyake S, Moriya H, Yamazaki M, Itoh F, Imai K, Kurosawa N, Owada E, Miyamoto A. Detection of the four

- sequence variations of MDR1 gene using TaqMan MGB probe based real-time PCR and haplotype analysis in healthy Japanese subjects. *Clin Biochem* 2003; 36: 511–8.
- 10 Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther* 2004; 75: 13–33.
 - 11 Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, John A, Cascorbi I, Gerloff T, Roots I, Eichelbaum M, Brinkmann U. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. *Proc Natl Acad Sci U S A* 2000; 97: 3473–8.
 - 12 Schwab M, Eichelbaum M, Fromm MF. Genetic polymorphisms of the human MDR1 drug transporter. *Annu Rev Pharmacol Toxicol* 2003; 43: 285–307.
 - 13 Sparreboom A, Danesi R, Ando Y, Chan J, Figg WD. Pharmacogenomics of ABC transporters and its role in cancer chemotherapy. *Drug Resist Updat* 2003; 6: 71–84.
 - 14 Lockhart AC, Tirona RG, Kim RB. Pharmacogenetics of ATP-binding cassette transporters in cancer and chemotherapy. *Mol Cancer Ther* 2003; 2: 685–98.
 - 15 Schaeffeler E, Eichelbaum M, Brinkmann U, Penger A, Asante-Poku S, Zanger UM, Schwab M. Frequency of C3435T polymorphism of MDR1 gene in African people. *Lancet* 2001; 358: 383–4.
 - 16 Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, Taylor A, Xie HG, McKinsey J, Zhou S, Lan LB, Schuetz JD, Schuetz EG, Wilkinson GR. Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther* 2001; 70: 189–99.
 - 17 Cattaneo D, Perico N, Remuzzi G. From pharmacokinetics to pharmacogenomics: a new approach to tailor immunosuppressive therapy. *Am J Transplant* 2004; 4: 299–310.
 - 18 Morris MC, Allanby CW, Toseland P, Haycock GB, Chantler C. Evaluation of a height/plasma creatinine formula in the measurement of glomerular filtration rate. *Arch Dis Child* 1982; 57: 611–5.
 - 19 Macphee IA, Fredericks S, Tai T, Syrris P, Carter ND, Johnston A, Goldberg L, Holt DW. Tacrolimus pharmacogenetics: polymorphisms associated with expression of cytochrome p4503A5 and P-glycoprotein correlate with dose requirement. *Transplantation* 2002; 74: 1486–9.
 - 20 Anglicheau D, Verstuyft C, Laurent-Puig P, Becquemont L, Schlageter MH, Cassinat B, Beaune P, Legendre C, Thervet E. Association of the multidrug resistance-1 gene single-nucleotide polymorphisms with the tacrolimus dose requirements in renal transplant recipients. *J Am Soc Nephrol* 2003; 14: 1889–96.
 - 21 Haufroid V, Mourad M, Van Kerckhove V, Wawrzyniak J, De Meyer M, Eddour DC, Malaise J, Lison D, Squifflet JP, Wallemacq P. The effect of CYP3A5 and MDR1 (ABCB1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. *Pharmacogenetics* 2004; 14: 147–54.
 - 22 Mai I, Perloff ES, Bauer S, Goldammer M, John A, Filler G, Budde K, Roots I. MDR1 haplotypes derived from exons 21 and 26 do not affect the steady-state pharmacokinetics of tacrolimus in renal transplant patients. *Br J Clin Pharmacol* 2004; 58: 548–53.
 - 23 Goto M, Masuda S, Saito H, Uemoto S, Kiuchi T, Tanaka K, Inui K. C3435T polymorphism in the MDR1 gene affects the enterocyte expression level of CYP3A4 rather than Pgp in recipients of living-donor liver transplantation. *Pharmacogenetics* 2002; 12: 451–7.
 - 24 Hebert MF, Dowling AL, Gierwatowski C, Lin YS, Edwards KL, Davis CL, Marsh CL, Schuetz EG, Thummel KE. Association between ABCB1 (multidrug resistance transporter) genotype and post-liver transplantation renal dysfunction in patients receiving calcineurin inhibitors. *Pharmacogenetics* 2003; 13: 661–74.
 - 25 Wei-lin W, Jing J, Shu-sen Z, Li-hua W, Ting-bo L, Song-feng Y, Sheng Y. Tacrolimus dose requirement in relation to donor and recipient ABCB1 and CYP3A5 gene polymorphisms in Chinese liver transplant patients. *Liver Transpl* 2006; 12: 775–80.
 - 26 Zheng H, Schuetz E, Zeevi A, Zhang J, McCurry K, Webber S, Iacono A, Lamba J, Burckart GJ. Sequential analysis of tacrolimus dosing in adult lung transplant patients with ABCB1 haplotypes. *J Clin Pharmacol* 2005; 45: 404–10.
 - 27 John A, Kopke K, Gerloff T, Mai I, Rietbrock S, Meisel C, Hoffmeyer S, Kerb R, Fromm MF, Brinkmann U, Eichelbaum M, Brockmoller J, Cascorbi I, Roots I. Modulation of steady-state kinetics of digoxin by haplotypes of the P-glycoprotein MDR1 gene. *Clin Pharmacol Ther* 2002; 72: 584–94.
 - 28 Arora-Gupta N, Davies P, McKiernan P, Kelly DA. The effect of long-term calcineurin inhibitor therapy on renal function in children after liver transplantation. *Pediatr Transplant* 2004; 8: 145–50.
 - 29 Mor E, Patel T, Glabman S, Sheiner P, Emre S, Guy S, Schwartz M, Miller C. Comparison of short and long-term renal function in liver transplant patients receiving cyclosporin or FK 506. *Transpl Int* 1994; 7 (Suppl. 1): S77–80.
 - 30 Evans HM, McKiernan PJ, Kelly DA. Mycophenolate mofetil for renal dysfunction after pediatric liver transplantation. *Transplantation* 2005; 79: 1575–80.