ABCB1 Genotype of the Donor but Not of the Recipient Is a Major Risk Factor for Cyclosporine-Related Nephrotoxicity after Renal Transplantation

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Cyclosporine (CsA) nephrotoxicity is a severe complication in organ transplantation because it leads to impaired renal function and chronic allograft nephropathy, which is a major predictor of graft loss. Animal models and in vivo studies indicate that the transmembrane efflux pump P-glycoprotein contributes substantially to CsA nephrotoxicity. It was hypothesized that the TT genotype at the ABCC1 3435C→T polymorphism, which is associated with decreased expression of P-glycoprotein in renal tissue, is a risk factor for developing CsA nephrotoxicity. In a case-control study, 18 of 97 patients developed CsA nephrotoxicity and showed complete recovery of renal function in all cases when switched to a calcineurin inhibitor–free regimen. Both recipients and donors were genotyped for ABCC1 polymorphisms at the positions 3435C→T and 2677G→T/A. For controlling for population stratification, two additional polymorphisms, CYP2D6*4 and CYP3A5*3, with intermediate allelic frequencies were studied. The P-glycoprotein low expressor genotype 3435TT only of renal organ donors but not of the recipients was overrepresented in patients with CsA nephrotoxicity as compared with patients without toxicity ($\chi^2 = 10.5; P = 0.005$). CsA dosage, trough levels, and the concentration per dose ratio were not different between the patient groups. In a multivariate model that included several other nongenetic covariates, only the donor’s ABCC1 3435TT genotype was strongly associated with CsA nephrotoxicity (odds ratio, 13.4; 95% confidence interval, 1.2 to 148; $P = 0.034$). A dominant role of the donor’s ABCC1 genotype was identified for development of CsA nephrotoxicity. This suggests that P-glycoprotein is an important factor in CsA nephrotoxicity.


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to be mainly involved in the pathogenesis. Hence, Bennett et al. (6) described chronic CsA nephropathy as the Achilles’ heel of immunosuppressive therapy. Therefore, identification of risk factors that predispose for chronic CsA nephrotoxicity could improve the treatment of patients.

There are two lines of evidence that support the notion that the multidrug resistance (ABCB1) 1 gene product P-glycoprotein (P-gp) could be a factor of major relevance. In animals, an inverse relationship among periglomerular and interstitial fibrosis, CsA deposits in renal tissue, and the level of P-gp expression in proximal tubular cells was observed (7). In agreement with these data, we have shown in a histopathologic study that low expression of P-gp in renal parenchymal cells was associated with the occurrence of CsA nephrotoxicity, namely chronic interstitial fibrosis, arteriolopathy, and tubular vacuolization (8). Because CsA is a P-gp substrate, these data indicate that factors that modulate P-gp expression could have an impact on CsA nephrotoxicity as a result of an accumulation of CsA within renal cells in patients with low expression of P-gp.

Several common single nucleotide polymorphisms that alter the expression and function of human P-gp were identified recently within the ABCB1 gene (9). For instance, in human renal proximal epithelial cells, the 3435C→T polymorphism was correlated to an approximately twofold lower P-gp amount in individuals homozygous for the 3435T allele (10). Furthermore, a significant reduction in the renal clearance of orally or intravenously administered digoxin (11) as well as of irinotecan (12), both well-characterized P-gp substrates, these data indicate that factors that modulate P-gp expression could have an impact on CsA nephrotoxicity as a result of an accumulation of CsA within renal cells in patients with low expression of P-gp.

These findings prompted us to test the hypothesis of whether patients with the P-gp low expressor genotype are more susceptible to developing CsA nephrotoxicity. Because recipient’s and donor’s genotype can be discordant and the donor’s genotype seems to be the relevant predictor for P-gp expression in the renal graft, genotyping for ABCB1 was performed in recipients and their corresponding donors. This assumption is corroborated by an earlier study that demonstrated clearly that the occurrence of CsA nephrotoxicity, namely chronic interstitial fibrosis, arteriolopathy, and tubular vacuolization (8). Because CsA is a P-gp substrate, these data indicate that factors that modulate P-gp expression could have an impact on CsA nephrotoxicity as a result of an accumulation of CsA within renal cells in patients with low expression of P-gp.

Materials and Methods

Patient Population

The study protocol specified the inclusion criteria as following: (1) primarily immunosuppressive therapy with CsA, (2) no transplant loss or death of patients within 3 mo posttransplantation, (3) no rejection episode in patients before diagnosis of CsA nephrotoxicity, (4) regular follow-up visits posttransplantation for at least a period of 12 mo, (5) white ethnicity to exclude spurious association as a result of unexpected differences in genetic background, and (6) availability of recipient’s and donor’s genomic DNA. Because donor’s DNA was available only from living kidney donors or cadaveric kidneys derived from the local transplantation center Frankfurt/Main between January 1986 and December 2000, a total of 97 patients were eligible. The study was approved by the ethics committee of the University Hospital Frankfurt/Main, and kidney recipients as well as living donors gave written informed consent when they were alive at the time of the screening date.

CsA nephrotoxicity was defined clinically by two experienced nephrologists on the basis of the following criteria (1) a persistently elevated serum creatinine of at least >1.6 mg/dl but <4.5 mg/dl over a period of >3 mo and/or a creeping serum creatinine of approximately 20% from baseline, (2) subsequent decrease of serum creatinine to baseline status within a period of at most 6 mo after conversion to a calcineurin inhibitor–free immunosuppression, (3) and exclusion of renal impairment as a result of acute rejection at the time of conversion either proved by biopsy and/or judged by clinical evaluation using standard criteria (e.g., acute rise in serum creatinine combined with volume retention, tenderness of the graft, and/or acute deterioration of renal perfusion diagnosed by Doppler ultrasound of the graft). In cases of CsA nephrotoxicity, patients were switched subsequently to a calcineurin inhibitor–free regimen that contained glucocorticosteroids and azathioprine or mycophenolate mofetil. Patients were seen for follow-up visits every 4 mo until their final visit in January 2003 to monitor renal transplant function.

According to local immunosuppressive protocol used in Frankfurt/Main, all patients received primarily after transplantation a triple therapy that consisted of CsA (starting dosage 8 mg/kg body wt), glucocorticosteroids, and azathioprine or mycophenolate mofetil. Only in highly immunized patients (18.6% of all patients) was T cell antibody therapy added. Long-term standard immunosuppressive therapy (>1.5 mo posttransplantation) consists of CsA or tacrolimus and low-dose glucocorticosteroids. CsA administration was adjusted to a target therapeutic window in the range 100 to 300 ng/ml. Delayed graft function was defined according to standard criteria: Creatinine clearance <10 ml/min during the first 3 d posttransplantation and/or the need for dialysis treatment during the first postoperative week (14).

Diagnosis of rejection episodes was established by histology-proven biopsies of renal allografts according to the Banff classification 1997 and/or by clinical evaluation as mentioned above (see [3] definition of CsA nephrotoxicity).

A total of 557 healthy unrelated individuals who were recruited randomly as described previously (15) were used as a control population for genetic analyses. In brief, all of these subjects were individuals of white (German) origin with a median age of 26 yr (range, 17 to 65 yr; 64% male and 36% female).

Genotyping of ABCB1 Polymorphisms

For recipients and living donors, blood was collected at the time of screening date, whereas in all cases of cadaveric kidney transplantation, donor’s DNA was provided by the Department of Transfusion Medicine, University Hospital Frankfurt/Main, in an anonymous manner. Leukocyte DNA was isolated by standard procedures (QiAamp DNA Blood Mini Kit). ABCB1 3435C→T and 2677T/3435T polymorphisms were detected by denaturing HPLC as described previously (16). Laboratory personnel were blinded to case status of the study participants.

Genomic Control

To exclude spurious association, we studied two polymorphisms of the genes cytochrome P450 2D6 (CYP2D6*4) and cytochrome P450 3A5 (CYP3A5*3C), both with intermediate allelic frequencies and not linked to ABCB1 (17,18). This is a commonly accepted approach to address population stratification involving genotyping for mutations distinct from the candidate gene under study. When allele and genotype frequencies of the control polymorphisms are not significantly different between index cases and controls, a chance finding in association
studies is very unlikely (19,20). The CYP2D6 key mutation 1846G→A representative for CYP2D6*4 was analyzed using the predeveloped TaqMan Assay-Reagents Allelic Discrimination Kit (Applied Biosystems, Foster City, CA), and the key mutation 6986A→G representing the most frequent allele CYP3A5*3C and other *3 allelic variants (http://www.imm.ki.se/CYPalleles/) was genotyped by a new established TaqMan assay (21).

### Statistical Analyses

Values are presented as median and range. The SPSS (version 12.0) and the GraphPad Prism (version 3.03) packages were used for all statistical analyses. Unpaired t test, Mann-Whitney test, χ² analysis, or Fisher exact test were used as appropriate to assess differences or proportions of clinical and genetic data, respectively, between study groups. To test whether variables in each patient before and after an intervention differ significantly, we used paired t test or Wilcoxon test. Odds ratios (OR) are given with 95% confidence intervals (CI) and two-sided P values. P < 0.05 was considered statistically significant.

Sample size calculation was performed using the program nQuery Advisor Release 4.0. At least 81 patients had to be enrolled to detect a significant difference between the mean calcineurin inhibitor levels at baseline and at 12 months. This sample size is based on a power of 80% and a 5% significance level. At least 3435TT genotype based on a power of 80% and a significance level of 0.05 was used to detect a significant difference between the mean calcineurin inhibitor levels at baseline and at 12 months. This sample size is based on a power of 80% and a significance level of 0.05. The designated end point was withdrawal of CsA as a result of CsA nephrotoxicity. Data on a patient were censored in the Kaplan-Meier analysis when the patient had shown CsA nephrotoxicity or was on calcineurin inhibitor treatment at the end of follow-up. The log-rank test was used to assess the prognostic value of a certain genotype in relation to CsA nephrotoxicity.

### Results

#### Demographic Data

The baseline characteristics of the 97 patients included and their allografts are summarized in Table 1. Underlying diseases for renal failure were classified as follows: chronic glomerulonephritis including autoimmune diseases (42.3%), nephrosclerosis and diabetic nephropathy (4.1%), chronic interstitial nephritis (16.5%), atrophic kidney of unknown causes (17.5%), polycystic kidney diseases (12.4%), and other causes (e.g., trauma, hemolytic uremic syndrome, Alport syndrome [7.2%]).

Eighteen of the 97 included patients met our criteria for CsA nephrotoxicity and were switched to a calcineurin inhibitor–free immunosuppressive regimen (glucocorticosteroids and azathioprine or mycophenolate mofetil) after receiving CsA therapy for a median time of 1.08 yr (0.36 to 2.71). In these patients, median serum creatinine levels increased significantly by 19% from baseline (third month posttransplantation) to the time point of CsA withdrawal (P = 0.0001; Figure 1A), decreased subsequently in all patients within 6 mo by 26% (P = 0.0002), and remained stable over a median follow-up time of 2.7 yr (1.2 to 13.9; NS). This significant improvement of renal function after conversion to a calcineurin inhibitor–free regimen was also mirrored by an increase of the median creatinine clearance by 41% (P = 0.0002). Over a median follow-up period of 4.4 yr (1.9 to 16.6) between baseline (third month posttransplantation) and time of inclusion, the remaining 79 recipients stayed on calcineurin inhibitor treatment (either CsA or tacrolimus) and steroids without a significant alteration of serum creatinine levels (P = 0.29; Figure 1B).

Demographic variables were not significantly different between both study groups (Table 1) except for the duration of calcineurin inhibitor treatment (P < 0.001). At 3 mo posttransplantation, a time point at which all patients were in a stable phase after their kidney transplantation, the median CsA dosage, trough levels, and concentration per dose ratio were comparable in patients with and without nephrotoxicity. Moreover, in patients with CsA nephrotoxicity, the CsA trough levels were not significantly different between the third month posttransplantation and the time point of CsA withdrawal (191 ng/ml [100 to 278] versus 221 [144 to 394]; P = 0.7). Finally, in the control group, CsA dosages were similar over the entire follow-up period.

In the median follow-up period of 2.7 yr (1.2 to 13.9), neither biopsy-proven rejection episodes nor graft loss was observed in all 18 patients after CsA withdrawal and subsequent calcineurin inhibitor–free immunosuppression. In contrast, 34 of the 76 patients of the control group were switched to tacrolimus therapy as a result of rejection episodes or side effects (e.g., gingival hyperplasia, hypertrichosis) under CsA treatment. In four cases, loss of renal grafts was observed and one patient died as a result of myocardial infarction. Moreover, when we stratified the control group between patients who stayed on CsA with a median follow-up time of 5.4 yr (1.9 to 16.6) after renal transplantation compared with patients who were switched to tacrolimus and a median follow-up of 4.3 yr (2.1 to 6.0), all clinical parameters (see Table 1) were not significantly different between both groups (data not shown).
Table 1. Demographic and clinical characteristics of patients and their allografts

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients with Nephrotoxicity (n = 18)</th>
<th>Patients without Nephrotoxicity (n = 79)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Graft-related factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age of donor (yr; n = 94)</td>
<td>55 (25 to 72)</td>
<td>53 (18 to 74)</td>
<td>0.59</td>
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<tr>
<td>gender of donor (n = 84)</td>
<td>female 11, male 6</td>
<td>female 40, male 27</td>
<td>0.79</td>
</tr>
<tr>
<td>living kidney donor (n)</td>
<td>6</td>
<td>40</td>
<td>0.20</td>
</tr>
<tr>
<td>cold-ischemia time (hr/min; n = 91)</td>
<td>9:15 (2:18 to 29:11)</td>
<td>10:34 (0:07 to 37:53)</td>
<td>0.51</td>
</tr>
<tr>
<td>delayed graft function (n = 94)</td>
<td>5</td>
<td>11</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>Patient-related factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age of recipient (yr; n = 97)</td>
<td>43 (19 to 73)</td>
<td>44 (11 to 72)</td>
<td>0.60</td>
</tr>
<tr>
<td>gender of recipient (n = 97)</td>
<td>female 6, male 12</td>
<td>female 34, male 45</td>
<td>0.60</td>
</tr>
<tr>
<td>arterial hypertension before transplantation (n = 83)</td>
<td>15</td>
<td>44</td>
<td>0.25</td>
</tr>
<tr>
<td>arterial hypertension 3 mo posttransplantation (n = 79)</td>
<td>15</td>
<td>60</td>
<td>1.0</td>
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<tr>
<td>blood pressure &gt;140/90 mmHg 3 mo posttransplantation with or without antihypertensive therapy (n = 79)</td>
<td>11</td>
<td>32</td>
<td>0.15</td>
</tr>
<tr>
<td>diabetes before transplantation (n = 80)</td>
<td>1</td>
<td>2</td>
<td>0.54</td>
</tr>
<tr>
<td>positive serology for hepatitis B and/or C (n = 85)</td>
<td>1</td>
<td>7</td>
<td>1.0</td>
</tr>
<tr>
<td>duration of dialysis before transplantation (yr; n = 97)</td>
<td>4.6 (0.5 to 19.2)</td>
<td>4.3 (0 to 18.5)</td>
<td>0.40</td>
</tr>
<tr>
<td>no. of mismatches at the HLA-A, B, and DR loci (n = 97)</td>
<td>4</td>
<td>13</td>
<td>0.51</td>
</tr>
<tr>
<td>duration of CNI treatment (yr; n = 97)</td>
<td>1.08 (0.36 to 2.71)</td>
<td>none</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tacrolimus application (n)</td>
<td>34d</td>
<td>34d</td>
<td>0.96</td>
</tr>
<tr>
<td>CsA dosage (mg/day) 3 mo posttransplantation</td>
<td>300 (200 to 510)</td>
<td>300 (130 to 800)</td>
<td>0.99</td>
</tr>
<tr>
<td>CsA trough concentration (ng/ml) 3 mo posttransplantation</td>
<td>221 (144 to 394)</td>
<td>216 (99 to 500)</td>
<td>0.39</td>
</tr>
<tr>
<td>CsA concentration/dose ratio (ng/ml per mg/day per kg body wt) 3 mo posttransplantation</td>
<td>55.9 (25.3 to 103.0)</td>
<td>51.2 (18.1 to 122.0)</td>
<td>0.39</td>
</tr>
</tbody>
</table>

*aCsA, cyclosporine; CNI, calcineurin inhibitor.  
bTotal number of available data in brackets.  
cEnd point time of inclusion, allograft failure as a result of causes other than CsA nephrotoxicity (n = 4), or death (n = 1).  
dSwitching of CsA treatment to tacrolimus as a result of rejection episodes or side effects under CsA treatment.

Association with ABCB1

The ABCB1 genotype frequencies of the 2677G→T/A and 3435C→T polymorphisms for all kidney recipients and donors, respectively, were in Hardy-Weinberg equilibrium. Overall, no significant differences between allele (data not shown) and genotype frequencies for the 2677G→T/A (data not shown) and the 3435C→T polymorphisms (Figure 2) were found when the total number of recipients and donors was compared with a large-scale healthy control group of 537 German whites. This holds true when recipients with and without CsA nephrotoxicity were compared (Table 2; χ² = 0.8; P = 0.66). In contrast, the allele and genotype frequencies of the ABCB1 3435C→T polymorphism between donors with and without CsA nephrotoxicity were significantly different with an overrepresentation of the P-gp low expressor genotype 3435TT in patients with CsA nephrotoxicity (Table 2; allele: OR, 3.2; 95% CI, 1.4 to 7.6; P = 0.005; genotypes: χ² = 10.5; P = 0.005). When individuals homozygous for CC and TT, respectively, were compared, again, patients with CsA nephrotoxicity were more likely to have the TT than the CC genotype (OR, 9.2; 95% CI, 1.1 to 79.4; P = 0.03). For the 2677G→T polymorphism, no significant differences were observed (allele: OR, 1.7; 95% CI, 0.8 to 3.5; P = 0.19; genotypes: χ² = 1.9; P = 0.38).

Using haplotype analysis by PHASE, the haplotypes 11
(2677G/3435C) and 22 (2677T/3435T) according to Johne et al. (22) were the most common and occurred in 47.6 and 32.5% of the recipients as well as in 38.2 and 38.7% of the donors, respectively. In recipients and donors, the haplotype 12 (2677G/3435T) occurred with intermediate frequencies of 13.9 and 17.9%, respectively, whereas the haplotype 21 (2677T/
3435C) was rare (6 and 5.1%, respectively). Only the donor’s haplotype 11 encoding for a higher P-gp function in human was significantly associated with a lower risk for developing CsA nephrotoxicity in carriers compared with noncarriers (OR, 0.29; 95% CI, 0.1 to 0.7; \(P = 0.0045\)). For all other haplotypes, no significant relationship to CsA nephrotoxicity was found.

Moreover, we evaluated the distribution of allele and genotype frequencies within our control group for the 45 patients who stayed on CsA therapy as compared with the 34 patients who were switched to tacrolimus. No statistical significant differences for frequency distribution of \(ABCB1\) geno-/haplotypes were observed.

Genomic Control
To determine whether patients with or without CsA nephrotoxicity might be covertly stratified, we analyzed two unlinked frequent genetic polymorphisms, \(CYP2D6*4\) allele and \(CYP3A5*3C\) allele. There were no significant deviations from Hardy-Weinberg equilibrium for both polymorphisms. The OR for comparison of allele and genotype frequencies of recipients and donors cross the 1.0 value, suggesting that the two cohorts were not significantly stratified (Table 2). Moreover, because \(CYP3A5\) is considered to be involved in drug metabolism of CsA (23,24), an association between the recipient’s \(CYP3A5\) genotype and CsA blood levels was investigated and no correlation was found.

Prediction of CsA Nephrotoxicity
Kaplan-Meier curves were calculated for the donor’s and recipient’s genotypes of the \(ABCB1\) polymorphisms \(2677G\rightarrow\text{T}\) and \(3435C\rightarrow\text{T}\) as well as both control gene polymorphisms \(CYP2D6*4\) and \(CYP3A5*3C\). No significant differences with respect to the recipient group were found for all polymorphisms (e.g., \(ABCB1 3435C\rightarrow\text{T}\); Figure 3A). In contrast, the donor’s genotype was highly predictive in that approximately 40% of all donors homozygous for the \(3435\text{T}\) allele developed CsA nephrotoxicity approximately 2.5 yr posttransplantation compared with only 10% of donors with the CT or CC genotype (Figure 3B). The \(2677G\rightarrow\text{T}\) polymorphism of the donor did not influence significantly Kaplan-Meier estimates (Figure 3, C and D). This holds true also for both control genes, \(CYP2D6*4\) and \(CYP3A5*3C\).

Correlations with Other Measures
Multivariate logistic regression analysis was carried out to establish whether the \(ABCB1 3435C\rightarrow\text{T}\) polymorphism was an independent prognostic marker for prediction of CsA nephrotoxicity. The model initially included coexisting conditions that were associated with chronic renal graft failure according to the literature (2,25). A backward stepwise procedure was adopted to obtain the final model of significant predictors for CsA nephrotoxicity after sequential exclusion of factors in the model. CsA nephrotoxicity was strongly associated only with the \(ABCB1 3435\text{TT}\) genotype with an estimated OR of 13.4 (95% CI, 1.2 to 148; \(P = 0.034\); regression coefficient 2.6). As a second predictor, a trend of significance (OR, 3.7; 95%, 0.9 to 16.4; \(P = 0.08\)) was observed for the parameter “graft from a cadaveric donor.”

Discussion
In this study, we found that the common polymorphism \(3435C\rightarrow\text{T}\) in the \(ABCB1\) gene encoding for the renal epithelial efflux transporter P-gp (26) was associated with an increased risk for CsA nephrotoxicity in patients after renal transplantation. Specifically, the \(3435\text{TT}\) genotype of renal organ donors...
was significantly overrepresented in a group of 18 recipients with CsA nephrotoxicity who were subsequently switched to a calcineurin inhibitor–free regimen that contained either azathioprine or mycophenolate mofetil, which are definitively not P-gp substrates (9,27). Approximately 40% of all organ donors with the TT genotype showed CsA nephrotoxicity approximately 2.5 yr posttransplantation in comparison with allografts with the CT or the CC genotype and an incidence of only 10% CsA nephrotoxicity. Our data suggest a dominant role of the donor’s $ABCB1$ genotype for developing CsA nephrotoxicity because no association with the recipient’s genotype was observed.

Several studies have addressed the relevance of a causal relationship among renal P-gp expression and function, intrarenal CsA accumulation, and drug-induced nephrotoxicity. CsA-treated renal transplant patients with acute tubular necrosis, acute allograft rejection, or chronic allograft nephropathy expressed P-gp in nephron structures that are predominately affected by CsA-induced nephrotoxicity (8,28). In rats that were treated chronically with CsA, intrarenal angiotensin II deposits, peritubular fibrosis, and CsA deposits were inversely related to renal P-gp expression, i.e., the lower the tubular P-gp expression, the higher the histologic signs of severe nephrotoxicity (7). These data indicate that CsA elimination from tubular cells by

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**Figure 3.** Kaplan-Meier curves for the proportion of individuals with CsA nephrotoxicity over the years posttransplantation in relationship to the donor’s and recipient’s genotypes $ABCB1$ 3435C→T (A and B) as well as $ABCB1$ 2677G→T (C and D). CNI, calcineurin inhibitor.
P-gp represents a detoxification mechanism (29). In our previous histopathologic study using immunostaining, we demonstrated that a significant lower P-gp expression was observed in allograft biopsies of patients with CsA nephrotoxicity compared with biopsies from patients with histology-proven acute tubular necrosis or acute vascular or chronic allograft rejection (8). This confirms the notion that a constitutional low expression of P-gp in individual renal grafts leads to an insufficient clearance of CsA and thereby is responsible for an accumulation of CsA in the kidney and consequently resulting in nephrotoxicity.

To the best of our knowledge, our study is the first to suggest an association between ABCB1 polymorphisms and CsA nephrotoxicity in renal transplant patients, which provides a new molecular explanation for CsA nephrotoxicity. Human P-gp expression shows pronounced interindividual differences in various tissues, and individuals homozygous for the T allele of a polymorphism, 3435C→T in exon 26, were associated with an on average twofold lower P-gp expression in renal proximal epithelial cells, intestinal mucosa, peripheral leukocytes, and human placenta (10,30–33). Although the 3435C→T polymorphism is a silent mutation, located in the wobble position of a codon, and the responsible molecular mechanism has not been elucidated so far, an association with a significant alteration of disease outcome under drug treatment with P-gp substrates has been demonstrated in several studies. For instance, the P-gp low expressor 3435TT genotype was significantly more frequent in patients who responded to antiepileptic drug treatment as compared with drug-resistant epilepsy (34), and TT carriers had shown a significantly better response rate to HIV therapy (e.g., viral load and increase of CD4+ cell count) than patients with the CC genotype (35). Furthermore, two independent studies demonstrated that only the 3435TT genotype was associated with an increased risk for developing ulcerative colitis (36,37), confirming the observation that P-gp knockout mice showed an increased risk for a human ulcerative colitis-like disease.

However, we are aware that conflicting results have been reported with regard to the 3435C→T polymorphism and its relationship to functional consequences such as drug plasma levels, expression of P-gp and mRNA, or therapy outcome (9,27). Kim et al. (38) proposed that the nonsynonymous ABCB1 single nucleotide polymorphism (SNP) at position 2677 (Ala893Ser) seems to be more sensitive for prediction of P-gp function. In this study, the 2677TT genotype was associated with significantly lower plasma concentrations of the P-gp substrate fexofenadine, which the authors explain by a higher intestinal P-gp expression. Because a tight linkage disequilibrium between both polymorphic sites 2677 and 3435 was demonstrated (39,40), at present it is unclear why in some studies a phenotype-genotype correlation was found only for the ABCB1 2677 polymorphism but not for the ABCB1 3435 polymorphism and vice versa. Furthermore, several other nonsynonymous (e.g., 1236C→T) as well as synonymous SNP located in introns or in the promoter region that are partly in linkage disequilibrium with the variants at positions 2677 and 3435, respectively, were identified (40–42). In several studies, specific ABCB1 haplotypes mostly between the 2677 and 3435 polymorphisms were investigated assuming that combinations between SNP may be more predictive than testing for single polymorphic sites (22,40). However, when considering various ABCB1 haplotypes in our study, we did not see better prediction for CsA nephrotoxicity. We found a relationship only between the donor’s haplotype 1 (2677G/3435C) and CsA nephrotoxicity. Because haplotype 1 is associated with higher P-gp function in humans (22), a protective effect of this haplotype for developing CsA nephrotoxicity can be suggested (OR, 0.29; 95% CI, 0.1 to 0.7; P = 0.0045).

Several explanations for why conflicting results were observed for the ABCB1 sequence variations at position 2677 and 3435 in relationship to P-gp expression, function including drug disposition of Pgp substrates, and disease outcome (see reviews 9,27,43) have been proposed. Confounding factors such as differences in study populations, particularly in sample size, ethnicities, or disease states, and differences in diet and environmental chemicals seem to be most important. Moreover, because commonly only the variants at positions 2677 and 3435 were tested without genotyping for additional intronic and exonic SNP including newly identified promoter mutations (41,42), it cannot be excluded with certainty that specific complex haplotypes may be more predictive.

In this context, it is interesting to note that Hebert et al. (44) recently investigated chronic renal dysfunction after liver transplantation in patients who received calcineurin inhibitor (CsA or tacrolimus). On the basis of these results, it was shown that individuals homozygous for the ABCB1 2677TT allele have a significant reduced risk for developing chronic renal dysfunction. This finding was interpreted by the authors to mean that the 2677T allele is associated with higher P-gp expression in renal epithelial cells, leading to an increased renal elimination of CsA. However, this is in contrast to the results by Siegsmund et al. (10) because in this study, a lower P-gp expression in human proximal epithelial cells was significantly associated with the 3435TT genotype, which is partly in linkage to 2677TT. Moreover, a significant reduction in the renal clearance of the P-gp substrate irinotecan (12) was demonstrated only in association with the P-gp low expressor geno-/haplotypes (e.g., 2677T/3435T). Therefore, Hebert et al. (44) proposed an alternative explanation for their results. Because only the recipient’s but not the donor’s genotype of the transplant liver was taken to account, alteration of hepatic metabolism of CsA on the basis of constitutional differences in expression of drug-metabolizing enzymes cannot be unequivocally excluded in their study. Nevertheless, this example demonstrates how complex the interpretation of ABCB1 phenotype-genotype correlation studies can be and that the knowledge of the underlying so-far-unknown mechanism resulting in alteration of P-gp expression/function is of great importance. Until now, only hypothetical mechanistic explanations have been postulated for how the synonymous 3435 polymorphism could influence expression and/or function of Pgp. The 3435 polymorphism may be in linkage disequilibrium with an unknown causal promoter/enhancer SNP in the ABCB1 gene or a neighbor gene affecting basal transcription of the gene (45). Moreover, SNP may affect
mRNA splicing through allele-specific differences in RNA folding (46), modify protein expression or function through RNA processing or translational control (47,48), or affect the rate of protein synthesis through reduced translational efficiency (9). In the case of the nonsynonymous SNP 2677T (Ser893), Kim et al. (38) demonstrated using a retrovirus expression system that cells expressing the Ser893 P-gp variant had roughly a twofold increased transport activity for digoxin relative to the corresponding cells expressing the reference protein. However, other authors have shown that the 2677 SNP did not significantly alter the in vitro function of P-gp using different expression systems (40,48,49). Thus, in case of the relevance of 2677, it is also questionable which causal mechanism is underlying.

Although our study was not a prospective clinical trial, several lines of evidence exclude a spurious association between ABCB1 genetics and CsA nephrotoxicity. The overall distribution of allele and genotype frequencies for both ABCB1 polymorphisms was not significantly different for recipients as well as donors in comparison with a large healthy control group. If P-gp expression in the kidney is responsible for CsA nephrotoxicity, then only the donor’s and not the recipient’s genotype plays the predominant role as a risk factor. This is confirmed by the results of our study. As an independent genetic control, we used the CYP2D6*4 and CYP3A5*3C gene polymorphisms. Both genes are not linked to ABCB1. Again, for alleles and genotypes, the frequencies were the same between patients with and without nephrotoxicity as well as in comparison with healthy control subjects. Moreover, demographic and medical conditions (Table 1) as possible confounding factors can be excluded because we did not find significant differences between both patient groups (50). This holds true when patients in the control group were stratified between those who stayed on CsA at follow-up and those who were switched to tacrolimus. The assumption that rejection episodes may entail substantial histologic damage of the transplant, thereby making the graft more susceptible for nephrotoxicity, can be definitively excluded in our study because a rejection episode before diagnosis of CsA nephrotoxicity was an exclusion criteria. Finally, in a multivariate model, only the donor’s ABCB1 3435TT genotype was strongly associated with CsA nephrotoxicity (OR, 13.4; 95% CI, 1.2 to 147.9; \( P = 0.034 \)). The observation that CsA dosage and dose-adjusted CsA trough blood concentrations in our study were not related to ABCB1 geno-/haplotypes is in agreement with several other studies that investigated CsA pharmacokinetics even more comprehensively (23,24,51).

There are, however, some limitations of this study. First, the number of study patients was small because the study population was limited by the availability of donors’ and recipients’ DNA. Second, the diagnosis of CsA nephrotoxicity was not proved by histologic criteria (52) because it is not common practice in our nephrology center to biopsy regularly renal grafts with suspected CsA nephrotoxicity. However, it should be taken into account that CsA-related histopathologic changes such as arteriolopathy, interstitial fibrosis, and tubulopathy can also occur in other forms of chronic allograft nephropathy of different origin (52,53). For strengthening the correctness of diagnosis of CsA nephrotoxicity in our study, significant improvement of renal function after conversion to a calcineurin inhibitor–free regimen was a prerequisite.

On the basis of our data, a more tailored immunosuppressive strategy in patients with CsA nephrotoxicity may be beneficial as previously proposed (54). The ABCB1 3435TT genotype of the allograft renders patients more susceptible for developing CsA nephrotoxicity. Therefore, we suggest that those patients should be monitored more closely. In the case of impaired renal function as consequence of CsA treatment, patients with the 3435TT genotype might benefit from early conversion to a calcineurin inhibitor–free regimen. Our study for the first time provides an objective marker for this decision process. Thus, P-gp seems to be a more general relevant target in CsA nephrotoxicity as so far considered. Most important, our data emphasize the necessity to consider the donor’s genotype in genetic studies on renal transplantation to elucidate causative factors for the development of renal dysfunction (e.g., chronic allograft nephropathy).

References


ABC1 Polymorphism and Cyclosporine Nephrotoxicity


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