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Original Article

The Impact of CYP3A5 and MDR1 Polymorphisms on Tacrolimus Dosage Requirements and Trough Concentrations in Pediatric Renal Transplant Recipients

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ABSTRACT. Previous international studies demonstrated significant heterogeneity in the tacrolimus (TAC) dose required to attain target blood concentrations, attributed to both genetic and ethnic factors. While the majority of previous reports on adult recipients of renal, heart and liver transplants have shown a significant effect of CYP3A5*3 single nucleotide polymorphisms (SNPs) on TAC pharmacokinetics (PKs), the impact of multidrug resistance protein 1 (MDR1) and SNPs remains controversial. Yet, similar data of TAC in pediatric populations, in whom the intra- and inter-subject variations are likely to be even greater, is currently limited. We aimed to examine the influence of various CYP3A5 and MDR1 genotypes on TAC dose requirements and PKs in the Jordanian pediatric renal transplant population. Thirty-eight patients were genotyped for CYP3A5*1 and *3 and MDR1 C3435T. Dose-adjusted trough concentrations (C₀/D) and daily doses (D) were compared among different CYP3A5 and MDR1 genotypes in the early and maintenance phases post-transplant. Surprisingly, there were no significant differences in D, C₀ or C₀/D among the genotypes of CYP3A5 or MDR1 polymorphisms in either the early or the maintenance phase after transplantation, whereas after combining the C₀/D levels of MDR1 C allele expressers, noticeably lower TAC levels were observed as compared with the TT genotype. However, the difference became not significant beyond 3 months. Based on a pharmacogenetic evaluation, the independent impact of CYP3A5 SNPs on TAC PKs was not evident, demonstrating the need for further large-scale studies.

Introduction

Tacrolimus (TAC), as a potent immunosuppressive agent, in combination with mycophenolate mofetil or azathioprine and corticosteroids is frequently employed in most of the immunosuppressive protocols post-transplantation. TAC is a macroline that has a narrow
therapeutic scope in terms of efficacy and safety. Previous international studies demonstrated significant heterogeneity in the dose required to attain target blood concentrations, which was attributed to both genetic and ethnic factors. However, initial dosing (priori) is still based on general guidelines. Subsequent (posteriori) individualization of the dosage regimen based on frequent therapeutic drug monitoring (TDM) is suggested to be crucial for avoiding under- and over-immunosuppression. However, a number of essential limitations are often associated with traditional TDM, but primarily because it can only be initiated when an immunosuppressant is administered; therefore, it is not informative for predicting the initial dosage. Additionally, the subsequent monitoring to adjust the dose during the long-term follow-up requires extensive blood testing for drug concentrations and expends a substantial amount of the clinician’s time and resources. Therefore, several conventional pharmacokinetic (PK) and population modeling studies based on the blood concentration of TAC have been conducted for about 20 years in an attempt to optimize TDM. However, many of these population PK models were shown to have only limited predictive value with regard to explaining the variability in TAC exposure/drug concentrations. Therefore, an alternative dose-individualization approach based on genetic information was suggested for post-transplant management using immunosuppressants, especially in the initial scenario of dose.

TAC is known to be a substrate of cytochrome P450 (CYP) 3A enzymes, in particular CYP3A4 and CYP3A5, which are encoded by the CYP3A4 and CYP3A5 genes, respectively. However, CYP3A5 may play a more dominant role than CYP3A4 in the metabolism of TAC. In addition, TAC is transported out of the intestine, liver and kidney cells via p-glycoprotein (P-gp), which is encoded by the multidrug resistance 1 (MDR1) gene. CYP3A5 is also found in these similar cells of CYP3A5 expressers. The difference in expression level and the bioactivity of these proteins due to several single nucleotide polymorphisms (SNPs) have been identified in their encoding genes and, therefore, were thought to partially explain the inter-individual variations of TAC PKs. In particular, the presence of an SNP in intron 3 CYP3A5 A6986G has been reported to result in the absence of functional CYP3A5 protein in homozygous carriers (CYP3A5 *3/*3). Moreover, a total of 50 SNPs have been identified in MDR1, including C1236T, G2677T/A and C3435T SNPs in exons 12, 21 and 26, respectively, which were assumed to form different haplotypes in different ethnic groups. Some of the genetic polymorphisms of MDR1 were found to influence the expression level and function of P-gp, yet conflicting effects were revealed. At first, the MDR1 cDNA C3435T SNP was found to reduce the expression level of P-gp in the intestinal mucosa, placenta and kidney. However, it was reported that the MDR1 mRNA level was actually higher in the intestinal mucosa carrying a T allele compared with the C allele.

During the last decade, pharmacogenetic studies involving kidney, heart and liver transplant recipients were conducted to examine the individual effects of known CYP3A4/5 and MDR1 SNPs on the TAC PK parameters [daily dose requirement (D), trough concentration (C0), C0/D, D/C0 and the area under the concentration–time curve (AUC)] and/or pharmacodynamic outcomes in these patients [acute rejection, long-term patient and graft survival, infection, nephrotoxicity, diabetogenesis and hypertension]. Yet, the influence of these SNPs remains unclear, with complex and inconsistent conclusions.

Because of the low frequencies in genetic polymorphisms, it was difficult to explain the large PK variation only by the CYP3A4 genotypes and so far the effect of CYP3A4-A392G SNP is considered limited and may be confounded by ethnicity or genetic linkage with CYP3A5 and MDR1 SNPs. However, the majority of previous reports on recipients of renal, heart and liver transplants have shown a significant effect of CYP3A5*3 SNP on the PK of TAC, and a lesser influence on outcomes. However, the impact of CYP3A5
polymorphism on TAC PK was found to be modified by time interval post-transplant.\textsuperscript{23-25} Similarly, the impact of MDR1 SNPs on either TAC PKs or pharmacodynamics remains controversial across different studies in various ethnic groups.\textsuperscript{8,9,12,22,26-30} While few investigations in some ethnic minorities displayed a significant or slight significant association with either PK\textsuperscript{12,22,26,27} or pharmacodynamic measures,\textsuperscript{28-30} several others have failed to show such associations.\textsuperscript{8,9}

Additionally, most of these studies were conducted to fully understand the molecular mechanisms related to the inter-individual variations of TAC PK in adults. Yet, similar data of TAC in pediatric populations in whom the intra- and inter-subject variations are likely to be even greater is currently limited. In fact, it is essential to consider the potential for age-related changes in CYP3A4/5 enzyme levels due to both environmental and biological maturation (physiological development) factors, particularly because younger children will be receiving immunosuppressive therapy throughout their lives.\textsuperscript{31}

The frequency of the CYP3A5*3 allele is highly dependent on ethnicity, with being mostly detected in Caucasian subjects, 60–90\% of whom do not express CYP3A5 protein. Conversely, much more than 50\% of African subjects have at least one CYP3A5*1 allele and express CYP3A5 protein.\textsuperscript{15} Andrews et al\textsuperscript{12} stated that African-European recipients of renal transplant needed twice the dose of TAC as Caucasian recipients to achieve the target blood concentration.

To be best of our knowledge, no pharmacogenetic studies, documented with international publications, have been performed on the Jordanian population to uncover the frequency as well as the types of polymorphisms found in CYP3A5 or MDR1 genes. The objectives of our study were therefore (1) to describe the prevalence of polymorphisms of CYP3A5 and MDR1 among the Jordanian pediatric renal transplant population, (2) to examine the influence of various CYP3A5 and MDR1 genotypes on TAC dose requirements and PKs in the early and maintenance phases post-transplant and (3) to examine the impact of these genotypes on the short- or long-term clinical outcomes during the prospective follow-up of the same population. The latter objective will be discussed in another article.

**Methodology**

**Patients and data collection**

Thirty-eight Jordanian pediatric renal transplant recipients who received kidney grafts between January 2007 and January 2009 were included in this study. The study protocol was approved by the Ethics Committee of Royal Medical Services. Informed consent was obtained from each patient and/or their parents. Details of the TAC-based immunosuppressive regimen and its routine therapeutic drug monitoring have been described elsewhere.\textsuperscript{33} The whole blood C\textsubscript{0} was measured 12 h post-dose at various time points using a microparticle enzyme immunoassay (Abbott IMx, Abbott Laboratories, Abbott Park, Illinois, USA). The target concentrations were 10–20 ng/mL during the first month after transplantation and 5–10 ng/mL thereafter.

**Genotyping**

**Genotyping of CYP3A5*3 and *1**

Genomic DNA was extracted from 400 L of whole blood using a phenol–chloroform kit (PIERCE, Rockford, IL, USA). The polymerase chain reaction (PCR) reaction was carried out in 20 L of a solution containing 2 L of 10x PCR Gold Buffer, 2 mM MgCL\textsubscript{2}, 80 M each of dNTPs, 50 pmol each of primers, 50 ng of genomic DNA and 0.6 U of AmpliTaq Gold (Applied Biosystem, Grand Island, NY, USA). The forward primer was 5’-ATGGAGAGT-GGCATAGGA-GATA-3’, but a modified reverse primer (5’-TGTGGTCCAAACAGGG-AAGAGAT-3’) was used on the basis of the reported sequence (GenBank accession number: AF355800). The PCR conditions were 8 min at 94°C, followed by 40 cycles of 30 s at 94°C, 30 s at 59°C and 30 s at 72°C, and a final extension for 10 min at 72°C. The PCR product was detected on a 2% agarose gel by means of
ethidium bromide staining.

Genotyping of MDR1 at exon 26

Genotyping of MDR1 at exon 26 C3435T SNP was performed by using forward: 5’-TGCTGGTCCTGAAGTTGATCTGTGAAC-3’ and reverse 5’-ACATTAIGGCAGTGAC-TCGATGAAGGCA-3’ primers and through the employment of Mbol endonuclease.

Statistical Analysis

All results were expressed as the mean ± SD. TAC dose-adjusted trough levels (C₀/D) during the early (7 and 14 days) and maintenance phases (1, 3, 6, 9 and 12 months post-transplant) were calculated as the trough level (ng/mL) divided by the dose (mg/kg/day). The distribution of continuous and categorical data across various genotypes was evaluated using parametric and non-parametric tests where suitable. Mean values of serum creatinine, creatinine clearance, D, C₀ and C₀/D associated with various genotypes were compared by Student’s t-test, Wilcoxon test and ANOVA test (with post hoc Tukey’s test) using the GraphPad Prism version 5.02 for windows (GraphPad Software, San Diego, CA, USA).

Results

The CYP 3A5 *1/*1, *1/*3 and *3/*3 genotypes were detected in two (5.3%), four (10.5%) and 32 (84.2%) of the 38 pediatric patients, respectively, while the MDR1 C3435T CC, 3435CT and 3435TT were detected in 15 (39.5%), 15 (39.5%) and eight (21.1%) pediatric patients, respectively. Both genotype distributions did not deviate from the Hardy–Weinberg equilibrium. The distribution of baseline patient characteristics (Table 1) did not differ significantly within the subcategories of the two genotypes. Additionally, serum albumin, hematocrit, hemoglobin, total bilirubin and liver enzymes did not change significantly and were comparable among the different genotypes (data not shown) throughout the observation period (22 ± 15 months).

Surprisingly, there were no significant differences in D, C₀ or C₀/D among each genotype group of CYP3A5 or MDR1 polymorphisms in either the early or the maintenance phase after transplantation (Table 2). Moreover, after combining the C₀/D levels of CYP3A5 expressers (CYP3A5 *1/*1 + *1/*3), the difference remained non-statistically significant in comparison with the CYP3A5 non-expressers (CYP3A5 *3/*3) at any time point (Figure 1), whereas after combining the C₀/D levels of MDR1 C allele expressers, noticeably higher TAC levels were observed in the TT genotype as compared with the combined group of TC and CC genotypes near the beginning of the maintenance phase post-transplantation (Figure 2). However, the difference became non-significant beyond 3 months.

To examine the combined effect of CYP3A5 and MDR1 polymorphisms, the patients were divided into three genotype groups: *3/*3 + MDR1 CC (n = 11), *3/*3 + MDR1 CT (n = 13) and *3/*3 + MDR1 TT (n = 8). Interestingly, pediatrics who had the combined mutations in CYP3A5 and MDR1 (*3/*3 + MDR1 TT) displayed significantly higher C₀/D levels (Figure 3), suggesting a possible additive effect due to interaction between the first two SNPs, whereas a comparison between the other available genotype combinations - *1/*1 + MDR1 CC (n=2), *1/*3 + MDR1 CT (n=2) and *1/*3 + MDR1 CC (n = 2) - did not demonstrate any statistical significance.

Discussion

Ethnic variation in TAC PKs has been well established. Therefore, a significant heterogeneity in the dosage required to achieve target blood concentrations was noted. This was partially attributed to genetic factors. To the best of our knowledge, there are no data demonstrating whether the Jordanian pediatric population has different PKs compared with Caucasians, African Americans or Asians. This is the first pharmacogenetic prospective follow-up study to be conducted involving Jordanian pediatric renal transplant recipients to evaluate the impact of CYP3A5 and MDR1 polymorphisms on TAC PKs and pharmacodynamics.
With regard to CYP3A5, our patients exhibited genotype frequencies more similar with those already described in Caucasians and Japanese populations; however, these genotype frequencies were different from the African-American populations.\textsuperscript{14,15,36,37} The frequency of wild-type CYP3A5 *1/*1 genotype was more comparable to Asians (7–12.2%), higher than Caucasians (0%), but less than African-Americans (25%). The frequency of heterozygote CYP3A5 *1/*3 genotype was lower than all populations (25–57.1%). However, the frequency of the homozygous *3/*3 genotype was more comparable to Caucasians (70.4–83.1%) but significantly higher than Asians (31.1–60.5%) and African-Americans (35%). For the MDR1 exon 26 polymorphism, the distribution of wild-type CC, heterozygote CT and homozygous TT was very similar to that reported for Caucasians (35.8, 42% and 22.2%, respectively),\textsuperscript{38} while it was different from Chinese (25, 43.8 and 31.3%, respectively) and Indians (18.4, 36.8 and 44.8%, respectively).\textsuperscript{15} Some studies in adults have reported a decrease in the TAC dosage requirements to achieve similar trough concentrations with increasing time post-transplant.\textsuperscript{23,25,39,40} Declines in corticosteroid dosage and recovered hematurcrit and albumin levels with time have been postulated as reasons for a decrease in TAC clearance,\textsuperscript{40} whereas in other studies the opposite effect of corticosteroid dosage on TAC clearance was demonstrated.\textsuperscript{41} However, the influence of time post-transplant on TAC PKs in pediatrics is not clear. While a previous study demonstrated significantly higher dosage requirements in the first month compared with that required to achieve the same TAC target 3.5 years later, others observed either decreased\textsuperscript{42} or no changes\textsuperscript{43} with elapsed time.

---

Table 1. The distribution of baseline demographic and clinical characteristics of the pediatric patients stratified by CYP3A5 and MDR1 genotypes.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>CYP3A5 genotypes</th>
<th>MDR1 genotypes (3435C&gt;T)</th>
<th>Genotype identification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*1/*1</td>
<td>*1/*3</td>
<td>*3/*3</td>
</tr>
<tr>
<td>Patient no. (%)</td>
<td>2 (5.3)</td>
<td>4 (10.5)</td>
<td>32 (84.2)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>11.5 ± 2.1</td>
<td>12 ± 2.7</td>
<td>11.2 ± 3.04</td>
</tr>
<tr>
<td>Male/female</td>
<td>1/1</td>
<td>1/3</td>
<td>15/17</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>28.9 ± 15.7</td>
<td>38.3 ± 13.3</td>
<td>28.6 ± 9.24</td>
</tr>
<tr>
<td>Cause of end-stage renal failure (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoplastic kidney</td>
<td>0 (0)</td>
<td>1 (2.6)</td>
<td>10 (26.3)</td>
</tr>
<tr>
<td>Reflux kidney disease</td>
<td>0 (0)</td>
<td>1 (2.6)</td>
<td>5 (13.2)</td>
</tr>
<tr>
<td>Primary glomerular disease</td>
<td>1 (2.6)</td>
<td>0 (0)</td>
<td>6 (15.8)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (5.3)</td>
<td>2 (5.3)</td>
<td>11 (29)</td>
</tr>
<tr>
<td>Co-medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azathioprine (%)</td>
<td>0 (0)</td>
<td>1 (2.6)</td>
<td>7 (18.4)</td>
</tr>
<tr>
<td>MMF (%)</td>
<td>2 (5.3)</td>
<td>2 (5.3)</td>
<td>23 (60.5)</td>
</tr>
<tr>
<td>MF (%)</td>
<td>1 (2.6)</td>
<td>1 (2.6)</td>
<td>2 (5.3)</td>
</tr>
<tr>
<td>Prednisone dose (mg/day)</td>
<td>45 ± 21.2</td>
<td>60 ± 0\textdagger</td>
<td>38.1 ± 14.95</td>
</tr>
<tr>
<td>Pre-emptive transplant (%)</td>
<td>0 (0)</td>
<td>1 (2.6)</td>
<td>10 (26.3)</td>
</tr>
<tr>
<td>Duration of RRT (months)</td>
<td>15 ± 12.7</td>
<td>14 ± 3.5</td>
<td>16.6 ± 10.1</td>
</tr>
<tr>
<td>Albumin (gm/L)</td>
<td>42 ± 4</td>
<td>38 ± 6</td>
<td>43.6 ± 1.8</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>28.5 ± 2.1</td>
<td>30.7 ± 4</td>
<td>25.62 ± 6.1</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.6 ± 2.2</td>
<td>13.8 ± 2.1</td>
<td>11.3 ± 1.9</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>2.7 ± 1.84</td>
<td>1.8 ± 0.63</td>
<td>3.7 ± 2.29</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>37.4 ± 31.1</td>
<td>42.3 ± 8.7</td>
<td>34.5 ± 35.1</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.55 ± 0.21</td>
<td>0.65 ± 0.35</td>
<td>0.37 ± 0.16</td>
</tr>
<tr>
<td>SGOT (U/L)</td>
<td>16 ± 12</td>
<td>16 ± 14</td>
<td>25.4 ± 22.1</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>21 ± 3</td>
<td>13 ± 4.2</td>
<td>23.8 ± 5.5</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD. AZA: Azathioprine, MMF: Mycophenolate mofetil, MF: Mycophenolate sodium, RRT: Renal replacement therapy, SGOT: Alanine aminotransferase, SGPT: Aspartate aminotransferase, †: statistically significant compared with *3/*3 (P <0.05).
Table 2. The impact of CYP3A5 and MDR1 genotypes on tacrolimus pharmacokinetics.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Time point</th>
<th>Genotype identification</th>
<th>CYP3A5 genotypes</th>
<th>MDR1 genotypes (3435C&gt;T)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>*1/*1</td>
<td>C/C</td>
<td>C/T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*1/*3</td>
<td>*1/*3</td>
<td>*1/*3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*3/*3</td>
<td>*3/*3</td>
<td>*3/*3</td>
</tr>
<tr>
<td>Dose (mg/day)</td>
<td>1 M</td>
<td>7 ± 1.4</td>
<td>8.5 ± 3</td>
<td>7.19 ± 2.74</td>
</tr>
<tr>
<td></td>
<td>3 M</td>
<td>7 ± 1.4</td>
<td>7.5 ± 4.12</td>
<td>6.67 ± 2.29</td>
</tr>
<tr>
<td></td>
<td>6 M</td>
<td>6 ± 2.8</td>
<td>5.8 ± 3.1</td>
<td>6.27 ± 2</td>
</tr>
<tr>
<td></td>
<td>9 M</td>
<td>6 ± 2.8</td>
<td>6.3 ± 2.6</td>
<td>6.17 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>12 M</td>
<td>6 ± 2.8</td>
<td>6.3 ± 2.6</td>
<td>6.23 ± 2.1</td>
</tr>
<tr>
<td>Dose (mg/kg/day)</td>
<td>1 M</td>
<td>0.251 ± 0.117</td>
<td>0.234 ± 0.039</td>
<td>0.231 ± 0.071</td>
</tr>
<tr>
<td></td>
<td>3 M</td>
<td>0.175 ± 0.067</td>
<td>0.167 ± 0.05</td>
<td>0.197 ± 0.068</td>
</tr>
<tr>
<td></td>
<td>6 M</td>
<td>0.152 ± 0.054</td>
<td>0.132 ± 0.05</td>
<td>0.178 ± 0.065</td>
</tr>
<tr>
<td></td>
<td>9 M</td>
<td>0.137 ± 0.037</td>
<td>0.143 ± 0.052</td>
<td>0.167 ± 0.059</td>
</tr>
<tr>
<td></td>
<td>12 M</td>
<td>0.138 ± 0.045</td>
<td>0.138 ± 0.048</td>
<td>0.164 ± 0.062</td>
</tr>
<tr>
<td>C₀ (ng/mL)</td>
<td>14 days</td>
<td>8 ± 0.71</td>
<td>5.4 ± 0.85</td>
<td>9.7 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>1 M</td>
<td>7.35 ± 1.2</td>
<td>7.7 ± 1.9</td>
<td>8.76 ± 4.34</td>
</tr>
<tr>
<td></td>
<td>3 M</td>
<td>5.2 ± 1.2</td>
<td>7.88 ± 3.82</td>
<td>9.2 ± 3.99</td>
</tr>
<tr>
<td></td>
<td>6 M</td>
<td>7.35 ± 0.05</td>
<td>5.68 ± 2.14</td>
<td>7.15 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>9 M</td>
<td>6.15 ± 1.2</td>
<td>5.6 ± 1.76</td>
<td>6.62 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>12 M</td>
<td>7.3 ± 0.42</td>
<td>7.5 ± 2.3</td>
<td>6.99 ± 2.35</td>
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<td>C₀/dose§)</td>
<td>14 days</td>
<td>29.9 ± 5.8</td>
<td>24.5 ± 4.1</td>
<td>44.1 ± 25.23</td>
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<td>1 M</td>
<td>35.2 ± 16.9</td>
<td>36 ± 12.4</td>
<td>36.3 ± 19.8</td>
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<td>41.2 ± 24.5</td>
<td>52.5 ± 28.5</td>
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<td>45.6 ± 17.9</td>
<td>33.9 ± 8.1</td>
<td>38.9 ± 19.2</td>
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<td>9 M</td>
<td>44.5 ± 23.6</td>
<td>52.96 ± 17.3</td>
<td>40.2 ± 17.1</td>
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<td>12 M</td>
<td>55.6 ± 18</td>
<td>59.4 ± 30.5</td>
<td>47.5 ± 22.6</td>
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Values presented as mean ± SD, C₀: Tacrolimus trough concentration, §: Dose expressed as mg/kg/day; †: statistically significant compared with T/T (mutant type) (P < 0.05); ‡: statistically significant compared with C/T (heterozygote type) (P < 0.05).

Table 2. The impact of CYP3A5 and MDR1 genotypes on tacrolimus pharmacokinetics.

After transplantation.

In the present study, the body weight-adjusted dose of TAC differed significantly between the early (<3 months) and maintenance stages (0.193 ± 0.07 vs 0.1597 ± 0.06; P-value Wilcoxon test = 0.0002); however, the C₀/D level was non-statistically significant during the long-term follow-up duration (P = 0.775), probably due to continuous dose modifications according to TDM. Several other possible explanations may have contributed to our current observation. First, the dose of corticosteroid, which is known to increase CYP3A5 activity resulting in reduction of TAC C₀, was significantly reduced during the first 3 months. In the current study, the mean initial dose of prednisolone and the dose in the 3rd month were 40.8 ± 15.7 and 9.7 ± 3.9 mg/day, respectively, while that in the 12th month was 5.18 ± 0.97 mg/day (P < 0.0001). This has resulted in higher trough levels despite the significant progressive reduction in TAC dose from 0.193 during the 3rd month to 0.1597 mg/kg/day 1 year post-transplant (P = 0.0002).

Secondly, hematocrit and albumin concentrations, which were found to have a significant impact on TAC clearance and level, did not increase significantly beyond the first 2 weeks post-transplant in our pediatrics and remained relatively constant during the studied period. In accordance with the findings in previous studies, the majority (>70%) of our pediatric patients had hematocrit values consistently higher than 33%, below which it was only found to significantly increase the TAC clearance. This may have contributed to less variability in our TAC levels with increase in time elapsed since transplantation.

Regardless of ethnicity, the majority of studies in the recipient of organ transplantation has demonstrated that the CYP3A5 *1 allele carriers (CYP3A5 expressers) needed a larger TAC dose and sometimes longer time to reach the target C₀ level compared with the CYP3A5 *3/*3 carriers (CYP3A5 non-expressers), despite TDM. Yet, in other reports, no significant association was found between CYP3A5 genotypes and TAC unadjusted or
dose-adjusted C₀ concentrations. In our pediatric study, no associations were found between dosage requirements or C₀/D levels and CYP3A5 polymorphism, and these findings were not subjected to time-dependent change. The masking of the effects of CYP3A5 genotypes on TAC PKs in our pediatrics may be attributed to the level of expression of intestinal or hepatic CYP3A5 rather than their metabolic capabilities, which may be modified by environmental conditions and are likely to undergo developmental changes (e.g., bowel length, gastric pH, hepatic blood flow) as a function of age. Moreover, CYP3A5 is expressed in almost 50% of all infants’ livers, but may possibly be found in only 29% of adult

![Figure 1](image1.png)

**Figure 1.** The tacrolimus whole blood concentrations (ng/mL) per dose (mg/kg/day) during the early and the maintenance phase post-transplant in pediatric renal transplant patients separated into CYP3A5 expressers (■) and CYP3A5 non-expressers (●). The points represent the mean values for each time interval. The difference was not statistically significant at any time point (P > 0.05).

![Figure 2](image2.png)

**Figure 2.** The tacrolimus whole blood concentrations (ng/mL) per dose (mg/kg/day) during the early and the maintenance phase post-transplant in pediatric renal transplant patients separated into their MDR1 C3435T TT (●) and CT/CC genotypes (■). The points represent the mean values for each time interval. The TT patients have significantly higher TAC concentrations per dose than the CT/CC patients at 1.5 (P = 0.018) and 3 months (P = 0.048).
livers. This could partially explain our contradictory findings to those obtained in adult renal transplant studies. Additionally, a fat-rich diet in Jordan, the small number of patients carrying the wild (*1) allele in our study, the difference of transplant etiologies, concurrent illnesses, higher corticosteroid and lower co-medication (MMF, MF, AZA) compared with other adult or pediatric immunosuppressive regimens could contribute to unrevealing of the impact of CYP3A5 polymorphisms on TAC level in our pediatric population. This controversy emphasized the need for further larger and more controlled prospective studies in pediatric transplant patients to confirm the independent impact of CYP3A5 on TAC PKs and long-term clinical outcomes. Another important fact that should be taken into account in designing new studies is the employment of more accurate exposure indices than C0, such as the area under the TAC blood concentration–time curve during one dosing interval at steady state (AUCss).

The impact of MDR1 polymorphisms on TAC PKs remains uncertain, with most studies performed in adults, whereas limited data are available for the pediatric population. In our study, the MDR1 3435TT variant genotype displayed higher TAC C0/D levels as compared with the heterozygote (CT) and wild type (TT) in the first part of the maintenance phase post-transplant. Such findings confirmed previous suggestions of possible lower functional activity of P-gp due to the lesser expression associated with the T allele rather than the C allele. Yet, the difference turned into statistically non-significant beyond the 3rd month. Conversely, one study in pediatric heart transplant patients has found a significant association of MDR1 C3435T with TAC C0/D at 6 and 12 months, but not at 3 months. This could be attributed to the dissimilarity in physiologic factors underlying the two disease conditions, causing a variable increase in the MDR1 expression level. Additionally, our results were in contrast to several data from adult patients as well as the inadequate information currently available from pediatrics, which demonstrated no association of MDR1 polymorphisms with any PK parameters of TAC at any time period. However, our observation was in line with some recent studies in adult ethnic minorities, reflecting the variable importance of MDR1 po-

Figure 3. The tacrolimus whole blood concentrations (ng/mL) per dose (mg/kg/day) during the early and the maintenance phase post-transplant in pediatric renal transplant patients separated into three combined SNP groups: *3/*3 + MDR1 CC (♦), *3/*3 + MDR1 CT ( ) and *3/*3 + MDR1 TT (●). The points represent the mean values for each time interval. The *3/*3 + TT group has a significantly higher TAC concentration/dose at 1.5 (P = 0.018) and 3 months (P = 0.048) and also beyond 2 years (P = 0.001).
lymorphisms to different populations.

Although the majority of previous investigations have only appraised the influence of individual SNPs, haplotype analyses involving calcineurin inhibitors have proposed that multiple polymorphism combinations may interact to accentuate or conceal the independent effect of an SNP. This may explain why individual SNP analysis may produce narrow and frequently contradictory impacts, particularly in small studies or those involving a population of low frequency of a variant allele. Previous studies reported high frequencies of both the MDR1 3435T and the CYP3A5*3 variant alleles in the same populations, which advocated a possible linkage disequilibrium between these polymorphisms. In agreement, all TT carriers in our population were CYP3A5 non-expressers (*3/*3). Interestingly, the obscured effect of the CYP3A5*3 allele was revealed after stratifying our pediatric group and comparing the PK parameters of TT and *3/*3 simultaneous carriers to different combination polymorphisms. Similarly, the association between various MDR1 gene SNPs and CYP3A5 SNP were evaluated in several studies. Anglicheau et al in a study of 81 renal transplant recipients declared a cumulative effect of MDR1 mutation at exon 26, 21 and 12 on TAC PKs compared with the wild-type haplotype. However, in contrast to our results, Loh et al recently reported higher TAC C/D levels in patients with a *3/*3 and CC genotype combination compared with a *1/*3 and CT genotype combination, suggesting that the difference is mostly due to the effect of CYTP3A5 SNP. Conversely, Wang et al in a study of 81 lung transplant recipients demonstrated that MDR1 haplotypes were associated with TAC requirements, independent of the CYP3A5 genotype. In consistent with our finding, while all patients were known to be CYP3A5 *3/*3 expressers, patients with the 1236T-2677T-3435T + 1236T-2677T-3435T genotype had a significantly higher C/D compared with the 1236C-2677G-3435C + 1236C-2677G-3435C genotype. Additionally, a recent population PK study revealed that the combination of MDR1 and CYP3A5*3 genetic polymorphisms has a relatively strong influence on the inter-individual variability of TAC clearance, with patients who are CYP3A5 expressers and simultaneously CC-GG-CC having three-fold higher rates than those who are *3/*3 and TT-TT-TT carriers.

In our study, we tested only the C3435T SNP of MDR1. Because this SNP was reported to be a silent polymorphism that did not result in any amino acid changes, the conflicting results of these studies and our own may be partly due to linkage disequilibrium with other functional polymorphisms within the MDR1 gene. However, our observed results are mostly attributed to further linkage disequilibrium with the MDR1 SNPs in exons 12 and 21 remain to be examined. However, at the moment, we cannot exclude that dose adjustments based on both SNPs (CYP3A5 and MDR1 C3435T) should, ideally, be considered.

In conclusion, we found no significant time-dependent variability in TAC PKs after renal transplantation in our pediatric patients. Based on pharmacogenetic evaluation in this Jordanian study, the independent impact of CYP3A5 SNPs on TAC PKs was not evident in either the early or the maintenance phase, demonstrating the need for further large-scale studies to increase our knowledge about their effects on clinical outcome before application in routine practice. Indeed, in the light of the currently observed very high prevalence of *3 allele (89.5%) in the Jordanian population, we speculate to obtain more significant inter-genotype differences in a larger sample size study. However, for the effect of MDR1 C3435T polymorphism, the TT patients required a lesser dosage of TAC to achieve similar blood levels compared with CC/CT patients, remarkably near the beginning of the maintenance phase. Interestingly, an additive effect on TAC PKs was revealed in our pediatric renal transplant recipients who had both TT and *3/*3 SNPs, resulting in higher TAC C/D levels compared with other *3/*3 patients who had other MDR1 C3435T genotypes. This suggested a possible linkage disequilibrium between the two polymorphisms and highlighted the need for considering do-
sage individualization based on both genotypes, particularly in pediatric patients. However, taken together, our data advocated that further molecular investigations are still required to explore the mutual relationship between CYP3A5 and MDR1 genotypes or haplotypes in affecting the immunosuppressant therapy in different ethnic and age categories.

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