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# Frequencies and roles of CYP3A5, CYP3A4 and ABCB1 single nucleotide polymorphisms in Italian teenagers after kidney transplantation

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**Abstract:**
The main genes involved in the pharmacokinetics of immunosuppressive drugs are those encoding cytochrome P450 (CYP) family enzymes and multidrug resistance 1 (ABCB1). In this study, 87 Italian teenagers with transplanted kidneys (mean age 11.6 ± 4.8 years) receiving calcineurin inhibitors (CNIs) were genotyped for the single nucleotide polymorphisms (SNPs) *CYP3A5*1/3* and *CYP3A4*1B* for *CYP3A*, and C1236T, G2677T/A, C3435T and IVS21+49 for *ABCB1*, and retrospectively evaluated for the influence of the screened SNPs on CNI blood level at different post-transplantation times. The *CYP3A5*1* allele was present in 7% of the patients, and the *CYP3A4*1B* allele was present in 3% of patients. The *ABCB1* C1236T, G2677T/A and C3435T SNPs C, G and T occurred frequently (55%, 53% and 54%, respectively). The frequency of the T allele of IVS21+49 was 86%. The frequency of SNPs in both genes was comparable with that reported in other European Caucasian populations but different from that found in Asians or Afro-Americans. None of the cyclosporine (CsA) pharmacokinetic parameters were associated with the *CYP3A5* genetic polymorphism, whereas the presence of the A allele in some patients was responsible for the required administration of a significantly increased dose of tacrolimus (Tac) that was necessary to reach therapeutic target levels. None of the Tac pharmacokinetic parameters were associated with *ABCB1* SNPs, but *ABCB1* SNPs had early effects on the CsA exposure index and dose requirements. In conclusion, because SNPs of the *CYP3A* and *ABCB1* genes may be associated with CNI pharmacokinetic parameters and exposure indices, pre-transplant genetic screening should be considered in order to avoid immunosuppressant-related adverse events.

**Key words:**
*CYP3A5, CYP3A4*1B, ABCB1* polymorphisms, allele distribution, kidney transplantation, calcineurin inhibitors

**Abbreviations:** CNI – calcineurin inhibitors, CsA – cyclosporine, Tac – tacrolimus

## Introduction

The possible influence of genetic polymorphisms in patients undergoing kidney transplantation has recently been indicated as one of the most important variables affecting the pharmacokinetics of immunosuppressive drugs. The genes that are primarily involved in metabolizing immunosuppressants are those encoding cytochrome P450 (CYP)3A family enzymes and multidrug resistance P-glycoprotein 1 (the *ABCB1* gene). As many drugs undergo substantial intestinal and liver metabolism after absorption from the gut lumen, it is believed that CYP3A and P-glycoprotein

(P-gp) are largely responsible for the poor oral bioavailability of calcineurin inhibitors [27]. The CYP3A subfamily is a cluster consisting of four isoenzymes. *CYP3A4* and *CYP3A5* are the main genes involved in the metabolism of cyclosporine (CsA) and tacrolimus (Tac) [37]. CYP3A5 is expressed more variably and at a relatively higher level in the proximal renal tubules [14, 27]. *CYP3A5*1* encodes an active protein and is responsible for the increased metabolism of many drugs including Tac but not CsA [27]. During the transcription phase, the *CYP3A5*3* (6986 A>G) allele introduces an alternative splicing site leading to protein truncation, thus resulting in the absence of full-length protein expression [31, 38]. CYP3A4 is abundantly and constitutively expressed in hepatic and intestinal epithelia [27], so the reported increase in transcriptional activity shown by the *CYP3A4*1B* allele *in vitro* would theoretically also reflect significant enzymatic activity *in vivo* [1].

P-gp, which is encoded by the *ABCB1* gene, is a large ATP-dependent transmembrane protein involved in the extracellular extrusion of many xenobiotics and drugs including calcineurin inhibitors (CNIs) [34, 41, 49]. CsA is a typical ABCB1 substrate, and intestinal P-gp may be responsible for the reduced intestinal absorption of CsA [43, 50]. More than 700 variations in the nucleotide sequences have been described (http:www.genecard.org), and, although nothing is known about the clinical impact of most of the sequence variations on P-gp function, some seem to be functionally relevant and substantially influence the pharmacokinetics of substrate drugs. The most extensively investigated single nucleotide polymorphisms (SNPs) of *ABCB1* are 3435C>T (rs1045642) in exon 26, 1236C>T (rs128503) in exon 12, and 2677G>T/A (rs 2032582) in exon 21 [29]. The 3435C>T polymorphism is a silent polymorphism that may be in linkage disequilibrium with other functional polymorphisms in the *ABCB1* gene, including 2677G>T/A. The 3435C>T polymorphism may also reduce *ABCB1* mRNA stability in the liver, and it could change protein folding and activity [36].

Another interesting SNP is IVS21+49 T>C (rs2032583) in intron 21 of *ABCB1*, which is involved in the pharmacokinetics of many drugs including antidepressants and methadone [42]. It is rarely considered in *ABCB1* studies because it is located 57 bp from SNP rs2032582 and was amplified by polymerase chain reaction (PCR) and sequenced with the same primers.

The aim of this study was to assess the frequency of variant alleles and the genotype distribution of *CYP3A5*1/3*, *CYP3A4*1B*, C3435T, G2677T/A, C1236T and IVS21+49 and their effects on CNI pharmacokinetics in a selected population of Italian teenagers undergoing kidney transplantation.

## Patients and Methods

### Patient population

The study involved 87 pediatric kidney transplant recipients (31 female and 56 male, mean age 11.6 ± 4.8 years), who received CsA (n = 61) or Tac (n = 26) as their main immunosuppressant. We considered a genetic control group of 50 healthy individuals that were age- and gender-matched to the subjects receiving transplants. The study was carried out in accordance with the requirements of our local Ethics Committee and the principles of the Declaration of Helsinki. All of the peripheral blood samples were obtained with parental consent.

### Immunosuppressive protocols and pharmacokinetic studies

CsA was administered orally six hours after transplantation at a dose of 500 mg/m$^2$ targeting a two-hour post-dosing blood concentration (C2) of 1400 ng/ml. Tac was administered at a dose of 0.3 mg/kg/day in order to achieve trough blood levels (C0) of 10–20 ng/ml in the first month after transplantation. Both CNIs were administered in combination with mycophenolate mofetil at a starting dose of 600–800 mg/m$^2$ aiming for a C0 of 1.5–3 μg/ml. Steroids (10–15 mg/kg/day) were given intravenously for the first two postoperative days and then orally at a dose of 1 mg/kg/day, which was gradually tapered to 0.125 mg/kg/day by six months after transplantation.

Whole blood samples for pharmacokinetic study were collected 6, 30 and 60 days after transplantation, and the following parameters were computed: trough level (C0: ng/ml), weight-adjusted daily dose (mg/kg); and dose-normalized trough level (C0 divided by the corresponding 24-hour dose). Tac and CsA blood concentrations were assessed using Syva® EMIT (Dade Behring, Eschborn, Germany).

**Tab. 1.** Primer sequences used to amplify PCR fragments containing SNPs

| SNP | FORWARD | REVERSE | bp | TM |
|---|---|---|---|---|
| *CYP3A5* | 5'-ATG GAG AGT GGC ATA GGA GAT A-3' | 5'-TGT GGT CCA AAC AGG GAA GAG AT-3' | 150 | 59 |
| *CYP3A4* | 5'-GCT CTG TCT GTC TGG GTT TGG-3' | 5'-CAC ACC ACT CAC TGA CCT CCT-3' | 280 | 64 |
| *ABCB1* C1236T | 5'-TAC CCA TCT CGA AAA GAA GTT AAG G-5' | 5'-GAA AGA TGT GCA ATG TGA CTG CTG AT-3' | 350 | 54 |
| *ABCB1* G2677T/A and IVS21+49 | 5'-TAC CCA TCA TTG CAA TAG CAA TAG CAG-3' | 5'-AAG ATT GCT TTG AGG AAT GGT-3' | 210 | 61 |
| *ABCB1* C3435T | 5'-TGC TGG TCC TGA AGT TGA TCT GTG AAC-3' | 5'-ACA TTA GGC AGT GAC TCG ATG AAF GCA-3' | 230 | 61 |

SNP = single nucleotide polymorphism, PCR = polymerase chain reaction. bp = base pair length; TM = annealing temperature

### Genotyping of *CYP3A* and *ABCB1* SNPs

DNA was extracted from 400 µl of fresh blood using the phenol-chloroform method as previously described [19] and then re-suspended in 120 µl of deoxyribonuclease-free water. PCR was performed using a Gene Amp PCR system 2400 (Perkin Elmer Italia S.p.A., Monza, Italy) under the conditions shown in Table 1. For *CYP3A4*1B*, 1X buffer (Applied Biosystems Roche, Brancbury, NJ, USA), 2-mM $MgCl_2$ (Applied Biosystems Roche), 0.14-µM primers (Thermo Electron GmbH, Ulm, Germany), 80-µM dNTPs (Euroclone, Pero, Italy), 0.12-U/µl Taq Gold (Applied Biosystems Roche), and 100 ng of DNA were added to a final volume of 20 µl; for *CYP3A5* and *ABCB1*, 1X buffer (Applied Biosystems Roche), 1.8-mM $MgCl_2$ (Applied Biosystems Roche), 0.28-µM of primers (Thermo Electron GmbH), 0.2-U/µl Taq Gold (Applied Biosystems Roche), and 100 ng of DNA were added to a final volume of 20 µl. The PCR products were purified using Microcon (Millipore Corporate, Billerica, MA, USA) in accordance with the manufacturer's protocol. Single strands of 1.5 µl of purified product were amplified using a Big Dye kit (Applied Biosystems, Foster City, CA, USA) as indicated in the manufacturer's protocol in a final volume of 10 µl. The PCR consisted of an initial denaturation step at 96°C for 2 s, 23 cycles at 96°C for 10 s, 44°C for 5 s, and 60°C for 4 min. The final product was sequenced using an Abi-Prism 3100 sequencer (Applied Biosystems).

### Statistical analysis

The data were analyzed using Prism 5.0 (GraphPad Software, San Diego CA, USA). A p-value of $p < 0.05$ was considered to be statistically significant. All of the data are expressed as the mean values ± standard deviation unless stated otherwise. One-way ANOVA was used to examine the differences between the genotype subgroups. The expected genotype distribution was calculated using the Hardy-Weinberg equilibrium. The chi-squared test was used to compare the *CYP3A* and *ABCB1* polymorphisms in different populations. The haplotypes were calculated by Helix Three software (Golden Helix, USA).

## Results

### Genetic frequencies

Table 2 shows the genetic frequencies of the *CYP3A5*, *CYP3A4*1B*, *ABCB1* C1236T, G2677T/A, C3435T and IVS21+49 polymorphisms in the transplanted and control groups. The expected frequency of each genotype was evaluated using Hardy-Weinberg equilibrium; none of the observed frequencies were significantly different from expected values except IVS21+49. In the transplanted patients, the G allele of *CYP3A5* (a non-expressing variant) was present in 92.5% of the subjects, and the A allele (wild-type variant) of *CYP3A4* was present in 97.1% of patients. Regarding

**Tab. 2.** Frequencies of *CYP3A* and *ABCB1* SNPs in transplant patients and healthy subjects

| | SNP | KT | HS |
|---|---|---|---|
| *CYP3A5* | G | 0.93 | 0.93 |
| | A | 0.07 | 0.07 |
| *CYP3A4* | A | 0.97 | 0.95 |
| | G | 0.03 | 0.05 |
| *ABCB1* | C | 0.55 | 0.51 |
| C3435T | T | 0.45 | 0.49 |
| *ABCB1* | G | 0.53 | 0.64 |
| G2677T/A | T | 0.43 | 0.36 |
| | A | 0.04 | 0.00 |
| *ABCB1* | C | 0.54 | 0.60 |
| C1236T | T | 0.46 | 0.40 |
| *ABCB1* | C | 0.14 | 0.20 |
| IVS21+49 | T | 0.86 | 0.80 |

SNP = single nucleotide polymorphism; KT = kidney transplant patients; HS = healthy subjects

the *ABCB1* polymorphism in the population receiving transplants, the C alleles of C1236T and C3435T were present at similar frequencies (54.0% and 54.5%, respectively). In the case of polymorphisms located at position 2677, the frequencies were 53.4% for the G allele and 4.0% for the A allele. The T allele of the IVS21+49 polymorphism displayed a frequency of 86.2%. The frequencies of the SNPs of the *CYP3A* and *ABCB1* genes were comparable in the two groups.

Table 3 compares the allelic frequencies of the studied polymorphisms with those observed in other ethnic groups. The distribution of *CYP3A5* alleles was similar to that found in other European Caucasian populations [3, 12, 21, 23, 25, 28] (particularly British and Spanish [21, 23]) but, as expected, there were significant differences compared to Asian and Afro-Americans populations due to the greater presence of the A allele ($p < 0.004$) [10, 20, 21, 30, 55]. The African and Asian populations also had significantly different distributions of *CYP3A4*1B* [11, 20, 30].

Interestingly, when we compared *ABCB1* allele frequencies, we noticed that African and South American populations had very high frequencies of wild-type alleles in comparison with our population [9, 15, 16, 20, 21, 35, 62], whereas Asian populations had higher frequencies of the T alleles of C1236T and G2677T/A [11, 30, 32]. The allele frequencies of *ABCB1* in our population were also statistically different from that observed in some Caucasian populations, like Belgian, Czech and Portuguese populations [8, 25, 47] and similar to other Caucasian populations [3, 7, 12, 21–24, 28, 39].

## Pharmacokinetic data

Table 4 summarizes the results of the influence of the screened polymorphisms on Tac pharmacokinetics. The presence of the A allele of *CYP3A5* greatly affected Tac pharmacokinetics beginning at one month after transplantation. Recipients bearing the A allele required an average Tac dose of 7.43 ± 3.11 mg (0.32 ± 0.14 mg/kg) to reach a normalized trough level of 27.84 ± 10.73 ng/ml per mg/kg throughout the observational period, whereas non-carriers required a smaller dose (0.22 ± 0.08 mg/kg; $p < 0.05$) to reach a higher normalized blood trough level (66.98 ± 32.02 ng/ml per mg/kg; $p < 0.05$). On the other hand, *CYP3A4* and *ABCB1* gene polymorphisms only marginally affected Tac pharmacokinetics. In fact, homozygous TT patients for SNP C1236T seemed to have a drug concentration that was significantly lower than the heterozygous patients but only during the immediate post-transplant period. Instead, a month after transplantation, we found that patients who were heterozygous for *CYP3A4* required a higher Tac dose, while patients who were heterozygotes for SNP 2677 showed decreased Tac blood concentrations. The haplotype analyses in the majority of patients (homozygous GG for *CYP3A5*) yielded four main haplotypes for *ABCB1*: TTTT (38,6%) CGTC (29,5%) CGCC (13.6%) and CGTT (6.8%) for genes C1236T, G2677T/A, IVS 21+49, C3435T, respectively. For these haplotypes any significant correlation with the considered pharmacokinetic parameters was recorded.

Table 5 shows that *CYP3A5* SNPs did not affect CsA pharmacokinetics; the effect of the *CYP3A4* polymorphism could not be investigated because none of the patients were heterozygotic.

*ABCB1* polymorphisms affected CsA pharmacokinetics during the immediate post-transplant period; on post-operative day six, IVS21+49 heterozygotes (CT) had higher CsA trough levels than homozygotes

**Tab. 3.** Comparison of the frequencies of *CYP3A* and *ABCB1* SNPs found in this study and in other Caucasian and non-Caucasian populations

| | | | *CYP3A5* | | | *CYP3A4* | | | *ABCB1* C3435T | | | *ABCB1* G2677T/A | | | | *ABCB1* C1236T | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | SUBJECT¤ | G | A | | A | G | | C | T | | G | T | A | | C | T | |
| THIS STUDY | 87 | KT | 0.92 | 0.08 | | 0.97 | 0.03 | | 0.55 | 0.45 | | 0.53 | 0.43 | 0.04 | | 0.54 | 0.46 | |
| GB CAUCASIAN [21] | 133 | KT | 0.89 | 0.11 | | | | | 0.41 | 0.59 | | 0.52 | 0.44 | 0.04 | | 0.56 | 0.44 | |
| FINNISH [28] | 449 | HEALTHY | 0.92 | 0.08 | | | | | 0.63 | 0.37 | | | | | | | | |
| SWISS [12] | 245 | UPT | 0.92 | 0.08 | | 0.96 | 0.04 | | 0.50 | 0.50 | | 0.55 | 0.45 | 0.00 | | | | |
| CZECH [47] | 189 | HEALTHY | | | | | | | 0.44 | 0.56 | * | 0.53 | 0.46 | 0.01 | * | 0.55 | 0.45 | |
| GERMAN [7] | 461 | HEALTHY | | | | | | | 0.46 | 0.54 | | 0.56 | 0.42 | 0.02 | | 0.59 | 0.41 | |
| POLISH [39] | 122 | HEALTHY | | | | | | | 0.63 | 0.38 | | 0.57 | 0.41 | 0.01 | | 0.58 | 0.41 | |
| PORTUGUESE [8] | 100 | HEALTHY | | | | | | | 0.36 | 0.65 | * | 0.53 | 0.48 | 0.00 | * | | | |
| SPANISH [23] | 177 | HEALTHY | 0.91 | 0.09 | | 0.96 | 0.04 | | 0.50 | 0.50 | | | | | | | | |
| FRENCH [3] | 149 | KT | 0.81 | 0.13 | | 0.90 | 0.10 | | 0.61 | 0.39 | | 0.61 | 0.36 | 0.02 | | 0.61 | 0.35 | |
| RUSSIAN [22, 24] | 290 | HEALTHY - CA. | | | | | | | 0.46 | 0.54 | | 0.53 | 0.42 | 0.03 | | 0.52 | 0.48 | |
| BELGIAN [25] | 50 | KT | 0.94 | 0.06 | | | | | 0.48 | 0.52 | | 0.61 | 0.39 | 0.00 | * | 0.59 | 0.41 | |
| ASIAN | | | | | | | | | | | | | | | | | | |
| CHINESE [11] | 96 | HEALTHY | | | | 1.00 | 0.00 | * | 0.47 | 0.53 | | 0.33 | 0.50 | 0.12 | * | 0.28 | 0.72 | * |
| INDIAN [11] | 87 | HEALTHY | | | | 0.99 | 0.01 | * | 0.37 | 0.63 | * | 0.29 | 0.60 | 0.07 | * | 0.33 | 0.67 | * |
| JAPANESE [32] | 114 | HEALTHY | | | | | | | 0.62 | 0.39 | | | | | | 0.39 | 0.61 | * |
| KOREAN [10] | 29 | HEALTHY | 0.69 | 0.31 | * | | | | 0.57 | 0.43 | | 0.40 | 0.45 | 0.12 | * | 0.31 | 0.69 | * |
| CHINESE [30] | 106 | HEALTHY | 0.78 | 0.22 | * | 0.99 | 0.01 | * | 0.47 | 0.53 | | | | | | | | |
| GB SOUTH ASIAN [21] | 49 | KT | 0.72 | 0.28 | * | | | | 0.45 | 0.55 | | 0.35 | 0.60 | 0.05 | * | 0.38 | 0.62 | * |
| CHINESE [30] | 106 | KT | 0.78 | 0.22 | * | 1.00 | 0.00 | * | 0.47 | 0.53 | | | | | | | | |
| SOUTH AMERICA | | | | | | | | | | | | | | | | | | |
| BRAZIL | | | | | | | | | | | | | | | | | | |
| WHITE [15] | 106 | HEALTHY | | | | | | | 0.55 | 0.45 | | 0.61 | 0.38 | 0.01 | * | 0.60 | 0.40 | |
| MIXED [15] | 114 | HEALTHY | | | | | | | 0.66 | 0.34 | * | 0.70 | 0.28 | 0.02 | * | 0.65 | 0.35 | * |
| BLACK [15] | 100 | HEALTHY | | | | | | | 0.71 | 0.30 | * | 0.82 | 0.18 | 0.01 | * | 0.67 | 0.34 | * |
| BRAZILIAN | | | | | | | | | | | | | | | | | | |
| WHITE [55] | 98 | HEALTHY | 0.78 | 0.22 | * | | | | | | | | | | | | | |
| MIXED [55] | 111 | HEALTHY | 0.64 | 0.36 | * | | | | | | | | | | | | | |
| BLACK [55] | 99 | HEALTHY | 0.32 | 0.68 | * | | | | | | | | | | | | | |
| CHILEAN | | | | | | | | | | | | | | | | | | |
| MESTIZA [62] | 104 | HEALTHY | | | | | | | 0.67 | 0.33 | * | 0.65 | 0.26 | 0.09 | * | 0.59 | 0.41 | |
| MAPUCHE [62] | 96 | HEALTHY | | | | | | | 0.65 | 0.35 | * | 0.69 | 0.16 | 0.15 | * | 0.40 | 0.60 | * |
| PACUENSE [62] | 52 | HEALTHY | | | | | | | 0.75 | 0.25 | * | 0.78 | 0.15 | 0.07 | * | 0.69 | 0.31 | * |
| AFRICAN | | | | | | | | | | | | | | | | | | |
| GHANAIAN [35] | 23 | HEALTHY | | | | | | | | | | 0.89 | 0.11 | 0 | * | 0.85 | 0.15 | * |
| TANZANIAN [20] | 103 | MALARIA | 0.14 | 0.75 | * | 0.30 | 0.67 | * | 0.84 | 0.16 | * | 0.97 | 0.03 | 0.00 | * | | | |
| ZULU [9] | 110 | HEALTHY | | | | | | | 0.86 | 0.14 | * | | | | | | | |
| GB BLACK [21] | 15 | KT | 0.53 | 0.47 | * | | | | 0.70 | 0.30 | * | 0.83 | 0.13 | 0.03 | * | 0.80 | 0.20 | * |

* Chi-squared test p-value < 0.005; kind of subjects: KT = kidney transplantation; CA = cancer; UPT = under pain treatment with methadone

**Tab. 4.** Relationships between SNPs in *CYP3A* and *ABCB1* genes and tacrolimus (Tac) pharmacokinetics

| | | | Tac trough concentration (ng/ml) | | | Tac dose (mg/kg per day) | | | Tac dose-adjusted concentration (ng/ml per mg/kg) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Post-transplantation day | | (n) | #6 | #30 | #60 | #6 | #30 | #60 | #6 | #30 | #60 |
| *CYP3A5* | AG | 6 | 10.50 | 10.42 | 14.56 | 0.28 | **0.44**[a] | **0.40**[a] | 27.95 | **21.78**[a] | **35.25**[a] |
| | | | (7.01) | (3.69) | (4.79) | (0.02) | (0.16) | (0.12) | (11.03) | (6.52) | (11.43) |
| | GG | 20 | 13.13 | 14.12 | 12.39 | 0.22 | 0.24 | 0.18 | 57.68 | 69.08 | 74.05 |
| | | | (5.61) | (5.64) | (5.38) | (0.10) | (0.10) | (0.08) | (35.53) | (30.29) | (30.17) |
| *CYP3A4* | AG | 5 | 11.53 | 11.28 | 14.95 | 0.20 | **0.42**[b] | 0.30 | 43.65 | 79.52 | 101.66 |
| | | | (8.91) | (3.63) | (5.19) | (0.14) | (0.30) | (0.22) | (36.58) | (107.93) | (125.97) |
| | AA | 21 | 12.64 | 13.70 | 12.43 | 0.24 | 0.24 | 0.20 | 54.27 | 64.05 | 70.77 |
| | | | (5.60) | (5.73) | (5.25) | (0.08) | (0.10) | (0.10) | (34.65) | (31.72) | (31.88) |
| *ABCB1* C1236T | CC | 10 | 13.11 | 15.39 | 14.41 | 0.22 | 0.26 | 0.18 | 53.96 | 83.94 | 95.17 |
| | | | (5.50) | (5.95) | (6.07) | (0.10) | (0.14) | (0.10) | (22.32) | (56.9) | (79.12) |
| | CT | 8 | 17.23 | 8.75 | 12.13 | 0.22 | 0.18 | 0.18 | 97.31 | 57.23 | 77.57 |
| | | | (6.06) | (3.69) | (1.88) | (0.08) | (0.10) | (0.08) | (61.06) | (35.57) | (29.17) |
| | TT | 8 | **8.94**[c] | 12.73 | 11.00 | 0.28 | 0.32 | 0.30 | **31.67**[d] | 49.02 | 50.22 |
| | | | (4.65) | (3.99) | (5.02) | (0.10) | (0.16) | (0.18) | (8.60) | (29.03) | (31.43) |
| *ABCB1* G2677T/A | GG | 11 | 13.11 | 15.39 | 14.41 | 0.22 | 0.26 | 0.18 | 53.96 | 83.94 | 95.17 |
| | | | (5.50) | (5.95) | (6.07) | (0.10) | (0.14) | (0.10) | (22.32) | (56.94) | (79.12) |
| | GT | 9 | 12.02 | **9.87**[e] | 12.68 | 0.24 | 0.30 | 0.30 | 60.65 | 45.73 | 57.55 |
| | | | (8.25) | (3.08) | (4.63) | (0.08) | (0.18) | (0.18) | (55.80) | (32.97) | (36.14) |
| | TT | 6 | 11.35 | 14.73 | 9.47 | 0.30 | 0.26 | 0.18 | 37.42 | 66.41 | 70.49 |
| | | | (4.79) | (3.74) | (2.15) | (0.12) | (0.14) | (0.16) | (5.41) | (26.59) | (34.47) |
| *ABCB1* C3435T | CC | 10 | 13.25 | 13.11 | 12.28 | 0.22 | 0.24 | 0.18 | 52.95 | 78.45 | 86.64 |
| | | | (5.88) | (5.08) | (4.21) | (0.10) | (0.14) | (0.10) | (24.44) | (60.59) | (84.65) |
| | CT | 10 | 12.90 | 12.66 | 13.90 | 0.22 | 0.24 | 0.22 | 64.7 | 59.07 | 75.42 |
| | | | (7.30) | (7.34) | (6.25) | (0.06) | (0.10) | (0.10) | (49.4) | (37.76) | (40.24) |
| | TT | 6 | 10.64 | 14.28 | 12.30 | 0.30 | 0.32 | 0.28 | 35.24 | 57.27 | 61.78 |
| | | | (4.44) | (3.39) | (5.93) | (0.10) | (0.20) | (0.24) | (6.93) | (30.08) | (33.10) |
| *ABCB1* IVS21+49 | CT | 9 | 10.48 | 11.83 | 12.87 | 0.22 | 0.32 | 0.22 | 44.70 | 45.83 | 54.67 |
| | | | (3.62) | (3.10) | (5.05) | (0.12) | (0.14) | (0.12) | (20.06) | (28.62) | (20.53) |
| | TT | 17 | 13.33 | 13.94 | 12.96 | 0.26 | 0.24 | 0.22 | 56.23 | 75.18 | 87.70 |
| | | | (6.58) | (6.23) | (5.50) | (0.08) | (0.14) | (0.16) | (39.17) | (49.84) | (69.68) |

The mean values and standard deviations (parentheses). Statistically significant results in bold. [a] $p < 0.05$ *vs.* GG homozygotes; [b] $p < 0.05$ *vs.* AA homozygotes; [c] $p < 0.05$ *vs.* CT heterozygotes. [d] $p < 0.05$ *vs.* CC and CT; [e] $p < 0.05$ *vs.* GG and TT homozygotes

**Tab. 5.** Relationships between SNPs in *CYP3A* and *ABCB1* genes and cyclosporine (CsA) pharmacokinetics

| | | | CsA trough concentration (ng/mL) | | | CsA dose (mg/kg per day) | | | CsA dose-adjusted concentration (ng/mL per mg/kg) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Post-transplantation day | | (n) | #6 | #30 | #60 | #6 | #30 | #60 | #6 | #30 | #60 |
| *CYP3A5* | AG | 7 | 258.00 | 252.67 | 191.83 | 9.12 | 10.20 | 8.06 | 30.60 | 29.44 | 25.68 |
| | | | (110.40) | (128.79) | (89.61) | (3.92) | (3.88) | (2.98) | (17.39) | (17.83) | (11.23) |
| | GG | 54 | 245.86 | 251.48 | 215.09 | 11.88 | 10.24 | 9.10 | 24.68 | 27.90 | 28.74 |
| | | | (157.90) | (117.82) | (119.13) | (5.22) | (5.82) | (6.06) | (24.06) | (13.66) | (15.44) |
| *ABCB1* C1236T | CC | 16 | 277.92 | 262.07 | 218.33 | 10.72 | 9.30 | 8.04 | 33.03 | 33.42 | 30.68 |
| | | | (180.23) | (102.16) | (112.17) | (5.14) | (3.70) | (2.98) | (38.27) | (17.25) | (13.65) |
| | CT | 33 | 253.96 | 239.30 | 210.04 | 12.90 | 10.88 | 10.12 | 20.13 | 25.34 | 25.42 |
| | | | (160.09) | (128.91) | (107.98) | (5.24) | (6.88) | (7.10) | (10.84) | (13.59) | (14.21) |
| | TT | 12 | 195.09 | 268.55 | 209.91 | **9.38**[a] | 9.76 | 6.78 | 27.43 | 28.28 | 32.49 |
| | | | (85.68) | (114.47) | (146.06) | (4.20) | (3.58) | (3.26) | (19.87) | (9.36) | (18.14) |
| *ABCB1* G2677T/A | GG | 14 | 271.58 | 239.17 | 223.29 | 11.60 | 9.36 | 11.02 | 31.22 | 26.73 | 28.57 |
| | | | (182.33) | (100.17) | (112.74) | (5.00) | (3.22) | (10.46) | (39.95) | (11.2) | (15.07) |
| | GT | 29 | 265.68 | 245.25 | 216.61 | 13.00 | 10.90 | 8.90 | 21.34 | 26.57 | 26.71 |
| | | | (167.08) | (134.41) | (112.95) | (5.40) | (7.30) | (2.80) | (11.02) | (13.85) | (13.92) |
| | TT | 12 | 195.09 | 268.55 | 209.91 | **9.38**[b] | 9.76 | **6.78**[b] | 27.43 | 28.28 | 32.49 |
| | | | (85.68) | (114.47) | (146.06) | (4.20) | (3.58) | (3.26) | (19.87) | (9.36) | (18.14) |
| | A | 6 | 214.50 | 274.80 | 157.00 | 9.74 | 10.28 | 8.04 | 22.43 | 40.35 | 26.4 |
| | | | (125.65) | (102.37) | (51.77) | (5.04) | (4.88) | (3.46) | (17.55) | (28.70) | (15.75) |
| *ABCB1* C3435T | CC | 19 | 272.87 | 250.47 | 209.71 | 11.56 | 8.98 | 10.30 | 31.97 | 31.97 | 27.77 |
| | | | (164.21) | (101.77) | (109.83) | (4.72) | (3.26) | (9.62) | (36.92) | (17.44) | (15.04) |
| | CT | 28 | 263.45 | 251.57 | 204.00 | 11.86 | 11.06 | 8.58 | 22.56 | 26.63 | 27.16 |
| | | | (178.08) | (140.16) | (100.47) | (5.54) | (7.30) | (2.66) | (13.16) | (14.11) | (13.25) |
| | TT | 14 | 196.14 | 252.93 | 228.29 | 11.10 | 10.14 | 8.08 | 22.53 | 26.49 | 30.96 |
| | | | (83.13) | (100.12) | (147.20) | (5.12) | (3.7) | (3.84) | (17.10) | (9.61) | (17.88) |
| *ABCB1* IVS21+49 | CT | 15 | **355.5**[c] | 277.80 | 189.00 | 13.22 | 10.28 | 8.76 | **40.38**[c] | 29.03 | 24.42 |
| | | | (178.41) | (165.45) | (127.74) | (4.60) | (3.22) | (2.74) | (44.53) | (14.17) | (12.21) |
| | TT | 46 | 219.31 | 245.38 | 217.98 | 11.14 | 10.22 | 9.02 | 21.82 | 27.88 | 29.25 |
| | | | (134.50) | (105.08) | (113.49) | (5.24) | (6.14) | (6.40) | (13.86) | (14.15) | (15.46) |

The mean values and standard deviations (parentheses). Statistically significant results in bold: [a] $p < 0.05$ *vs.* CT heterozygotes; [b] $p < 0.05$ *vs.* GT heterozygotes; [c] $p < 0.05$ *vs.* TT homozygotes

(355.5 ± 178 ng/ml *vs*. 219.3 ± 134; $p < 0.05$), and C1236T and G2677T/A homozygotes (TT) required a lower daily CsA dose than CT and GT heterozygotes (18.76 ± 8.42 mg/kg *vs*. 25.82 ± 10.48 mg/kg; $p < 0.05$; 18.60 ± 8.42 mg/kg *vs*. 23.20 ± 10 mg/kg; $p < 0.05$). In this specific patient sub-population, in individuals who were homozygous for *CYP3A5* GG, we calculated *ABCB1* haplotypes. Three major haplotypes were reported: CGTC (40%), TTTT (27.3%), and CGCC (20%) for genes C1236T, G2677T/A, IVS 21+49 and C3435T, respectively. None of those showed a significant correlation with the considered pharmacokinetic parameters.

## Discussion

We analyzed 87 Italian teenagers undergoing kidney transplantation for the presence of *CYP3A5*, *CYP3A4*1B* and *ABCB1* (C1236T, G2677T/A, C3435T, IVS21+49) genetic polymorphisms. To the best of our knowledge, this is the first study in such a select population. This population was chosen due to the fact that the influence of CYP [53] on the metabolism of many drugs is greatest in this age group. Recently, Fanta and colleagues [17] suggested that age-related polymorphisms of *ABCB1* could explain the different bioavailability of CsA. Therefore, when dealing with a pediatric population, the blind use of pharmacokinetic data extrapolated from an adult population can be rather risky, especially with regard to immunosuppressive agents.

The allele frequencies of *CYP3A* and *ABCB1* are also greatly influenced by ethnicity, so it is not uncommon to find some "continental" peculiarities, especially in regards to *ABCB1* polymorphisms. The allelic distribution in our cohort receiving transplants was generally comparable with that observed in other Caucasian populations. Interestingly, the patients with allele C in IVS21+49 were not homozygous for allele T in *ABCB1* C1236T and/or G2677T/A and/or C3435T. As this did not reflect Hardy-Weinberg equilibrium, it is possible that the IVS21+49 polymorphism may be a recent mutation appearing on a CC-GG-CC strand rather than on the expected TT-TT-TT strand.

The CNIs Tac and CsA are characterized by a narrow therapeutic window and large inter- and intra-individual differences in both pharmacokinetics and pharmacodynamics [16, 33, 51]. Drug- to-drug interactions have been identified as one of the main factors responsible for significant alterations in immunosuppressive drug behavior in an individual patient [16, 54]. Many clinically relevant pharmaceutical interactions have been described between CNIs and other drugs used after transplantation [45] including other immunosuppressive agents. Steroids, which are still one of the cornerstones of current immunosuppressive protocols, display a significant pharmacokinetic interaction with Tac [2], whereas conflicting results are reported in regards to CsA [59]. In our patient population, we enforced strict therapeutic drug monitoring as part of our standard clinical practice when CNIs are used [18, 46] thereby minimizing possible drug-to-drug interactions.

A number of published studies have shown that genotype affects the metabolism of the immunosuppressive drugs that are usually used to control graft rejection [4, 26, 44, 48], and SNPs may be associated with inter-individual variations in Tac and CsA pharmacokinetics in transplant recipients. Our results confirmed the pivotal role of the *CYP3A5* polymorphism in Tac pharmacokinetics: carriers of the A allele required a higher Tac dose to reach therapeutic blood concentrations than non-carriers. The same was found by Press et al. [48] in a cohort of adult kidney transplant recipients: patients with the *CYP3A5*1/*3* genotype required a Tac loading dose that was 1.5 times higher than *CYP3A5*3/*3* patients to reach the predefined target level. *ABCB1* and *CYP3A4* did not seem to influence Tac pharmacokinetics, as has been previously reported [4, 26, 40, 44, 48] when larger post-transplant populations were considered.

However, we observed a significant reduction in Tac concentration, and we dose-adjusted Tac concentrations in the early post-transplant period for the TT polymorphism of C1236T; this suggests that there is higher metabolism of the drug in subjects with this genotype. This could be substantiated by the fact that 1236 and 3435 polymorphisms of *ABCB1* influence the shaping and stability of mRNA and, consequently, gene expression [60].

Few data are available concerning the effect of *CYP3A5* on cyclosporine metabolism [5], and the roles of *CYP3A5* and *CYP3A4* have not yet been clarified. However, our data are in line with those from other studies showing that they have no effect [11, 58].

In relation to the effects of *ABCB1* polymorphisms on CsA pharmacokinetics, it is necessary to bear in

mind that the drug is simultaneously both a substrate and an inhibitor of *ABCB1* [6, 13], which is why the influence of *ABCB1* polymorphisms on CsA pharmacokinetics is rather controversial [4]. We found that *ABCB1* polymorphisms affected CsA pharmacokinetics in the immediate post-transplant period, but not later, and that many authors have reported the lack of influence of *ABCB1* SNPs on CsA excretion in kidney transplant patients with stable renal function [11, 57]. The time-related effect of *ABCB1* polymorphisms on CsA metabolism in our patients may be explained by the fact that the drug's potent inhibitory effect on P-gp function requires an adequate pharmacological load before it is fully expressed [52, 56]. Finally, the relatively small number of patients included in this study could also explain the lack of a relationship between the most frequent haplotypes and the immunosuppressive drugs. Wang et al. [61] showed that, in lung transplant recipients, *ABCB1* haplotypes derived from three common polymorphisms were associated with Tac dosing when limited to subjects with *CYP3A5 *3/*3* nonexpressors. Future studies involving the genetic predisposition to adverse effects of immunosuppressants that are substrates for P-gp and CYP3A5, such as CsA, should consider the combined effects of multiple gene polymorphisms including the *CYP3A5* genotype and *ABCB1* haplotype. A large study population should be planned in the near future to adequately address this issue.

In conclusion, as inter-racial influences in the Italian population are not as frequent as in other European countries, it can be expected that approximately 20% of Italian kidney transplant patients treated with CNIs may experience immunosuppressant-related adverse events due to *CYP3A* and *ABCB1* polymorphisms. The pre-transplant screening of the most relevant polymorphisms should therefore be considered in order to tailor immunosuppressive therapies to an individual patient's metabolism.

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