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Influence of *CYP3A5* polymorphism on tacrolimus maintenance doses and serum levels after renal transplantation: Age dependency and pharmacological interaction with steroids

Ferraris JR, Argibay PF, Costa L, Jimenez G, Coccia PA, Ghezzi LFR, Ferraris V, Belloso WH, Redal MA, Larriba JM. Influence of *CYP3A5* polymorphism on tacrolimus maintenance doses and serum levels after renal transplantation: Age dependency and pharmacological interaction with steroids.
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Abstract: TAC, MMF and MP are used in pediatric kidney tx. The cytochrome P450 (CYP)3A5 enzyme appears to play a role in TAC metabolism. The aims of this study were to investigate *CYP3A5* polymorphism's effect on TAC dosing and the age dependency of TAC dosing by testing blood concentrations, and the interaction between steroids and TAC during the first year after tx. Genomic DNA was extracted and amplified with specific primers. *CYP3A5* alleles were confirmed by direct sequencing of PCR products on an automated AB13100 capillary sequencer. We studied 48 renal transplant patients (age at tx 12 ± 0.5 yr, 22 boys) receiving TAC, MMF, MP. Of these, 79% were *CYP3A5**3/*3 (non-expressers homozygotes) and 21% were *CYP3A5**1/*3 (expressers). TAC trough levels were 7.1 ± 0.4 ng/mL in *CYP3A5**3/*3 patients and 6.5 ± 0.7 ng/mL in *CYP3A5**1/*3 group ($p = 0.03$). *CYP3A5**1/*3 patients had lower levels of dose-adjusted TAC (36.7 ± 5.8 ng/mL/mg/kg/day) to achieve target blood concentration and required higher daily dose per weight (0.21 ± 0.03 mg/kg/day) than *CYP3A5**3/*3 patients, 72.4 ± 8.0 ng/mL/mg/kg/day and 0.13 ± 0.01 mg/kg/day ($p < 0.001$). Prepubertal patients with different *CYP3A5* polymorphisms required significant higher TAC doses and achieved lower dose-normalized concentration compared with pubertal patients. Both TAC dose and adjusted-dose correlated with daily MP dose in *CYP3A5**1*3 (r: 0.4, $p < 0.03$ and r: 0.4, $p < 0.03$) and in *CYP3A5**3*3 (r: 0.6, $p < 0.01$ and r: 0.47, $p < 0.001$) patients. *CYP3A5* polymorphism performed before tx could contribute to a better individualization of TAC therapy. The higher TAC dose in prepubertal patients and the pharmacological interactions between MP and TAC may not be fully explained by different *CYP3A5* polymorphisms.

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Abbreviations: DD, deceased donor; eGFR, estimated glomerular filtration rate; LRD, living-related donor; MMF, mycophenolate mofetil; MP, methylprednisone; PCR, polymerase chain reaction; s.e., standard error; SNP, single-nucleotide polymorphism; TAC, tacrolimus; tx, transplantation.

Calcineurin inhibitors, MMF and MP are drugs largely used to prevent allograft rejection in pediatric kidney tx. TAC is the most common drug used for solid organ tx in adults and pediatric patients (1, 2). But TAC has a narrow

therapeutic range and its pharmacokinetics shows wide inter- and intra-individual variability (3). At present, periodic monitoring of TAC blood concentration is required in renal transplant patients in order to maintain TAC levels within the target concentration range and thus, reduce the risk of over- and under-immunosuppression (4, 5). This type of dose adjustment requires time because a TAC steady-state level must be reached first. Balancing the exposure to this narrow therapeutic index agent becomes a critical aspect in the management of patients. Moreover, young children need a higher TAC dose than older patients (6). Therefore, it is difficult to determine the optimal TAC dosage during the first days/months after kidney tx.

Among cytochrome P450 (CYP) 3A isoenzymes, CYP3A5 has been identified as the major enzyme responsible for the metabolism of TAC (7). The *CYP3A5* gene located at 7p21 displays polymorphisms. An important SNP A6986G in intron 3 presents A and G alleles designated as *CYP3A5*1* and *CYP3A5*3*, respectively. The *CYP3A5*3* causes alternative splicing and protein truncation, resulting in the absence of CYP3A5 (8). The presence of *CYP3A5*3*/**3* is associated with the absence of protein activity (non-expressers), whereas individuals with at least one *CYP3A5*1* express the CYP3A5 isoenzyme (expressers). In addition, CYP3A is involved in the metabolism of steroids which are inducers of CYP members (9). Therefore, the use of glucocorticoids would be expected to necessitate a higher dosage of TAC. Studies in adults and pediatric patients (10, 11) have documented increased TAC levels upon steroid reduction or withdrawal, but data about this pharmacological interaction in renal transplant patients with different *CYP3A5* polymorphisms are not available. Moreover, except for a few reports (12–14), the previous studies about TAC and *CYP3A5* were performed in adult kidney transplant patients (15–18).

Finally, our study was undertaken to answer the following questions: (i) Does *CYP3A5* polymorphism affect TAC dosing in pediatric transplant patients? (ii) Does *CYP3A5* polymorphism play a role in the age-dependency of TAC dosing? and (iii) Does *CYP3A5* polymorphism have an influence on the interaction between MP and TAC?

Patients and methods

Patient data

Patients were treated and studied at the Pediatric Department of Hospital Italiano of Buenos Aires. We studied 48 kidney transplant patients who were considered eligible for our study if they met the following inclusion criteria: (i) having started a post-transplant immunosuppressive protocol with TAC associated with MMF and MP, (ii) having a functional graft beyond the first year following kidney tx, (iii) having received an LRD or DD graft without delayed function, and (iv) having no clinical history of taking medications known to interact with calcineurin inhibitors, such as calcium channel blockers (diltiazem, nicardipine, and verapamil), antiepileptics (phenytoin and carbamazepine), antimycotics (fluconazole and ketoconazole) and macrolide antibiotics (erythromycin and clarithromycin).

Immunosuppressive regimen

TAC was started at 0.15 mg/kg/day twice a day and then adjusted to maintain whole-blood levels between 5–10 ng/mL (IMx; Abbott, Buenos Aires, Argentina). All patients were followed at weekly intervals during the first four - months, every two wk from month 5 to 8, and finally every three wk from month 9 to 12. In this study, the analysis regarding renal allograft function, TAC whole blood concentration, office blood pressure, height, weight and weight-adjusted TAC, MMF, and MP over a one-yr period was arbitrarily performed at only 13 different time points: half, one, two, three, four, five, six, seven, eight, nine, 10, 11 and 12 months after tx. Therefore, we have used 13 measurements from each patient for statistical comparisons.

Intravenous MP 4 mg/kg/day was administered during the first three postoperative days and then tapered from 0.5 to 0.15 mg/kg/day by postoperative month 4. MMF was given at a dose of 600 mg/m^2/day divided in two doses. LRD graft patients were treated with anti-CD25 (daclizumab, five doses of 1 mg/kg), and DD graft patients were treated with antithymocyte globulin (1.2 mg/kg/day, during seven days).

Acute and chronic rejection, suggested by clinical and laboratory parameters, were confirmed by renal biopsy, unless contraindicated.

Clinical and laboratory studies

Control blood samples were drawn after overnight fasting from 9:00 PM to 8:00 AM, and clinical visits were performed as usual, between 9:00 AM and 11:00 AM. TAC trough blood concentrations were measured and then dose-normalized, using the TAC concentration/dose ratio obtained by dividing TAC trough concentration by the corresponding 24-h dose in mg/kg.

eGFR was calculated for each patient based on the modified Schwartz formula that includes adjustments for height, weight, and patient age. This formula has also been used by the North American Pediatric Renal Transplant Cooperative Study (19) and is as follows:

$$\text{Weight} < 10\,\text{kg}: \quad \text{eGFR} = \frac{(0.45 \times \text{height})}{\text{serum creatinine}}$$

$$\text{Weight} > 10\,\text{kg} < 70\,\text{kg}: \quad \text{eGFR} = \frac{(0.55 \times \text{height})}{\text{serum creatinine}}$$

$$\text{Weight} > 70\,\text{kg}: \quad \text{eGFR} = \frac{([1.55 \times \text{age}] + [0.55 \times \text{height}])}{\text{serum creatinine}}$$

The height is expressed in centimeters, serum creatinine (determined by picric acid method) in mg/dL and age in years.

Genotyping

CYP3A measurements were performed 1–4 yr after kidney tx. Genomic DNA was extracted from 200 μL of whole blood using QIAmp DNA Mini kit (Qiagen, GmbH, Hilden, Germany). The PCR was performed in a final volume of 20 μL. The forward primer was 5′-ATGGAGAT-GGCATAGAAGATA-3′, and the reverse primer was 5′-TGTG-GTCCAAACAGGGAAGAGAT-3′. The PCR conditions were three min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 59 °C, 30 s at 72 °C, and final extension for 10 min at 72 °C. The PCR product was detected on 2% agarose gels by means of ethidium bromide staining. The presence of variant *CYP3A5* alleles was confirmed by direct sequencing of PCR products on an automated ABI 3100 capillary sequencer (Applied Biosystems, Foster City, CA, USA), using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems) (20).

Statistical analysis

The analysis of results was performed retrospectively, and the approval for the study was granted by the Institutional Ethics Committee.

The statistical evaluation of data was performed using commercially available SPSS Statistics 17.0 software (SPSS Inc., Chicago, IL, USA). Values are expressed as mean ± s.e. and, when necessary, 95% confidence intervals and ranges are shown. Data were evaluated by one-way ANOVA and when variables did not follow normal distribution, alternative nonparametric tests such as Mann–Whitney *U*-test and Kruskal–Wallis were used. The Bonferroni *post hoc* test was applied in the case of significant difference between groups. Data were also analyzed by linear regression. Comparison of linear correlations was performed using the one-way ANCOVA test. p values <0.05 were considered to be statistically significant.

Results

Clinical features of patients

Every patient was followed up for at least one yr. The 48 kidney transplant patients were divided in two groups according to their *CYP3A5* polymorphism. Thirty-eight patients (79%) bear the *CYP3A5*3/*3* allele (non-expressers homozygotes) and 10 patients (21%) carry the *CYP3A5*1/*3* allele (expressers heterozygotes). The *CYP3A5*1/*1* genotype was not find in our patients. Demographic characteristics of both groups are shown in the Table 1.

Mean age at tx, gender, etiology of end stage renal disease, weight, number of DD or LRD, and HLA-AB and HLA-DR mismatches were not different between both groups. No patient developed acute or chronic rejection during the first-year post-transplant. Moreover, serum creatinine levels and eGFR were not significantly different between groups. One-yr mean serum creatinine and eGFR values for *CYP3A5*3/*3* and *CYP3A5*1/*3* patients were 0.8 ± 0.05 mg/dL and 105.6 ± 4.9 mL/min/1.73 m^2; and 0.8 ± 0.13 mg/dL and 108.2 ± 7.6 mL/min/1.73 m^2, respectively.

Table 1. Clinical features of patients, according to *CYP3A5* polymorphism

	*CYP3A5*3/*3* (n = 38)	*CYP3A5*1/*3* (n = 10)	p
Age at transplant (yr)	12.6 ± 0.9	11.6 ± 1.9	NS
Male/female (n)	26/12	3/7	NS
Etiology of ESRD			
Dysplasia/uropathy	15	2	NS
Glomerulopathy	15	6	NS
Other causes	8	2	NS
Prepubertal/pubertal (≤ 12/>12 yr)	16/22	5/5	NS
Weight at transplant (kg)	33.5 ± 3.2	32.2 ± 7.3	NS
DD/LRD	8/30	3/7	NS
HLA-AB mismatches	1.4 ± 0.2	1.5 ± 0.2	NS
HLA-DR mismatches	0.5 ± 0.2	0.5 ± 0.2	NS
Acute rejection (first year)	None	None	NS
Chronic rejection (first year)	None	None	NS

Effects of *CYP3A5* polymorphism on TAC dose and concentration

TAC dose requirement was more than two-fold higher in *CYP3A5*1/*3* than in *CYP3A5*3/*3* patients (Fig. 1a). One-year mean TAC doses in *CYP3A5*1/*3* and *CYP3A5*3/*3* patients were 0.21 ± 0.03 mg/kg/day and 0.13 ± 0.01 mg/kg/day, respectively ($p < 0.001$).

Significant differences in TAC blood trough levels and dose-normalized trough levels were observed between groups. *CYP3A5*3/*3* patients displayed higher TAC blood trough levels and dose-normalized trough levels than *CYP3A5*1/*3* patients (Fig. 1b,c).

It should be emphasized that TAC blood trough levels were significantly lower in the *CYP3A5*1/*3* group during the first 2.5 months post-tx (Fig. 1b).

One-yr mean TAC blood trough levels and dose-normalized trough levels in *CYP3A5*3/*3* patients and *CYP3A5*1/*3* patients were 7.1 ± 0.4 ng/mL and 72.4 ± 8.0 ng/mL/mg/kg/day and 6.5 ± 0.7 ng/mL and 36.7 ± 5.8 ng/mL/mg/kg/day ($p < 0.03$ and $p < 0.001$, respectively).

MP and MMF doses were not statistically different between groups throughout the study protocol. One-yr mean MP and MMF doses in *CYP3A5*3/*3* and *CYP3A5*1/*3* patients were 0.24 ± 0.04 and 15.7 ± 1.0 mg/kg/day, and 0.29 ± 0.08 and 16.9 ± 2.5 mg/kg/day, respectively.

Effects of age and *CYP3A5* polymorphism on TAC dose and dose-normalized trough level

*CYP3A5*3/*3* and *CYP3A5*1/*3* patients were divided in two age subgroups: prepubertal

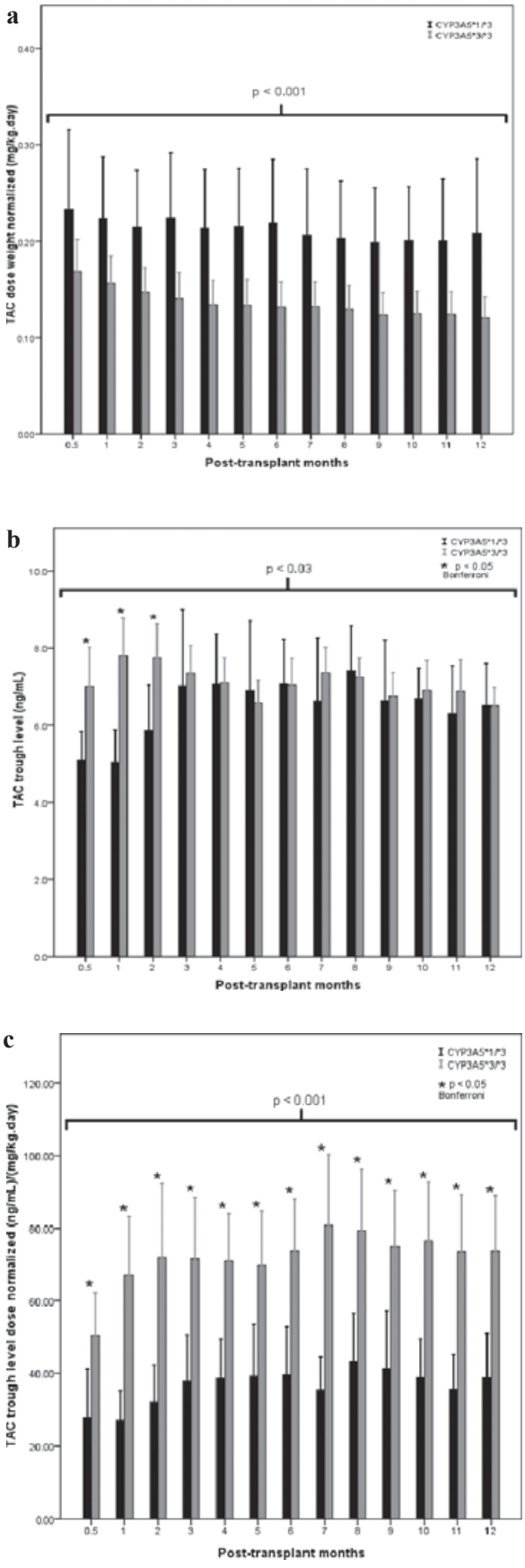

Fig. 1. Effects of *CYP3A5* genotype on TAC dose/weight normalized (a), TAC blood trough levels (b) and TAC trough level/dose normalized (Co/dose) (c), during the first-year post-tx. Black columns are for *CYP3A5*1/*3* patients, and gray columns, for *CYP3A5*3/*3* patients. Columns and error bars represent mean values and 95% CI, respectively.

(≤ 12 yr) and pubertal (> 12 yr). There were no significant differences among subgroups in hematocrit or albumin levels.

One-yr mean TAC dose was significantly higher ($p < 0.001$) in prepubertal than in pubertal patients. At the same time, one-yr mean TAC dose was significantly higher ($p < 0.001$) in *CYP3A5*1/*3* patients (Fig. 2a), independently of age. Consequently, one-yr mean TAC

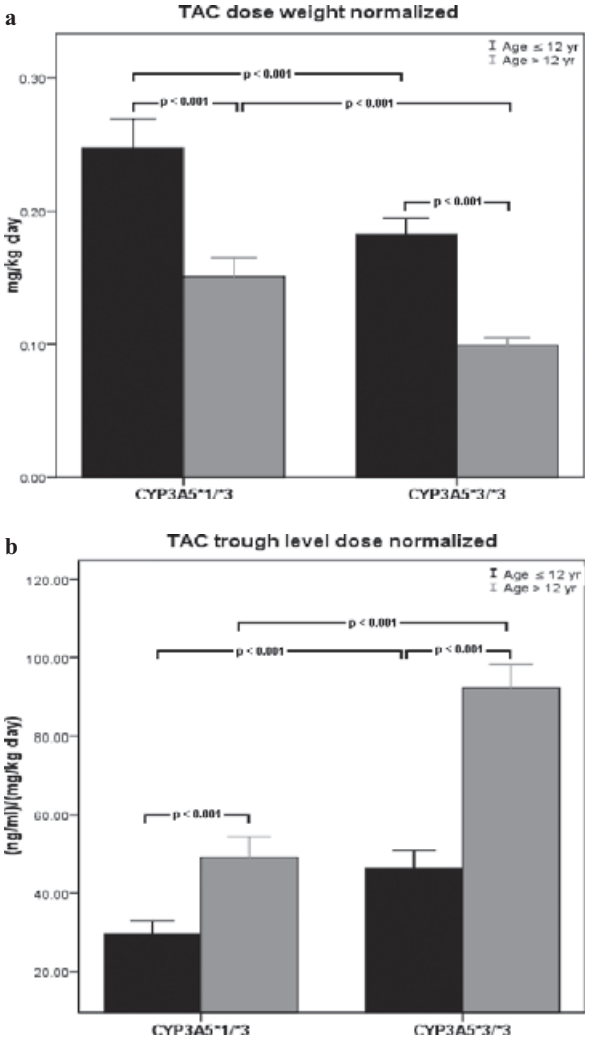

Fig. 2. One-yr mean TAC dose/weight normalized (a) and mean TAC trough level/dose normalized (Co/dose) (b) in prepubertal (≤ 12 yr) and pubertal (> 12 yr) patients with different *CYP3A5* genotype. Black columns correspond to prepubertal patients, and gray columns, to pubertal patients. Columns and error bars represent mean values and 95% CI.

dose-normalized trough level was significantly lower ($p < 0.001$) in prepubertal than in pubertal patients. As expected, one-yr mean TAC dose-normalized trough level was significantly lower ($p < 0.001$) in *CYP3A5*1/*3* patients (Fig. 2b), also independently of age.

Effects of MP dose on TAC treatment and its relation with *CYP3A5* polymorphism

There was a significant positive correlation between TAC and MP daily doses in both groups of patients, during the first three months post-transplant. The higher the MP dose, the higher the TAC dose required to achieve target trough blood concentrations (Fig. 3a,b). The ANCOVA test revealed no significant difference between both groups.

Finally, there was a significant negative correlation between TAC trough level/dose normalized and MP daily dose in both groups of patients. The higher the MP dose, the lower the TAC trough level/dose normalized needed to achieve target TAC trough blood concentration (Fig. 3c,d). Again, the ANCOVA test showed no significant difference between both groups.

Discussion

Our findings in children who underwent successful renal tx treated with TAC, and with different *CYP3A5* alleles demonstrated that prepubertal patients required higher daily TAC doses to achieve target blood and dose-normalized trough levels, and that TAC-MP pharmacological interaction is independent of *CYP3A5* polymorphism. Also, this paper confirmed previous reports stating that *CYP3A5 *1/*3* pediatric patients need higher daily TAC dose than *CYP3A5 *3/*3* children to achieve adequate blood concentrations (12–14).

The recent identification of genetic polymorphisms among drug-metabolizing enzymes and drug-transporters has confirmed the hypothesis that genetic factors influence the interindividual pharmacokinetic variability of immunosuppressive drugs, potentially affecting their major side effects and efficacy.

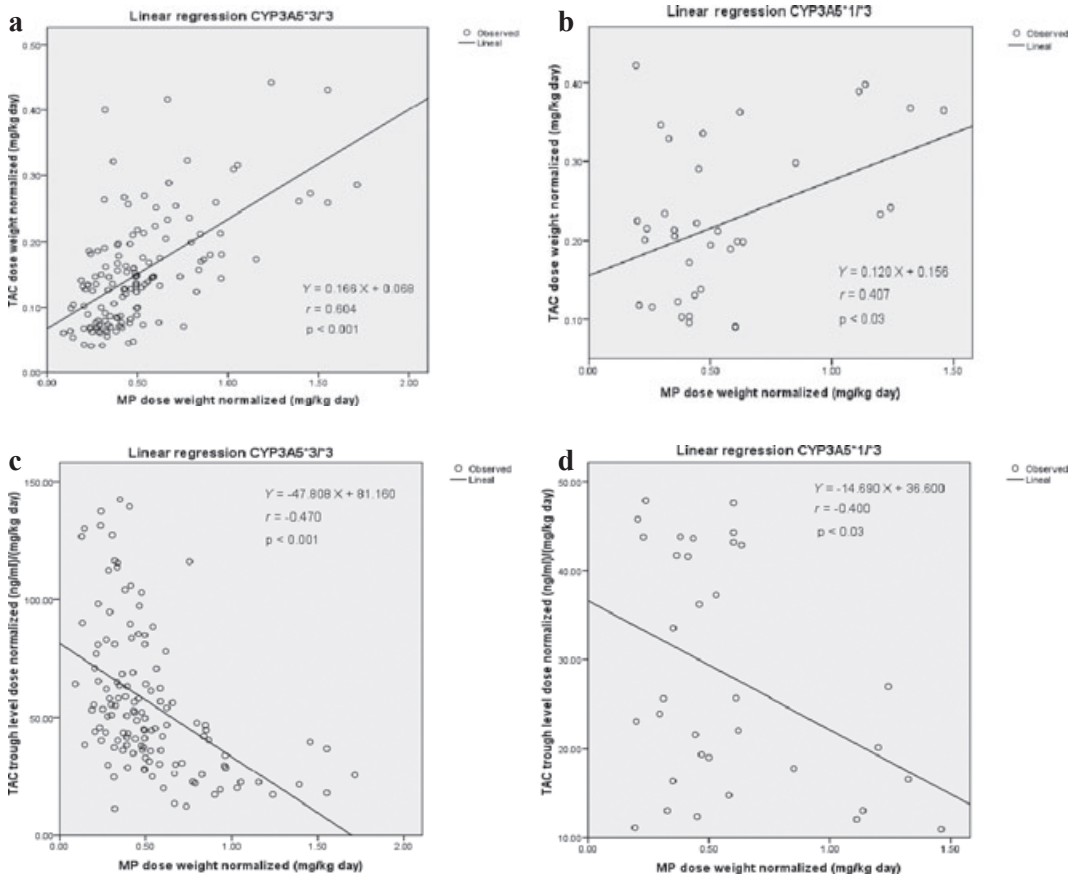

Fig. 3. Correlations between TAC and MP doses/weight normalized in *CYP3A5*3/*3* (a) and *CYP3A5*1/*3* (b) patients; and between TAC trough level/dose normalized (Co/dose) and MP dose/weight normalized in *CYP3A5*3/*3* (c) and *CYP3A5*1/*3* (d) patients, during the first three months post-tx.

TAC is known to be a substrate of CYP3A4, CYP3A5, and P-glycoprotein (21–23). The presence of *CYP3A5*3* allele is associated with the absence of protein activity, whereas individuals with at least one *CYP3A5*1* allele express the CYP3A5 isoenzyme.

The allelic frequencies of *CYP3A5* in our population were similar to those reported for other pediatric Caucasian populations (12–14), but different from African American, Hispanic and Asian individuals. The most common allele in our patients (*CYP3A5*3*) had a frequency of 79%, much higher than the wild-type allele (*CYP3A5*1*).

The uniform initial TAC dose was 0.15 mg/kg twice daily, ignoring the *CYP3A5* genotype because our study was retrospective, but later we observed that blood TAC trough concentrations showed significant differences according to *CYP3A5* genotype. TAC blood trough and dose-normalized trough levels were lower and TAC dose higher in the *CYP3A5 *1/*3* group. In contrast, TAC blood trough and dose-normalized trough levels were higher and TAC dose lower in *CYP3A5*3/*3* patients. The almost two-fold higher doses found in our *CYP3A5*1/*3* patients is in agreement with data from adults (24) and pediatric patients (12–14). Therefore, the *CYP3A5*3/*3* patients may have both increased TAC absorption, because of decreased metabolism in the gastrointestinal tract, and decreased drug elimination, because of lower hepatic metabolism. Although the allelic difference among patients could predispose to deficient immunosuppression in *CYP3A5*1/*3* patients, it was not associated with the appearance of any sign of rejection. Moreover, serum creatinine and eGFR were similar in both groups of patients. Therefore, with the implementation of therapeutic drug monitoring that we used, the effects of significant differences in pharmacogenetics can be carefully observed and adjustments to therapy can be introduced. Also, other studies found that *CYP3A5* genotype shows no impact on acute rejection (25, 26). Further on, we do not observe side effects associated with TAC in any patient group.

Previous studies revealed that younger and/or low-weight patients showed higher TAC dose requirements as compared with older patients. To answer the question about the role of *CYP3A5* genotype in the age dependency of TAC dosing, *CYP3A5*3/*3* and *CYP3A5*1/*3* patients were divided in two age subgroups: prepubertal ($\leq$ 12 yr) and pubertal ($>$ 12 yr). We found that, in order to achieve target blood trough concentration, the TAC dose per kilogram in the youngest group was almost two-fold higher than in the older group, and the TAC trough level/dose normalized were lower in the youngest group. Also, an expected finding was that in prepubertal and pubertal patients, the daily TAC dose was higher and the dose-normalized trough levels were lower in *CYP3A5*1/*3* compared to *CYP3A5*3/*3* patients. Therefore, the reason for higher dosing requirements in younger children could be explained by the increased expression and activity of both intestinal and hepatic CYP3A isoenzymes because of the age dependency of enzymatic activity (27–30). It could be hypothesized that the effect of intestinal and hepatic CYP3A4 enzyme was clearly shown in *CYP3A5*3/*3* patients (non-expressers), and that the sum of CYP3A4 and CYP3A5*1/*3 enzymatic activity could explain why these patients needed higher TAC doses than *CYP3A5*3/*3* patients. In agreement with these findings, it was demonstrated that, in patients carrying the *CYP3A5*1/*3* allele, higher TAC dosages should be recommended in children with body weight $<$ 20 kg (14).

Next, we investigated the impact of *CYP3A5* genotype on the interaction between TAC and MP. We showed that MP tapering during the first three months after tx was associated with a decrease in TAC maintenance dosing, and that higher MP doses were associated with lower TAC trough level/dose normalized in pediatric patients, independently of *CYP3A5* polymorphism. This observation is in line with a previous report in renal transplant adult patients (10). Although glucocorticoids are metabolized by CYP3A, and a preliminary report found no differences in methylprednisolone plasma concentrations in control subjects who were *CYP3A5*1/*3* compared with *CYP3A5*3/*3* subjects (31), glucocorticoids are potent inducers of CYP3A isoenzymes and this action is likely to be superimposed on genetic influences during the early period after tx when higher MP doses are used. The interaction between MP and TAC studied in *CYP3A5*3/*3* patients would exclude the potential confounding factor of *CYP3A5* polymorphism, indicating that the polymorphism alone does not fully explain MP-TAC interaction and the age dependency of TAC dosing. Therefore, the role of CYP3A4 and P-glycoprotein could be important in both situations.

The P-glycoprotein efflux pump in the gut may be activated by glucocorticoids (32, 33) and may affect TAC disposition by reducing its absorption from the intestine and enhancing its secretion into the bile and urine. Unfortunately, there are no data regarding intestinal P-glycoprotein

expression in pediatric patients but, based on experimental data, it seems that P-glycoprotein representation is likely similar to that of an adult shortly after birth (34). Moreover, the question of whether P-glycoprotein is implicated in TAC pharmacokinetics remains controversial (16, 17, 35, 36). This may reflect the possibility that multiple genes (loci) govern the pharmacokinetics of these immunosupressants.

The main limitations of this study are the small number of patients included, the retrospective design, and no identification of other genes such as *CYP3A5* and *ABCB1*, which encode the P-glycoprotein. However, the new information we show and the comparison of the data with the literature reinforce the validity of the findings.

Conclusions

CYP3A5 polymorphism performed before tx could contribute to a better individualization of TAC therapy, thus avoiding a delay in achieving target TAC blood concentration. However, *CYP3A5* testing will not replace therapeutic monitoring because many genetic and non-genetic factors can modulate the pharmacokinetic phenotype. Finally, the higher TAC dose in prepubertal patients and the pharmacological interaction between TAC and MP may not be fully explained by different *CYP3A5* polymorphisms.

Further large prospective, randomized, and multicenter studies will be necessary to compare the effects of CYP3A5 genotype vs. therapeutic monitoring after organ tx in children.

Authors' contributions

Jorge R. Ferraris: concept, design, interpretation, drafting article; Pablo F. Argibay: critical revision of article; Lucas Costa: statistics; Graciela Jimenez: data collection; Paula A. Coccia: data collection; Lidia F.R. Ghezzi: data collection; Verónica Ferraris: data collection; Waldo Belloso: critical revision of article; María A. Redal: measurement of *CYP*; Julián M. Larriba: measurement of *CYP*.

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