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# Frequency of CYP3A5 genetic polymorphisms and Tacrolimus pharmacokinetics in Pediatric Liver Transplantation

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**Abstract:** The body of evidence available in paediatrics population is limited for making clinical decisions regarding pharmacotherapy optimization of tacrolimus. The objective of this study was to estimate the frequency of CYP3A5 genetic polymorphisms and their relationship with tacrolimus requirements in paediatric population. This was a longitudinal cohort study, with two-year follow-up of 77 patients under 18 who had liver transplant over the period 2009-2012 at the Paediatric Hospital J. P Garrahan. Tacrolimus levels from day 5 to 2-year post-transplant were obtained from hospital records of routine therapeutic drug monitoring. The genotyping of CYP3A5 (CYP3A5*1/*3 or *3/*3) were performed in liver biopsies of both the donor and the recipient. Recipients frequency of CYP3A5 *1 expression was 37.1% and 32.2% for Donors. Patient who received an organ expresser showed lower Co/dose especially after 90 days post-surgery. **The** role of each polymorphism is different according to days after transplantation proceeds and it must be taken into account to optimize the benefits of TAC therapy during the post-transplant induction and maintenance phase.

Keywords: tacrolimus, CYP3A5 , liver transplant , pharmacokinetics

## 1. Introduction

Tacrolimus (TAC) is a calcineurin inhibitor widely used in solid organ transplantation. TAC has a narrow therapeutic margin and a large intra- and inter -individual variability (1, 2). Incidence of rejection and adverse effects remains as problems despite therapeutic drug monitoring of TAC (3). There is growing interest in developing markers those will allow to individualize treatment of TAC. Within this group of potential biomarkers a remarkable example are single nucleotide polymorphisms of CYP3A5 (3-5). This enzyme has a highly polymorphic expression with at least 11 single nucleotide polymorphisms (SNPs) documented (3). The SNP most studied is the transition from adenine to guanine at position 6986- intron 3 - CYP3A5 gene (rs 776746), also called CYP3A5*1. This allele is associated with high levels of CYP3A5-mRNA and full functional CYP3A5-protein (6, 7). Caucasian population expresses CYP3A5*1 between 10-40% while Asian population expresses between 50-70 % (8). The CYP3A5*1 (homozygotes and heterozygotes ) expressers require much higher daily doses of TAC as well as more time to reach desired serum

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levels of TAC. What is more, expressers have three times the risk of acute rejection within the first month after transplant than no-expressers (9).

After liver transplant , simultaneous expression of CYP3A5*1 in both the intestine and the implanted liver may occur (3). In a previous study of adult population we showed the interaction does occur: Expression of CYP3A5*1 present in liver donor has great impact on TAC levels adjusted by dose in long-term concentrations; while also the expression of this SNP in the receiver has a greater impact but in time just after transplantation (8). However, kinetic and pharmacodynamic are very different comparing paediatric to adult populations. This can be explained by the greater variability of specific enzymes, which are acquired by the child during growth and altering the clinical response to TAC (3). The body of evidence available in paediatrics population is limited for making clinical decisions regarding the therapeutic optimization of TAC. Thus it is essential to generate more information to optimize and customize monitoring strategies to liver transplant in this population. The objective of this study was to estimate the frequency of CYP3A5 genetic polymorphisms and their relationship with pharmacokinetics in Pediatric Liver Transplantation

## **2.** Materials and Methods

A longitudinal study was conducted in f 77 patients under 18 who after liver transplantation over the period 2009-2012 at the Paediatric Hospital J. P Garrahan (PHJPG)

Were included patients with full or partial liver graft, from either living donor or cadaveric donor. All patient were receiving tacrolimus with or without steroids and with or without mofetil mycophenolate. Were excluded HIV infected patients, who suffered early death before receiving immunosuppressive regimen with TAC in the immediate postsurgical and patients with partial or total loss of medical records.

### *2.1. Dosage and treatment scheme:*

Patient information was collected immediately after liver transplantation . Below it is described the scheme of immunosuppression performed in patients according to the Clinical Practice Guidelines of PHJPG for patients after liver transplantation. In induction phase all patients received basiliximab. Patient under 30 kg received 10 mg/dose and over 30 kg received 20 mg/dose. Both doses were administered as an intravenous bolus; the former within 8 hours after reperfusion of the graft and the later the fourth post-surgery day. TAC was dispensed in the maintenance phase which started 24 hours after reperfusion. The initial oral regimen was 0.1 mg/kg/day every 12 hours. Afterwards the dose of TAC was adjusted to tacrolimus blood levels, liver parameters, kidney function and the viral load of Epstein Barr Virus (10). In patients without infectious activity (viral load less than 4000 copies/ ug DNA) and creatinine clearance less than the expected range for your age, the initial desired TAC blood levels were 8-12 ng/ml during the first month after transplantation (11). It was proceeded a quick immunosuppression reduction in patients with viral load above 4000 copies / ug DNA in 2 consecutive samples or clinical evidence of EBV infection. No antiviral therapy was implemented. In patients who developed renal toxicity, regardless viral load, monitoring of TAC was decreased a 25%. In those cases mycophenolate mofetil (MMF) was added as rescue therapy with an initial dose of 20 mg/kg/day and then it was increased up to 40 mg/kg/day after a week of treatment.

### *2.2. Monitoring and quantification of tacrolimus blood levels.*

TAC levels from day 5 to 2-year post-transplant were obtained from hospital records of routine therapeutic drug monitoring. The values recorded correlate to monitoring blood levels from samples drawn prior to the morning dose (Co) (C0:is a concentration measured in t=0, before the first dose of the drug).

Quantification of TAC was performed by chemiluminescence immunoassay by Architect i1000 of Abbott according to the manufacturer's instructions. The low quantification limit was 2.0 ng/ml.

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The linearity was observed from 2-30 ng/ml. The variation coefficient for Quality Control Samples was below 6%.

*2.3. Information collected.*

Demographic information (date of birth, gender), anthropometric data (weight, height), indication of transplant, post- transplantation follow-up time; current medication and doses, concomitant medications (The effect of drug drug interaction was analyzed in a previously published article) (12-13), amount of transplanted graft; , amount of postsurgical days, data related to donor type were collected. We registered clinical laboratory results including hematology (hemoglobin , hematocrit, red blod cells, white cells and platelets, RIN) and clinical chemistry results (creatinine, urea nitrogen, total and direct bilirubin, alkaline phosphatase, alanine aminotransferase- GPT or ALT- aspartate aminotransferase- GOT or AST- gamma glutamyl transpeptidase-GGT- and albumin).

*2.4. DNA isolation and genotyping.*

The genotyping of CYP3A5 were performed in liver biopsies of both the donor and the recipient. The donor's DNA was obtained from liver biopsies or surgical specimens obtained from the Pathology Service of PHJPG. Each of them were tissue fixed in formalin-buffer, paraffin embedded and sectioned by 10 microns thick.

DNA extraction was performed using commercial kits QIAamp DNA Blood Kit and QIAamp DNA FFPE Tissue following the manufacturer's instructions. We obtained from 20 to 100 ng of DNA in each case. The CYP3A5*3 (rs776746) polymorphism was detected by PCR and directly sequenced. Patients with variants (CYP3A5*1/*1 or CYP3A5*1/*3) were called 'expressers' while those with variants CYP3A5*3/*3 were called 'not expressers'.

*2.5. Ethical aspects*.

The proper Informed Consent was signed by a parent or legal guardian before starting any specific evaluations. The study was approved by the office of Teaching and Research of PHJPGand by the Ethics Committee of the Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

*2.6. Statistical analysis*.

We compared daily doses of TAC, Co (TAC levels prior to the morning dose) and Co/dose (concentration adjusted by dose) according to CYP3A5 *1 allele expression between donors and recipients. All values were expressed as mean ± standard deviation. U Mann -Whitney test was used to determine differences between continuous variables among groups. The chi- square test was used to analyze differences between discrete variables. Analysis were performed using STATA 11.0 ©.

## 3. Results

We evaluated 77 paediatric patients medicated with TAC during the first 2 years after transplantation. **Table 1** shows the characteristics of the population studied. We observed 45 patients (58.44 %) with adverse events associated with tacrolimus, 51 patients (66.23 %) had at least one acute cellular rejection episode and 8 patients died ( 10.39% ) during follow-up.

CYP3A5 *1 expression was 37.1% in recipients and 32.2%for Donors. There were not shown statistically significant deviations in the distribution of polymorphisms according to the Hardy-Weinberg principle ( $p > 0.05$ ).

A total of 3670 blood concentrations of TAC were analysed during the study period, with a mean of 47.8 samples per patient. We observed a greater difference in expressers recipients

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regarding not expressers, especially in the first two weeks postoperative, and tend to reduce those differences over time, see **Figure 1**.

When adjusted dose by concentrations according to the genotype of the donor, those who received an organ expresser showed lower Co/dose especially after 90 days post-surgery. See **Figure 2**. A statistically significant reduction in the Co / dose of 0.00063 ng/ml mg/kg/day was observed in comparison with those receiving an organ not expresser (p=0.001).

**Table 1** Characteristics of the studied population (n=77).

| Feature | n(%) |
|---|---|
| Female | 46 (59.74) |
| Age at transplantation (years,± DE) | 5.32 (5.42) |
| Weight ( Kg , ± DE) | 21.84( 17.89) |
| Origin | |
| Argentina | 64(83.11) |
| Bolivia | 2(2.60) |
| Paraguay | 9(11.69) |
| Other | 2(2.59) |
| **Primary illness** | |
| Biliary atresia | 32(41.55) |
| Fulminant hepatitis | 16(20.77) |
| Autoimmune hepatitis | 11(14.28) |
| Hepatoblastoma | 8(10.38) |
| Others | 10(12.98) |
| **Kind of Donor** | |
| Cadaveric | 55(71.42) |
| Alive | 22(28.57) |
| **Kind of Graft** | |
| Full | 26(33.76) |
| Technical variant | 51(66.23) |

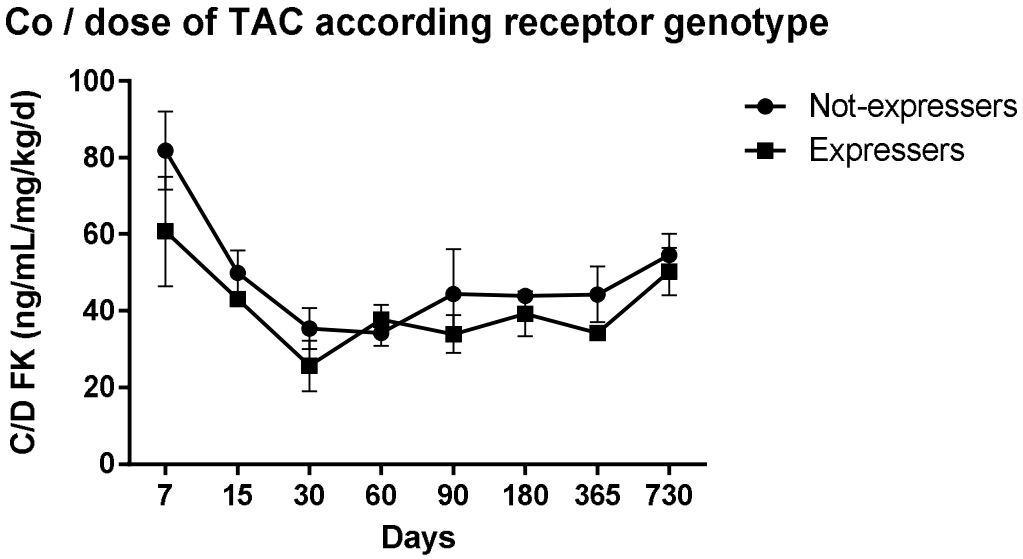


**Figure 1**. Temporal behaviour of Co / dose of TAC according recipient genotype.

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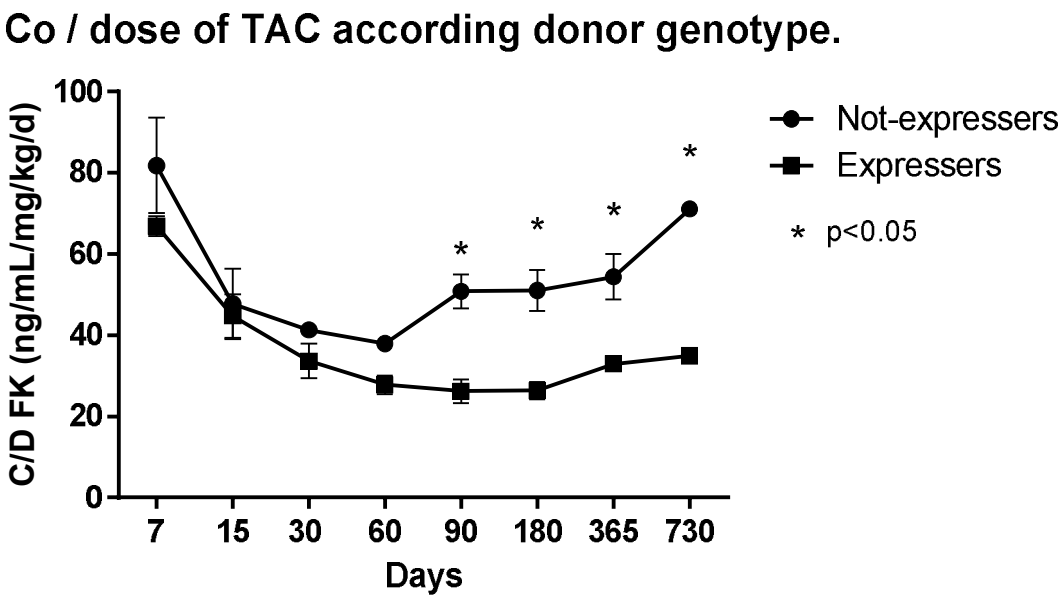


**Figure 2**. Temporal behaviour of Co / dose of TAC according donor genotype.

## 4. Discussion

The CYP3A5 polymorphisms has a differential impact in the pharmacokinetics of tacrolimus according it expression in donors and recipients . In contrast to previous studies this is the first study in the paediatric patients that evaluates the effect of polymorphisms on TAC pharmacokinetics at long term. Other works considered shorter periods and generally not Hispanic population.

Patients with CYP3A5 allele A (CYP3A5 * 1 or wild type) have a normal splicing of the whole 13 exons in this gene. This results in a normal transcript and producing high levels of mRNA, thus expressing the enzyme metabolizing TAC. Patients with allele G (CYP3A5 * 3) have a point mutation (A / G) resulting in the insertion of an inappropriate 'exon' 3B within the transcript. This new exon introduces an early termination codon, leading to a non-functional protein fragment (14). The frequency of expressers (CYP3A5 * 1) in our study was reported to be intermediate between Asian frequency (33% to 66%) and Caucasian (9% to 15%) populations. These estimates are consistent with previous results in studies in Argentine renal transplant patients ,which reported values ranging from 9% to 27%(15) (16) (17). These differences between the caucasian and asian frequencies, reveal the genetic diversity present in latin america as a result from the colonial stage, African (slaves to the 19th century), and post-independence immigrants (the majority of Spain, Italy, France, Europe from the east) (18).

Similar results have been found in studies focused on the frequency of variations in other genes related to antineoplastic metabolism (19). Continue to building this pharmacogenetic map in latin america improve the understanding of the variations in the metabolism and the effect of the different medicines, without the need to extrapolate results obtained from other populations.

In liver transplanted patients, both donor and recipient carrying the CYP3A5 polymorphisms are associated with changes in the pharmacokinetics of TAC. However, the role of each polymorphism is different according to days after transplantation. We have shown that the recipient CYP3A5 genotype plays a more important role than the donor genotype. Recipients with CYP3A5 * 1 achieved lower blood concentrations of TAC and lower dose-adjusted concentrations despite the medical pharmacotherapeutic follow-up (based on adjusting the blood concentrations to the reference therapeutic margins). These findings are consistent with a recent study of 64 post-transplant children with 1 year follow-up (20). It was shown that lower dose-

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adjusted ($p < 0.05$) concentrations are required in patients who are expressers, without correlation with donor genotype, especially in the first 7 days after transplantation (20).

To recognize the role played by the recipient CYP3A5 genotype in the first weeks after transplantation is essential to avoid excessive dose increases to patients who are expressers of this genotype (21).

In contrast to receipt, the donor genotype alter significantly kinetics of TAC increasing along with time after transplantation. The effect of CYP3A5 expression on the recipient is an augmented hepatic clearance of the liver implanted with the polymorphism. This tendency was evidenced in our study; a reduction of dose-adjusted concentrations was documented statistically significant after 60 to 90 postoperative days. Such observation might be attached to the time needed by the organ to recover from the ischemia and reperfusion injury, regeneration and graft growth as the months after transplantation occur (22). Our results indicate the importance of know the genotype present in the organ previously to be implanted. This is a priority during the ambulatory follow-up to select patients with greater hepatic clearance, who will get a lower concentration and which may require different medical follow-up to avoid sub-immunosuppression.

Our study has limitations mainly given the retrospective nature. Among which are biases due to misclassification of patients either by memory bias or problems to record information in clinical histories- by omitting information or incorrectly record said documents-. There biases could be minimized obtaining always information from primary registers (physical or electronic medical history) and checking with other clinical records (nursing records, pharmacy Hospital). We only analyse concentration at time 0 (C0) per patient due to we use data hospital therapeutic monitoring, and for TAC this concentration is used to clinical monitoring for this drugs. Also effect of other variables in the pharmacokinetics of TAC such as age, drugs interaction and length of the event related to dose-adjusted concentrations were not evaluated , and should be analyse by other studies.

In conclusion, patients after liver transplantation, both donor and recipient carrying CYP3A5 polymorphisms are susceptible to suffer changes in TAC pharmacokinetics. However, the role of each polymorphism is different according to days after transplantation proceeds and it must be taken into account to optimize the benefits of TAC therapy during the post-transplant induction and maintenance phase.

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