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Donor *CYP3A5* genotype influences tacrolimus disposition on the first day after paediatric liver transplantation

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Running head

Donor *CYP3A5* genotype in paediatric liver transplantation

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What is already known about this subject

- Tacrolimus is a drug widely used in paediatric solid organ transplantation.
- Therapeutic drug monitoring (TDM) was introduced to reach the target blood concentration as early as possible after transplantation, to avoid over- or under-dosing however, on the first day after transplantation, the tacrolimus dose is calculated according to body weight or body surface and it is not TDM guided.
- Liver donor *CYP3A* genotypes influence tacrolimus blood concentration in adult recipients.

What this study adds

- On the first day after paediatric liver transplantation, the donor *CYP3A5* genotype influences tacrolimus blood concentration more than the donor *CYP3A4* genotype
- Physiological factors, mainly recipient age, may also play a role in tacrolimus disposition on the first day after paediatric liver transplantation.
- There is a quick recovery of CYP3A enzyme activity in the liver graft after transplantation
- The tacrolimus therapeutic range may be reached more quickly if the initial, not yet TDM guided, doses are doubled in paediatric patients under 6 years of age who receive a graft from a male extensive metabolizer.

Tables of Links

TARGETS
Enzymes
CYP3A4
CYP3A5

LIGANDS
Tacrolimus

These Tables list key protein targets and ligands in this article that are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY, and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [1].

Summary

AIM

The aim of our study was to investigate the influence of the *CYP3A4/5* genotype in paediatric liver transplant recipients and donors and what contribution age and gender make to tacrolimus disposition on the first day after transplantation.

METHODS

The contribution of the *CYP3A4/5* genotype in paediatric liver transplant recipients and donors to the tacrolimus blood trough concentrations (C_0) and tacrolimus concentration/weight-adjusted dose ratio on day one was evaluated in 67 liver transplanted children: 33 boys and 34 girls, mean age 4.5 years.

RESULTS

Donor *CYP3A5* genotype appears to be significantly associated with tacrolimus disposition on the first day after liver transplantation ($P < 0.0002$). Other physiological factors, such as the recipient age and donor gender may also play a role and lead to significant differences in tacrolimus C_0 and tacrolimus concentration/weight-adjusted dose ratio on day one. However, according to the linear general model, only recipient age appears independently associated with tacrolimus disposition on the first day after liver transplantation ($P < 0.03$). Indeed, there was a faster tacrolimus metabolism in children under 6 years of age ($P < 0.02$).

CONCLUSIONS

Donor *CYP3A5* genotype, recipient age and, to a lesser extent, donor gender appear to be associated with tacrolimus disposition on day one after transplant. This suggests that increasing the starting tacrolimus doses in paediatric patients under 6 years of age, who receive a graft from a male extensive metabolizer, may enhance the possibility of their tacrolimus levels reaching the therapeutic range sooner.

Introduction

Tacrolimus is a calcineurin inhibiting immunosuppressant drug widely used in solid organ transplantation [2] which is effective in the management both of adult and paediatric transplant patients [3]. However, this drug has a narrow therapeutic index, exposing patients to a risk of acute graft rejection or toxicity if its blood concentrations are respectively too low or too high. Moreover, tacrolimus concentrations and pharmacokinetics vary considerably on the first day after transplantation, thus complicating dose calculations. Therefore, therapeutic drug monitoring (TDM) was introduced in order to reduce variability and avoid over- or under-dosing. TDM may be carried out by measuring the tacrolimus trough concentration in peripheral blood 12 hours after a dose (C_0) or by the area under the concentration-time curve (AUC), with the primary goal of maintaining C_0 and AUC within a predefined therapeutic range [4]. Thus TDM is crucial for optimizing treatment efficacy, reducing rejection episodes and preventing adverse effects in daily practice. However, despite the undoubted contribution by TDM in improving long-term patient and graft survival, it has been demonstrated that reaching quickly the immunosuppressant target concentrations is crucial [5]. Recent literature has reported that patients should reach target blood concentrations as early as possible in the first week after transplantation to minimize early rejection risk [6]. This is especially important in paediatric recipients, where certain factors, such as growth, age-related changes, as well as time from transplantation, co-medications and hepatic and renal function, may influence the response to immunosuppressants. Therefore, choosing the correct first oral dose of tacrolimus, when it is not yet TDM guided, may well reduce the time needed to reach the drug therapeutic window and prove beneficial particularly in paediatric liver transplant patients.

To meet this goal, numerous authors have suggested that some genetic factors, such as the polymorphisms in genes coding for biotransformation enzymes (cytochrome P450 (CYP) isoenzymes 3A4 and 3A5) should be taken into account to guide the initial tacrolimus dosing [7]. Indeed, it has been consistently demonstrated that CYP3A5 expressers (*CYP3A5*1/*1* and **1/*3*

carriers) have lower C_0 levels and higher oral clearance (CL/F), thus requiring higher tacrolimus doses to reach the same C_0 at steady-state than CYP3A5 non-expressers (*CYP3A5*3/*3* carriers). These findings support the case for a strategy of pharmacogenetic-based dosing of tacrolimus [8]. A new SNP in intron 6 (15289C>T; *CYP3A4*22*) has recently been described, which demonstrates decreased CYP3A4 mRNA hepatic expression and a lower microsomal CYP3A4 enzymatic activity in the T-variant allele [9]. Although the effect of this newly described *CYP3A4* polymorphism on tacrolimus dosing requirements and trough blood concentrations has been extensively investigated in adults, data in children are scanty [10, 11].

A retrospective study was carried out to evaluate whether the *CYP3A4/5* combined genotype approach in paediatric liver recipients and donors could achieve target blood tacrolimus concentrations more quickly. The contribution of other factors, such as age and gender, on tacrolimus disposition on the first day after transplantation was also investigated.

Patients and methods

A total of 67 paediatric patients (aged <18 years at transplant) managed on oral tacrolimus after a liver transplant performed at A.O.U. Città della Salute e della Scienza, Torino, Italy, were enrolled into the study. DNA samples from the recipients and their donors were provided by the Regional Transplantation Center (CRT Piemonte e Valle d'Aosta, Italy) (Table 1).

Approval of the local ethics committee (protocol identification number 15386/28.3) was obtained, as was a written informed consent from the patients' parents or guardians. A population study for *CYP3A4*22* single nucleotide polymorphism (SNP) was also performed on peripheral blood samples collected from 262 unrelated healthy adult volunteers of Italian-Caucasian descent, recruited from blood donors at the Centre's blood bank, A.O.U. Città della Salute e della Scienza, Torino, Italy. This population study also was approved by the local ethics committee (protocol identification number 8802/A5.4) and written informed consent was obtained from all participants.

Induction therapy with an interleukin-2 receptor antagonist (basiliximab) was administered to 52/67 patients and the first tacrolimus dose was given perioperatively in all (Table 1). Tacrolimus was then administered orally or via naso-gastric tube on the first day in all transplanted patients, to reach a total daily dose of 0.15 mg/Kg/day as per protocol. TDM was used to adjust the subsequent tacrolimus doses aiming at reaching a target C_0 of 10-12 ng/ml. Steroids were added in 41/67 patients (Table 1).

Tacrolimus concentration measurement

Tacrolimus blood trough concentrations were quantified according to the Waters MassTrak Immunosuppressants XE Kit protocol, taking into account the Lake Louise Consensus document for therapeutic drug monitoring. Instrumental analysis was performed on Waters Acquity^{TM} Ultra Performance Liquid Chromatography (UPLC) coupled to a Waters Acquity Tandem Quadrupole Mass Spectrometry Detector (TQD) [12].

Criteria adopted to satisfy the guidelines on tacrolimus therapeutic drug monitoring in whole-blood samples take into account the measured quality control values within 10% of the values stated in the certificate of analysis, performed at least once per day at the initial run batch.

Accuracy of tacrolimus quantification was checked by participating in an external proficiency-testing scheme, set up by Bionalytics, UK.

The tacrolimus trough concentration for the effect of dose adjustments were normalized to obtain the tacrolimus trough concentration/weight-adjusted dose ratio, as the tacrolimus trough concentration, in itself, does not well define the variability in doses and blood levels of tacrolimus between different transplant patients [7, 13, 14].

Genotyping

Donor and recipient DNA samples were genotyped for *CYP3A4*22* and *CYP3A5*3* using the allelic discrimination reaction performed with TaqMan (Thermo Fisher Scientific). The *CYP3A4*22* assay was validated by sequencing homozygous wild-type (**1/*1*), heterozygous (**1/*22*), and homozygous mutant (**22/*22*) samples. When whole body metabolism was considered, the patients were classified as rapid metabolizers (RM) if the donor/recipient pair carried all, or at least five, *CYP3A4/5*1;* as extensive CYP3A4/5 metabolizers (EM), if they carried four *CYP3A4/5*1* alleles; as intermediate CYP3A4/5 metabolizers (IM), if they carried none, or up to three *CYP3A4/5*1* alleles in their recipient and donor DNA (Table 2).

When only recipient or donor metabolism were considered, the patients were classified as RM if they carried all or three *CYP3A4/5*1* alleles, as EM if they carried two *CYP3A4/5*1* alleles and as IM if they carried none, or one *CYP3A4/5*1* allele.

Statistical analysis

Data are presented as mean and standard deviation (SD) or as median and interquartile range (IQR). Differences were compared using either the Kruskal-Wallis or the Mann-Whitney tests, as

appropriate, and a P value of less than 0.05 was taken as statistically significant. A general linear model was performed to test the overall contribution of *CYP3A5* genotype, donor gender and recipient age to tacrolimus levels. All data analyses were carried out using SPSS 23.0 software (SPSS, Chicago, IL).

The Hardy-Weinberg equilibrium was calculated with the method described by Rodriguez et al. [15].

Results

Patient characteristics

Table 1 reports on the 67 paediatric liver transplant patients who were eligible for the study, with the demographic details of recipients and donors

Tacrolimus disposition

Data for tacrolimus concentration (ng/ml) and concentration/weight-adjusted dose ratio (ng/ml/dose) on the first day after liver transplantation were available for all patients. The median tacrolimus blood trough concentration was 7.8, IQR 9.1 ng/ml and the median concentration/weight-adjusted dose ratio was 95, IQR 111 ng/ml/dose (Figures 1A and B). A total of 67.2% of all analyzed concentrations fell below the target range of 10-12 ng/ml, whilst 25.4% were higher than the upper desired limit (Figure 1A).

Influence of other drugs, graft-recipient weight ratio, gender and age on tacrolimus disposition on the first day after liver transplantation

No influences of glucocorticoids, antifungal drugs or graft-recipient weight ratio (GRWR) on tacrolimus trough concentrations or on the concentration/weight-adjusted dose ratios were observed in our paediatric population on the first post-transplant day, whereas the donor gender and the age of the recipient did seem to play a major role in tacrolimus disposition. On the first day after transplant, recipients of a male donor graft had a median C_0 of 5.6, IQR 7 ng/ml, whilst recipients of a female organ had a median C_0 of 9.7, IQR 10 ng/ml ($P < 0.02$) and median concentration/weight-adjusted dose ratios of 87, IQR 125 *vs* 147, IQR 113 ng/ml/dose ($P < 0.04$).

Age related differences in our patients, stratified for an age at transplant under or above 6 years, were observed only in the recipients and exclusively for the tacrolimus concentration/weight-adjusted dose ratios (86, IQR 123 ng/ml/dose under 6 years of age *vs* 139, IQR 100 ng/ml/dose

above 6 years of age ($P < 0.02$) (Figure 2). The discrepancy observed in the statistical significance when comparing the groups only according to tacrolimus concentration/weight-adjusted dose ratio might well be found in how the dose-normalization better defines the wide intervariability in the tacrolimus trough concentration among paediatric transplant patients compared to tacrolimus trough concentration without dose normalization.

Frequency of *CYP3A4* and *CYP3A5* variants in paediatric liver transplant recipients and donors

DNA samples for *CYP3A4* and *CYP3A5* genotyping were available for all 67 patients and their donors.

Taking into consideration the recipients' *CYP3A4* genotype, fifty-nine (88%) carried the *CYP3A4*1/*1* genotype, six (9%) carried the *CYP3A4*1/*22* genotype and two (3%) carried the *CYP3A4*22/*22* genotype.

The *CYP3A4* genotyping of donors showed that fifty-five (82%) carried the *CYP3A4*1/*1* genotype, eleven (16%) carried the *CYP3A4*1/*22* genotype and one (1.5%) carried the *CYP3A4*22/*22* genotype.

Taking into consideration the recipients' *CYP3A5* genotype, one (1.5%) carried the *CYP3A5*1/*1* genotype, eight (12%) the *CYP3A4*1/*3* genotype and fifty-eight (87%) of the patients carried the *CYP3A5*3/*3* genotype. The *CYP3A5* donor genotyping showed that one (1.5%) carried the *CYP3A5*1/*1* genotype, twenty-eight (33%) carried the *CYP3A5*1/*3* genotype and forty-four (66%) of the patients carried the *CYP3A5*3/*3* genotype. According to the frequency of *CYP3A4* and *CYP3A5* variants in our paediatric liver transplant patients, an higher frequency of *CYP3A5*1* allele in donors was observed, suggesting that some liver donors might come from ethnic groups where the *CYP3A5*1* allele frequency is higher than in Caucasian subjects [16]. We cannot rule out this possibility since individuals from Asia or Africa are nowadays a significant demographic presence in Italy.

Both recipients' and donors' *CYP3A4* and *CYP3A5* genotypes were tested by the Hardy-Weinberg equilibrium test, and only the recipients' *CYP3A4* genotype showed equilibrium deviations (chi-square = 8.28, P = 0.05). Sequencing confirmed the *CYP3A4* genotype in the paediatric liver transplant recipients.

In order to determine whether the deviation from equilibrium observed in the recipient paediatric population may have been due to genotyping errors, or to biological factors, *CYP3A4*22* SNP was confirmed by sequencing the PCR products and genotyping for *CYP3A4*22* SNP 262 was carried out on a healthy unrelated adult population of identical extraction. No deviation from the Hardy-Weinberg equilibrium (chi-square = 0.83, P = 0.05) was observed in the control population. Therefore, the deviation from the Hardy-Weinberg equilibrium observed in the *CYP3A4* genotyping of the paediatric recipient population may well be due to the small number of patients.

Influence of *CYP3A4/5* combined-genotype approach on tacrolimus disposition on the first day after liver transplantation

Whole body metabolism on the first day after liver transplantation was investigated in the donor/recipient pairs through a *CYP3A4/5* combined-genotype approach. Statistically different tacrolimus trough concentrations (C_0) were detected between rapid, extensive and intermediate metabolizers (4.1, IQR 6.3 RM *vs* 9.2, IQR 8.9 EM ng/ml $P < 0.005$; 4.1, IQR 6.3 RM *vs* 8.5, IQR 8.5 IM ng/ml; $P < 0.02$) (Figure 3), whilst there were no statistically significant differences in the tacrolimus concentration/weight-adjusted dose ratios. The difference in behavior between tacrolimus C_0 and concentration/weight-adjusted dose ratio prompted the investigation of the influence the *CYP3A4/5* combined-genotype approach on tacrolimus disposition had in the recipients (extrahepatic metabolism) and in donors (hepatic metabolism) on the first day after transplantation. In our opinion, the lack of statistical significance, when we compared the whole body metabolism groups according to the tacrolimus trough concentration/weight-adjusted dose, was possibly due to the physiological variables included in our statistical analysis.

Indeed, no statistical differences in extrahepatic metabolism were observed between rapid, extensive or intermediate metabolizers, stratified by the *CYP3A4/5* combined-genotype approach. Instead, statistical differences were observed in hepatic metabolism between rapid, extensive and intermediate metabolizers both for the tacrolimus C_0 (3.0, IQR 6.6 RM *vs* 8.5, IQR 9.0 EM ng/ml $P < 0.003$; 3.0, IQR 6.6 RM *vs* 9.5, IQR 7.1 IM ng/ml $P < 0.004$) and for the concentration/weight-adjusted dose (62, IQR 68 RM *vs* 122, IQR 103 EM ng/ml $P < 0.003$; 62, IQR 68 RM *vs* 184, IQR 148 PM ng/ml/dose $P < 0.02$).

Effect of *CYP3A4* and *CYP3A5* liver genotypes on tacrolimus disposition on the first day after liver transplantation.

Since the recipient *CYP3A4/5* combined-genotypes did not seem to play an important role in tacrolimus disposition in our paediatric population, we turned our attention to the donors' *CYP3A4* and *CYP3A5* genotypes, investigating the contribution of these metabolic enzymes to tacrolimus disposition on the first day after liver transplantation.

Patients carrying both liver *CYP3A4*22/*22* and **1/*22* were pooled, and a comparison was made between *CYP3A4*1/*1* and *CYP3A4*1/*22-*22/*22*. No statistically significant differences were observed between the two groups, either in tacrolimus C_0 or in concentration/weight-adjusted dose ratios. When the same statistical analysis was performed on liver *CYP3A5*1/*1-*1/*3* and *CYP3A5*3/*3* patients, statistically significant differences were observed between the two groups in tacrolimus C_0 (3.0, IQR 5.3 (**1/*1-*1/*3*) *vs* 9.1, IQR 8.6 (**3/*3*) ng/ml; $P < 0.0002$) and concentration/weight-adjusted dose ratios (62, IQR 65 (**1/*1-*1/*3*) *vs* 147, IQR 123 (**3/*3*) ng/ml/dose; $P < 0.0002$) (Figure 4A and B).

Interplay of recipient age, donor gender and donor *CYP3A5* genotype on tacrolimus disposition on the first day after liver transplantation.

Since in our paediatric population recipient age, donor gender and donor *CYP3A5* genotype seem to play a role in tacrolimus disposition on the first day after liver transplantation, we performed a statistical analysis to show the overall contribution of the donor *CYP3A5* genotype, donor gender and recipient age on tacrolimus disposition.

According to the linear general model, only the donor *CYP3A5* genotype and the recipient age were independently associated with tacrolimus disposition, $P < 0.03$ and $P < 0.02$, respectively, whilst the donor gender seemed to play a minor role.

Discussion

Our data show that, on the first day after transplantation, less than 8% of our paediatric liver transplant patients had a tacrolimus trough blood concentration within the therapeutic range (10-12 ng/ml). In keeping with the assumption that an appropriate early systemic tacrolimus exposure is clinically relevant for a smooth post-transplant course, it could be argued that a majority of our paediatric population may have been exposed to the risk of adverse drug-related events, ranging from graft rejection to drug toxicity [5] .

Tacrolimus dosage was TDM monitored in all patients in order to reduce the time lag required to reach a stable tacrolimus trough blood concentration within the therapeutic range. However, relying on TDM guided dose adjustments means that the desired target concentrations are reached only days or even weeks after transplantation. To overcome this drawback, many authors have suggested that factors such as recipient age, baseline histological score of the graft, co-medication, creatinine clearance, *CYP3A5* genetic polymorphism and CYP3A4 expression rates of donors [14, 17-19] should be taken into account and guide the daily tacrolimus administration. This is particularly relevant on the first day after transplantation, when the tacrolimus dose is calculated according to body weight or body surface area and TDM has not yet been started.

A novel SNP located in intron 6 of the *CYP3A4* gene, *CYP3A4*22*, has been associated with reduced *CYP3A4* mRNA expression in the liver [9] and its contribution to tacrolimus metabolism has been confirmed in kidney, heart and liver transplantation [10, 11, 20]. Our study investigated the role that *CYP3A4/5* combined genotypes play on tacrolimus trough blood concentration and tacrolimus concentration/weight-adjusted dose ratio on the first day after liver transplantation in children. Concomitant drugs, graft-recipient weight ratio, gender and age were also taken into account. Graft-recipient weight ratio and co-medications did not affect tacrolimus disposition on the first day after liver transplantation in our paediatric liver transplant recipients. Our findings on the effect of co-medications differ from those of other studies, where co-administered drugs, in

particular fluconazole, were reported to be the most important covariate influencing tacrolimus CL/F [20]. However, these studies investigated tacrolimus disposition in transplant patients over a longer time period, when drug induction and inhibition on the CYP450 system are more consistent. On the first day after liver transplantation the co-medications effect on tacrolimus disposition may not be so relevant due to the time these drugs need to exert their effect on the CYP450 enzymes.

Conversely, a gender effect was observed from the first post-transplant day, as male donor grafts engendered lower tacrolimus trough concentrations and lower tacrolimus concentration/weight-adjusted dose ratios than female donors, with median values below the therapeutic range. These data are in contrast with the results reported by others [21, 22], who observed significantly lower areas under the curve values in women than in men in adult kidney transplanted patients. Thangavel et al. also reported a female predominant expression of human CYP3A5 according to different secretion of growth hormone (GH) in healthy male and female adult individuals [23]. However, the differences between our results and those of Kuyper et al. [21] and Velicković-Radovanović et al. [22] may well be due to the differences in the study protocol: sampling time points, children vs adults, liver vs kidney transplantation. It must also be considered that liver dysfunction often leads to endocrine perturbations through the abolition of the balanced crosstalk between the endocrine system and the hepatic microenvironment. Indeed, some studies have shown anomalies in the GH levels, possibly due to GH resistance, before liver transplantation, whereas the patients achieved an almost normal GH range only after a week from the liver transplantation [24-27]. Therefore, we could speculate that at a very early stage after liver transplantation, GH hormonal perturbation might affect the CYP3A5 enzyme expression in the donor liver, changing the physiological status of CYP3A5 enzyme induction. This alterations may have induced a higher CYP3A5 enzyme expression in male than in female liver grafts in our paediatric transplant population. However, according to our linear general model analysis, donor gender does not seem to influence tacrolimus disposition as much as donor *CYP3A5* genotype and recipient age.

The effect of age on tacrolimus disposition observed in our study are in agreement with previous

studies on paediatric renal and liver transplant recipients, with pre-pubertal children needing 2 to 3 times higher doses than adults [28, 29]. Indeed, when we compared the tacrolimus concentration/weight-adjusted dose ratios in children who were younger or older than 6 years, we found a faster tacrolimus disposition in the younger group (Figure 2).

When recipient and donor tacrolimus metabolism (whole body metabolism) were considered, *CYP3A4/5* combined genotypes were strongly related to tacrolimus disposition on the first day after liver transplantation, but only to tacrolimus trough blood concentration (Figure 3). Rapid CYP3A4/5 metabolizers exhibited approximately 50% lower tacrolimus trough blood concentrations than did intermediate or extensive metabolizers on the first day after liver transplantation, while most of the rapid metabolizers were below the therapeutic range (Figure 3). These data are in agreement with those reported in paediatric heart transplant recipients, where slow CYP3A4/5 metabolizers required almost 20% less tacrolimus than did extensive metabolizers and approximately 50% less than rapid metabolizers [11].

This discrepancy between tacrolimus blood trough concentration and tacrolimus concentration/weight-adjusted dose ratio in the tacrolimus whole body metabolism lead us to analyze the *CYP3A4/5* genotypes in recipients (extrahepatic metabolism) and donors (hepatic metabolism) separately. A significant contribution in tacrolimus disposition attributable to the donor liver was observed. Conversely, recipient metabolism did not provide any significant contribution, thus confirming the crucial role that hepatic metabolism plays in tacrolimus disposition at a very early stage after liver transplantation [7, 30]. Nevertheless, CYP-dependent metabolism in children exceeds adult CYP activity, decreasing to adult levels around puberty. Therefore, our finding that children under 6 need higher doses of tacrolimus than adults, might be influenced by extrahepatic metabolism and not by hepatic metabolism, as also reported by Uesugi et al. [31]. This may been explained by the quick recovery of CYP3A enzyme activity in the liver graft after transplantation [32] and by different maturation changes in ontogeny of CYP3A5 activity between children and adults [33-35], together with the unique pathophysiological condition

occurring in patients undergoing a liver transplantation, which may also play an important role [36]. Lastly, the *CYP3A4* genotype and *CYP3A5* genotype contribution to tacrolimus hepatic metabolism on the first day after liver transplantation were investigated separately. The importance of the *CYP3A5* genotype in tacrolimus disposition was confirmed, as most *CYP3A5*1/*1* and **1/*3* paediatric patients had tacrolimus concentrations under the therapeutic range, even at this very early stage after liver transplantation (Figure 4A and B), while the *CYP3A4* genotype played only a minor role. Our results partially contrast with those of Guy-Viterbo et al. who reported that the presence of the *CYP3A4*22* genotype in the liver donor decreased tacrolimus clearance by 29% [20]. This incongruity on the *CYP3A4* genotype role in tacrolimus disposition may be due to differences between the study protocols. Our study investigated the tacrolimus trough blood concentration and concentration/weight-adjusted dose ratio by just one point blood sampling at a very early stage after liver transplantation, whereas Guy-Viterbo et al. [20] studied the *CYP3A4* genotype contribution by blood sampling at several points up to 90 days after liver transplantation. Therefore, in our opinion, a phenomenon such as phenoconversion [37] or difference in CYP3A5 and CYP3A4 activity recovery after liver transplantation, may change the relative importance of CYP3A5 and CYP3A4 enzymes for tacrolimus disposition, the latter becoming more relevant over time. Consequently, it may be assumed that the tacrolimus metabolism over time might be related to liver function recovery rather than to the CYP3A5 enzyme activity [32].

In conclusion, our study shows that the initial disposition of tacrolimus in paediatric liver transplant patients is influenced by the liver donor *CYP3A5* genotype, thus indicating that not only does the CYP3A5 enzyme play a major role over the CYP3A4 enzyme in the tacrolimus metabolism at this very early stage, but also that other physiological factors, mainly the recipient age, may also play a role. This finding is in agreement with data reported in paediatric heart transplant recipients, where age and *CYP3A5* genotype were independently associated with the tacrolimus concentration/dose ratio [10].

We acknowledge that the comparatively small size of our paediatric population is a limiting factor

and that further research is required in support of these preliminary findings. However, this study suggests that, in paediatric patients under 6 years of age who receive a graft from a male extensive metabolizer, at least doubling the initial tacrolimus doses, when they are not yet TDM guided, may enhance the efficacy of immunosuppression.

References

1. Alexander SP, Kelly E, Marrion N, Peters JA, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Southan C, Buneman OP, Catterall WA, Cidlowski JA, Davenport AP, Fabbro D, Fan G, McGrath JC, Spedding M, Davies JA, Collaborators C. The Concise Guide to PHARMACOLOGY 2015/16: Overview. Br J Pharmacol 2015; 172: 5729-43.

2. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. Clin Pharmacokinet 2004; 43: 623-53.

3. Reyes J, Jain A, Mazariegos G, Kashyap R, Green M, Iurlano K, Fung J. Long-term results after conversion from cyclosporine to tacrolimus in pediatric liver transplantation for acute and chronic rejection. Transplantation 2000; 69: 2573-80.

4. Venkataramanan R, Shaw LM, Sarkozi L, Mullins R, Pirsch J, MacFarlane G, Scheller D, Ersfeld D, Frick M, Fitzsimmons WE, Virji M, Jain A, Brayman KL, Shaked A. Clinical utility of monitoring tacrolimus blood concentrations in liver transplant patients. J Clin Pharmacol 2001; 41: 542-51.

5. Borobia AM, Romero I, Jimenez C, Gil F, Ramirez E, De Gracia R, Escuin F, Gonzalez E, Sansuan AJ. Trough tacrolimus concentrations in the first week after kidney transplantation are related to acute rejection. Ther Drug Monit 2009; 31: 436-42.

6. Matas AJ, Gillingham KJ, Chavers BM, Nevins T, Kashtan C, Mauer SM, Payne WD, Gruessner R, Najarian JS. The importance of early cyclosporine levels in pediatric kidney transplantation. Clin Transplant 1996; 10: 482-6.

7. Goto M, Masuda S, Kiuchi T, Ogura Y, Oike F, Okuda M, Tanaka K, Inui K. CYP3A5*1-carrying graft liver reduces the concentration/oral dose ratio of tacrolimus in recipients of living-donor liver transplantation. Pharmacogenetics 2004; 14: 471-8.

8. Haufroid V, Wallemacq P, VanKerckhove V, Elens L, De Meyer M, Eddour DC, Malaise J, Lison D, Mourad M. CYP3A5 and ABCB1 polymorphisms and tacrolimus pharmacokinetics in renal transplant candidates: guidelines from an experimental study. Am J Transplant 2006; 6: 2706-13.

9. Wang D, Guo Y, Wrighton SA, Cooke GE, Sadee W. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. Pharmacogenomics J 2011; 11: 274-86.

10. Elens L, Bouamar R, Hesselink DA, Haufroid V, van der Heiden IP, van Gelder T, van Schaik RH. A new functional CYP3A4 intron 6 polymorphism significantly affects tacrolimus pharmacokinetics in kidney transplant recipients. Clin Chem 2011; 57: 1574-83.

11. Gijsen V, Mital S, van Schaik RH, Soldin OP, Soldin SJ, van der Heiden IP, Nulman I, Koren G, de Wildt SN. Age and CYP3A5 genotype affect tacrolimus dosing requirements after transplant in pediatric heart recipients. J Heart Lung Transplant 2011; 30: 1352-9.

12. Volosov A, Napoli KL, Soldin SJ. Simultaneous simple and fast quantification of three major immunosuppressants by liquid chromatography--tandem mass-spectrometry. Clin Biochem 2001; 34: 285-90.

13. Stratta P, Quaglia M, Cena T, Antoniotti R, Fenoglio R, Menegotto A, Ferrante D, Genazzani A, Terrazzino S, Magnani C. The interactions of age, sex, body mass index, genetics, and steroid weight-based doses on tacrolimus dosing requirement after adult kidney transplantation. Eur J Clin Pharmacol 2012; 68: 671-80.

14. Provenzani A, Santeusanio A, Mathis E, Notarbartolo M, Labbozzetta M, Poma P, Provenzani A, Polidori C, Vizzini G, Polidori P, D'Alessandro N. Pharmacogenetic considerations for optimizing tacrolimus dosing in liver and kidney transplant patients. World J Gastroenterol 2013; 19: 9156-73.

15. Rodriguez S, Gaunt TR, Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am J Epidemiol 2009; 169: 505-14.

16. Hesselink DA, Bouamar R, Elens L, van Schaik RH, van Gelder T. The role of pharmacogenetics in the disposition of and response to tacrolimus in solid organ transplantation. Clin Pharmacokinet 2014; 53: 123-39.

17. Lancia P, Jacqz-Aigrain E, Zhao W. Choosing the right dose of tacrolimus. Arch Dis Child 2015; 100: 406-13.

18. Thervet E, Loriot MA, Barbier S, Buchler M, Ficheux M, Choukroun G, Toupance O, Touchard G, Alberti C, Le Pogamp P, Moulin B, Le Meur Y, Heng AE, Subra JF, Beaune P, Legendre C. Optimization of initial tacrolimus dose using pharmacogenetic testing. Clin Pharmacol Ther 2010; 87: 721-6.

19. Monostory K, Toth K, Kiss A, Hafra E, Csikany N, Paulik J, Sarvary E, Kobori L. Personalizing initial calcineurin inhibitor dosing by adjusting to donor CYP3A-status in liver transplant patients. Br J Clin Pharmacol 2015; 80: 1429-37.

20. Guy-Viterbo V, Baudet H, Elens L, Haufroid V, Lacaille F, Girard M, Debray D, Chardot C, Reding R, Wallemacq P, Musuamba F. Influence of donor-recipient CYP3A4/5 genotypes, age and fluconazole on tacrolimus pharmacokinetics in pediatric liver transplantation: a population approach. Pharmacogenomics 2014; 15: 1207-21.

21. Kuypers DR, Claes K, Evenepoel P, Maes B, Coosemans W, Pirenne J, Vanrenterghem Y. Time-related clinical determinants of long-term tacrolimus pharmacokinetics in combination therapy with mycophenolic acid and corticosteroids: a prospective study in one hundred de novo renal transplant recipients. Clin Pharmacokinet 2004; 43: 741-62.

22. Velickovic-Radovanovic R, Mikov M, Paunovic G, Djordjevic V, Stojanovic M, Cvetkovic T, Djordjevic AC. Gender differences in pharmacokinetics of tacrolimus and their clinical significance in kidney transplant recipients. Gend Med 2011; 8: 23-31.

23. Thangavel C, Boopathi E, Shapiro BH. Inherent sex-dependent regulation of human hepatic CYP3A5. Br J Pharmacol 2013; 168: 988-1000.

24. Maes M, Sokal E, Otte JB. Growth factors in children with end-stage liver disease before and after liver transplantation: a review. Pediatr Transplant 1997; 1: 171-5.

25. Infante D, Tormo R, Castro de Kolster C, Potau N, Martinez V, Broto J, Lloret J. Changes in growth, growth hormone, and insulin-like growth factor-I (IGF-I) after orthotopic liver transplantation. Pediatr Surg Int 1998; 13: 323-6.

26. Bassanello M, De Palo EF, Lancerin F, Vitale A, Gatti R, Montin U, Ciarleglio FA, Senzolo M, Burra P, Brolese A, Zanus G, D'Amico DF, Cillo U. Growth hormone/insulin-like growth factor 1 axis recovery after liver transplantation: a preliminary prospective study. Liver Transpl 2004; 10: 692-8.

27. Greer RM, Quirk P, Cleghorn GJ, Shepherd RW. Growth hormone resistance and somatomedins in children with end-stage liver disease awaiting transplantation. J Pediatr Gastroenterol Nutr 1998; 27: 148-54.

28. Kim JS, Aviles DH, Silverstein DM, Leblanc PL, Matti Vehaskari V. Effect of age, ethnicity, and glucocorticoid use on tacrolimus pharmacokinetics in pediatric renal transplant patients. Pediatr Transplant 2005; 9: 162-9.

29. MacFarlane GD, Venkataramanan R, McDiarmid SV, Pirsch JD, Scheller DG, Ersfeld DL, Fitzsimmons WE. Therapeutic drug monitoring of tacrolimus in pediatric liver transplant patients. Pediatr Transplant 2001; 5: 119-24.

30. Wei-lin W, Jing J, Shu-sen Z, Li-hua W, Ting-bo L, Song-feng Y, Sheng Y. Tacrolimus dose requirement in relation to donor and recipient ABCB1 and CYP3A5 gene polymorphisms in Chinese liver transplant patients. Liver Transpl 2006; 12: 775-80.

31. Uesugi M, Masuda S, Katsura T, Oike F, Takada Y, Inui K. Effect of intestinal CYP3A5 on postoperative tacrolimus trough levels in living-donor liver transplant recipients. Pharmacogenet Genomics 2006; 16: 119-27.

32. Thorn M, Lundgren S, Herlenius G, Ericzon BG, Loof L, Rane A. Gene expression of cytochromes P450 in liver transplants over time. Eur J Clin Pharmacol 2004; 60: 413-20.

33. Stevens JC, Hines RN, Gu C, Koukouritaki SB, Manro JR, Tandler PJ, Zaya MJ. Developmental expression of the major human hepatic CYP3A enzymes. J Pharmacol Exp Ther 2003; 307: 573-82.

34. Blake MJ, Castro L, Leeder JS, Kearns GL. Ontogeny of drug metabolizing enzymes in the neonate. Semin Fetal Neonatal Med 2005; 10: 123-38.

35. Ince I, Knibbe CA, Danhof M, de Wildt SN. Developmental changes in the expression and function of cytochrome P450 3A isoforms: evidence from in vitro and in vivo investigations. Clin Pharmacokinet 2013; 52: 333-45.

36. Sarlis NJ, Gourgiotis L. Hormonal effects on drug metabolism through the CYP system: perspectives on their potential significance in the era of pharmacogenomics. Curr Drug Targets Immune Endocr Metabol Disord 2005; 5: 439-48.

37. Shah RR, Smith RL. Addressing phenoconversion: the Achilles' heel of personalized medicine. Br J Clin Pharmacol 2015; 79: 222-40.

Figure Legends

Figure 1. (A) Tacrolimus trough concentration (C_0) on the first day after liver transplantation in the whole paediatric population. The grey zone denotes the target therapeutic range in the first days after liver paediatric transplantation. (B) Tacrolimus concentration/weight-adjusted dose ratio on the first day after liver transplantation in the whole paediatric population. The horizontal line in the middle indicates the median; the top and the bottom lines mark the 75th and 25th percentiles, respectively.

Figure 2. Tacrolimus concentration/weight-adjusted dose ratio on the first day after liver transplantation in paediatric patients who were younger and older than 6 years old. The horizontal line in the middle indicates the median; the top and the bottom lines mark the 75th and 25th percentiles, respectively. $P < 0.05$ statistically significant.

Figure 3. Tacrolimus trough concentration (C_0) on the first day after liver transplantation in paediatric patients according to the *CYP3A4/5* combined-genotype approach on whole body metabolism. The grey zone denotes the therapeutic range in the first days after liver paediatric transplantation. The horizontal line in the middle indicates the median; the top and the bottom lines mark the 75th and 25th percentiles, respectively. RM: Rapid Metabolizer; EM: Extensive Metabolizer; IM: Intermediate Metabolizer. $P < 0.05$ Statistical significant.

Figure 4. (A) Tacrolimus trough concentration (C_0) on the first day after liver transplantation in paediatric patients according to liver *CYP3A5* genotype. The grey zone denotes the therapeutic range in the first days after liver paediatric transplantation. (B) Tacrolimus concentration/weight-adjusted dose ratio on the first day after liver transplantation in paediatric patients according to liver *CYP3A5* genotype. The horizontal line in the middle indicates the median; the top and the bottom lines mark the 75th and 25th percentiles, respectively. $P < 0.05$ Statistically significant.

Table 1 Recipient and donor demographics

	N	%
Patients	67	100
Recipient gender (male/female)	33/34	49/51
Donor gender (male/female)	41/26	61/39
Recipient age at transplantation (years, mean ± SD)	4.5 ± 5.2	
Donor age (years, mean ± SD)	14 ± 12	
Weight at transplantation (kg, mean ± SD)	18.1 ± 15.7	
GRWR (median, IQR)	3.4 IQR 2.2	
Ethnicity:		
Caucasian	52	78
Unknown	15	22
Indication for liver transplantation:		
Biliary atresia	33	49
Primary liver tumour	14	21
Primary sclerosing cholangitis	6	9
Metabolic disease	4	6
Vascular malformation	3	5
Alagille syndrome	2	3
Miscellaneous	5	7
Additional therapy:		
Basiliximab	52	78
Steroids	41	61
Patients taking drugs potentially interfering with CYP3A (fluconazole and/or steroids)	50	75

GRWR: Graft-Recipient Weight Ratio; IQR: Interquartile Range; CYP: Cytochrome P450

Table 2A *CYP3A4/5* recipient and donor genotype profiles

CYP3A4/5 Rapid metabolizers

Number	17	5	1	1
CYP3A4 recipient	**1/*1*	**1/*1*	**1/*1*	**1/*1*
CYP3A5 recipient	**3/*3*	**1/*3*	**1/*1*	**3/*3*
CYP3A4 donor	**1/*1*	**1/*1*	**1/*1*	**1/*1*
CYP3A5 donor	**1/*3*	**3/*3*	**3/*3*	**1/*1*

Table 2B *CYP3A4/5* recipient and donor genotype profiles

CYP3A4/5 Extensive metabolizers

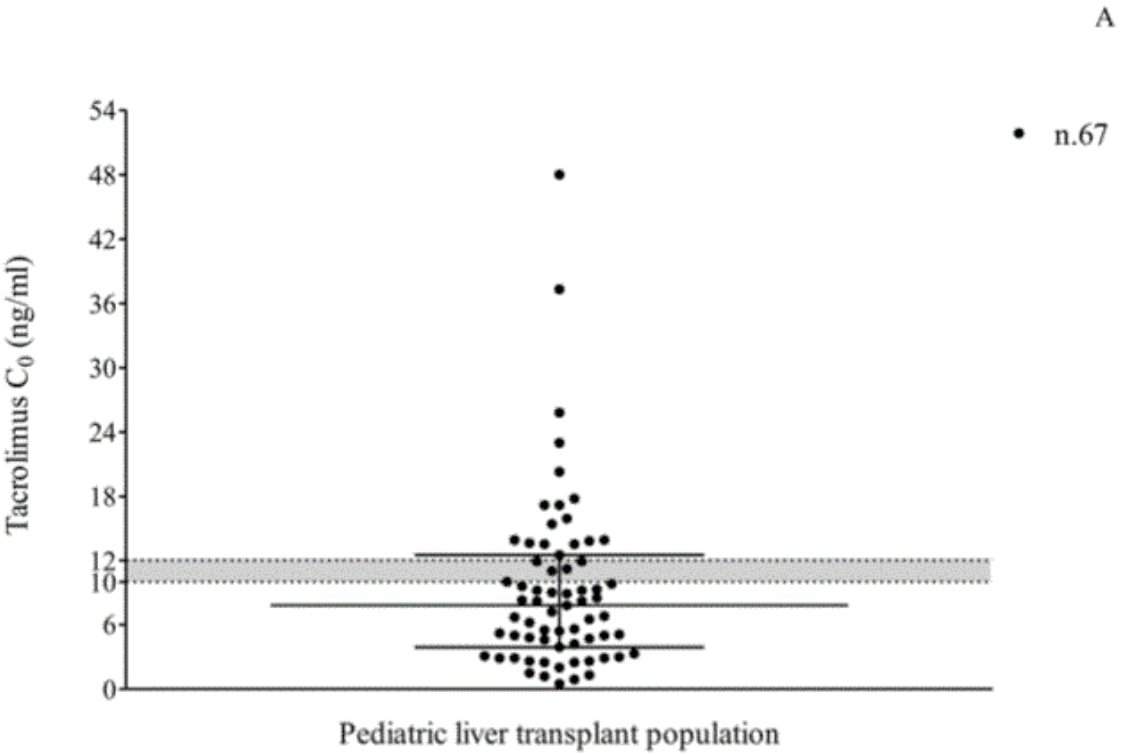
Number	24	3	2	2	1
CYP3A4 recipient	**1/*1*	**1/*22*	**1/*1*	**1/*1*	**1/*22*
CYP3A5 recipient	**3/*3*	**3/*3*	**1/*3*	**3/*3*	**1/*3*
CYP3A4 donor	**1/*1*	**1/*1*	**1/*22*	**1/*22*	**1/*1*
CYP3A5 donor	**3/*3*	**1/*3*	**3/*3*	**1/*3*	**3/*3*

Table 2C *CYP3A4/5* recipient and donor genotype profiles

CYP3A4/5 Intermediate metabolizers

Number	6	2	1	1	1
CYP3A4 recipient	**1/*1*	**22/*22*	**1/*22*	**1/*22*	**1/*1*
CYP3A5 recipient	**3/*3*	**3/*3*	**3/*3*	**3/*3*	**3/*3*
CYP3A4 donor	**1/*22*	**1/*1*	**1/*1*	**1/*22*	**22/*22*
CYP3A5 donor	**3/*3*	**3/*3*	**3/*3*	**3/*3*	**3/*3*

Figure 1


A
n.67
Tacrolimus C_0 (ng/ml)
54
48
42
36
30
24
18
12
10
6
0
Pediatric liver transplant population

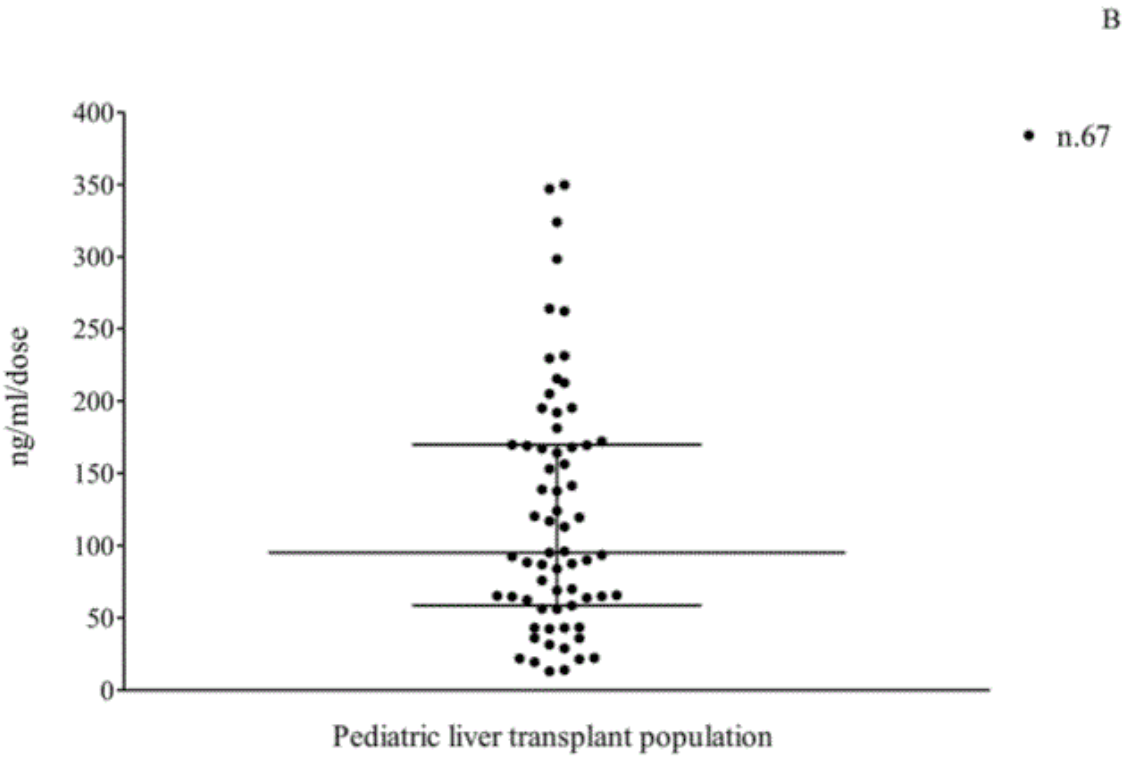

B
n.67
ng/ml/dose
400
350
300
250
200
150
100
50
0
Pediatric liver transplant population

Figure 2

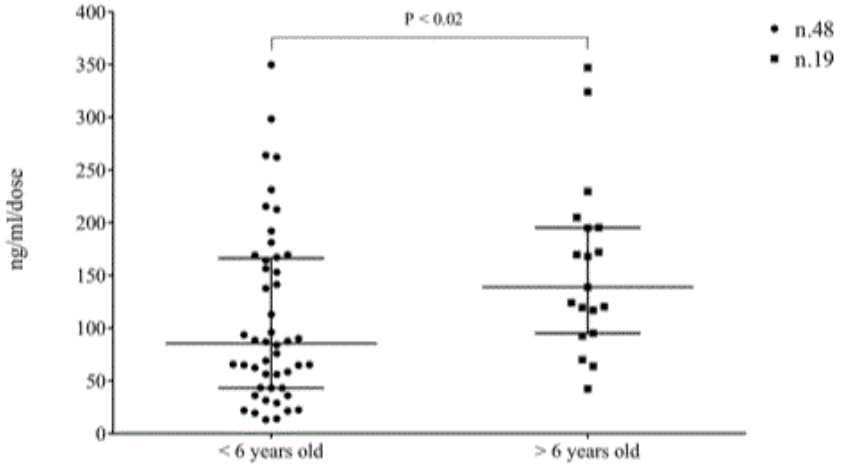

P < 0.02
n.48
n.19
ng/ml/dose
400
350
300
250
200
150
100
50
0
< 6 years old
> 6 years old

Figure 3

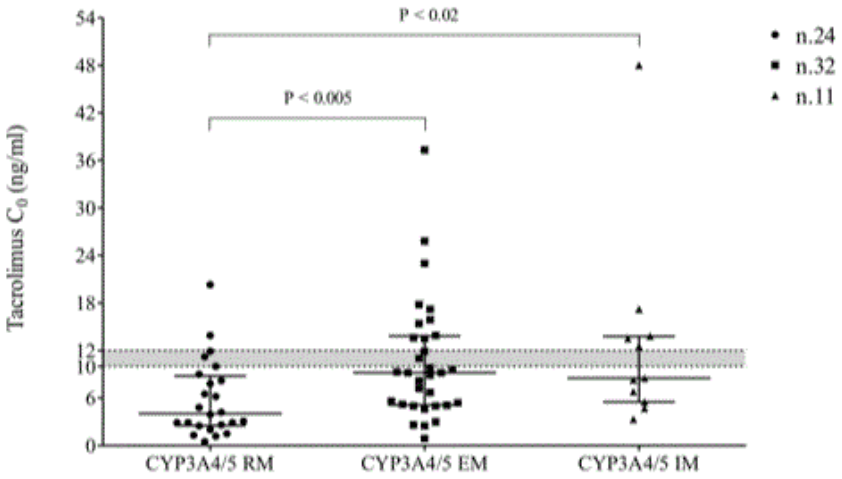
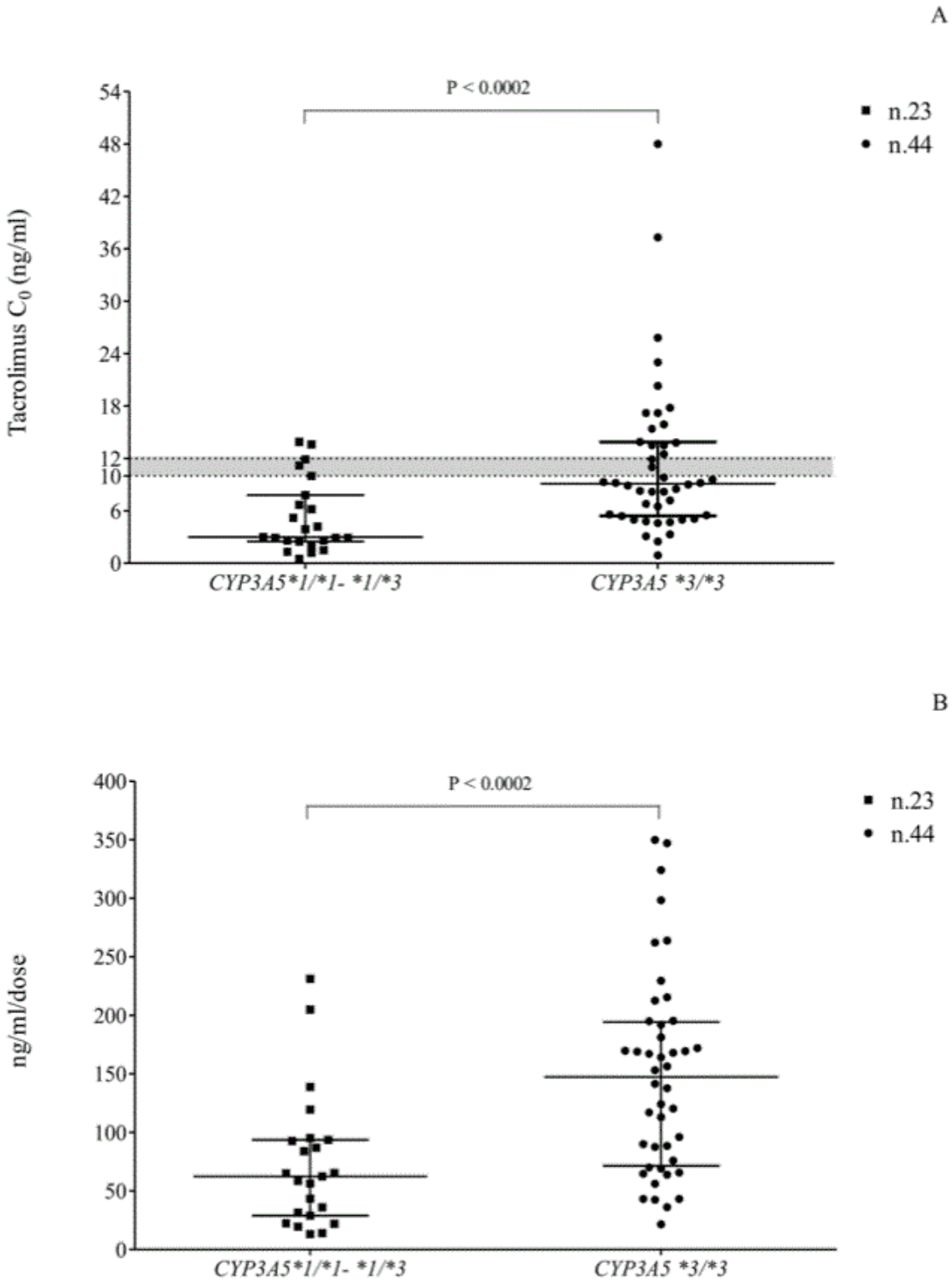

P < 0.02
P < 0.005
n.24
n.32
n.11
Tacrolimus C0 (ng/ml)
54
48
42
36
30
24
18
12
10
6
0
CYP3A4/5 RM
CYP3A4/5 EM
CYP3A4/5 IM

Figure 4


A
P < 0.0002
n.23
n.44
Tacrolimus C_0 (ng/ml)
54
48
42
36
30
24
18
12
10
6
0
CYP3A5*1/*1- *1/*3
CYP3A5 *3/*3
B
P < 0.0002
n.23
n.44
ng/ml/dose
400
350
300
250
200
150
100
50
0
CYP3A5*1/*1- *1/*3
CYP3A5 *3/*3