Haufroid Vincent (Orcid ID: 0000-0001-5040-9806)

Tacrolimus pharmacokinetics is associated with gut microbiota diversity in kidney transplant patients: results from a pilot cross-sectional study.

Alexandra L. Degraeve, PhD[1,2], Laure B. Bindels, PhD[2], Vincent Haufroid, PhD[3,4], Serge Moudio, PhD[1], Lidvine Boland, PharmD[1,3,4], Kevin-Alexandre Delongie, BSc[4], Joseph P. Dewulf, PhD[3,4,5,6], Djamila Chaib Eddour[7], Michel Mourad, PhD[7,8], Laure Elens, PhD[1,3,8,#]

[1]Department of Integrated PharmacoMetrics, PharmacoGenomics and PharmacoKinetics, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium.

[2]Metabolism and Nutrition Research Group, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium.

[3]Louvain centre for Toxicology and Applied Pharmacology, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Brussels, Belgium.

[4]Department of Clinical Chemistry, Cliniques Universitaires Saint-Luc, Brussels, Belgium.

[5]Institute of Rare Diseases, Cliniques Universitaires Saint-Luc, Brussels, Belgium.

[6]Department of Biochemistry, de Duve Institute, Université catholique de Louvain, Brussels, Belgium.

[7]Kidney and Pancreas Transplantation Unit, Cliniques Universitaires Saint-Luc, Brussels, Belgium.

[8]Contributed equally to this work

#Correspondence: Laure Elens, Université catholique de Louvain (UCLouvain). Louvain Drug Research Institute (LDRI), Integrated Pharmacometrics, pharmacoGenomics and pharmacoKinetics (PMGK). Avenue Emmanuel Mounier 72 B01.72.10, 1200 Bruxelles, Belgium, Email : laure.elens@uclouvain.be. Phone number : 0032 2 764 72 27. ORCID ID: 0000-0002-0039-3583.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/cpt.3077

Conflict of Interest

The authors declared no competing interests for this work.

Funding

This work was supported by the Fonds de la Recherche Scientifique – FNRS under Grant No F450919F. This work was also completed with the financial support of the Biocodex Microbiota Foundation and the French community of Belgium (WBI program) through FSR action (UCLouvain). A.L.D. is a PhD Research Fellow from the Fonds de la Recherche Scientifique – FNRS.

ORCID

Alexandra Degraeve : https://orcid.org/0009-0002-7388-9579

Laure Bindels : https://orcid.org/0000-0003-3747-3234

Vincent Haufroid : https://orcid.org/0000-0001-5040-9806

Serge Moudio : NA

Lidvine Boland : https://orcid.org/0000-0002-6390-829X

Kevin-Alexandre Delongie : NA

Joseph Dewulf : https://orcid.org/0000-0001-7223-2706

Djamila Chaib Eddour : NA

Michel Mourad : NA

Laure Elens : https://orcid.org/0000-0002-0039-3583

Keywords

Translational pharmacology, Pharmacokinetics, Tacrolimus, Gut microbiota, Inter-individual variability, Gut barrier, New-onset diabetes after transplantation.

Abstract

Clinical use of tacrolimus, an essential immunosuppressant following transplantation, is complexified by its high pharmacokinetic variability. The gut microbiota gains growing interest but limited investigations have evaluated its contribution to tacrolimus pharmacokinetics. Here, we explore the associations between the gut microbiota composition and tacrolimus pharmacokinetics. In this pilot cross-sectional study (Clinicaltrial.gov NCT04360031), we recruited 93 CYP3A5 non-expressers stabilized kidney transplant patients. Gut microbiota composition was characterized by 16S rRNA gene sequencing, tacrolimus pharmacokinetic parameters were computed, and additional demographical and medical covariates were collected. Associations between pharmacokinetic parameters or diabetic status and the gut microbiota composition, as reflected by α- and β-diversity metrics, were evaluated. Patients with higher tacrolimus AUC/(dose/kg) had higher bacterial richness, and tacrolimus pharmacokinetic parameters were associated with specific bacterial taxa (e.g., *Bilophila*) and amplicon sequence variant (ASV) (e.g., ASV 1508 and ASV 1982 [*Veillonella*/unclassified *Sporomusaceae*]; ASV 664 [unclassified *Oscillospiraceae*]). Building a multiple linear regression model showed that ASV 1508 (co-abundant with ASV 1982) and ASV 664 explained respectively 16.0% and 4.6% of the inter-individual variability in tacrolimus AUC/(dose/kg) in CYP3A5 non-expressers patients, when adjusting for haematocrit and age. *Anaerostipes* relative abundance was decreased in diabetic patients. Altogether, this pilot study revealed unprecedented links between the gut microbiota composition and diversity and tacrolimus pharmacokinetics in stable kidney transplant patients. It supports the relevance of studying the gut microbiota as an important contributor to tacrolimus pharmacokinetic variability. Elucidating the causal relationship will offer new perspectives to predict tacrolimus inter- and intra-pharmacokinetic variability.

Introduction

Kidney transplantation (KT), the gold standard solution for patients with end-stage renal disease, reduces mortality and improves the patient's health and quality of life, thereby inducing many physiological changes. Furthermore, nutritional strict restrictions related to dialysis are alleviated[1], whereas a lifelong immunosuppressive (IS) regimen is initiated to prevent graft rejection[2]. Each of these events is likely impacting the gut microbiota. The human gut microbiota refers to all microorganisms residing in the gastrointestinal tract, with bacteria being the most prevalent kingdom. Health status, nutritional habits and medications (antibiotics as well as host-targeted drugs) are important parameters known to affect its composition[3, 4], here referring to the bacterial component. Characterization of the intestinal microbiota of KT patients has boomed since 2014 when Fricke *et al.*[5] and Lee *et al.*[6] compared for the first time patients' microbiota pre- and post-KT.

Concerning the impact of IS therapy on the gut microbiota composition, there has been several clinical studies on this topic and reviewed nicely by Przybyciński *et al.*[7] and by Winichakoon *et al.*[8]. The major limitation of those studies is that they compare the gut microbiota of KT patients under IS strategy versus KT patients before transplantation or versus healthy controls. Therefore, it is not possible to differentiate the specific effect of the KT on the gut microbiota composition from the changes associated with the inherent IS therapy. Other clinical investigations have explored the gut microbiota composition to explain frequent side-effects associated with KT and IS treatment, namely diarrhoea[9], infection[10], hypertension[11], new-onset diabetes after transplantation (NODAT)[12] or the risk of graft rejection[13].

Besides, limited attention has been placed on its involvement in the pharmacokinetics (PK) of IS drugs. Importantly, intestinal bacteria are known to either bioaccumulate[14] or biotransform drugs[15] or affect the host expression of important drug-processing genes[16]. Tacrolimus (TAC), a key element in IS strategies, is characterized by a high inter- and intra-individual PK variability affecting

the long-term prognosis of KT and requiring regular therapeutic drug monitoring[17]. Progression in the understanding of the genetic component of the PK inter-individual variability (IIV) has already led to the establishment of new genotype-based dosage recommendations improving the attainment of TAC target levels (reviewed in[18]). However, approximately 50% of the PK IIV remains unexplained[19], and pharmacogenetics cannot account for the important intra-individual PK variability, a major risk factor for graft rejection[20]. Importantly, in our recent preclinical study, we have demonstrated that the gut microbiota increases TAC absorption through the downregulation of ATP Binding Cassette Subfamily B, Member 1 (*ABCB1*) intestinal expression[21]. Therefore, it seems particularly interesting to characterize the role of the gut microbiota in TAC PK as it could contribute to both sources of PK variability.

In the present cross-sectional study, TAC-treated KT recipients were recruited in a stable period after engraftment to evaluate whether the gut microbiota composition associates with the observed IIV in TAC PK, not explained by the patient's genetic or pathophysiological status.

Methods

Ethics statement

The protocol of the present clinical cross-sectional study was approved the Ethical Committee of UCL Saint-Luc (unique Belgian registration number B403201941607). All patients provided written informed consent before taking part to the study. The trial was carried out in accordance with the Good Clinical Practice (GCP) as required by the following regulations: the Belgian law of 7 May 2004 regarding experiments on the human persons and the EU Directive 2001/20/EC on Clinical Trials. The study has been registered at Clinicaltrial.gov (identifier NCT04360031) before starting the recruitment.

Study design and population

This study aimed to characterize the relationship between the faecal microbiota composition and the variabilities observed in TAC PK response in KT patients. Adult KT recipients were recruited between March 2020 and June 2021 at the Cliniques Universitaires Saint-Luc (Belgium) during their routine follow-up visit. To be eligible, patients must had been transplanted since at least one year and maximum eight years and under a stabilized IS drug regimen consisting of oral TAC (Advagraf°, Astellas) administered once-a-day in combination with twice-daily mycophenolate mofetil (Cellcept°, Roche). Details of the eligibility criteria and sample collection are provided in the *Supplementary Methods*.

Tacrolimus assay

Blood quantification of TAC was performed on a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method, as fully described in the *Supplementary Methods*.

Pharmacokinetic analysis

For all patients, TAC daily doses were retrieved from their medical record and adjusted on a kilogram of body weight basis (dose/kg). TAC trough concentrations (C_0) were determined for all patients from blood sample collected during a routine visit. For some patients that agreed to stay longer, additional time points were collected at 1h and/or 3h after TAC dose intake. Area under the curve (AUC) from 0 to 24h were estimated for each patient using a machine-learning algorithm developed by *Woillard et al.*[22]. When a PK point was missing, the individual value was substituted by the median of the whole cohort. Dose-adjusted C_0 and AUC were calculated by dividing them by the corresponding dose/kg (C_0/(dose/kg) and AUC/(dose/kg), respectively).

Genotyping

A description of genotyping methods is given in the *Supplementary Methods*.

Gut microbiota analysis

Genomic DNA was extracted from feces following the protocol Q described by Costea *et al*.[23]. Absolute quantification of the total bacteria was performed by quantitative PCR using the primers Bacteria Universal P338f (ACTCCTACGGGAGGCAGCAG) and P518r (ATTACCGCGGCTGCTGG)[24]. 16S ribosomal RNA (16S rRNA) gene sequencing and ensuing bioinformatics analyses (including amplicon sequence variant (ASV)[25] identification and α-diversity indexes calculation) were performed as previously described[26]. Full details of those sections are provided in the *Supplementary Methods*.16S rRNA gene sequencing data supporting the findings of this study are openly available in the SRA repository, at https://dataview.ncbi.nlm.nih.gov/object/PRJNA929904?reviewer=cl28pdhnt5sucu24n08gg49qap (project ID: PRJNA929904, will be made public upon acceptance).

Biochemical analysis

A description of the methods used for citrulline, and lipopolysaccharide-binding protein (LBP) quantification is given in the *Supplementary Methods*.

Data and statistical analysis

Statistical analyses were carried out using GraphPad Prism v9.1.2 for Windows (GraphPad Software, USA) and R v3.6.3. Data are presented as mean ± SD or as box-and-whiskers plots with extreme values. Concerning the microbiota data, unrarefied data were filtered to limit statistical analyses to taxa with a minimal mean relative abundance of 0.01% and a minimal prevalence of 20% (resulting in 11 phyla, 34 families, 73 genera and 799 ASV, respectively). The normality was not inspected for every taxonomic level. Therefore, non-parametric tests were used for all analyses. For univariate analyses, a *P* value ≤ 0.05 was considered statistically significant, whereas a *Q* value (i.e., *P* value adjusted for multiple testing) ≤ 0.1 was considered significant in multivariate analyses. The detailed statistical plan is given in the *Supplementary Methods*.

Results

Clinical characteristics of the patients

All KT patients had a stabilized IS treatment including TAC in combination with mycophenolate mofetil, with low dose corticosteroid, except for five patients. Table 1 describes the main characteristics of the cohort. All patients were cytochrome P450 (CYP) 3A5 non-expressers (*CYP3A5*3/*3*) per protocol. This selection was based on the observation that IIV is exacerbated in CYP3A5 non-expressers when compared to CYP3A5 expressers. Retrieving data from a previous published study[27], we found that the variance of the AUC/(dose/kg) distribution was significantly higher in the CYP3A5 non-expresser group when compared to the CYP3A5 expressers (Figure S1, P = 0.037). Furthermore, quantitatively, CYP3A5 non-expressers represent more than 70% of European KT patients in previous studies[27-29]. Therefore, we recruited only patients not expressing CYP3A5 to remove the variability in our PK data that would be explained by the genetics (and *de facto* not be explained by the gut microbiota composition).

Bacterial composition overview

The faecal microbiota composition of the patients was characterized using 16S rRNA gene sequencing (Figure 1, S2, S3). At the highest taxonomic level, the *Firmicutes* and *Bacteroidetes* were the more abundant intestinal phyla, consistent to what has been previously described for the healthy human population[4, 30]. Among the different phyla, the *Verrucomicrobia* showed the broadest inter-patient variability (relative abundance: mean 1.86%, ranging from 0 to 19.4%). 13 *Firmicutes*, 3 *Bacteroidetes*, 2 *Actinobacteria* and 1 *Proteobacteria* composed the core microbiota, corresponding to the genera shared by at least 95% of samples. A similar core microbiota was observed by Falony *et al*.[4] in the Flemish Gut Flora Project population, a cohort of healthy human adults originating from the same geographical area (Belgium) as our population.

TAC PK parameters distribution

For all patients, three TAC PK parameters were determined based on their medical record and TAC blood dosages: the dose/kg (mean 0.0625 mg/kg, ranging from 0.0214 to 0.1754 mg/kg) reflecting the intestinal exposure to the drug, the C_0/(dose/kg) (mean 111.6 ng/ml per mg/kg, ranging from 30.2 to 285.9 ng/ml per mg/kg) and the AUC/(dose/kg) (mean 3975 ng.h/mL per mg/kg, ranging from 1214 to 9567 ng.h/mL per mg/kg) reflecting the drug whole blood exposure for a specified dose. To evaluate how the gut microbiota composition distinguishes patients according to their PK parameters, patients were first classified into low, medium, and high-range groups as delineated by the tertiles of the PK parameters distributions, called here "PK phenotypes" (Figure 2 A, B). TAC C_0 correlated strongly with the estimated AUC (Figure 2C, *rho* = 0.95, $P < 0.05$). Therefore, results were highly similar for both PK parameters. We thus presented analyses made with AUC/(dose/kg), with similar tendencies observed for C_0/(dose/kg).

α-diversity metrics differ between TAC PK phenotypes

First, we compared the bacterial α-diversity of patients when grouped according to their PK phenotypes. Patients with the highest TAC AUC/(dose/kg) showed significantly higher number of observed ASV ($P < 0.05$) and a tendency to an increased Chao1 index ($P = 0.056$) when compared to the low-range group, while no difference in evenness indexes were observed between groups (Figure 3). Shannon and Simpson indexes were also increasing in patients with increasing TAC AUC/(dose/kg) without reaching statistical significance for the Simpson index ($P = 0.072$). As the AUC/(dose/kg) is inversely proportional to the dose/kg, similar results were observed in the opposite direction when patients were grouped according to their dose/kg (Figure S4).

TAC PK phenotypes associate with variability in β-diversity at the ASV level

We evaluated whether patients with different PK phenotypes differ in terms of β-diversity, meaning the bacterial diversity between samples. We performed principal component analysis (PCA) and computed permutational multivariate analysis of variance (PERMANOVA) at the family, genus and

ASV levels. Bacterial ASV refers to inferred individual DNA sequences of the 16S rRNA gene[25] and is often considered the equivalent to the species taxonomical level.

At family and genus levels, we observed no major shift in the bacterial composition between the different patient phenotypes whatever the PK parameter considered (Figure 4A, B, D, E). Despite no major shift in bacterial composition between low and high groups on the PCA, the permutation test reached the statistical significance at the ASV level when considering the AUC/(dose/kg) phenotype ($P < 0.05$). This PK regrouping explained 2.6% of the overall bacterial community variation (Figure 4C, G), while a similar trend was observed for the dose/kg phenotype (Figure 4F, G). The sex, haematocrit and age were also significantly correlated with the inter-individual bacterial variability but had lower explanatory power when compared to the AUC/(dose/kg) parameter (Figure 4G).

TAC PK parameters associate with bacterial taxa and ASV

Although the overall bacterial composition was significantly different between AUC/(dose/kg)-based phenotypes, differences in individual taxa were limited. Indeed, differential relative abundance analyses did not reveal any family, genus or ASV which distinguished significantly low from high-range groups after correction for multiple testing, neither for the AUC/(dose/kg) nor for the dose/kg.

Importantly, some of the collected variables are known or suspected to influence drug PK and/or the gut microbiota composition. These covariables might thus confound the analysis and mask difference between PK groups. Therefore, associations were evaluated between those variables and the PK parameters to weight whether adjusting for identified covariables can control the intra-group variability; thus, revealing significant correlations between specific taxa/ASV relative abundance and TAC PK parameters. Sex, age, self-reported race and ethnicity and haematocrit were significantly associated with both the AUC/(dose/kg) and the dose/kg, whereas other variables such as the diabetic status or tested genotypes were not (Table S1). Consequently, significant covariables were considered to refine the identification of taxa/ASV associated with

TAC PK parameters. After correcting for haematocrit, which had the most significant correlation with both PK parameters, only age remained significantly correlated. Consequently, only haematocrit and age were selected in the multivariate analyses, performed through partial Spearman rank-based correlations (pSRBC).

When adjusting for haematocrit and age, pSRBC analyses revealed strong positive correlations between the AUC/(dose/kg) and the genus *Bilophila*, the ASV 1982, ASV 1508 (both identified as a *Veillonella* or as a member of the *Sporomusaceae* family) and ASV 664 (identified as belonging to the *Oscillospiraceae* family) (Figure 5). ASV 1982, ASV 1420 and ASV 219 (identified as a *Bacteroides* or a *Phocaeicola*) were negatively correlated with the dose/kg. Table S2 fully provides the ASV taxonomic assignment. The pSRBC approach was applied on the α-diversity indexes revealing no association with neither the AUC/(dose/kg) nor the dose/kg.

Bilophila has been shown to increase gut permeability upon certain conditions[31], thus we measured two markers of the intestinal barrier function (i.e., citrulline and LBP[32]). No difference was observed neither for citrulline nor for LBP between patients of the different PK phenotypes (Figure S5). Citrulline and LBP levels were also analysed with adjustment for haematocrit and age, and no association with the PK parameters were found.

Identified ASV explain a large part of IIV in TAC AUC/(dose/kg)

Next, we aimed at estimating the percentage of IIV imputable to each variable by building a multiple linear regression model. Only the parameters previously identified as associated to the AUC/(dose/kg) were used, namely haematocrit, age, *Bilophila*, ASV 664, ASV 1508 and ASV 1982. Significant covariates retained during model building explained together 52.1% of the variability in TAC AUC/(dose/kg) of this cohort. Identified significant regressors were haematocrit, ASV 1508 (coded as present or absent), age, and ASV 664 (coded as present or absent), and they contributed respectively to 24.1%, 16.0%, 7.4% and 4.6% of the explained variability as assessed by the semi-partial r^2 (Δr^2) (Table 2). Of note, ASV 1982 and ASV 1508, both identified as a *Veillonella* or a member of the *Sporomusaceae* family, had highly correlated relative

abundances (Figure S6). Thus, only one of both ASV was retained in the final model, as the second one could not bring additional explanatory contribution. Building a similar model using continuous parameters for taxa/ASV also revealed a contribution of ASV 1508 or 1982.

We estimated quantitatively the specific effect of each of the 2 ASV retained in the model on TAC AUC/(dose/kg). When fixing haematocrit and age values to their respective mean, the presence of ASV 1508 and ASV 664 resulted in a 47.9% and 28.8% increase in the AUC/(dose/kg) value, respectively.

Anaerostipes *relative abundance is reduced in diabetic KT patients*

NODAT is one of the most frequent adverse effects associated with TAC treatment[12, 33]. In this cohort, 17 patients had diabetes at the time of inclusion, including 12 cases of NODAT. In terms of α-diversity, no difference was observed between diabetic and non-diabetic KT patients whatsoever the index considered (Figure S7). Despite no difference in overall β-diversity as evaluated by PCA and PERMANOVA analyses, differential relative abundance analysis identified one genus, *Anaerostipes*, with a significantly reduced relative abundance in diabetic versus non-diabetic KT patients (Figure S8). The relative abundance of genera previously identified as significantly affected by the diabetic status in KT patients (i.e., *Akkermansia*, *Faecalibacterium* and *Lactobacillus*)[12] was not different between the two groups.

Discussion

In the last decade, the gut microbiota has gained increasing attention as a factor modulating the graft outcome and the occurrence of associated adverse events in KT[7]. Recent advances provided robust evidence that the gut microbiota is involved in TAC PK through the regulation of *ABCB1* intestinal expression[21]. Therefore, in the present pilot study, we evaluated, in a cohort of ambulatory stable KT patients, whether the gut microbiota composition associates with the observed IIV in TAC PK.

In meta-analyses of large human cohorts, drugs are consistently associated with variation in intestinal microbial communities[4, 34]. In KT, not only TAC but well the entire IS drug regimen might affect the gut microbiota composition. Isolating the specific effect of TAC on the gut microbiota composition is highly complex in human, as it is not ethically possible to compare the same healthy individual with or without the drug. Here we compared only KT patients, all treated with a TAC-based IS therapy but with individually adjusted TAC daily dose to achieve the therapeutic target. With this design, we found that patients with a lower TAC AUC/(dose/kg) phenotype showed a lower α-diversity, mainly mediated by a decrease in richness. Our result is coherent with previous observations indicating a decreased α-diversity, evaluated through the Shannon index, in KT patients as compared to pre-KT and healthy controls[8] and suggest that TAC contributes, at least to some extent, to this reduction. Also, when evaluating the consequences of kidney transplantation on β–diversity, many previous clinical studies reported an increase in *Proteobacteria* in KT patients as compared to pre-KT and healthy controls (e.g. 13% in KT patients vs 5% in healthy controls in[35]) whatsoever the time post-KT[8]. In our cohort, the relative abundance of this phylum is on average 7% but, even if not strictly comparable, it is not associated with the investigated TAC PK parameters. Our data indirectly indicate that the rise in *Proteobacteria* observed after KT is unlikely a direct and specific result of TAC administration or dose escalation but is probably due to the transplantation itself or the associated cares.

Besides, although not observed at the family and genus levels, a shift in microbiota composition was observed at the lowest analysed level, namely the ASV level, between the different phenotypes. Coherently, most of the associations between bacteria and PK parameters involved ASV. Two of these ASV were retained in the final multiple linear regression model which explained 52.1% of IIV in TAC AUC/(dose/kg). For a similar blood exposure, the required dose/kg is lower in presence of ASV 1508 and ASV 664.

Even if commonly accepted to normalize the required TAC dose, contrarily to genetic association studies, the inclusion of the body weight in the calculation of this TAC PK parameter might

constitutes a statistical bias in the case of gut microbiota associations. Indeed, the body weight has been previously associated to human gut microbiota composition[4, 30, 36]. Nevertheless, applying a similar multiple linear regression model approach to explain IIV in TAC dose (mg) still identified ASV 1508 and ASV 664 as significant regressors. However, the explained variability was lower as they contributed respectively to 8.6% and 3.2% of the explained variability in TAC dose (Table S3).

Previously, 45% of IIV in TAC PK parameters were attributed to *CYP3A5*3* genotypes[19]. Here, focusing on CYP3A5 non-expressers exclusively, ASV 1508 and ASV 664 explained together between 11.8% and 20.6% of the remaining 55% of unexplained IIV in TAC PK, depending on the considered PK parameter (AUC/(dose/kg) or dose (mg)). Thus, these two ASV could explain between 6.5% and 11.3% of the overall TAC PK IIV. This suggests that these bacteria could be complementary to genotypes to explain PK IIV, at least in CYP3A5 non-expressers where higher IIV is observed.

To our knowledge only two clinical studies evaluated the link between TAC PK parameters and the gut microbiota composition. Consistent with our results, Lee *et al.*[37] described a positive correlation between the relative abundance of *Faecalibacterium prausnitzii* in the first week post-KT and the future TAC dosing at one-month. However, in our study and in theirs, correction for both age and haematocrit (or haemoglobin in their case) removes the significance of the association. As they analysed only taxa with a relative abundance above 2%, they might have missed changes in less abundant bacteria, such as *Bilophila* identified here (mean relative abundance of 0.29%). More recently, Jennings *et al.*[38] reported 37 taxa enriched in the high-dose group (meaning above the median dose/kg) (including *Subdoligranulum* genera and members of the *Lachnospiraceae* family), whereas *Bacteroides* was less abundant. When correcting for haematocrit, we showed a similar positive association between the *Lachnospiraceae* family and the dose/kg. This is the only similitude with our results. The divergences could result from differences in study design. First, Jennings *et al*.[38] studied heart transplant recipients and not KT

patients. Additionally, these studies occurred within one to three months after transplantation, where patients were still under prophylactic therapy including the use of antibiotics[37, 38]. Finally, despite its recognised importance to predict TAC PK[18], the patient *CYP3A5* genotype status was not controlled in both studies[37, 38].

Regards to the current knowledge on TAC PK, as expected, haematocrit and age were identified as covariables, and were thus introduced as correcting factors in the pSRBC analysis. Aging induces many physiological changes affecting the different PK processes: absorption, distribution, metabolism and excretion of drugs (reviewed in[39]). Elderly KT patients are characterized by higher TAC dose- and weight-adjusted blood exposure[40, 41], notably explained by a reduced drug clearance itself resulting from the decline in liver function[42]. Therefore, age is a valuable factor integrated in some models of TAC population PK[28, 29, 43]. Additionally, the gut microbiota composition evolves with advancing age due to, among others, the deterioration of dentition (and subsequent changes in dietary habits) and the lowering of intestinal motility[44]. Overall, aging comes with decreasing bacterial stability and α-diversity and, inversely, a wider inter-individual diversity[44]. Henceforth, age, intestinal microbiota and PK processes constitute an intimately related triangle where it is complex to disentangle the specific effect of age on one from the other to explain TAC PK variability. Similarly, as TAC is extensively bound to erythrocytes, haematocrit has been already identified as an important criterion to predict TAC whole blood exposure[43], and Falony *et al.*[4] identified both red blood cell count and haemoglobin as covariates contributing to the microbiome variation in a human population. Altogether, it highlights the importance of including both age and haematocrit as contributing factors in such analyses.

We identified one genus, *Bilophila* (belonging to the order of *Deltaproteobacteria*) and 3 ASV (ASV 664, ASV 1508 and ASV 1982), that were positively associated with TAC AUC/(dose/kg) when adjusting for haematocrit and age. The species *Bilophila wadsworthia* is the only one described so far for this genus[31]. Natividad *et al.*[31] studied this bacterium in the context of high-fat diet-induced metabolic syndrome and showed that *B.wadsworthia* contributes to the high-fat diet-

induced intestinal barrier dysfunction in mice. Contrarily to this observation, we found no association between citrulline or LBP and TAC PK parameters that could support an alteration of the gut barrier function in patients with higher TAC AUC/(dose/kg). For citrulline, a concentration above 26 µM is associated with a lower risk of severe gastrointestinal graft-vs-host disease and considered as the reflect of a good enterocytic mass[45]. All patients except four had a citrulline level above this threshold, suggesting of a good gut barrier function in our cohort. ASV 1982 and ASV 1508 were confidently assigned to either the *Veillonella* genus or the *Sporomusaceae* family, and they showed a collinear abundance. Some *Sporomusaceae* members are known to ferment lactate[46] similarly to *Veillonella* that produces the short-chain fatty acids propionate and acetate from lactate[47]. Finally, the ASV 664 was only assigned down to the *Oscillospiraceae* family, whose some members are short-chain fatty acids producers[48] and involved in the bacterial synthesis of secondary bile acids[49]. Recently, we demonstrated that *ABCB1* intestinal expression is reduced by polar bacterial metabolites, thereby affecting TAC absorption. These important findings coincide with our observation that the required dose is lower when the identified taxa are present in the intestine. Both short-chain fatty acids and bile acids are abundant microbiota-associated intestinal metabolites and they have been shown to modulate ABCB1 expression[50]. Altogether, this suggests that differences in microbiota function and metabolism, rather than an alteration of the intestinal barrier, may underlie the associations between TAC AUC/(dose/kg) and the gut microbiota, possibly involving polar bacterial metabolites able to regulate *ABCB1* intestinal expression.

Recently, Lecronier *et al.*[12] identified associations between the abundance of *Lactobacillus sp*., *Akkermansia muciniphila*, and *Faecalibacterium prausnitzii* and the glycaemic status of KT patients. We did not observe difference in these three genera according to diabetic status but a reduction in *Anaerostipes* in diabetic as compared to non-diabetic patients. As they performed targeted analyses of nine bacteria/bacterial groups by qPCR, whether *Anaerostipes* levels were also affected in this cohort was not reported. The risk of NODAT increases with higher TAC blood

levels[33] but we found no association between the relative abundance of *Anaerostipes* and TAC PK parameters. Interestingly, the genus *Anaerostipes* belongs to the *Lachnospiraceae*, one of the main butyrate-producing families, and several studies have shown a decrease relative abundance of butyrogenic bacteria in diabetic patients[51]. Jiao *et al.*[52] suggested that gut microbiota contributes to TAC-induced glycaemic disorders in mice, while a butyrate-supplementation in those TAC-treated mice allowed a normalisation of the glycaemia, offering new therapeutic perspectives[52].

Our study has several limitations due to its pilot nature. First, as a cross-sectional design, we were not able to evaluate the relationship between changes in microbiota along time and TAC intra-patient PK variability. Second, not all patients provided samples for C_{1h} and C_{3h} TAC blood measurements and AUC had to be estimated by interpolating the data. Third, this study does not control for all potential confounding effects. Fourth, our cohort does not include any self-identified Black patient and, ethnicity and race were based on the patient self-identification. Consequently, the conclusions of our study regarding impact of the race and ethnicity of the participant and how it confounds our associations is limited to our study population and narrowed to the definition used by the investigators. Fifth, no validation cohorts were available to confirm our findings. Sixth, the characterization of the bacterial composition using 16S rRNA gene sequencing is often limited to the family or genus level; metagenomics (i.e., full sequencing of the bacterial metagenome) would provide a more precise taxonomic assignment of the identified bacterial units. Our results require thus further confirmation and associations found shall be fully characterized in longitudinal studies involving more patients, starting in the early period after transplantation and recording more detailed PK. Indeed, some of our patients were not able to provide the 3 PK sampling points for AUC computation. AUC estimations were thus performed by substituting the C_{1h} and/or C_{3h} missing values by the median of the distribution. This could have impacted our results as it can lead to over-/under-estimation, especially for extreme PK profiles. The resulting effect is that the magnitude of the PK variability's in AUC might have been underestimated. Our data indicates that the absolute bias in estimated AUC was on average 6.8% with values ranging from -20.4% to

31.0%. Nevertheless, as the estimated AUC and the measured C_{0h} values were highly correlated and because the associations found with the AUC/(dose/kg) were similar to those observed with C_{0h}/(dose/kg), it does not seem to have altered the observations from a qualitative point of view.

Altogether, this study revealed unprecedented links between the gut microbiota and TAC PK in KT patients. Although at this stage the causal links cannot be established, it strengthens the importance to study the gut microbiota as a contributor to TAC PK IIV. Combining a longitudinal approach with preclinical strategies will help to decipher the cause from the consequence in the associations highlighted in this work. Moreover, our work opens the door to the identification of new factors influencing TAC intra-individual PK variability. Indeed, even if *CYP3A* genotypes are undoubtedly significant predictors of TAC PK IIV, they cannot take part in intra-individual variability, unlike the gut microbiota. Finally, our work may also contribute to a wider extent to the better understanding of the role of the gut microbiota in graft rejection risk. Indeed, antibody-mediated rejection has been recently linked to changes in gut microbiota composition[13]. In this context, the main hypothesis brought forward was the impact of the gut microbiota on the host immune system, which may modulate the rejection risk. Here, with our work, we enriched the potential mechanisms linking the gut microbiota and the risk of rejection by uncovering a link between the gut microbiota and TAC PK. Modulations of TAC PK could be, beyond a modulation of the immune system, a mechanism by which the gut microbiota impeaches on risk rejection. However, graft rejection is an outcome that was not assessed in the current study. Future longitudinal study will be needed to decipher the exact mechanisms surrounding the impact of the gut microbiota on both TAC PK and the rejection risk.

Study Highlights

- What is the current knowledge on the topic?

Tacrolimus (TAC) is central for immunosuppressive (IS) strategy in kidney transplantation (KT) but presents high inter- and intra-patient pharmacokinetic (PK) variability. Contribution of the gut microbiota to TAC PK variability is underexplored in KT patients.

- What question did this study address?

The main objective of this pilot cross-sectional study was to investigate whether the gut microbiota composition associates with the observed inter-individual variability (IIV) in TAC PK, not explained by the patient's genetic or pathophysiological status.

- What does this study add to our knowledge?

KT patients with opposing pharmacokinetic phenotypes have differences in α-diversity (mainly in richness) and β-diversity. Some bacterial units, ASV 1508, ASV 1982 (identified as a *Veillonella* or a unclassified *Sporomusaceae*) and ASV 664 (assigned to the *Oscillospiraceae* family), explain a significant part of IIV in TAC dose requirement.

- How might this change clinical pharmacology or translational science?

This study identifies new associations between the gut microbiota composition and TAC PK parameters. Elucidating the causal relationship will offer new perspectives to predict TAC inter- and intra-PK variability.

Acknowledgements

The authors thank all patients for their participation in the study. We are indebted to the clinical research nurses for their help in patient recruitment and data collection. We thank Halima El-Hamdaoui, Bouazza Es Saadi, Martin Nicolas and Nadtha Panin for skilled assistance. We also thank the University of Minnesota Genomics Centre for performing the 16S rRNA gene sequencing.

Author contributions

A.L.D., L.B.B., and L.E. wrote the manuscript; L.E., L.B.B., M.M., and V.H. designed the research; A.L.D., S.M., D.C.E., and L.B. performed the research; A.L.D. and L.E. analyzed the data; V.H., K-A.D., and J.P.D. contributed analytical tools.

References

1. Sabbatini, M. *et al.* Nutritional management in renal transplant recipients: A transplant team opportunity to improve graft survival. *Nutr Metab Cardiovasc Dis*. 2019;29(4):319-24.
2. Pilch, N. A., Bowman, L. J. and Taber, D. J. Immunosuppression trends in solid organ transplantation: The future of individualization, monitoring, and management. *Pharmacotherapy*. 2021;41(1):119-31.
3. Clarke, G., Sandhu, K. V., Griffin, B. T., Dinan, T. G., Cryan, J. F. and Hyland, N. P. Gut reactions: Breaking down xenobiotic-microbiome interactions. *Pharmacol Rev*. 2019;71(2):198-224.
4. Falony, G. *et al.* Population-level analysis of gut microbiome variation. *Science*. 2016;352(6285):560-64.
5. Fricke, W. F., Maddox, C., Song, Y. and Bromberg, J. S. Human microbiota characterization in the course of renal transplantation. *Am J Transplant*. 2014;14(2):416-27.
6. Lee, J. R. *et al.* Gut microbial community structure and complications after kidney transplantation: A pilot study. *Transplantation*. 2014;98(7):697-705.
7. Przybyciński, J., Drożdżal, S., Wilk, A., Dziedziejko, V., Szumilas, K. and Pawlik, A. The effect of the gut microbiota on transplanted kidney function. *Int J Mol Sci*. 2023;24(2):1260.
8. Winichakoon, P., Chaiwarith, R., Chattipakorn, N. and Chattipakorn, S. C. Impact of gut microbiota on kidney transplantation. *Transplant Rev (Orlando)*. 2022;36(1):100668.
9. Lee, J. R. *et al.* Gut microbiota dysbiosis and diarrhea in kidney transplant recipients. *Am J Transplant*. 2019;19(2):488-500.
10. Lee, J. R. *et al.* Butyrate-producing gut bacteria and viral infections in kidney transplant recipients: A pilot study. *Transpl Infect Dis*. 2019;21(6):e13180.
11. Liu, X. Y. *et al.* Kidney microbiota dysbiosis contributes to the development of hypertension. *Gut Microbes*. 2022;14(1):2143220.
12. Lecronier, M. *et al.* Gut microbiota composition alterations are associated with the onset of diabetes in kidney transplant recipients. *PLoS One*. 2020;15(1):e0227373.
13. Li, X., Li, R., Ji, B., Zhao, L., Wang, J. and Yan, T. Integrative metagenomic and metabolomic analyses reveal the role of gut microbiota in antibody-mediated renal allograft rejection. *J Transl Med*. 2022;20(1):614.
14. Klünemann, M. *et al.* Bioaccumulation of therapeutic drugs by human gut bacteria. *Nature*. 2021;597(7877):533-38.
15. Zimmermann, M., Zimmermann-Kogadeeva, M., Wegmann, R. and Goodman, A. L. Mapping human microbiome drug metabolism by gut bacteria and their genes. *Nature*. 2019;570(7762):462-67.
16. Kuno, T., Hirayama-Kurogi, M., Ito, S. and Ohtsuki, S. Effect of intestinal flora on protein expression of drug-metabolizing enzymes and transporters in the liver and kidney of germ-free and antibiotics-treated mice. *Mol Pharm*. 2016;13(8):2691-701.
17. Brunet, M. *et al.* Therapeutic drug monitoring of tacrolimus-personalized therapy: Second consensus report. *Ther Drug Monit*. 2019;41(3):261-307.
18. Degraeve, A. L. *et al.* Predictors of tacrolimus pharmacokinetic variability: Current evidences and future perspectives. *Expert Opin Drug Metab Toxicol*. 2020;16(9):769-82.
19. Haufroid, V. *et al.* The effect of cyp3a5 and mdr1 (abcb1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. *Pharmacogenetics*. 2004;14(3):147-54.
20. Gonzales, H. M. *et al.* A comprehensive review of the impact of tacrolimus intrapatient variability on clinical outcomes in kidney transplantation. *Am J Transplant*. 2020;20(8):1969-83.
21. Degraeve, A. L. *et al.* Gut microbiome modulates tacrolimus pharmacokinetics through the transcriptional regulation of abcb1. *Microbiome*. 2023;11(138):1-20.

22. Woillard, J. B., Labriffe, M., Debord, J. and Marquet, P. Tacrolimus exposure prediction using machine learning. *Clin Pharmacol Ther*. 2021;110(2):361-69.
23. Costea, P. I. *et al.* Towards standards for human fecal sample processing in metagenomic studies. *Nat Biotechnol*. 2017;35(11):1069-76.
24. Ovreås, L., Forney, L., Daae, F. L. and Torsvik, V. Distribution of bacterioplankton in meromictic lake saelenvannet, as determined by denaturing gradient gel electrophoresis of pcr-amplified gene fragments coding for 16s rrna. *Appl Environ Microbiol*. 1997;63(9):3367-73.
25. Callahan, B. J., McMurdie, P. J. and Holmes, S. P. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME Journal*. 2017;11(12):2639-43.
26. Pötgens, S. A. *et al.* Multi-compartment metabolomics and metagenomics reveal major hepatic and intestinal disturbances in cancer cachectic mice. *J Cachexia Sarcopenia Muscle*. 2021;12(2):456-75.
27. Elens, L. *et al.* Impact of cyp3a4*22 allele on tacrolimus pharmacokinetics in early period after renal transplantation: Toward updated genotype-based dosage guidelines. *Ther Drug Monit*. 2013;35(5):608-16.
28. Andrews, L. M. *et al.* A population pharmacokinetic model to predict the individual starting dose of tacrolimus in adult renal transplant recipients. *Br J Clin Pharmacol*. 2019;85(3):601-15.
29. Lloberas, N. *et al.* The combination of cyp3a4*22 and cyp3a5*3 single-nucleotide polymorphisms determines tacrolimus dose requirement after kidney transplantation. *Pharmacogenet Genomics*. 2017;27(9):313-22.
30. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486(7402):207-14.
31. Natividad, J. M. *et al.* Bilophila wadsworthia aggravates high fat diet induced metabolic dysfunctions in mice. *Nat Commun*. 2018;9(1):2802.
32. Schoultz, I. and Keita Å, V. The intestinal barrier and current techniques for the assessment of gut permeability. *Cells*. 2020;9(8):1909-38.
33. Jouve, T., Noble, J., Rostaing, L. and Malvezzi, P. An update on the safety of tacrolimus in kidney transplant recipients, with a focus on tacrolimus minimization. *Expert Opin Drug Saf*. 2019;18(4):285-94.
34. Zhernakova, A. *et al.* Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science*. 2016;352(6285):565-69.
35. Souai, N. *et al.* Impact of the post-transplant period and lifestyle diseases on human gut microbiota in kidney graft recipients. *Microorganisms*. 2020;8(11):1724-42.
36. Crovesy, L., Masterson, D. and Rosado, E. L. Profile of the gut microbiota of adults with obesity: A systematic review. *Eur J Clin Nutr*. 2020;74(9):1251-62.
37. Lee, J. R. *et al.* Gut microbiota and tacrolimus dosing in kidney transplantation. *PLoS One*. 2015;10(3):e0122399.
38. Jennings, D. L. *et al.* Gut microbial diversity, inflammation, and oxidative stress are associated with tacrolimus dosing requirements early after heart transplantation. *PLoS One*. 2020;15(5):e0233646.
39. Krenzien, F. *et al.* A rationale for age-adapted immunosuppression in organ transplantation. *Transplantation*. 2015;99(11):2258-68.
40. Jacobson, P. A. *et al.* Lower calcineurin inhibitor doses in older compared to younger kidney transplant recipients yield similar troughs. *Am J Transplant*. 2012;12(12):3326-36.
41. David-Neto, E. *et al.* Longitudinal pharmacokinetics of tacrolimus in elderly compared with younger recipients in the first 6 months after renal transplantation. *Transplantation*. 2017;101(6):1365-72.
42. Tornatore, K. M., Attwood, K., Venuto, R. C. and Murray, B. Age associations with tacrolimus and mycophenolic acid pharmacokinetics in stable black and white kidney transplant recipients: Implications for health inequities. *Clin Transl Sci*. 2023;
43. Størset, E., Holford, N., Midtvedt, K., Bremer, S., Bergan, S. and Åsberg, A. Importance of hematocrit for a tacrolimus target concentration strategy. *Eur J Clin Pharmacol*. 2014;70(1):65-77.

44. Buford, T. W. (dis)trust your gut: The gut microbiome in age-related inflammation, health, and disease. *Microbiome*. 2017;5(1):80.
45. Hueso, T. *et al.* Association between low plasma level of citrulline before allogeneic hematopoietic cell transplantation and severe gastrointestinal graft vs host disease. *Clin Gastroenterol Hepatol*. 2018;16(6):908-17.e2.
46. Aoyagi, T. *et al.* Draft genome sequence of a novel lactate-fermenting bacterial strain of the family sporomusaceae within the class negativicutes. *Microbiol Resour Announc*. 2019;8(10):e01735-18.
47. Wicaksono, D. P., Washio, J., Abiko, Y., Domon, H. and Takahashi, N. Nitrite production from nitrate and its link with lactate metabolism in oral veillonella spp. *Appl Environ Microbiol*. 2020;86(20):e01255-20.
48. Louis, P. and Flint, H. J. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol Lett*. 2009;294(1):1-8.
49. Kim, K. H., Park, D., Jia, B., Baek, J. H., Hahn, Y. and Jeon, C. O. Identification and characterization of major bile acid 7α-dehydroxylating bacteria in the human gut. *mSystems*. 2022;7(4):e0045522.
50. Foley, S. E. *et al.* Microbial metabolites orchestrate a distinct multi-tiered regulatory network in the intestinal epithelium that directs p-glycoprotein expression. *mBio*. 2022;13(4):e0199322.
51. Qin, J. *et al.* A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*. 2012;490(7418):55-60.
52. Jiao, W. *et al.* Butyric acid normalizes hyperglycemia caused by the tacrolimus-induced gut microbiota. *Am J Transplant*. 2020;20(9):2413-24.

Figure Legends

Figure 1. Overview of the faecal microbiota composition of stable kidney transplant (KT) patients. (A) Barplots showing the relative abundance of phyla of all KT patients (n = 93) classified according to the tacrolimus dose/kg (ascending order from left to right). The far right column corresponds to the mean of the cohort. (B) Core microbiota (i.e., genera present in at least 95% of samples) of the cohort at the genus level, clustered by phylum. (C) Legend of the global figure.

Figure 2. Distribution of the tacrolimus (TAC) pharmacokinetic (PK) parameters in the cohort. (A, B) Distribution histogram of the TAC dose/kg (A) and AUC/(dose/kg) (B). Red brackets identify the *shortest half* representing the most dense 50% of the observations. The middle of the diamond identifies the mean, whereas left and right corners represent the lower and upper bounds of the 95% confidence interval of the mean, respectively. The cohort has been divided in tertiles (as indicated by P33 and P66) into low, medium and high-range PK phenotypes (blue, yellow, and orange, respectively, n = 31/group). (C) Spearman's correlation between TAC estimated individual AUC and TAC trough concentration (C_0) (n = 93).

Figure 3. α-diversity metrics differ between TAC PK phenotypes. Evolution of α-diversity metrics (in arbitrary units, a.u.) of patients divided in low, medium and high groups according to the TAC AUC/(dose/kg) phenotype (n = 31/group). *P < 0.05.

Figure 4. Tacrolimus (TAC) pharmacokinetic (PK) phenotypes associate with variability in β-diversity at the amplicon sequence variant (ASV) level. (A-F) Principal component analysis (PCA) of the taxa relative abundances in low, medium and high groups when clustered according to TAC AUC/(dose/kg) (A-C) or dose/kg (D-F) (n = 31/group). Data are presented for the family (A, D), the genus (B, E) and the ASV (C, F) levels respectively. (G) Identification of microbiome-associated variables with their respective effect size. *P < 0.05 (green).

Figure 5. Tacrolimus (TAC) pharmacokinetic (PK) parameters correlate with only few specific taxa, when correcting for haematocrit and age. Presentation of the partial Spearman's

rank-based correlation (pSRBC) analyses performed between TAC AUC/(dose/kg) or dose/kg values and the relative abundance of different bacterial taxonomic levels (family, genus and ASV) while correcting for haematocrit level and age (n = 93). Only taxa with at least one *rho* value ≤ -0.25 or ≥ 0.25 are presented, ordered by *rho* value for the AUC/(dose/kg). *Q < 0.1.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

Table 1. Main characteristics of the 93 kidney transplant (KT) patients at day of inclusion.

	GLOBAL POPULATION
Sex n (% total): Male / Female	55 (59.1%) / 38 (40.9%)
Age (years) [a]	51 ± 14 (19-78)
Self-reported race and ethnicity n (%total): Arabic Asian Caucasian Hispanic	 15 (16.1%) 6 (6.4%) 62 (66.7%) 10 (10.8%)
Body weight (kg) [a]	71.8 ± 12.5 (46.5-100.0)
BMI (kg/m^2) [a]	24.6 ± 3.6 (17.8-33.5)
Haematocrit (%) [a]	40.9 ± 5.3 (25.0-53.0)
Creatinine clearance (mg/dL) [a]	1.42 ± 0.56 (0.37-3.20)
CYP3A4*22 genotype n (% total): Homozygote CC / Heterozygote CT	87 (93.5%) / 5 (5.4%) *(1 missing data)*
ABCB1 1199 genotype n (% total): Homozygote GG / Heterozygote GA	87 (93.5%) / 5 (5.4%) *(1 missing data)*
ABCB1 3435 genotype n (% total): Homozygote TT / Homozygote CC / Heterozygote CT	11 (11.8%) / 21 (22.6%) / 60 (64.5%) *(1 missing data)*
Time from KT (months) [a]	52.9 ± 26.7 (12-96)
Primary kidney disease n (% total):	30 (32.2%)

Glomerulonephritis	26 (28.0%)
Polycystic kidney disease	8 (8.6%)
Interstitial nephritis	16 (17.2%)
Others	13 (14.0%)
Unknown	
Number of KT n (% total):	
1st	77 (82.8%)
2nd and more	16 (17.2%)
RRT prior to KT n (% total):	
Haemodialysis	60 (64.5%)
Peritoneal dialysis	11 (11.8%)
Pre-emptive	22 (23.7%)
Donor-type n (% total):	
Living-related	19 (20.4%)
Living-unrelated	9 (9.7%)
Deceased heart beating	42 (45.2%)
Deceased non-heart-beating	23 (24.7%)
Cytomegalovirus status +/- n (% total):	
D- / R-	17 (18.3%)
D- / R+	30 (32.2%)
D+ / R-	12 (12.9%)
D+ / R+	34 (36.6%)
Ischemia time (min) [a]	535.9 (25.0-1352) *(5 missing data)*
HLA mismatch n (% total):	
0 – 3	66 (71.0%)
4 – 6	17 (18.3%)
Unknown	10 (10.7%)
Antibody induction therapy n (% total):	
None	55 (59.1%)
Basiliximab	22 (23.7%)
Immunoglobulin	10 (10.8%)
Anti-thymocyte globulin	3 (3.2%)
Others	3 (3.2%)
Bristol stool scale n (% total):	
2	7 (7.5%)
3	31 (33.3%)
4	28 (30.1%)
5	17 (18.3%)
6	10 (10.8%)
Diabetic status n (% total): No / Yes	76 (81.7%) / 17 (18.3%), including 12 NODAT
Corticosteroid maintenance n (% total): No / Yes	5 (5.4%) / 88 (94.6%)
Mycophenolate mofetil daily dose (mg) n (% total):	1 (1.1%)

360	13 (14.0%)
500	4 (4.3%)
720	75 (80.6%)
1000	

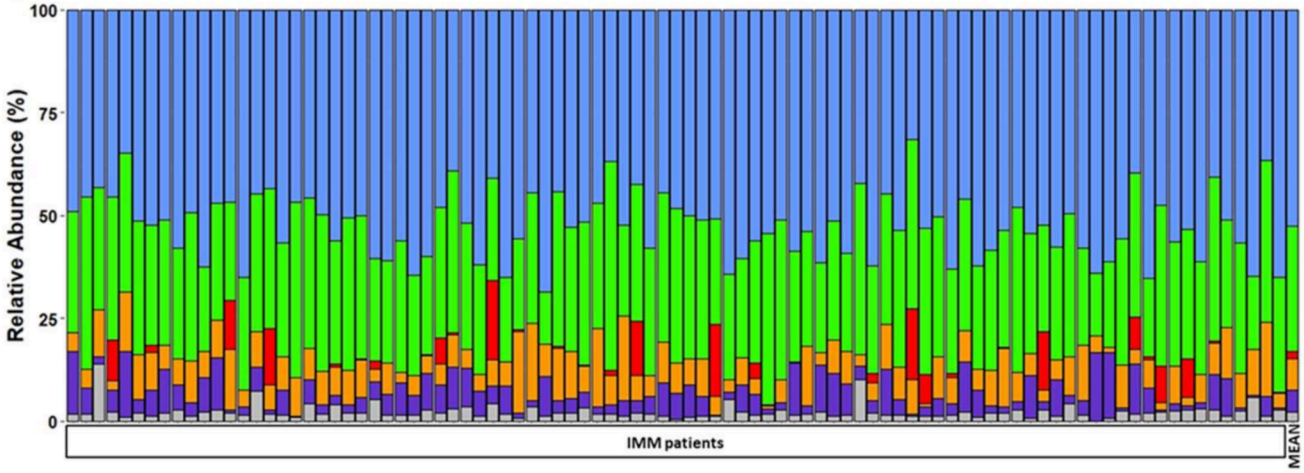
[a]Data are presented as mean ± SD, with minimal and maximal values in brackets. (RRT: renal replacement therapy ; D: donor, R: recipient ; HLA: Human Leukocyte Antigen ; NODAT: New-onset diabetes after transplantation).

Table 2. Identified amplicon sequence variants (ASV) explain a large part of inter-individual variability (IIV) in tacrolimus (TAC) AUC/(dose/kg).

Iterative model	Covariates	β (regression coefficient)	p-value of β	Δr^2	Total R^2	p-value of the model
1	Constant	2.760	* *P* < 0.001	-	24.1%	
	Haematocrit	0.019	* *P* < 0.001			
2	Constant	2.588	* *P* < 0.001	16.0%	40.1%	* *P* < 0.001
	Haematocrit	0.024	* *P* < 0.001			
	ASV 1508[a]	0.087	* *P* < 0.001			
3	Constant	2.480	* *P* < 0.001	7.4%	47.5%	* *P* < 0.001
	Haematocrit	0.021	* *P* < 0.001			
	ASV 1508[a]	0.082	* *P* < 0.001			
	Age	0.004	* *P* < 0.001			
4	Constant	2.487	* *P* < 0.001	4.6%	52.1%	* *P* < 0.001
	Haematocrit	0.022	* *P* < 0.001			
	ASV 1508[a]	0.083	* *P* < 0.001			
	Age	0.004	* *P* < 0.001			
	ASV 664[a]	0.053	* *P* < 0.005			

Summary of the stepwise inclusion of covariates during model building (n = 93). [a]: indicates that taxa were coded as absent or present.

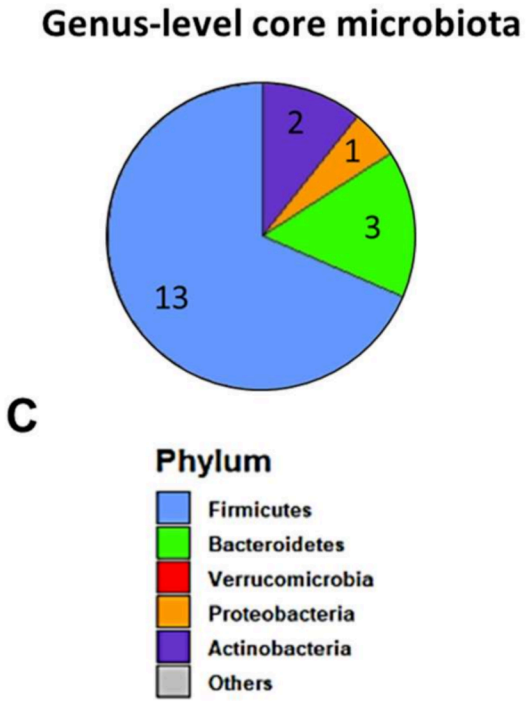
A



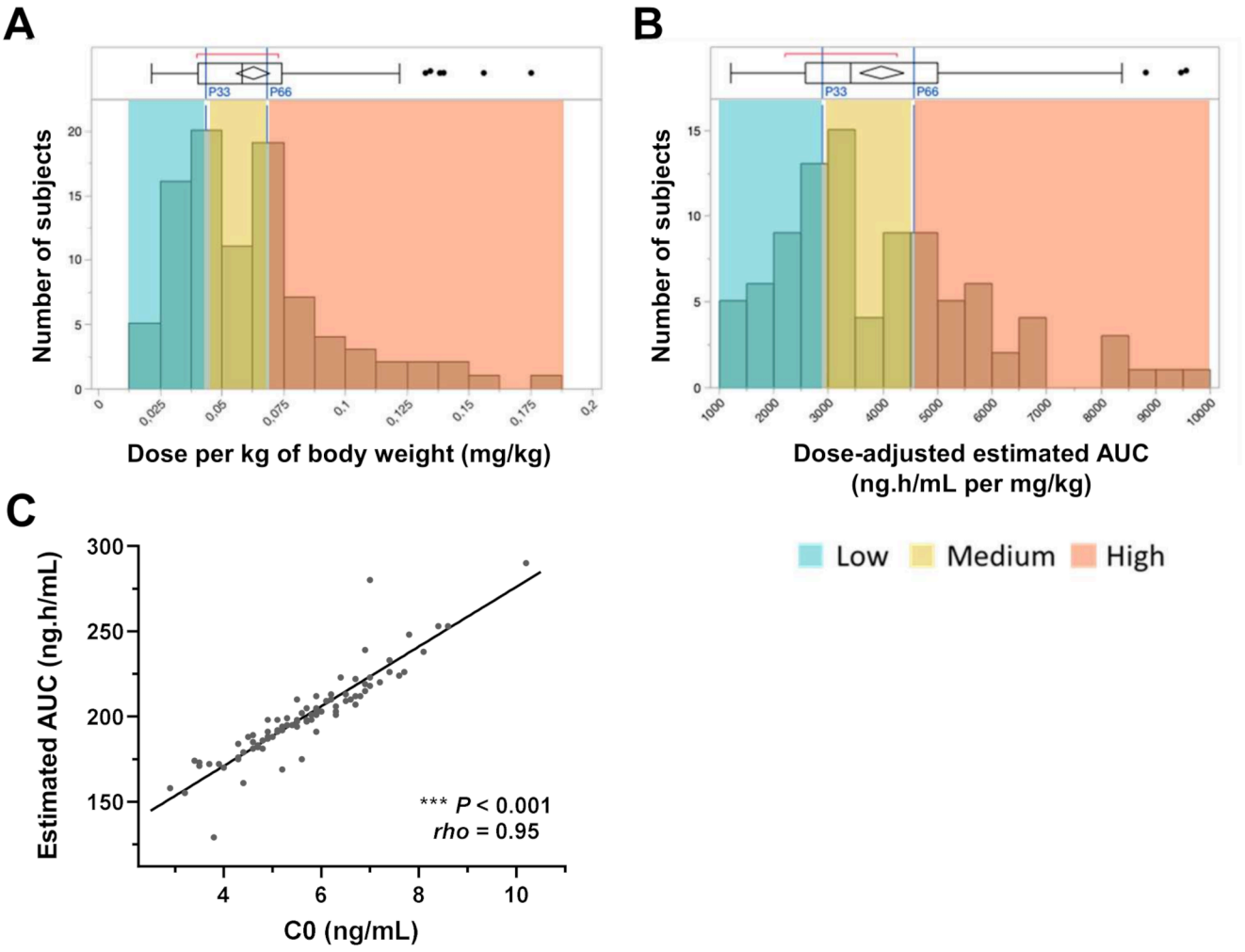
B

Genus-level core microbiota	Mean relative abundance
Anaerostipes	0.60 %
Blautia	1.91 %
Clostridium IV	0.70 %
Clostridium XIVa	0.53 %
Clostridium XVIII	0.29 %
Faecalibacterium	3.92 %
Fusicatenibacter	0.57 %
Gemmiger	1.81 %
Lachnospiracea incertae sedis	3.57 %
Oscillibacter	1.38 %
Roseburia	3.09 %
Ruminococcus 2	0.41 %
Streptococcus	1.13 %
Alistipes	2.62 %
Bacteroides	14.96 %
Parabacteroides	2.99 %
Escherichia Shigella	0.76 %
Actinomyces	0.15 %
Bifidobacterium	3.20 %
Sum:	**44.59 %**

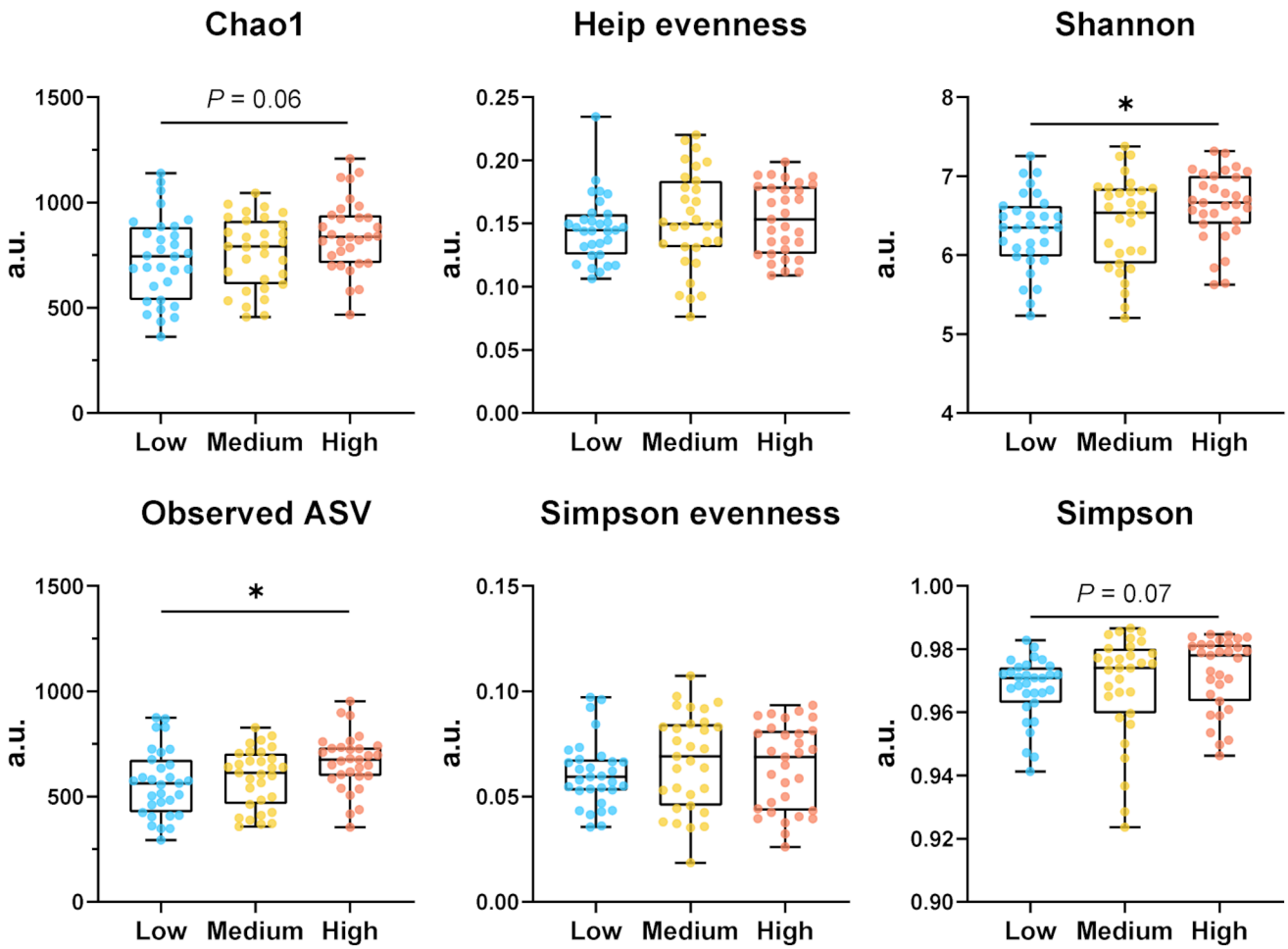
C



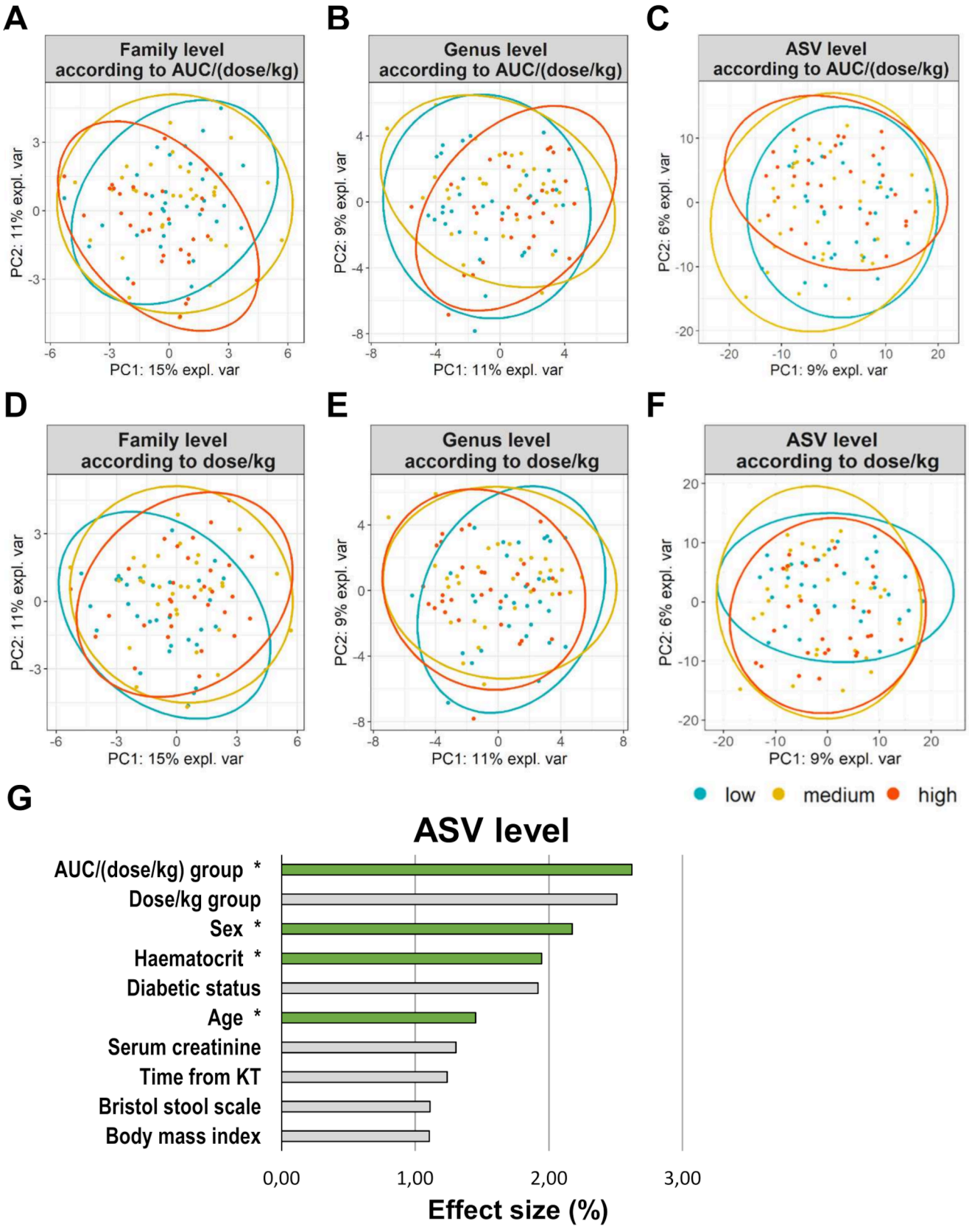
2023-0506-f01-z-.tif



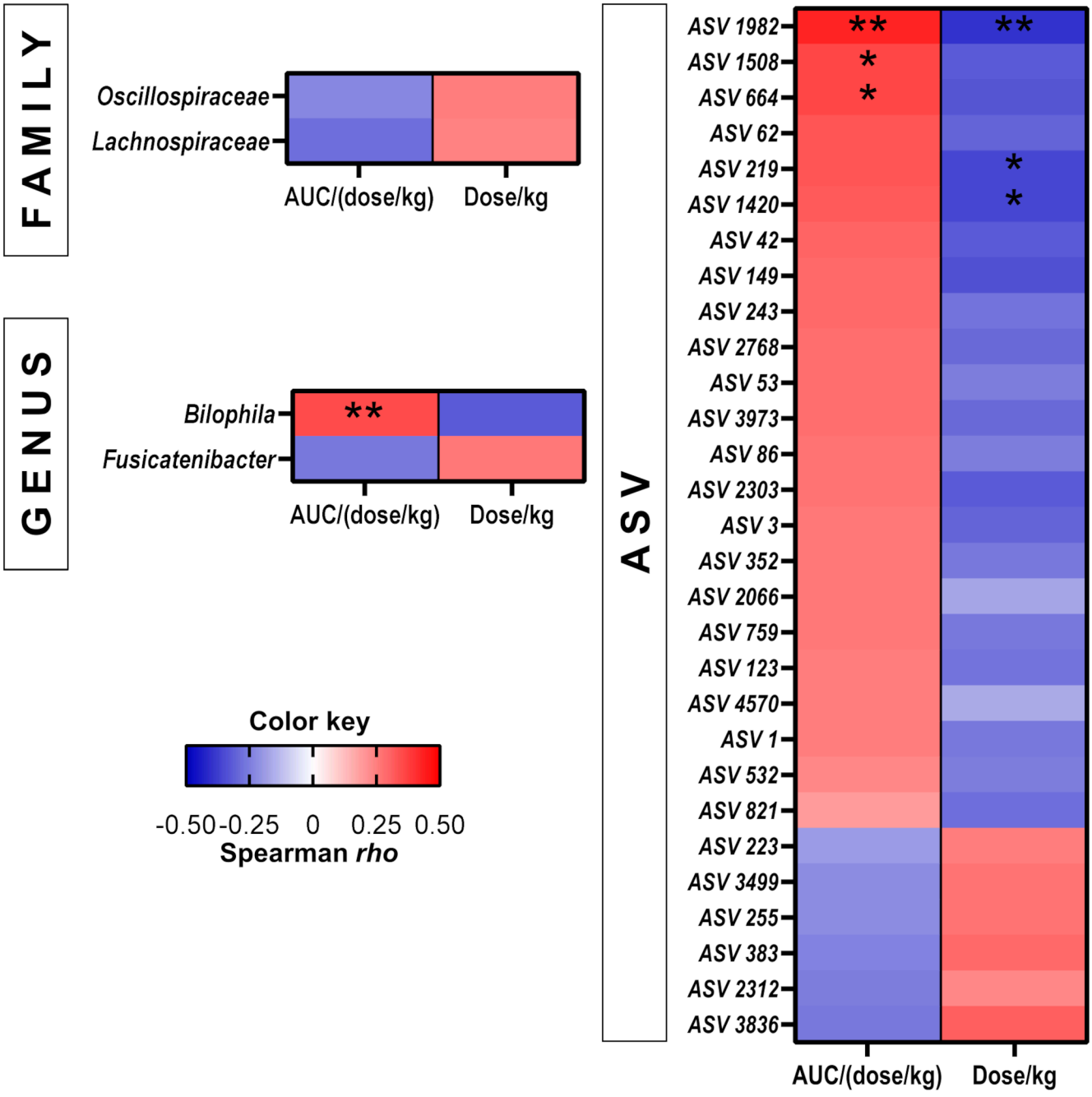
2023-0506-f02-z-.tif



2023-0506-f03-z-.tif



2023-0506-f04-z-.tif



2023-0506-f05-z-.tif