# The role of organic anion-transporting polypeptides and their common genetic variants in mycophenolic acid pharmacokinetics.

Nicolas Picard, Sook Wah Yee, Jean-Baptiste Woillard, Yvon Lebranchu, Yannick Le Meur, Kathleen M. Giacomini, Pierre Marquet

HAL Id: inserm-00415314

https://inserm.hal.science/inserm-00415314

Submitted on 7 May 2010

# The role of Organic Anion Transporting Polypeptides (OATPs) and their common genetic variants in mycophenolic acid pharmacokinetics

N Picard[1,2,3], SW Yee[4], J-B Woillard[1,3], Y Lebranchu[5], Y Le Meur[1,6], KM Giacomini[4] and P Marquet[1,2,3]

[1]INSERM, U850, Limoges, France; [2]CHU Limoges, Department of Pharmacology and Toxicology, Limoges, France; [3]Univ Limoges, Laboratory of Medical Pharmacology, Limoges, France; [4]Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, California, USA; [5]CHU Tours, Department of Nephrology, Tours, France; [6]CHU Brest, Department of Nephrology, Brest, France.

Correspondence should be addressed to N. PICARD, INSERM U850, 2 rue du Dr Marcland, 87025 Limoges, France

Tel: +33555435970; fax: +33555435936

Email. nicolas.picard@unilim.fr

**Abstract**

The goal of this study was to determine the role of the Organic Anion Transporting Polypeptides, OATP1A2, OATP1B1 and OATP1B3 and their genetic variants in the pharmacokinetics of the immunosuppressive drug mycophenolate mofetil (MMF). Using OATP-transfected HEK cells, we measured the uptake of mycophenolic acid (MPA) and its glucuronide (MPAG). MPAG, but not MPA, significantly accumulated in cells expressing OATP1B3 or OATP1B1 ($p<0.05$). MPA and MPAG pharmacokinetics were significantly influenced by the *OATP1B3* polymorphism, 334T>G/699G>A, in 70 renal transplant patients co-treated with tacrolimus or sirolimus but not in 115 patients co-treated with cyclosporine. The decrease in MPA dose-normalized exposure and concomitant increase in the MPAG/MPA metabolic ratio are consistent with reduced enterohepatic cycling in patients carrying the *OATP1B3* 334G-699A haplotype. Further studies demonstrated that this variant of OATP1B3 exhibited a reduced $V_{max}$ in transfected HEK cells, providing functional support of our clinical findings.

**Introduction**

Mycophenolic acid (MPA), the active moiety of mycophenolate mofetil (MMF), is a non-nephrotoxic, cornerstone immunosuppressant in kidney transplantation. MPA undergoes extensive entero-hepatic recirculation initiated by almost complete hepatic glucuronidation into the inactive MPA-7-O-glucuronide (MPAG) (1) and active biliary excretion of this metabolite by the Multidrug Resistance-associated Protein-2 (MRP2) (2). It is thought that, after MPAG hydrolysis by intestinal flora, the amount of MPA reabsorbed could represent 10 to 60% of total MPA exposure (1). Consequently, inhibitors of MPA entero-hepatic cycling (including cyclosporine) affect MPA levels (3, 4). Although significant associations between polymorphisms in enzymes involved in MPA glucuronidation and MMF pharmacokinetics were described, results differed depending on the post-transplantation period and comedications and could not always be replicated (see supplementary Table 1). The hepatic uptake of MPA and/or MPAG being a prerequisite for MPA entero-hepatic recirculation, it could contribute to the pharmacokinetic variability and drug-drug interactions of MPA-based formulations. There has been only one indirect hint about the implication of the Organic Anion Transporting Polypeptides (OATPs) localized to the sinusoidal membrane of the hepatocytes in the disposition of MMF. Specifically, Miura et al. reported an association between *OATP1B3* and *OATP1B1* polymorphisms and MPAG and MPA pharmacokinetics in Japanese renal transplants (5, 6).

This study aimed to determine whether MPA and MPAG are substrates of the main OATP expressed in hepatocytes (OATP1B1 and 1B3) or enterocytes (OATP1A2) and the effect of genetic variations in competent transporters on MPA pharmacokinetics in renal transplant recipients receiving MMF combined with different other immunosuppressants, taking into account other SNPs previously reported to affect MPA pharmacokinetics.

**Results**

*Cellular uptake of MPA and MPAG in OATP-expressing cells*

After 5 min incubation in cells expressing OATP1A2, 1B1 or 1B3, accumulation of MPA was not affected as compared to that observed in cells transfected with an empty vector (Figure 1a). In contrast, MPAG uptake was significantly and substantially enhanced by OATP1B3 and to a lesser extent by OATP1B1, but not by OATP1A2 (Figure 1b).

Experiments with radiolabeled model substrates confirmed the activity of each transporter (see supplementary data).

*Identification and frequency distribution of patient genotypes*

Our clinical pharmacogenetic investigation was primarily based on our aforementioned *in-vitro* results and thus aimed to assess the effect of *SLCO1B3* and *SLCO1B1* polymorphisms on MMF pharmacokinetics. In addition, patients were genotyped for polymorphisms in *UGT1A9*, *UGT1A8* and *ABCC2*, previously reported to significantly affect MPA pharmacokinetics.

SNPs to be investigated in *SLCO* genes were selected from the literature and from the SOPHIE (Study of Pharmacogenetics in Ethnically Diverse Populations) database (http://pharmacogenetics.ucsf.edu/index.html). Two frequent coding, non-synonymous SNPs in complete linkage disequilibrium were reported in *SLCO1B3* (T334G, G699A) (7). In the Caucasian group of the SOPHIE cohort one additional common non-synonymous SNP was found in *SLCO1B3* (G767C) with allele frequency of 0.18, and three in *SLCO1B1* (A388G, C463A and T521C) with allelic frequencies of 0.44, 0.16 and 0.17, respectively.

No linkage disequilibrium was observed between the *SLCO1B3* T334G and G767C SNPs. In contrast, for *SLCO1B1*, the A388G SNP showed a strong linkage disequilibrium with the

C463A (D'=1; $r^2$=0.21) and the T521C (D'=0.79; $r^2$=0.17) SNPs, while there was no linkage between the C463A and the T521C SNPs. This lead to six *SLCO1B1* haplotypes: A-C-T (0.53), G-C-C (known as *SLCO1B1\*15;* 0.15), G-C-T (0.14), G-A-T (0.13), A-C-C (0.021), and G-A-C (0.004).

All genotypes were in Hardy-Weinberg equilibrium. Their distributions were compared to those observed in the HapMap Caucasian panel (www.hapmap.org; Release 27, Feb 09), except for *UGT1A9* C-440T, T98C and *SLCO1B3* C767G, not reported in HapMap. No significant difference was found, except for *ABCC2* C-24T (p=0.01592) whose allelic distribution was however not significantly different from that reported in the literature for Caucasians (8, 9).


*Influence of comedications on MPA pharmacokinetics*

As the nature of the immunosuppressant coadministered with MMF influences MPA pharmacokinetics, this effect was evaluated before the pharmacogenetic association study. Since no difference in MMF dose (1416±528 vs 1304±478 mg; p=0.3633), MPA dose-normalized (dn) $AUC_{0-12h}$ (64.6±34.7 vs 66.0±29.7 mg.h/l/g, p=0.6401), $dn$-$C_0$ (4.4±3.5 vs 3.9±2.5 mg/l/g, p=0.6958) and $dn$-$C_{max,}$ (20.1±9.4 vs 27.2±21 mg/l/g, p=0.2674) or MPAG $dn$-$AUC_{0-9h}$ (1011±595 vs 1183±611 mg.h/l/g; p=0.1220), $dn$-$C_0$ (99±75 vs 108±59 mg/l/g, p=0.3436) and $dn$-$C_{max}$ (152±84 vs 177±95 mg/l/g, p=0.2059) was observed between patients receiving the drug in combination with sirolimus or tacrolimus, the two sub-groups of patients were combined for the pharmacogenetic study. In contrast, patients co-treated with cyclosporine were studied independently as they received significantly higher doses of MMF (1890±679mg) and had lower MPA $dn$-$AUC_{0-12h}$ (44.3±20.8 mg.h/l/g) as compared to patients on sirolimus or tacrolimus (p<0.0001).

*SLCO genotypes and MMF pharmacokinetics*

In patients receiving MMF in combination with cyclosporine (n=115), no significant association between *SLCO1B3* or *1B1* genotypes or haplotypes and MPA dn-$AUC_{0-12h}$ was found (Table 1).

In 70 patients co-treated with sirolimus or tacrolimus, MPAG dn-$AUC_{0-9h}$ showed a trend to a gradual decrease with the number of *SLCO1B3* T334 alleles (Table 2). Although not significant, important differences in MPAG dose-normalized levels were observed at all the sampling time points (Figure 2b). The metabolic ratio of MPAG to MPA showed a very significant decrease in carriers of the *SLCO1B3* T334 allele (p=0.0001) (Figure 2c).

In these patients, MPA dose-normalized exposure was significantly different depending on *SLCO1B3* T334G genotype (Table 2): the *SLCO1B3* T334 allele resulted in significantly higher MPA dose-normalized levels at every concentration-time points, except $T_{20min}$ (p=0.0636) and $T_{120min}$ (p=0.0975) (Figure 2a). Consequently, carriers of a least one *SLCO1B3* T334 allele (n=22) had 1.42-fold higher (IC95%: 1.39-1.45; p=0.0003) MPA dn-$AUC_{0-12h}$ and a 1.38-fold higher (IC95%: 1.32-1.49; p=0.0010) dn-$C_{max}$ as compared to carriers of the 334GG genotype (n=48). There was no difference in $T_{max}$ (Table 2). MPA $AUC_{4-9h}$ was studied as a surrogate for enterohepatic cycling: patients heterozygous or homozygous for the *SLCO1B3* T334 allele (n=22) had significantly higher MPA dn-$AUC_{4-9h}$ than other patients (n=48; p=0.0027). However, they also had higher dn-$AUC_{0-4h}$ (p=0.0008), resulting in unchanged $AUC_{4-9h}/AUC_{0-9h}$ ratio (p=0.4945).

The *SLCO1B3* T334 allele was associated with a similar increase in MPA dn-$AUC_{0-12h}$ (p=0.0074 and p=0.0651, respectively) and decrease in MPAG/MPA metabolic ratio (p=0.0517 and 0.0002, respectively) in the subgroups of patients co-treated with sirolimus (n=42) and tacrolimus (n=28).

There was no association between the *SLCO1B3* T334G genotype and MMF daily dose, categorized as low ($\leq$1g), intermediate (=1.5g) or standard/high ($\geq$2g) (p=0.1080). However, a significant association was observed between this genotype and MPA $AUC_{0-12h}$ categorized as below, in, or above the generally admitted therapeutic range (30-60 mg.h/l; p=0.03548). One third of the patients with the 334 TT or TG genotype (n=7/22) had MPA $AUC_{0-12h}$ higher than the admitted therapeutic range, as compared to only 8% of patients of the 334GG genotype (n=4/48). Conversely, in carriers of the 334GG genotype, 40% (n=19/48) had MPA $AUC_{0-12h}$ below the therapeutic range as compared to 23% (n=5/22) patients of the TT or TG genotype. There was no association of the second *SLCO1B3* SNP tested (G767C) with any of the exposure indices.

Despite a trend to higher MPA dn-$AUC_{0-12h}$ in heterozygous carriers of the *SLCO1B1* T521C SNP as compared to homozygous TT521 carriers (p=0.0534), this SNP was not associated with MPAG exposure (Table 2). The other *SLCO1B1* SNP tested (A388G and C463A) did not show any association with MPA or MPAG pharmacokinetics (Table 2).

Thesias® haplotype analysis revealed an association between the *SLCO1B1\*15* allele and MPA dn-$AUC_{0-12h}$ (p=0.0246) or MPAG/MPA ratio (p=0.0267), but not with MPAG dn-$AUC_{0-9h}$.

No statistical interaction between the effect of *SLCO1B3* T334G genotype and *SLCO1B1\*15* allele was found using ANOVA, but the effect of the *SLCO1B1\*15* allele did not remain significant regarding MPA dn-$AUC_{0-12h}$ (p=0.0553) and only showed a trend regarding MPAG/MPA ratio (p=0.0302, NS after Bonferroni correction). In contrast, the effect of *SLCO1B3* T334G genotype remained highly significant (p values of 0.0008 and 0.0043 for MPA dn-$AUC_{0-12h}$ and MPAG/MPA ratio, respectively).

*UGT and ABCC2 genotypes and MMF pharmacokinetics*

In patients co-treated with cyclosporine, no influence of *UGT1A8* and *1A9* polymorphisms was observed on MPA dn-$AUC_{0-12h}$ (Table 1). There was a trend towards an association between the *ABCC2* C-24T SNP and MPA dn-$AUC_{0-12h}$, however, without apparent additivity: heterozygotes for this SNP showed higher MPA dn-$AUC_{0-12h}$ than homozygotes for the reference or the variant genotype.

No significant association of *ABCC2*, *UGT1A8* or *UGT1A9* genotypes with MPAG exposure was found in patients co-treated with tacrolimus or sirolimus (Table 3). Regarding MPA pharmacokinetics, a trend to lower MPA dn-$C_{max}$ was found in two patients heterozygous for the *UGT1A9* T98C genotype as compared to patients of the reference genotype (p=0.0344; NS after Bonferroni correction). However, no association between this polymorphism and other MPA (or MPAG) exposure indices or with MPAG/MPA ratio was found (Table 3).

The *UGT1A8* C518G SNP tended to be associated with MPA dn-$C_0$ (p=0.0130), $C_{max}$ (p=0.0368) and dn-$AUC_{0-12h}$ (p=0.0171; Table 3) but not with MPAG exposure indices (Table 3) or MPAG/MPA ratio. Finally, patients homozygous for the *UGT1A9* C-440T variant genotype (n=4) had significantly lower MPA $T_{max}$ than patients heterozygous or homozygous for the reference allele, but similar MPA or MPAG exposure indices.


*Transport activity of OATP1B3 reference and variant proteins*

The effect of the *SLCO1B3* variant found to be associated with MPA clinical pharmacokinetics was investigated *in-vitro*.

The uptake of MPAG into cells expressing the OATP1B3 variant protein (i.e., 334G-699A haplotype) was markedly lower than in cells expressing the reference sequence. OATP1B3 variant protein showed a similar $K_m$ (122±109 vs 114±69.8 µM) with the reference OATP1B3 but a reduced $V_{max}$ (313±188 vs 508±205 pmol/min/mg protein, respectively) (Figure 3).

**Discussion**

This study provides direct evidence that MPAG is a substrate of the liver-specific uptake transporter OATP1B3 and that the pharmacokinetics of MPA is significantly influenced by the common *SLCO1B3* T334G polymorphism of this gene in renal transplant patients receiving MMF in a cyclosporine-free immunosuppressive regimen. Reduced transport of MPAG was observed in cells expressing this particular OATP1B3 variant, which is consistent with the trend towards a gene-dose decrease in MPAG exposure with the number of variant alleles observed in these patients. We found a significant decrease in MPA dn-$C_{max}$ and dn-$AUC_{0-12h}$ in carriers of the 334G allele. Based on our *in-vitro* findings and on the change in MPAG exposure in transplant patients, we suggest that this is secondary to reduced MPAG hepatic uptake, hence reduced re-absorption of MPA via the entero-hepatic cycling.

Entero-hepatic cycling is indeed a major feature of MPA pharmacokinetics and its contribution to MPA exposure is well-known. MPAG is extensively produced in the liver, partly excreted into the bile and substantially hydrolysed to MPA in the small intestine, leading to MPA re-absorption estimated to contribute 10 to 60% to total MPA exposure (1). It is generally thought that MPAG biliary excretion occurs just after its production in the liver. Here we suggest that, additionally, the active transport of circulating MPAG into hepatocytes substantially contributes to MPA enterohepatic cycling and then in MPA systemic exposure. Indeed, MPAG plasma concentrations are by far higher than those of MPA and this study strongly suggests that MPAG is actively taken up by the hepatocyte. The trend towards higher MPAG plasma levels in patients carrying the *SLCO1B3* G-allele is consistent with our *in-vitro* findings and supports the hypothesis that reduced OATP1B3 activity would decrease MPAG hepatic uptake. Nonetheless, the change in MPAG levels was not significant, maybe because renal elimination is the main disposition pathway for this metabolite. Thus, despite

reducing MPA re-circulation, the decrease in MPAG biliary excretion might have a limited effect on MPAG overall elimination.

We did not observe any association between this SNP and MPA exposure in 115 patients co-treated with cyclosporine, which might be due to the inhibitory effect of cyclosporine on MPA biliary excretion. Inhibition of MRP2 by cyclosporine will indeed reduce MPA recirculation, whatever the activity of OATP1B3 is. Looking at the means ± SD in the three genotype groups (Table 1) as well as to the p value obtained, it seems that the number of patients in this comedication subgroup was sufficient to exclude any clinically relevant influence of this particular SNP on MPA exposure in patients receiving cyclosporine. In contrast, increasing the number of patients cotreated with tacrolimus or sirolimus might have allowed us to investigate a potential additive gene-dose effect.

Human OATP1B3 is mainly expressed in hepatocytes where it mediates, together with OATP1B1, the uptake of a number of drugs or endogenous compounds from blood to hepatocytes (10). In this study, we showed that cell expression of either transporter resulted in the facilitated uptake of the main metabolite of MPA (MPAG). However, OATP1B1 activity with respect to MPAG was relatively modest as compared to that of OATP1B3, suggesting limited implication of OATP1B1 in MPAG uptake *in-vivo* as compared to OATP1B3. The finding that none of the OATP1B1 polymorphisms or haplotypes tested in this study was significantly and markedly associated with MPA or MPAG pharmacokinetics supports this hypothesis.

We did not observe any difference between HEK293T native and OATP-expressing cells regarding the uptake of the parent drug (MPA). We cannot totally exclude that MPA is a substrate of OATP. Indeed, passive diffusion (or transport via some unknown transporter in HEK) apparently plays a much higher role in MPA than MPAG cellular transport, with a net uptake rate approximately 3-fold higher for MPA than MPAG in HEK293T native cells

(0.021 vs 0.008 pmol/mg/min). This may mask the active transport of the drug (if any) in our cellular model.

OATP1A2 is highly expressed in the intestine, the kidney, in cholangiocytes and at the blood-brain barrier (10, 11) where it is supposed to play an important role in drug absorption and drug-drug interactions (11). We observed no difference between native and OATP1A2-expressing HEK293T cells regarding MPA or MPAG uptake, suggesting that these compounds are not substrates of OATP1A2.

The first study investigating the functional consequences of *SLCO1B3* polymorphisms showed in stably transfected MDCKII and HEK293 cells that variant OATP1B3 proteins resulting from either of the two coding non-synonymous SNPs T334G (S122A) and G699A (M233I) had similar basolateral membrane localization with the reference protein (12). They did not find differences in uptake activities between these proteins using several model substrates (Estrone-3-sulfate, bromosulfophthalein and 17-β-glucuronosyl estradiol). However, the authors did not investigate the consequence of the combined amino acid substitutions resulting from these two SNPs (in complete linkage disequilibrium). Hamada et al. found that COS-7 cells transfected with the variant *SLCO1B3* haplotype (334G-699A) had a significantly reduced ability to uptake testosterone as compared to cells transfected with the reference *SLCO1B3* gene or a sequence containing either the 334G or the 699A SNP (7). In the present study, our *SLCO1B3* plasmid integrated simultaneously the two SNPs and we obtained results in line with those of Hamada et al., with a decreased uptake of MPAG in cells transfected with the 334G-699A variant as compared to the reference sequence. Our clinical results are compatible with decreased activity of the OATP1B3 variant protein but surprisingly different from those recently published for the same drug in Japanese patients. Miura el al. described higher dn-$AUC_{6-12}$ (regarded a marker of MPA recirculation) in patients carrying the 334GG (or 699AA) genotype as compared to carriers of the 334TT genotype (6).

Based on this pharmacokinetic observation, the authors proposed that MPA uptake into hepatocytes and excretion into bile might be increased in patients with *SLCO1B3* 334GG genotype. This is in contradiction with previous (12) and the present *in-vitro* evidence that the 334G allele reduces OATP1B3 activity. Also, the present results do not support this hypothesis as MPA is not a substrate of OATP1B3 *in-vitro*, and yet we found a lower dose-normalized MPA exposure in carriers of the 334GG genotype. It should be noted that Miura et al. recently reported, in accordance with our hypothesis, that patients homozygous for the *SLCO1B3* 334G-699A allele had significantly higher MPAG dn-AUC$_{0-12}$ than patients heterozygous or homozygous for the reference allele (5).

We observed an important decrease of MPA exposure in patients receiving MMF combined with cyclosporine as compared to tacrolimus or sirolimus. This difference is well-known and is thought to result from the inhibitory effect of cyclosporine on MPAG biliary excretion. The mechanisms presumably involved in this drug-drug interaction and in the pharmacogenetic association we observed are very close. It is even tempting to hypothesise that the inhibitory effect of cyclosporine might not only concern MRP2 (as generally admitted) but also OATP1B3. Indeed, *in-vitro* studies showed that cyclosporine is a potent inhibitor of OATP1B1 and OATP1B3 (13). This inhibitory effect was associated with drug-drug interactions of clinical relevance, in particular with statins (14, 15).

Most of the pharmacogenetic studies performed regarding MMF focused on UGT enzymes or MRP2 but weak associations were mainly reported (see supplementary Table 1). In the present study, we tested the effect of SNPs previously associated with MPA pharmacokinetics using stringent criteria for statistical analysis (i.e., Bonferroni correction): none of the SNPs evaluated significantly influenced MPA pharmacokinetics, whatever the comedication. It is possible that this absence of effect, in particular for UGT1A9 SNPs previously associated *in-vitro* with a decreased enzyme activity or expression (16, 17) is related to differences in the

post-transplantation periods at which patients were studied. Association between the *UGT1A9* C-2152T, T98C or UGT1A8 C518G SNPs were indeed observed in patients studied at early post-transplantation periods (<30 days) (18-20), when MPA apparent clearance (CL/F) is known to be higher than at later time-points (21), presumably owing to several time-dependent factors such as the renal function, albumin and haemoglobin levels, decreasing cyclosporine predose levels (22) and inductive effect of corticosteroids on UGT (23).

However, this cannot explain the discrepancy with the significant influence of *UGT1A9* C-440T/T-331C on MPA dn-$AUC_{0-12h}$ found by Baldelli et al. in stable renal transplants receiving a steroid-free regimen (9).

Our study certainly has some limitations: in addition to those mentioned above, potential covariates such as serum albumin, previously associated with MPAG disposition in renal transplants (9), could not be taken in account in multivariate analyses to ponder the associations (or the absence of associations) found here, contrary to what van Shaik et al. recently did (20), because they were not available in our databases.

Apart from the association of *SLCO1B3* genotype with MPA pharmacokinetics (of which the relevance for MMF efficacy or tolerance remains to be investigated in a larger population), our study demonstrates for the first time that OATP proteins are involved in the pharmacokinetics of MPA. It gives new hints regarding the mechanism of drug-drug interactions, in particular with cyclosporine whose known effects on MPA and MPAG plasma levels are similar to those of the mutant *SLCO1B3* allele herein.

In conclusion, the pharmacokinetics of MPA in stable renal transplant patients receiving a cyclosporine-free regimen is influenced by genetic polymorphisms of the hepatic uptake transporter OATP1B3, of which MPAG is a substrate. Cyclosporine might inhibit the transporter function and thus attenuate the effect of its polymorphism.

**Methods**

*Cellular assays*

*SLCO1B3*-pcDNA5/FRT plasmids containing the reference sequence (NM_019844) or a sequence with two nucleotide exchanges corresponding to the *SLCO1B3* T334G (rs4149117) and *SLCO1B3* G699A (rs7311358) polymorphisms were constructed as described in supplementary data. *SLCO1A2*-PSG5 and *SLCO1B1*-PCMV6-XL4 plasmids were a generous gift from Theo J. Visser, Department of Internal Medicine, Erasmus University Medical Center.

Human embryonic kidney (HEK) 293T/17 cell lines (American Type Culture Collection, ATCC) were routinely cultured at 37°C with 5% $CO_2$ and 95% humidity in low-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 100U/ml penicillin, and 100μg/ml streptomycin. Cells were seeded in 10-cm dishes and transfected with 10μg of *SLCOs* cDNA (or empty vector), 20μl of lipofectamine LTX (Invitrogen, Carlsbad, CA) and 2ml of Optimem (Invitrogen). 24h after transfection, cells were trypsinized and seeded in 24-well poly-d-lysine-coated plates (BD Discovery Labware, Bedford, MA) at a density of $1.5 \times 10^5$ cells/well in order to reach confluence within 24h for uptake assays.

Transport activities were analyzed through measuring intra-cellular accumulation of MPA and MPAG. (20μM). Uptake was initiated by adding the compounds in Hank's Buffer Salt Solution (HBSS, Invitrogen) after cells had been washed twice and preincubated with HBSS at 37°C for 15 min. After 5 to 10 minutes, the cells were washed twice with ice-cold uptake buffer, lysed in 250μl of NaCl 0.2N (1 hour at 4°C) and lysates neutralized by adding 25μl of 2N HCl. MPA and MPAG were determined using a previously described validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay (3). Results were normalized

to per-well protein content as measured using the bicinchoninic acid protein assay (Pierce, Rockford, IL).

OATP1B3-mediated net uptake kinetics was studied after 10 min of incubation with MPAG, after subtracting the uptake in empty vector-transfected cells. $K_m$ and $V_{max}$ were obtained by fitting the Michaelis–Menten equation (GraphPad Software, San Diego, CA).


*Clinical pharmacogenetic analysis*

A first group of 70 kidney transplant patients enrolled in one of three phase 4 clinical trials gave their informed consent for providing blood samples for pharmacogenetic investigations related to the pharmacokinetics of MMF. 42 patients received MMF in combination with sirolimus and 28 with tacrolimus. MMF was administered at an initial fixed dose of 1g twice daily, which was reduced if necessary based on clinical criteria. Tacrolimus was administered twice daily and sirolimus once daily, targeting a whole blood trough concentration between 0.05 to 0.015 mg/l and 0.010 to 0.012 mg/l, respectively. For MMF pharmacokinetics, blood samples were collected in each patient between 3 and 6 months post-grafting at 0, 20, 40, 60, 90, 120, 180, 240, 360 and 540 min after the morning dose. MPA and MPAG plasma concentrations were determined by use of a validated LC-MS/MS method (24). Area under the concentration-time curve ($AUC_{0-12h}$) were calculated using validated Bayesian models. In the absence of an adequate model for MPAG, $AUC_{0-9h}$ were estimated using the linear trapezoidal rule. The plasma metabolic ratio of MPAG to MPA was estimated as the $AUC_{0-9h}$ ratio. There was no difference in renal function between patients receiving tacrolimus or sirolimus as assessed by comparing serum creatinine levels at the time of blood sampling (p=0.7659).

A second group of 115 kidney transplants out of 137 patients enrolled in the multicenter, open-label, randomized trial Apomygre (Clinical Trial Registry No. NCT0019967) provided

written informed consent for pharmacogenetic investigations related to MMF pharmacokinetics. These patients received the drug in combination with cyclosporine. Study aim and design, patients population and primary results were recently published (25). Blood samples were collected at the same times as in the first group at 3 or 6 months post-transplantation. MPA $AUC_{0-12h}$ were estimated using concentration at three sampling time (20, 60 and 180min) using previously published, validated Bayesian models (21, 26).

All these trials were approved by ethic committees.

There was no statistical difference in corticosteroid dose between patients co-treated with cyclosporine and tacrolimus or sirolimus at the time of blood sampling (p>0.05).

Patients' DNA was isolated from EDTA-treated blood as previously described in details (27) and was characterized for SNPs in *SLCO1B3*, *SLCO1B1*, *UGT1A8*, *UGT1A9* and *ABCC2* genes using validated TaqMan allelic discrimination assays on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Courtaboeuf, France).

*Statistical analysis*

Pairwise linkage disequilibrium between polymorphisms was evaluated by means of D' and r² (calculated using Thesias® software (28)) and patients' most probable haplotypes inferred using the PHASE program (29). Categorical variables were compared using the Fisher's exact test and continuous variables using the Kruskal–Wallis or Mann-Whitney tests, or ANOVA when studying the interaction between variables. A Bonferroni correction was applied leading to a *p* threshold for significance of 0.005 (analysis of 10 SNPs). All statistical comparisons were performed using the StatView program (SAS Institute, Cary, NC, version 5.0).

**Conflict of Interest/Disclosure:** The authors declared no conflict of interest.

**References**

(1)     Bullingham, R.E., Nicholls, A.J. & Kamm, B.R. Clinical pharmacokinetics of mycophenolate mofetil. *Clin. Pharmacokinet.* **34**, 429-455 (1998).

(2)     Hesselink, D.A. *et al.* Cyclosporine interacts with mycophenolic acid by inhibiting the multidrug resistance-associated protein 2. *Am. J. Transplant.* **5**, 987-994 (2005).

(3)     Picard, N., Premaud, A., Rousseau, A., Le Meur, Y. & Marquet, P. A comparison of the effect of ciclosporin and sirolimus on the pharmokinetics of mycophenolate in renal transplant patients. *Br. J. Clin. Pharmacol.* **62**, 477-484 (2006).

(4)     Smak Gregoor, P.J., van Gelder, T., Hesse, C.J., van der Mast, B.J., van Besouw, N.M. & Weimar, W. Mycophenolic acid plasma concentrations in kidney allograft recipients with or without cyclosporin: a cross-sectional study. *Nephrol. Dial. Transplant.* **14**, 706-708 (1999).

(5)     Miura, M. *et al.* Influence of Drug Transporters and UGT Polymorphisms on Pharmacokinetics of Phenolic glucuronide Metabolite of Mycophenolic Acid in Japanese Renal Transplant Recipients. *Ther. Drug Monit.* **30** (2008).

(6)     Miura, M. *et al.* Influence of SLCO1B1, 1B3, 2B1 and ABCC2 genetic polymorphisms on mycophenolic acid pharmacokinetics in Japanese renal transplant recipients. *Eur. J. Clin. Pharmacol.* **63**, 1161-1169 (2007).

(7)     Hamada, A. *et al.* Effect of SLCO1B3 haplotype on testosterone transport and clinical outcome in caucasian patients with androgen-independent prostatic cancer. *Clin Cancer Res.* **14**, 3312-3318 (2008).

(8)     Naesens, M., Kuypers, D.R., Verbeke, K. & Vanrenterghem, Y. Multidrug resistance protein 2 genetic polymorphisms influence mycophenolic acid exposure in renal allograft recipients. *Transplantation* **82**, 1074-1084 (2006).

(9)     Baldelli, S. *et al.* C-440T/T-331C polymorphisms in the UGT1A9 gene affect the pharmacokinetics of mycophenolic acid in kidney transplantation. *Pharmacogenomics* **8**, 1127-1141 (2007).

(10)    Niemi, M. Role of OATP transporters in the disposition of drugs. *Pharmacogenomics* **8**, 787-802 (2007).

(11)    Franke, R.M., Scherkenbach, L.A. & Sparreboom, A. Pharmacogenetics of the organic anion transporting polypeptide 1A2. *Pharmacogenomics* **10**, 339-344 (2009).

(12)    Letschert, K., Keppler, D. & Konig, J. Mutations in the SLCO1B3 gene affecting the substrate specificity of the hepatocellular uptake transporter OATP1B3 (OATP8). *Pharmacogenetics* **14**, 441-452 (2004).

(13)    Treiber, A., Schneiter, R., Hausler, S. & Stieger, B. Bosentan is a substrate of human OATP1B1 and OATP1B3: inhibition of hepatic uptake as the common mechanism of its interactions with cyclosporin A, rifampicin, and sildenafil. *Drug Metab. Dispos.* **35**, 1400-1407 (2007).

(14)    Shitara, Y., Itoh, T., Sato, H., Li, A.P. & Sugiyama, Y. Inhibition of transporter-mediated hepatic uptake as a mechanism for drug-drug interaction between cerivastatin and cyclosporin A. *J. Pharmacol. Exp. Ther.* **304**, 610-616 (2003).

(15)    Simonson, S.G. *et al.* Rosuvastatin pharmacokinetics in heart transplant recipients administered an antirejection regimen including cyclosporine. *Clin. Pharmacol. Ther.* **76**, 167-177 (2004).

(16)    Bernard, O. & Guillemette, C. The main role of UGT1A9 in the hepatic metabolism of mycophenolic acid and the effects of naturally occurring variants. *Drug Metab. Dispos.* **32**, 775-778 (2004).

(17)     Girard, H. *et al.* Identification of common polymorphisms in the promoter of the UGT1A9 gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver. *Pharmacogenetics* **14**, 501-515 (2004).

(18)     Kuypers, D.R., Naesens, M., Vermeire, S. & Vanrenterghem, Y. The impact of uridine diphosphate-glucuronosyltransferase 1A9 (UGT1A9) gene promoter region single-nucleotide polymorphisms T-275A and C-2152T on early mycophenolic acid dose-interval exposure in de novo renal allograft recipients. *Clin. Pharmacol. Ther.* **78**, 351-361 (2005).

(19)     Johnson, L.A., Oetting, W.S., Basu, S., Prausa, S., Matas, A. & Jacobson, P.A. Pharmacogenetic effect of the UGT polymorphisms on mycophenolate is modified by calcineurin inhibitors. *Eur. J. Clin. Pharmacol.* **64**, 1047-1056 (2008).

(20)     van Schaik, R. *et al.* UGT1A9 -275T>A/-2152C>T Polymorphisms Correlate With Low MPA Exposure and Acute Rejection in MMF/Tacrolimus-Treated Kidney Transplant Patients. *Clin. Pharmacol. Ther.* **86**, 319-327 (2009).

(21)     Premaud, A. *et al.* Maximum a posteriori bayesian estimation of mycophenolic acid pharmacokinetics in renal transplant recipients at different postgrafting periods. *Ther. Drug Monit.* **27**, 354-361 (2005).

(22)     van Hest, R.M., Mathot, R.A., Pescovitz, M.D., Gordon, R., Mamelok, R.D. & van Gelder, T. Explaining variability in mycophenolic acid exposure to optimize mycophenolate mofetil dosing: a population pharmacokinetic meta-analysis of mycophenolic acid in renal transplant recipients. *J. Am. Soc. Nephrol.* **17**, 871-880 (2006).

(23)     Cattaneo, D., Perico, N., Gaspari, F., Gotti, E. & Remuzzi, G. Glucocorticoids interfere with mycophenolate mofetil bioavailability in kidney transplantation. *Kidney Int.* **62**, 1060-1067 (2002).

(24)     Premaud, A., Rousseau, A., Le Meur, Y., Lachatre, G. & Marquet, P. Comparison of liquid chromatography-tandem mass spectrometry with a commercial enzyme-multiplied immunoassay for the determination of plasma MPA in renal transplant recipients and consequences for therapeutic drug monitoring. *Ther Drug Monit* **26**, 609-619 (2004).

(25)     Le Meur, Y. *et al.* Individualized mycophenolate mofetil dosing based on drug exposure significantly improves patient outcomes after renal transplantation. *Am J Transplant* **7**, 2496-2503 (2007).

(26)     Premaud, A. *et al.* A double absorption-phase model adequately describes mycophenolic acid plasma profiles in de novo renal transplant recipients given oral mycophenolate mofetil. *Clin Pharmacokinet* **44**, 837-847 (2005).

(27)     Le Meur, Y. *et al.* CYP3A5*3 influences sirolimus oral clearance in de novo and stable renal transplant recipients. *Clin Pharmacol Ther* **80**, 51-60 (2006).

(28)     Tregouet, D.A. & Tiret, L. Cox proportional hazards survival regression in haplotype-based association analysis using the Stochastic-EM algorithm. *Eur J Hum Genet* **12**, 971-974 (2004).

(29)     Stephens, M. & Donnelly, P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* **73**, 1162-1169 (2003).

**Table 1:** Mycophenolic acid (MPA) dose-normalized (dn) $AUC_{0-12h}$ according to *SLCO*, *UGT* or *ABCC2* Single Nucleotide Polymorphisms (SNPs) in patients co-treated with cyclosporine

| Gene | SNP | Patients genotypes (n) | MPA dn-$AUC_{0-12h}$ (mg.h/l)/g | P values |
|---|---|---|---|---|
| *SLCO1B3* | T334G | TT (n=6) | 42.7±18.4 | 0.4747 |
| | | TG (n=32) | 48.6±22.9 | |
| | | GG (n=76) | 42.7±20.2 | |
| | G767C | GG (n= 79) | 47.1±23.2 | 0.0469 |
| | | GC (n= 36) | 38.1±12.4 | |
| *SLCO1B1* | T521C | TT (n=75) | 44.8±20.7 | 0.7507 |
| | | TC (n=36) | 43.7±22.1 | |
| | | CC (n=4) | 38.6±10.9 | |
| | A388G | AA (n=37) | 45.5±17.1 | 0.2800 |
| | | AG (n=52) | 44.1±22.2 | |
| | | GG (n=26) | 42.8±23.3 | |
| | C463A | CC (n=83) | 43.6±18.3 | 0.9717 |
| | | CA (n=30) | 46.3±27.0 | |
| | | AA (n=2) | 42.8±25.0 | |
| *UGT1A8* | C518G | CC (n=59) | 43.7±18.9 | 0.9066 |
| | | CG (n=50) | 44.1±22.1 | |
| | | GG (n=6) | 51.4±29.3 | |
| *UGT1A9* | C-440T | CC (n=69) | 40.8±15.3 | 0.2721 |
| | | CT (n=39) | 49.2±27.1 | |
| | | TT (n=7) | 50.7±24.0 | |
| | T-275A and/or C-2152T* | TT and CC (n=101) | 44.1±21.2 | 0.4498 |
| | | TA and CT or CC (n=12) | 47.8±19.1 | |
| | | AA and TT (n=2) | 31.8±7.9 | |
| | T98C | TT (n=105) | 43.7±21.1 | 0.0634 |
| | | TC (n=10) | 50.7±17.1 | |
| *ABCC2* | C-24T | CC (n=68) | 40.2±14.5 | 0.0087 |
| | | CT (n=35) | 53.6±26.0 | |
| | | TT (n=12) | 39.9±26.7 | |

*SNPs in strong linkage disequilibrium: D'=1 ; $r^2$=0.87

**Table 2:** Pharmacokinetic parameters of mycophenolic acid (MPA) and its glucuronide (MPAG) according to *SLCO* Single Nucleotide Polymorphisms (SNPs) in patients co-treated with tacrolimus or sirolimus

| Gene | SNP | Genotypes (n) | MPA $dn\text{-}C_0$ (mg/l)/g | MPA $dn\text{-}C_{max}$ (mg/l)/g | MPA $T_{max}$ (min) | MPA $dn\text{-}AUC_{0-12h}$ (mg.h/l)/g | MPAG $dn\text{-}C_0$ (mg/l) | MPAG $dn\text{-}C_{max}$ (mg/l) | MPAG $T_{max}$ (min) | MPAG $dn\text{-}AUC_{0-9h}$ (mg.h/l) |
|---|---|---|---|---|---|---|---|---|---|---|
| *SLCO1B3* | T334G | TT (n= 2) | 3.4±0.6 | 21.4±10.5 | 65.0±35.3 | 73.9±30.2 | 79.6±42.7 | 111.6±32.8 | 90.0±42.4 | 621.7±235.3 |
| | | TG (n=20) | 5.3±2.8 | 28.6±10.7 | 71.5±111.6 | 82.6±32.6 | 91.0±51.0 | 137.4±54.0 | 114.5±112.1 | 964.8±457.6 |
| | | GG (n=48) | 3.7±3.1 | 20.6±16.7 | 69.6±86.7 | 57.5±30.2 | 108.8±74.7 | 173.7±98.7 | 123.3±75.6 | 1146.2±644.2 |
| | | *p values* | *0.0413* | ***0.0012*** | *0.8113* | ***0.0040*** | *0.6296* | *0.3664* | *0.3297* | *0.2477* |
| | | *p values* (TT+TG vs GG) | *0.0150* | ***0.0004*** | *0.8233* | ***0.0009*** | *0.3774* | *0.1947* | *0.1364* | *0.2394* |
| | G767C | GG (n=49) | 4.4±3.2 | 23.5±13.2 | 63.5±74.5 | 68.5±33.4 | 103.9±72.1 | 157.2±82.7 | 117.9±88.1 | 1042.6±524.5 |
| | | GC (n=21) | 3.7±2.7 | 21.5±19.8 | 85.2±126.0 | 57.4±30.0 | 100.9±60.2 | 171.7±101.7 | 124.3±83.5 | 1165.1±741.9 |
| | | *p values* | *0.3153* | *0.1644* | *0.9183* | *0.2234* | *0.9365* | *0.5010* | *0.6570* | *0.4769* |
| *SLCO1B1* | T521C | TT (n=47) | 3.6±2.3 | 22.1±17.0 | 64.0±75.7 | 60.0±30.0 | 112.3±76.3 | 170.0±96.7 | 116.6±84.1 | 1137.9±639.8 |
| | | TC (n=23) | 5.3±4.1 | 24.6±11.6 | 82.2±121.0 | 75.8±35.6 | 84.8±44.7 | 144.3±66.6 | 126.5±91.8 | 959.7±482.2 |
| | | *p values* | *0.0942* | *0.0902* | *0.6753* | *0.0534* | *0.1168* | *0.2685* | *0.9695* | *0.2578* |
| | A388G | AA (n=19) | 4.0±2.5 | 18.5±9.0 | 80.0±115.2 | 58.2±30.4 | 123.9±93.7 | 167.8±95.9 | 117.4±114.9 | 1079.4±545.2 |
| | | AG (n=43) | 4.0±3.3 | 23.7±16.3 | 71.6±89.9 | 65.9±33.0 | 91.0±55.7 | 156.6±87.1 | 127.4±75.5 | 1054.0±634.9 |
| | | GG (n=8) | 5.2±3.1 | 29.4±20.5 | 37.5±16.7 | 77.9±34.8 | 118.9±52.3 | 173.2±85.5 | 85.0±58.1 | 1164.6±542.6 |
| | | *p values* | *0.4849* | *0.1966* | *0.1858* | *0.2692* | *0.0883* | *0.6920* | *0.2154* | *0.5720* |
| | C463A | CC (n=52) | 3.9±2.3 | 23.3±15.1 | 74.8±106.4 | 65.7±33.0 | 96.7±70.2 | 154.2±86.1 | 119.2±90.5 | 1015.1±562.9 |
| | | CA (n=17) | 4.6±4.6 | 22.1±16.9 | 58.2±21.9 | 63.5±33.2 | 119.5±62.6 | 185.2±96.2 | 127.6±72.3 | 1291.0±672.6 |
| | | AA (n=1) | 8.6 | 18.3 | 20.0 | 62.9 | 137.0 | 145.6 | 20.0 | 824.9 |
| | | *p values* | *0.2799* | *0.7794* | *0.1598* | *0.9049* | *0.1393* | *0.3723* | *0.1249* | *0.1680* |

dn: dose-normalized

**Table 3:** Pharmacokinetic parameters of mycophenolic acid (MPA) and its glucuronide (MPAG) according to *UGT or ABCC2* Single Nucleotide

Polymorphisms (SNPs) in patients co-treated with tacrolimus or sirolimus

| Gene | SNP | Patients genotypes (n) | MPA $dn\text{-}C_0$ (mg/l)/g | MPA $dn\text{-}C_{max}$ (mg/l)/g | MPA $T_{max}$ (min) | MPA $dn\text{-}AUC_{0\text{-}12h}$ (mg.h/l)/g | MPAG $dn\text{-}C_0$ (mg/l) | MPAG $dn\text{-}C_{max}$ (mg/l) | MPAG $T_{max}$ (min) | MPAG $dn\text{-}AUC_{0\text{-}9h}$ (mg.h/l) |
|---|---|---|---|---|---|---|---|---|---|---|
| *UGT1A9* | C-440T (T-331C) | CC (n=39) | 4.1±3.7 | 22.0±18.3 | 75.6±93.8 | 63.1±35.0 | 108.5±88.3 | 171.9±104.2 | 130.0±95.1 | 1143.5±692.2 |
| | | TC (n=27) | 4.1±2.3 | 23.3±11.3 | 68.5±97.4 | 68.7±30.1 | 95.0±50.6 | 144.7±59.0 | 111.5±76.5 | 974.3±429.7 |
| | | TT (n=4) | 4.4±2.1 | 29.0±6.7 | 25.0±10.0 | 61.5±17.7 | 102.2±46.6 | 174.7±86.4 | 77.5±35.0 | 1163.1±559.7 |
| | | *p values* | *0.5049* | *0.0753* | *0.0330* | *0.5080* | *0.7807* | *0.7867* | *0.3018* | *0.6777* |
| | T-275A or C-2152T* | TT(CC) (n=63) | 4.3±3.1 | 23.3±15.8 | 71.9±97.2 | 65.7±30.4 | 105.8±69.8 | 163.4±90.4 | 118.4±88.9 | 1090.6±605.2 |
| | | TA (CT) (n=7) | 2.8±2.8 | 19.3±11.7 | 52.8±22.1 | 60.3±51.2 | 79.7±50.6 | 144.9±69.9 | 132.8±59.6 | 978.2±523.4 |
| | | *p values* | *0.0912* | *0.3325* | *0.8469* | *0.1799* | *0.5119* | *0.9143* | *0.2858* | *0.9143* |
| | T98C | TT (n=68) | 4.1±3.0 | 23.3±15.4 | 70.1±93.8 | 66.2±32.5 | 103.7±68.9 | 163.4.2±88.7 | 119.0±87.2 | 1089.2±599.4 |
| | | TC (n=2) | 6.1±6.5 | 8.9±3.1 | 65.0±35.3 | 30.9±8.7 | 78.9±38.3 | 98.0±51.0 | 150.0±42.4 | 745.6±371.3 |
| | | *p values* | *0.5671* | *0.0344* | *0.5957* | *0.0570* | *0.6764* | *0.2172* | *0.2358* | *0.4175* |
| *UGT1A8* | C518G | CC (n=40) | 4.2±3.2 | 25.4±18.1 | 69.0±94.5 | 66.8±29.6 | 105.1±75.5 | 166.8±95.4 | 109.7±73.8 | 1066.9±587.5 |
| | | CG (n=26) | 4.5±3.0 | 21.0±9.8 | 73.5±97.8 | 67.9±36.8 | 105.1±60.9 | 160.7±41.9 | 139.6±105.6 | 1040.4±643.9 |
| | | GG (n=4) | 1.1±0.4 | 10.1±6.0 | 57.5±28.7 | 30.9±8.3 | 68.4±30.5 | 114.2±41.8 | 92.5±37.7 | 807.7±263.5 |
| | | *p values* | *0.0130* | *0.0368* | *0.4694* | *0.0171* | *0.5331* | *0.5350* | *0.5786* | *0.6701* |
| *ABCC2* | C-24T | CC (n=48) | 4.1±3.2 | 22.7±17.6 | 60.4±51.6 | 64.1±33.0 | 98.4±72.5 | 158.1±93.7 | 127.1±78.0 | 683.1±299.7 |
| | | CT (n=19) | 4.7±3.0 | 23.0±9.8 | 96.8±157.5 | 67.4±33.6 | 112.8±60.1 | 165.1±75.9 | 103.15±110.05 | 1107.0±648.1 |
| | | TT (n=3) | 2.6±1.2 | 26.2±4.7 | 53.3±11.5 | 68.0±27.7 | 116.2±49.4 | 193.9±95.4 | 110.0±17.3 | 1341.8±577.8 |
| | | *p values* | *0.5066* | *0.1804* | *0.7746* | *0.8393* | *0.3411* | *0.2371* | *0.0913* | *0.3835* |

*SNPs in complete linkage disequilibrium

**Figure legends**

**Figure 1**: Mycophenolic acid (MPA; a) and mycophenolic acid phenyl-glucuronide (MPAG; b) (20µM) uptake into HEK293T cells transiently transfected with OATP1B1, 1B3 and 1A2. Empty vector (EV) transfected cells served as control. Data are mean ± SD of three independent experiments performed in triplicate. Uptake time was 5 min. (*p<0.05 for comparison with control cells).

**Figure 2**: Mean ± SEM of dose normalized concentration-time profiles of mycophenolic acid (MPA; a), MPA-phenyl glucuronide (MPAG; b) and distribution of MPA to MPAG metabolic ratio (MPAG/MPA $AUC_{0-9h}$ ratio; c) as a function *SLCO1B3* T334G genotype in patients co-treated with tacrolimus or sirolimus. In panel c, the lines represent the mean values. *p<0.05; **p<0.005

**Figure 3:** Kinetics of mycophenolic acid phenyl-glucuronide (MPAG) transport by reference (T334-G699) and variant (334G-699A OATP1B3. HEK293 cells transiently expressing either the OATP-reference or the OATP-variant proteins were incubated in the presence of increasing concentrations of MPAG for 10 min. Data were fitted to the Michaelis–Menten equation after subtracting background uptake. Data points represent the mean ±SD from n=3 experiments.
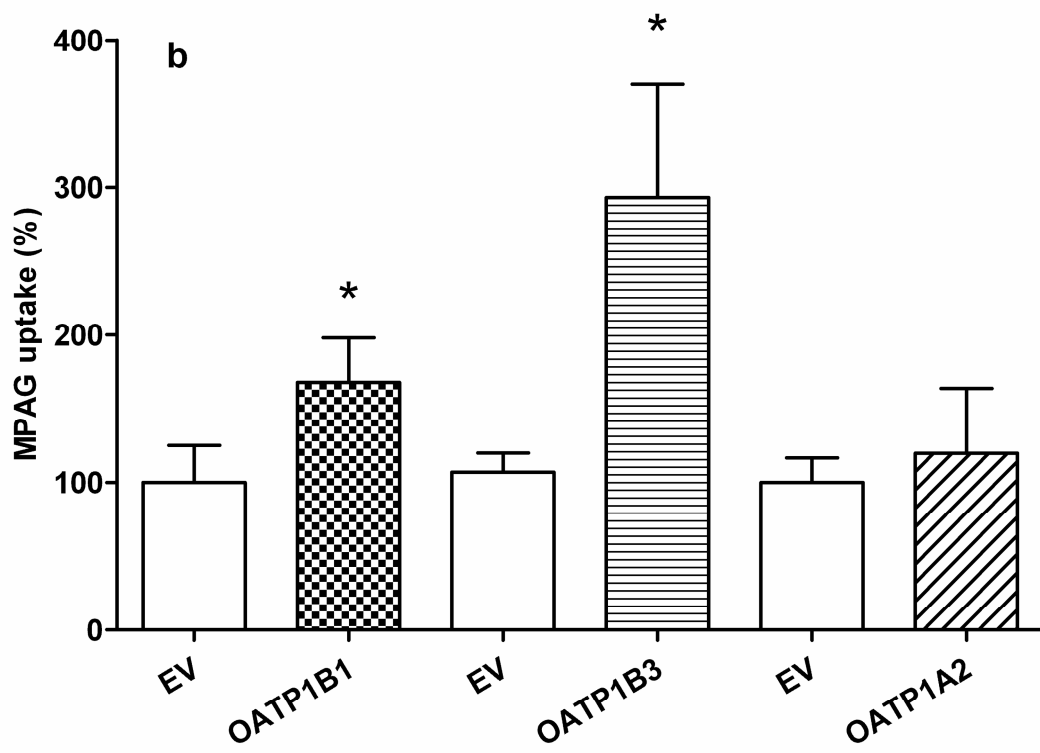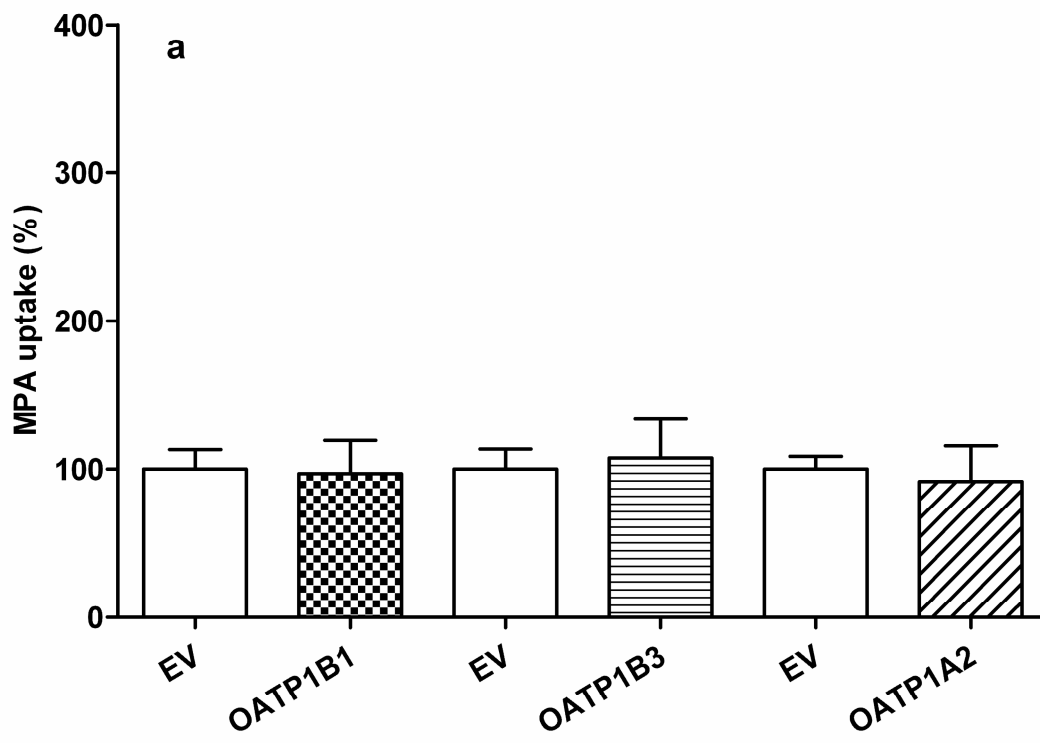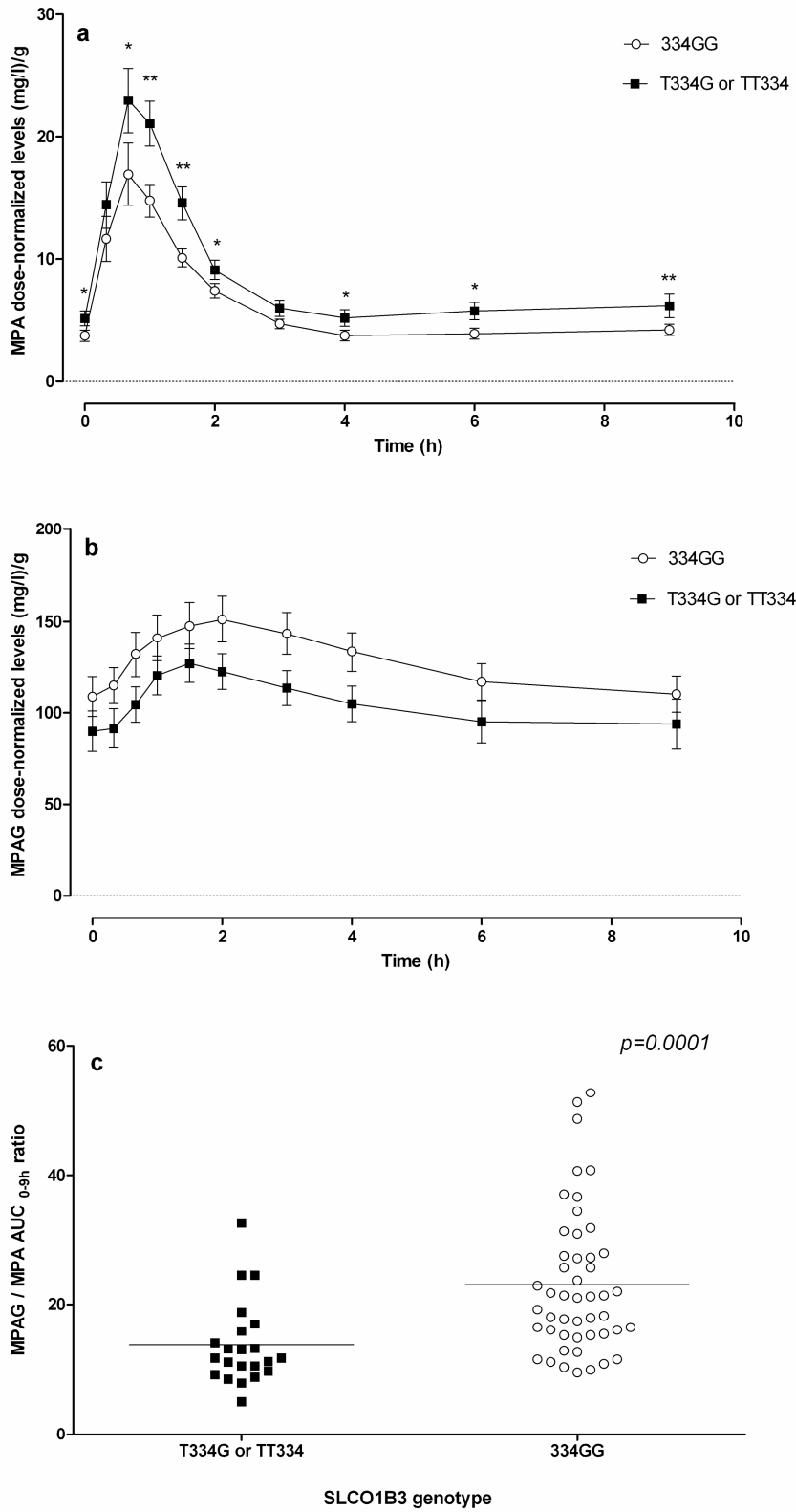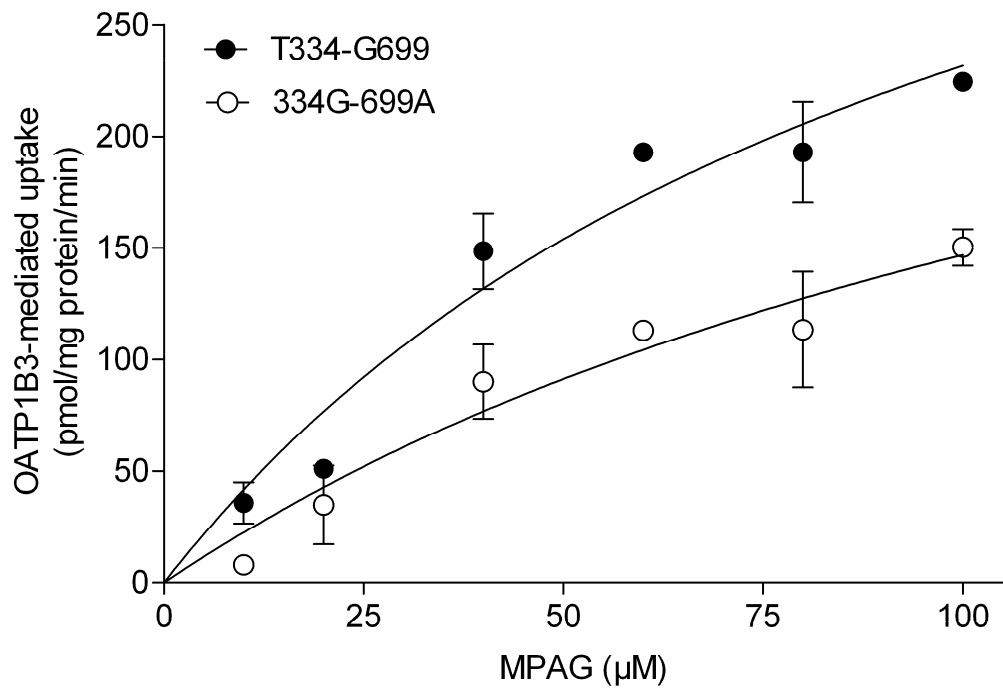
FIGURE 1

FIGURE 2

FIGURE 3