

# Steady-state pharmacokinetics of mycophenolic acid in renal transplant patients: exploratory analysis of the effects of cyclosporine, recipients' and donors' *ABCC2* gene variants, and their interactions

N. Božina<sup>1,2</sup> · Z. Lalić<sup>3</sup> · S. Nađ-Škegro<sup>4</sup> · A. Borić-Bilušić<sup>5</sup> · T. Božina<sup>6</sup> · Ž. Kaštelan<sup>7</sup> · V. Trkulja<sup>2</sup>

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## Abstract

**Purpose** The study aims to evaluate the impact of recipients' and donors' polymorphisms in multidrug resistance-associated protein 2 (MRP2) gene *ABCC2* -24C>T and 1249G>A on disposition of mycophenolic acid (MPA) and their interaction with cyclosporine (CsA) (compared to tacrolimus, TAC) in stable de novo adult renal transplant patients of Croatian origin.

**Methods** A total of 68 recipient-donor pairs were genotyped. Steady-state pharmacokinetics of MPA was assessed by the model-independent method.

**Results** Adjusted for MPA formulation, renal function, type of calcineurin inhibitor and recipients' and donors' genotypes at the two loci, donors' A-allele at 1249G>A was associated with a reduced peak (29%) and early ( $AUC_{0-2}$ , 33%) exposure and increased MPA clearance (26%). Donors' A-allele combined with CsA was associated with 78% higher MPA clearance, 49% lower early and 48% lower total exposure as compared to wild type homozygosity + TAC. Recipients' SNPs per se

did not reflect on MPA disposition. However, A-allele at 1249G>A + CsA (compared to wild type + TAC) was associated with a numerically greater increase in MPA clearance (59 vs. 41%), reduction in total exposure (36 vs. 27%) and increase in absorption rate ( $C_{max}/AUC$ ) (56 vs. 37%) than observed for the main effect of CsA. Less pronounced effects were observed for the combination of variant allele at -24C>T and CsA.

**Conclusion** Considering MPA disposition, data indicate: donors' *ABCC2* 1249G>A polymorphism increases clearance and reduces exposure; CsA increases clearance and reduces exposure by inhibiting MRP2 in the gut, the liver, and the kidney; donors' *ABCC2* 1249G>A polymorphism enhances the renal CsA effect, while recipients' polymorphism seems to enhance the liver and the gut CsA effects.

**Keywords** Renal transplantation · Immunosuppression · Mycophenolic acid · Cyclosporine · Tacrolimus · *ABCC2* protein

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✉ N. Božina  
nbozina@kbc-zagreb.hr

<sup>1</sup> Division of Pharmacogenomics and Therapy Individualization, Department of Laboratory Diagnostics, University Hospital Center Zagreb, Kišpatičeva 12, 10000 Zagreb, Croatia

<sup>2</sup> Department of Pharmacology, Zagreb University School of Medicine, Zagreb, Croatia

<sup>3</sup> Department of Laboratory Diagnostics, Analytical Toxicology and Pharmacology Division, University Hospital Center Zagreb, Zagreb, Croatia

<sup>4</sup> Department of Urology, University Hospital Center Zagreb, Zagreb, Croatia

<sup>5</sup> Agency for Medicinal Products and Medical Devices of Croatia, Zagreb, Croatia

<sup>6</sup> Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Zagreb University School of Medicine, Zagreb, Croatia

<sup>7</sup> Department of Urology, University Hospital Center Zagreb, Zagreb University School of Medicine, Zagreb, Croatia

## Introduction

Mycophenolic acid (MPA) is an antimetabolite immunosuppressant of choice in solid organ transplantation. Regardless of the (oral) formulation (immediate-release ester pro-drug mycophenolate mofetil, MMF, or enteric-coated sodium salt, EC-MPS), pharmacokinetics (PK) of MPA is complex and variable; hence, MPA is subject to therapeutic drug monitoring (TDM) [1, 2].

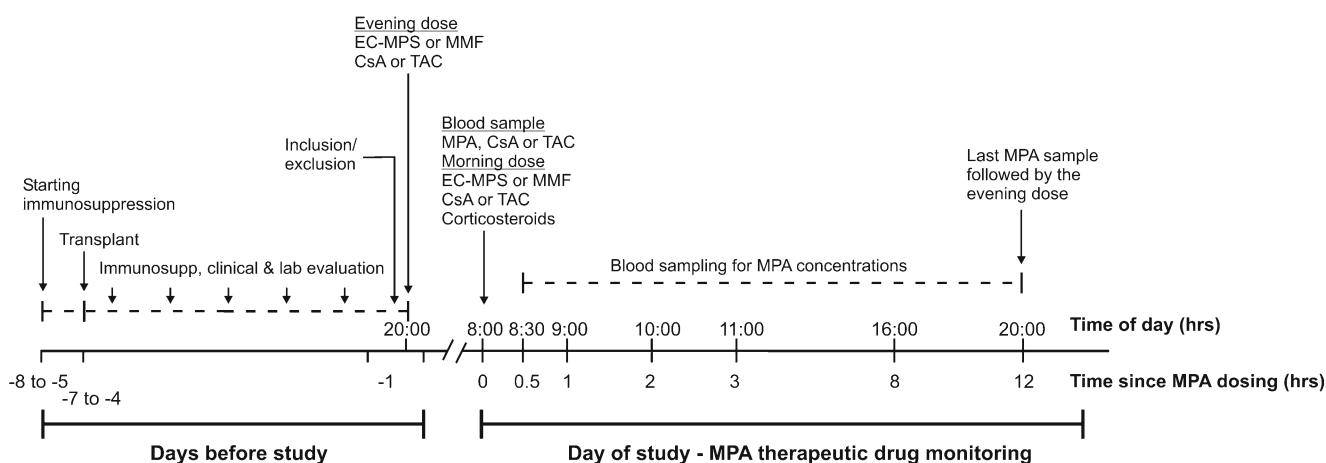
MPA is extensively metabolized in the liver by glucuronidation to inactive MPA-phenyl-glucuronide (MPAG) and a minor acyl-MPAG. Glucuronides are largely excreted into the bile, deglucuronidated in the gut, and contributed to MPA enterohepatic re-circulation accounting for 10–61% of total MPA exposure (a second increase in concentration 6–12 h after dosing). Final elimination is by the kidney apparently by active secretion (MPAG) involving organic anion transporters [1]. Reduced renal function reduces MPAG/MPA elimination and increases total circulating MPA. Exposure to MPA is strongly related to its effects in renal transplant recipients: low(er) area under the concentration-time curve over dosing interval and pre-dose levels increase the risk of acute rejections [3, 4].

Variability in MPA PK is due to a number of factors including gender, serum albumin, renal function, co-medication, and genetics [1]. Regarding the latter two, calcineurin inhibitors (CNIs) included in standard immunosuppressant protocols in renal transplantation, cyclosporine A (CsA) and tacrolimus (TAC), affect exposure to MPA with more profound effects of CsA [1, 5]. CsA increases MPA clearance and reduces pre-dose levels. Inhibition of the multidrug resistance-associated protein 2 (MRP2/*ABCC2*) resulting in a reduced enterohepatic re-circulation of MPAG/MPA is considered to be the main CsA effect [5–7]. MRP2, a member of the ATP-binding

cassette (ABC) transporter superfamily, is expressed at the apical hepatocyte membrane, the luminal membrane of the proximal renal tubular cells, in the intestinal epithelium, placenta, and some other organs. Both MPA and MPAG are substrates for MRP2, and MRP2 is considered the main transporter in MPAG excretion in the liver [8], although this role was suggested also for the breast cancer resistance protein (BCRP/*ABCG2*) [9]. Animal studies indicate the role of MRP2 in MPAG/MPA transport in the kidneys. In vitro, CsA potently inhibits MRP2-mediated MPAG transport, while TAC has no effect [10].

Several SNPs in the *ABCC2* gene encoding MRP2 might partly explain variability in MPA PK. In vitro, *ABCC2* -24C>T variant (rs717620) decreases the *ABCC2* promoter activity [11]. Recipients' variant allele carriage at this locus had no major impact on MPA disposition in several studies in solid organ transplant patients [12–16], but an “additive” effect to the effect of SNPs in glucuronidation enzymes has been suggested [17]. Moreover, the *ABCC2* -24C>T might modify the effect of CsA on MRP2 [18–20]. The *ABCC2* 1249G>A SNP in exon 10 (rs2273697) (valine-to-isoleucine at position 417) in vitro has no effect on MRP2 expression or activity [21] but appears associated with a reduced expression of MRP2 in preterm placenta [22]. To our knowledge, there have been no reports to indicate relevance of this SNP for disposition of MPA.

Little is known about potential effects of the donors' *ABCC2* SNPs in renal transplantation. Certain variant alleles, but not -24C>T or 1249G>A, were reported associated with delayed graft function [23]. However, there have been no reports on effects of donors' *ABCC2* SNPs on exposure to MPA. Here, we investigate the impact of recipients' and donors' *ABCC2* -24C>T and 1249G>A on steady-state exposure to MPA and their interaction with CsA, an inhibitor of several transporter proteins [24] in stable de novo renal transplant patients.



**Fig. 1** Study flow. See “Patients and Methods” for details. Renal transplant recipients on immunosuppressive therapy (based on mycophenolic acid and cyclosporine or tacrolimus) for 5–8 days meeting predefined criteria were included in the present analysis of steady-state pharmacokinetics of mycophenolic acid during a dosing

interval based on data obtained through routine therapeutic drug monitoring. *EC-MPS* enteric-coated mycophenolate sodium, *MMF* mycophenolate mofetil immediate-release formulation, *CsA* cyclosporine, *TAC* tacrolimus, *MPA* mycophenolic acid

**Table 1** Patient characteristics in respect to recipients' and donors' *ABCC2* genotype. Data are median (range), mean  $\pm$  SD, or count (%)

	Recipient <i>ABCC2</i> -24C>T genotype		Recipient <i>ABCC2</i> 1249G>A genotype		Donor <i>ABCC2</i> -24C>T genotype		Donor <i>ABCC2</i> 1249G>A genotype	
	CC ( <i>n</i> = 47)	T-carrier ( <i>n</i> = 21)	GG ( <i>n</i> = 41)	A-carrier ( <i>n</i> = 27)	CC ( <i>n</i> = 39)	T-carrier ( <i>n</i> = 29)	GG ( <i>n</i> = 41)	A-carrier ( <i>n</i> = 27)
Age (years)	52 (15–71)	53 (22–65)	54 (22–71)	50 (15–67)	53 (25–69)	52 (15–71)	51 (22–67)	55 (15–71)
Male	24 (51.1)	11 (52.4)	20 (48.8)	15 (55.6)	21 (53.9)	14 (48.3)	20 (48.8)	15 (55.6)
Weight (kg)	69 (37–101)	65 (48–92)	67 (48–101)	69 (37–100)	66 (37–101)	69 (50–100)	72 (41–101)	66 (37–100)
Height (cm)	168.1 $\pm$ 11.4	167.9 $\pm$ 9.0	165.9 $\pm$ 8.8	171.4 $\pm$ 12.5	167.7 $\pm$ 9.5	168.7 $\pm$ 12.2	168.9 $\pm$ 12.0	166.9 $\pm$ 8.3
BMI (kg/m <sup>2</sup> )	24 (14–39)	24 (17–28)	24 (17–39)	23 (14–31)	24 (14–39)	24 (18–31)	25 (17–39)	24 (14–29)
Creatinine ( $\mu$ mol/L)	154 (89–266)	165 (89–302)	152 (89–302)	165 (93–262)	157 (93–302)	161 (89–248)	161 (89–266)	152 (93–302)
eGFR (mL/min/1.73 m <sup>2</sup> )	34 (14–102)	34 (17–67)	37 (14–102)	34 (20–65)	37 (14–67)	29 (19–102)	32 (14–102)	40 (17–65)
Corticosteroid-treated	68 (100)	68 (100)	41 (100)	27 (100)	39 (100)	29 (100)	41 (100)	27 (100)
Prednis. eq. dose (mg)	40 (20–75)	30 (20–60)	40 (20–60)	30 (20–75)	30 (20–60)	40 (20–75)	40 (20–75)	30 (20–63)
EC-MPS-treated	30 (63.8)	15 (71.4)	30 (73.2)	15 (55.6)	26 (66.7)	19 (65.5)	26 (63.4)	19 (70.4)
EC-MPS dose (mg) <sup>a</sup>	All 720 bid	All 720 bid	All 720 bid	All 720 bid	All 720 bid	All 720 bid	All 720 bid	All 720 bid
MMF-treated	17 (36.2)	6 (28.6)	11 (26.8)	12 (44.4)	13 (33.3)	10 (34.5)	15 (36.6)	8 (29.6)
1000 mg (739 MPA) bid	12	6	10	8	11	7	12	6
750 mg (554 MPA) bid	2	0	0	2	1	1	1	1
500 mg (370 MPA) bid	3	0	1	2	1	2	2	1
CsA-treated	28 (59.6)	15 (71.4)	26 (63.4)	17 (29.6)	27 (69.2)	16 (55.2)	27 (65.9)	16 (59.3)
Morning dose (mg/kg)	2.47 (1.03–3.65)	2.38 (1.28–3.52)	2.43 (1.28–3.52)	2.44 (1.03–3.65)	2.38 (1.28–3.65)	2.50 (1.03–3.52)	2.43 (1.03–3.52)	2.47 (1.28–3.65)
Evening dose (mg/kg)	2.59 (1.29–4.00)	2.14 (0.96–3.52)	2.44 (0.96–4.00)	2.44 (1.29–4.00)	2.30 (0.96–3.66)	2.59 (1.29–4.00)	2.44 (1.29–4.00)	2.37 (0.96–3.65)
CsA C <sub>trough</sub> ( $\mu$ g/L)	201 (70–341)	193 (139–279)	196 (133–341)	203 (70–341)	199 (70–341)	198 (133–341)	199 (70–341)	200 (143–341)
TAC-treated	19 (40.4)	6 (28.6)	15 (36.6)	10 (70.4)	12 (30.8)	13 (44.8)	14 (34.1)	11 (40.7)
Morning dose (mg/kg)	0.04 (0.02–0.08)	0.04 (0.03–0.05)	0.04 (0.02–0.06)	0.04 (0.02–0.08)	0.04 (0.02–0.08)	0.04 (0.03–0.06)	0.04 (0.02–0.05)	0.04 (0.02–0.08)
Evening dose (mg/kg)	0.04 (0.02–0.11)	0.05 (0.04–0.07)	0.05 (0.02–0.07)	0.04 (0.03–0.11)	0.04 (0.02–0.11)	0.05 (0.03–0.07)	0.05 (0.02–0.07)	0.05 (0.03–0.11)
TAC C <sub>trough</sub> ( $\mu$ g/L)	10.4 (5.2–27.4)	9.1 (3.4–13.6)	9.6 (3.7–20.1)	9.6 (3.4–27.4)	10.0 (3.4–27.4)	9.0 (5.2–20.1)	8.9 (3.4–15.4)	10.9 (6.6–27.4)

MPA mycophenolic acid, EC-MPS enteric-coated MPA sodium salt, MMF mycophenolate mofetil, CsA cyclosporine, TAC tacrolimus, BMI body mass index, eGFR estimated glomerular filtration rate, bid bis in die (twice daily)

<sup>a</sup> 720 mg of MPA sodium salt corresponds to 674 mg of MPA

## Patients and methods

### Study outline

This prospective observational study was approved by the institutional Ethics Committee and is outlined in Fig. 1. Study procedures were described in detail previously [25]. Briefly, renal transplant recipients on a standard immunosuppressant treatment including MPA, a CNI (CsA or TAC), and corticosteroids for 5–8 days (i.e., 4–7 days after transplantation) (attained steady-state of MPA, CsA, and TAC) were included if meeting the predefined criteria: (1) informed consent; (2) stable clinical condition based on a close monitoring over the pre-study days (Fig. 1): (a) no surgical complications, (b) no signs of graft dysfunction/rejection, (c) no severe comorbidity (cardiovascular, hepatic, metabolic, infections, gastrointestinal), (d) low immunological risk, (e) serum creatinine  $\leq 300$   $\mu\text{mol/L}$  (or estimated glomerular filtration rate  $\geq 14$  mL/min/1.73 m<sup>2</sup>) and by at least 1/3 lower than the initial value (first postoperative day) and stable diuresis at around 60 mL/h, (f) serum albumin  $>31$  g/L; (3) not treated with proton pump inhibitors, antacids, phosphate binders, oral iron, magnesium or calcium, rifampicin, or any antibiotics during the pre-study and study days. A standard immunosuppressive protocol consisted of (a) EC-MPS (Myfortic<sup>®</sup>, Novartis, Switzerland) or MMF (CellCept<sup>®</sup>, Roche, Switzerland), (b) CsA (microemulsion formulation, Neoral<sup>®</sup>, Novartis, Switzerland) or TAC (Prograf<sup>®</sup>, Astellas Pharma Ireland), (c) prednisone (Decortin<sup>®</sup>, Merck, Germany) or methylprednisolone (Medrol<sup>®</sup>, Pfizer, Italy) starting post transplantation at 60 mg/day prednisone-equivalent dose for 3 days with a rapid reduction to 30 mg/day. All drugs were obtained commercially and their assignment was at the discretion of the attending physician.

### Bioanalytical methods

Blood samples were drawn immediately before 08:00 H to determine trough concentrations of CsA, TAC, and MPA, followed by drug administration and six serial blood samples for determination of the MPA profile during the dosing interval at 0.5, 1, 2, 3, 8, and 12 h post-dose (Fig. 1). Whole blood CsA and TAC were determined by a validated affinity column-mediated immunoassay (Siemens, Germany). Total plasma MPA was determined by high pressure liquid chromatography (HPLC) with UV/VIS spectrophotometric detector and Turbochrom software (Perkin Elmer, USA) using a commercially available HPLC kit and reverse-phase column for MPA in plasma (Chromsystems, Germany). All analytes were included in the external proficiency testing scheme (RfB and UK NEQUAS). Serum creatinine and estimated glomerular filtration rate (eGFR) were determined as previously described [25].

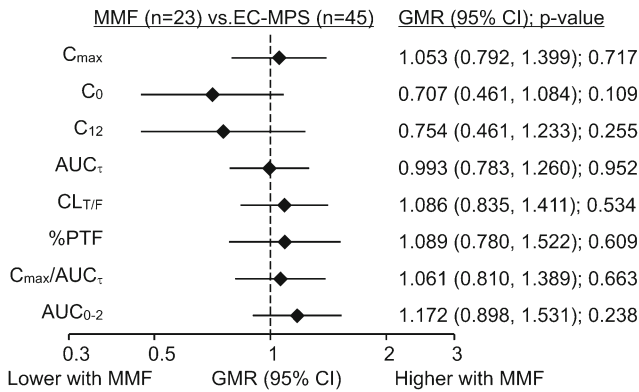
**Table 2** Dose-normalized (per mycophenolic acid dose) pharmacokinetic parameters of mycophenolic acid at steady-state in respect to recipients' and donors' *ABCC2* genotype. Data are median (range)

	Recipient <i>ABCC2</i> -24C>T genotype		Recipient <i>ABCC2</i> 1249G>A genotype		Donor <i>ABCC2</i> -24C>T genotype		Donor <i>ABCC2</i> 1249G>A genotype	
	CC (n = 47)	T-carrier (n = 21)	GG (n = 41)	A-carrier (n = 27)	CC (n = 39)	T-carrier (n = 29)	GG (n = 41)	A-carrier (n = 27)
$C_{\text{max,ss}}$ (mg/L)	15.5 (3.1–51.2)	17.9 (11.2–44.7)	16.6 (3.1–51.2)	16.7 (5.8–39.3)	15.5 (3.1–34.9)	17.9 (6.8–51.2)	18.8 (6.8–51.2)	13.5 (3.1–39.3)
$T_{\text{max,ss}}$ (h)	1 (0–12)	2 (0.5–3)	2 (0–8)	1 (0–12)	2 (0–12)	1 (0–2)	1 (0–2)	2 (0–12)
$C_0$ (mg/L)	3.6 (0.6–17.4)	3.2 (0.5–12.4)	3.1 (0.5–17.4)	3.7 (0.6–13.8)	4.0 (0.6–17.4)	2.9 (0.5–8.4)	3.1 (0.5–17.4)	3.6 (0.6–13.8)
$C_{12}$ (mg/L)	1.8 (0.15–16.9)	2.3 (0.14–9.6)	2.4 (0.15–16.9)	1.8 (0.14–10.3)	2.3 (0.14–16.9)	1.9 (0.15–8.6)	2.2 (0.14–16.9)	1.8 (0.15–10.3)
$AUC_{0-12}$ (mg·h/L)	50.7 (22.8–153)	58.3 (25.0–177)	53.5 (23.8–177)	52.4 (22.8–153)	50.2 (22.8–177)	53.5 (23.8–115)	53.8 (23.9–153)	50.2 (22.8–153)
$CL_{\text{T/F,ss}}$ (mL/min/kg)	3.5 (0.8–9.1)	2.6 (1.0–8.1)	3.1 (0.8–9.1)	3.5 (0.8–8.1)	3.5 (0.8–8.1)	3.0 (0.9–9.1)	3.1 (0.8–8.1)	3.5 (0.8–9.1)
%PTF	334 (52–1120)	365 (124–1054)	333 (52–1120)	341 (108–1053)	332 (52–1053)	365 (124–1120)	366 (94–1053)	217 (52–1120)
$C_{\text{max}}/AUC_{\tau}$ (1/h)	0.30 (0.10–0.94)	0.32 (0.15–0.88)	0.29 (0.10–0.84)	0.31 (0.14–0.88)	0.30 (0.10–0.88)	0.32 (0.15–0.94)	0.33 (0.13–0.88)	0.22 (0.10–0.94)
$AUC_{0-2}$ (mg·h/L)	16.1 (3.5–47.2)	18.6 (6.4–47.0)	15.4 (3.5–49.7)	19.1 (5.1–42.8)	16.8 (3.5–49.7)	16.1 (5.2–47.2)	19.9 (5.2–49.7)	12.6 (3.5–42.8)

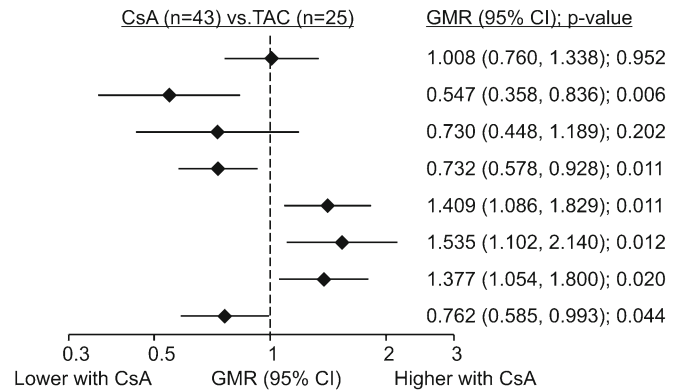
720 mg of mycophenolic acid (MPA) sodium salt corresponds to 674 mg of MPA, and 1000 mg, 750 or 500 mg of mycophenolate mofetil correspond to 739, 554, and 370 mg of MPA

$C_{\text{max,ss}}$  peak plasma concentration at steady-state,  $T_{\text{max,ss}}$  time of peak concentration,  $C_0$  morning pre-dose trough,  $AUC_{0-2}$  area under the concentration-time curve during dosing interval at steady-state, %PTF peak-trough fluctuation,  $AUC_{0-2}$  partial AUC over the first 2 h

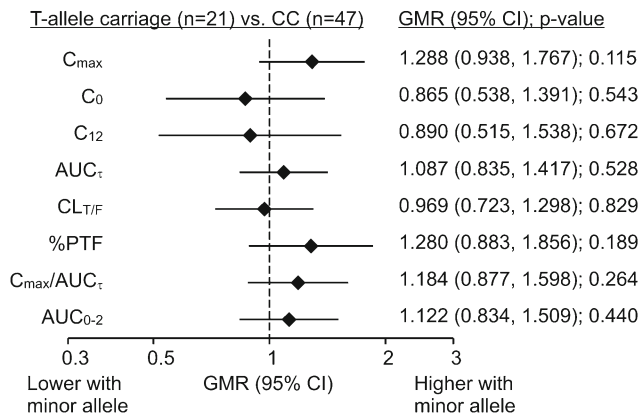
**MPA formulation effects**



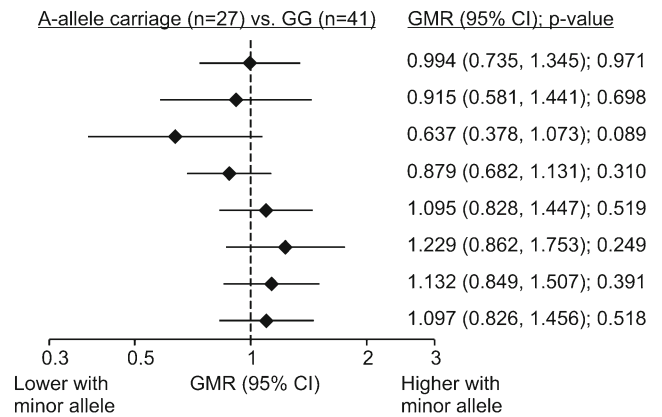
**Cyclosporin effects**



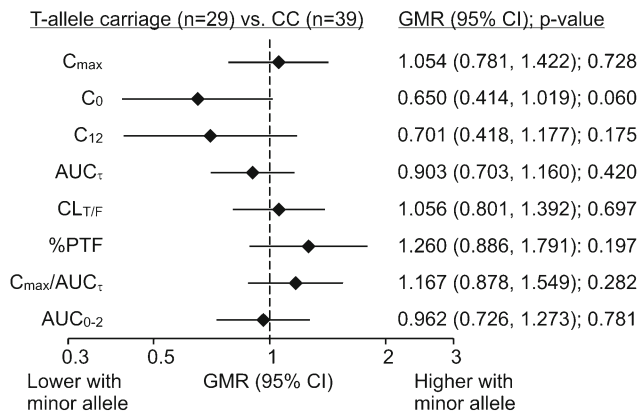
**Recipient -24C>T genotype effects**



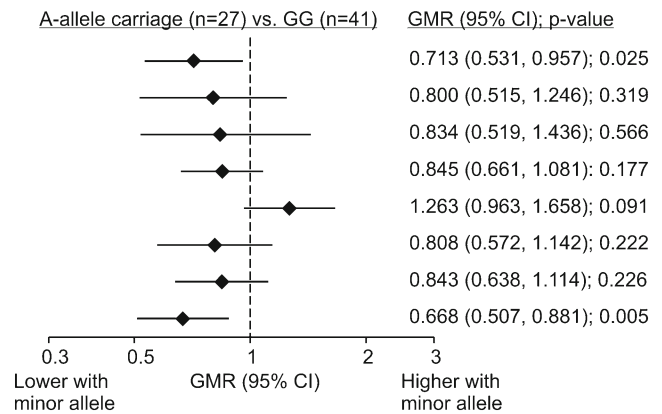
**Recipient 1249G>A genotype effects**



**Donor -24C>T genotype effects**



**Donor 1249G>A genotype effects**



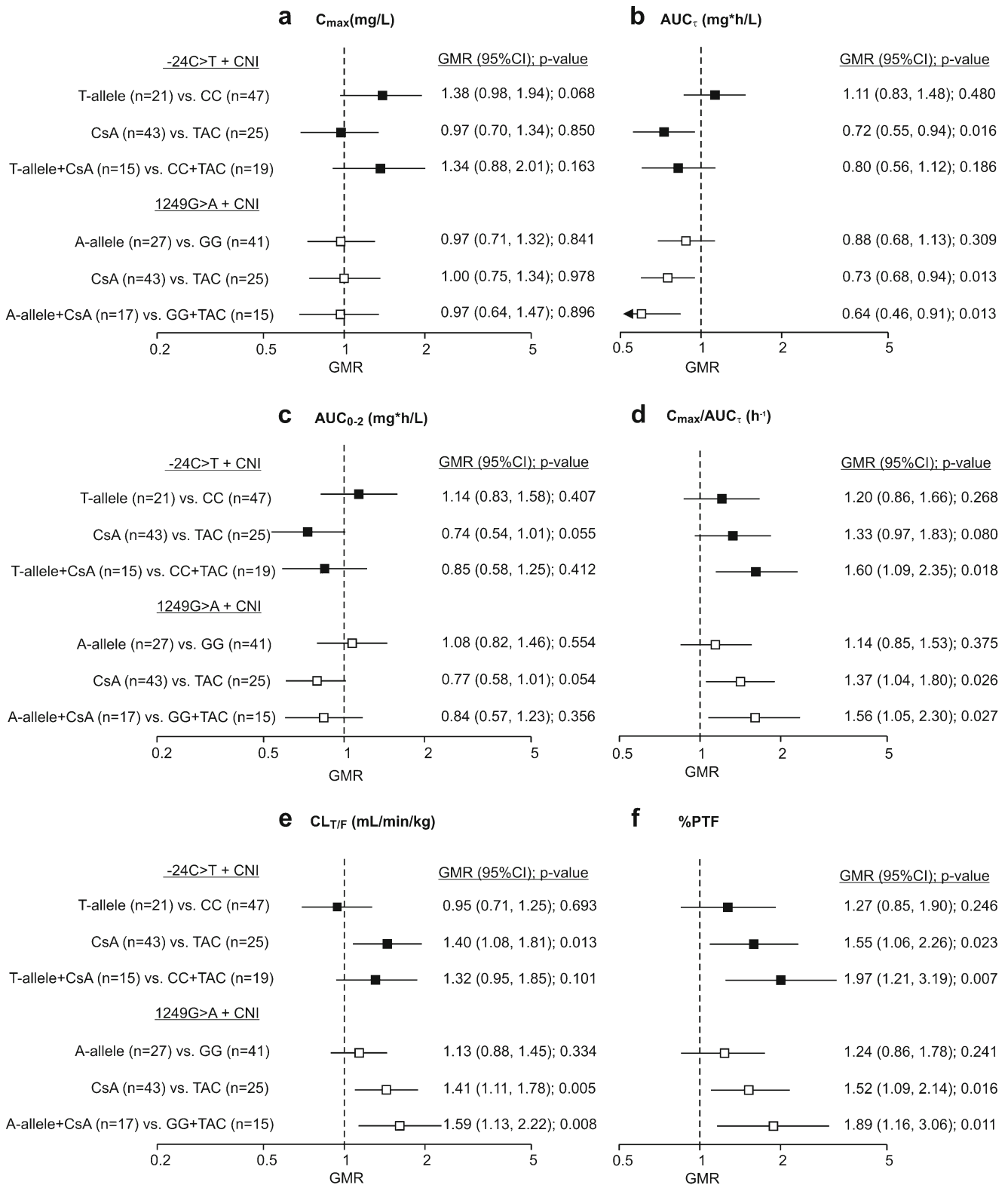
**Fig. 2** Adjusted effects of mycophenolic acid (MPA) formulation (mycophenolate mofetil, MMF vs. enteric-coated sodium salt, EC-MPS), cyclosporine (CsA) compared to tacrolimus (TAC) and recipients' and donors' *ABCC2* genotypes at locus -24C>T and locus 1249G>A on steady-state pharmacokinetics of MPA. Ln-transformed data for each pharmacokinetic parameter were analyzed in a separate general linear model (effects, MPA formulation, calcineurin inhibitor, estimated glomerular filtration rate, recipients' genotype at *ABCC2* locus -24 dichotomized as CC or T-allele carriage, recipients' genotype

at *ABCC2* locus 1249 dichotomized as GG or A-allele carriage and donors' genotypes at the two loci dichotomized in the same way) and derived contrasts are expressed as geometric means ratios (GMR) with 95% confidence intervals.  $C_{max,ss}$  peak plasma concentration at steady-state,  $C_0$  morning pre-dose trough,  $C_{12}$  evening trough,  $AUC_{t,ss}$  area under the concentration-time curve during dosing interval at steady-state, %PTF peak-trough fluctuation,  $AUC_{0-2}$  partial AUC over the first 2 h

## Genotyping

Genomic DNA was isolated from whole blood using BioSprint 15 DNA Blood Kit (Qiagen, Hilden, Germany) on

KingFisher mL System (Thermo Labsystems, Vantaa, Finland). Analysis of the *ABCC2* -24C>T and 1249G>A was performed on an Applied Biosystems 7500 Real-Time PCR System according to the manufacturer's instructions (Applied



◀ **Fig. 3** Joint effects of the recipients' minor allele carriage at locus -24 or locus 1249 in *ABCC2* and treatment with cyclosporine (CsA) on steady-state pharmacokinetics of mycophenolic acid. Ln-transformed data for each pharmacokinetic parameter were analyzed in a separate general linear model (effects, MPA formulation, calcineurin inhibitor (CNI; CsA or tacrolimus, TAC), estimated glomerular filtration rate, donors' genotypes at *ABCC2* locus -24 dichotomized as CC or T-allele carriage, donors' genotype at *ABCC2* locus 1249 dichotomized as GG or A-allele carriage, recipients' genotypes at the two loci dichotomized in the same way and an interaction term between the recipients' minor allele carriage (each locus) and calcineurin inhibitor) and derived contrasts are expressed as geometric means ratios (GMR) with 95% confidence intervals. Adjustments for multiple comparisons were by the simulation method.  $C_{max,ss}$  peak plasma concentration at steady-state,  $T_{max,ss}$  time of peak concentration,  $AUC_{t,ss}$  area under the concentration-time curve during dosing interval at steady-state, %PTF peak-trough fluctuation,  $AUC_{0-2}$  partial AUC over the first 2 h

Biosystems, CA, USA) using a validated TaqMan® Drug Metabolism Genotyping Assays (Applied Biosystems, CA, USA). Assay IDs were for *ABCC2* -24C>T C\_2814642\_10 and for 1249G>A C\_22272980\_20.

### Pharmacokinetic analysis

Steady-state PK parameters of MPA were determined by non-compartmental method (Kinetica 4.1, InnaPhase Corp., USA): peak concentration ( $C_{max,ss}$ ) (mg/L); time to peak concentration ( $T_{max,ss}$ ) (hours); area under the concentration-time curve ( $AUC_{\tau,ss}$  by the log-linear method) (mg × h/L); pre-dose concentrations ( $C_0$ ,  $C_{12}$ ) (mg/L); apparent total body clearance ( $CL_{T/F,ss}$ ) (mL/min/kg), and peak-trough fluctuation (%PTF). Partial area under the curve  $AUC_{0-2}$  and  $C_{max}/AUC_{\tau}$  ratio as an indicator of the absorption rate [26] were also determined. The analysis was based on dose-normalized concentrations accounting for the fact that 1000 mg of MMF corresponded to 739 mg of MPA and 1000 mg of EC-MPS corresponded to 936 mg of MPA.

### Sample size and data analysis

No exact a priori sample size calculation was done since assignment of CNI (CsA or MPA) was not directed and variability of the PK outcomes and prevalence of variant alleles in the population were largely unknown. The enrollment continued until the smallest CNI (CsA or MPA)-by-genotype subset (wild-type or variant allele carriage) attained 12 subjects. A comparison between any two 12 vs. 12 patients subset has 80% power to detect a difference of at least 50% (geometric means ratio  $\geq 1.5$  or  $\leq 0.67$ ) at two-sided alpha level assuming a relatively conservative coefficient of variation of 35%. Such a difference could be reasonably considered as potentially clinically relevant. Ln-transformed PK parameters were analyzed by fitting general linear models to obtain the adjusted effects of CsA (vs. TAC), recipients' and donors' genotype, and their interactions. Results are expressed as geometric mean ratios

(GMRs). Adjustment for multiple comparisons was by the simulation method. We used SAS 9.4 (SAS Inc., Cary, NC).

## Results

### Patient characteristics

A total of 68 patients were included. Recipients' and donors' *ABCC2* genotypes indicated no departure from Hardy-Weinberg equilibrium and no linkage disequilibrium between the loci (electronic supplementary material Table S1). Table 1 summarizes demographic, renal function, and other characteristics in respect to recipients' and donors' genotypes.

### Effects of MPA formulation, CNI, and recipients' and donors' *ABCC2* SNPs on steady-state pharmacokinetics of MPA

Concentration-time MPA profiles by formulation, CNI, and *ABCC2* genotypes are depicted in electronic supplementary material (Fig. S1). Table 2 summarizes pharmacokinetic parameters by genotype.

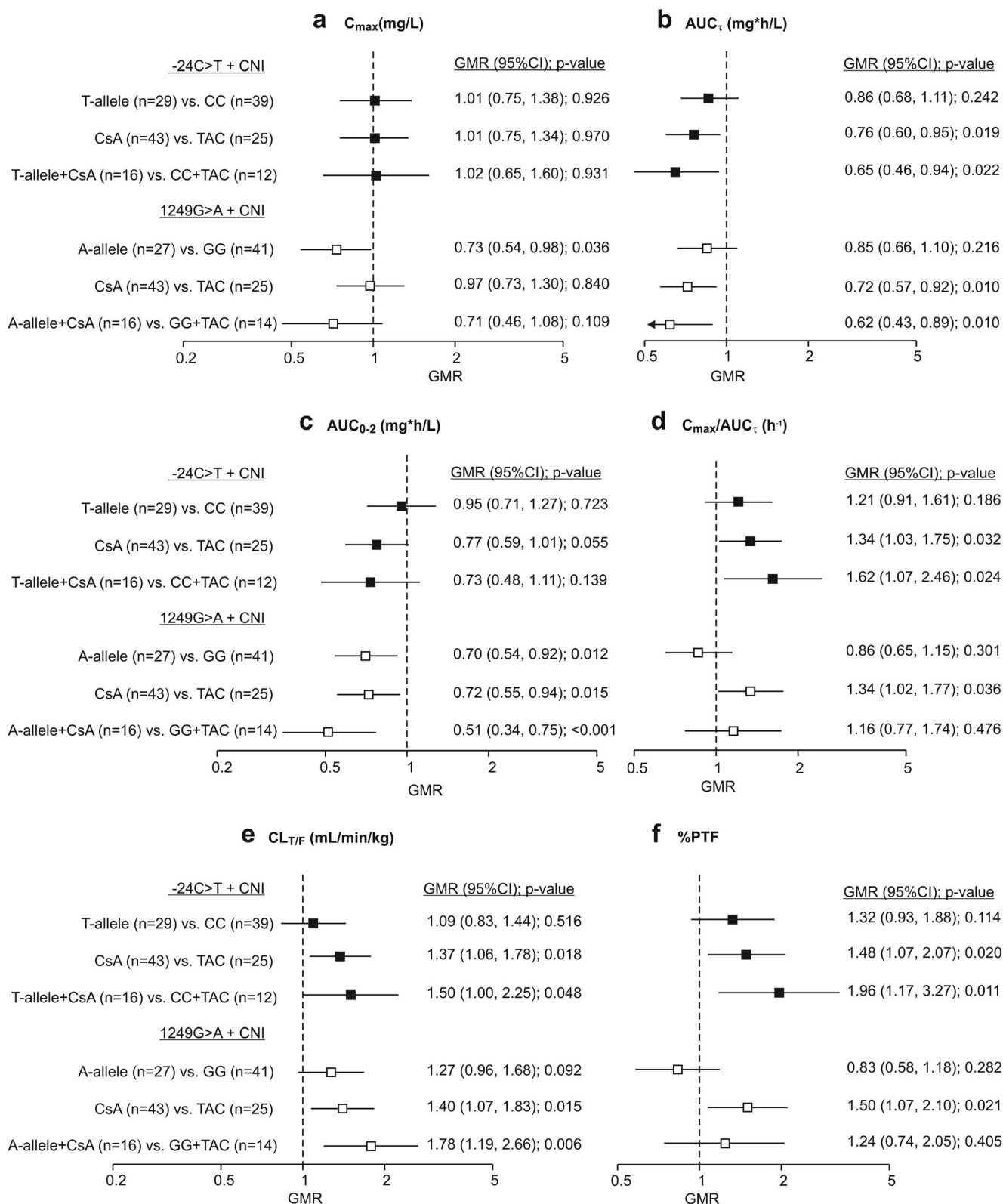
PK parameters were analyzed with the following effects: MPA formulation, CNI type, eGFR, recipients' and donors' *ABCC2* genotypes (both loci) dichotomized as minor allele carriage vs. wild type. The adjusted main effects are depicted in Fig. 2. There appeared no effect of MPA formulation except for a trend of lower troughs ( $C_0$ ,  $C_{12}$ ) with MMF. CsA-treated patients had significantly lower  $C_0$  (45%),  $AUC_{\tau}$  (27%), and  $AUC_{0-2}$  (24%) and higher  $CL_{T/F}$  (41%), %PTF (54%), and  $C_{max}/AUC_{\tau}$  (38%) than those of TAC-treated patients. The CsA effect was consistent by MPA formulation (not shown). Recipients' minor allele carriage at either locus had no apparent effect except for a tendency of lower  $C_{12}$  in A-allele carriers at 1249G>A, but the estimate was imprecise (GMR=0.637, 95% CI 0.378–1.073). Donors' T-allele carriage at -24C>T was associated with a tendency of lower  $C_0$ , but the estimate was imprecise (GMR=0.650, 95% CI 0.414–1.019). However, donors' A-allele at 1249G>A was associated with a significantly lower  $C_{max}$  (29%) and a lower  $AUC_{0-2}$  (33%) (Fig. 2).

### Joint effects of CsA and recipients' *ABCC2* SNPs

PK parameters were analyzed with the same effects as in Fig. 2 plus interactions between recipients' -24C>T or 1249G>A genotype and CNI. The adjusted effects of the recipients' minor allele carriage (vs. wild type), CsA (vs. TAC), and of minor allele combined with CsA (vs. wild type + TAC) are depicted in Fig. 3. A combination of CsA and 1249A was associated with a reduction in  $AUC_{\tau}$  and an increase in clearance that were numerically greater than the main effects of

CsA (36 vs. 27, 59 vs. 41%, respectively). Similar additive effects were observed regarding increased  $C_{max}/AUC_{\tau}$  and %PTF, whereas the effect on  $AUC_{0-2}$  appeared numerically “opposing” to the main CsA effect (Fig. 3). CsA and -24T

combination effect appeared numerically “opposing” to the main CsA effect regarding  $AUC_{\tau}$ , clearance and  $AUC_{0-2}$ , and additive regarding increased  $C_{max}/AUC_{\tau}$  and %PTF (Fig. 3).





◀ **Fig. 4** Joint effects of the donors' minor allele carriage at locus -24 or locus 1249 in *ABCC2* and treatment with cyclosporine (CsA) on steady-state pharmacokinetics of mycophenolic acid. Ln-transformed data for each pharmacokinetic parameter were analyzed in a separate general linear model (effects, MPA formulation, calcineurin inhibitor (CNI; CsA or tacrolimus, TAC), estimated glomerular filtration rate, recipients' genotypes at *ABCC2* locus -24 dichotomized as CC or T-allele carriage, recipients' genotype at *ABCC2* locus 1249 dichotomized as GG or A-allele carriage, donors' genotypes at the two loci dichotomized in the same way and an interaction term between the donors' minor allele carriage (each locus) and calcineurin inhibitor) and derived contrasts are expressed as geometric means ratios (GMR) with 95% confidence intervals. Adjustments for multiple comparisons were made by the simulation method.  $C_{max,ss}$  peak plasma concentration at steady-state,  $T_{max,ss}$  time of peak concentration,  $AUC_{t,ss}$  area under the concentration-time curve during dosing interval at steady-state, %PTF peak-trough fluctuation,  $AUC_{0-2}$  partial AUC over the first 2 h

### Joint effects of CsA and donors' *ABCC2* SNPs

PK parameters were analyzed with the same effects as in Fig. 3, but interactions were between donors' -24C>T or 1249G>A genotype and CNI. The adjusted effects of the donors' minor allele carriage (vs. wild type), CsA (vs. TAC), and of minor allele combined with CsA (vs. wild type + TAC) are depicted in Fig. 4. A combination of CsA and 1249A was associated with a reduction in  $AUC_{\tau}$  (38%) and  $AUC_{0-2}$  (49%) and an increase in clearance (78%) that were numerically greater than the main effects of CsA (28, 28, and 40%, respectively), while the effects of the combination appeared numerically "opposing" to the main CsA effects regarding  $C_{max}/AUC_{\tau}$  and %PTF (Fig. 4). A combination of CsA and -24T was associated with a reduction in  $AUC_{\tau}$  (35%) and increase in clearance (50%) that was numerically greater than the main CsA effects (24 and 37%, respectively). Similar additivity was observed regarding  $C_{max}/AUC_{\tau}$  and %PTF (increase), but not regarding  $AUC_{0-2}$  (Fig. 4).

### Discussion

The effects of CNIs and of genetic factors have attracted much attention in investigations of the variability of MPA pharmacokinetics. Currently, only UGT1A9 genotype is considered a relevant biomarker to predict initial dosing of MPA in patients co-treated with TAC [27]. However, *ABCC2* variants have been extensively evaluated as potential sources of MPA PK variability, per se, as well as molecular basis for the effects of CsA. Present study is specific in that it accounted for both the recipients' and donors' genotypes, bearing in mind MRP2-mediated renal MPAG/MPA transport. The study has limitations; as it was observational (routine TDM), it did not include MPAG measurements and did not account for glucuronidation enzyme or other transporter genotypes. The PK blood sampling schedule was guided by the TDM requirement of an

$AUC_{\tau}$  based on at least six sampling points and might have been suboptimal for detection of  $t_{max}$ . However, it has several strengths—by inclusion/exclusion criteria and simultaneous adjustments for renal function and recipients' and donors' genotypes at both loci, a fair control of confounding was achieved; all treatment-by-genotype subsets were large enough to detect potentially relevant effects (the smallest compared subsets contained 16 vs. 12 patients, Fig. 4). Under these circumstances, there appeared no major main effect of the donors' variant allele -24T on PK of MPA. However, donors' variant allele 1249A was associated with a reduced peak ( $C_{max}$ , 29%) and early exposure ( $AUC_{0-2}$ , 33%) co-incident with an increased clearance (26%,  $p = 0.091$ ) (Fig. 2). These findings are indicative of an increased renal elimination. The fact that total exposure and trough values were not proportionally reduced (15–20% lower  $AUC_{\tau}$ ,  $C_0$  and  $C_{12}$ ,  $p > 0.05$ ) might be explained by the (assumed) "second wave of MPA exposure" due to MPAG/MPA enterohepatic re-circulation— $AUC_{\tau}$  might have been reduced less than  $AUC_{0-2}$  on the account of an "input" occurring after the 2nd post-dose hour. Unfortunately, "late partial AUC" was not informative since there were only three sampling points over 10 later hours (Fig. S1). Still, a trend towards lower %PTF (by 20%,  $p > 0.05$ ) indirectly supports such a possibility as it reflects a difference between  $C_{max}$  (reduced) and  $C_{min}$  (reduced, but less so). In mechanistic terms, this is in line with the role of MRP2 as a major efflux protein and with association of the variant allele with lower MRP2 expression [22]. In such a scenario, MPAG/MPA would be more available for tubular secretion resulting in an increased clearance. Indirectly, this possibility is supported by additive effects of CsA (an MRP2 inhibitor as opposed to TAC) and variant allele carriage: reductions in  $AUC_{\tau}$  and  $AUC_{0-2}$  and increase in clearance were all numerically greater in 1249A carriers co-treated with CsA (vs. wild-type homozygosity + TAC) than the main effects of CsA (Fig. 4). Similar effects regarding  $AUC_{\tau}$  (reduction) and clearance (increase) were observed also for the combination of CsA and donors' variant allele carriage at *ABCC2* -24C>T but are difficult to interpret since no main effect of the T-allele was observed (Fig. 4). However, the differences between the T-allele + CsA combination and CC + TAC combination were numerically greater than the main CsA effects suggesting a greater MRP2 inhibition by CsA when variant allele was present. Overall, data suggest that the donors' *ABCC2* polymorphisms may affect exposure to MPA per se and/or by increasing susceptibility to inhibition of tubular MRP2 by CsA.

The lack of the main effects of either *ABCC2* -24C>T or *ABCC2* 1249G>A recipients' SNPs on MPA is in line with previous reports [9, 12–16], but present data suggest that the recipients' *ABCC2* SNPs could moderate the effect of CsA on MRP2. The observed main effects of CsA (compared to TAC) are generally consistent with the reported [6, 24] increased

clearance and reduced exposure to MPA. Increased clearance (41%) is in agreement with reduced morning trough (45%) total ( $AUC_{\tau}$ , 27%) and early ( $AUC_{0-2}$ , 24%) exposure (Fig. 2). This is consistent with the reported CsA inhibition of the MRP2-mediated hepatic MPAG excretion and inhibited enterohepatic re-circulation of MPAG/MPA [6, 7]. As already discussed, present data suggest a relevant contribution of the renal MRP2 inhibition to the increased MPA clearance. The fact that  $C_{\max}$  was not affected should be viewed in parallel with the increased  $C_{\max}/AUC_{\tau}$  (38%) (Fig. 2)—increased absorption rate suggests MRP2 inhibition at the intestinal level and could have “compensated” for the effect of increased clearance on peak exposure. The increased %PTF (53%) (Fig. 2) is an understandable consequence of an increased clearance and unchanged peak exposure. Therefore, the present observations appear plausible when viewed in the light of the (assumed) simultaneous effects of CsA on MRP2 at the intestinal, hepatic, and renal levels. Such a view is further supported by the apparent additive effects of the recipients’ variant allele 1249A and CsA (Fig. 3): The differences between the A-allele + CsA combination and wild-type homozygosity + TAC combination in respect to clearance, total exposure, absorption rate, and %PTF were all numerically greater than the main CsA effects. Lack of such an effect on  $AUC_{0-2}$  should be viewed in the light of the (additively) increased  $C_{\max}/AUC_{\tau}$ —additionally increased absorption rate might have “compensated” for the effect of an increased clearance not only in respect to  $C_{\max}$  but also, in part, in respect to early exposure. To our knowledge, this is the first indication that the recipients’ *ABCC2* 1249G>A polymorphism might moderate the effects of CsA on MRP2 since previous studies in renal transplant recipients [18–20] focused on the -24C>T SNP. The results have been in part conflicting. In a study in patients co-treated with TAC [19], variant allele was associated with higher MPA trough, while in a study in CsA co-treated patients, no such effect was seen [20]. One study included [18] both genetic and co-treatment patient subsets (CsA vs. TAC or sirolimus). Raw  $AUC_{0-12}$  values on day 7 for T-allele overall (50.0,  $n = 14$ ) were similar to wild type overall (46.6,  $n = 23$ ); for CsA overall (40.2,  $n = 22$ ) were 27% lower ( $p = 0.009$ ) than for TAC/sirolimus overall (55.2,  $n = 17$ ); whereas values for CsA+T-allele (51.9,  $n = 7$ ) were 17% lower than those of TAC/sirolimus + wild type (62.8,  $n = 9$ ). The present data also suggest that the recipients’ -24T allele could actually “act in opposition” to CsA regarding MPA clearance/disposition (Fig. 4): Adjusted differences between T-allele carriers co-treated with CsA and wild-type homozygotes co-treated with TAC regarding clearance,  $AUC_{\tau}$ , and  $AUC_{0-2}$  were all numerically smaller than the main CsA effects indicating a reduced CsA effect. The seemingly additive effect on  $C_{\max}/AUC_{\tau}$  (consistent with a trend of increased  $C_{\max}$  for the T-allele + CsA combination vs. wild-type homozygosity + TAC combination, 34%,  $p = 0.163$ ; Fig. 3) suggests a possibility of a

different CsA–T-allele interaction at the intestinal vs. the hepatic level.

High inter-individual variability in MPA pharmacokinetics in renal transplant recipients is largely due to genetic factors: polymorphisms of MPA metabolizing enzymes (UGT1A9 [27], UGT2B7 [14, 16]) and various transporter proteins (MRP2 [8, 10], BCRP [9], SLCO1B3 or 1B1 [12, 28]); CNIs are substrates of polymorphic CYP3A4/5 and P-glycoprotein/ABCB1 [29]; CsA in particular inhibits CYPs and UGTs and a range of drug transporters [7, 8, 10, 30] where the extent of CsA effects might be affected by their polymorphisms. Hence, a polygenic approach is needed to achieve a comprehensive understanding of the gene-drug and gene-moderated drug-drug interactions. In this respect, the present study is clearly limited and its results should be viewed within these limitations.

## Conclusions

The present study is an initial exploration of a potential interaction between effects of CsA and of recipients’ and donors’ *ABCC2* gene variants on disposition of MPA in renal transplant recipients. Since it is based only on plasma MPA pharmacokinetics, arising inferences are largely indirect. With these limitations in mind, the present results are in agreement with the view that CsA reduces MPA disposition by inhibiting enterohepatic cycle (MRP2 inhibition). However, they indicate that the recipients’ *ABCC2* -24T>C and 1249G>A SNPs might moderate the effects of CsA on MRP2. Data also indicate that CsA might reduce MPA disposition at the renal level as well, by inhibiting MRP2. Finally, to our knowledge, the present results are first to indicate that the donors’ *ABCC2* genotype may influence disposition of MPA—variant allele carriage at 1249G>A per se appears to reduce the exposure to MPA (likely by increasing renal MPAG/MPA clearance) and apparently acts in the “same direction” as CsA at the renal level.

**Authors’ contributions** BN, LZ, VT and KŽ were responsible for the study concept and design. LZ, NŠS, and KŽ were responsible for acquisition of subjects. BT and LZ performed research. BBA and NŠS analyzed and interpreted the data. TV and BN analyzed and interpreted the data and prepared the manuscript. All authors participated in critical revision of the manuscript, contributed comments, and approved the final version.

**Compliance with ethical standards** This prospective observational study was approved by the institutional Ethics Committee and was conducted in line with the Declaration of Helsinki (2008 version). Laboratory analyses were performed in line with the ISO-15189 standard of good practice.

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