The reduced function allele SLCO1B1 c.521T>C is of no practical relevance for the renal graft function over the first post-transplant year in patients treated with mycophenolic acid

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Abstract

Aim. To estimate the effect of the reduced-function polymorphism SLCO1B1 c.521T>C on the renal graft function (estimated glomerular filtration rate, eGFR) over 12 months in patients treated with mycophenolic acid (MPA). Methods. Consecutive eligible adults ([?]16 years of age) engrafted over a 6-year period who received MPA as a part of maintenance immunosuppression were assessed for eGFR on 9 occasions over 12 post-transplant months. The SLCO1B1 c.521C>T variant allele carriers (treated) and wild-type subjects (controls) were balanced on a range of demographic, medical, and genetic variables at baseline, and the development of eGFR (slope) was estimated with further adjustment for time-varying covariates. A subset of patients were assessed for exposure to MPA 5-7 days after the transplantation. Results. The adjusted eGFR slopes from day 1 to day 28 (peak), and from day 28 to day 365 were practically identical in treated (n=86) and control (n=168) patients (GMR=0.99, 95%CI 0.92-1.06, and GMR=0.98, 0.94-1.01, respectively). The rates of adverse renal outcomes and possible MPA-related adverse effects were low, and similar in treated and controls (adjusted RR=0.94, 0.49-1.84 and RR=1.08, 0.74-1.58, respectively). The pharmacokinetic substudy did not signal that treated and control patients differed with respect to MPA clearance, peak, trough or total exposure, overall (treated n=23, control n=45), if cotreated with cyclosporine (n=17 vs. n=26) or with tacrolimus (n=8 vs. n=17). Discussion. In patients treated with MPA, variant allele SLCO1B1 c.521T>C has no effect on the 12-month renal graft function. It does not seem to affect exposure to- and safety of MPA.

Introduction

The organic anion transporting polypeptide 1B1 (OATP1B1) mediates hepatic uptake and is important for the pharmacokinetics of several drugs.[1-4] The encoding gene – solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) – has a number of variants.[1,5] Currently, 41 alleles (haplotypes) forming 990 genotypes have been proposed (with **1* and **1/*1* indicating the "reference" haplotype/genotype)[6]. For most of them, the functional consequences are unknown,[6] but some result in an increased and some in a reduced function (may depend on the substrate).[5,6] Regarding the latter, two common single nucleotide polymorphisms (SNPs) - *SLCO1B1 c.521T>C*(rs4149056) (OATP1B1 V174A), and *c.388A>G*(rs2306283) (OATP1B1 N130D) - are in partial linkage disequilibrium (LD) and form four haplotypes: **1* (TA), **37* (TG) (previously *1B), **5* (CA), and **15* (CG) (includes also *17 from the legacy nomenclature, that includes two promoter polymorphisms g.*-11187G>A* and g.*-10499A>C*)[1,2,5]. The presence of the variant c.*521T>C* allele (**5* , **15,*17*) is decisive for the reduced function. In Europeans, the cumulative prevalence of genotypes that include these alleles is around 35%.[6]

Calcineurin inhibitors (CNI) [cyclosporine A (CsA), tacrolimus][1] and mTOR inhibitors (sirolimus, everolimus)[7] inhibit OAT1B1 *in vitro* , whereas the two major metabolites of mycophenolic acid (MPA), standardly used in combination with CNIs/mTOR inhibitors for immunosuppression in renal transplantation – MPA glucuronide (MPAG) and acyl-glucuronide (AcMPAG) - are substrates of OAT1B1.[8,9] The *c.521T>C* variant results in a reduced MPAG/AcMPAG uptake[8]. The pharmacokinetics of MPA is complex (enterohepatic recirculation of MPAG/AcMPAG), variable [regardless of the formulation, i.e., mycophenolate mofetil (MMF) or enteric-coated MPA sodium salt (EC-MPS)], and is closely related to efficacy and tolerability[10,11]. Efforts toward the improvement of clinical outcomes in MPA-treated renal transplant recipients consider "classical" [e.g., age, sex, body mass index, comorbidities, hepatic function, drug-drug interactions (particularly with CNIs)],[11] but also pharmacogenetic factors: polymorphisms in genes encoding enzymes (uridine 5'-diphospho-glucoronosyltransferases, UGTs, primarily UGT1A9, also UGT2B7), and transporters (OATP1B1, 1B3, ABCB1, ABCG2, ABCC2) involved in pharmacokinetics of MPA, and polymorphisms in the MPA target enzymes (inosine-5'-monophosphate dehydrogenases, IMPDH type 1 and 2).[11-13] Regarding *SLCO1B1* SNPs and outcomes in MPA-treated renal transplant patients, practically all the studies thus far addressed *c.521T>C* or haplotypes *5, *15, *17. Other SNPs were sporadically addressed [as recently reviewed[13], rs11045819 in three studies, one intronic SNP (rs4149036) in one study[15]] (see Appendix A, Table A1 for studies on *c.521T>C*). One analysis of 218 European patients[8] reported a lower incidence of MPA-related adverse events in *c.521T>C* variant carriers (41%) vs. wild-type subjects (63%) cotreated with tacrolimus,[8] implying better tolerability due to lower MPA exposure (reduced enterohepatic recirculation).[8]Counterintuitively, a coincident report on 185 Europeans suggested higher total steady-state MPA exposure (area under the concentration-time curve, $AUC_{0\text{-}12}$) in 23*c.521T>C* variant carriers vs. 47 wild-type subjects cotreated with tacrolimus or sirolimus. In patients cotreated with CsA (40 variant vs. 75 wild-type), the difference was not apparent[9]. It was implied that under CsA inhibition, the "effect" of the SNP was not "visible"[9], although both tacrolimus[1] and sirolimus[7] also inhibit OATP1B1. In the largest study on the topic (European patients)[16], MPA $AUC_{0\text{-}12}$ was repeatedly (over 12 months) closely similar in variant and wild-type subjects regardless of co-treatment (CsA or tacrolimus), with a similar incidence of diarrhea and leukopenia[16] (Table A1). Three further studies signaled no association between this SNP and exposure to MPA/risk of leukopenia, but were small, with different clinical and ethnic particulars[17-19] (Table A1 for details).

Apparently, there has been no clear-cut signal to relate the variant*SLCO1B1 c.521T>C* allele to the outcomes in MPA-treated renal transplant recipients, but this could be due to insufficient data[20]: (i) thus far, no relevant study addressed the relationship between this SNP and graft function; (ii) pharmacokinetic data is exclusively crude, and two larger analyses yielded contradictory results.[9,16] In an attempt to contribute to the question of whether carriage of the *SLCO1B1 c.521T>C* variant had practically relevant repercussions for the renal transplant patients treated with MPA, we aimed to estimate the effect of the variant allele on the graft function over the 1st post-transplant year, and on the steady-state exposure to MPA.

Patients and Methods

Outline

We conducted two observational studies in consecutive adults (age [?]16 years) of European (Slavic) descent engrafted over 6 years at a single center (University Hospital Zagreb, Zagreb, Croatia), who experienced stable, uncomplicated first-week recovery.

The main study focused on graft function over the first 12 months. As a standard procedure, patients were assessed for estimated glomerular filtration rate (eGFR) weekly over the first month and at 4 later time points ($\pm$7 days) up to one year (Appendix B, Figure B1). eGFR is an appropriate indicator of graft function[21] and is strongly predictive of its long-term survival[22]. We emulated a target trial[23] with renal transplant recipients treated with MPA as the target population, *SLCO1B1 c.521T>C* variant allele carriage (TC or CC) as the evaluated treatment vs. the wild-type control (TT), and the difference in eGFR slopes between the treated and controls[21] as the outcome. We expected deaths to be sporadic, and that post-baseline interruption, withdrawal, or replacement (e.g., sirolimus/everolimus) of MPA was possible. If MPA is not

in use, the setting cannot inform about the posted question. However, interruptions/replacements of MPA (for any reason) occasionally happen in daily life and the question that motivated the study was a question of "general strategy" - analogous to the concept of "treatment policy" estimand in randomized trials.[24,25] Therefore, we included patients who received MPA for at least a month over the first two months, including at least two weeks of the first post-transplant month. In addition to *SLCO1B1 c.521T>C* (rs4149056), patients were genotyped for the enzyme [*UGT1A9 c.-275T>A*(rs6714486) and c.*-2152C>T* (rs17868320);*IMPDH2 c.3757T>C* (rs11706052)] and transporter [*ABCB1 c.2677G>T/A* (rs2032582), c.*1236C>T* (rs1128503) and c.*3435C>T* (rs1045642); and *ABCG2 c.421C>A* (rs2231142)] polymorphisms that have been suggested (although not unequivocally) associated with exposure to MPA and/or clinical outcomes in MPA-treated patients. *SLCO1B1 c.521T>C* variant carriers (treated) and wild-type subjects (controls) were balanced on a range of baseline covariates. Variables that could have changed over time and affected the outcome (MPA use, use and type of CNI, trough CNI concentrations, and body mass index) were modeled as time-varying covariates (Appendix B, Figure B1).

A subset of patients from the main study who did not require induction treatment, and some additional patients not included in the main study, were, early in the course of treatment, i.e., 5-7 days after the transplantation when steady-state had been achieved, evaluated for exposure to MPA over the dosing interval (Appendix B, Figure B2). They were genotyped for several further SNPs considered relevant for covariate adjustment, i.e., *UGT2B7 -161C>T*(rs7668258), *ABCC2 -24C>T* (rs717620) and*1249G>A* (rs2273697). Data were initially used to explore the relationship between the *ABCC2* and *ABCG2*polymorphisms with exposure to MPA.[26,27] In the present analysis, we emulated a target trial as in the main study:*SLCO1B1 c.521T>C* variant carriers (treated) and wild-type subjects (controls) were balanced on a range of baseline covariates, and the 12-hour (dosing-interval) MPA concentration-time profile was determined (7 sampling time points) (Appendix B, Figure B2) to estimate differences between treated and controls in steady-state pharmacokinetic parameters. All estimates were assessed for sensitivity to unmeasured confounding. The studies were approved by the Institutional Ethics Committee (University Hospital Center Zagreb, Approval Kl:8.1-16/119-4).

Patients

Patients were included if they provided informed consent for genotyping and the use of anonymized data for research purposes. The uncomplicated recovery over the initial week was defined by (i) lack of surgical complications and signs of graft dysfunction or rejection; (ii) stably improving renal function (serum creatinine by at least 1/3 lower on day 7 than on the 1st postoperative day, with stable diuresis of at least 50 mL hr^{-1}); (iii) serum albumin >31 gL^{-1}. Patients with HIV infection were not included. For the pharmacokinetic study, patients had to meet additional criteria: a) started on MPA (either formulation) and a CNI, but not mTOR inhibitors; b) no severe comorbidity (cardiovascular, hepatic, metabolic, infectious, gastrointestinal); c) judged to be of low immunological risk with no induction treatment; d) serum creatinine [?]300 μmol/L at the start of the sampling period; e) not treated with drugs that affect exposure to MPA (proton pump inhibitors, antacids, phosphate binders, oral iron, magnesium or calcium, rifampicin or any antibiotics) during the prestudy and study days.

Immunosuppression

Induction was applied based on standard criteria, predominantly with interleukin-2 antagonists, occasionally with thymoglobulin or rituximab. Patients were started on 60 mg prednisone equivalents, a CNI (CsA or tacrolimus), and MPA (either formulation) (Appendix B, B.3). A few patients started on sirolimus or everolimus were also included in the main study since the initial treatment was shortly switched to MPA.

Bioanalytical procedures and genotyping

Whole blood CsA and tacrolimus, and total plasma MPA measurements, as well as genotyping, were performed as described previously[27] (Appendix B, B.4). We calculated eGFR (mL min^{-1} 1.73 $m2^{-1}$) by the CKD-EPI 2021 equation based on serum creatinine quantified by an enzymatic assay on an automated analyzer (Cobas c 501; Roche, Germany), validated by isotope dilution mass spectrometry.

Main study - primary outcomes

The *co-primary* outcomes were differences between treated and controls in the (i) slope of the eGFR from day 1 to day 28 (expected peak eGFR); and (ii) slope of the eGFR from day 28 to day 365. Reported are also eGFR values at days 1, 28, and 365, but as complementary data with no intended inference. Although the in-house protocol anticipates that eGFR is determined at each regular visit (Appendix B, Figure B1), we assumed that some values could be intermittently missing due to skipped visits (for any reason, but independent of "treatment"). However, no data imputation was planned: we considered that 2-3 values over the 1st month were sufficient to determine the slope of increasing eGFR, and also that 2 or 3 values were sufficient to estimate the slope after day 28. Hence, all available data by time point were used.

Main study - other outcomes

We determined the rates (first and repeated events) of (i) adverse renal outcomes as cumulative number of deaths, graft failures, and renal biopsies regardless of the findings; and (ii) adverse events (graded by the Terminology Criteria for Adverse Events, CTCAE) as cumulative number of deaths, new-onset carcinomas, any cytopenia grade[?]3, any adverse event (gastrointestinal, urinary or respiratory tract infections, central nervous system adverse events) grade [?]2, or cytomegalovirus (CMV) or herpes virus infections.

Pharmacokinetic indicators

Standard steady-state pharmacokinetic parameters based on dose-adjusted MPA concentrations were determined (Appendix B, B.5). Total exposure over the dosing interval (area under the concentration-time curve 0-12 hours, $AUC_{\tau,\sigma\varsigma}$) was of primary interest.

Control of confounding

For the main (see Appendix B, B.6) and the pharmacokinetic study (Appendix B, B.7) we generated directed acyclic graphs to identify variables to account for in order to achieve reasonable control of confounding and identify potential sources of unmeasured confounding. We used energy balancing with average treatment effect as the estimand (package *WeightIT* [28] in R[29]) to achieve the balance between the *SLCO1B1 c.521T>C*variant carriers (treated) and wild-type controls regarding a range of covariates at baseline (no missing data). Energy balancing is a weighting method that achieves (where possible) a distributional balance of covariates between groups[30,31]. Standardized differences <0.1 indicated an adequate balance. In the main study, further adjustment was made for time-varying covariates (all were considered external). Data on MPA use (binary), CNI use (no, tacrolimus or CsA), and BMI were regularly recorded, and were missing for skipped visits. CNI troughs were categorized as "low" (below target values), "target", "high" or "missing" – except over the period with weekly assessments, where values were imputed based on adjacent values (if possible). In the pharmacokinetic study, covariate balancing between treated and controls was done in the overall sample, and also separately in patients cotreated with CsA and those cotreated with tacrolimus, to estimate the variant allele effect in each subset. Since the pharmacokinetic study was relatively limited in size, the achieved balance for occasional covariates was suboptimal (d[?]0.1) – these covariates were included in multivariable models. Appendix B, B.6 and B.7 provide details on sources, control of- and residual confounding in both studies.

Data analysis (Appendix B, B.8 for details)

We fitted a weighted piecewise random intercept and time linear mixed-model with a knot at day 28 to ln-transformed eGFR to generate: (a) adjusted eGFR slopes before and after the knot and (b) adjusted (geometric mean) eGFR values at days 1, 28 and 365, and differences between treated and controls (geometric means ratios, GMR). Weighted Poisson models were fitted to recurrent adverse renal outcomes and adverse events to generate adjusted event rates and differences between treated and controls as rate ratios (RR). Pharmacokinetic parameters were analyzed by fitting weighted frequentist and Bayesian models. We used SAS 9.4 for Windows (SAS Inc., Cary, NC) and package*rstanarm* [32] in R. We used CubeX[33] to test for linkage disequilibrium (LD).

Sensitivity to residual confounding (Appendix B, B.9 for details)

Although the existing data did not clearly point out any of the known factors as a likely source of residual confounding in the main (Appendix B, B.6) and the pharmacokinetic study (Appendix B, B.7), we conceived strong hypothetical biasing factors (or sets of factors): (i) for the main study, a biasing factor with an effect of GMR=0.60 or 0.79 and a total prevalence of 58%; (ii) for the pharmacokinetic study, a biasing factor with an effect of GMR=1.50 and a total prevalence of 10%. We then corrected[34] (package*episensr* [35] in R) the present estimates: (i) in the main study, for the effect of GMR=0.79 and a presumed chance imbalance in the prevalence of the biasing factor in treated and controls of 2.0:1.0 or 1.0:2.0, and for the effect of GMR=0.60, and the chance imbalance of 1.5:1.0 or 1.0:1.5; (ii) in the pharmacokinetic study, for the effect of GMR=1.50, and a chance imbalance of 4:1 or 1:4 between treated and controls.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[36]

Results

Main study

We enrolled 254 patients, 86 (33.9%) *SLCO1B1 c.521T>C* variant carriers [genotype TC (n=77) or CC (n=9), treated) and 168 wild-type patients (genotype TT, controls) (Table 1) (see Appendix C, C.1, Table C1 for all genotyped SNPs). Two control patients died during the first year, both related to graft failure (Figure 1A). Among the treated, 80 (93.0%) were exposed to MPA throughout, three (3.6%) most of the time (5-7 visits), and three for at least a month at the start of treatment. Among the controls, 152 (90.5%) were exposed to MPA throughout, 10 (6.0%) most of the time, and six (3.6%) for at least a month at the start of treatment. Of the 9 post-baseline eGFR values, 8-9 values were provided by 74 (86.0%) treated and 149 (88.7%) controls, whereas 7-9 values were provided by 80 (93.0%) treated and 159 (94.6%) controls. Only 6 treated (7.0%) and 9 controls (5.4%) provided 5 or 6 (mainly) eGFR values.

Treated and controls moderately differed with respect to a number of baseline covariates (Table 1). After covariate balancing, all standardized differences were <0.1 (Table 2). Treated and controls displayed almost identical eGFR values over time – both considering the raw data (Figure 1A), and data after baseline covariate balancing and additional account for time-varying covariates (MPA use, use/type of CNI, CNI troughs and body mass index) (Figure 1B). Adjusted eGFR slopes to day 28 (GMR=0.99, 95%CI 0.92-1.06), and from day 28 to day 365 (GMR=0.98, 95%CI 0.94-1.01) indicated no relevant difference between the treated and controls (Table 3). The rates of adverse renal outcomes and possible MPA-related adverse events were low and closely similar in treated and controls (RR=0.94, 95%CI 0.49-1.84 and 1.08, 0.74-1.58, respectively) (Table 4). The estimates for all outcomes were virtually identical when 22 patients not exposed to MPA continuously over 12 months were excluded from the analysis (not shown).

Pharmacokinetic study

We enrolled 68 patients, 25 (36.8%) *SLCO1B1 c.521T>C* variant allele carriers (treated) and 43 (63.2%) wild-type controls (see Appendix C, C.2, Table C2 for all genotyped SNPs). The use of CsA (68.0% vs. 60.5%) and of tacrolimus (32.0% vs. 39.5%) was generally similar in treated and controls (Table 5; Appendix C, C.3, Table C3 provides additional data). MPA concentration-time profiles were similar in treated and controls overall and in subsets cotreated with CsA or tacrolimus (Appendix C, C.4, Figure C1). After matching/balancing, treated and controls were closely similar with respect to all covariates (Table 5), with minor differences regarding the prevalence of the highest number of variant alleles across the*ABCB1* diplotype, urine output, and CNI troughs (d=0.100-0.115) (Table 5) – these variables were included in multivariable models. The raw data did not indicate relevant differences between treated and controls regarding the total exposure to MPA ($AUC_{\tau,\sigma\varsigma}$) – neither overall, nor in the CsA or tacrolimus cotreated subsets (no SNP*CNI interaction) (Table 6). The same applies to other pharmacokinetic parameters (Appendix C, C.4, Table C4).

The analysis of a fully adjusted total sample also did not indicate relevant differences between treated and controls regarding $AUC_{\tau,\sigma\varsigma}$ (Table 6), or other pharmacokinetic parameters (Appendix C, C.5, Table C5). The CsA and tacrolimus cotreated subsets were relatively small, hence matching/balancing was done on a reduced number of covariates (see Appendix C, C.6, Table C6-C7 for details) – there appeared no relevant effect of the variant allele on $AUC_{\tau,\sigma\varsigma}$ (Table 6), or on other pharmacokinetic parameters (Appendix C, C.6, Table C8) in either CsA or tacrolimus cotreated patients. However, we observed a numerical tendency of somewhat higher morning (C_0) and evening (C_{12}) trough concentrations: overall and comparably in CsA and tacrolimus-cotreated subjects, in raw and adjusted data (Appendix C: C.4, Table C4; C.5, Table C5; C.6, Table C8).

Sensitivity to unmeasured confounding

The estimated effects (GMRs) of the variant *SLCO1B1 c.521T>C* allele on eGFR slope to day 28, and between days 28 and 365, as well as those on $AUC_{\tau,\sigma\varsigma}$ were only mildly altered when corrected for a strong hypothetical unmeasured (residual) confounding (Table 7).

Discussion

The OATP1B1 transporter is a known point at which clinically relevant drug-drug interactions may occur.[5] It is less clear whether its genotype-defined activity is of practical importance for pharmacological treatments.[5] In renal transplantation, the latter has attracted some attention since OAT1B1 participates in enterohepatic recirculation of a commonly used immunosuppressant mycophenolic acid (MPA).[8,9] Among the variants of the encoding gene for which functional consequences are known, by far the most common genotypes are those defined by the presence of the*SLCO1B1 c.521T>C* variant allele resulting in a reduced transporter activity.[5,6] Thus far, investigations of the relationship between this polymorphism and safety of- or exposure to MPA reported exclusively crude data with contradictory findings (and some with serious methodological limitations, Appendix A, Table A1). Having in mind the potential consequences of pharmacokinetic and clinical inter-subject variability of MPA,[11] we considered it a worthwhile effort to try to contribute to the resolution of a dilemma about practical relevance of this polymorphism in renal transplant recipients.

In the main study, we focused on the graft function over 12 months (eGFR slope) rather than on the incidence of biopsy-proven acute rejection (BPAR): (i) eGFR slope is a recommended outcome in clinical trials in this setting;[21] (ii) BPAR is relatively uncommon. In one of the largest studies evaluating the association of any SNP with renal graft outcomes[37], the 12-month incidence of BPAR was 14.6% - a rate by far too low for detection or exclusion of any non-dramatic but possibly clinically relevant effect in studies with 350-400 patients. For example, assume 120 variant carriers with 19% BPAR and 240 wild-type controls with 12% BPAR – power (two-sided alpha=0.05) is only 43% to detect this RR=1.58; (iii) at our center (as in many others[38,39]), biopsies are performed only when clinically indicated. We therefore evaluated the rate of renal biopsies (as repeating events), and used a composite outcome to account for competing events of death/manifest graft failure. Finally, to account for interference of intercurrent infections, severe cytopenias or other (possibly) MPA-related adverse events (AEs), we evaluated the rate of a (composite) of death, infections, cytopenias and clinical AEs of at least moderate severity. While controlling for a wide range of potential demographic, comorbidity, genetic and treatment confounders, we observed no effect of the variant allele on the eGFR slope. The generated estimates were not substantially changed after correction for a hypothetical unmeasured confounding and stayed within the conventional limits of equivalence (i.e., +/- 20% difference in slope). The correction was based on thoroughly considered potential sources of residual confounding (Appendix B, sections B.6 and B.9). However, even if unknown (and not considered) unmeasured confounders existed, the hypothesized biasing effect for which the estimates were corrected was of a size that does not seem likely to exist regardless of its potential sources. For other outcomes, we expected low incidence, hence intended to draw no inference, but closely similar rates of renal and other adverse outcomes in treated and controls did not signal any relevant effect of the variant allele. Finally, we did not *a priori*calculate the number of patients required for the current purpose. However, the coefficient of variation (CV) in the eGFR analysis was 14.2% - with the effective sample sizes of 72 treated and 141 controls, and assuming a CV of 15.0%, we achieved almost 100% power to detect a difference between them of only 10% in the eGFR slopes

(i.e., GMR 0.90, or, reciprocally, 1.11, two-sided alpha=0.05). Therefore, present data strongly suggest that the *SLCO1B1 c.521T>C* polymorphism is of no practical relevance in MPA + CNI-treated renal transplant recipients.

It has been suggested[8] that the effect of this SNP was "visible" in patients cotreated with tacrolimus but not with CsA, due to the inhibition of OATP1B1 by CsA. The present study could not address the issue of possible CNI type*SNP interaction, since CNIs (CsA, tacrolimus) were commonly switched over time (or occasionally transiently replaced by mTOR inhibitors). This, however, does not seem as a likely possibility: (i) tacrolimus and sirolimus also inhibit OATP1B1;[1,7] (ii) in a larger study, no association between this SNP and exposure to MPA or incidence of diarrhea/leukopenia was observed in (also) European patients regardless of whether cotreated with CsA or tacrolimus;[16] (iii) the present pharmacokinetic analysis did not signal effects of the variant allele, neither in the CsA- nor in the tacrolimus cotreated patients.

The pharmacokinetic study was relatively small, but, to the best of our knowledge, it is the first one that attempted to achieve a reasonable control of confounding. In the entire sample, we consistently (raw data, data adjusted for a range of covariates, frequentist and Bayesian estimates) observed no relevant effect of the variant allele on $AUC_{\tau,\sigma\varsigma}$, and no substantial change in the estimates after correction for a strong hypothetical residual confounding. For the raw and fully adjusted data, CVs were 21.2% and 22.0%, respectively. Assuming a CV of 25%, the effective sample sizes of 21 treated and 30 control subjects in the fully adjusted analysis provided 88% power (two-sided alpha 0.05) to detect a difference of 20% (GMR 0.80 or, reciprocally, 1.25) between the treated and controls – hence, the observed lack of a "relevant difference" is not due to a small sample size. The subsets of patients cotreated with CsA or tacrolimus, however, were small. Still, the numerical consistency of the estimates in the total sample and in the CsA/tacrolimus subsets strongly suggest no effect modification by the CNI type. The study was also limited by the fact that MPA metabolites were not quantified. The numerical tendency of higher trough concentrations in *SLCO1B1 c.521T>C* variant carriers than in wild-type controls appears to be in line with the effect of the SNP on MPAG/AcMPAG recirculation. Hence, while it is possible that this SNP reflects on the MPA pharmacokinetics, its overall effect on exposure to MPA does not seem to be practically relevant.

In conclusion, the present study strongly suggests that the reduced-function polymorphism *SLCO1B1 c.521T>C* conveys no practical consequences for the renal outcomes, safety or exposure to MPA in renal transplant recipients cotreated with CNIs.

References

1. Oshiro C, Mangravite L, Klein T, Altman R. PharmGKB very important pharmacogene: *SLCO1B1* . *Pharmacogent Genomics* . 2010; 20(3):211-216.
2. Kalliokoski A, Niemi M. Impact of OATP transporters on pharmacokinetics. *Br J Pharmacol* . 2009; 158:693-705.
3. Sandoval P, Hagenbuch B. Drug transporter – uptake. In: Kenakin T, ed. *Comprehensive pharmacology* . Cambridge, MA: Elsevier; 2022: 586-607
4. Lam YWF. Principles of pharmacogenomics: pharmacokinetics, pharmacodynamics and clinical implications. Polymorphisms in drug transporter genes. OAT1B1. In: Lam YWF, Scott SA, eds.*Pharmacogenomics.* 2nd ed. London, UK: Academic Press; 2019:24-26.
5. Ramsey LB, Gong L, Lee S et al. PharmVar GeneFocus: *SLCO1B1* .*Clin Pharm Ther.* 2023; 113(4): 782-793.
6. Gene-specific information tables for *SLCO1B1* . PharmGKB. https://www.pharmgkb.org/page/slco1b1RefMaterials. Accessed June 25, 2023.
7. Farasyn T, Crowe A, Hatley O et al. Preincubation with everolimus and sirolimus reduces organic anion-transporting polypeptide (OATP)1B1 – and 1B3 – mediated transport independently of mTOR kinase inhibition: implication in assessing OATP1B1 – and OAT1B3 mediated drug-drug interactions. *J Pharm Sci* . 2019; 108(10)3443-3456.
8. Michelon H, Konig J, Durrbach A et al. SLCO1B1 genetic polymorphism influences mycophenolic and tolerance in renal transplant recipients.*Pharmacogenomics* . 2010; 11(12):1703-1713.

9. Picard N, Yee SW, Woillard JB et al. The role of organic anion-transporting polypeptides and their common genetic variants in mycophenolic acid pharmacokinetics. Clin Pharmacol Ther. 2010; 87(1): 100-108.
10. Tett SE, Saint-Marcoux F, Staatz CEet al. Mycophenolate, clinical pharmacokinetics, formulations, and methods for assessing drug exposure. *Transplant Rev* . 2011; 5(2):47-57.
11. Bergan S, Brunet M, Hesselink DA et al. Personalized therapy for mycophenolate: consensus report by the International association on therapeutic drug monitoring and clinical toxicology. *Ther Drug Monit* . 2021; 43(2):150-200.
12. Lamba V, Sanhavi K, Fish A, Altman RB, Klein TE. PharmGKB summary: mycophenolic acid pathway. *Pharmacogenet Genomics* . 2014; 24(1):73-79.
13. Dalla Vecchia Genvigir F, Cerda A, Dominguez Crespo Hirata T, Hirata MH, Dominguez Crespo Hirata R. Mycophenolic acid pharmacogenomics in kidney transplantation. *J Transl Genet Genom* . 2020; 4:320-355.
14. Takuathung MN, Sakuludomkan W, Koonrungsesomboon N. The impact of genetic polymorphisms on the pharmacokinetics and pharmacodynamics of mycophenolic acid: a systematic review and meta-analysis. *Clin Pharmacokinet* . 2021; 60(10):1291-1302.
15. Liu J, Zhu Y, Zhang J et al. Influence of SLCO1B1 polymorphisms on the pharmacokinetics of mycophenolic acid in renal transplant recipients. *Curr Drug Metab* . 2023; 24(2)114-123.
16. Baumar R, Hesselink DA, van Schaik RHN et al. Mycophenolic acid-related diarrhea is not associated with polymorphisms in SLCO1B nor with ABCB1 in renal transplant recipients. *Pharmacogenet Genomics* . 2012; 22(6):399-407.
17. Miura M, Satoh S, Knoue K et al. Influence of SLCO1B1, 13B, 2B1 and ABCC2 genetic polymorphisms on mycophenolic acid pharmacokinetics in Japanese renal transplant patients. *Eur J Clin Pharmacol* . 2007; 63(12):1161-1169.
18. Sun SS, Shao K, Lu JQ et al. Influence of calcineurin inhibitors and genetic polymorphism of transporters on enterohepatic circulation and exposure of mycophenolic acid in Chinese adult renal allograft patients. *J Clin Pharmacol* . 2023; 63(4):410-420.
19. Varnell CD, Fukuda T, Kirby CL et al. Mycophenolate mofetil-related leukopenia in children and young adults following kidney transplantation: influence of genes and drugs. *Pediatr Transplant* . 2017; 21(7): https://doi.org//10.1111/petr.13033
20. Altman DG, Bland MJ. Absence of evidence is not evidence of absence. BMJ 1995; 311/7003):485.
21. Hilbrands L, Budde K, Bellini MI et al. Allograft function as endpoint for clinical trials in kidney transplantation. *Transpl Int* . 2022; 35:10139. doi: 10.3389/ti.2022.10139
22. Schold JD, Nordyke RJ, Wu Z, Corvino F, Wang W, Mohan S.Clinical events and renal function in the first year predict long-term kidney transplant survival. *Kidney360* . 2022; 3(4):714-727.
23. Hernan MA, Robins JM. Using big data to emulate a target trial when a randomized trial is not available. *Am J Epidemiol* . 2016; 183 (8):758-764.
24. Ratitich B, Goel N, Mallinckrodt C et al. Defining efficacy estimands in clinical trials: examples illustrating ICH E9 (R1) guidelines. *Ther Innov Regul Sci* . 2020; 54(2):370-384.
25. Ratitch B, Bell J, Mallinckrodt C et al. Choosing estimands in clinical trials: putting the ICH E9(R1) into perspective. *Ther Innov Regul Sci* . 2020; 54(2):324-341.
26. Božina N, Lalić Z, Nađ Škegro S et al. 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. *Eur J Clin Pharmacol* . 2017; 73(9)1129-1140.
27. Borić Bilušić A, Božina N, Lalić Z et al. Loss of function ABCG2 c.421C>A (rs2231142) polymorphism increases steady-state exposure to mycophenolic acid in stable renal transplant recipients: an exploratory matched cohort study. *Adv Ther* . 2023; 40(2):601-618.
28. Greifer N. WeightIt: Weighting for Covariate Balance in Observational Studies. 2023. https://github.com/ngreifer/WeightIt
29. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing,2020. Vienna, Austria

30. Huling JD, Mak S. Energy balancing of covariate distributions. arXiv:2004.13962. https://doi.org/10.48550/arXiv.2004.13962.
31. Huling JD, Greifer N, Chen G. Independence weights for causal inference with continuous treatments. *J Am Stat Assoc* . 2023; https://doi.org/10.1080/01621459.2023.2213485
32. Goodrich B, Gabry J, Ali I, Brilleman S. rstanarm: Bayesian applied regression modeling via Stan. R package version 2.21.4, 2023, https://mc-stan.org/rstanarm/.
33. Gaunt TR, Rodriquez S, Day INM. Cubic exact solution for the estimation of pairwise haplotype frequencies: implications for linkage disequlibrium analysesand a web tool 'CubeX'. *BMC Bioinformatics* . 2007; 8:428.doi:10.1186/1471-2105-8-428
34. Schneeweiss S. Sensitivity analysis and external adjustment for unmeasured confounders in epidemiologic database studies of therapeutics. *Pharmacoepidemiol Drug Saf* . 2006; 15(5):291-303.
35. Heine D. The episensr package: basic sensitivity analysis for epidemiological results. doi:10.5281/zenodo.7942249, R package version 1.2.0, 2023, https://dhaine.github.io/episensr/
36. Alexander SPH, Fabbro D, Kelly E, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: Enzymes. *Br J Pharmacol* . 2019;176 Suppl:S297-S396.
37. Shah S, Harwood SM, Dohler B, Opelz G, Yaqoob MM. Inosine monophosphate dehydrogenase polymorphisms and renal allograft outome. *Transplantation.* 2012; 94:486-491.
38. Mehta Cherikh W, Sood P, Hariharan S. Kidney allograft surveillance biopsy practices across US transplant centers: a UNOS survey. *Clin Transplant.* 2017;31: https://doi.org/10.1111/ctr.12945.
39. Lee DM, Abecassis MM, Friedewald JJ, Rose S, First MR. Kidney graft surveillance biopsy utilization and trends: results from a survey of high-volume transplant centers. *Transplant Proc* . 2020; 52:3085-3089.

Table 1 . Baseline patient characteristics: raw data, overall, and by *SLCO1B1 c.521T>C* genotype - variant carriers, TC or CC (treated), and wild-type subjects (TT) (controls). Data are median (min-max), mean±SD or count (%). Shown are standardized differences (d, values <0.1 indicate minor and irrelevant differences) for variant carriers vs. wild type subjects.

	All patients	Variant allele	Wild type	d
N	254	86	168	—
SLCO1B1 c.521T>C variant carrier	86 (33.9)	—	—	—
SLCO1B1 c.521T>C wild-type	168 (66.1)	—	—	—
Age (years)	52 (16-73)	51 (16-73)	52 (16-73)	-0.039
Men	147 (57.9)	43 (50.0)	104 (61.9)	-0.242
Body mass index (kg $m^{2\,-1}$)	25.5±4.2	25.6±4.4	25.5±4.1	0.010
Underlying renal disease				

	All patients	Variant allele	Wild type	d
Congenital	75 (29.5)	27 (31.4)	48 (28.6)	0.062
Glomerulonephritis	74 (29.1)	22 (25.6)	52 (40.0)	-0.119
Diabetic nephropathy	13 (5.1)	5 (5.8)	8 (4.8)	0.047
Hypertensive nephropathy	18 (7.1)	6 (7.0)	12 (7.1)	-0.006
Pyelonephritis	15 (5.9)	5 (5.8)	10 (6.0)	-0.006
Other & unknown	59 (23.3)	21 (24.4)	38 (22.6)	0.083
Hemodialysis vintage (months)	29 (0-180)	27 (5-168)	30 (0-180)	-0.029
Hemodialysis vintage >24 months	141 (55.5)	45 (52.3)	96 (57.1)	-0.097
Living donor	6 (2.4)	0	6 (3.6)	—
Second (vs. first) transplantation	29 (11.4)	6 (7.0)	23 (13.7)	-0.222
HLA A mismatch				
None	80 (31.5)	28 (32.6)	52 (30.0)	0.034
Partial	138 (54.3)	46 (53.5)	92 (54.8)	-0.026
Complete	36 (14.2)	12 (13.9)	24 (14.2)	-0.009
HLA B mismatch				
None	30 (11.8)	6 (7.0)	24 (14.3)	-0.238
Partial	141 (55.5)	47 (54.6)	94 (56.0)	-0.026
Complete	83 (32.7)	33 (38.4)	50 (29.7)	0.182
HLA DR mismatch				
None	55 (21.6)	18 (20.9)	37 (22.0)	-0.027
Partial	179 (70.5)	61 (70.9)	118 (70.2)	0.015
Complete	20 (7.9)	7 (8.1)	13 (7.7)	0.015

	All patients	Variant allele	Wild type	d
Severe mismatch[1]	111 (43.7)	42 (48.8)	69 (41.1)	0.157
Cold ischemia duration (hours)	13 (0.1-35)	12 (3.1-24.0)	13 (0.1-35)	-0.223
Ischemia >14 hours	107 (42.1)	31 (38.1)	76 (41.1)	-0.188
Null biopsy				
Not done	74 (29.1)	17 (19.8)	57 (33.9)	-0.323
No pathology	108 (42.5)	39 (45.4)	69 (41.1)	0.086
Any pathology	72 (28.4)	30 (34.8)	42 (25.0)	0.217
Graft function delayed	68 (26.8)	25 (29.1)	43 (25.6)	0.078
ABCB1 2677/1236/3435 diplotype				
0-1 variant alleles	72 (28.3)	27 (31.4)	45 (26.8)	0.102
2-3 variant alleles	103 (45.6)	35 (40.7)	68 (40.5)	0.004
4-6 variant alleles	79 (31.1)	24 (27.9)	55 (32.7)	-0.105
IMPDH2 3757T>C variant allele	52 (20.5)	17 (19.8)	35 (20.8)	-0.026
UGT1A9 -275/-2152 diplotype				
Variant	19 (7.5)	5 (5.8)	14 (8.3)	-0.098
ABCG2 c.421C>A variant allele	46 (18.1)	15 (17.4)	31 (18.5)	-0.026
Hypertension	191 (75.2)	66 (76.7)	125 (74.4)	0.054

	All patients	Variant allele	Wild type	d
Other cardio-/cerebrovascular	50 (19.7)	16 (18.6)	34 (20.2)	-0.041
Diabetes	15 (5.9)	4 (4.6)	11 (6.5)	-0.083

Continues

Table 1 continued

Autoimmune disease	7 (2.8)	1 (1.2)	6 (3.6)	-0.159
History of malignancy	8 (3.2)	5 (5.8)	3 (1.8)	0.211
Immunosuppressant induction				
None	40 (15.7)	8 (9.3)	32 (19.1)	-0.282
Interleukin 2 antagonists	199 (78.4)	74 (86.1)	118 (70.2)	0.343
Other (thymoglobulin, rituximab)	15 (5.9)	4 (4.6)	18 (10.7)	-0.163
Maintenance immunossuppression				
Calcineurin inhibitor				
Cyclosporine A	80 (31.4)	25 (29.1)	55 (32.7)	-0.079
Tacrolimus	174 (68.5)	61 (70.9)	113 (67.3)	0.079
Mycophenolic acid				
None[2]	5 (2.0)	2 (2.3)	3 (1.8)	0.038
Mycophenolate mofetil	88 (34.6)	31 (36.1)	57 (33.9)	0.044
Enteric-coated[3]	161 (63.4)	53 (61.6)	108 (64.3)	-0.055
Other (sirolimus, everolimus)	5 (2.0)	2 (2.3)	3 (1.8)	0.038
Corticosteroids	254 (100)	86 (100)	168 (100)	0.000
Leukocyte count (x 10^9 L^{-1})	6.8 (3.3-17.8)	6.3 (3.5-17.8)	6.9 (3.3-16.9)	-0.150

Immunosuppressant induction				
Hemoglobin ($g\ L^{-1}$)	118±14	116±13	119±15	-0.209
Platelets (x 10^9 L^{-1})	195 (85-771)	197 (86-771)	194 (85-396)	0.042

[1]At least one locus complete + one locus partial mismatch

[2]Everolimus or sirolimus to start with, shortly switched to mycophenolic acid

[3]Enteric-coated sodium mycophenolate

Table 2 . Baseline patient characteristics after covariate balancing between the *SLCO1B1 c.521T>C* variant allele carriers (treated) and wild-type subjects (controls). Data are mean±SD or count (%). Standardized differences (d) <0.1 indicate minor and irrelevant differences.

	Variant	Wild type	d
N	86	168	—
Age (years)	49±13	50±12	-0.020
Men	45.9 (53.7)	95.7 (57.1)	-0.069
Body mass index (kg/m^2)	25.5±4.4	25.5±4.0	-0.017
Underlying glomerulonephritis	25.0 (29.0)	52.4 (31.3)	-0.050
Other chronic kidney diseases	61.0 (71.0)	115.6 (68.7)	0.050
Hemodialysis vintage up to 24 months	37.5 (43.7)	73.1 (43.6)	0.002
Hemodialysis vintage > 24 months	48.5 (56.3)	94.9 (56.4)	-0.002
First transplantation	77.0 (89.8)	148.9 (88.9)	0.029
Second transplantation	9.0 (10.2)	19.1 (11.1)	-0.029
Severe mismatch[1]	39.0 (45.6)	73.3 (43.8)	0.037
Ischemia duration (hours)	12.8±4.3	13.0±4.8	-0.041
Null biopsy			
Not done	22.0 (25.7)	47.7 (28.5)	-0.063

	Variant	Wild type	d
No pathological findings	38.1 (44.5)	72.6 (43.3)	0.025
Any pathology	25.9 (29.8)	47.7 (28.2)	0.033
Graft function delayed	23.1 (27.0)	44.6 (26.6)	0.009
ABCB1 2677/1236/3435 diplotype			
0-1 variant alleles	25.5 (29.4)	48.7 (29.0)	0.008
2-3 variant alleles	35.3 (41.2)	68.5 (40.7)	0.010
4-6 variant alleles	25.2 (29.4)	50.8 (30.3)	-0.019
IMPDH2 3757T>C			
Variant carriers	18.0 (21.0)	34.9 (20.5)	0.010
Wild type	68.0 (79.0)	133.1 (79.5)	-0.010
UGT1A9 -275T>A /-2152C>T diplotype			
Variant	80.5 (93.8)	11.6 (6.9)	-0.028
Wild type	5.5 (6.2)	156.4 (93.1)	0.028
ABCG2 c.421C>A			
Variant carriers	70.0 (81.7)	31.0 (18.5)	-0.006
Wild type	16.0 (18.3)	137.0 (81.5)	0.006
Hypertension or cardio-/cerebrovascular incidents	67.1 (78.6)	132.1 (78.9)	-0.008
Diabetes, autoimmune disease or cancer history	9.9 (11.6)	19.1 (11.4)	0.006
Immunosuppressant induction			
None	12.0 (13.8)	26.0 (15.4)	-0.047
Interleukin 2 antagonists	69.8 (81.3)	133.0 (79.2)	0.055
Other (thymoglobulin, rituximab)	4.1 (4.9)	9.0 (5.4)	-0.024

	Variant	Wild type	d
Maintenance immunosuppression			
Cyclosporine A	27.2 (31.7)	53.0 (31.4)	0.006
Tacrolimus	58.8 (68.3)	115.0 (68.6)	-0.006
Mycophenolic acid			
No (used sirolimus or everolimus)	1.6 (1.9)	3.1 (1.9)	0.001
Yes (any formulation)	84.4 (98.1)	164.9 (98.1)	-0.001

[1]At least one locus complete + one locus partial mismatch

Effective sample sizes were 72.5 treated and 141.3 controls; mean±SD weights were 1.0±0.432 (range 0.20-2.35) and 1.0±0.424 (range 0.20-2.35), respectively, and entropies were 0.098 and 0.094, in the treated and controls, respectively.

Table 3 . Co-primary and complementary outcomes: adjusted estimates of the estimated glomerular filtration rate (eGFR) rate of change (slope) from day 1 to day 28, and from day 28 to day 365 after transplantation; and eGFR values at days 1, 28, and 365 in *SCLO1B1 c.521T>C* variant allele carriers (treated) and wild-type controls. The rate of change is expressed as a relative (percent) change: per 1 day for the period between days 1 and 28, and per 28 days for the period between days 28 and 365. Differences between treated and controls are expressed as geometric means ratios (GMR) for treated vs. controls.

	Variant allele (95%CI)	Wild type (95%CI)	GMR (95%CI)
Co-primary outcomes			
Slope Day 1-28 (eGFR/day) (%)	5.4 (4.7, 5.9)	5.5 (5.1, 5.9)	0.99 (0.92-1.06)
Slope Day 28-365 (eGFR/28 days) (%)[1]	-0.8 (-1.6, -0.1)	-1.4 (-0.9, -2.0)	0.98 (0.94-1.01)
Complementary data			
eGFR Day 1 (mL/min/1.73m^2)	12.2 (10.1-14.6)	12.2 (10.4-14.3)	1.00 (0.82-1.21)
eGFR Day 28 (mL/min/1.73m^2)	49.9 (43.2-57.6)	51.3 (44.7-58.8)	0.97 (0.86-1.09)
eGFR Day 365 (mL/min/1.73m^2)	45.2 (40.0-51.1)	43.2 (38.0-49.0)	1.05 (0.95-1.15)

[1]The slope from day 28 to day 365 had a negative sign in both patient groups. The difference (GMR) refers to absolute values, hence the slopes of -0.8%/28 days and of -1.4%/28 days yield a GMR=0.98.

Table 4 . Kidney-related adverse outcomes and adverse events (raw data and adjusted estimates) in *SLCO1B1 c.521T>C* ariant allele carriers (treated) and wild-type subjects (controls). Differences between treated and controls are expressed as rate ratios (RR).

	Variant allele	Wild type	RR (95%CI)
N	86	168	
Kidney-related adverse outcomes			
Death	0	2 (graft failure)	—
Graft failure	0	3 (2 died)	—
N with an indication for renal biopsy	15 (17.4)	26 (15.5)	—

	Variant allele	Wild type	RR (95%CI)
Total number of renal biopsies	16	31	—
Died or graft failure or biopsy	15 (17.4)	28 (16.7)	—
Total deaths, failures, or biopsies	16	34	—
Adjusted annual rate of deaths, failures, biopsies	0.22 (0.11, 0.45)	0.23 (0.10, 0.53)	0.94 (0.49-1.84)
Adverse events (AE)			
Cytopenia grade [?]3[1]	14 (16.3)	22 (13.1)	—
Other AE grade [?]2[2]	33 (38.4)	64 (38.1)	—
New onset carcinoma	2	1	—
Died or at least one AE	42 (48.8)	76 (45.2)	—
Total AEs (deaths, AEs, new onset carcinoma)	79	132	—
Adjusted annual rate of AEs	0.81 (0.54, 1.22)	0.75 (0.48, 1.18)	1.08 (0.74-1.58)

[1]Anemia or thrombocytopenia or leukopenia CTCAE grade 3 or higher

[2]Gastrointestinal adverse events, respiratory infections, urinary tract infections, or central nervous system adverse events CTCAE grade 2 or higher. No patient was diagnosed with CMV or herpes simplex virus infections.

Table 5 . Baseline characatersistics of *SLCO1B1 c.521T>C* variant allele carriers (TC or CC genotype) and wild-type controls (TT genotype) in the pharmacokinetic study: before (raw data) and after matching and covariate balancing. Data are mean±SD or count (%). Standardized differences (d) <0.1 indicate irrelevant differences (additional data in Appendix C, C.3, Table C3).

	Before matching & balancing	Before matching & balancing	Before matching & balanci
	Variant		Wild-type
N	25		43
MMF	9 (36.0)		14 (32.6)
EC-MPS	16 (64.0)		29 (67.4)
Cyclosporine	17 (68.0)		26 (60.5)
Tacrolimus	8 (32.0)		17 (39.5)
ABCG2 c.421 variant	4 (16.0)		8 (18.6)
ABCG2 c.421 wild type	21 (84.0)		35 (81.4)
ABCC2 -24 variant	11 (44.0)		10 (23.3)
ABCC2 -24 wild type	14 (56.0)		33 (76.7)
ABCC2 1249 variant	9 (36.0)		18 (41.9)
ABCC2 1249 wild type	16 (64.0)		25 (58.1)
UGT2B7 -161 variant	21 (84.0)		35 (81.4)
UGT2B7 -161 wild type	4 (16.0)		8 (18.6)
UGT1A9 variant diplotype	0		3 (7.0)
UGT1A9 wild type diplotype	25 (100)		40 (93.0)
ABCB1 wild type/ 1 var allele	9 (36.0)		9 (20.9)
ABCB1 2 -3 variant alleles	13 (52.0)		18 (41.9)
ABCB1 4 -6 variant alleles	3 (12.0)		16 (37.2)
Age (years)	50.2±14.5		49.5±12.1
Men	10 (40.0)		26 (60.5)
Women	15 (60.0)		17 (39.5)
Body mass index (kg $m^{2\ -1}$)	23.8±4.6		24.4±3.5
Urine output (L day^{-1})	2.8±0.9		2.6±0.7
eGFR (mL min^{-1} 1.73 $m^{2\ -1}$)	37±12		38±17

	Before matching & balancing	Before matching & balancing	Before matching & balanci
Ln(CNI trough) (μg L $^{-1}$)[2]	5.19±0.40		5.32±0.31

[1]Variant and wild-type subjects were first exactly matched on mycophenolic acid (MPA) formulation (mycophenolate mofetil, MMF, or enteric-coated MPA sodium salt, EC-MPA), type of calcineurine inhibitor (CNI), sex and *UGT1A9 -275T>A /-2152C>T* diplotype. Three wild-type subjects were pruned since the only ones with the variant diplotype. Covariate balancing of the matched data followed. Effective sample sizes: 21.3 variant and 30.4 controls; weights: mean (SD) 1.0 (0.42) variant and 1.0 (0.57) controls.

[2]Linear transformation to ln(cyclosporine trough) scale

eGFR – estimated glomerular filtration rate

Table 6 . Differences (geometric means ratios, GMR) between*SLCO1B1 c.521T>C* variant allele carriers (TC or CC genotype) and wild-type controls (TT genotype) in the main pharmacokinetic outcome (area under the concentration-time curve of mycophenolic acid over dosing interval at steady-state, $AUC_{\tau,\sigma\varsigma}$): raw (unadjusted) data with a test of interaction between the polymorphism and calcineurine (CNI) type (cyclosporine A, CsA, or tacrolimus, TAC); fully adjusted data in the overall sample[1]; partly adjusted data, separately in patients cotreated with CsA and with tacrolimus for the test of the interaction polymorphism * CNI type[2]. Shown are frequentist and Bayesian estimates, and respective P-values / probabilities (Prob., %).

	Frequentist	Frequentist	Frequentist	Bayes	Bayes	Bayes
	GMR (95%CI)		P/Prob (%)	GMR (95%CrI)		Prob (%)/P
Raw data						
Overall TC/CC (n=25) vs. TT (n=43)	1.18 (0.94-1.48)		0.155/92.2	1.16 (0.93-1.45)		91.4/0.1
SLCO1B1 c.521T>C * CNI			0.903/54.8			60.2/0.7
CsA: TC/CC (n=17) vs. TT (n=26)	1.22 (0.93-1.59)			1.21 (0.93-1.55)		
TAC: TC/CC (n=8) vs. TT (n=17)	1.18 (0.82-1.72)			1.15 (0.86-1.53)		
Fully adjusted data[1]						
Overall TC/CC vs. TT	1.11 (0.97-1.27)		0.115/94.2	1.04 (0.80-1.39)		61.6/0.7

	Frequentist	Frequentist	Frequentist	Bayes	Bayes	Bayes
Partly adjusted data[2] *SLCO1B1 c.521T>C* * CNI			0.770/61.5			51.7/0.9
CsA: TC/CC vs. TT	1.19 (0.94-1.50)			1.19 (0.79-1.75)		
TAC: TC/CC vs. TT	1.29 (0.78-2.14)			1.16 (0.79-1.79)		

[1] Analysis in the matched and balanced (as shown in Table 5) variant allele carriers and wild-type controls with additional adjustment for suboptimally matched variables (d[?]0.1) in Table 5.

[2] Variant carriers and wild-type subjects were matched and balanced separately in the subset cotraeted with CsA and in the subset cotreated with tacrolimus. Since these subsets were limited in size, a reduced number of covariates were considered – see Appendix C, C.6, Tables C6-C7 for details.

Table 7 . Sensitivity of the estimated effects of the*SLCO1B1 c.521T>C* variant allele (genotype TC or CC, treatment) vs. wild-type genotype (TT, control) on the primary outcomes in the main study - slope of the estimated glomerular filtration rate (eGFR) to day 28, and from day 28 to day 365 – and on the total exposure ($AUC_{\tau,\sigma\varsigma}$) in the pharmacokinetic study. For the main study, we assumed a hypothetical uncontrolled (unmeasured) covariate (or a set of covariates) with an overall prevalence of 58%, and a strong biasing effect expressed as geometric means ratio (GMR) of 0.60 or 0.79. We corrected the observed effects (GMRs) for the biasing effect assuming a high chance imbalance in the prevalence of the biasing effect between treated and controls of 1.5:1.0 or 2.0:1.0 and*vice-versa* . For the pharmacokinetic (PK) study, we assumed a hypothetical unmeasured covariate (or a set of covariates) with an overall prevalence of 10%, and a strong biasing effect expressed as GMR=1.50. We corrected the observed GMRs for this effect, assuming a high chance imbalance in its prevalence between treated and controls of 4:1 and *vice-versa* (for details see Appendix B, B.9).

Outcome	Observed GMR	Biasing GMR	Prevalence of biasing variable(s)	Correct GMR
Main study				
Slope to day 28	0.99 (0.92-1.06)	0.60	Variant 64/86 (75%); wt 83/168 (50%) Imbalance ratio variant vs. wt: 1.5:1.0	1.13 (1.05-1.

Outcome	Observed GMR	Biasing GMR	Prevalence of biasing variable(s)	Correct GMR
			Variant 37/86 (43%); wt 110/168 (65%) Imbalance ratio variant vs. wt: 1.0:1.5	0.89 (0.82-0.
		0.79	Variant 76/86 (86%); wt 73/168 (43%) Imbalance ratio variant vs. wt: 2.0:1.0	1.10 (1.02-1.
			Variant 30/86 (35%); wt 117/168 (70%) Imbalance ratio variant vs. wt: 1.0:2.0	0.91 (0.85-0.
Slope day 28-365	0.98 (0.94-1.01)	0.60	Variant 64/86 (75%); wt 83/168 (50%) Imbalance ratio variant vs. wt: 1.5:1.0	1.12 (1.07-1.
			Variant 37/86 (43%); wt 110/168 (65%) Imbalance ratio variant vs. wt: 1.0:1.5	0.88 (0.84-0.

Outcome	Observed GMR	Biasing GMR	Prevalence of biasing variable(s)	Correct GMR
		0.79	Variant 76/86 (86%); wt 73/168 (43%) Imbalance ratio variant vs. wt: 2.0:1.0	1.09 (1.04-1.
			Variant 30/86 (35%); wt 117/168 (70%) Imbalance ratio variant vs. wt: 1.0:2.0	0.90 (0.87-0.
PK study $AUC_{\tau,\sigma\varsigma}$				
Raw data, overall	1.18 (0.94-1.48)	1.50	Variant 5/25 (20%); wt 2/43 (5%) Imbalance ratio variant vs. wt: 4.0:1.0	1.10 (0.88-1.
Freq., Bayes	1.16 (0.93-1.45)			1.08 (0.87-1.
			Variant 1/25 (4%); wt 6/43 (14%) Imbal-ance ratio variant vs. wt: 1.0:4.0	1.24 (0.98-1.55) 1.22 (0.97-1.52)

Outcome	Observed GMR	Biasing GMR	Prevalence of biasing variable(s)	Correct GMR
Adjusted, overall Freq., Bayes	1.11 (0.97-1.27) 1.04 (0.80-1.39)		Variant 5/25 (20%); wt 2/40 (5%) Imbal-ance ratio variant vs. wt: 4.0:1.0	1.03 (0.90-1.18) 0.97 (0.75-1.30)
			Variant 1/25 (4%); wt 6/40 (15%) Imbal-ance ratio variant vs. wt: 1.0:4.0	1.17 (1.02-1.34) 1.10 (0.84-1.46)

Figure 1 . Estimated glomerular filtration rate (eGFR) over time after transplantation in *SLCO1B1 c.521T>C* variant allele carriers (n=86, treated) and wild-type controls (n=168).**A** . Raw data. Depicted are numbers of patients providing eGFR values at each time point. **B** . Adjusted data (after balancing of baseline covariate and adjustment for time-varying covariates). Depicted are adjusted geometric mean eGFR values at days 1, 28 and 365.

Symbols are geometric means, bars are 95% confidence intervals.

List of Appendices

Appendix A – Additional Introduction

Appendix B – Additional Methods

Appendix C – Additional Results