Supplemental Digital Content 2 – Additional Methods

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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1. Outline of the main study

As illustrated in Figure S1, *SLCO1B1 c.521T>C* variant carriers (TC or CC genotype) (exposed) and wild-type (TT) subjects (controls) started on maintenance immunosuppression with glucocorticoids, mycophenolic acid (MPA) and a calcineurin inhibitor (CNI) were balanced on a number of baseline covariates (energy balancing). The estimated glomerular filtration rate was determined weekly over the first month and on four further occasions. Trough CNI

concentrations and body mass index were also repeatedly recorded. Over time, MPA could have been interrupted, withdrawn or transiently replaced (sirolimus, everolimus); CNIs could have been switched, interrupted, withdrawn or replaced. The use of MPA, use/type of CNI, trough concentrations and body mass index were modeled as time-varying covariates.

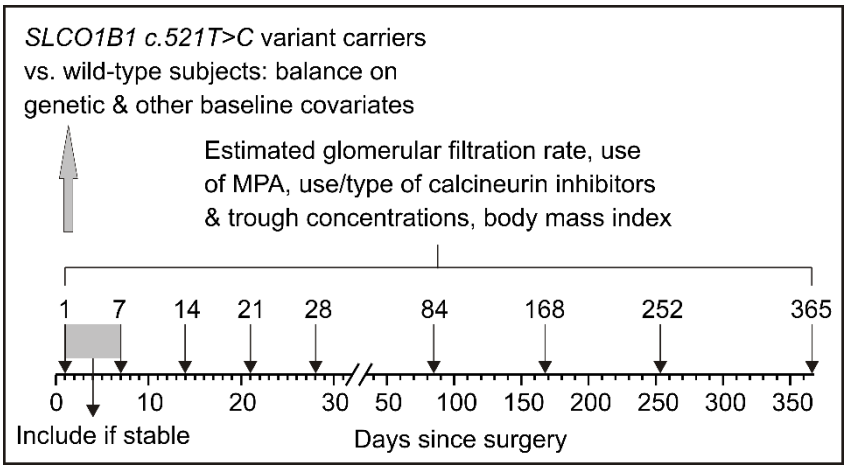

Figure S1. Outline of the main study.

2. Outline of the pharmacokinetic study

Patients started on standard immunosuppression with corticosteroids, MPA – mycophenolate mofetil (MMF) or enteric-coated MPA sodium salt (EC-MPS) – and a CNI [cyclosporine A (CsA) or tacrolimus (TAC)] were closely monitored over 5-7 post-transplant days; on the subsequent day (steady-states of MPA and CNIs achieved), after overnight fast, at 08:00 hours blood samples were taken for quantification of MPA and CNIs, treatments were administered and 6 blood samples were taken over the 12-hour dosing interval (at 0.5, 1, 2, 3, 8 and 12 hours post-dose) for quantification of MPA. Those meeting the eligibility criteria (see the main text) were included in the analysis. *SLCO1B1 c.521T>C* variant carriers (exposed) and wild-type subjects (controls) were matched/ balanced on a number of baseline covariates (exact matching, energy balancing).

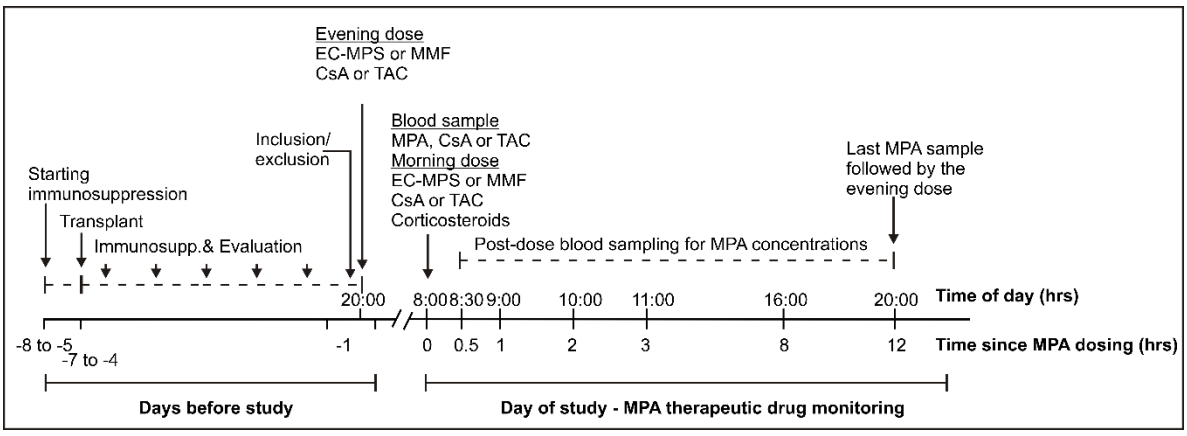

Figure S2. Outline of the pharmacokinetic study.

3. Patients

Since the pharmacokinetic evaluation was planned on a limited number of patients, inclusion/exclusion criteria additional to that employed in the main study (see Main text, "Patients") were defined in order to reduce sources of variability. Patients had to: (a) be judged as "low immunological risk", thus not exposed to induction immunosuppression; (b) be free of significant cardiovascular, gastro-intestinal, hepatic and metabolic comorbidity, since such conditions, and treatments used for such conditions, might interfere with pharmacokinetics of MPA; (c) have serum albumin values (on the morning of the study day) >31 g/L, since hypoalbuminemia affects exposure to MPA (both MPA and MPAG bind to- and compete for serum albumin); (d) have steadily improving renal function, as indicated by (i) serum creatinine <300 μmol/L (corresponds to eGFR of ≥14 mL/min/1.73 m^2) on the morning of the study day, and by at least 1/3 lower than the initial value (1st postoperative day), and (ii) stable diuresis of around 60 mL/h; (e) be free of treatments that might affect absorption and/or clearance of MPA during the pre-study (and study) days (antacids, proton pump inhibitors, phosphate binders, oral iron, magnesium or calcium, antibiotics, rifampicin).

4. Immunosuppression

The standard immunosuppressant protocol consisted of: a) MPA: 1st dose before the transplantation (1x720 mg EC-MPS or 1x1000 mg MMF), continued as EC-MPS 2x720 mg day^{-1} or as MMF 2x500-1000 mg day^{-1} (based on body weight); b) a CNI: 1st dose before the transplantation (CsA 1x3.0 mg kg^{-1} or tacrolimus 1x4.5 mg), continued as CsA 2x3 mg kg^{-1} day^{-1} or tacrolimus 2x4.5 mg day^{-1} with adjustments based on the trough concentrations; c) 60 mg prednisone equivalents starting post-transplantation, with a rapid reduction to 30 mg day^{-1}. Long-term, by far the most patients were kept on 5-10 mg/day prednisone equivalents.

5. Bioanalytical procedures and genotyping

Whole blood CsA and tacrolimus were determined by a validated affinity chrome-mediated immunoassay (ACMIA, Siemens, Germany) Total plasma MPA was determined by high-pressure liquid chromatography (HPLC) with UV/VIS spectrophotometric detection (at 215 nm, 25°C, workflow 1 mL min^{-1}) using a commercially available HPLC kit for MPA in plasma (Chromsystems, Germany). All analytes were included in the external proficiency testing schemes (RfB, Instand, and UK NEQUAS).

Genomic DNA was isolated from whole blood using BioSprint 15 DNA Blood Kit (Qiagen, Hilden, Germany) on KingFisher mL System (Thermo Labsystems, Vantaa, Finland). Genotyping was performed on an Applied Biosystems 7500 Real-Time PCR System, according to manufacturer's

instructions (Applied Biosystems, CA, USA) using a validated TaqMan® Drug Metabolism Genotyping Assays (Life Technologies, Carlsbad, CA, USA) for the following polymorphisms: *IMPDH2* c.*3757T>C* (ID C_1842928_10), *ABCG2 c.421C > A* (rs2231142, ID C_15854163_70); *ABCC2 -24C>T* (rs717620, ID C_2814642_10) and *1249G>A* (rs2273697; ID C_22272980_20); *SLCO1B1 c.521T > C* (rs4149065, ID C_30633906_10); *UGT2B7 –161C>T* (rs7668258, ID C_27827970_40); *UGT1A9-275 T>A* (rs6714486, ID C__27843087_10) and *-2152C>T* (rs17868320, ID C__34418857_10); *ABCB1 c.3435C>T* (rs1045642, ID C___7586657_20) and c.*1236C>T* (rs1128503, ID C___7586662_10). Genotyping of *ABCB1 c.2677G > T/A* (rs2032582) was performed by real-time PCR genotyping on the LightCycler® instrument (Roche Diagnostics, Mannheim, Germany). *ABCB1* and *SLCO1B1* are included in the external proficiency testing schemes (RfB and EMQN respectively).

6. Pharmacokinetic indicators

Standard MPA steady-state measures [peak exposure ($C_{max,ss}$ mg/L), area under the concentration-time curve over the dosing interval of 12 hours ($AUC_{\tau,ss}$ mg x h/L), morning and evening pre-dose concentrations (C_0, C_{12}, mg/L), apparent total body clearance ($CL_{T/F,ss}$ mL/min/kg)] were determined by the non-compartmental method (Kinetica 4.1, InnaPhase Corp., USA). The analysis was based on dose-normalized concentrations (per 1000 mg) 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.

7. Confounding in the main study

Sources of confounding

We aimed to estimate the effect of the *SLCO1B1 c.521T>C* variant allele carriage on graft function in the target population. Considering the complexity of the setting, a number of factors could confound the assessed causal path (variant allele → graft function) due to their known or potential effects on the graft function (Table S1). Considering the specifics of the present study, several of these were of no relevance. All patients were of European (Slavic) descent, hence recipient's race/ethnicity was not a factor to account for. Next, there were only 6 (of 254) living donations – too few (6/168 *SLCO1B1 c.521T>C* wild-type subjects) to reflect on the outcome. Hence, there was no need to account for the type of donation. Regarding the maintenance immunosuppression protocol and compliance: by definition of the target population, MPA had to be included, but could have varied over time (interruption, withdrawal, replacement with mTOR inhibitors), and the same was the case with CNIs (tacrolimus or CsA), which also could have varied over time (switched, interrupted, replaced). Hence, it was not needed to specifically

account for the "use of mTOR inhibitors" – where neither CNI was in use, mTOR inhibitors were used; where MPA was not in use, mTOR inhibitors were used. A large part of the variability in the MPA efficacy and safety/tolerability is ascribed to variable pharmacokinetics. Consequently, it is recommended that MPA is therapeutically monitored (AUC over the dosing interval), but regularly repeated MPA AUC determination over time is still not a widespread practice. Hence, exposure to MPA throughout the observed period remained unmeasured. Based on the knowledge about possible confounders listed in Table S1 [1-11], we generated a directed acyclic graph (DAG) in Figure S3. It depicts several sets of biasing variables. Finally, post-baseline conditions (e.g., infections) could affect the graft function/outcome [1,2]. However, it is also possible that their occurrence could be affected by the evaluated "exposure" – hence, such events could be mediators of the treatment effect. To avoid bias arising from adjustment on post-baseline events [12] intercurrent events were considered as a separate outcome – adverse events (leukopenia episodes, infections, new onset carcinoma, etc.).

Elements depicted in Figure S3:

1. *SLCO1B1* SNPs – other than the investiaged *c.521T>C*. Some of the other *SLCO1B1* SNPs result in a reduced and some in increased OATP1B1 function *in vitro* – this could affect the MPA exposure. **2**. **Demographics** – age, sex, and BMI. It is assumed that they (could): (i) affect the activity of enzymes and transporters known to affect exposure to CNIs (CYP3A4/5, ABCB1). This may reflect on the activity of the immune system (M4) → graft function, or, directly on the graft function (e.g., CNI toxicity). Also, exposure to CNIs may reflect on exposure to MPA, since both CNIs (and particularly CsA) affect exposure to MPA. MPA exposure reflects on IMPDH enzyme activity (M3), and consequently on the immune system activity (M4) → graft function; or, directly on the graft function (e.g, toxicity); (ii) affect the activity of enzymes and transporters known to participate in MPA pharmacokinetics (UGT1A9 [key] with the contribution of other UGT enzymes, ABCB1, ABCC2, OATP1B1 and other OATP1B transporters [e.g., OATP1B3], and ABCG2), with consequences as explained; (iii) affect some peritransplantational events, for example, delayed onset of graft function (with consequences for the outcome); (iv) affect CNI and/or MPA exposure, IMPDH activity, immune system activity, and graft function (eGFR) "directly", i.e, through some other potential/hypothetical mechanisms.; (v) contribute to the "general health status" (or frailty), which, however, is primarily defined by other variables.

Table S1. Factors considered as potential sources of confounding bias in the estimation of the causal effect of the *SLCO1B1 c.521T>C* variant allele on graft function over the first 12 months after renal transplantation.

Potential sources of confounding bias	Ref.
Donor & recipient characteristics, transplantation & peritransplantation-related factors: (i) donor's age, health status, whether living (related/unrelated) or cadaveric donation; (ii) recipient's demographics, history of dialysis, underlying kidney disease, race/ethnicity, overall or "general health status" (fitness, frailty); (iii) first or a repeated transplantation, extent of HLA mismatch; (iv) duration of cold ischemia, delayed onset of the graft function, findings of the null biopsy.	1
Immunosuppression-related factors: (i) induction (yes/no) and used treatments; (ii) maintenance protocol and compliance (e.g., type of CNI, use of mTOR inhibitors; interruption/switching over time).	1
Intercurrent (post-transplant) events: infections, cardio-/cerebrovascular incidents, trauma, new-onset malignancies, and similar.	2
"Classical" factors with known/potential effects on exposure to MPA (regardless of the formulation): recipient's age, sex, BMI, food, gut microbiota, hypoalbuminemia, possibly comorbidities reflecting on the renal and hepatic function; drug-drug interactions(CNIs – mainly CsA; UGT enzyme inhibitors/inductors; possibly also ABCB1 inhibitors/substrates and drugs affecting gastrointestinal pH, or drugs that contain metal ions).	3-5
"Classical" factors with known/potential effects on exposure to CNIs: demographics, BMI, possibly comorbidities, drug-drug interactions (particularly CYP3A4/5 inhibitors/inducers, ABCB1 inhibitors)	6
Polymorphisms associated with MPA exposure (apart from *SCLO1B1 c.521T>C*). Considering the complex PK or MPA, a large number of polymorphisms (enzymes, transporters) have been evaluated. Only some of them have been suggested associated with MPA exposure – but not unambiguously (e.g., opposing results, both "positive" and "negative" reports, questionable study quality, etc.). The following might be at least to some extent relevant: (i) *UGT1A9:* -275T>A (rs6714486), -2152C>T (rs1786832), c.98T>C (*3, rs72551330); (ii) *UGT2B7*: c.802T>C (rs7439366); (iii) *SLCO1B3*: c.334T>G (rs4149117); (iv) *ABCB1*: *ABCB1c. 2677G>T/A* (rs2032582), *c.3435C>T* (rs1045642), c.*1236C>T* (rs1128503); (v) *ABCC2*: -24C>T (rs717620); (vi) *ABCG2*: c.421C>A (rs2231142).	5, 7-9
Polymorphisms associated with CNI exposure. For both CsA and tacrolimus, SNPs defining the CYP3A5 phenotype, possibly also *ABCB1 c.2677G>T/A* (rs2032582), *c.3435C>T* (rs1045642), c.*1236C>T* (rs1128503).	6,10
Polymorphisms in the *IMPDH* genes. *IMPDH2 – 3757T>C* (rs11706052); *IMPDH1* – rs2278293, 2278294.	7, 8
Polymorphisms in genes encoding proteins involved in immunity/inflammation. Around 60 SNPs in genes encoding eicosanoids, interferons, interleukins, other cytokines, their receptors, signaling molecules, cell-cycle regulating molecules, transcription factors, adhesion molecules toll-like receptors, and other inter- and intracellular molecules have been sporadically reported associated with adverse graft outcomes in renal transplantation.	11

3. Major background (non-renal) diseases. Non-renal comorbidities might preclude indication for renal transplantation, but generally, renal transplant recipients may suffer a number of concomitant diseases. We considered that for the current setting (with eGFR as the primary outcome), hypertension, diabetes, autoimmune diseases, history of malignancy, and cardio-/cerebrovascular incidents should be of particular interest. This subsumes also treatments received for these conditions. We considered that these conditions cumulatively were one of the major factors defining the **"general health status"** (frailty). The other major contribution comes from the underlying **renal pathology**, which includes the type of primary

kidney disease and dialysis vintage. Demographics and **other background diseases** contribute. It is assumed that the general health status (could): (i) affect the activity of enzymes and transporters relevant for exposure to CNIs and MPA (with downstream consequences); (ii) MPA/CNI levels by possible other mechanisms; (iii) peritransplantational events, e.g., delayed graft function; and (iv) directly, i.e., through other possible mechanisms – IMPDH activity (M3), immune system activity (M4), and the graft function (eGFR). **4. Other background (non-renal) diseases** – minor contribution to the "general health status"; possible interactions between the prescribed drugs and CNIs/MPA**. 5. Renal pathology**. Apart from contributing to the "general health status" (particularly history of/duration of dialysis; broader contexts of some underlying disease, e.g., polycystic kidney disease), underlying renal pathology might reflect on the graft function: glomerulonephritis, for example, might recur. **6. Transplantation-related factors** – the type of donor (cadaveric or live) is not an issue in the present setting (as explained). Donor's age and health status might reflect directly on the graft function. The extent of HLA mismatch, and whether it was the first or repeated transplantation are each (and jointly) considered to potentially affect peritransplantational events (e.g., delayed onset of graft function; findings of the null biopsy) and/or the activity of the immune system (M4), all of which could, through further mediators, affect the graft function, or, graft function could be affected "directly", i.e,. through some other potential mechanisms.

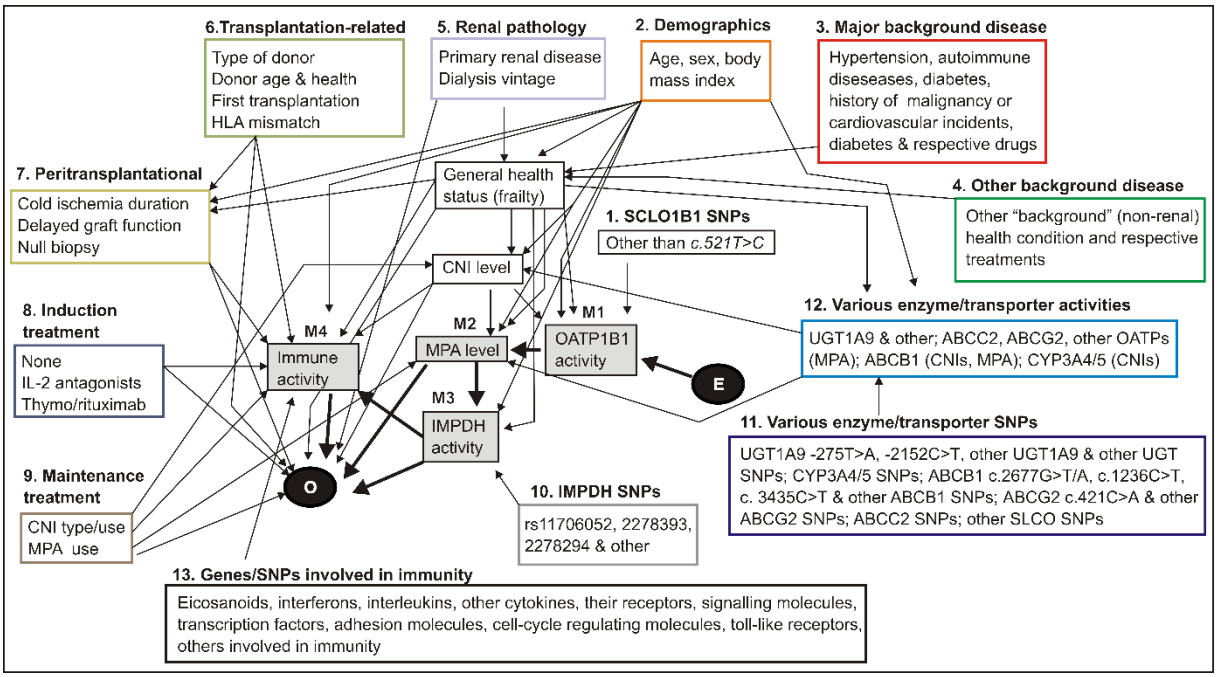

Figure S3. DAG illustrates the setting regarding the primary outcome (package *daggity* [13] in R). The evaluated causal path extends between the two black circles (E, O) connected with thick black arrows. Gray-filled squares indicate mediator variables that were not directly quantified. The exposure (E) is the *SLCO1B1 c.521 T>C* variant allele (TC/CC), and control is the wild-type genotype (TT). The outcome (O) is the estimated glomerular filtration rate (continuous, longitudinal). The path includes 4 mediators (M1-M4): treatment has no other means to affect the outcome but to affect OATP1B1 activity (M1), which in turn reflects on the MPA level (M2) that affects IMPDH activity (M3) to result in the changed activity of the immune system (M4) – presumably affecting the outcome. MPA level (M2) might affect the outcome directly (e.g., toxicity), and IMPDH activity (M3) (since present in a variety of cells) might to some extent affect the graft function by some other mechanism(s) ("directly"). Since the exposure is a genotype, there are no classical confounders that cause both the exposure and the outcome[12]. There are, however, many factors that may affect the outcome and the mediators, and could be non-causally associated with the treatment. DAG does not include a depiction of non-causal associations, but these are assumed for all shown covariates. Covariates could be classified into groups. Although numerous causal relationships could exist between the individual variables within and between such groups, marked are known or biologically plausible causal effects on a "group level". All such effects are depicted by thin black arrows. See text. ABCB1 ATP binding cassette subfamily B member 1 (P-glycoprotein); ABCC2 – ATP binding cassette subfamily C member 2 (multidrug resistance-associated protein 2); ABCG2 – ATP binding cassette subfamily C member 2 (breast cancer resistance protein); CNI – calcineurin inhibitors; CYP3A4/5 – cytochrome P-450 enzymes 4/5; HLA – human leukocyte antigen; IL – interleukin; IMPDH – inosine monophosphate dehydrogenase; MPA – mycophenolic acid; SLCO1B1 – solute carrier organic anion transporter family member 1B1 (OATP1B1); Thymo – thymoglobulin; UGT – uridine 5'-diphospho-glucuronosyltransferases

7. Peritransplantational events. While the duration of the cold ischemia might reasonably be expected to contribute to pathological findings already at null biopsy, and/or to the delayed graft function, these factors are considered jointly. It is presumed that they could be affected by several factors (see above) and that they could have effects on the immune system activity (M4), or "directly" (i.e., through some other potential mechanism) on the graft function. **8. Induction treatment** –whether applied or not, and treatments used, are considered to affect the activity of the immune system (M4), or, "directly" (e.g., toxicity) the graft function. **9. Maintenance treatment**. Type of CNI – although both CsA and tacrolimus are valid options for maintenance immunosuppression in renal transplantation, they might differently affect the outcome: (i) "directly" (e.g., different renal toxicity); (ii) by (slight) differences in the level of immune suppression. Both these effects depend on the level of exposure – but the two drugs, with the recommended posology, might differ also in how "successful" they are in achieving the desired (target) exposure (represented by the trough concentrations). MPA – the two formulations (MMF, EC-MPS) are considered therapeutically equivalent, hence we made no distinction in this respect. Our objective was to estimate the effect of the particular *SLCO1B1* SNP on the outcome specifically in MPA-treated patients. Generally, this implies that MPA was used throughout the observed period. However, in daily life, in patients started on MPA, over time (and for any reason) MPA could be transiently interrupted or permanently withdrawn or replaced with other immunosuppressants. With respect to the present objective, such changes represent "changes in patients' state". However, considering the defined estimand, we reasoned that all patients treated for at least a month during the early post-transplant phase should be included, and MPA use modeled as an external time-varying covariate (regardless of the reasons for potential interruption/withdrawal). MPA might affect the outcome through the effect on IMPDH2 activity (M3), or, potentially "directly" (e.g., toxicity). All effects are likely dependent on the level of MPA exposure, but MPA exposure was not quantified. **10. *IMPDH* SNPs** – depicted are three SNPs (one *IMPDH2* – affects the IMPDH2 activity *in vitro*; two *IMPDH1*) that have been indicated as related to outcomes in renal transplant patients. Theoretically, any of the (many) other *IMPDH2* or *IMPHD1* SNPs could have influenced the enzyme activity, hence the MPA effect on the outcome. **11. Various enzyme/transporter SNPs /12. Respective enzyme/transporter activity**. Although not without inconsistencies, several SNPs have been suggested to affect the activity of the respective proteins (enzymes: primarily CYP3A4/5 regarding CNIs, primarily UGT1A9 [with possible contribution of some other UGTs] regarding MPA; transporters: ABCB1 regarding both CNIs and MPA, ABCC2, SLCO1B1, [other than the evaluated SNP] possibly other SLCO transporters, and ABCG2 regarding MPA) that may reflect on the exposure to CNIs and/or MPA. Considering their physiological functions other than drug metabolism and transport, the activity of at least some of these enzymes/transporters could potentially affect the graft function

by some other mechanism. However, we considered this to be highly unlikely – hence enzymes/transporters and respective SNPs are viewed exclusively from the standpoint of possible effects on exposure to immunosuppressants. **13. Genes/SNPs encoding proteins involved in the immune system activity**. As depicted in Table S1 and recently reviewed [11[, a number of genes/polymorphisms directly or indirectly involved in immune system activity/cell cycle regulation have been sporadically implicated (in candidate gene studies or in [still limited] genome-wide association studies) in renal adverse outcomes.

Control of confounding

We combined balancing of the exposed and controls on baseline covariates with statistical modeling of BMI, MPA use, type of CNI and CNI troughs as time-varying covariates. As already stated, owing to the particulars of the present setting, recipient's race/ethnicity, cadaveric/live donation and (non)use of mTOR inhibitors were not an issue. Figure S4 presents a DAG that incorporates measures undertaken to control the confounding.

Fully controlled variables are depicted in Figure S4 by the removal of black arrows starting from them (indicating that no causal effects emerge from these variables) and the strikethrough text. *Demographics* – exposed (*SLCO1B1 c.521T>C* TC or CC genotype) and control (wild-type, TT) subjects were balanced at baseline regarding age, sex, and body mass index. Body mass index was also modeled as a time-varying covariate. *Major background (non-renal) disease* – exposed and control subjects were balanced at baseline regarding all of the listed diseases. They were not balanced specifically with respect to treatments delivered for these background diseases, but these are considered to be generally similar. *Renal pathology* – exposed and controls were balanced at baseline for primary renal disease (glomerulonephritis vs. other) and dialysis vintage (up to or longer than 24 months). *Transplantation –related variables*. Exposed and control subjects were balanced at baseline for the proportion of first and second transplantations, as well as regarding the severe HLA mismatch (at least one locus complete + one locus partial mismatch). *Peritransplantational events* – exposed and controls were balanced at baseline for the duration of cold ischemia, delayed graft function and the results of the null biopsy (not done, no pathology, some pathology). *Induction treatment* – exposed and controls were balanced at baseline for implementation of induction therapy and used drugs. *Maintenance treatment* – exposed and control were balanced at baseline for the type of CNI and MPA use – these variables were also modeled as time-varying covariates. *Exposure to CNIs (CNI troughs)* – CNI troughs were modeled as a time-varying covariate categorized as explained in the main text.

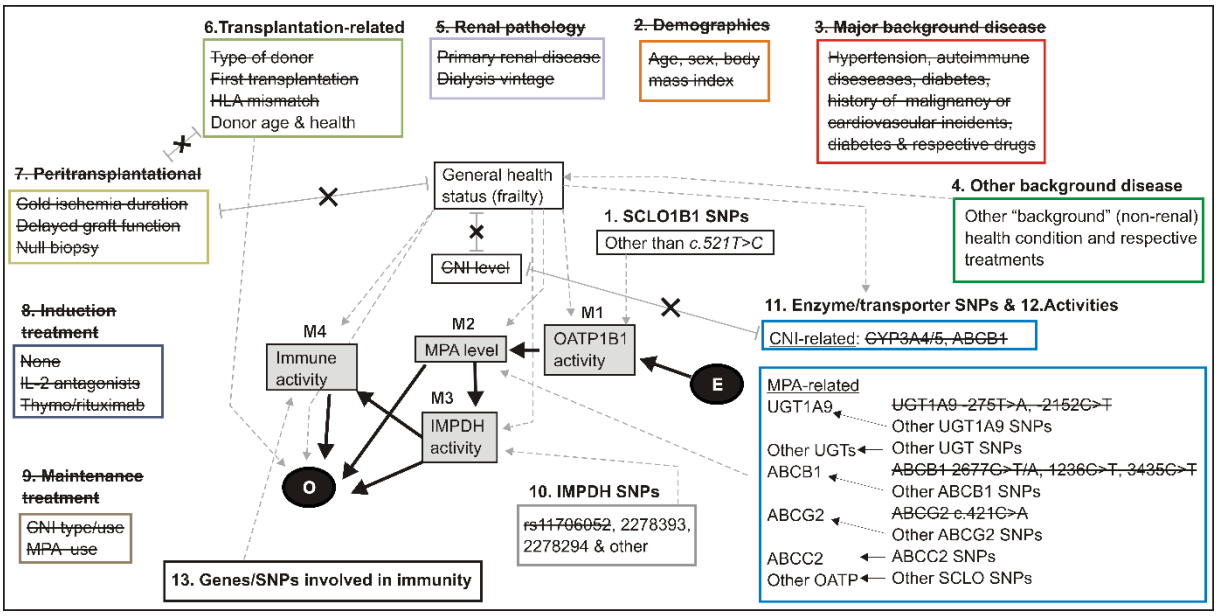

Figure S4. Slightly reorganized (for clarity) DAG from Figure S3 depicts variables (confounders) that were conditioned on (controlled for, blocked) in the present study, those that could be considered "partly" controlled, and those that remained as potential sources of residual (unmeasured) confounding. Gray-shaded rectangles indicate variables that were not directly measured but were considered "driven" by other variables. See text for details.

The activity of enzymes/transporters relevant for exposure to CNIs - by controlling for CNI troughs, any impact that CYP3A4/5 or ABCB1 activity could have had on exposure to CNIs was "retrogradely" blocked (depicted by crossed gray "block" line between CNI level and enzymes/transporters). The activity of CYP3A4/5 and ABCB1 was controlled also by controlling for the health conditions; ABCB1 was additionally controlled by balancing exposed and control subjects with respect to the 3 common *ABCB1* SNPs.

Partly controlled variables are depicted with gray dashed arrows indicating their "attenuated" effects. *General health status* – all causal effects emerging from variables that were considered major drivers of the "general health status" in Figure S3 were fully blocked. Additionally, one of its potential mediators (peritransplantational events) was blocked. Hence, the only unblocked potential effect on "general health status" was that of "other (minor) non-renal diseases", unlikely to generate any relevant difference between the exposed and control subjects in that respect. As a "worst case" scenario, exposed and controls might have differed mildly in the "general health status", which could have affected the outcome through mild and unlikely relevant effects on exposure to MPA (directly, or through the effects on enzymes and transporters involved in MPA pharmacokinetics), or on the mediators (M1, M2, M3) and/or the outcome "directly" (i.e., through other potential mechanisms). *Factors that could affect OATP1B1 activity (M1).* OATP1B1 activity is the "starting" mediator (M1) of the presumed effect of the

evaluated treatment on graft function. As outlined in the main text, other *SLCO1B1* SNPs have been only sporadically evaluated in this setting, without any clear signal of their relevance for clinical or pharmacokinetic outcomes. Still, we considered that – theoretically – "other SNPs" might have also affected the OATP1B1 activity. However, we assumed this effect to be mild. Exposed and controls were balanced on other, non-genetic, variables that could have affected the OATP1B1 activity. *Factors that could affect MPA level (M2)*. MPA level is a further mediator (M2) of the presumed effect of the evaluated treatment on the graft function. Exposed and control patients were balanced on all major non-genetic factors that could have affected MPA via general health status – by affecting transporter/enzyme activities, or by any other potential mechanism. Also, exposed and controls were balanced with respect to several SNPs that (relative to other SNPs of the respective genes) could be viewed as "major" in this setting: *UGT1A9* SNPs (*-257T>A* and *-2152C>T*), the three common *ABCB1* SNPs (c.2677G>T/A, c.1236C>T and c.3435C>T) and the common *ABCG2 c.421C>A* SNP. The only not fully controlled determinants of MPA exposure remained: (i) in part, "general health status", i.e., the part driven by "other (minor) non-renal diseases", with likely only a minor effect, if any; (ii) in part, the activity of the UGT1A9 enzyme and other UGTs potentially contributing to MPA metabolism, on the account of "other" *UGT1A9* SNPs and SNPs in genes encoding "other" UGTs that were not controlled; (iii) in part, the activity of ABCB1, on the account of "other" *ABCB1* SNPs that were not controlled; (iv) in part, the activity of ABCC2. Exposed and controls were balanced with respect to all non-genetic factors with a potential effect on ABCC2 activity, of which the use and level of exposure to CsA is the major contributor, but no *ABCC2* SNP was genotyped; (v) in part, the activity of ABCG2, on the account of "other" *ABCG2* SNPs that were not controlled and might have – theoretically – affected the transporter function; (vii) in part, the activity of OATP1B3 - exposed and controls were balanced with respect to all non-genetic factors that could have affected the OATP1B3 activity, but no *SLCO1B3* SNP was genotyped. *Factors that could affect IMPDH activity (M3)*. IMPDH activity is a mediator (M3) of the presumed effect of the assessed treatment on the outcome. The immunosuppressant effect of MPA is due to the inhibition of this enzyme. Of the two forms (IMPDH type 1, type 2), type 2 is considered of primary interest in this setting – its activity in the lymphocytes is several-fold higher than that of the type 1 isoform. Exposed and controls were balanced with respect to a number of non-genetic variables that could have reflected on the enzyme activity, and with respect to the *IMPDH2 3575T>C* (rs11706052) SNP. This polymorphism results in increased IMPDH activity in the peripheral mononuclears, and the enzyme is less susceptible to inhibition by MPA *in vitro* [14, 15]. However, two *IMPDH1* SNPs (Figure S4) that have been suggested related to the renal outcomes in this setting (as well as "other" *IMPDH* SNPs) were not controlled. Taken together, we considered it reasonable to assume that hypothetical confounding arising from differences in

IMPDH activity between exposed and controls was "attenuated/limited". *Donor's age and health* were not (directly) controlled and could have affected the outcome. We considered this hypothetical effect to be "attenuated" since the exposed and controls were balanced on several factors (delayed onset of graft function, null biopsy findings) that could be reasonably viewed as mediators of at least a part of the effect of the donor's age and health. *Genes/SNPs involved in immunity.* We did not control for any of at least 60 [11] (see Table S1) SNPs in genes regulating the synthesis/turnover of various extracellular and intracellular molecules involved in the immune/inflammatory mechanisms and/or cell-cycle control. However, we considered their potential effect on the outcome to be "limited/attenuated" for two main reasons: a) reports on their (potential) association with the adverse graft outcomes after kidney transplantation have been inconsistent and came mostly from methodologically limited studies [11]; b) it is reasonable to consider that all factors that would fall into this category are inter-related with other patients' characteristics, e.g, renal pathology, major non-renal comorbidity, peritransplantational events, i.e., that by balancing "other variables", some level of balance is achieved with respect to these factors, as well.

Sources of residual confounding

Figure S4 indicates several potential sources of residual confounding (i.e., factors that were not directly controlled, but whose hypothetical confounding effect was considered "attenuated").

"Other(minor) background (non-renal) diseases" – as already explained, this uncontrolled factor was highly unlikely to have confounded the estimated effect.

Donor's age and health status. Older donor's age and health status, particularly hypertension and obesity, have been shown as important predictors of reduced long-term graft survival in the analyses of several large database [1]. In the present setting, we considered these factors as reasonable sources of residual confounding, however, we assumed that the "effect" might not be as pronounced as suggested by some earlier registry analyses [1]: (i) we assumed that some "attenuation" would come from adjustment for peritransplantational factors (e.g., delayed onset of the graft function, results of the null biopsy); (ii) the actual effect of the donor's age might be "significant" but moderate. A recent analysis of data from the Eurotransplant database for brain-dead deceased donors (2006-2018, follow-up to July 2020) [16] (of interest for the present study) referred to 32958 transplantations in 8 European countries. In a donor-recipient model of death-censored graft survival (on a 10-year horizon), the "effect" of donor's age was HR=1.02, 1.02-1.02; i.e., around 21% higher immediate risk of graft failure for 10 years higher donor's age [16]. Much stronger predictors in the model were transplant number, recipient's diabetes, cystic disease, waiting time, and HLA matches.

IMPDH2 SNPs other than 3757T>C. A total of 25 variants (including *3757T>C*) of the *IMPDH2* have been identified [17]. As recently comprehensively reviewed [7], apart from *3757T>C*, the relationship to clinical outcomes has been investigated for rs121434586 (results in a reduced protein level and enzymatic activity) and rs4974081 – in a total of 3 studies, neither SNP was associated with any clinical outcome. Overall, theoretically, any of the non-typed *IMPDH2* SNPs might have confounded the present estimates – but this is highly unlikely.

IMPDH1 SNPs. A total of 73 *IMPDH1* variants have been identified [17]. As recently reviewed [7], at least 17 have been evaluated for potential association with different clinical outcomes (includes various adverse events) – but with inconsistent and unconvincing reports. Three of those, two intronic (rs2278293, rs 2278294), and one synonymous (rs2228075) SNP have been evaluated in at least two studies for their potential association with the graft outcomes: biopsy proven acute rejection (BPAR) or graft survival (Table S2). The studies varied grately in design and quality, as well as in quality of reporting (Table S2). Two studies [18,19] that addressed rs2228075 reported just odds ratios with a P-value (no confidence intervals), or stated "not significant" (Table S2), and both concluded "no association". Studies assessing rs227893 consistently indicated no association between the SNP and the outcomes (Table S2). The largest number of studies (n=7) (Table S2) evaluated rs2278294, and four of them were reasonably clearly presented regarding data analysis and reporting (Table S2): (i) two studies, with at least some account for potential confounding, suggested lower odds of BPAR over 12 months in variant carriers [19,20]; (ii) the largest one with the most comprehensive control of confounding reported no association with death-censored 5-year graft survival, although numerically (HR=0.79), it appeared that the risk was somewhat lower in variant carriers [21]; and (iii) one [22] suggested no association between the variant carriage and BPAR at 6 months, although numerically, odds appeared slightly lower in variant carriers (OR=0.86). Three other studies [24-26] were either of poor quality or data reporting was extremely vague – but none suggested association between the variant allele carriage and the graft outcomes (Table S2). Despite these ambiguities, we considered it possible that the variant allele rs227893 was in a way "protective" for the graft: the four "better" studies indicated (although with much uncertainty) a possibly reduced risk of graft failure [21], or a reduced risk of BPAR over 6-12 months [19,20,22]. We used the latter three studies to generate a pooled (meta-analytical) estimate of the odds ratio of BPAR (6-12 months) associated with the variant allele (vs. wild-type genotype), and all four studies to estimate the prevalence of variant allele carriage (Figure S5).

Table S2. Summary of studies evaluating *IMPDH1* SNPs rs2278293, rs2278294 and rs2228075 with respect to renal graft outcomes (biopsy-proven acute rejection, BPAR, or graft survival) in patients on MPA immunosuppression. Studies were identified from recent systematic reviews [7,8] and an additional search of PubMed Medline.

Study	Brief study outline	rs2278293	rs2278294	rs2228075
Grinyo 2008 [18]	Patients from an RCT. Outcome: BPAR by 12 months. Reports OR (no CIs) for variant carriers (n=82) vs. wt (n=134) adjusted for age (dichotomized), sex, CsA dosing regimen (3 variants), type of donor (3 types).	---	---	OR=0.80, p=0.571
Wang 2008 [19]	169 patients engrafted over 5 years. Outcome: BPAR by 12 months. Reports OR for variant carriers vs. wt, (presumably adjusted for age, sex, race, but unclear).	Variant n=121 wt n=56 OR=0.34 (0.15-0.76)	Variant n=121 wt n=69 OR=0.40 (0.18-0.89)	Variant n=85 wt= 105, OR=0.50 (NS)
Gensburger 2010 [20]	Patients from 2 RCTs. Outcome: BPAR by 12 months. Reports OR for variant carriers vs. wt, adjusted for CNI troughs and MPA exposure.	Variant n=324 wt n=132 "no association"	Variant n=268 wt n=188 OR=0.54 (0.34-0.85)	---
Shah 2012 [21]	Collaborative Transplant study. Outcome: 5-year death-censored graft survival. Reports HR for variant carriers vs. wt, adjusted for age, sex, transplant year and number, cold ischemia time, HLA mismatch, primary renal disease, initial immunosuppressant regimen and type of CNI..	Variant n=612 wt n=314 HR=0.91 (0.64-1.29)	Variant n=401 wt n=300 HR=0.79 (0.53-1.15)	---
Woillard 2014 [22]	Patients from an RCT. Outcome: BPAR by 6 months. Reports univariate OR for variant carriers vs. wt..	Variant n=117 wt n=52 OR=0.91 (0.50-1.68)	Variant n=104 wt n=85 OR=0.86 (0.47-1.58)	---
Ciliao 2018 [23]	Patients assessed at mean 7 years after transplantation. Outcome: rejection. Reports raw proportions.	Variant 35/95 (37%); wt 14/46 (30%)	---	---
Michelon 2010 [24]	Patients from the Transgene study. Outcome: BPAR by 12 months. Vague reporting – numbers not discernible (total N=218). Reports raw proportions..	Variant 19.6% vs. wt 21.6%	Variant 20.1% vs. wt 21.1%	---
Kagaya 2010 [25]	Japanese patients (N=82). Outcome: subclinical BPAR at day 28. Reports raw proportions of for wt, heterozygous and variant homozygous patients across the levels of exposure to MPA (based on AUC).	"no association"	"no association"	---
Thishya 2021 [26]	255 patients (31% wt, 55% heterozygous, 14% variant homozygous). Outcomes: death, AE, infection (but no time-frame provided). Reports adjusted ORs (age, donor type,diabetes, body mass index, several transporter SNPs).	---	"no association" (any outcome)	---

As depicted in Figure S5, meta-analysis of ORs indicated a considerable heterogeneity across the studies (best illustrated by extremely wide prediction intervals). The pooled point-estimate indicated a 40% reduction in the odds of graft rejection associated with the variant allele carriage (Figure S5). The four studies consistenly indicated a high prevalence of the variant allele carriage – the pooled proportion estimate indicated 58% with narrow CIs and a narrow prediction interval (Figure S5).

Overall, we considered it possible that a hypothetical marked chance imbalance in prevalence of rs2278294 variant carriers between the exposed and controls might have confounded the present estimates of the effect of the variant *SLCO1B1 c.521T>C* allele on graft function.

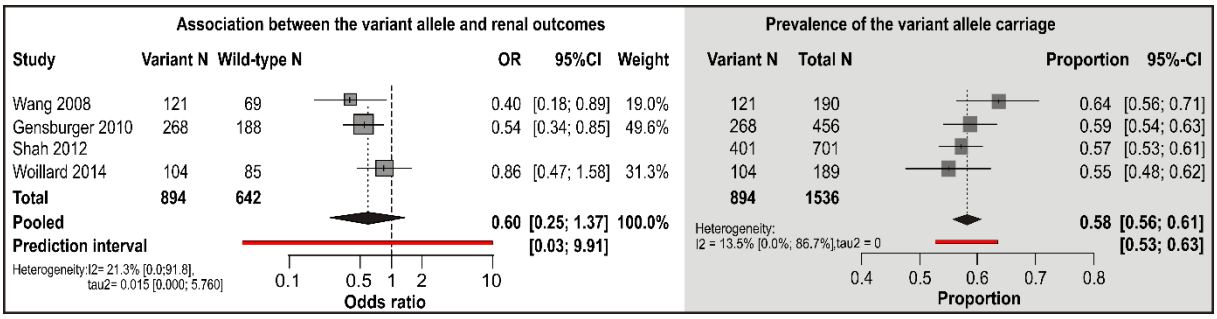

Figure S5. Meta-analysis of association of the carriage of the *IMPDH1* variant rs2278294 allele and acute rejection over 6-12 months in patients treated with MPA, and meta-analysis of the prevalence of variant allele carriage based on four studies outlined in Table S2 (Shah 2012 also included). We used package *meta* [27] in R – generic inverse variance method for the reported ORs; random intercept logistic regression model fitted to logit-transformed proportions for prevalence. Both procedures included Hartung-Knapp-Sidik-Jonkman adjustment.

SNPs in genes encoding enzymes and transporters involved in MPA pharmacokinetics. Selection of the enzyme/transporter SNPs considered as covariates in the present analysis was a result of a compromise between their previously reported associations with MPA and CNI exposure [5-9] and feasibility. Potential relevance of those that were not genotyped is addressed here.

UGT1A9 SNPs other then the typed -275T>A and -2152C>T. ***UGT1A9*3* (*c.98T>C*, rs72551330)** results in reduced enzyme activity *in vitro* [28]. We used recent systematic reviews [7,8] and additionally searched PubMed Medline to identify 3 studies (European renal transplant patients) [29-32] reporting crude (unadjusted for potential covariates) dose-adjusted steady-state AUCs ($AUC_{\tau,ss}$) in variant (TC) vs. wild-type (TT) patients on MMF cotreated with CsA (2 cohorts) or with macrolactams (3 cohorts), and additional studies reporting prevalence of the variant allele in (predominantly or exclusively) patients of European descent: French [24,32],Italian [33] and Polish [34, 35]. We performed random-effects meta analysis (package *meta* [27]) of the AUCs using ratio of means (ROM) as an effect measure [36], and of the proportions of the variant allele carriers (Figure S6). The two cohorts cotreated with CsA did not indicate association between the variant allele and AUC, but the number of variant allele carriers was very low (15 in total) (Figure S6). It was very low also in the three cohorts cotreated with macrolactams (10 in total) (Figure S6), and the outcomes were completely contradictory: 2 French variant carriers apparently had 50% lower AUCs vs. 68 wild-type subjects (3-6 months postoperatively) (Figure S6), while 3 Belgian and 5 Dutch variant carriers had 60-80% higher AUCs vs. 92 and 158 wild-type subjects, respectively, as assessed 7-10 days postoperatively (Figure S6). Across both subsets, reported values were extremely heterogeneous resulting in very wide confidence

intervals (extending from around twice lower to twice higher AUC in variant carriers) (Figure S6), and extremely wide 95% prediction intervals (extending from around 6-fold lower to 7-fold higher exposure in variant carriers) (Figure S6).

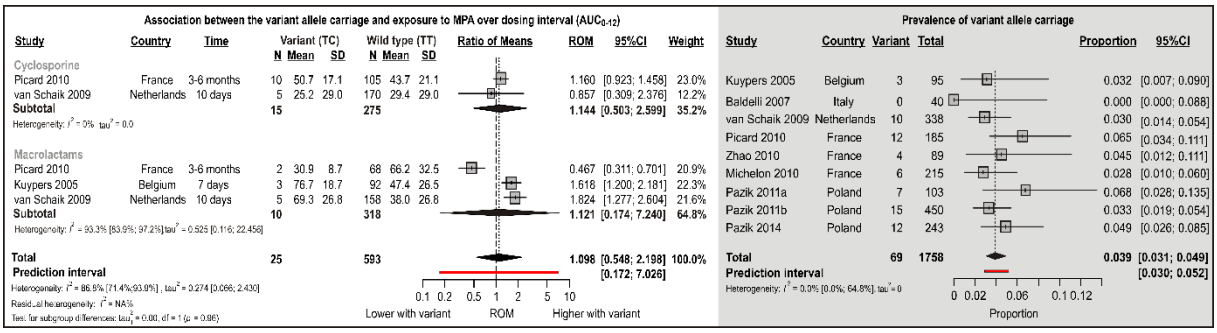

Figure S6. **Left**: meta-analysis (random-effects, REML variance estimator) of studies reporting mean±SD MPA $AUC_{0\text{-}12}$ at steady-state in renal transplant recipients treated with MMF and cotreated with CsA or macrolactams (tacrolimus or sirolimus) and genotyped for *UGT1A9*3 (c.98T>C)* SNP, classified as variant carriers (heterozygous, TC) vs. wild type homozygous subjects [the effect measure is ratio of means (ROM)]. **Right**: meta-analysis of prevalence of the variant allele carriage in renal transplant patients of (exclusively or predominantly) European desent (random intercept logistic regression model fitted to logit-transformed proportions). Pazik 2011a and 2011b refer to the same study [34] – Pazik 2011a shows data for renal transplant recipients, Pazik 2011b shows data for the background population from which these patients originated. Both meta-analyses employed the Hartung-Knapp-Sidik-Jonkman correction.

The larger (van Schaik [29]) study in the macrolactam subset evaluated 5 variant carriers vs. 158 wild-type patients over one year: a reduced sampling schedule (at pre-dose, 0.5 and 2 hours post-dose) was used to project $AUC_{0\text{-}12}$ at 3 and 10 days, and at 1, 3, 6 and 12 months postoperatively. The geometric mean AUCs (no measures of spread or precision) at these time points were displayed graphically (Figure 3 in ref. 29). The reported time-averaged difference (geometric means ratio, GMR) between TC and TT subjects was around 50%. We graph-read (using digitizing software) the geometric mean values at Day 10 [we considered this time-point to be "untainted" by possible intercurrent (post-baseline) events that might have interfered with the "SNP effect"] and used p-values to recover common SD – this is shown in Figure s6 as ROM (corresponds to GMR) of 1.824 (i.e., higher than the time-averaged value). The prevalence of the variant allele carriers was rather precisely and consistently estimated at 3-5% (Figure s6).

Overall, we considered it highly unlikely that the fact that we did not control for the *UGT1A9*3* (*c.98T>C*) SNP biased the present estimates of the effect of *SLCO1B1 c.521T>C* variant allele on the graft function: (i) all reported associations are unadjusted for any potential confounding; (ii) there is a huge uncertainty whether the *UGT1A9*3* is of any relevance for the exposure to MPA regardless of the type of CNI, and, particularly, whether any such "effect" would be relevant for the graft function over 12 months; (iii) the prevalence of *UGT1A9*3* variant in our catchment population should be reasonably viewed as very low – too low, even if one were to assume a huge chance imbalance between exposed and controls, to affect the present estimates, particularly since only a part of the patients in the present cohort were cotreated with macrolactams, i.e., exclusively tacrolimus, and the use and type of CNI over time was modeled as a time-varying covariate. Finally, population pharmacokinetic studies in French [32] and Chinese [37] patients found no association of this SNP with MPA clearance.

Other *UGT1A9 SNPs*. Three *UGT1A9* promoter SNPs [beyond *-275T>A* (rs6714486) and *-2152C>T* (rs17868320) that we controlled for] are associated with increased UGT1A9 levels in the liver: *- 440C>T* (rs2741045), *-331T>C* (rs2741046) and *-665C>T* (rs10176426) [7, 38]. However, studies have failed to provide consistent signals about association of any of these SNPs and exposure to MPA [7]; moreover, rs6714486 and rs17868320 are in complete LD with these SNPs and form two haplotypes (*UGT1A9*1l* and **1n*) [38]. Therefore, by controlling for rs6714486 and rs17868320, one controls also for several SNPs that were not directly genotyped. No consistent signal of association with MPA exposure has been found for several other *UGT1A9* SNPs (rs6731242, rs13418420, rs3832043, rs2741049, rs13418420, rs17868323) [7,8,37]. Moreover, rs6714486 and rs17868320 are in LD with some of them (haplotypes *UGT1A91v* and **1w*) [38].

Overall, it is highly unlikely that the *UGT1A9* SNPs that were ***not genotyped*** (controlled for) have individually or cumulatively introduced any relevant bias to the estimates of the *SLCO1B1 c.521T>C* variant effect on eGFR.

SNPs of other UGT enzymes. Recent systematic reviews and evaluations [7, 8, 32, 37] found no reasonable/replicated evidence of association between any of a number of evaluated ***UGT2B7*** SNPs (rs7439366, rs7668258, rs7438135, rs7662029, rs73823859, rs62298861, rs28365063) and haplotypes with MPA exposure, renal or safety outcomes. The same applies to ***UGT1A1*, *UGT1A7*** and ***UGT1A8*** SNPs. Hence, it is highly unlikely that the present estimates were confounded by the fact that they were not considered as covariates.

ABCC2 SNPs. Activity of the ABCC2 efflux transporter is an important mechanism in the pharmacokinetics of MPA, particularly the enterohepatic recirculation – the two major MPA metabolits, MPAG and AcMPAG are ABCC2 substrates [5]. The well documented and practically

relevant effect of CsA on exposure to MPA is largely due to inhibition of ABCC2 by CsA. Understandably, *ABCC2* SNPs have attracted much attention. Two recent systematic reviews [8,8] identified a total of 21 studies, 20 of which pertained, among other SNPs, to *ABCC2 c.-24C>T* (rs717620) and *c.1249G>A* (rs2273697) SNPs and their potential relationship to exposure to MPA and/or clinical outcomes in renal transplant recipients. Other addressed SNPs included rs1885301, rs7910642, rs2804402, rs717620, rs113646094, rs113646094, rs8187694, rs17222723, rs3740066, rs8187710, rs12762549, rs280402. These studies failed to provide reasonable/replicated evidence that any of these SNPs were relevant for exposure to MPA or for graft outcomes or safety in MPA-treated patients. One more recent cross-sectional study [39] in 147 patients (around 3-4 years after transplantation), 82 Caucasians and 65 African-American, treated with MPA and CsA (n=80) or tacrolimus (n=67) suggested that *ABCC2 c.-24C>T*, *c.1249G>A* and *3972C>T* (rs3740066) – when considered as haplotypes – were associated with exposure to MPA. While c.*-24C>T* and *c.1249G>A* are known not to be linked, strong LD was reported between each of these two SNPs and *3972C>T*. In patients cotreated with CsA, MPA AUC and clearance were similar between the wild-type haplotype subjects (CGC) and (combined) 3 variant haplotypes subjects (CGT, CAC, TGT), which appears logical considering the CsA effects on ABCC2. In tacrolimus cotreated patients, however, wild-type haplotype was associated with higher AUC (40 mg*h/L) vs. variant haplotypes (20 mg*h/L), while total body clearance was proportionally lower. However, no covariate adjustments (e.g., age, sex, race, BMI, albumin levels, comedication, comorbidities etc.) were implemented. Considering individual SNPs, only the wild-type *3972C>T* genotype appeared associated with mildly higher MPA AUC vs. variant allele carriers/variant homozygotes [39]. In contrast, a recent population pharmacokinetic analysis [37] based on 917 MPA concentrations from 191 adult patients cotreated with tacrolimus did not signal any relevance of any of these three (and a number of other enzyme/transporter SNPs) SNPs for the clearance of MPA. Overall, we considered it highly unlikely that not accounting for *ABCC2* SNPs have biased the present estimates: a) based on the existing data, it is not very likely that any of the *ABCC2* SNPs/haplotypes affects exposure to MPA to any relevant extent; b) CsA is probably the major "regulator" of the role of ABCC2 in MPA exposure. In the present study, we controlled (over time) for the use of CsA or tacrolimus, as well as for the CNI trough concentrations, and by this virtue we likely "balanced" the exposed and controls with respect to ABCC2 activity to the extent sufficient to "prevent" any (if existing) effect of *ABCC2* SNP(s).

ABCB1 SNPs other than 1236C>T, 2677G>T/A and 3435C>T. MPA is a substrate of the efflux protein ABCB1 – SNPs in *ABCB1* have attracted quite some attention regarding the variability of MPA pharmacokinetics. The three typed SNPs are by far the most prevalent coding SNPs in Caucasians (minor allele prevalence around 45-55%). The only other coding SNP with a minor

allele prevalence >1% in Causians is *61A>G* (rs9282564) – 8% [40]. Of the 8 studies evaluating *ABCB1* SNPs related to MPA exposure/effects identified in recent systematic reviews [7,8], all addressed one of- or all three common SNPs, and only one assessed rs9282564 – and reported no association with either exposure to MPA or with clinical events. We therefore considered it unlikely that not accounting for "other" *ABCB1* SNPs have confounded the present estimates.

ABCG2 SNPs other than c.421C>A. Besides *c.421C>A*, reduced *ABCG2* function has been reported for three further SNPs (rs34783571, rs192169062 and rs34264773), for three SNPs no effect on function is reported and for the rest functional consequences are unknown [41]. Cumulative minor allele prevalence of all "reduced function" and "unknown" SNPs is around 1.0% [41] – hence, not accounting for "other" *ABCG2* SNPs most likely did not confound the present estimates.

Other SLCO1B1 SNPs. The Introduction section of the main text elaborates: the major MPA metabolites MPAG and AcMPAG are substrates of OATP1B1; both CsA and tacrolimus inhibit OATP1B1; *SLCO1B1* gene is highly polymorphic and the presence of the variant allele *c.521T>C* (rs4149056) defines several reduced-function genotypes (diplotypes) with a cumulative prevalence of around 35%. A total of 990 *SLCO1B1* genotypes (diplotypes) have been predicted, 81 of which have been shown or predicted to result in a "reduced" or "poor" transporter function: 58 of those do not contain *c.521T>C*. Most of these genotypes are theoretically possible, and prevalence in Europeans is currently known for 8 of them: their cumulative prevalence is 4.1% [42]. On the other hand, "increased function" has been designated to 3 genotypes with a cumulative prevalence in Europeans of 2.8% [42].A recent retrospective analysis of 71 Chinese patients (MPA blood samples taken in the period up to 36 or after 36 months; AUC projected from a model) suggested that an *SLCO1B1* SNP in intron 4 (*g.33232C>A*, rs4149036) was (quote) "the most relevant SNP to MPA AUC…" [43]. This was concluded based on a multivariable model that included 3 other SNPs (two *ABCC2* SNPs and one cytochrome P-450 oxidoreductase SNP), age, sex, albumin levels, time elapsed since transplantation, lymphocyte counts and whether patients experienced acute rejection. However, it is more reasonable to conclude that the reported association was an artefact arising from an overfitted model (71 patients, 68 strata based on sex, elapsed time [dichotomized], history of acute rejection [dichotomized – also, possibly a concequence of some of the included "covariates" – hence, a collider that introduced bias], and 3 further dicohotomized SNPs) – it is impossible to fit a reasonable model with that many categorical and several continuous covariates in a set of 71 patients. Hence, this report should best be disreagarded. Overall, although theoretically any of the numerous *SLCO1B1* SNPs (genotypes) that were not controlled might have affected the OATP1B1 activity and, hence,

confounded the present estimates of the effect of *SLCO1B1 c.521T>C* – this seems highly unlikely considering the current knowledge on the topic.

Other SLCO SNPs. ***SLCO1B3***. *In vitro*, MPAG is a substrate of OATP1B3 [27]. CsA inhibits OATP1B3 (tacrolimus also, CsA is a prominent inhibitor) [44]. *SLCO1B3* is less polymorphic than *SLCO1B1* [45]. As recently systematically reviewed [7,8] four *SCLO1B3* SNPs have been evaluated with respect to MPA (exposure, clinical outcomes): *c.334T>G* (rs4149117), *c.699G>A* (rs7311358), *c.767G>C* (rs60140950) and rs1104585. The latter two were evaluated in only one study (analysis of patients from the Transgene study [24]) and did not appear associated with any outcome. The former two, *c.334T>G* and *c.699G>A* are in a complete LD and form a wild-type (TG) and variant (GA) haplotype – hence, identification of the *c.334T>G* variant carriers identifies the variant haplotype. Based on the published systematic reviews [7,8] and PubMed Medline search, we identified 4 studies reporting AUC of MPA over dosing interval at steady-state (AUC_{0-12}) calculated based on dose-adjusted MPA concentrations in renal transplant patients classified with respect to the *SLCO1B3 c.334T>G* SNP as combined TT/TG subjects vs. GG subjects: one French (Picard 2010 [30]) and one Dutch (Bouamar 2012 [46]), each reporting (separately) values in patients on MMF cotreated with either CsA or macrolactams (mostly tacrolimus, less commonly sirolimus); one Chinese study (Geng 2012 [47]) in which MMF was combined with CsA; and one Japanese study (Miura 2007 [48]) in which MMF was combined with tacrolimus. All values were crude, unadjusted mean±SD AUC_{0-12} quantified at different post-transplantation times. With two further studies reporting prevalence of TT/TG genotype in European patients [22, 24], we performed random-effects meta-analysss (the same methods as in Figure S6) to (i) generate a pooled estimate of the "effect" of the TT/TG genotype on MPA AUC (quantified as ROM), (ii) generate an estimate of the prevalence of this genotype in European patients (Figure S7). In CsA cotreated subjects, pooled estimated indicated a tendency of slightly higher exposure to MPA in TT/TG (total N=110) than in GG patients (total N=208), with a reasonable level of heterogeneity/inconsistency (Figure S7). The same was the case in macrolactam cotreated patients (TT/TG N=131, GG N=192), but heterogeneity/inconsistency was higher and CIs around the estimate rather wide (imprecision) (Figure S7). The overall pooled estimate indicated the same trend, but htereogeneity was high (tau^2=0.021), as was inconsistency (I^2=86%) – and, hence, the prediction intervals were rather wide (Figure S7). The prevalence of the TT/TG genotypes was rather consistently estimated at 31% (Figure S7).

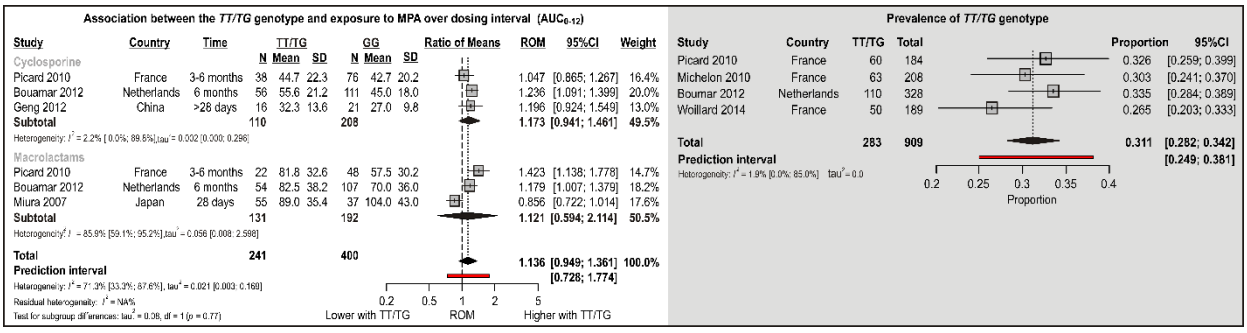

Figure S7. **Left**: meta-analysis of mean±SD MPA $AUC_{0\text{-}12}$ at steady-state in renal transplant recipients treated with MMF and cotreated with CsA or macrolactams and genotyped for *SLCO1B3 c.334T>G* SNP, classified as TT/TG vs. GG (association between the genotype and MPA AUC). **Right**: meta-analysis of prevalence of the TT/TG genotype in patients of (exclusively or predominantly) European desent. The methods are the same as those used in the meta-analyses depicted in Figure B6.

Overall, we considered that the fact that we did not control for the *SLCO1B3* c.*334T>G* SNPs was very unlikely to confound the present estimates of the effect of *SLCO1B1 c.521T>C* variant on the graft function: (i) all data on the relationship between this SNP and exposure to MPA were crude, with no control of confounding; (ii) the existing results are burdened with uncertainty about the esimates (limited precision, heterogeneity). It seems that even if the effect of this SNP on the MPA exposure existed, it should be considered very mild; (iii) CsA is a "reference" drug for OATB1B3 inhibition in *in vitro* functional studies. It is reasonable to assume that under the CsA inhibition (and we controlled for the use and trough concentrations of CsA) such effects are unlikely to be relevant; (iv) One population pharmacokinetic study reported no association between *c.334T>G* and MPA clearance in tacrolimus cotreated patients [37].

***SLCO2B1* SNPs**. One study evaluated *1457C>T* (rs2306168) and one evaluated *c.-71T>C* (rs2851969) SNPs – none associated with any MPA-related outcome [7].

Overall, it does not seem likely that the present estimates were confounded by the fact that we did not account for *SLCO1B3* or *2B1* SNPs.

<u>*Genes/SNPs coding proteins involved in immune system activity and/or cell-cycle regulation*</u>. As already outlined, reports on the relationship between any of these SNPs and clinical outcomes in renal transplant patients have been inconsistent and came mostly from methodologically limited studies – the uncertainty about their relevance is considerable.[11] It is reasonable to assume that any "imbalance" in prevalence of such "effects" between *SLCO1B1 c.521T>C* variant carriers and wild-type controls is "attenuated" by balancing them on characteristics that might have been affected by these hypothetical effects. We therefore considered this element as an unlikely source of residual confounding.

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8. Confounding in the pharmacokinetic study: sources, controlled and residual

Sources of confounding

The assessed causal path (*SLCO1B1 c.521T>C* → OAT1B1 activity → MPA level) is a part of the causal path evaluated in the main study (Figure S3). Hence, the same confounding factors should be considered, except those that may be viewed as not related to MPA pharmacokinetics e.g., IMPDH enzyme activity/ *IMPDH1* and *IMPDH2* SNPs, genes-SNPs "involved in immunity/cell cycle control" (Figure S3). In agreement, factors that were not considered relevant in the main study (given the study specifics), i.e, race, ethnicity, type of donation and use of mTOR inhibitors were of no relevance in the pharmacokinetic study, as well. DAG in Figure S8 depicts the setting. It was generated based on the knowledge about factors suggested [with more or less (un)certainty] to potentially affect pharmacokinetics of mycophenolic acid [1-6] and calcineurin inhibitors [7], and those pertaining to pharmacokinetic relevance of various transporter proteins [9-12]. It also includes elements that could be reasonably suspected to be of potential relevance based on biological/physiological reasoning: (i) *age, sex and BMI* (individually or as a "group") might reflect on the activity of OATP1B1 (by any hypothetical mechanism), as well as the activity of any UGT relevant for MPA pharmacokinetics (primarily UGT1A9, possibly contributing UGT2B, theoretically – any other UGT), or CPY3A4/5 relevant for CNIs, and activity of any or all transporters implicated in the pharmacokinetics of MPA or CNIs (SLCO1B3, ABCC2, ABCB1, ABCG2), and function of the renal graft; (ii) the same is applicable for "*non-renal comorbidities and related treatments*" (e.g., enzyme-transporter interfering drugs, any other potential mechanism); (iii) *transplantation-related /peritransplantational factors and induction treatment* are considered relevant primarily for the graft function that affects the outcome; (iv) *steroids* are of no direct relevance for exposure to MPA, but could affect acitivty of CYP3A4/5 that metabolize CNIs; (v) *type of CNI and CNI troughs*. This factor is highly relevant for exposure to MPA: both CsA and tacrolimus inhibit ABCB1, ABCG2, OATP1B1 and 1B3, but might differ in the extent of the effect. CsA also inhibits ABCC2. It is assumed that the extent of the effect of CNIs depends on their concentration – both are monitored and titrated based on trough concentrations. However, the two might differ with respect to probability of achieving certain troughs over the first week of treatment with the recommended dosing schedule; (vi) *MPA formulation/dosing*. MMF and EC-MPA are therapeutically equivalent. Their dosing is based on the MPA content, but slight differences in exposure after the initial week of treatment are theoretically possible; (vii) *CYP3A4/5 polymorphisms* are relvant for the CYP3A4/5 phenotype; (viii) UGT and transporter gene SNPs – depicted are SNPs for which it has been suggested that they might be associated with exposure to MPA. Depicted are also "other SNPs" in their respective genes, as a theoretical possibility.

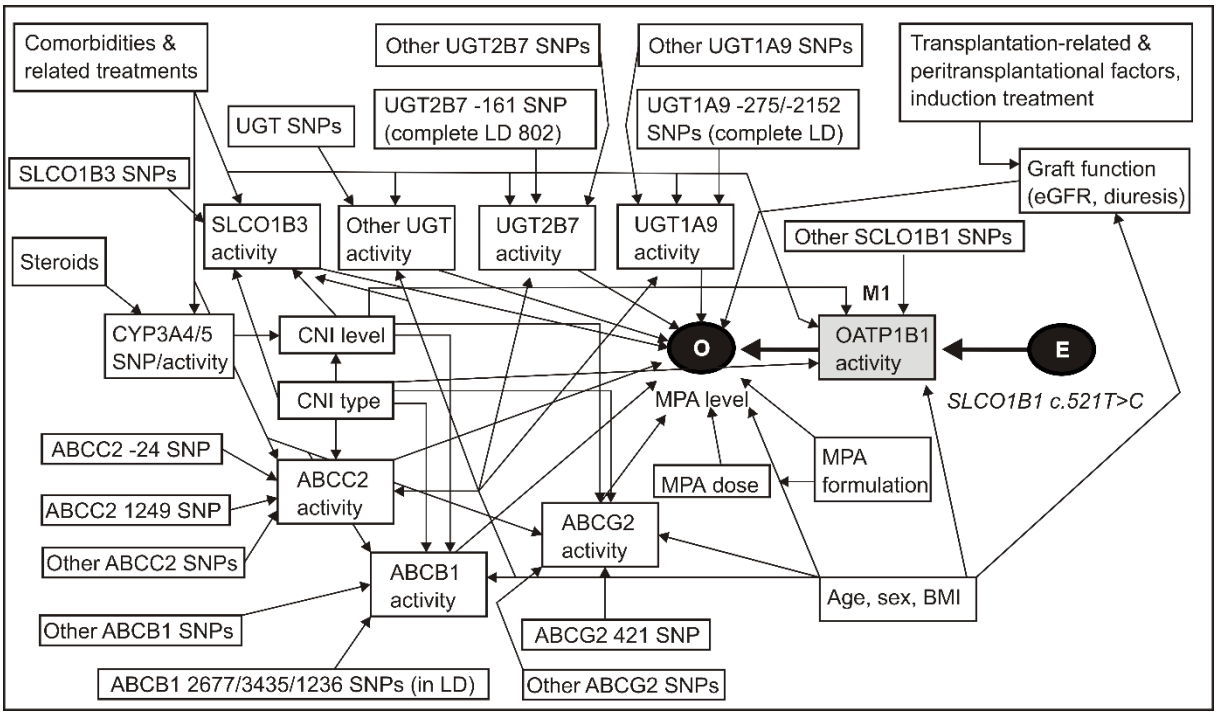

Figure S8. DAG illustrates the setting regarding the pharmacokinetic outcome (package *daggity* [13]in R). The evaluated causal path extends between the two black circles connected with thick black arrows: exposure (E) is the *SLCO1B1 c.521 T>C* variant allele (TC/CC), and control is the wild-type genotype (TT); outcome (O) is depicted as "MPA level" and represents the standard steady-state pharmacokinetic parameters for MPA. The path includes one mediator (M1) – OATP1B1 activity (gray square). Since the treatment/exposure is a genotype, there are no classical confounders that cause both the treatment and the outcome. There are, however, many factors that might (at least theoretically) affect the outcome and the mediator, and could be non-causally associated with the treatment. DAG does not include a depiction of non-causal associations, but these are assumed for all shown covariates. All presumed causal paths are indicated by thin black arrows. Factors depicted in more detail in Figure S3 as "transplantation related, peritransplantational, induction treatment" are here considered as a "group", just as are "comorbidities and related treatments". See text. ABCB1 ATP binding cassette subfamily B member 1 (P-glycoprotein); ABCC2 – ATP binding cassette subfamily C member 2 (multidrug resistance-associated protein 2); ABCG2 – ATP binding cassette subfamily C member 2 (breast cancer resistance protein); CNI – calcineurin inhibitors; CYP3A4/5 – cytochrome P-450 enzymes 4/5; MPA – mycophenolic acid; SLCO1B1 – solute carrier organic anion transporter family member 1B1 (OATP1B1); UGT – uridine 5'-diphospho-glucuronosyltransferases

Control of confounding

As outlined in the main text, inclusion/exclusion criteria for the pharmacokinetic analysis were somewhat more stringent than those for the main study. In addition to the "common" criteria,

patients in the pharmacokinetic analysis: (i) were all started on MPA, (ii) were explicitly free of cardiovascular, hepatic, metabolic, infectious and gastrointestinal disturbances and drugs known to interfere with MPA absorption/metabolism (apart from CsA), (iii) did not receive the induction treatment. By this virtue, these potential sources of confounding were blocked. Exposed *SLCO1B1 c.521T>C* variant carriers) and controls (wild-type subjects) were exactly matched and balanced on a range of baseline covariates (baseline: status on the morning of the MPA sampling day at 07:30 a.m. prior to drug dosing [see Figure S2]). In addition to factors used for covariate balancing in the main study, for the pharmacokinetic analysis patients were balanced also for the MPA formulation, graft function (eGFR, daily diuresis), and common *ABCC2* [*c.-24C>T* (rs717620) and *c.1249G>A* (rs2273697)] SNPs and *UGT2B7 -161C>T* (rs7668258). DAG in Figure S9 illustrates factors that were fully or partially controlled.

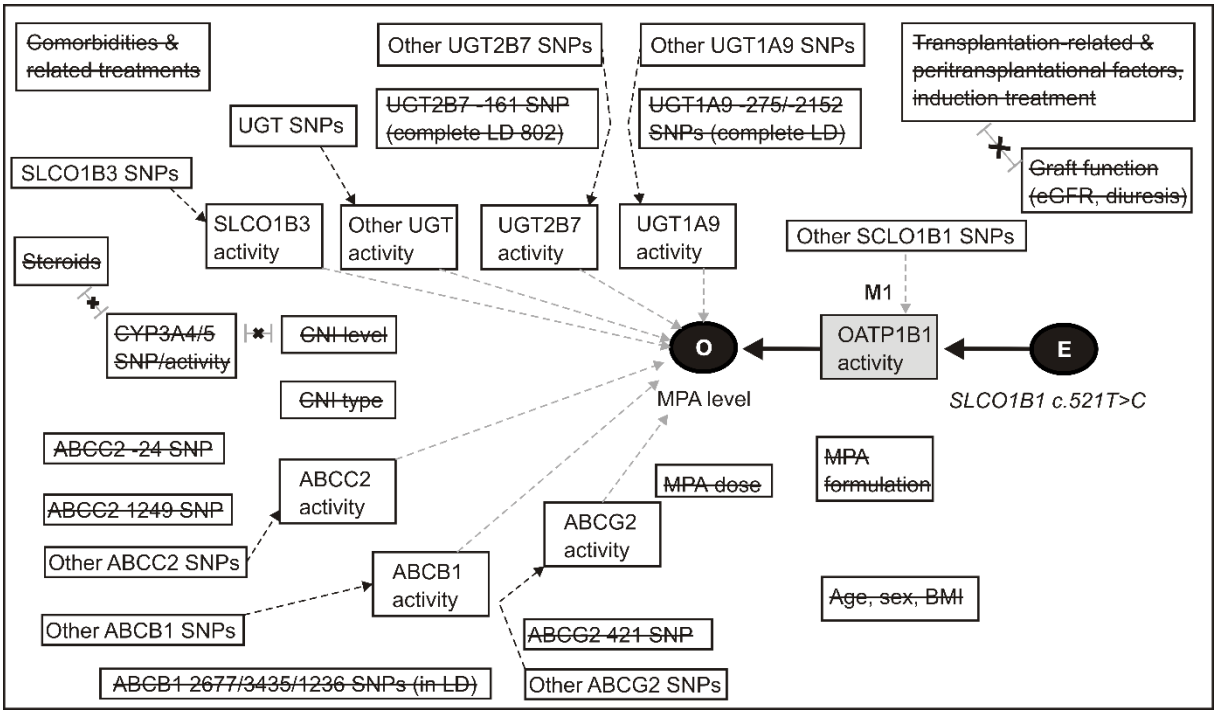

Figure S9. Modified DAG from Figure S8 – depicts variables (confounders) that were conditioned on (controlled, blocked) in the present analysis, those that could be considered "partly" blocked and those that remained as potential sources or residual (unmeasured) confounding. Gray-shaded rectangle depicts the mediator (M1) variable that was not directly quantified, but is considered "driven" by other variables. See text for details.

Fully controlled variables are depicted by removal of black arrows starting from them (indicating that no causal effects emerge from these variables) and the strikethrough text. *Demographics* – exposed and controls were balanced on age, sex and body mass index. *(Non-renal) comorbidities and related treatments* – by inclusion/exclusion criteria. *Transplantation-*

related and peritransplantational events, induction treatment – by inclusion/exclusion criteria. These variables were additionally ("retrogradly") blocked by balancing exposed and controls on their mediator – *graft function* (eGFR, diuresis), as indicated by a crossed "blocked" line. *MPA formulation and dose* – patients were balanced with respect to the MPA formulation (MMF or EC-MPA). All pharmacokinetic calculations were based on dose-adjusted (regarding the MPA content) MPA concentrations. *CNI type* – exposed and controls were balanced with respect to type of CNI. *CNI level (troughs)* –exposed and controls were balanced with respect to CNI troughs (log-transformed) determined on the morning of the MPA sampling day (before dosing). For this purpose, tacrolimus concentartions were "projected" onto the CsA range by linear transformation. *Steroids, CYP3A4/5 activity* – all patients were started on 60 mg prednisolone equivalents on Day 1 post-transplatnation, and were tapered to 30 mg day over the subsequent 3 days. Also, by balancing patients on CNI troughs, the potential impact of steroids on CYP3A4/5 activity was "retrogradly" blocked (indicated by crossed "blocked" lines). *UGTA9 -275/-2152, UGT2B7 -161 (and 802 in complete LD), the three common ABCB1 SNPs, ABCG2 c.421 SNP and ABCC2 c.24 and c.1249 SNP* – patients were balanced with respect to these genotypes.
Partly controlled variables are "reduced" to activities of enzymes/transporters involved in pharmacokinetics of MPA (UGT1A9, possibly UGT2B7, theoretically – other UGTs, ABCB1, ABCG2, ABCC2, SLCO1B1 and 1B3): these are considered to be partly controlled ("attenuated") since exposed and controls were balanced with respect to all relevant non-genetic factors that could have (potentially) affected their activity, and also on common polymorphisms in their respective genes for which at least some indication exists of (potential) relevance for exposure to MPA. Their "reduced" or "attenuated" hypothetical confounding effect is indicated by dashed gray arrows, and arises from the fact that not all theoretically possible SNPs were controlled.

Sources of residual confounding
As discussed related to the main study, none of these potential and "attenuated" pharmacokinetic factors appeared a likely source of residual confounding – and this is applicable for the pharmacokinetic study, as well.

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9. Data analysis

Main study

Primary outcome. Ln-transformed eGFR values taken over time were analyzed in the covariate-balanced exposed and control subjects by fitting a weighted piecewise random intercept and time linear mixed-model with a knot at day 28, with treatment, time, treatment*time interaction (before and after the knot), and BMI, CNI use, CNI troughs, and MPA use as (time-varying) fixed effects, with restricted maximum likelihood estimation and robust (sandwich) variance estimator. We estimated (i) adjusted eGFR slopes before and after the knot (as percent change/day before the knot, and per 28 days for the period up to 365 days), and (ii) adjusted eGFR values at days 1, 28 and 365, and differences between treated and controls expressed as geometric means ratios (GMRs).

Other outcomes. Recurrent adverse renal outcomes and adverse events in the covariate-balanced exposed and control subjects were analyzed by fitting Poisson models with ln-time as an offset, with treatment, time, BMI, MPA use, and CNI use as (time-varying) fixed effects, with robust sandwich variance estimator to generate adjusted event rates/patient-year, and differences between treated and controls as rate ratios (RR). We used SAS 9.4 for Windows (SAS Inc., Cary, NC) *proc mixed* and *proc genmod*.

Pharmacokinetic study

All analyses were performed on ln-transformed pharmacokinetic parameters, and all analyses were performed by fitting frequentist and Bayesian models. Differences between *SLCO1B1 c.521T>C* variant allele carriers and wild-type controls are expressed as geometric means ratios (GMR) with 95% confidence/highest posterior density credible intervals. All frequentist models employed maximum likelihood estimation. In all Bayesian models (4 chains, 4000 iterations, 8000 samples of the posterior) we defined a moderately informed skeptical normal prior for the effect [ln(GMR)] of primary interest - centered at 0.0, with standard deviation 0.355 [$N(0,0.355)$]. It assigns equal probability to a GMR <1.0 and a GMR >1.0, and 95% of the probability to GMRs between 0.50 and 2.00. It is in agreement with the *a priori* hypothesis of no treatment effect. For all other effects included in multivariable models, we assigned vaguely informed neutral normal prirors [$N(0, 0.70)$]: assign 95% probability within the range from GMR=0.25 to GMR=4.0.

Since the number of patients in the pharmacokinetic study was limited, we combined several procedures to achieve conditional exchangeability between *SLCO1B1 c.521T>C* variant allele carriers and wild-type controls: (i) patients were first exactly matched (package *MatchIt* [1]in R) on several categorical covariates that we considered of high importance: MPA formulation (MMF

or EC-MPS), type of CNI (CsA or tacrolimus), sex, *UGT1A9 -275T>A / -2152C>T* diplotype and *ABCG2 c.421G>A* genotype [2]; (ii) matched sets were then subjected to covariate energy balancing (package *WeightIt* [3]in R), and patients were weighted by the final weights (exact weights*energy balancing weights); (iii) if any of the covariates remained suboptimally balanced (standardized differences, d≥0.1), it was included in a multivariable model. Hence, all "adjusted" models were weighted, and subclass formed by exact matching was a random effect.
Several models were fitted: a) simple general linear models to raw (unadjusted) data to (i) estimate the (raw) difference between variant carriers and wild-type controls, and (ii) to test the interaction between the *SLCO1B1* SNP and CNI type (variant vs. wild-type differences in CsA and tacrolimus cotreated patients); b) generalized linear mixed models to "adjusted" data, frequentist (with cluster robust variance estimator) and Bayesian (with priors on the terms of a decomposition of the covariance matrices [Gamma shape=1, scale=1; LKJ for correlation matrix, regularization=1; Dirichlet for the simplex vectors, concentration=1]), to estimate the main effect the *SLCO1B1 c.521T>C* variant allele. To test the SNP*CNI interaction term in the "adjusted" data, variant allele carriers and wild-type controls were matched/balanced separately in the subset cotreated with CsA and subset cotreated with tacrolimus. The test of interection was performed on merged data. We used SAS 9.4 for Windows (SAS Inc., Cary, NC) *proc glimmix* and package *rstanarm* in R.

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10. Correcting the estimates for unmeasured confounding

Main study

As elaborated in section 6, it appeared that, based on the current knowledge, none of the addressed potential sources of residual confounding was very likely to affect the graft function and thus confound the present estimates. Nevertheless, we conceived a hypothetical confounder (a single factor or a set of factors) that biased the present estimates. Based on the existing evidence, we considered that it would be most straightforward to use *IMPDH1* SNP rs2278294 to "represent" the biasing effect: (i) of all the considered factors, it appears to be the one most "difficult to discard" as irrelevant (despite the inconsistent reports and uncertainties outlined in Table S2 and Figure S5); (ii) based on the existing data (Figure S5), prevalence of this SNP in the present sample could be reasonably well predicted; (iii) the existing data provide the basis for a reasonable approximation of the size of this (hypothetical) biasing effect. One approximation is based on the study reporting the 5-year death-censored graft survival (study Shah 2012 in Table S2): adjusted HR=0.79 (0.53-1.15) for the variant allele carriage vs. wild-type. Graft function over the first 12 months (the outcome in the present study) is a strong predictor of the long-term graft survival – hence, the estimated HR=0.79 suggests a "protective" effect of the variant allele that could be "translated" into "better graft function (eGFR development)" over the first year. Another approximation comes from the pooled estimate of OR for BPAR over the first year (variant carriers vs. wild-type subjects; Figure S5). While the link between the odds of BPAR and graft function (eGFR) might not be straightforward, the pooled estimate of OR=0.60 (0.26-1.37) could also be viewed as an indicator of a "protective effect". The two reported measures (HR, OR) are ratio effect measures, just as is the GMR used in the present analysis to quantify potential differences between *SLCO1B1 c.521T>C* variant carriers and wild-type subjects regarding the eGFR slope – they all inform about relative (%) differences. Hence, it seems reasonable to "translate" the biasing effect of the *IMPDH1* SNP into GMR=0.79 and GMR=0.60. We assumed: (i) a large (hypothetical) chance imbalance in prevalence of the *IMPDH1* variant carriers among "exposed" (*SLCO1B1 c.521T>C* variant carriers) and "controls" (wild-type subjects) of 2:1 and *vice-versa* (1:2), and corrected the present estimates [1,2] for the biasing effect of GMR=0.79; and (ii) a large chance imbalance of 1.5:1.0 (or 1.0:1.5) and corrected the present estimates for GMR=0.60.

Pharmacokinetic study

As elaborated in sections 6 and 7, it appeared that, based on the current knowledge, none of the addressed potential sources of residual confounding was very likely to affect the present pharmacokinetic estimates. Nevertheless, we conceived a hypothetical biasing factor (a single factor or a set of factors). Based on the existing evidence, we considered that it would be most

straightforward to use *UGT1A9*3* (*c.98T>C*, rs72551330) to "represent" the biasing effect: as shown in Figure S6, raw data from two studies suggested higher MPA AUC at 7-10 days after transplantation in variant carriers vs. wild-type subjects (cotreated with macrolactams). Despite the inconsistencies vs. other studies and small number of subjects (Figure S6), we deemed that – as unlikely as it might be - this appeared the most reasonable potential source of "pharmacokinetic confounding". Based on the larger of the two studies shown in Figure S6 that suggested a difference between *UGT1A9 c.98T>C* variant carriers and wild-type subjects (van Schaik 2009), we assumed that the biasing effect was GMR=1.50 (corresponds to the GMR for variant vs. wild-type subjects for time-avaraged AUC), and that the prevalence of variant carriers was 10% overall, i.e., 2.5-fold higher than estimated in Figure S6. We then assumed a huge chance imbalance in prevalence of *UGT1A9 c.98T>C* variant carriers among treated and controls of 4:1 and *vice-versa*, and corrected [1,2] the present estimates for this biasing effect.

References

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