Risk of diarrhoea in a long-term cohort of renal transplant patients given mycophenolate mofetil: the significant role of the UGT1A8 2 variant allele.

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Risk of diarrhea in a long-term cohort of renal transplant patients given mycophenolate mofetil: the significant role of the *UGT1A8*2* variant allele.

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Running head: Pharmacogenetics of mycophenolate-induced diarrhea

Keywords: Kidney transplantation, mycophenolate mofetil, pharmacogenetics, diarrhea, uridine diphosphate-glucuronosyltransferase, *UGT1A8*2*

Word count: 3709 words

3 tables and 4 figures

What is already known about this subject

- Mycophenolate mofetil (MMF), the most widely used drug in allograft transplantation, is subject to hepatic and intestinal glucuronidation and entero-hepatic cycling.
- Diarrhea is its most frequent adverse event leading to non-compliance, treatment interruption and ultimately to an increased rate of acute rejection.
- Cyclosporine reduces the biliary excretion of mycophenolate metabolites, presumably by inhibiting the efflux transporter MRP2
- When combined with MMF, cyclosporine reduces the incidence of diarrhea, suggesting the role played by biliary excretion of mycophenolate glucuronides in this adverse event.

What this study adds

- In a long term cohort of renal transplant patients on MMF, the two factors significantly associated with a reduced incidence of diarrhea were: the co-medication with cyclosporine (as opposed to tacrolimus or sirolimus), and the *2 variant allele of the intestinal *UGT1A8.*
- Polymorphisms in the others UDP-glucuronosyl-transferase and MRP2 were not significant.

Summary

Aim: In renal transplant patients given mycophenolate mofetil (MMF), we investigated the relationship between the digestive adverse events and polymorphisms in the UGT genes involved in mycophenolic acid (MPA) intestinal metabolism and biliary excretion of its phase II metabolites.

Methods: Clinical data and DNA from 256 patients transplanted between 1996 and 2006 and given MMF with cyclosporine (CsA, n=185), tacrolimus (TAC, n=49) or sirolimus (SIR, n=22), were retrospectively analyzed. The relationships between diarrhea and polymorphisms in *UGT1A8* (*2 518C>G, *3 830G>A), *UGT1A7* (622C>T), *UGT1A9* (-275T>A), *UGT2B7* (-840G>A) and *ABCC2* (-24C>T, 3972C>T) or the co-administered immunosuppressant were investigated using the Cox proportional hazard model.

Results: Multivariate analysis showed that patients on TAC or SIR had a 2.8 higher risk of diarrhea than patients on CsA (HR=2.809; 95%CI (1.730-4.545); p<0.0001) and that non-carriers of the *UGT1A8*2* allele (CC518 genotype) had a higher risk of diarrhea than carriers (C518G and 518GG genotypes) (HR=1.876; 95%CI (1.109-3.175); p=0.0192). When patients were split up with respect to the immunosuppressive co-treatment, a significant effect of *UGT1A8*2* was found in those co-treated with cyclosporine (HR=2.414; 95%CI (1.089-5.354); p=0.0301) but not TAC or SIR (p=0.4331).

Conclusion: These results suggest that a possible inhibition of MPA metabolites biliary excretion by cyclosporine and a decreased intestinal production of these metabolites in *UGT1A8*2* carriers may be protective factors against MMF-induced diarrhea.

Introduction

Mycophenolate mofetil (MMF), the prodrug of mycophenolic acid (MPA), is an immunosuppressive drug widely used in combination therapy with cyclosporine (CsA), tacrolimus (TAC) or sirolimus (SIR) for the prevention or the treatment of acute rejection following kidney, heart and liver allograft transplantation.

The main adverse events (AE) reported for MPA are gastrointestinal (GI) disorders (in particular diarrhea), bone marrow suppression and anemia (1, 2). MMF would be discontinued in 20% of the patients because of such adverse events (3). It was first hypothesized that MMF digestive adverse events could be related to MMF dose and/or to MPA plasma levels (4, 5) but this was not confirmed by a further study (6). Other hypotheses include the possible predisposition of patients to MMF diarrhea in relation to MPA metabolism, and drug-drug interactions. The metabolism of MPA is mainly by conjugation of its phenol group to give the inactive MPA-phenyl-glucuronide (MPAG) (7) which involves UGT1A9, and to a lower extent UGT1A7, 1A8 and 1A10 (8, 9). The conjugation of MPA carboxylic acid moiety leads to a second glucuronide, namely MPA-acyl-glucuronide (AcMPAG) (10), which is mainly produced by UGT2B7 in the liver and, to a lower extent, in other tissues including the intestine and the kidneys (8). MMF induces a particular type of diarrhea, the exact mechanism of which remains unknown. Several authors reported that the normal villous structure of the small bowel was lost (11-13). Owing to the reactivity of AcMPAG (14),(15), it was suggested that AcMPAG could be involved in this adverse event through a secondary immunological mechanism (16). However, neither MPAG nor AcMPAG plasma exposures were associated with diarrhea in a study in kidney transplants patients (6), where the only significant factor found was the calcineurin inhibitor associated to MMF: a lower

incidence of diarrhea was observed in patients co-treated with CsA than in those co-treated with TAC. As CsA inhibits the Multidrug Resistance Protein 2 (MRP2)-mediated excretion of MPA metabolites into the bile (17), it suggests that biliary excretion of MPA metabolites, hence intestinal exposure to these metabolites would be more closely linked with diarrhea than systemic exposure.

The aim of this study was to investigate in a long-term cohort of renal transplant patients on MMF the influence on digestive adverse events of: (i) polymorphisms of the genes encoding the UGTs involved in MPA intestinal metabolism (UGT1A7, UGT1A8, UGT1A9 and UGT2B7); (ii) polymorphisms of the gene encoding the efflux transporter involved in the biliary excretion of MPA metabolites (*ABCC2*); and (iii) co-administered immunosuppressants.

Methods

Patients

The clinical data-on-file and banked DNA samples from patients transplanted between 1996 and 2006, routinely followed as outpatients at Limoges University hospital were retrospectively studied. The ethics committee of Limoges hospital approved the protocol. Informed consent was obtained from each living patient, while the French Health Authorities have waived the requirement for consent for deceased patients. The following inclusion criteria were used: recipient age > 18 years; functioning graft after more than one year posttransplantation; kidney graft from a cadaveric donor; constitutional DNA available; deceased patient or signed informed consent. Exclusion criteria were as follows: patient age < 18 years, pregnancy, graft survival < 1 year and kidney and pancreas, heart or liver combined transplantation. For each patient, the

following clinical data were recorded from the medical file by the same nephrologist (JPR): date of birth, sex, HLA mismatches between donor and recipient, duration of cold ischemia, induction therapy, immunosuppressive drug regimens and gastrointestinal adverse events (GI AEs), with their starting and ending dates. The GI AEs were classified as diarrhea, abdominal pain, nausea/vomiting and anorexia. In order to reduce the number of statistical tests and because of the low frequencies of abdominal pain (11.0%), nausea/vomiting (5.5%) and anorexia (7.0%), only diarrhea (27.7%) and the global incidence of all GI AEs (35.1%) were finally analyzed. Clinical data collection was performed before the initiation of the genetic study to avoid any bias. Diarrhea was taken into consideration when sufficient and convincing data was available in the clinical file, in particular regarding the duration, severity and resolution of the diarrhea episode, and when it most likely fulfilled the following definition: more than 2 loose, watery stools per day persisting for more than five days, without fever or inflammatory disease, or any other patent etiology (positive viral or cytobacterial or parasitological examination of the stools when available and treatments known to provoke diarrhea, other than immunosuppressive therapy) and/or when the episode stopped after MMF dose reduction or discontinuation. Patients were treated following the medical practice at that time in Limoges University Hospital.

Genomic DNA bank

DNA collection and conservation was performed by the immunogenetic laboratory of Limoges University Hospital. Genomic DNA was extracted from EDTA-treated blood using a previously described manual method (18).

Identification of genotypes

Genomic DNA was used to characterize the genotypes of each patient for SNPs in the *UGT1A7*, *UGT1A8*, *UGT1A9*, *UGT2B7* and *ABCC2* (MRP2) genes (table 1). Genotypes were determined using Real-Time Quantitative Polymerase Chain Reaction (ABI PRISM 7000 Sequence Detection System; Applied Biosystems, Courtaboeuf, France) and validated allelic discrimination assays (TaqMan Custom or Drug Metabolism Genotyping assays®, Applied Biosystems).

Briefly, 1 to 20 ng of genomic DNA were mixed with each assay and PCR universal master mix (Applied Biosystems, Foster City, CA USA) in a total volume of 14 µL. Thermal cycler parameters included 10 minutes at 95°C and 40 cycles of denaturation at 92°C for 15 seconds and annealing/extension at 60°C for 1 minute, except for *UGT1A8* and *UGT1A7* assays which required 1.5 min elongation steps and 45 PCR cycles.

Statistical analysis

Deviations from the Hardy-Weinberg equilibrium were studied using the Fisher exact test. The effect of the polymorphisms (SNPs or haplotypes) on phenotypes was investigated using the Cox proportional hazard model, considering successively all GI AEs and diarrhea only. P values less than 0.05 were considered significant and 95% confidence intervals provided when relevant. For SNP and haplotype association analyses, the most frequent allele was considered as the reference. When the frequency of variant homozygous patients was lower than 5%, these patients were gathered with the heterozygotes. For multivariate analysis, the significance of variables in the final model was tested by a backward stepwise process using the likelihood ratio to evaluate the effect of omitting variables. After studying the effect of each polymorphism independently, the association of haplotypes with GI AEs was analyzed using the THESIAS program (http://genecanvas.ecgene.net) (19) when appropriate.

The *ABCC2* polymorphism was investigated in 2 subgroups of treatment independently: CsA and TAC /SIR because of the hypothesis of *ABCC2* biliary inhibition by CsA.

In order to investigate the effect of MMF dose on the incidence of diarrhea, the dose at the time of the first episode of diarrhea in "case" patients was compared to the dose collected at a similar time after initiation of MMF in "control" patients paired on follow-up duration while on MMF. Dose was classified into 4 groups (≤750, 1000, 1500 or ≥2000) and compared using the Fisher exact test, then in two groups (<2g or >=2g) to be analyzed using the Cox model. For significant covariates, time-to-event data (first episode) were estimated using Kaplan-Meier analysis for patients with or without the factor of interest, and groups were compared by the log-rank test.

Except when stated otherwise, all statistical analyses were performed using Statview 5.0 (SAS Institute Inc, Cary, NC, USA).

Results

Clinical Data

The clinical and demographic characteristics of the 256 patients who fulfilled the inclusion and exclusion criteria (out of 386 patients transplanted at Limoges University Hospital over the period 1996-2006) are described in table 2. Patients' follow up was 41.0 months on average (ranging from 0.6 to 115.0). Each patient was taken into consideration as from the initiation of MMF treatment, which corresponded to the first days posttransplantation in 222 patients (86.7%). The 34 others were switched from azathioprine to MMF in the stable post-transplantation period. During the study period, a total of 194 episodes of gastrointestinal adverse events (GI AEs) were observed in 90 patients (35.1%), including 118 episodes of diarrhea in 71 patients (27.7%). Twenty

five patients had more than 2 episodes of diarrhea. The mean MMF dose at the time of the episode was 1750±699 g in the group with diarrhea (cases) and 1768±443 g in the group without (controls) (ns). Among patients with diarrhea, there was a significant difference in MMF dose between patients co-treated with TAC (1278±521 mg) and those co-treated with either SIR (1889±333 g) or CsA (1927±738 g) (p=0.0026).

Linkage disequilibrium study

All the genotype distributions were in conformity with the Hardy-Weinberg equilibrium and similar to those reported in the literature (table 1). A strong linkage disequilibrium (LD) was observed between the *ABCC2* -24C>T and the 3972C>T Single Nucleotide Polymorphisms (SNPs) (D'=0.93, R^2=0.41). Four haplotypes were found: -24C/3972C (59.6%), -24T/3972T (22.5%), -24C/3972T (16.9%) and -24T/3972C (1.0%). No LD was observed between the *UGT* SNPs.

Effects of co-administered immunosuppressant and MMF dose

The percentage of patients with diarrhea was 17.8%, 54.5% and 53.1% in the CsA, SIR and TAC subgroups, respectively (table 2). There was no difference in the incidence of diarrhea between patients on TAC or SIR (Cox model: p=0.5789) (Fig.1). Consequently, due to the rather small number of patients in these subgroups (n = 49 and 22, respectively), they were combined for comparison to CsA co-treated patients. Univariate analysis using the Cox model showed a highly significant association of the co-administered immunosuppressant with diarrhea (TAC/SIR vs CsA: Hazard Ratio (HR)=4.251; 95%CI (2.637-6.853); p<0.0001) (table 3), as well as with the GI AEs studied globally (TAC/SIR vs CsA: Hazard Ratio (HR)=3.788; 95%CI (2.457-5.848); p<0.0001).

No significant association was found between GI AEs and MMF dose at the time of the event, whether the dose was considered in four groups (≤750, 1000, 1500, ≥2000; Fisher exact test p=0.095) (Fig. 2), or in two groups (<2000 and ≥2000: Hazard Ratio 1.146; CI95% (0.702-1.869); p=0.5861) (table 3).

Survival analysis

For patients with more than 1 episode of diarrhea, only the first episode was taken into account. Kaplan Meier analysis of the time to the first episode of diarrhea demonstrated a significantly higher incidence in carriers of at least one *UGT1A8*2* variant allele (Fig. 3A; p=0.0101). Similar results (Fig. 3B; p=0.0352) were obtained when only the patients who started MMF in the first days posttransplantation (n=222) were considered, while a similar trend, with a seemingly even larger difference (Fig. 3C; p=0.1448) was observed in the others, who started MMF later after transplantation (n=34). The Kaplan Meier analysis of the time to the first episode of diarrhea shows a significantly higher incidence in patients co-treated with TAC/SIR than in patients co-treated with CsA (Fig. 1; p<0.0001). Similar results were obtained for the GI AEs studied globally (data not shown, p<0.0001). No difference in the Kaplan Meier analysis of the time to graft loss was found between patients with and without diarrhea (p=0.3016), nor with and without GI AEs studied globally (p=0.1641).

Pharmacogenetic association

Considering again the first episode of diarrhea, univariate analysis showed non-carriers of *UGT1A8*2* had a significantly higher incidence of diarrhea than heterozygous or homozygous carriers of the allele (HR=1.968; 95%CI (1.163-3.322); p=0.0117).

However, this SNP was not associated with GI AEs considered as a whole (HR=0.803, 95%CI (0.559-1.154); p=0.2365). No significant associations were found between *UGT1A7* (622C>T), *UGT1A8*3* (830G>A), *UGT1A9* (-275T>A), *UGT2B7* (-840G>A) or *ABCC2* (-24C>T, 3972C>T) SNPs and all GI AEs (data not shown) or diarrhea (table 3). The influence of the main *ABCC2* haplotypes compared to the most frequent haplotype (-24C/3972C), called here CC, was not significant on diarrhea (table 3) or GI AEs as a whole (data not shown), neither when considering all patients together, nor when considering patients on CsA or patients on TAC or SIR separately (data not shown).

A multivariate Cox model taking into consideration in the same model the co-administered immunosuppressant and the *UGT1A8* genotype showed that the patients on tacrolimus or sirolimus had a 2.8-fold higher risk of diarrhea than the patients on CsA (HR=2.809; 95%CI (1.730-4.545); p<0.0001), while non-carriers of *UGT1A8*2* had a 1.9-fold higher risk of diarrhea as compared to homozygous or heterozygous carriers (C518G or 518GG genotypes) (HR=1.876; 95%CI (1.109-3.175); p=0.0192).

In order to evaluate the respective role of the *UGT1A8**2 allele and the immunosuppressive co-treatment over time, four groups were set up and further compared using the Cox model: the patients co-treated with CsA and carrying at least one *UGT1A8*2* allele had the lowest incidence of diarrhea, followed in increasing order of incidence by patients with: CsA and no *UGT1A8*2* allele (HR=2.414; 95%CI (1.089-5.354); p=0.0301); TAC or SIR and one or two *UGT1A8*2* allele (HR=6.287; 95%CI (2.503-15.792); p<0.0001); and TAC or SIR and no *UGT1A8*2* allele (HR=8.332; 95%CI (3.769-18.417); p<0.0001) (Fig. 4). However, there was no significant association between the risk of diarrhea and *UGT1A8*2* in patients co-treated with TAC

or SIR (*UGT1A8*2* carriers vs. non-carriers: HR=0.755; 95%CI (0.373-1.526); p=0.4331).

Discussion

Based on the data collected retrospectively in 256 renal transplant patients receiving MMF, we found that patients given tacrolimus or sirolimus or non-carriers of *UGT1A8*2* had a higher incidence of diarrhea than those given cyclosporine or carrying the *UGT1A8*2* allele, respectively. Several studies investigated the relations between gene polymorphisms of the UGTs or efflux transporters and interindividual variability of MMF exposure, but only a few focused on the direct association of these polymorphisms with MMF-related AEs. Here we studied the potential link of these genes with the occurrence of MMF-related diarrhea episodes. The low numbers of other kinds of AEs prevented us from performing statistical analyses for each of them.

The co-administered calcineurin was also taken into consideration, since cyclosporine is known to influence MMF pharmacokinetics through drug-drug interactions and tacrolimus to induce diarrhea. To the best of our knowledge, this is the first pharmacogenetic study of MMF-related toxicity in a long-term cohort of renal transplant patients. The number of patients studied over this extended period (1996 to 2006) represents a large sample of the renal transplant population in our center.

MMF-related diarrhea was the major digestive AE reported in the patients' files.

Diarrhea was previously described as the main digestive AE of MMF (20), with frequencies close to those observed herein (15% when associated with CsA and 38% with TAC) (6). The role of MMF in diarrhea episodes occurring in transplant patients is difficult to ascertain since numerous etiologies could result in similar symptoms. In this

study, all the clinical files were retrospectively screened by one individual nephrologist, allowing a homogenous definition and report of this AE. It excluded clinically evident infectious diarrheas. Moreover, CMV antigen or PCR were negative at the time of diarrhea in all patients. Because examination of the stools or extensive biological work-up was not systematically performed, we cannot exclude misclassification in some cases as suggested by the study of Maes et al.(13). However, diarrheas of infectious origin usually do not disappear after MMF dose reduction. Furthermore, misclassification in the present study, if any, must have been similar in the different genotypic groups and would only result in a loss of statistical power and not in a statistical bias. Similarly, the environmental factors which can also be associated with diarrhea (not investigated herein) may also have resulted in a loss of power but in no bias, which can only emphasize our findings.

Before MMF release, it had already been reported that patients under TAC had a higher incidence of diarrhea than patients under CsA: in a multicenter randomized trial comparing TAC versus CsA in association with azathioprine and steroids, the one year incidence of diarrhea was twice as much in TAC patients (21.8% vs 10.3%; $p<0.005$) (21). In comparison, the incidence here was higher, at 53.1%, 54.5% and 17.8% for TAC, SIR and CsA, respectively, suggesting that MMF represents an independent risk factor of diarrhea. This study shows that the associated immunosuppressant is the main factor associated with diarrhea in patients on MMF: CsA was associated with approx. a 2.8-fold lower risk of diarrhea as compared to SIR or TAC. Similar results were previously reported by Heller et al. who found that renal transplant patients receiving MMF in combination with TAC have a 2.4 higher incidence of diarrhea than those on CsA (n=110). It was also previously demonstrated that patients receiving MMF in

combination with SIR or TAC were exposed to higher plasma concentrations of MPA than those with CsA (22-24). CsA presumably decreases MPAG biliary excretion by inhibiting MRP2, as suggested by data from mutant rats not expressing MRP2 (17). Consequently, less MPAG is subject to deconjugation by the intestinal flora, resulting in decreased re-circulation of MPA, impacting its plasma levels. However, several studies failed to demonstrate any direct association between plasma levels of MPA or MPA metabolites and MMF related AE (6), including the Apomygre trial where the incidence of MMF related GI AEs was identical in the two protocol arms despite significantly higher MPA exposure over the first three months post-transplantation in the concentration-controlled group (25). This suggests that the decreased risk of MMF-related diarrhea in patients receiving CsA as compared to SIR or TAC would be more related to a local mechanism. CsA might decrease intestinal exposure to MPAG/AcMPAG, as well as to MPA (derived from intestinal hydrolysis of these metabolites), which could result in a lower risk of diarrhea by a yet unknown mechanism. A potential limitation of the present work is that we did not investigate MPA exposure as a covariate in the multivariate analysis, due to the fact that, apart from patients included in clinical trials, the determination of MPA levels was not regularly performed in this retrospective cohort, in particular before 2002. Another limitation is that no detailed information was available about the diarrhea intensity and its evolution after treatment modification (if any).

Noteworthy, the sex ratio was different between the 3 groups of associated immunosuppressant ($p=0.01$). This difference may be explained by the physicians' prescription habits as they usually prefer to prescribe tacrolimus instead of cyclosporine to female patients because of the well-known risk of hypertrichosis associated with the

latter. However, there was no difference in the incidence of diarrhea between males and females.

We observed a longer follow up period for patients co-treated with CsA as compared to those co-treated with TAC or SIR, which is due to the fact that cyclosporine was more often prescribed than tacrolimus or sirolimus over the first years of the follow-up period.

The daily dose of MMF itself does not seem to influence the risk of diarrhea, although in clinical practice, a dose decrement is sometimes used to stop or reduce it. The relative risk of the MMF dose, estimated from 121 renal transplants by Borrows et al., was very low (1.17 per 1g-increase of MMF dose) (5). In this study, we compared the dose received before the adverse events in patients with diarrhea to that of paired patients without. Although the pairing strategy did not allow for more than one control per case, resulting in a loss of statistical power, the effect of MMF dose was not significant in this sub-group of 136 patients, which is consistent with another study where patients who suffered from diarrhea had not received a significantly different MMF daily dose than those who did not (6). Moreover, we found that patients on tacrolimus had the highest incidence of diarrhea although they received a lower MMF dose at the time of diarrhea than patients on SRL of CsA. The high reactivity of AcMPAG could possibly contribute to MMF GI AEs. Alternatively, the amount of MPA produced in the gut from the hydrolysis of MPAG during MPA enterohepatic cycling could also possibly trigger local inflammation, although MMF is already partly hydrolyzed to MPA in the gut lumen or in intestinal epithelial cells, due to the ubiquity of esterases in the body. In this study we investigated the major polymorphisms of the isoforms thought to be involved in MPA intestinal metabolism or metabolites excretion and found that patients carrying

the *UGT1A8*2* allele had a lower risk of diarrhea than homozygous wild-type carriers. Bernard et al found that UGT1A8 produces both MPAG and AcMPAG using stably-expressed enzyme in HEK-293 cells. In their study, cells transfected with *UGT1A8*2* had a decreased capability to produce AcMPAG as compared to *UGT1A8*1* (V_{max} and Cl_{int} values were divided by 2) but similar activity for MPAG formation (26) . Thus, the lower incidence of MMF-related diarrhea found here in patients carrying the *UGT1A8*2* allele could possibly be linked to a lower local production of AcMPAG, which would prevent its toxicity on the intestinal mucosa. The relative risk of diarrhea linked with the co-administered immunosuppressant is greater than that of the *UGT1A8* polymorphism, but the UGT activity might be an important factor in CsA treated patients (whose biliary excretion of metabolites is reduced) contrary to patients receiving SIR or TAC, as suggested by the ranking of their combined effects (Fig. 4).

We observed no effect of *UGT1A8*2* when GI AEs were studied as the whole, which included diarrhea, abdominal pain, nausea/vomiting and anorexia. It was already suggested that MMF diarrhea can be due to a local mechanism involving MPA glucuronidation. This hypothesis is probably less likely for the other GI AEs. This can explain why the results were not similar when studying diarrhea alone or together with the other GI AEs. We hypothesized that GI AEs as a whole represented a too heterogeneous phenotype, masking the association between diarrhea and the genotype.

ABCC2 was another good candidate gene in line with our hypothesis, but we did not find any significant effect of its SNPs or haplotype on the risk of diarrhea in the whole population, or when considering separately patients on CsA and patients on TAC/SIR. This last result shows that this absence of genotypic effect is not due to a masking MPR2 inhibition, which has been reported for CsA (17) but not for TAC or SIR.

In conclusion, the inhibition of the biliary excretion of MPA metabolites by CsA and the local production of these metabolites depending on the activity of UGT1A8 may be important risk factors of MMF-related diarrhea. The exact mechanisms underlying these findings deserve further investigation.

Acknowledgements

We thank the Limoges University Hospital and the University of Limoges for their support. We are also grateful to J. H. Comte for his excellent laboratory work and to Karen Poole with manuscript editing.

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Table 1- Frequency and distribution of the polymorphisms studied.

Gene	Polymorphism	Frequency of the variant allele	Genotype		
			wt/wt§	wt/m¤	m/m
UGT	622C>T (*UGT1A7*)	0.627	37/251*	113/251	101/251
	518C>G (*UGT1A8*2*)	0.226	151/256	94/256	11/256
	830G>A (*UGT1A8*3*)	0.017	247/256	9/256	0/256
	-275T>A (*UGT1A9*)	0.055	228/256	28/256	0/256
	-840G>A (*UGT2B7*)	0.482	73/256	117/256	65/256
ABCC2	-24C>T	0.235	148/251*	88/251	15/251
	3972C>T	0.394	82/251*	140/251	29/251

§wt: wild type. ¤m: variant

*5 patients remained undetermined for *UGT1A7* and *ABCC2* genotypes

Table 2- Patients' characteristics according to the immunosuppressant associated to MMF.

	CsA (n=185)	**SIR (n=22)**	**TAC (n=49)**
Male/Female	129/56	9/13	24/25
Age	48.7±14.01	55.3±13.95	47.8±13.03
(min/max)	(17.5/74.3)	(20.6/72.7)	(21.8/70.5)
follow-up	48.7±2.5	19.1±4.7	21.9±3.2
(min/max)	(0.7/114.6)	(0.6/95.0)	(0.7/115.1)
Number of patients (%) with ≥ 1 episode of diarrhea	33 (17.8%)	12 (54.5%)	26 (53.1%)

Parameters are expressed as mean±SD, age is expressed in years, follow-up period is expressed in months and the frequency is given for the number of patients with adverse events; CsA: Cyclosporine, TAC: Tacrolimus and SIR: Sirolimus

Table 3- Univariate analysis (Cox Model) of the influence of the different variables studied on the incidence of diarrhea

Variable	***Category¤***	***Hazard Ratio****	***CI 95%***	***p***	***Number of patients***
MMF dose	<2 vs>=2	1.146	0.702-1.869	0.5861	68 vs 68*
Co-administered immunosuppressant	TAC vs CsA	3.817	2.262-6.452	<0.0001	49 vs 185
	SIR vs CsA	4.808	2.463-9.434	<0.0001	22 vs 185
	SIR vs TAC	1.214	0.612-2.408	0.5789	22 vs 49
	SIR/TAC vs CsA	4.251	2.637-6.853	<0.0001	71 vs 185
*UGT1A8*2 518C>G*	CC vs CG/GG	1.968	1.163-3.322	0.0117	151 vs 94/11
*UGT1A8*3 830G>A*	GA/AA vs GG	1.190	0.374-3.788	0.7679	9/0 vs 247
UGT1A7 622C>T	CC vs TT	0.962	0.465-1.987	0.9161	37 vs 101
	CT vs TT	1.065	0.640-1.772	0.8084	113 vs 101
UGT1A9 -275T>A	TA/AA vs TT	1.389	0.712-2.712	0.3355	28/0 vs 228
UGT2B7 -840G>A	AA vs GG	1.104	0.569-2.142	0.7700	65 vs 73
	AG vs GG	1.245	0.705-2.200	0.4496	117 vs 73
ABCC2 -24C>T	CC vs CT/TT	1.085	0.673-1.751	0.7376	148 vs 88/15
ABCC2 3972C>T	CC vs TT	0.765	0.353-1.657	0.4964	82 vs 29
	CT vs TT	0.774	0.374-1.602	0.4901	140 vs 29
ABCC2 Haplotype -24C>T/3972C>T	C-T vs C-C	1.271	0.813-1.989	0.2930	Haplotype frequency in 251 patients : CC : 0.47 CT : 0.30 TC : 0.14 TT : 0.09
	T-T vs C-C	0.980	0.523-1.544	0.9324	
	T-C vs C-C	0.644	0.098-4.215	0.6463	

*Case-control sub-study. NB. in five patients with diarrhea, the dose could not be taped off. ¤For the most frequent category of the variable, taken as reference, HR=1.

Legends to figures

Figure 1 - Kaplan-Meier analysis of the time until the first episode of diarrhea in patients on either CsA/MMF, SIR/MMF or TAC/MMF (TAC: Tacrolimus, SIR: Sirolimus, CsA: Cyclosporine).

Figure 2 - MMF dose repartition in patients with diarrhea (n=68) and in controls (n=68).

Figure 3 - Kaplan-Meier analysis of the time until the first episode of diarrhea according to *UGT1A8* 518C>G genotype (*UGT1A8*2*). 3A: all patients (n=256); 3B: patients who started MMF in the first days posttransplantation (n = 222); 3C: patients who were switched from azathioprine to MMF in the stable post-transplantation period (n=34).

Figure 4 - Kaplan-Meier analysis of the time until the first episode of diarrhea according to the *UGT1A8* genotypic groups and immunosuppressive co-treatments (TAC: Tacrolimus, SIR: Sirolimus, CsA: Cyclosporine). Cox proportional hazard models showed significant differences between: (i) CsA co-treated/*UGT1A8*2* carriers and CsA co-treated /*UGT1A8*2* non-carriers (*p=0.03); (ii) CsA co-treated/*UGT1A8*2* carriers and TAC or SIR co-treated/*UGT1A8*2* carriers or non-carriers (§ $p<0.0001$); (iii) CsA co-treated /*UGT1A8*2* non-carriers and TAC or SIR co-treated/*UGT1A8*2* carriers or non-carriers (# $p<0.01$). In patients co-treated with TAC or SIR, no significant difference was found between *UGT1A8*2* carriers and non-carriers.

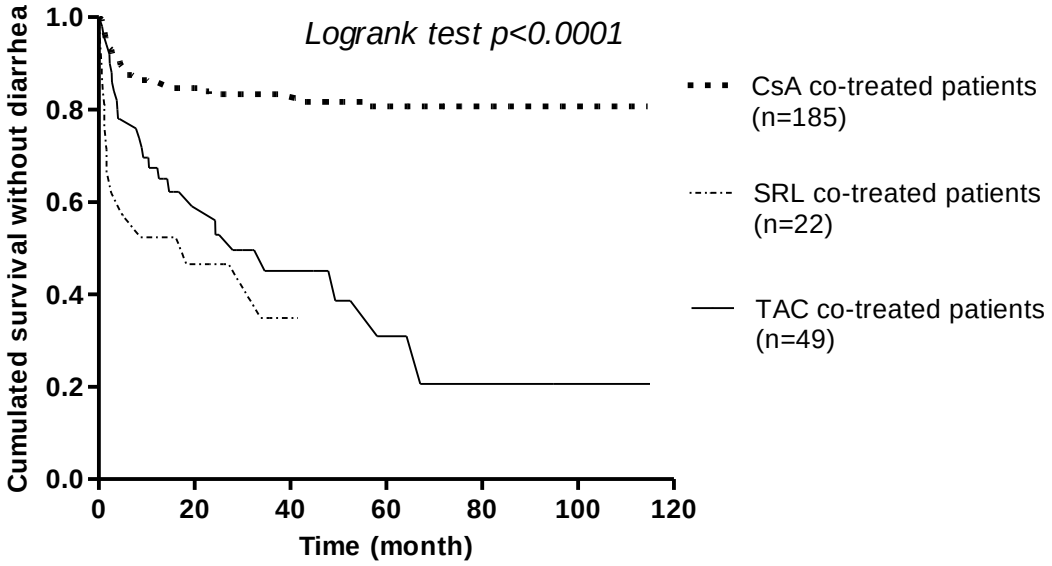
Logrank test p<0.0001
Cumulated survival without diarrhea
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
Time (month)
CsA co-treated patients (n=185)
SRL co-treated patients (n=22)
TAC co-treated patients (n=49)

Figure 1

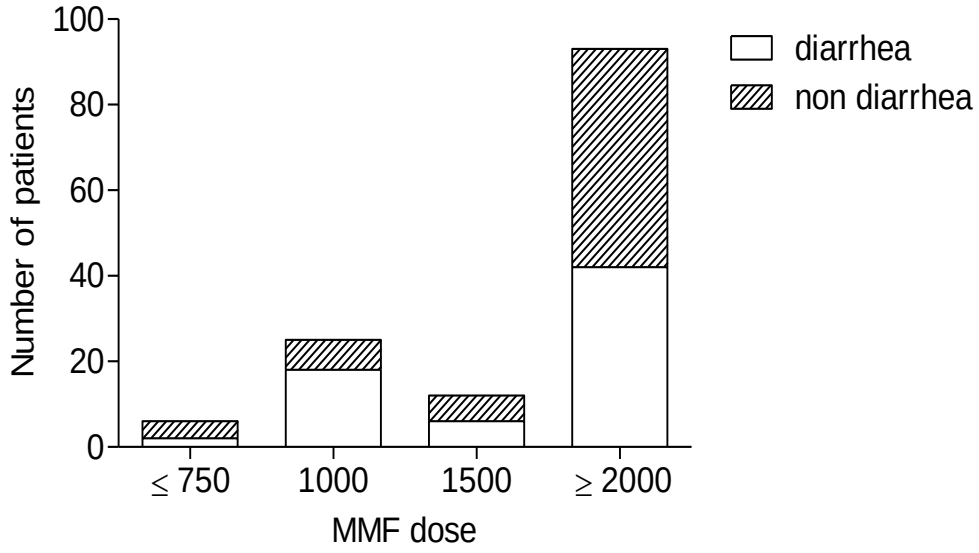
100
80
60
40
20
0
Number of patients
≤ 750
1000
1500
≥ 2000
MMF dose
diarrhea
non diarrhea

Figure 2

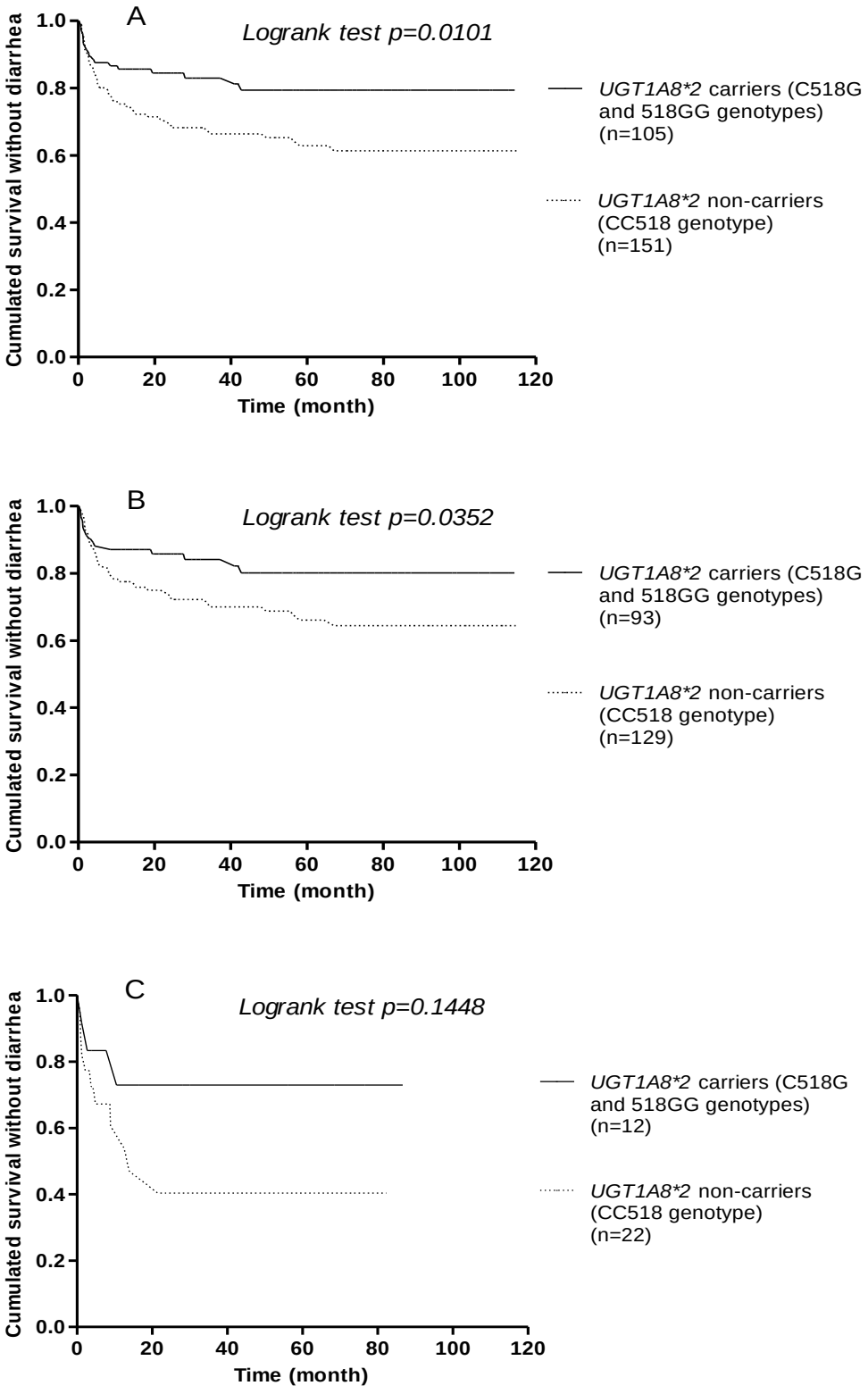
A
Logrank test p=0.0101
Cumulated survival without diarrhea
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
Time (month)
UGT1A8*2 carriers (C518G and 518GG genotypes) (n=105)
UGT1A8*2 non-carriers (CC518 genotype) (n=151)
B
Logrank test p=0.0352
Cumulated survival without diarrhea
Time (month)
UGT1A8*2 carriers (C518G and 518GG genotypes) (n=93)
UGT1A8*2 non-carriers (CC518 genotype) (n=129)
C
Logrank test p=0.1448
Cumulated survival without diarrhea
Time (month)
UGT1A8*2 carriers (C518G and 518GG genotypes) (n=12)
UGT1A8*2 non-carriers (CC518 genotype) (n=22)

Figure 3

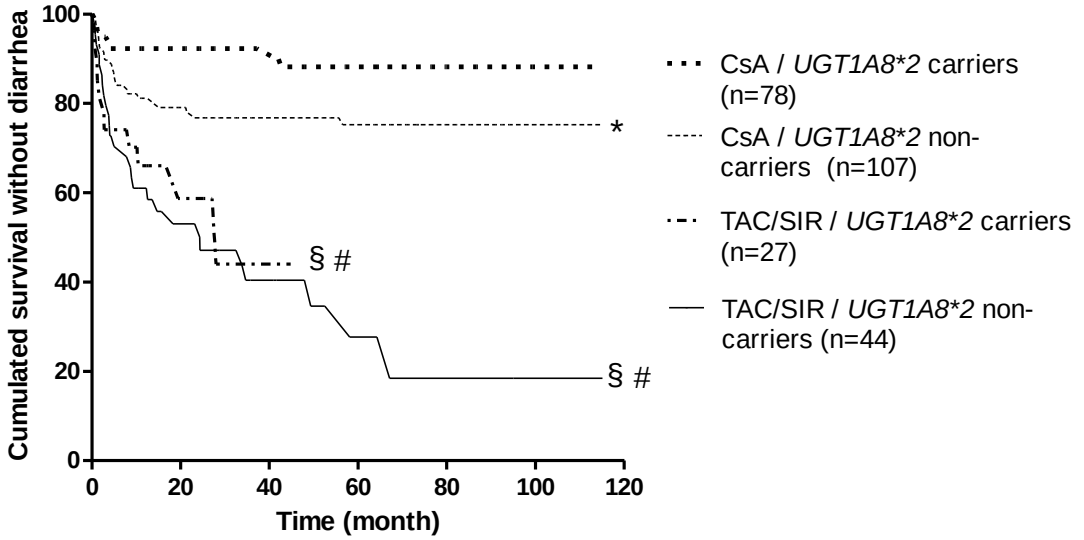
Cumulated survival without diarrhea
100
80
60
40
20
0
0
20
40
60
80
100
120
Time (month)
*
§ #
§ #
CsA / UGT1A8*2 carriers (n=78)
CsA / UGT1A8*2 non-carriers (n=107)
TAC/SIR / UGT1A8*2 carriers (n=27)
TAC/SIR / UGT1A8*2 non-carriers (n=44)

Figure 4