Polymorphisms in type I and II inosine monophosphate dehydrogenase genes and association with clinical outcome in patients on mycophenolate mofetil.

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Polymorphisms in type I and II Inosine Monophosphate Dehydrogenase (IMPDH) genes and association with clinical outcome in patients on mycophenolate mofetil.

Olivier Gensburger[1,2], Ron H.N. van Schaik[3], Nicolas Picard[1,2,4], Yannick Le Meur[5], Annick Rousseau[1,6], Jean-Baptiste Woillard[1,2], Teun van Gelder[7], Pierre Marquet[1,2,4].

[1]INSERM U850, Limoges, France; [2]Univ Limoges, Laboratory of Medical Pharmacology, Limoges, France; [3]Department of Clinical Chemistry, Erasmus University Medical Centre, Rotterdam, The Netherlands; [4]CHU Limoges, Department of Pharmacology-Toxicology, Limoges, France; [5]CHU Brest, Hôpital de la Cavale Blanche, Service de Néphrologie, Brest, France; [6]Univ Limoges, Laboratory of Biophysics, [7]Departments of Hospital Pharmacy and Internal Medicine, Erasmus Medical Centre, Rotterdam, The Netherlands.

(Running title) MPA target polymorphism involvement in its TDM

Address for correspondence:

Prof. Pierre Marquet,

Service de Pharmacologie-Toxicologie, CHU de Limoges, 2 avenue Martin-Luther King

87042 Limoges cedex

Tel. +33 555 05 60 17

Fax. +33 555 05 61 62

E-mail: pierre.marquet@unilim.fr

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Abstract:

Background: Type I and II inosine monophosphate dehydrogenases (IMPDH) are the targets of mycophenolic acid (MPA), a widely used immunosuppressant. The aims of this study were: to check the presence of controversial polymorphisms in the *IMPDH II* gene; to look for new ones; and to investigate potential associations between the most frequent SNPs in both *IMPDH* genes and clinical outcome in renal transplant recipients.

Methods: The DNA and clinical data of 456 patients from two clinical trials were collected. We sequenced the *IMPDH II* gene in 80 patients and we genotyped the 456 patients' DNA for the *IMPDH II* rs4974081, rs11706052, 787C>T and the *IMPDH I* rs2278293 and rs2278294 SNPs, all of which were previously reported to be potentially involved in MPA treatment related outcome. We investigated the associations of biopsy proven acute rejection (BPAR), leucopenia, CMV infections and other infections with these *IMPDH* polymorphisms, as well as with demographic, biological and treatment data using multivariate analysis.

Results: Many *IMPDH II* variant alleles referenced in Genbank were not detected and no new polymorphisms were identified. In the whole group of 456 patients, the *IMPDH I* rs2278294 SNP was associated with a lower risk of BPAR and a higher risk of leucopenia over the first year post-transplantation. No other IMPDH I or IMPDH II polymorphism was significantly associated with any clinical outcome. Interestingly, CNI and MPA exposures below the therapeutic range increased the risk of BPAR. CMV infection was the factor most closely linked with leucopenia, whereas tacrolimus was associated with fewer infections than cyclosporine.

Conclusion: *IMPDH II* genotyping may not improve MPA treatment outcome over the first year post-transplantation, in contrast to MPA and CNI therapeutic drug monitoring and *IMPDH I* genotyping.

Introduction

Mycophenolic acid (MPA) is an immunosuppressive drug widely used in liver and kidney transplantation [1-3]. Its effect is essentially attributed to the inhibition of type II Inosine MonoPhosphate DeHydrogenase (IMPDH) [4-6], a rate-limiting enzyme involved in the *de novo* purine synthesis pathway [7, 8]. Lymphocytes are devoid of the salvage pathway that allows other cells to recycle purines, and are therefore dependent on IMPDH II for DNA synthesis and GMP/AMP controlled pathways [9]. MPA therapy is not devoid of treatment failures and side effects [10-12], which indicates inter-individual pharmacokinetic or pharmacodynamic variability. The potential benefit of mycophenolate mofetil (MMF) dose adjustment in decreasing the risk of acute rejection is still controversial, as two studies yielded divergent results [13, 14]. There have been many attempts to identify pharmacogenetic parameters that could be taken into account in order to control MPA pharmacokinetic variability, with limited success so far [15, 16]. As IMPDH I and II are the target proteins of MPA, polymorphisms in their genes might explain part of the inter-individual variability in MPA effects [17]. Wang et al. found an association between two intronic *IMPDH I* SNPs (rs2278293 and rs2278294) and the incidence of BPAR in kidney transplant recipients, and concluded that these *IMPDH I* polymorphisms should be considered in further pharmacogenetic studies [18]. In a recent study on IMPDH II expression, Vannozzi F et al. identified *IMPDH II* gene as another good candidate for MPA pharmacogenetic studies [19]. Several studies demonstrated that both isoforms of IMPDH are transcribed in response to T cell activation [11] and that the increase of IMPDH activity is linked to activation of both IMPDH isoforms [20-21].

The human *IMPDH II* gene is located on chromosome 3 locus 3p21.2 and is approximately 5.2 kb in length (accession number: NC_000003.10). It has 14 exons encoding a 1.7 kb mRNA (NM_000884.2) leading to a 514 amino-acid protein of 56 kDa

(NP_000875.2) [22]. This protein shares 84% homology with IMPDH I and its exonic sequence is highly conserved among species, especially in mammalians [23]. The 3D structure has been published, and crucial amino-acids for the enzyme activity (Cys-331, Asp-364 and Ser-329) and MPA binding site (Ser-276 Thr-333 and Gln-441) have also been reported [22, 23]). Polymorphisms in these regions could potentially be linked with inter-individual variations in MPA effects. A random mutagenesis study has found three IMPDH II mutations (L30F/Q227R, A462T and F465S/D470G) conferring resistance to MPA [24]. However, none of them have yet been reported *in vivo*.

Little is known about the consequences of *IMPDH II* gene polymorphisms and their involvement in patients' response to MPA. Only two non-synonymous polymorphisms are referenced in the NCBI database (the AAG/- rs5848860 deletion and the rs11557540 A/G single nucleotide polymorphism) with no frequency mentioned. A recent study [25] based on genotyping of more than 400 DNA samples reported no mutated allele for these two previously reported polymorphisms (rs5848860 and rs11557540) and suggested these reported polymorphisms to be sequencing artifacts. Using *in silico* techniques, the same team predicted a possible effect of a mutation in the promoter region (rs4974081) on *IMPDH II* transcription [25], which has not yet been confirmed by *in vitro* or clinical studies. Wang J. *et al.* reported the previously non-referenced 787C>T SNP in exon 7, leading to a Leu-Phe substitution and a 10-fold decrease in basal enzyme activity *in vitro* [16]. However, this mutation showed a low frequency (~1%) in the population and the authors were not able to demonstrate any association between this mutation and clinical outcomes [18].

Grinyó J. *et al* recently reported an association of the 3757T>C SNP (rs11706052) in intron 7 on the *IMPDH II* gene with a higher risk of acute rejection in renal transplant recipients [15], whereas Wang et al. did not find any association between this SNP and clinical outcomes. A recent study in a cohort of 80 renal transplanted patient treated by MPA

found increased IMPDH activity in variant carriers of the 3757T>C *IMPDH II* SNP [26], but no association with acute rejection incidence was found, which may have been due to an underpowered sample size.

The goals of the present study in a large group of renal transplants (n=456) receiving mycophenolate mofetil in combination with calcineurin inhibitors were to look for possible new polymorphisms in exons, the flanking intron-exon regions and the proximal promoter of the *IMPDH II* gene in 80 renal transplant patients and for associations between clinical outcomes and the previously reported or new *IMPDH* polymorphisms, taking in account demographic, biological and treatment data as potential confounders.

Material and methods

Patient population:

This study was conducted in adult, de novo renal transplant patients enrolled in the pharmacogenetic substudies of two clinical trials: a first group of 121 (out of 137) patients enrolled in of the Apomygre trial (Clinical Trial Registry No. NCT0019967); a second group of 335 patients from the international FDCC trial (NCT00166244). Both studies were prospective, randomized, open-labeled and aimed to compare concentration-controlled MMF with the approved fixed-dose over the first 12 months posttransplantation. All patients signed a specific written informed consent for pharmacogenetic investigations. The study design, patient populations and primary results were recently reported [13, 14]. Briefly, patients enrolled in Apomygre received MMF in combination with cyclosporine, whereas those in FDCC were either on tacrolimus or cyclosporine (Table 1). MPA $AUC_{0\text{-}12h}$, the associated calcineurine inhibitor (CNI), CNI concentrations, side effects (leucopenia, infection, diarrhea), and biopsy proven acute rejection (BPAR) episodes were collected at D7, D14, M1 M3, M6 and M12 post-transplantation in both trials. Leucopenia was defined as a white blood

cell count < $3.10^9.l^{-1}$. Infections were defined as clinical infectious syndromes with positive identification of an organism by microbiological culture, serology, antigen detection, PCR techniques or histology. Patients' demographic characteristics are reported in Table 1.

DNA samples:

In the FDCC study [27], genomic DNA was isolated from 200 µl of EDTA whole blood using a total nucleic acid extraction kit (MagNA Pure LC; Roche Diagnostics, Mannheim, Germany). In the Apomygre study, genomic DNA was extracted from EDTA-treated blood using a previously described manual ethanol precipitation method [28].

DNA from 80 randomly selected APOMYGRE patients were used for *IMPDH II* sequencing. The genomic DNA samples from 121 APOMYGRE and 335 FDCC patients were genotyped and used for the pharmacogenetic association study.

IMPDH II sequencing:

To tentatively identify novel *IMPDH II* mutations, 10 targets covering the proximal promoter, 14 exons and intron/exon flanking regions from 80 patients DNA samples were sequenced. Table 2 lists the SNPs identified. Ten genomic DNA regions (target 1 to 10) covering the proximal promoter region, the 14 exons and the flanking intron-exon regions of the *IMPDH II* gene were PCR-amplified using the AmpliTAQ gold PCR master mix (Applied Biosystems, Courtaboeuf, France) (covered regions are reported in supplemental Table 1). Reactions were carried out in a final volume of 100 µl using 150 ng genomic DNA and 10 µM of reverse and forward primers, designed using the Primer3 website (http://frodo.wi.mit.edu/) and reported in supplemental Table 1. Amplification consisted of an initial denaturation for 6 minutes at 95°C followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 15 seconds and extension at 72°C for 1 minute. Terminal elongation was performed at 72°C for 7 minutes. The PCR-amplified products were purified

using the QiaVac96 kit (Qiagen, Courtaboeuf, France) and forward sequenced using the Bigdye terminator v1.1 kit (Applied Biosystems) according to the manufacturers' procedures. All the potential SNPs were confirmed by reverse sequencing. The extension products were purified using a standard Ethanol/EDTA/sodium acetate precipitation procedure and the nucleotide sequences determined on an ABI 3100 automated sequencer (Applied-Biosystems). The Sequencer v4.8 program (Gene Codes Corp. Ann arbor, MI, US) was used for data processing using the *IMPDH II* reference sequence (Genbank NC_000003.10) for automatic alignment. All SNPs and unclear regions were visually checked.

Genotyping assays:

The *IMPDH II* rs11706052 and 787C>T SNPs were genotyped in patients whose DNA had not been sequenced at the previous step. The previously reported rs4974081, rs2278293 and rs2278294 SNPs were genotyped in all 456 patients because *IMPDH II* rs4974081 was out of our sequencing coverage and both rs2278293 and rs2278294 are located on the *IMPDH I* gene. Genotyping was performed using validated TaqMan allelic discrimination assays on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems), using the manufacturer protocol.

Statistical analyses:

Deviation from the Hardy-Weinberg equilibrium was investigated for each SNP separately using the Fisher exact test. Statistical analyses were performed using R software version 2.10.1 (R foundation for statistical computing, http://www.r-project.org). Each clinical outcome criterion (BPAR, CMV infections, other infections and leucopenia episodes occurring over the first year post transplantation) was used in turn as the dependent variable for stepwise logistic regression analysis using the "Epicalc" R package. Different genetic models (co-dominant, dominant, recessive and log-additive) were tested using the

"SNPassoc" R package. The log-addidive model was retained for all the dependent variables on the basis of the lowest Akaike criterion. Patients' genotypes, sex, age, MPA inter-dose area under the concentration-time curve ($AUC_{0\text{-}12h}$), the associated CNI and its representative blood levels (C_{2h} for cyclosporine, C_0 for tacrolimus), were used as independent variables. In addition, CMV infections were considered as an independent variable for the multivariate regression analysis considering leucopenia as the dependant variable. The CNI concentration and MPA $AUC_{0\text{-}12h}$ values used were those closest to the event or, when the dose administered or patient's exposure might have been influenced by the event, just prior to it. Concentration values in the stable period (M6-M12) were used when no events occurred. Patients were divided into the following categorized groups: below (group 1), within (group 2), or above (group 3) the target range for either CNI concentration (CNI concentration groups) or MPA $AUC_{0\text{-}12h}$ (MPA AUC groups). Target ranges were 30 to 60 mg.h/l for MPA $AUC_{0\text{-}12h}$; 1300-1500 (D7-M1), 1100-1300 (M1-M3), 800-1100 (M3-M6) and 700-900 µg/l (M6-M12) for cyclosporine C_{2h} [13]; 10-15 µg/l (D7-M3) and 5-15 µg/l (M3-M12) for tacrolimus C_0 [29]. The final multivariate model was determined by stepwise regression. Variables with p values > 0.20 were not included into the final model; p values < 0.05 in the final model were considered as significant. Linkage disequilibrium estimation and haplotype analysis were performed using the THESIAS program (http://genecanvas.ecgene.net).

Results

IMPDH II polymorphisms:

Within exonic regions, no SNP was detected. Specifically, no variant allele for the two non-synonymous (rs11557540 and rs5848860) or the seven synonymous (rs11557543, rs11557547, rs11557541, rs11557545, rs11557542, rs11557544 and rs11557546) SNPs referenced in Genbank (http://www.ncbi.nlm.nih.gov/SNP; June 2009) were found. Similarly,

the 787 C>T (Leu236Phe) and 417 C>T SNPs previously reported by Wang *et al.* [16] could not be found in these 80 samples. Within the intronic regions sequenced, the SNPs referenced in Genbank were not found either, except for the rs11706052 SNP (i.e., IVS7+10T>C or 3757T>C; Table 2), detected in 9.3% of the sequenced alleles (Table 2). In addition, we observed, although at low frequencies, 3 previously reported intronic SNPs (IVS1-162C>T: 1.2%; IVS1+91 T>G: 0.6%; and IVS12-23C>T: 2.5%; Table 2). The IVS4+197 C>T and IVS5-62 G>A SNPs reported by Wang et al. were located out of our target boundaries and the IVS8-7G>A SNP was not present in the samples analyzed. No other SNP was found in the untranslated (intronic, proximal promoter and 5'/3' UTR) regions sequenced.

The minor rs4974081 allele located in the *IMPDH II* distal promoter region was genotyped and found at an allelic frequency of 24% in our 456 patients (Table 3). The intronic 3757T>C (rs11706052) SNP detected by gene sequencing was further investigated in the remaining patients by genotyping. The frequency of its variant allele was 9% (Table 3). No variant 787C>T (Leu236Phe) allele was found in the group of 456 patients (Table 3). No linkage disequilibrium was found between *IMPDH II* rs4974081 and rs11706052 SNPs (r^2 = 0.02).

***IMPDH I* polymorphisms:**

For the *IMPDH I* rs2278293 and rs2278294 SNPs, the minor allele frequencies were 46% and 36%, respectively (Table 3). The allelic distributions of all the SNP detected were in the Hardy-Weinberg equilibrium. The SNPs were in strong linkage disequilibrium (D'=0.78; r^2=0.41) leading to 4 haplotypes with the following frequencies: G-G (0.50); G-A (0.04); A-G (0.14), A-A (0.32).

For those previously reported, the allelic frequency found here was consistent with the literature: rs4974081, 21% in Caucasians [25]; 3757T>C (rs11706052) 7-10.2% [15, 16];

787C>T (Leu236Phe) < 1% [16, 26] ; rs2278293 42.7% ; and rs2278294 40.6% [18]. It is noteworthy that the patients' ethnicity or the allelic distribution between ethnic subpopulations was not mentioned in these last four papers.

Association between clinical outcome and genetic, demographic and pharmacologic parameters:

No association was found between the four tested clinical outcomes and any of the *IMPDH II* genotypes tested. Neither variants of the distal promoter (rs4974081) nor of the 3757T>C (rs11706052) SNPs were significantly associated with an increased risk of side effects or acute rejection (OR for BPAR was 0.66 (95% CI [0.39-1.13]; p = 0.13) for rs4974081 variant allele carriers, and 0.89 (95% CI [0.44-1.78]; p = 0.74) for rs11706052 carriers). The *IMPDH I* rs2278293 variant allele was not significantly associated with any tested outcomes either. The final regression model using acute rejection (BPAR) as the dependent variable included *IMPDH I* rs2278294, MPA $AUC_{0\text{-}12h}$ and CNI blood levels as significant factors (Table 4). The risk of BPAR was lower for the rs2278294 variant allele carriers, with a gene-additive effect (OR 0.54 [0.34-0.85]; p = 0.0075). Patients whose exposure was below the target range for both immunosuppressive drugs presented a significantly higher risk of acute rejection than patients within or above the target range for at least one of the drugs (OR: 2.22, 95% CI [1.20-4.11]; p = 0.0109 and OR: 2.27, 95% CI [1.272-4.06]; p = 0.0056 for MPA $AUC_{0\text{-}12h}$ and CNI blood level respectively) (Table 4). Leucopenia was significantly associated with *IMPDH I* rs2278294, the type of CNI used, their categorized blood levels and CMV infections. However, the CNI type may be a confounding factor, as it showed a significant interaction with CMV (p = 0.0369). The association with *IMPDH I* rs2278294 showed an additive risk for each variant allele carried (OR 1.66, 95% CI [1.11-2.48]; p = 0.0139) (Table 4).

Haplotype analysis of *IMPDH I* showed no significant association with the clinical outcomes.

Discussion

We did not discover any new *IMPDH II* polymorphism in the 80 patients whose DNA was sequenced. In addition, we did not observe the previously reported, non-synonymous *IMPDH II* polymorphisms rs11557540, rs5848860 and 787C>T. The first two mutations were already indicated as potential sequencing errors by Mohamed *et al.* [25] who studied a cohort of more than 400 patients, whereas the third (787C>T) polymorphism was found at a low frequency (1% or less) by Wang *et al.* in two different studies [16, 18]. However, we did not find this last variant allele either when genotyping our 456 DNA samples. This difference may be due to the different ethnical origins of the populations studied. Even though Wang *et al.* reported that the Leucine to Phenylalanine amino acid change resulting from this SNP induced a drastic decrease in IMPDH II catalytic activity [16], they also wrote in a second paper that a putative association with leucopenia would be difficult to prove due to its very low frequency [18]. Furthermore, such a rare allele would probably not significantly contribute to clinical management of the MMF benefit/risk balance.

The present sequencing study did not retrieve any mutated allele of the *IMPDH II* intronic SNPs listed in GenBank, except for rs11706052 (3757T>C) which was present at a frequency similar (9% of our 456 patients) to those reported by Grinyo *et al.* [15] and Wang *et al.* [16]. In our cohort, this SNP was not significantly associated with BPAR, which is consistent with the results previously reported by Wang *et al.* [18] who found no association in 191 kidney transplant recipients using univariate analysis. However, Grinyo *et al* [15] did find a significant association between the 3757T>C SNP and BPAR at 3 and 12 months post-transplantation in 237 patients of the CAESAR study. They used multivariate logistic

regression but only considered a few demographic covariates (age, sex, treatment groups – cyclosporine standard dose, low-dose or withdrawal- and donor type), but not MPA dose (that was significantly linked with BPAR in their study) or MPA exposure. They also admitted that they had very few variant allele carriers and that they had no mechanistic explanation for this association. The present study has the largest group of patients prospectively followed for one year post-transplantation so far, and used multivariate logistic regression considering many possible confounders. We were not able to confirm the association between this genotype and acute rejection.

We also found that this 3757T>C SNP was not associated with leucopenia (which was consistent with Wang et al.'s results) [18], nor with CMV infections or other infections, which were not tested in the other two studies. A recent paper [26] reported a higher IMPDH activity in carriers of the variant 3757T>C SNP, without any association with clinical outcomes. As we did not measure IMPDH activity in the present patient cohort, we were unable to check the functional consequence of this polymorphism.

We were also interested in the promoter variant rs4974081 for which an effect on *IMPDH II* transcription and thus possibly on MMF effects was forecasted *in silico* [25]. The variant allele was present in 25% of our 456 patients, but no statistically significant association was found with any clinical outcome tested. In vitro studies of *IMPDH II* transcription may give more information about the potential influence of this promoter mutation.

We found three *IMPDH II* intronic SNPs, which were previously reported by Wang et al [16] at the same low frequencies. These SNPs showed no significant association with patient outcomes. Although the study may have been underpowered to find any significant association with these rare mutated alleles, it is less likely that intronic SNPs have a large influence on MPA pharmacodynamics.

We also investigated two *IMPDH I* polymorphisms (rs2278293 and rs2278294) which were previously reported to be associated with a decreased risk of BPAR [18]. No association with either BPAR or other tested outcomes was found for the rs2278293 variant in our large sample of patients. This discrepancy with Wang et al. is not explained by the fact that we did not group heterozygous and homozygous variant carriers (51% and 20% of patients, respectively) to perform our analyses, because when we did there was no significant association between the rs2278293 variant and BPAR either (data not shown). On the other hand, we did find a significant, protective effect on BPAR of the rs2278294 variant allele as compared to the wild-type allele (log-additive model; OR 0.54 95% CI [0.34-0.85]; p = 0.0075; Table 4). Nevertheless, no explanation on the mechanism behind this decreased risk of BPAR with an *IMPDH I* intronic SNP could be inferred. The mutant allele carriers also had a 1.6-fold increased risk of leucopenia, again with a gene-dose effect. The fact that BPAR and leucopenia are inversely associated to the same rs2278294 SNP reinforces the pertinence of this finding. A factor favoring low lymphocyte levels, sometimes leading to leucopenia episodes, is expected to protect the patient from developing an immunological reaction against the allograft.

One limitation of this study is that we missed information about the patients' ethnicity. This is partly due to the fact that the APOMYGRE study was conducted in France, where it is not legally possible to ask patients about their ethnic origins, unless it is the main aim or a main determinant of the study and provided one obtains a special authorization from the ministry of health. However, all patients enrolled in this sub-study were recruited in Europe, which suggests that a vast majority were of European descent, although this cannot be ascertained.

When combined with MPA, cyclosporine was associated with more frequent side effects (leucopenia, CMV and other infections) than tacrolimus, with no difference in acute rejection

incidence. Nevertheless, the association between CNI type and infections could be biased by the fact that all tacrolimus co-treated patient were enrolled in the FDCC trial where reporting infections could have been slightly different from the APOMYGRE trial.

Currently, the benefit of MPA TDM in transplant recipient is still under debate, as highlighted in a recent review by Knight & Morris [30]. Many retrospective studies found significant relationships between MPA $AUC_{0\text{-}12h}$ and BPAR or side effects in various types of populations [31-35], but the few prospective studies lead to diverging conclusions. The FDCC study found no statistically significant differences in outcome between the fixed-dose group and the dose-adjusted group [14], whereas the Apomygre trial did [13]. In the current retrospective study based on patients from these two trials we found statistically significant evidence that being in the target range for both the CNI (whether cyclosporine or tacrolimus) and MPA strongly protected against acute rejection, with no increased risk of side effects.

Conclusions

We did not find new variant alleles in the *IMPDH II* gene when sequencing DNA from 80 patients, nor associations between the known *IMPDH II* polymorphisms and clinical outcome. We found a significant association of the *IMPDH I* rs2278294 SNP with BPAR and leucopenia, but the previously reported association between *IMPDH I* rs2278293 and outcomes was not confirmed. MPA $AUC_{0\text{-}12H} < 30$ mg.h/L was significantly associated with an increased risk of BPAR, while the nature of and the exposure to the associated CNI were associated with BPAR, and leucopenia.

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Table 1: demographic characteristics of the studied patients enrolled either in the Apomygre or FDCC trials.

	Apomygre (n=121)	**FDCC (n=335)**	**Total (n=456)**
Sex			
Female	42	129	171
Male	79	206	285
Age (years)	49.5 ± 13.7	48.6 ± 13.0	48.8 ± 13.2
Co treatment			
Cyclosporine	121	156	277
Tacrolimus	0	179	179

Table 2: Summary of all known *IMPDH II* (NC_000003.10) polymorphisms and their localization. Positions are presented relatively to ATG set as +1.

Position (ATG as +1)	Genebank accession number	Other names	Synony-mous (Yes/No)	Found in this study (minor allele frequency)*
# - 3624 A>G / Promoter[1]	rs4974081	NA°	NA	Out of bounds
# - 162 C>T / Promoter[2]	NA	IVS1 - 162 C>T	NA	Yes (1.2%)
# 189 T>G / Intron 1[2]	NA	IVS1+91 T>G	NA	Yes (0.6%)
# 834 C>T / Exon 3	rs11557543	NA	Yes	No
# 863 A>G / Exon 3	rs11557547	NA	Yes	No
# 1049 C>T / Exon 4	rs11557541	NA	Yes	No
# 1331 C>T / Intron 4[2]	NA	IVS4+197 C>T	NA	Out of bounds
# 1373 G>A / Intron 4[2]	NA	IVS5-62 G>A	NA	Out of bounds
# 1455 C>T / Exon 5	rs11557545	NA	Yes	No
# 1527 C>T / Exon 5[2]	NA	417 C>T Phe139Phe	Yes	No
# 1570 T>C / Exon 5	rs11557542	NA	Yes	No
# 2565 C>T / Exon 7	rs11557544	NA	Yes	No
# 2632 C>T / Exon 7[2]	NA	787 C>T Leu236Phe	N (Leu>Phe)	No
# 2674 T>C / Intron 7[2,3]	rs11706052	IVS7+10 T>C/3757 T>C	NA	Yes (9.3%)
# 2735 G>A / intron 7[2]	NA	IVS8-7G> A	NA	No
# 2936 C>G / Exon 9	rs11557546	NA	Yes	No
# 4413 A>G / Exon 11[1]	rs11557540	NA	No (asp>Gly)	No
# 4526 C>T / intron 11[2]	NA	IVS12-23 C>T	NA	Yes (2.5%)
# 4833 AAG/- / Exon 13[1]	rs5848860	NA	No	No
# 4965 C>T / Exon 14	rs104981	NA	Yes	No

*Calculated from sequencing data. °NA= not applicable

[1]Mohamed et al. [2]Wang et al. [3]Grinyo et al.

Table 3. Observed frequencies of the genotyped *IMPDH I* and *IMPDH II* SNPs.

SNP	Genotype	Frequencies (n=456)	MAF*
IMPDH I gene			
rs2278293	Homozygous wt (GG)	28.9%	
	Heterozygous (AG)	51.1%	46%
	Homozygous var (AA)	20.0%	
rs2278294	Homozygous wt (GG)	41.2%	
	Heterozygous (AG)	45.6%	36%
	Homozygous var (AA)	13.2%	
IMPDH II gene			
rs4974081	Homozygous wt (AA)	56.4%	
	Heterozygous (AG)	37.9%	24%
	Homozygous var (GG)	5.7%	
rs11706052	Homozygous wt (TT)	82.1%	
	Heterozygous (TC)	17.4%	9%
	Homozygous var (CC)	0.4%	
787C>T	Homozygous wt (CC)	100.0%	
	Heterozygous (CT)	0%	0%
	Homozygous var (TT)	0%	

(*MAF = Minor allele frequency; wt = wild type; var = variant)

Table 4: Results of multivariate, stepwise logistic regression. Only the significant dependent variables kept in the final model are shown.

Variable	Category		Adjusted OR	95%CI	P Value
1. Leucopenia over the first year post-transplantation					
CMV	Yes		6.06	[3.03-12.11]	< 0.0001
	No		-	-	-
CNI	Cyclosporine		-	-	-
	Tacrolimus		0.49	[0.24-1.00]	0.0503
CNI groups	1. Below TR		0.37	[0.18-0.74]	0.0052
	2. Within TR 3. Above TR	Grouped	-	-	-
IMPDH I	G (WT)		-	-	-
rs2278294	A		1.66	[1.11-2.48]	0.0139
*Interaction CNI*CMV*					0.0369
2. CMV infections					
Age	Per year increase		1.02	[1.01-1.04]	0.0330
Sex	Female		-	-	-
	Male		0.61	[0.38-0.99]	0.0467
CNI	Cyclosporine		-	-	-
	Tacrolimus		0.33	[0.19-0.57]	< 0.0001
3. Other Infections					
CNI	Cyclosporine		-	-	-
	Tacrolimus		0.22	[0.13-0.37]	< 0.0001
4. BPAR					
AUC MPA groups	1.Below TR		2.22	[1.20-4.11]	0.0109
	2.Within TR 3.Above TR	Grouped	-	-	-
CNI groups	1. Below TR		2.27	[1.27-4.06]	0.0056
	2. Within TR 3. Above TR	Grouped	-	-	-
IMPDH I	G (WT)		-	-	-
rs2278294	A		0.54	[0.34-0.85]	0.0075

(TR = therapeutic range, WT = wild type)

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Supplemental Table 1: Primers, sequences and coverage of *IMPDH II* proximal promoter, exons, and flanquing intronic/exonic regions.

Primer name	Sequence (5' to 3')	IMPDH II gene coverage (bp)
Target n°1		
T1Left	GGCTTGGACTGTGAAAAGAAA	Proximal Promoter (-500 to -160)
T1Right	CCGCGCCAATATAAACCA	
Target n°2		
T2Left	CGTCTCTTTATTTTGGGGAGGA	Proximal Promoter + 5'UTR + Exon 1 + flanking region Intron1/Exon1 (-160 to 270)
T2Right	CATGTGTCTGGAGCATGGAA	
Target n°3		
T3Left	GAGATTCCATGCTCCAGACAC	Flanking region Intron1/Exon1 + Exon 1 + Flanking region Exon 1/Intron 2 (440 to 620)
T3Right	GAAAGCATCCCTTACACCTCA	
Target n°4		
T4Left	ATCACCAGATTGGGCTTGG	Flanking region Intron 2/Exon 3 & Exon 4/Intron 5 + Exon 3 + Intron 3 + Exon 4 + (730 to 1110)
T4Right	GACCAAATCACACCAACACATC	
Target n°5		
T5Left	GTTGGTGTGATTTGGTCTTGTG	Flanking region Intron 4/Exon 5 & Exon 5/ Intron 5 + Exon 5 (1400 to 1860)
T5Right	CAGTATGATTGAGTGAGGGATGAG	
Target n°6		
T6Left	TCAGCTACTTGGGAAGGAAAGA	Flanking region Inton 5/Exon 6 + Exon 6 to 8 + intron 7 and 8 (2280 to 2830)
T6Right	CTTACCCACTCCCACCACAC	
Target n°7		
T7Left	TCCAAAGATGCCAAGAAACA	Flanking region Intron 7/Exon 8 & Exon 9/Intron 9 + Exon 8 + Intron 8 + Exon 9 (2700 to 3160)
T7Right	GCAGAGCAGGAGCAAGAAGG	
Target n°8		
T8Left	AGCAGCCATCCCAGACAC	Flanking region Intron 9/Exon 10 + Exon 10 (3770 to 4220)
T8Right	CTTGCCTGTCCCACCTGAA	
Target n°9		
T9Left	CATGTGTTCCTCCATCTCAACA	Exon 10 + Flanking region Exon 10/Intron 11 + Intron 10 & 11 + Exon 11 & 12(4090 to 4640)
T9Right	ATGCCAGCAATCAGGTAAGG	
Target n°10		
T10Left	ATTTGTCCCTTACCTAGATTGCT	Exon 12 to 14 + intron 12 & 13 + 3'UTR (4650 to 5110)
T10Right	CAGGAGGAACTTTTTGGACCTGGAA	