The influence of pharmacogenetics and cofactors on clinical outcomes in kidney transplantation.

Nicolas Picard, Pierre Marquet.

INSERM UMR-S850, France; Univ. Limoges, France; CHU Limoges, France

Correspondence should be sent to:

Prof. Pierre Marquet

CHU de Limoges

Service de pharmacologie, toxicologie et pharmacovigilance

2 avenue Martin-Luther King

87025 Limoges, France

Tel. +33 555 05 60 17

Fax. +33 555 05 61 62

pierre.marquet@unilim.fr

Abstract

Introduction Immunosuppressive drugs have a narrow therapeutic range and a large inter-individual response variability, which has prompted pharmacogenetic studies, mostly with regard to their dose-concentration relationships, but also about proteins involved in their pharmacodynamics. Some polymorphisms in genes involved in their disposition pathways were shown to affect their dose-concentration relationships. The impact of pharmacogenetics on their tissue distribution and the resulting clinical effects have less often been studied. More importantly, a few single nucleotide polymorphisms seem to have a significant impact on the incidence of acute rejection or the adverse effects of immunosuppressants. Environmental factors often interact with such genotype-phenotype relationships.

Areas covered Current knowledge about the impact of genetic polymorphisms of the metabolic enzymes, membrane transporters and target proteins of mycophenolic acid, calcineurin inhibitors and mTOR inhibitors on clinical outcomes in kidney transplantation.

Expert opinion The current level of evidence is not yet high enough to recommend pharmacogenetic personalization of immunosuppressive regimens in transplant recipients. The prevention of cellular toxicity associated with local metabolism or transport, which cannot be addressed by routine monitoring, is worth investigating further.

Keywords: Immunosuppressants, pharmacogenetics, kidney transplantation.

1. Introduction

By definition, pharmacogenetics describes the influence of variations in the DNA sequence on drug response. This includes: (i) the study of the pharmacogenetic-pharmacokinetic relationships (i.e., the influence of the genome on the fate of the drug in the body); (ii) the study of the pharmacogenetic-pharmacodynamic relationships (i.e., the influence of the genome on the molecular or cellular effects of the drug); and (iii) pharmacogenomics that studies the global influence of genes involved in the pharmacokinetic (PK) and pharmacodynamic (PD) phases on drug effects, i.e. a complex and multigenic phenotype.

The vast majority of pharmacogenetic studies in general have been focused on cytochrome P450 enzymes, and then on phase II (conjugation) enzymes. More recently, research has been oriented towards the role of membrane transporters in drug intestinal absorption, passage in the metabolising organs or accumulation in target tissues. Pharmacogenetic-pharmacodynamic studies have been less explored, but this field is now booming with specific approaches and models, including: the definition of networks of genes involved in drug response (so-called "pharmacogenes") through the analysis of electronic databases to generate a "Genome-scale candidate gene lists for pharmacogenomics" [1]; and high-throughput genomic technologies such as RNA chips ("transcriptomics"), permitting the identification of potential "pharmacogenes", whose expression is modified in response to a drug. Finally, the rather recent pharmacogenomic dimension has been primed by the success of the so-called "genome-wide" approaches, or Genome-Wide Association Studies (GWAS), in the field of complex diseases [2]. Only a few pharmacogenomic, genome-wide studies have been performed so far and their results, although validating this paradigm, may appear to be disappointing. For instance, the two genome-wide studies on warfarin published in 2008 [3] and 2009 [4] could only confirm the major role, already evidenced by classical pharmacogenetic-pharmacokinetic and

pharmacogenetic-pharmacodynamic studies, of the VKORC1 and CYP2C9 genes on warfarin dose requirements in patients.

Almost all immunosuppressive drugs (IS) exhibit high inter-individual pharmacokinetic variability and narrow therapeutic ranges, which prompted pharmacogenomic research in order to understand the sources of this variability, and then to use genotyping to try and limit it. Many *in vitro* studies showed that polymorphisms in genes involved in the IS disposition pathways (metabolic enzymes, influx or efflux transporters) were able to affect their pharmacokinetics, which was partly confirmed by observational clinical trials showing that a few of these polymorphisms actually had a significant impact on the IS dose-concentration relationships and explained part of their pharmacokinetic variability.

The pharmacogenetic variability of their target proteins has less been studied. Actually, the study of such pharmacogenetic-pharmacodynamic associations requires different and more complex approaches, both *in vitro* and *in vivo*, than pharmacogenetic-pharmacokinetic associations.

Also, transplantation is a special condition in that the transplanted organ, often involved in drug metabolism (liver), elimination (liver, kidney), distribution (heart) or even absorption (small bowel), but also in drug effects carries a different genome than that of the recipient.

In any case, the genome(s) alone cannot account for the whole inter-individual variability, as environmental factors, including the patients' medical history, food or the associated drugs also have strong impact on the inter-individual variability of IS drug effects.

This review article aims at synthesizing current knowledge on the influence of pharmacogenetics and pharmacogenomics and clinical covariates on clinical outcomes in kidney transplantation.

2. Mycophenolic acid

Mycophenolic acid (MPA) is a selective inhibitor of the de novo purine synthesis via inosine monophosphate dehydrogenase (IMPDH) enzyme inhibition. It is widely used in combination therapy with calcineurin inhibitors for the prevention or the treatment of acute rejection following organ transplantation. In kidney transplant recipients, two mycophenolic acid-based formulations are available, the 2-morpholinoethyl ester of MPA (mycophenolate mofetil; MMF) and a sodium-salt of MPA, formulated as delayed-release tablets. Current official recommendations are to administer both at fixed doses. However, several studies have demonstrated a marked relationship between MPA exposure (AUC_{0-12h} or trough concentration) and the incidence of acute rejection [5-7]. Nevertheless, the potential benefit of MPA therapeutic drug monitoring is still controversial, as two studies yielded divergent results [8-10]. 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. Further attempts concerned the identification of relevant genetic variations in MPA cellular targets, namely IMPDH1 and IMPDH2 which could similarly influence drug efficacy and toxicity. The main adverse events reported for MPA are gastrointestinal disorders, bone marrow suppression and anemia [11, 12]. MMF would be discontinued in 20% of the patients because of such adverse events [13]. Identification of pharmacogenetic markers of predisposition is thus particularly awaited.

2.1 MPA metabolism and disposition

MPA is mainly metabolized by glucuronidation to its inactive hydroxy-β-glucuronide (MPA-phenyl-glucuronide; MPAG) [14]. The reaction is catalyzed by several members of the UDP-glucuronosyltransferase 1A family (UGT1A1, UGT1A7, 1A8, 1A9, 1A10) [15-19], with a predominant role of UGT1A9 (highly expressed in human kidney and liver) [20] and UGT1A8 (exclusively expressed in intestinal cells) [16, 18]. A minor carboxyl-linked glucuronide (acyl-

MPA-glucuronide; AcMPAG) produced by UGT2B7 and UGT1A8 was also described [18, 21]. In contrast to MPAG, AcMPAG inhibits inosine-monophosphate dehydrogenase activity with the same uncompetitive mechanism as MPA [22] and is a highly reactive metabolite that can form covalent adducts with plasma albumin *in vivo* [23, 24]. Significant amounts of the glucuronides produced in hepatocytes are excreted into bile [14] but the glucuronides may also be transported back into blood by active transporters, to be further eliminated by the kidneys, the major disposition pathway for MPA [14]. The biliary excretion of MPAG is mediated by the Multi-Drug Resistance Protein 2 (MRP2), while that of AcMPAG involves not only MRP2 but also another unidentified canalicular transporter, at least in Wistar rats [25]. MPAG is a substrate for the organic anion transporting polypeptides (OATP) 1B1 and 1B3, two uptake transporters located on the sinusoidal side of the hepatocytes [26]. Circulating MPAG may thus partly be taken up by hepatocytes to be eliminated through the bile. MPAG contributes to mycophenolic acid enterohepatic circulation after deglucuronidation in the gut. This feature accounts for 10% to 61% of total MPA exposure and is reflected as a second increase in the MPA time concentration curve, occurring 6 to 12 hours after oral dosing [14].

2.2 Pharmacogenetics of MPA and digestive adverse events.

MPA 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 [27-29]. It was first hypothesized that MPA digestive adverse events could be related to MMF dose and/or to MPA plasma concentrations [5, 30], but this was not confirmed by a further study [31]. A lower incidence of diarrhea was observed in patients co-treated with ciclosporin than in those co-treated with tacrolimus [31]. As ciclosporin inhibits MRP2-mediated excretion of MPA metabolites into bile [32], it suggests that the biliary excretion of and intestinal exposure to these metabolites would be more closely linked with diarrhea than systemic exposure. In particular,

it was suggested that the reactive AcMPAG metabolite could be involved through a secondary immunological mechanism [33].

ABCC2 (which encodes MRP2) was the first candidate gene whose relation to MPA digestive adverse events was studied. In most ethnic groups, the more frequent SNPs in this gene are located 1549 (G>A; rs rs1885301), 1410 (A>G; rs1885301), 1023 (G>A; rs7910642), 1019 (A>G; rs2804402), and 24 (C>T; rs717620) bases upstream the ATG initiation codon, or in exon 10 (c.1249G>A; rs2273697) and exon 28 (c.3972C>T; rs3740066). There is no experimental evidence that the SNPs in the non-coding region are functional, except for the -24C>T polymorphism which was found to decrease *ABCC2* promoter activity *in vitro* [34]. The c.1249G>A SNP in exon 10, leading to a valine-to-isoleucine substitution at position 417, was associated with a reduced expression of MRP2 in preterm placentas [35]. However, no effect of this SNP was found *in vitro* on MRP2 expression or activity [36]. The synonymous c.3972 C>T SNP in exon 28 ($I^{1324}I$) is not expected to be functional. However, its linkage disequilibrium with the c.-24C>T SNP may explain certain indirect associations.

Seven studies investigated the effect of the *ABCC2* c.-24C>T SNP on MPA exposure [26, 37-41], with only one reporting a positive result. This study in 95 renal transplants on tacrolimus showed that the MRP2 c.-24C>T SNP was associated with significantly higher dose-corrected MPA trough concentrations between day 42 and one year, but not at day 7 post-transplantation [39]. More important, the authors reported that this SNP was associated with a higher incidence of diarrhea within the first-year post-transplantation (29% vs. 13%; p=0.049) [39]. However, three studies found no such association [38, 42, 43], while the others only concerned the dose-concentration relationships.

As highlighted before, UGT2B7 only catalyzes the production of AcMPAG, the minor but reactive metabolite of MPA. The association between *UGT2B7* polymorphisms and MMF-related digestive adverse effects was thus investigated. Several SNPs have been identified in *UGT2B7*. Among them, the nonsynonymous c.802C>T SNP (rs7439366; *UGT2B7*2*) in exon 2 results in a histidine-to-tyrosine substitution at codon 268. At least 6 other SNPs have been identified, in the *UGT2B7* promoter region (c.-1248A>G, c.-1241T>C, c.-1054T>C, c.-842G>A, c.-268A>G, c.-102T>C) [44]. It was reported that two of these SNPs (c.-842G>A and c.802C>T), in complete linkage disequilibrium, result in a significant increase of AcMPAG production by human liver microsomes [45]. A study conducted in 67 renal transplant recipients of different ethnicities (Caucasian, African American, Hispanic, others) employing a self-administered questionnaire, "*the gastrointestinal symptom rating scale*", suggested that patients with the *UGT2B7* c.802C>T variant genotype were protected from the gastro-intestinal adverse effects of MPA regardless of the mycophenolic acid formulation or concurrent calcineurin inhibitor administered [43]. In the specific case of diarrhea, the scale score was however not associated with the *UGT2B7* genotypes. At least two other studies have reported no association between *UGT2B7* c.-842G>A and the occurrence of MMF related-diarrhea in renal transplant recipients [42, 46]. In the study from van Agteren et al., AcMPAG concentrations were included in the analysis, and no association was found either. The role of the UGT2B7-catalysed formation of AcMPAG in the digestive side effects of MPA thus remains unclear.

As an intestinal MPA metabolizing enzyme, *UGT1A8* was also identified as a candidate gene that might have an impact on MMF digestive adverse events. Two main variant alleles were described in this gene (*UGT1A8*2* and *UGT1A8*3*), each bearing a missense SNP at nucleotides 518 (C>G; $A^{173}G$) and 830 (G>A; $C^{277}Y$), respectively [47]. *UGT1A8*3* is rare (e.g.,

1.2% in Caucasians) but experiments with human intestinal microsomes and HEK293-transfected cells predicted a reduced production of MPAG and or AcMPAG for this allele [21] [16]. *UGT1A8*2* is much more frequent (e.g., 23.8 % in Caucasians) and in HEK293 transfected cells it was associated with a decreased capability to produce AcMPAG as compared to *UGT1A8*1* (V_{max} and Cl_{int} values were twice lower), but with similar activity for MPAG formation [21]. In a long-term cohort of renal transplants *UGT1A8*2* (but not *UGT1A8*3*) was associated with a reduced incidence of diarrhea, as was the administration of ciclosporin (as opposed to tacrolimus or sirolimus). These results suggest that a possible inhibition of the biliary excretion of MPA metabolites by ciclosporin and a decreased intestinal production of these metabolites in *UGT1A8*2* carriers may be protective factors against MMF-induced diarrhea [42].

2.3 Pharmacogenetics of MPA and efficacy in kidney transplant recipients.

Given the high expression of UGT1A9 in the liver and the kidney, and its major role in MPAG formation, genetic polymorphisms in this enzyme are expected to influence MPA total clearance and thus patient exposure to the drug. A change in MPAG production may also indirectly affect MPA pharmacokinetics, both by increasing its disposition and by modifying the extent of its enterohepatic cycling.

The *UGT1A9* SNPs which were studied in relation to MPA pharmacokinetics are the promoter c.-275T>A/-2152C>T, c.-1887T>G and c.-440C>T/-331T>C SNPs, the c.98T>C coding SNP, and the IVS1+399 intronic SNP. The significant effects of two common SNPs in the *UGT1A9* promoter (c.-275T>A; rs6714486 and c.-2152C>T; rs17868320) on MPA exposure are probably the most clinically pertinent, but the exact mechanism associated with the change in MPA pharmacokinetics is unclear [48]. These SNPs were associated with increased UGT1A9 protein content in human liver microsomes [49]. They are in almost

complete linkage disequilibrium and have a variant allele frequency of 6% in Caucasians [49]. Kuypers et al. showed that reduced MPA exposure in carriers of these SNPs was not associated with a higher incidence of acute graft rejection [50]. Conversely, using a logistic regression model which simultaneously took into account other factors (e.g., tacrolimus concentrations, *CYP3A5* genotypes, HLA mismatches, age …), van Schaik et al. found that these SNPs significantly predicted acute rejection in patients on tacrolimus and a fixed dose of MMF (OR=13.3, 95%CI 1.1-162.3; $P < 0.05$) [40].

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].

Seventy-two SNPs have been referenced in the NCBI SNP database (gene ID : 3615) [51] for *IMPDH2*, including introns, exons and the 3' and 5' UTRs regions. However, these SNPs have inconstantly been confirmed in resequencing projects [52, 53].

Functional data is available for two *IMPDH2* SNPs. A non-synonymous SNP located in *IMPDH2* exon 7 (c.787C>T; $L^{263}F$) was reported to dramatically decrease the catalytic activity of the enzyme *in vitro* [54] through accelerated protein degradation [53]. A promoter SNP (c.-95C>T), located in the CRE(A) (cyclic adenosine monophosphate [cAMP] response element) transcription factor binding site was found to decrease luciferase activity in two different cell lines [55]. No clinical association study has been published so far regarding these two functional SNPs. Given their extremely low allelic frequency (<1%) [54, 55], any clinically-relevant contribution to interpatient variability in MPA effects is unlikely. Another SNP located in intron 7 (IVS7+10T>C; rs11706052) has been extensively studied. Evidence from two different studies indirectly suggests that it may be associated with a poorer response to MPA. The first one, conducted in 80 renal transplant patients treated with MMF, reported an increased IMPDH activity in carriers of the variant allele as compared to non-carriers [56]. In

the second study, conducted in healthy volunteers, the presence of the rs11706052 polymorphism was associated with an antiproliferative effect of MPA on lymphocytes reduced by approximately 50% (n=8 carriers of rs11706052 *versus* 12 non-carriers) [57]. Given the intronic localization of this SNP, the exact molecular mechanism involved is unclear. This particular SNP was also associated with a higher risk of acute rejection in renal transplant recipients in a cohort of 237 renal transplant recipients [58]. However, two other studies, similarly conducted in large groups of kidney graft recipients (191 and 456 patients) were unable to confirm the association of this particular SNP with acute rejection [52, 59].

Seventy-three SNPs have been identified in *IMPDH1* [53]. These SNPs vary widely depending on the ethnic origins of individuals. Among the four non-synonymous *IMPDH1* SNPs described, the c.824C>T SNP in exon 8 ($S^{275}L$) was found to be associated with a drastic decrease in enzyme activity *in vitro* (< 25% of the wild-type enzyme), caused by accelerated protein degradation [53]. No study has examined the influence of this SNP on MPA clinical effects. Two other SNPs within *IMPDH1* intron 7 (rs2278293 and rs2278294) were reported to be associated with a decreased risk of Biopsy-Proven Acute Rejection (BPAR) over the first year after renal transplantation [59]. In another study in renal transplant recipients, the protective effect of the rs2278294 variant allele regarding BPAR was confirmed (Odds Ratio: 0.54 95% CI [0.34-0.85]; p=0.0075), while no association between rs2278293 and BPAR was found [52]. In this study, the rs2278294 variant allele carriers also had a 1.6-fold increased risk of leucopenia. The fact that BPAR and leucopenia were inversely associated to the same SNP reinforces the pertinence of this finding: the rs2278294 may protect patients from developing an immunological reaction against the allograft by favoring low lymphocyte levels, hence leucopenia.

A third study conducted in 82 Japanese renal transplant recipients has investigated the association of these two SNPs with the incidence of subclinical acute rejection diagnosed by a biopsy examination 29 days after transplantation [60]. Day-time and night-time MPA pharmacokinetic data obtained within 24 h of the biopsy (day 28) was included in the analysis. The rs2278293 or rs2278294 SNPs were not found to be associated with the incidence of subclinical acute rejection. However, when the authors stratified their analysis based on the AUC range of MPA during day and night-time periods, a significant influence of the rs2278293 genotype on the incidence of subclinical acute rejection was found in patients with high night-time exposure to MPA ($>60\mu g.h.l^{-1}$), while a similar trend was observed in patients with high day-time exposure to MPA. The authors mentioned in their discussion the limitations of their study, which include the unique period of AUC measurement at day 28 and the lack of statistical power due to the small sample size. One can also regret that exposure data and genotypes were not tested simultaneously using multivariate analysis rather than in stratified analyses.

3. Calcineurin inhibitors

Ciclosporin and tacrolimus have considerably improved graft outcome in solid organ transplantation. However, their clinical benefits are balanced by their adverse effects, mainly their nephrotoxicity which remains a major problem in all types of solid organ transplantation.

3.1 Metabolism and disposition

Ciclosporin is subject to extensive phase 1 metabolism by CYP3A4 and CYP3A5 [61] with approximately 90% of the oral dose eliminated through the bile as metabolites and only 1% as unchanged ciclosporin [62]. Ciclosporin is a substrate for the P-glycoprotein (permeability glycoprotein, abbreviated as P-gp), a member of the ATP-binding cassette (ABC)

transporters, highly expressed in enterocytes, hepatocytes and renal proximal tubular cells. At the intestinal level, the protein forms a cooperative barrier with CYP3A by pumping the drug out of enterocytes. P-gp, as well as MRP2 [63], may also contribute to the biliary excretion of ciclosporin or its metabolites.

Tacrolimus is almost completely metabolized by intestinal and hepatic CYP3A prior to elimination: only 0.5% of the dose is found unchanged in urine or feces [64, 65]. At least 15 metabolites of first or second generation have been identified. Most of them have no pharmacological activity and may thus not contribute to tacrolimus therapeutic effects. In contrast to ciclosporin, the *in vitro* intrinsic clearance of tacrolimus is approximately 2-fold higher with CYP3A5 than with CYP3A4 [66]. Tacrolimus is a substrate of P-gp, which influences both its intestinal absorption and hepatic clearance [67, 68]. It is not a substrate, nor a potent inhibitor of MRP2 [69].

3.2 Impact of CYP3A polymorphisms on the efficacy and toxicity of calcineurin inhibitors

CYP3A5 gene expression is the main factor affecting CYP3A overall metabolism. CYP3A5 is expressed in only approximately 10 % of Caucasians as a result of a frequent mutation of adenosine to guanosine at position 6986 within intron 3 of the *CYP3A5* gene (rs776746). This SNP results in a splicing defect, leading to a truncated protein with no enzyme activity [70]. Therefore, only individuals with at least one *CYP3A5*1* allele actually express the CYP3A5 protein at a significant level, with large differences in prevalence across ethnicities (5-30% CYP3A5 expressers in Caucasians, 50-80% in African Americans and Chinese people) [71]. A very large number of studies investigated the association of the *CYP3A5*1/*3* polymorphism and ciclosporin or tacrolimus pharmacokinetics in renal transplantation.

Two recent meta-analyses [72, 73] concluded that the *CYP3A5*3* allele was associated, to a moderate extent, with increased ciclosporin C_0/dose or C_2/dose and reduced mean daily doses.

The second meta-analysis also studied the association of the variant with the incidence of acute rejection and found no such association. The consequence of *CYP3A5*3* on ciclosporin pharmacokinetics may not be of major extent and is likely to be compensated by routine monitoring and dose adjustment.

In contrast to ciclosporin, a strong association between *CYP3A5*1/*3* and tacrolimus pharmacokinetics has been demonstrated in multiple studies conducted in renal transplant patients [73-80]. The dose required to reach the therapeutic concentration range was estimated to be twice as much in carriers of at least one active *CYP3A5* allele than in non-carriers [74-77]. In 2009, a European Consensus Conference acknowledged that there is a significant impact of CYP3A5 polymorphisms on tacrolimus disposition, but concluded that the clinical role of any pharmacogenetic intervention remained unclear, and that further large-scale trials were needed before reaching relevant recommendations [78]. Thervet et al. recently provided an important piece of information by conducting a prospective multicenter clinical trial named "TACTICS" (Dose individualization of TACrolimus in renal transplantation through pharmacogeneTICS) [79]. Renal transplant patients (n=280) were randomly assigned to receive tacrolimus at an initial dose either based on the *CYP3A5* genotype or according to the recommended daily regimen. Further dose adjustments based on tacrolimus C_0 were allowed in both arms. After six doses, a significantly higher proportion of patients had reached the therapeutic range in the adapted than in the control group (43.2% vs. 29.1%, p=0.030) but there was no difference in the rate of biopsy-proven acute rejection in relation to the CYP3A5 genotype. As previously discussed by van Gelder T [80] and Kuypers DR [81], the lack of clinical benefit may partly have been caused by the design of the TACTICS study: a low risk population was considered and the patients received induction therapy with anti-thymoglobulin or basiliximab for 7 days (which is not common clinical practice) and a rather

high MMF dose (3 g per day). In addition, tacrolimus initiation was delayed until day 7 post-transplantation. The benefit resulting from prospective genotyping is more likely to translate into outcome improvement in common clinical practice.

These different studies suggest that genotyping *CYP3A5*1/*3* may help detect CYP3A5 expressers at risk of tacrolimus underexposure, although the compensation by routine drug monitoring may partly abrogate its clinical consequences, as shown by the absence of genotype-outcome association.

3.3 Impact of ABCB1 polymorphisms on the efficacy and toxicity of calcineurin inhibitors

The influence of *ABCB1* SNPs on ciclosporin or tacrolimus pharmacokinetics remains uncertain. Again, any genetic variability in P-gp-mediated drug absorption or elimination is most probably compensated for by routine drug monitoring [82]. However, *ABCB1* polymorphisms may directly influence the efficacy or toxicity of calcineurin inhibitors. Crettol et al. described higher ciclosporin concentrations in the lymphocytes of carriers of the *ABCB1* c.3435C>T SNP [83], suggesting that ciclosporin activity may be affected by this polymorphism independently of its effect on the drug bioavailability or clearance. In a very large scale study involving 832 renal transplant recipients, it was found that the recipient *ABCB1* haplotype gathering the exon 12, 21 and 26 alleles, regarded as a more reliable genetic marker than any of these three SNPs alone [84], predicted acute graft rejection [85].

In addition, P-gp activity in the kidney graft, which carries the donor's and not the recipient's genome, may contribute to the nephrotoxicity of calcineurin inhibitors. This was initially shown by Hauser *et al.* (n=97), who found that the donor but not the recipient *ABCB1* 3435 variant genotype was associated with cyclosporin nephrotoxicity (OR=13.4; CI95%, 1.2-148, p=0.034) [86]. Recently, in a long-term follow-up of a cohort of 259 renal transplant patients on

ciclosporin, we found that the *ABCB1* 1236T, 2677T and 3435T variant alleles and the corresponding (1236T-2677T-3435T) variant haplotype in graft donors were associated with a higher risk of graft loss, visible beyond the 4th year post-transplantation on the survival curves. Among several clinical characteristics, only this haplotype and previous episodes of acute rejection were identified as significant predictors of long-term graft survival. The decrease in renal function over the follow-up period (estimated as delta creatinine clearance per year) was also more pronounced when the donor was carrier of the *ABCB1* TTT haplotype [87].

A recent study in renal transplant patients treated with tacrolimus showed that a higher IF/TA grade was found over the first 3 years post-transplantation when both the donor and the recipient were homozygous for the *ABCB1* c.3435C>T SNP (OR=3.9; CI95% 2.0-7.6, p<0.001), while there was no association with tacrolimus exposure [88]. Degradation of the renal graft function was also quicker when the donor, the recipient and above all both were carriers of the 3435T variant. The authors proposed that P-gp in tubular epithelial cells influences local tacrolimus accumulation and, in addition, suggested that the recipient *ABCB1* polymorphisms might also contribute to graft injury because of the high prevalence of epithelial chimerism after kidney transplantation. However, the only significant determinants of graft survival were acute T cell-mediated and antibody-mediated rejections. We refer the reader to a recent review article on the pharmacogenetics of calcineurin inhibitors-associated nephrotoxicity by Hesselink DA et al. [89] for further reading.

3.4 Pharmacogenetics of the calcineurin pathway

Calcineurin is a calmodulin-regulated protein phosphatase composed of two subunits (calcineurin A; CAN and calcineurin B; CNB) which regulates the nuclear import of NF-AT (Nuclear Factors of Activated T-cells), required for expression of the genes involved in T-cell

activation (IL-2, mainly). Ciclosporin and tacrolimus inhibit calcineurin after association with intracellular binding proteins called immunophilins (i.e., cyclophilin A and FK506 Binding Protein-12: FKBP-12, respectively). Theoretically, polymorphisms in each of these different proteins may affect the cellular response to calcineurin inhibitors. For instance, it was shown *in vitro* that mutations generated by site-directed mutagenesis in the CNB subunit were associated with a lower phosphatase activity [90], while other mutations seemed to block the binding of ciclosporin on cyclophilin A or of tacrolimus on FKBP-12, thereby confering cell resistance to the effects of the drugs [91]. In humans, two SNPs were described in the cyclophilin A gene using single strand conformational analysis PCR and sequencing: one located in the first exon (c.36A>G) and the second in the gene promoter (c.-11C>G). No correlation between those SNPs and acute rejection was found, whereas the SNP c.-11C>G was associated with an increased risk of nephrotoxicity (OR=3.49; CI95% 1.47-8.24, p=0.006) [92]. The authors showed using a reporter assay that the -11G promoter allele resulted in higher luciferase activity than the -11C allele. The pro-nephrotoxic effect of c.-11C>G SNP may thus result from increased expression of cyclophilin A.

Numerous SNPs have also been described in the different proteins involved in the cellular effect of calcineurin inhibitors through the international HapMap project [93]. However, to the best of our knowledge, none was studied in the context of ciclosporin or tacrolimus effects.

4. mTOR inhibitors (ImTORs)

Sirolimus (also known as rapamycin) is currently indicated as a preventive treatment of graft rejection in renal transplantation, as part of calcineurin inhibitor sparing or avoidance regimens. Everolimus (40-O-(2-hydroxyethyl)—rapamycin), developed in an attempt to improve the pharmacokinetic characteristics of sirolimus, particularly to increase its oral bioavailability, was recently approved in Europe with the same indication in renal

transplantation [94]. Sirolimus showed a poorer risk-benefit profile than expected, in particular with inherent nephrotoxicity not identified in pre-clinical studies [95, 96]. Consequently, the use of ImTORs has actually been restricted to particular conditions so far. The potential of pharmacogenetics for these drugs is important.

4.1 Metabolism and disposition

Sirolimus is metabolized by the intestinal [97] and hepatic CYP3A enzymes [98], with no significant contribution of the metabolites to the pharmacological activity of the drug. Experiments using recombinant P450 showed that CYP3A4 is a more efficient catalyst of sirolimus metabolism than CYP3A5 (intrinsic clearance of sirolimus depletion: 2.34 vs. 0.66 µl/min/pmol P450) [99].

Everolimus is similarly metabolized by CYP3A4 and to a lesser extent CYP3A5 [100].

Both drugs are P-gp substrates [101], which may limit their intestinal absorption [101]. The primary elimination route of ImTORs is through the bile. Hepatic extraction may not involve active transporters [102], so much so that everolimus and sirolimus have very low affinities for OATP1B1 and 1B3 (personal data). Canalicular excretion of these two drugs or their metabolites probably involves P-gp. In the kidney, P-gp is not expected to play a significant role in the pharmacokinetics of ImTORs since renal elimination is not their primary disposition pathway. However, its inhibition by sirolimus enhances the nephrotoxicity of ciclosporin when the two drugs are coadministered [103].

4.2 Impact of CYP3A polymorphisms on the efficacy and toxicity of ImTORs

The association between *CYP3A5*3* alleles and sirolimus dose requirement was investigated in 149 renal transplant recipients, mostly of European descent [104]. The authors only found a significant association with sirolimus C_0/dose in the subgroup of 69 patients undergoing a sirolimus-based rescue therapy with low-dose corticosteroids and taking no calcineurin inhibitor. No association was found in patients on ciclosporin or tacrolimus suggesting that

the pharmacogenetic effect may be abrogated by pharmacokinetic drug-drug interactions. Acute rejection, anemia, dyslipidemia, thrombocytopenia or graft function were not significantly influenced by this polymorphism. However, patients had to be on sirolimus for three months before being enrolled, which means that patients in whom sirolimus had to be interrupted because of side effects (if any) were not studied.

Another study was conducted in 85 renal transplant recipients on sirolimus, of whom 38 received sirolimus as de novo therapy and 47 were switched from a calcineurin inhibitor to sirolimus, mainly for chronic allograft nephropathy or neoplasia [76]. No association between *CYP3A5*3* and sirolimus C_{trough}/dose was found, neither in the whole population nor in the sub-groups. We also conducted a prospective study in 47 renal transplant recipients on sirolimus and mycophenolate mofetil, without calcineurin inhibitor [105]. In all patients, a detailed pharmacokinetic profile was collected over the first nine hours post-dose, at 3 months or more after transplantation. Lower AUC_{0-9h}/dose, C_{trough}/dose and C_{max}/dose values were found in CYP3A5 expressers (p = 0.008, 0.01 and 0.02, respectively). These significant differences between *CYP3A5* genotypes were still obtained when normalizing sirolimus concentrations for hemoglobin levels (owing to the high affinity of sirolimus for red blood cells). However, there was no association between the *CYP3A5* genotype and clinical findings, except for a trend towards a higher leucopenia incidence in non-expressers.

In summary, these *in vivo* results suggest that the *CYP3A5*3* genotype has a strong influence on sirolimus bioavailability in both de novo and stable renal transplant patients, provided they are not combined with calcineurin inhibitors, which may abolish this effect (although this still needs to be confirmed). Despite the fact that no association with clinical outcome was found, the determination of this genotype for a priori dose adjustment of sirolimus may be useful given the long half-life of this drug [105].

Although less data are currently available regarding everolimus, it seems that the *CYP3A5*3* genotype has no marked influence on everolimus pharmacokinetics. We recently investigated this association in 28 stable renal transplants and found that the *CYP3A5*3* polymorphism did not influence everolimus dose requirement, exposure or dose-normalized exposure [100]. Further experimental studies with genotyped human liver microsomes confirmed that the *CYP3A5*3* polymorphism has no significant influence on everolimus metabolism [100]. No association with clinical outcome is thus expected.

4.3 Impact of the ABCB1 polymorphisms on the efficacy and toxicity of ImTORs

Two studies in renal transplant recipients of Chinese (n=47) [106] and (mostly) Caucasian (n=149) [104] origins showed no significant association of the *ABCB1* c.3435C>T SNP with sirolimus C_{trough}/dose. In another study conducted in 85 patients, no association was found between sirolimus C_{trough}/dose and any of the *ABCB1* exon 12, exon 21, and exon 26 SNPs, nor with their haplotype [76]. Also, there was no association between these three SNPs and any clinical outcome (acute rejection, anemia, dyslipidemia, thrombocytopenia or graft function) [104]. In summary, there does not seem to be any *ABCB1* pharmacogenetic effect on sirolimus pharmacokinetics or effects *in vivo*, although P-gp may limit its intracellular passage, as suggested by *in vitro* experiments. Other experimental data suggest that ciclosporin or another strong P-gp inhibitor such as verapamil might unveil the influence of the 3435C>T SNP on sirolimus pharmacokinetics or intracellular transport, owing to altered P-gp folding in the membrane [107]. Hence, association studies in patients coadministered sirolimus and ciclosporin might yield more interesting results.

To the best of our knowledge, no study of the consequences of genetic polymorphisms in *ABCB1* regarding everolimus pharmacokinetics or effects has been reported.

4.4 Pharmacogenetics of the mTOR pathway

The mTOR, also known as the FRAP (FKBP–rapamycin-associated) protein regulates protein synthesis through the phosphorylation and inactivation of the repressor of m-RNA translation "eukaryotic initiation factor 4E binding protein", and through the phosphorylation and activation of the phosphatidyl-inositol 3-kinase-p70 ribosomal S6 protein kinase (p70s6K). In particular, p70s6K regulates the cellular response to IL-2. mTOR forms a stoichiometric complex with RAPTOR which has a positive role in signaling to the downstream effector p70sS6K, maintenance of cell size, and mTOR protein expression. The association of RAPTOR with mTOR also negatively regulates the mTOR kinase activity. Finally, ImTORs act by forming an inhibitory complex with the intracellular receptor FK-BP12 which subsequently binds a region in the C-terminus of m-TOR termed FRB (FKBP12-Rapamycin Binding). This causes dephosphorylation and inactivation of the p70s6 kinase activity.

Mutations of the gene encoding mTOR, FK-BP12, P70s6K or RAPTOR might confer a resistant phenotype to these drugs as was demonstrated in mammalian cell lines [108]. However, to our knowledge no association study between such polymorphisms and sirolimus or everolimus effects has been published so far.

5. Conclusion

Current knowledge about the impact of pharmacogenetics or pharmacogenomics and clinical covariates on the clinical outcome of renal transplant recipients can be summarized as follows:

- Genotyping *CYP3A5*1/*3* may help detect patients at risk of tacrolimus underexposure, although the clinical benefit of genotyping is still unclear.

- Genotyping a couple of *UGT1A9* promoter SNPs may help reduce the acute rejection rate in kidney transplant recipients on mycophenolate-mofetil, whether combined with

ciclosporin or tacrolimus, provided mycophenolate is not dose-adjusted. Indeed, MPA AUC monitoring is expected to compensate for differences in exposure and abolish this pharmacogenetic-outcome association.

- *The UGT1A8*3* polymorphism is linked with the incidence of diarrhea in patients on mycophenolate mofetil, which in turn may lead to drastic dose decreases or to drug discontinuation. Consequently, genotyping this gene might be useful to either avoid giving MMF in *UGT1A8*2* carriers, or to combine MMF with ciclosporin rather than tacrolimus or ImTORs in these patients, as this drug has apparently a protective effect against mycophenolate-induced diarrhea.

- The *IMPDH I rs2278294* SNP, and possibly also the *rs2278293* SNP are significantly associated with a lower acute rejection rate and a higher incidence of leucopenia, which might be used to select a lower MMF dose, or target a lower MPA exposure in carriers of either of these SNPs.

- Genotyping for the *ABCB1 1236T/2677T/3435T* haplotype, and maybe for the cyclophilin A *c.-11C>G* SNP, should help detect patients with an increased risk of ciclosporin nephrotoxicity and hence avoid this drug, provided the other immunosuppressants are devoid of such associations.

- There are already hints that tacrolimus nephrotoxicity may also be enhanced by the donor and recipient *ABCB1* polymorphisms.

- The influence on ciclosporin or tacrolimus efficacy or side effects of polymorphisms in the proteins of the calcineurin pathway has not been investigated so far, apart from that of cyclophilin A polymorphisms.

- The influence on sirolimus or everolimus efficacy or side effects of polymorphisms in the proteins of the mTOR pathway, or even in CYP3A5 which strongly affects sirolimus pharmacokinetics, has not yet been investigated either.

However, for the pharmacogenetics of immunosuppressive drugs to come to the clinics, comparative, randomized clinical trials evaluating the impact on patient outcome of treatment personalization based on the abovementioned pharmacogenetic tests versus standard of care will be necessary to demonstrate their clinical relevance and convince physicians caring for the patients to prescribe them … and regulatory agencies to authorize them. Moreover, such pharmacogenetic trials, as well as routine pharmacogenetic treatment personalization may require analyzing the donor, or both the donor and recipient DNA, which renders matters more complex both technically and ethically.

Pharmacogenetics has been envisaged as a possible tool for treatment personalization, in addition to, or sometimes as a replacement for, therapeutic drug monitoring. However, this literature review shows that the current level of clinical evidence is not high enough yet to recommend pharmacogenetic personalization of immunosuppressive regimens in kidney transplant recipients.

6. Expert Opinion

The vast majority of pharmacogenetic studies in general have been focused up to now on drug metabolizing enzymes and membrane transporters. It is noticeable that pharmacogenetic variability in the metabolizing enzymes and transporters of IS drugs was sometimes found to have consequences on drug effects, as shown for mycophenolate mofetil or ciclosporin for instance. The influence of polymorphisms of transporters will definitely have to be studied further, as they may facilitate the onset of cellular toxicity. Indeed, membrane transporters are involved not only in drug intestinal absorption or passage in the metabolism organs, but also in the long overlooked accumulation in target or other tissues, with potential consequences on drug therapeutic or adverse effects. New transporter families, such as that of the MATE

(Multidrug And Toxin Extrusion) transporters, identified in 2005 [109, 110] will have to be taken into consideration.

Very few pharmacogenetic studies on IS drugs concerned target proteins, whose polymorphisms might have a greater impact on patient outcome, in particular for drugs routinely dose-adjusted based on exposure, i.e. whose pharmacokinetic variability is partly compensated for. However, as shown in this review article, some of these studies already reported significant influences of such polymorphisms on dose-effects or concentration-effects relationships. A few even showed the relevance of the donor genome, and revealed that drug-drug interactions may temper or abolish the impact of certain pharmacogenetic variations.

The fact that pharmacogenetic-pharmacodynamic relationships of IS drugs have not been investigated that much could partly be explained by the relative complexity of IS drug signaling pathways. Indeed, a pharmacodynamic effect usually results from the interaction of multiple proteins, sometimes made of several sub-units. This clearly multiplies the number of genetic polymorphisms to investigate as compared to pharmacogenetic studies of drug metabolizing enzymes or transporters where, most usually, a single gene (i.e., a single protein) has to be considered. Moreover, drug metabolizing enzymes and/or transporters have been extensively studied in the past and most of their SNPs are well known and the corresponding genotyping assays easily accessible. Even the functional consequences of these SNPs are often perfectly known, or else easy to study using *in vitro* models. The data published by the international HapMap project [93] suggests that most of the immunosuppressive drug target proteins also have a substantial genetic variability. However, in most cases, no functional study of the SNPs has been conducted so far.

Studying the roles of these polymorphisms of immunosuppressive drug targets in drug efficacy or toxicity represents a new, very promising research field, but requires beforehand a

rigorous selection of the proteins (i.e., pharmacogenes) and polymorphisms to investigate and the study of their functional consequences (*in vitro* or *ex vivo*).

Finally, genome-wide associations studies (GWAS) may help find genetic factors involved in the response of, or tolerance to, immunosuppressive drugs and concerning genes coding for proteins involved in the regulation of the immunological response at large, which should also be regarded as pharmacogenetics.

Article highlight box

- Introduction: immunosuppressants are drugs with a narrow therapeutic range and a large inter-individual response variability, which has prompted pharmacogenetic studies, mostly with regards to their dose-concentration relationships. However, some studies have dealt with genetic variability in IS efficacy and response, which are reviewed herein.

- Mycophenolic acid: polymorphisms in UGT1A9 and IMPDH I were found to have a significant influence on the incidence of acute rejection, while a polymorphism in UGT1A8 protected patients from drug-induced diarrhea.

- Calcineurin inhibitors: polymorphisms in the recipient and above all the donor *ABCB1* genes (coding for the P-glycoprotein) were reported to enhance the nephrotoxicity of these drugs and, in patients on ciclosporin, favor graft loss.

- ImTORs: the influence of polymorphisms in metabolizing enzymes and proteins of the mTOR pathway on the clinical outcome of patients on sirolimus or everolimus has not yet been investigated.

- Conclusion: Before these pharmacogenetic associations can be used for treatment personalization, they will have to be validated prospectively.

References

1.   Hansen NT, Brunak S, Altman RB. Generating genome-scale candidate gene lists for pharmacogenomics. Clin Pharmacol Ther 2009 Aug;86(2):183-9.

2.   Consortium WTCC. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007 Jun 7;447(7145):661-78.

3.   Cooper GM, Johnson JA, Langaee TY et al. A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose. Blood 2008 Aug 15;112(4):1022-7.

4.   Takeuchi F, McGinnis R, Bourgeois S et al. A genome-wide association study confirms VKORC1, CYP2C9, and CYP4F2 as principal genetic determinants of warfarin dose. PLoS Genet 2009 Mar;5(3):e1000433.

5.   van Gelder T, Hilbrands LB, Vanrenterghem Y et al. A randomized double-blind, multicenter plasma concentration controlled study of the safety and efficacy of oral mycophenolate mofetil for the prevention of acute rejection after kidney transplantation. Transplantation 1999 Jul 27;68(2):261-6.

6.   Pillans PI, Rigby RJ, Kubler P et al. A retrospective analysis of mycophenolic acid and cyclosporin concentrations with acute rejection in renal transplant recipients. Clin Biochem 2001 Feb;34(1):77-81.

7.   Kiberd BA, Lawen J, Fraser AD et al. Early adequate mycophenolic acid exposure is associated with less rejection in kidney transplantation. Am J Transplant 2004 Jul;4(7):1079-83.

8.   Gaston RS, Kaplan B, Shah T et al. Fixed- or controlled-dose mycophenolate mofetil with standard- or reduced-dose calcineurin inhibitors: the Opticept trial. Am J Transplant 2009 Jul;9(7):1607-19.

9.   Le Meur Y, Buchler M, Thierry A et al. Individualized mycophenolate mofetil dosing based on drug exposure significantly improves patient outcomes after renal transplantation. Am J Transplant 2007 Nov;7(11):2496-503.

10.   van Gelder T, Silva HT, de Fijter JW et al. Comparing mycophenolate mofetil regimens for de novo renal transplant recipients: the fixed-dose concentration-controlled trial. Transplantation 2008 Oct 27;86(8):1043-51.

11.   Fulton B, Markham A. Mycophenolate mofetil. A review of its pharmacodynamic and pharmacokinetic properties and clinical efficacy in renal transplantation. Drugs 1996 Feb;51(2):278-98.

12.   Placebo-controlled study of mycophenolate mofetil combined with cyclosporin and corticosteroids for prevention of acute rejection. European Mycophenolate Mofetil Cooperative Study Group. Lancet 1995 May 27;345(8961):1321-5.

13.   Mathew TH. A blinded, long-term, randomized multicenter study of mycophenolate mofetil in cadaveric renal transplantation: results at three years. Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group. Transplantation 1998 Jun 15;65(11):1450-4.

14.   Bullingham RE, Nicholls AJ, Kamm BR. Clinical pharmacokinetics of mycophenolate mofetil. Clin Pharmacokinet 1998 Jun;34(6):429-55.

15.   Basu NK, Kole L, Kubota S et al. Human UDP-glucuronosyltransferases show atypical metabolism of mycophenolic acid and inhibition by curcumin. Drug Metab Dispos 2004 Jul;32(7):768-73.

16.   Bernard O, Guillemette C. The main role of UGT1A9 in the hepatic metabolism of mycophenolic acid and the effects of naturally occurring variants. Drug Metab Dispos 2004 Aug;32(8):775-8.

17.   Mackenzie PI. Identification of uridine diphosphate glucuronosyltransferases involved in the metabolism and clearance of mycophenolic acid. Ther Drug Monit 2000 Feb;22(1):10-3.

18.   Picard N, Ratanasavanh D, Premaud A et al. Identification of the UDP-glucuronosyltransferase isoforms involved in mycophenolic acid phase II metabolism. Drug Metab Dispos 2005 Jan;33(1):139-46.

19.   Mojarrabi B, Mackenzie PI. The human UDP glucuronosyltransferase, UGT1A10, glucuronidates mycophenolic acid. Biochem Biophys Res Commun 1997 Sep 29;238(3):775-8.

20.   Albert C, Vallee M, Beaudry G et al. The monkey and human uridine diphosphate-glucuronosyltransferase UGT1A9, expressed in steroid target tissues, are estrogen-conjugating enzymes. Endocrinology 1999 Jul;140(7):3292-302.

21.   Bernard O, Tojcic J, Journault K et al. Influence of nonsynonymous polymorphisms of UGT1A8 and UGT2B7 metabolizing enzymes on the formation of phenolic and acyl glucuronides of mycophenolic acid. Drug Metab Dispos 2006 Sep;34(9):1539-45.

22.   Gensburger O, Picard N, Marquet P. Effect of mycophenolate acyl-glucuronide on human recombinant type 2 inosine monophosphate dehydrogenase. Clin Chem 2009 May;55(5):986-93.

23.   Shipkova M, Wieland E, Schutz E et al. The acyl glucuronide metabolite of mycophenolic acid inhibits the proliferation of human mononuclear leukocytes. Transplant Proc 2001 Feb-Mar;33(1-2):1080-1.

24.   Shipkova M, Armstrong VW, Weber L et al. Pharmacokinetics and protein adduct formation of the pharmacologically active acyl glucuronide metabolite of mycophenolic acid in pediatric renal transplant recipients. Ther Drug Monit 2002 Jun;24(3):390-9.

25.   Westley IS, Brogan LR, Morris RG et al. Role of Mrp2 in the hepatic disposition of mycophenolic acid and its glucuronide metabolites: effect of cyclosporine. Drug Metab Dispos 2006 Feb;34(2):261-6.

26.   Picard N, Yee SW, Woillard JB et al. The role of organic anion-transporting polypeptides and their common genetic variants in mycophenolic acid pharmacokinetics. Clin Pharmacol Ther 2010 Jan;87(1):100-8.

27.   Ducloux D, Ottignon Y, Semhoun-Ducloux S et al. Mycophenolate mofetil-induced villous atrophy. Transplantation 1998 Oct 27;66(8):1115-6.

28.   Kamar N, Faure P, Dupuis E et al. Villous atrophy induced by mycophenolate mofetil in renal-transplant patients. Transpl Int 2004 Sep;17(8):463-7.

29.   Maes BD, Dalle I, Geboes K et al. Erosive enterocolitis in mycophenolate mofetil-treated renal-transplant recipients with persistent afebrile diarrhea. Transplantation 2003 Mar 15;75(5):665-72.

30.   Borrows R, Chusney G, James A et al. Determinants of mycophenolic acid levels after renal transplantation. Ther Drug Monit 2005 Aug;27(4):442-50.

31.   Heller T, van Gelder T, Budde K et al. Plasma concentrations of mycophenolic acid acyl glucuronide are not associated with diarrhea in renal transplant recipients. Am J Transplant 2007 Jul;7(7):1822-31.

32.   Hesselink DA, van Hest RM, Mathot RA et al. Cyclosporine interacts with mycophenolic acid by inhibiting the multidrug resistance-associated protein 2. Am J Transplant 2005 May;5(5):987-94.

33.   Shipkova M, Beck H, Voland A et al. Identification of protein targets for mycophenolic acid acyl glucuronide in rat liver and colon tissue. Proteomics 2004 Sep;4(9):2728-38.

34.   Haenisch S, Zimmermann U, Dazert E et al. Influence of polymorphisms of ABCB1 and ABCC2 on mRNA and protein expression in normal and cancerous kidney cortex. Pharmacogenomics J 2007 Feb;7(1):56-65.

35.   Meyer zu, Schwabedissen HE, Jedlitschky G et al. Variable expression of MRP2 (ABCC2) in human placenta: influence of gestational age and cellular differentiation. Drug Metab Dispos 2005 Jul;33(7):896-904.

36.   Hirouchi M, Suzuki H, Itoda M et al. Characterization of the cellular localization, expression level, and function of SNP variants of MRP2/ABCC2. Pharm Res 2004 May;21(5):742-8.

37.   Baldelli S, Merlini S, Perico N et al. C-440T/T-331C polymorphisms in the UGT1A9 gene affect the pharmacokinetics of mycophenolic acid in kidney transplantation. Pharmacogenomics 2007 Sep;8(9):1127-41.

38.   Miura M, Satoh S, Inoue K et al. Influence of SLCO1B1, 1B3, 2B1 and ABCC2 genetic polymorphisms on mycophenolic acid pharmacokinetics in Japanese renal transplant recipients. Eur J Clin Pharmacol 2007 Dec;63(12):1161-9.

39.   Naesens M, Kuypers DR, Verbeke K et al. Multidrug resistance protein 2 genetic polymorphisms influence mycophenolic acid exposure in renal allograft recipients. Transplantation 2006 Oct 27;82(8):1074-84.

40.   van Schaik RH, van Agteren M, de Fijter JW et al. UGT1A9 -275T>A/-2152C>T polymorphisms correlate with low MPA exposure and acute rejection in MMF/tacrolimus-treated kidney transplant patients. Clin Pharmacol Ther 2009 Sep;86(3):319-27.

41.   Zhang WX, Chen B, Jin Z et al. Influence of uridine diphosphate (UDP)-glucuronosyltransferases and ABCC2 genetic polymorphisms on the pharmacokinetics of mycophenolic acid and its metabolites in Chinese renal transplant recipients. Xenobiotica 2008 Nov;38(11):1422-36.

42.   Woillard JB, Rerolle JP, Picard N et al. Risk of diarrhoea in a long-term cohort of renal transplant patients given mycophenolate mofetil: the significant role of the UGT1A8 2 variant allele. Br J Clin Pharmacol 2010 Jun;69(6):675-83.

43.   Yang JW, Lee PH, Hutchinson IV et al. Genetic polymorphisms of MRP2 and UGT2B7 and gastrointestinal symptoms in renal transplant recipients taking mycophenolic acid. Ther Drug Monit 2009 Oct;31(5):542-8.

44.   Duguay Y, Baar C, Skorpen F et al. A novel functional polymorphism in the uridine diphosphate-glucuronosyltransferase 2B7 promoter with significant impact on promoter activity. Clin Pharmacol Ther 2004 Mar;75(3):223-33.

45.   Djebli N, Picard N, Rerolle JP et al. Influence of the UGT2B7 promoter region and exon 2 polymorphisms and comedications on Acyl-MPAG production in vitro and in adult renal transplant patients. Pharmacogenet Genomics 2007 May;17(5):321-30.

46.   van Agteren M, Armstrong VW, van Schaik RH et al. AcylMPAG plasma concentrations and mycophenolic acid-related side effects in patients undergoing renal transplantation are not related to the UGT2B7-840G>A gene polymorphism. Ther Drug Monit 2008 Aug;30(4):439-44.

47.   Huang YH, Galijatovic A, Nguyen N et al. Identification and functional characterization of UDP-glucuronosyltransferases UGT1A8*1, UGT1A8*2 and UGT1A8*3. Pharmacogenetics 2002 Jun;12(4):287-97.

48.   Barraclough KA, Lee KJ, Staatz CE. Pharmacogenetic influences on mycophenolate therapy. Pharmacogenomics 2010 Mar;11(3):369-90.

49.   Girard H, Court MH, Bernard O et al. Identification of common polymorphisms in the promoter of the UGT1A9 gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver. Pharmacogenetics 2004 Aug;14(8):501-15.

50.   Kuypers DR, Naesens M, Vermeire S et al. The impact of uridine diphosphate-glucuronosyltransferase 1A9 (UGT1A9) gene promoter region single-nucleotide polymorphisms T-275A and C-2152T on early mycophenolic acid dose-interval exposure in de novo renal allograft recipients. Clin Pharmacol Ther 2005 Oct;78(4):351-61.

51.   NCBI SNP database; http://www.ncbi.nlm.nih.gov/projects/SNP/ (accessed September 2010).

52.   Gensburger O, Van Schaik RH, Picard N et al. Polymorphisms in type I and II inosine monophosphate dehydrogenase genes and association with clinical outcome in patients on mycophenolate mofetil. Pharmacogenet Genomics 2010 Sep;20(9):537-43.

53.   Wu T-Y, Peng Y, Pelleymonter L et al. Pharmacogenetics of the mycophenolic acid targets inosine monophosphate dehydrogenase IMPDH1 and IMPDH2: gene sequence variation and functional genomics. Br J Pharmacol 2010;accepted article; DOI: 10.111/j.1476-5331.

54.   Wang J, Zeevi A, Webber S et al. A novel variant L263F in human inosine 5'-monophosphate dehydrogenase 2 is associated with diminished enzyme activity. Pharmacogenet Genomics 2007 Apr;17(4):283-90.

55.   Garat A, Cauffiez C, Hamdan-Khalil R et al. IMPDH2 genetic polymorphism: a promoter single-nucleotide polymorphism disrupts a cyclic adenosine monophosphate responsive element. Genet Test Mol Biomarkers 2009 Dec;13(6):841-7.

56.   Sombogaard F, van Schaik RH, Mathot RA et al. Interpatient variability in IMPDH activity in MMF-treated renal transplant patients is correlated with IMPDH type II 3757T > C polymorphism. Pharmacogenet Genomics 2009 Aug;19(8):626-34.

57.   Winnicki W, Weigel G, Sunder-Plassmann G et al. An inosine 5'-monophosphate dehydrogenase 2 single-nucleotide polymorphism impairs the effect of mycophenolic acid. Pharmacogenomics J 2010 Feb;10(1):70-6.

58.	Grinyo J, Vanrenterghem Y, Nashan B et al. Association of four DNA polymorphisms with acute rejection after kidney transplantation. Transpl Int 2008 Sep;21(9):879-91.

59.	Wang J, Yang JW, Zeevi A et al. IMPDH1 gene polymorphisms and association with acute rejection in renal transplant patients. Clin Pharmacol Ther 2008 May;83(5):711-7.

60.	Kagaya H, Miura M, Saito M et al. Correlation of IMPDH1 gene polymorphisms with subclinical acute rejection and mycophenolic acid exposure parameters on day 28 after renal transplantation. Basic Clin Pharmacol Toxicol 2010 Aug;107(2):631-6.

61.	Kronbach T, Fischer V, Meyer UA. Cyclosporine metabolism in human liver: identification of a cytochrome P-450III gene family as the major cyclosporine-metabolizing enzyme explains interactions of cyclosporine with other drugs. Clin Pharmacol Ther 1988 Jun;43(6):630-5.

62.	Kelly P, Kahan BD. Review: metabolism of immunosuppressant drugs. Curr Drug Metab 2002 Jun;3(3):275-87.

63.	Kato R, Nishide M, Kozu C et al. Is cyclosporine A transport inhibited by pravastatin via multidrug resistant protein 2? Eur J Clin Pharmacol 2010 Feb;66(2):153-8.

64.	Venkataramanan R, Swaminathan A, Prasad T et al. Clinical pharmacokinetics of tacrolimus. Clin Pharmacokinet 1995 Dec;29(6):404-30.

65.	Moller A, Iwasaki K, Kawamura A et al. The disposition of 14C-labeled tacrolimus after intravenous and oral administration in healthy human subjects. Drug Metab Dispos 1999 Jun;27(6):633-6.

66.	Dai Y, Hebert MF, Isoherranen N et al. Effect of CYP3A5 polymorphism on tacrolimus metabolic clearance in vitro. Drug Metab Dispos 2006 May;34(5):836-47.

67.	Wu CY, Benet LZ. Disposition of tacrolimus in isolated perfused rat liver: influence of troleandomycin, cyclosporine, and gg918. Drug Metab Dispos 2003 Nov;31(11):1292-5.

68.   Jeong H, Chiou WL. Role of P-glycoprotein in the hepatic metabolism of tacrolimus. Xenobiotica 2006 Jan;36(1):1-13.

69.   Kobayashi M, Saitoh H, Kobayashi M et al. Cyclosporin A, but not tacrolimus, inhibits the biliary excretion of mycophenolic acid glucuronide possibly mediated by multidrug resistance-associated protein 2 in rats. J Pharmacol Exp Ther 2004 Jun;309(3):1029-35.

70.   Kuehl P, Zhang J, Lin Y et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet 2001 Apr;27(4):383-91.

71.   Warrington JS, Shaw LM. Pharmacogenetic differences and drug-drug interactions in immunosuppressive therapy. Expert Opin Drug Metab Toxicol 2005 Oct;1(3):487-503.

72.   Tang HL, Ma LL, Xie HG et al. Effects of the CYP3A5*3 variant on cyclosporine exposure and acute rejection rate in renal transplant patients: a meta-analysis. Pharmacogenet Genomics 2010 Sep;20(9):525-31.

73.   Zhu HJ, Yuan SH, Fang Y et al. The effect of CYP3A5 polymorphism on dose-adjusted cyclosporine concentration in renal transplant recipients: a meta-analysis. Pharmacogenomics J 2010 Apr 6.

74.   Haufroid V, Mourad M, Van Kerckhove V et al. The effect of CYP3A5 and MDR1 (ABCB1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. Pharmacogenetics 2004 Mar;14(3):147-54.

75.   Macphee IA, Fredericks S, Mohamed M et al. Tacrolimus pharmacogenetics: the CYP3A5*1 allele predicts low dose-normalized tacrolimus blood concentrations in whites and South Asians. Transplantation 2005 Feb 27;79(4):499-502.

76.   Mourad M, Mourad G, Wallemacq P et al. Sirolimus and tacrolimus trough concentrations and dose requirements after kidney transplantation in relation to CYP3A5 and MDR1 polymorphisms and steroids. Transplantation 2005 Oct 15;80(7):977-84.

77.   Tsuchiya N, Satoh S, Tada H et al. Influence of CYP3A5 and MDR1 (ABCB1) polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. Transplantation 2004 Oct 27;78(8):1182-7.

78.   Wallemacq P, Armstrong VW, Brunet M et al. Opportunities to optimize tacrolimus therapy in solid organ transplantation: report of the European consensus conference. Ther Drug Monit 2009 Apr;31(2):139-52.

79.   Thervet E, Loriot MA, Barbier S et al. Optimization of initial tacrolimus dose using pharmacogenetic testing. Clin Pharmacol Ther 2010 Jun;87(6):721-6.

80.   van Gelder T, Hesselink DA. Dosing tacrolimus based on CYP3A5 genotype: will it improve clinical outcome? Clin Pharmacol Ther 2010 Jun;87(6):640-1.

81.   Kuypers DR. Pharmacogenetic vs. concentration-controlled optimization of tacrolimus dosing in renal allograft recipients. Clin Pharmacol Ther 2010 Nov;88(5):595-6; author reply 97.

82.   Staatz CE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: Part II. Clin Pharmacokinet 2010 Apr 1;49(4):207-21.

83.   Crettol S, Venetz JP, Fontana M et al. Influence of ABCB1 genetic polymorphisms on cyclosporine intracellular concentration in transplant recipients. Pharmacogenet Genomics 2008 Apr;18(4):307-15.

84.   Johne A, Kopke K, Gerloff T et al. Modulation of steady-state kinetics of digoxin by haplotypes of the P-glycoprotein MDR1 gene. Clin Pharmacol Ther 2002 Nov;72(5):584-94.

85.   Bandur S, Petrasek J, Hribova P et al. Haplotypic structure of ABCB1/MDR1 gene modifies the risk of the acute allograft rejection in renal transplant recipients. Transplantation 2008 Nov 15;86(9):1206-13.

86.   Hauser IA, Schaeffeler E, Gauer S et al. ABCB1 genotype of the donor but not of the recipient is a major risk factor for cyclosporine-related nephrotoxicity after renal transplantation. J Am Soc Nephrol 2005 May;16(5):1501-11.

87.   Woillard JB, Rerolle JP, Picard N et al. Donor P-gp polymorphisms strongly influence renal function and graft loss in a cohort of renal transplant recipients on cyclosporine therapy in a long-term follow-up. Clin Pharmacol Ther 2010 Jul;88(1):95-100.

88.   Naesens M, Lerut E, de Jonge H et al. Donor age and renal P-glycoprotein expression associate with chronic histological damage in renal allografts. J Am Soc Nephrol 2009 Nov;20(11):2468-80.

89.   Hesselink DA, Bouamar R, van Gelder T. The pharmacogenetics of calcineurin inhibitor-related nephrotoxicity. Ther Drug Monit 2010 Aug;32(4):387-93.

90.   Feng B, Stemmer PM. Interactions of calcineurin A, calcineurin B, and Ca2+. Biochemistry 1999 Sep 21;38(38):12481-9.

91.   Zhu D, Cardenas ME, Heitman J. Calcineurin mutants render T lymphocytes resistant to cyclosporin A. Mol Pharmacol 1996 Sep;50(3):506-11.

92.   Moscoso-Solorzano GT, Ortega F, Rodriguez I et al. A search for cyclophilin-A gene variants in cyclosporine A-treated renal transplanted patients. Clin Transplant 2008 Nov-Dec;22(6):722-9.

93.   HapMap Database; www.hapmap.org (accessed February 2011).

94.   Ruiz JC, Sanchez A, Rengel M et al. Use of the new proliferation signal inhibitor everolimus in renal transplant patients in Spain: preliminary results of the EVERODATA registry. Transplant Proc 2007 Sep;39(7):2157-9.

95.   Kahan BD. Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. The Rapamune US Study Group. Lancet 2000 Jul 15;356(9225):194-202.

96.   Cravedi P, Ruggenenti P, Remuzzi G. Sirolimus to replace calcineurin inhibitors? Too early yet. Lancet 2009 Apr 11;373(9671):1235-6.

97.   Lampen A, Zhang Y, Hackbarth I et al. Metabolism and transport of the macrolide immunosuppressant sirolimus in the small intestine. J Pharmacol Exp Ther 1998 Jun;285(3):1104-12.

98.   Sattler M, Guengerich FP, Yun CH et al. Cytochrome P-450 3A enzymes are responsible for biotransformation of FK506 and rapamycin in man and rat. Drug Metab Dispos 1992 Sep-Oct;20(5):753-61.

99.   Picard N, Djebli N, Sauvage FL et al. Metabolism of sirolimus in the presence or absence of cyclosporine by genotyped human liver microsomes and recombinant cytochromes P450 3A4 and 3A5. Drug Metab Dispos 2007 Mar;35(3):350-5.

100.   Picard N, Rouguieg-Malki K, Kamar N et al. CYP3A5 genotype does not influence everolimus in vitro metabolism and clinical pharmacokinetics in renal transplant recipients. Transplantation accepted.

101.   Crowe A, Lemaire M. In vitro and in situ absorption of SDZ-RAD using a human intestinal cell line (Caco-2) and a single pass perfusion model in rats: comparison with rapamycin. Pharm Res 1998 Nov;15(11):1666-72.

102.   Oswald S, Nassif A, Modess C et al. Pharmacokinetic and pharmacodynamic interactions between the immunosuppressant sirolimus and the lipid-lowering drug ezetimibe in healthy volunteers. Clin Pharmacol Ther 2010 Jun;87(6):663-7.

103.   Anglicheau D, Pallet N, Rabant M et al. Role of P-glycoprotein in cyclosporine cytotoxicity in the cyclosporine-sirolimus interaction. Kidney Int 2006 Sep;70(6):1019-25.

104.   Anglicheau D, Le Corre D, Lechaton S et al. Consequences of genetic polymorphisms for sirolimus requirements after renal transplant in patients on primary sirolimus therapy. Am J Transplant 2005 Mar;5(3):595-603.

105.   Le Meur Y, Djebli N, Szelag JC et al. CYP3A5*3 influences sirolimus oral clearance in de novo and stable renal transplant recipients. Clin Pharmacol Ther 2006 Jul;80(1):51-60.

106.   Miao LY, Huang CR, Hou JQ et al. Association study of ABCB1 and CYP3A5 gene polymorphisms with sirolimus trough concentration and dose requirements in Chinese renal transplant recipients. Biopharm Drug Dispos 2008 Jan;29(1):1-5.

107.   Kimchi-Sarfaty C, Oh JM, Kim IW et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 2007 Jan 26;315(5811):525-8.

108.   Huang S, Bjornsti MA, Houghton PJ. Rapamycins: mechanism of action and cellular resistance. Cancer Biol Ther 2003 May-Jun;2(3):222-32.

109.   Masuda S, Terada T, Yonezawa A et al. Identification and functional characterization of a new human kidney-specific H+/organic cation antiporter, kidney-specific multidrug and toxin extrusion 2. J Am Soc Nephrol 2006 Aug;17(8):2127-35.

110.   Otsuka M, Matsumoto T, Morimoto R et al. A human transporter protein that mediates the final excretion step for toxic organic cations. Proc Natl Acad Sci U S A 2005 Dec 13;102(50):17923-8.