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

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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.

MESH Keywords Calcineurin ; antagonists & inhibitors ; Cytochrome P-450 CYP3A ; genetics ; Dose-Response Relationship, Drug ; Enzyme Inhibitors ; pharmacokinetics ; toxicity ; Genotype ; Glucuronosyltransferase ; genetics ; Humans ; IMP Dehydrogenase ; genetics ; Immunosuppressive Agents ; pharmacokinetics ; Kidney Transplantation ; Mycophenolic Acid ; pharmacokinetics ; therapeutic use ; P-Glycoprotein ; genetics ; Pharmacogenetics ; Polymorphism, Single Nucleotide ; drug effects ; Randomized Controlled Trials as Topic ; TOR Serine-Threonine Kinases ; antagonists & inhibitors ; Treatment Outcome

Author Keywords Immunosuppressants ; pharmacogenetics ; kidney transplantation

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.

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.

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].

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 gastrointestinal 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].

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 (>60μg.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.

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.

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].

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 pharmacogene TICS) [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.

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.

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.

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.

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].

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.

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.

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.

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.

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.

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