Clinical implementation of pharmacogenetics in kidney transplantation: calcineurin inhibitors in the starting blocks

Laure Elens,1,2 Rachida Bouamar,3 Nauras Shuker,3 Dennis A. Hesselink,4 Teun van Gelder3,4 & Ron H. N. van Schaik2

1Louvain Drug Research Institute (LDRI), Université Catholique de Louvain (UCL), Brussels, Belgium, 2Department of Clinical Chemistry, Erasmus MC, University Medical Center, Rotterdam, 3Department of Hospital Pharmacy, Erasmus MC, University Medical Center, Rotterdam and 4Department of Internal Medicine, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

Correspondence
Professor Ron H. N. van Schaik,
Department of Clinical Chemistry,
Erasmus MC, P.O. Box 2040, 3000 CA
Rotterdam, The Netherlands.
Tel.: +311 0703 3119
Fax: +311 0436 7894
E-mail: r.vanschaik@erasmusmc.nl

Keywords
calcineurin inhibitors, ciclosporin, kidney transplantation, pharmacogenetics, tacrolimus

Received
31 May 2013
Accepted
3 September 2013
Accepted Article
Published Online
10 October 2013

Pharmacogenetics has generated many expectations for its potential to individualize therapy proactively and improve medical care. However, despite the huge amount of reported genetic associations with either pharmacokinetics or pharmacodynamics of drugs, the translation into patient care is still slow. In fact, strong evidence for a substantial clinical benefit of pharmacogenetic testing is still limited, with a few exceptions. In kidney transplantation, established pharmacogenetic discoveries are being investigated for application in the clinic to improve efficacy and to limit toxicity associated with the use of immunosuppressive drugs, especially the frequently used calcineurin inhibitors (CNIs) tacrolimus and ciclosporin. The purpose of the present review is to picture the current status of CNI pharmacogenetics and to discuss the most promising leads that have been followed so far.

Introduction

The first successful kidney transplantation was performed in the mid-1950s and marked the advent of a new effective therapy for end-stage renal disease. Since then, the field of kidney transplantation has witnessed improved graft outcomes, with reduced rates of early acute rejection (AR) and increased patient survival [1]. The reason for this success can be attributed to the use of drugs that inhibit the immune response and thus prevent rejection. Nowadays, most patients are treated with combined immunosuppressive therapy consisting of a calcineurin inhibitor [CNI; either ciclosporin A (CsA) or tacrolimus (Tac)] in association with an anti-proliferative agent (most often mycophenolate) and glucocorticoids. Although the use of CNI-based immunosuppressive therapy has resulted in improved patient and graft survival, the adverse effects of chronic CNI treatment remain an important problem. In addition, the use of CNIs is complicated by their narrow therapeutic window. The difference between the concentrations exerting an adequate therapeutic effect and the concentrations causing adverse events is small [2, 3]. This is further complicated by a substantial between-subject variability in pharmacological response [4]. The consequences of both under- and over-exposure are severe. Not achieving effective concentrations increases the risk of immunologically-mediated graft rejection, potentially resulting in graft loss or damage, and ultimately, a reduction in patient survival [2, 3, 5]. By contrast, too high CNI exposure can lead to over-immunosuppression and thus increases the risk of infections and malignancies. The use of CNIs is also associated with drug-related complications mainly represented by nephrotoxicity and new-onset diabetes mellitus after transplantation (NODAT) [2, 3, 5]. Individualizing a patient’s drug therapy to balance therapeutic efficacy and adverse events has become an important goal for transplant physicians. Therapeutic drug monitoring (TDM) with
subsequent dose adaptation is an indispensable tool to target CNIs to their therapeutic window and is now universally applied. However, although the use of TDM limits the time a patient is exposed to supra- or sub-therapeutic concentrations, it essentially remains a reactive, trial-and-error approach and has no predictive value. Pharmacogenetics, on the other hand, may be of help in predicting an individual’s response to a given drug and can be applied before the start of pharmacotherapy [6–8]. The major promise of pharmacogenetics lies in its potential to identify high risk patients who would benefit from an alternative dosage and/or drug regimen prior to the start of therapy. Given its pro-active nature, pharmacogenetics may thus be a potential complementary tool to TDM for optimizing immunosuppressive treatment thereby improving effectiveness and reducing adverse drug reactions (ADRs). Given the many genetic associations with CNI pharmacokinetics (PK) and pharmacodynamics (PD) made to date [6–11], the purpose of the present review is to discuss the most promising findings for pharmacogenetic applications for CNIs in kidney transplantation.

**Ciclosporin A**

**CYP3A**

The major elimination route for CsA is an extensive phase I metabolism by the cytochrome P450 (CYP450) system, more precisely by the CYP3A subfamily [12]. Functional activity of CYP3A is determined through three isoenzymes, CYP3A4, CYP3A5 and CYP3A7. CsA is primarily metabolized by CYP3A4 with a limited role for CYP3A5 [13]. CYP3A7 is predominantly expressed during fetal life, suggesting a minor contribution in adults. Both CYP3A4 and CYP3A5 are characterized by huge variations in their activity and expression, either caused by genetics or by induction or inhibition by other substances. On a pharmacogenetic level, 22 and 11 different variant alleles have been reported for CYP3A4 and CYP3A5, respectively (http://www.cypalleles.ki.se/). However, only a few of them appear important when considering clinical pharmacogenetics since most variant alleles are either very rare [<0.1% minor allelic frequency (MAF)] or because clinical effects have not clearly been established [14]. The only exceptions with respect to low frequency are CYP3A4*1B [MAF 20% in all ethnicities confined (source 1000 Genomes)] and CYP3A4*22 [MAF 2%, in all ethnicities confined (source 1000 Genomes)]. For CYP3A5, the CYP3A5*3 allele is now fully characterized and has been largely considered as a potential candidate to explain differences in CsA metabolism because of the functional defect associated with this allele. The major highlights that have been reported up to now when considering CsA pharmacogenetics are summarized in Table 1.

In renal transplant recipients, for CYP3A4, different studies have explored the impact of CYP3A4*1B [−392G>A rs2740574, MAF in the Caucasian population = 3% (source 1000 Genomes)] on CsA PK. In a study involving 14 healthy volunteers, CYP3A4*1/*1 homozygous individuals showed a higher dose-adjusted CsA area under the concentration vs. time curve (AUC) and a lower oral clearance (CL/F) when compared with individuals homozygous for the variant allele after a single drug administration [15]. This finding is in agreement with the suggested increased expression associated with this single nucleotide polymorphism (SNP) [16]. On the contrary, the CYP3A4*1B allele was neither associated with CsA daily dose requirement nor with CsA predose concentrations (C0) in a study of 224 North Indian kidney transplant patients receiving CsA when considering data at month 1 and month 3 after surgery [17]. Hesselin et al. [18] showed no relationship between CYP3A4*1B genotype and CsA dose requirement in a cohort involving 110 CsA-treated kidney transplant patients, confirming the observation made in two other earlier studies [19, 20]. However, in a population PK analysis involving 151 kidney and heart transplant patients, a 9% higher CsA CL/F was observed for carriers of the CYP3A4*1B allele compared with CYP3A4*1/*1 homozygous [21]. The authors suggested that these contrasting results may have arisen through the use of a too crude PK analysis in other studies (CL/F vs. dose-adjusted C0). Alternatively, they explained these discrepancies by the fact that a lot of studies that performed more detailed PK profiling were possibly not powered enough to demonstrate this weak CYP3A4*1B influence on Tac CL/F. Nevertheless, it was concluded that even if patients carrying a CYP3A4*1B variant allele are characterized by a slightly higher oral CsA clearance, the effect on CsA disposition is minor and probably clinically irrelevant. New findings have recently been published for CYP3A4*1B, being associated with a higher CsA dose requirement in CYP3A4*1B carriers compared with non-carriers [22]. In this study, only 55 CsA-treated kidney transplant patients were investigated and all patients who carried the CYP3A4*1B allele also carried a CYP3A5*1 allele. Consequently, it can be rationally assumed that the continuous inconsistency observed for the associations between CsA PK and the CYP3A4*1B variant allele might also potentially be ascribed to the linkage disequilibrium (LD) observed with the CYP3A5*1-expressing allele. Indeed, despite the fact that one cannot exclude definitively that CYP3A4*1B has a weak effect on CsA CYP3A4-mediated metabolism, this linkage can potentially account for the fluctuating associated clinical phenotype. The CYP3A5*3 allele is defined by the presence of a SNP [6986A>G, rs776746, MAF in the Caucasian population = 5% (source 1000 Genomes)] that creates a cryptic splicing site resulting in the introduction of an additional exon and the production of an inactive truncated protein [23]. As a result, only carriers of one CYP3A5*1 active allele are expressing a functional isoenzyme and are therefore categorized as CYP3A5 expressers. Regarding CYP3A5*3 and its impact on CsA PK in Asian populations, a small
### Table 1
Key study findings for CsA (not exhaustive)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Allele/SNP</th>
<th>Rs number</th>
<th>Effect on CsA PK/PD</th>
<th>n</th>
<th>Population</th>
<th>Ethnicity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A4</td>
<td>CYP3A4*1B</td>
<td>Rs2740574</td>
<td>Dose-adjusted AUC for CYP3A4*1B carriers</td>
<td>14</td>
<td>Healthy volunteers</td>
<td>Mixed</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>CYP3A4*18B</td>
<td></td>
<td>CUF for CYP3A4*18B carriers</td>
<td>151</td>
<td>Kidney and heart transplant patients</td>
<td>Mixed</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>CYP3A4*18B</td>
<td>Rs28371759</td>
<td>Dose-adjusted G for CYP3A4*18B carriers</td>
<td>26</td>
<td>Healthy volunteers</td>
<td>Asian</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>CYP3A4*18B</td>
<td></td>
<td>CUF for CYP3A4*18B carriers</td>
<td>55</td>
<td>Kidney transplant patients</td>
<td>Chinese</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>CYP3A4*18B</td>
<td></td>
<td>Dose-adjusted G for CYP3A4*18B carriers</td>
<td>103</td>
<td>Kidney transplant patients</td>
<td>Chinese</td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td>CYP3A4*22</td>
<td>Rs35599367</td>
<td>Dose-adjusted G for CYP3A4*22 carriers</td>
<td>50</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>CYP3A5</td>
<td>Rs776746</td>
<td>Dose-adjusted G for CYP3A5 carriers</td>
<td>106</td>
<td>Kidney transplant patients</td>
<td>Chinese</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>CYP3A5</td>
<td></td>
<td>Dose-adjusted G for CYP3A5 carriers</td>
<td>224</td>
<td>Kidney transplant patients</td>
<td>North Indian</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td>CYP3A5</td>
<td></td>
<td>Dose-adjusted G for CYP3A5 carriers</td>
<td>126</td>
<td>Kidney transplant patients</td>
<td>Chinese</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>CYP3A5</td>
<td></td>
<td>Dose-adjusted G for CYP3A5 carriers</td>
<td>103</td>
<td>Kidney transplant patients</td>
<td>Chinese</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>CYP3A5</td>
<td></td>
<td>Dose-adjusted G for CYP3A5 carriers</td>
<td>24</td>
<td>Healthy volunteers</td>
<td>Mixed</td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td>CYP3A5</td>
<td></td>
<td>Urinary clearance for CYP3A5 carriers</td>
<td>110</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[18]</td>
</tr>
<tr>
<td></td>
<td>CYP3A5</td>
<td></td>
<td>Dose-adjusted G for CYP3A5 carriers</td>
<td>50</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td>CYP3A5</td>
<td></td>
<td>Dose-adjusted G for CYP3A5 carriers</td>
<td>1821</td>
<td>Kidney transplant patients</td>
<td>Mixed (mostly Caucasians)</td>
<td>[34]</td>
</tr>
<tr>
<td>POR</td>
<td>POR*28</td>
<td>Rs1057868</td>
<td>Dose-adjusted G for POR<em>28</em>28 carriers</td>
<td>174</td>
<td>Kidney transplant patients</td>
<td>Mixed (mostly Caucasians)</td>
<td>[59]</td>
</tr>
<tr>
<td>ABCB1</td>
<td>1236C &gt; T</td>
<td>Rs1128508</td>
<td>Risk of nephrotoxicity for 3435TT donor genotype carriers</td>
<td>97</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[43]</td>
</tr>
<tr>
<td></td>
<td>2677G &gt; T/A</td>
<td>Rs121918023</td>
<td>Risk of graft loss for 1236T-2677T-3435T donor haplotype carriers</td>
<td>259</td>
<td>Kidney transplant patients</td>
<td>Not specified</td>
<td>[50]</td>
</tr>
<tr>
<td></td>
<td>3435C &gt; T</td>
<td>Rs1045640</td>
<td>Graft survival for 3435CC donor genotype carriers</td>
<td>811</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Graft survival for 3435CC donor genotype carriers</td>
<td>675</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Risk of BPAR for 1236C-2677G-3435T recipient haplotype carriers</td>
<td>407</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[54]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intralymphocytic concentrations for 3435T variant allele carriers (recipient)</td>
<td>64</td>
<td>Kidney, liver and lung transplant patients</td>
<td>Caucasian</td>
<td>[55]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Risk of BPAR for 1236C-2677G-3435C recipient haplotype carriers</td>
<td>236</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[55]</td>
</tr>
</tbody>
</table>
effect was demonstrated by Hu et al. during the first week after transplantation in 106 Chinese renal transplant recipients [24] and in a cohort of 224 North Indian kidney transplant recipients where CYP3A5 expressers demonstrated higher dose requirement at month 1 and month 3 after transplantation when compared with non-expressers [17]. In that study, the dose-adjusted CsA concentration 2 h after administration ($C_0$) were also lower in the CYP3A5 expresser group but only at month 1 and not at month 3 after kidney transplantation. Very recently, in two recent studies involving Chinese transplant recipients, CYP3A5*3/*3 was associated with higher dose-adjusted $C_0$ of CsA [25, 26] although this was not confirmed by others [27, 28]. Zheng et al. [28] reported that the mean oral CsA CL/F was similar between CYP3A5 expressers and non-expressers. However, compared with CYP3A5 non-expressers, the average AUCs for the AM19 and AM1c9 CsA metabolites were 47.4% and 51.3% higher in CYP3A5 expressers, corresponding to 30% higher metabolite ratios for AM19 and AM1c9 in CYP3A5 expressers. Given the fact that CYP3A5 is expressed in the kidney [29] and because they found a mean apparent urinary clearance that was lower among CYP3A5 expressers compared with non-expressers, the authors speculated that intra-renal accumulation of CsA and its metabolite might depend on the CYP3A5 expression status. This observation might thus indicate that CYP3A5 genotype can be important to consider for its impact on CsA-related nephrotoxicity (see below). When considering the Caucasian population, in the study of Hesselink et al. no significant relationship was observed between the different CYP3A5*3 genotypes with the CsA PK parameters at 3 and 12 months after kidney transplantation [18]. They also demonstrated that, when only Caucasian patients were considered, a significant influence of CYP3A5 genotype on CsA dose requirement was found but only at month 12 after transplantation. This effect was replicated in the study of Haufrroid et al. who showed that CYP3A5*1 carriers were characterized by a somewhat lower CsA dose-adjusted $C_0$ when compared with CYP3A5*3/*3 patients [30]. These observations further support that CYP3A5 expressers require slightly higher CsA doses to reach the same $C_0$ than non-expressers. In their population PK analysis, Hesselink et al. demonstrated also that patients not expressing CYP3A5 had a reduced oral CsA clearance (~6%) compared with carriers of the CYP3A5*1 allele, but this difference was not statistically significant [21]. Some other studies failed to associate the CYP3A5*3 allele with CsA dose requirement [31–33] illustrating the low clinical relevance of taking into account the CYP3A5*3 genotype when considering CsA-based immunosuppressive regimens after kidney transplantation. Tang et al. recently performed a meta-analysis aiming to determine whether the CYP3A5*3 variant could affect CsA therapy by pooling data from 14 independent studies involving a total of 1821 kidney transplant patients [34]. The results showed that there were indeed significant differences in the CsA dose-adjusted $C_0$ and in the mean CsA daily dose between CYP3A5 expressers and non-expressers but these differences were not very substantial. After stratification for ethnicity, it was observed that the effect was not detected in the Caucasian sub-population but remained present in Asian patients. In conclusion, the meta-analysis suggested that the CYP3A5*3 variant at best has a small effect on CsA blood concentrations and is thus not likely to be of clinical relevance, as illustrated by the lack of association with AR [34].

A novel interesting lead is related to the effect of the CYP3A4*18B allele [rs28371759, MAF in the Asian population = 2% (source 1000 Genomes)] in the Asian population [35]. The reported data consistently suggest an increased CsA clearance for healthy Asian individuals carrying this gain of function allele [24, 35, 36]. This effect of the CYP3A4*18B allele on CsA PK has been recently confirmed in Chinese renal transplant recipients but only when considering CsA dose-adjusted $C_0$ and not dose-adjusted $C_0$. Larger clinical studies are thus needed to validate the importance of the CYP3A4*18B allele in Asian kidney transplant recipients treated with CsA. Another new lead involves the association between the CYP3A4*22 decrease-of-function (DOF) allele [rs35599367, MAF in the Caucasian population = 5% (source 1000 Genomes)], and the response to CsA [14, 37–39]. In a single centre cross-sectional study, CYP3A4*22 carriership was associated with 1.6-fold higher CsA dose-adjusted $C_0$, suggesting that variant carriers need lower CsA doses to achieve stable drug concentrations when compared with homozygous wild-type patients [40]. This observation was not confirmed in a longitudinal multicentre study considering multiple time points after kidney transplantation but in that latter study, the CYP3A4*22 allele was shown to constitute a risk factor for delayed graft function (DGF) and worse creatinine clearance [41]. It can be hypothesized that the lower dose requirement, the higher risk of DGF and the poorer renal function in carriers of the CYP3A4*22 allelic variant result from reduced CYP3A4 enzymatic activity. This possible reduced metabolizing activity was, however, not associated with a reduced incidence of biopsy-proven AR (BPAR), showing that this allele might narrow the therapeutic window by increasing the risk for nephrotoxicity without providing any profit in term of treatment efficacy. Prospective confirmatory studies are needed to confirm these new findings.

With the possible exception of CYP3A4*22, the inconsistency observed in the results concerning the influence of CYP3A recipient genotype on CsA-mediated nephrotoxicity makes it difficult to draw final conclusions. The situation in kidney transplantation is special as the donor kidney CYP3A genotype might differ from the recipient genotype. As local drug concentrations within the targeted organ are probably more important to explain individual susceptibility to ADRs, the CYP3A5 donor geno-
type (i.e. that of the transplanted kidney) may influence a kidney transplant patient’s susceptibility to the nephrotoxic effects of CNIs more than the recipient CYP3A5 genotype. Indeed, not only is CYP3A5 the predominant CYP3A enzyme expressed in the kidney [29] but CYP3A5 expression in distal renal tubules appears to be protective against arteriolar hyalinosis while, in proximal tubules, CYP3A5 is overexpressed in biopsies with signs of CNI nephrotoxicity [42]. Most studies have looked at recipient genotype only and very few data exist on the impact of the donor CYP3A5 genotype on CsA-related nephrotoxicity. Contrasting with our hypothesis, in one study, it was observed that the CYP3A5*3 polymorphism of the donor did not influence significantly the proportion of patients presenting with CsA-related nephrotoxicity several years after transplantation [43].

**ABCB1**

CsA is also a substrate for efflux transporters belonging to the ATP-binding cassette (ABC) family [44] which is constituted by a group of proteins responsible for the active transport of multiple compounds across cell membranes from the intra to the extracellular matrix. Given their localization mainly at the surface of cells implicated in excretory functions, ABC transporters are considered to play a key role in CsA clearance and bioavailability. However, as they are also expressed in other organs, their activity impacts on the distribution of the drug thereby affecting tissue concentrations. This matter appears as very important because it can directly impact on intracellular concentrations within targeted organs, like the kidney (toxicity) and/or lymphocytes (effectiveness). ABCB1 (formerly known as P-glycoprotein) is currently the most studied ABC transporter and has been strongly considered as a pharmacogenetic tool because of its central role in the absorption, distribution and excretion of a huge panel of drugs, among which are CNIs. The different results arising from the literature are conflicting and it is difficult to interpret the data in order to deduce the potential clinical relevance of considering SNPs in **ABCB1** for CsA treatment optimization (for a complete review see [45]). This can be explained by the fact that when a genetic variant affects weakly the functionality of a transporter, some compensatory mechanisms counterbalance this change in activity resulting in a minor impact of SNPs on CsA systemic exposure. As a consequence, and given the lack of consistent association between **ABCB1** SNPs, mostly 1236C>T [rs1128503, MAF in the Caucasian population = 43% (source 1000 Genomes)], 2677G>T/A [rs2032582, MAF in the Caucasian population = 40%, (source 1000 Genomes)] and 3435C>T [rs1045642, MAF in the Caucasian population = 53% (source 1000 Genomes)], and CsA PK, at first sight, it is not likely that **ABCB1** genotyping has a future in the field of pharmacogenetic clinical implementation. However, like CYP3A5, **ABCB1** is also expressed in the kidney and might thus act as a safeguard against CNI-associated nephrotoxicity by actively extruding the drugs from the tissue into urine, thereby limiting their accumulation in the renal parenchyma. This hypothesis is substantiated by the observation made in the study of Naessens et al. who showed that the absence of **ABCB1** expression in the tubular epithelium was associated with a higher grade of Tac-related histological lesions [46]. In another histologic study, an association between CsA toxicity, addressed by arteriolar hyalinosis and tubular vacuolization, and reduced tubular **ABCB1** expression has been observed [47]. This is in line with the fact that the 3435TT donor genotype was over-represented in patients with CsA-induced nephrotoxicity [43]. As the 3435T variant allele has been associated with decreased expression and activity [48, 49], one could speculate that patients engrafted with a kidney carrying the 3435TT allele are accumulating CsA more intensively when compared with 3435CC kidneys and are thus more at risk of presenting signs of nephrotoxicity. Accordingly, it has been found that the **ABCB1** 1236T-2677T-3435T variant haplotype in graft donors was associated with a higher risk of graft loss [50]. Moreover, it was shown that the decrease in renal function over the follow-up period was more pronounced in **ABCB1** TTT haplotype donor carriers, showing that SNPs in **ABCB1** could influence CsA long term related nephrotoxicity and eventually, graft lost. By contrast, Moore et al. showed in a population of 811 CsA-treated patients that the kidney donor 3435CC genotype was associated with a worse death-censored graft survival [51]. This observation was replicated in a subsequent cohort of 675 renal transplant patients but not in a third cohort of 2985 patients [51]. In that latter cohort, it was shown that the 3435CC donor genotype was indeed associated with a lower death-censored graft survival but only in the subgroup of 452 Tac-treated patients and not in the 2533 CsA-treated patients. The authors suggested that the contradictions observed across the studies might be due to differences in study designs. Alternatively, they speculated that the deleterious effect of the donor 3435CC genotype is related to an increased renal **ABCB1** expression based on the fact that this expression has been associated with an increased propensity to renal injury in animal models of ischaemic injury [52, 53]. Concerning the risk of AR and as it appears that **ABCB1** activity impacts more on intracellular accumulation than on CsA systemic exposure, it has been hypothesized that **ABCB1** lymphocyte activity could limit the access of CsA within targeted immunocompetent cells without affecting blood concentrations. In a large study of 832 renal transplant recipients treated with either CsA (n = 407) or Tac (n = 425), it was found that the recipient **ABCB1** haplotype pooling the 1236C, 2677G and 3435T alleles predicted a higher risk of AR [54]. The authors suggested that the haplotype causing increased **ABCB1** activity might limit the access of the drugs within immunocompetent cells. As a result, this haplotype might increase the risk of BPAR. However, this is contrasting with
the results reported by Crettol et al. showing increased CsA intralymphocytic concentrations for carriers of the 3435T allele [55]. Grinyo et al. demonstrated in a cohort of 237 Caucasian de novo renal transplant recipients an increased incidence of BPAR in patients carrying the C-G-C haplotype which is in line with the observation of Crettol et al. suggesting that the 3435T allele would lead to more efficient immunosuppression and thus protect against rejection [56]. The inconsistencies observed in the different associations can potentially be explained by the high LD that exists for the three SNPs 1236C>T 2677G>T/A and 3435C>T and the different haplotype combinations. For instance, the degree of LD differs among populations and can impact on the association but overall, it seems that these SNPs can eventually serve as candidate risk markers for AR. Also, in the study of Crettol et al. another coding ABCB1 SNPs, i.e. the 1199G>A, was associated with a 18-fold decreased CsA intralymphocytic concentrations [55] but again, this association needs to be replicated and deeply characterized before being considered for clinical implementation.

**P450 oxidoreductase**

P450 oxidoreductase (POR) is a protein containing both flavin adenine dinucleotide (FAD) and flavin mononucleotide moieties and constitutes an indispensable element of all microsomal (type II) CYP enzymes [57]. The 1508C>T SNP [rs1057868; POR*28, MAF in the Caucasian population = 30% (source 1000 Genomes)], is the most common SNP in POR and encodes the amino acid variant Ala503Val [58]. In a recent longitudinal study including 174 CsA-treated kidney transplant patients, a moderate but significant 15% decrease in the dose-adjusted CsA \( C_0 \) for patients homozygous for the POR*28 allele was shown compared with patients carrying a POR*1 allele during the first year after transplantation [59]. This suggests that this SNP causes a small increase of CYP3A-mediated CsA metabolism.

**Tacrolimus**

**CYP3A**

Both CYP3A4 and CYP3A5 are involved in Tac oxidative metabolism. Contrary to CsA, for Tac, CYP3A5 is a more competent catalyst than CYP3A4. CYP3A5 shows an in vitro catalytic activity towards Tac which is 1.6-fold higher than CYP3A4 [60]. Like for CsA, the most studied CYP3A SNPs towards Tac are CYP3A4*1B and CYP3A5*3. When looking at the controversies regarding the effect of CYP3A4*1B [6–10], it is most likely that the fluctuating relationships observed between the CYP3A4*1B allele and Tac dose requirement are resulting from its high LD with the CYP3A5*1 allele, for which the effect on Tac metabolism is now fully recognized both in vitro and in vivo. It has been consistently demonstrated that CYP3A5 expressers require two-fold higher Tac doses to reach the same steady-state \( C_0 \) when compared with CYP3A5 non-expressers, reflecting a higher Tac CYP3A5-mediated metabolism [18, 30, 33, 61–71]. Table 2 summarizes the most important associations made for Tac pharmacogenetics. Interestingly, it has also been shown that there is a delay in achieving target blood Tac \( C_0 \) for CYP3A5 expressers when compared with non-expressers, despite the use of the same TDM scheme [72]. This supports a possible benefit of a pharmacogenetic dosing strategy, implying the administration of two-fold higher Tac initial doses to CYP3A5 expressers to achieve the targeted concentrations more rapidly. This appears as particularly promising as achieving optimal drug exposure in the first days after transplantation is important as this is the period with the highest risk of AR [73, 74]. Indeed, some data suggest that rejection occurred earlier for CYP3A5 expressers than for non-expressers, i.e. only when analyzing the first or the third month period after transplantation [17, 75]. This was further supported by a recent meta-analysis [76]. As a conclusion, CYP3A5 expressers seem at increased risk of early episodes of AR (<3 months after transplantation) because of the longer time delay needed to achieve optimal Tac concentrations in the first week after start of therapy in comparison with CYP3A5 non-expressers. The evidence of a Tac metabolism defect caused by the CYP3A5*3/*3 carriehship is substantiated by the fact that this association is found whatever the transplant population or the PK parameter analyzed (AUC, CL/F, \( C_0 \)) but is also independent from the age, ethnicity, the time after transplantation and/or the gender of the patients. The ascendency of the explicative value of the CYP3A5*3 SNP has been recently illustrated by Birdwell et al. who showed that, among a panel of more than 2000 SNPs (in which the CYP3A4*22 allele was not included, see below) of the absorption, distribution, metabolism and excretion (ADME) pathway, no other variants than CYP3A5*3 significantly correlated with the dose-adjusted Tac \( C_0 \) [77]. Despite the convincing evidence of a genotype–PK relationship, it is not yet clear if the genotype is associated with an altered clinical response (reviewed in [6, 78, 79]). There is no evidence that a CYP3A5 genotype-based initial Tac dosage will improve the treatment outcome.

It has been proposed to decrease the dose for CYP3A5 non-expressers to 0.075 mg kg\(^{-1}\) and to increase the dose for CYP3A5 expressers to 0.150 mg kg\(^{-1}\) [63]. Thervet et al. conducted a randomized clinical trial (RCT) to evaluate whether these new dosing guidelines according to CYP3A5 genotype would allow earlier achievement of target blood concentrations, defined by a therapeutic window ranging from 10 to 15 ng ml\(^{-1}\), and result in an amelioration of the overall clinical response [80]. In that study, it was shown that an a priori CYP3A5 genotyping to adapt Tac starting dose is beneficial as it results in a more rapid achievement of the Tac target \( C_0 \) and with less dose adjustments than
# Table 2

## Key study findings for Tac (not exhaustive)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Allele/SNP</th>
<th>Rs number</th>
<th>Effect on Tac PK/PD</th>
<th>n</th>
<th>Population</th>
<th>Ethnicity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP3A4</strong></td>
<td>CYP3A4*18</td>
<td>Rs2740574</td>
<td>Dose-adjusted C0 for CYP3A4*18 carriers</td>
<td>136</td>
<td>Kidney transplant patients</td>
<td>Mixed (mostly Caucasian)</td>
<td>[64]</td>
</tr>
<tr>
<td></td>
<td>CYP3A4*22</td>
<td>Rs3559367</td>
<td>Dose requirement for CYP3A4*18 carriers</td>
<td>63</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[90]</td>
</tr>
<tr>
<td></td>
<td>CYP3A5*3</td>
<td>Rs776746</td>
<td>Dose-adjusted C0 for CYP3A4*18 carriers</td>
<td>185</td>
<td>Kidney transplant patients</td>
<td>Mixed</td>
<td>[41]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted C0 for CYP3A4*22 carriers</td>
<td>49</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted C0 for CYP3A4*22 carriers</td>
<td>60</td>
<td>Pediatric heart transplant patients</td>
<td>Caucasian</td>
<td>[85]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CL/F for CYP3A4*22 carriers</td>
<td>96</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[84]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Time of overexposure for CYP3A4*22 carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Creatinine clearance for CYP3A4*22 carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CYP3A5</strong></td>
<td>CYP3A5*3</td>
<td>Rs776746</td>
<td>Dose-adjusted C0 for CYP3A5*1 carriers</td>
<td>136</td>
<td>Kidney transplant patients</td>
<td>Mixed (mostly Caucasian)</td>
<td>[64]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted AUC(0,12 h) for CYP3A5*1 carriers</td>
<td>63</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[90]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted Cmax for CYP3A5*1 carriers</td>
<td>26</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[91]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted C0 for CYP3A5*1 carriers</td>
<td>118</td>
<td>Kidney transplant patients</td>
<td>Chinese</td>
<td>[92]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted C0 for CYP3A5*1 carriers</td>
<td>18</td>
<td>Kidney transplant patients</td>
<td>Mixed Asian</td>
<td>[93]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted C0 for CYP3A5*1 carriers</td>
<td>180</td>
<td>Kidney transplant patients</td>
<td>Mixed</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Time to achieve therapeutic C0 for CYP3A5*1 carriers</td>
<td>134</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[94]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CL for CYP3A5*1 carriers</td>
<td>50</td>
<td>Kidney transplant patients</td>
<td>Japanese</td>
<td>[95]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted C0 for CYP3A5*1 carriers</td>
<td>44</td>
<td>Kidney transplant patients</td>
<td>Mixed (mostly Caucasian)</td>
<td>[96]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted AUC(0,12 h) for CYP3A5*1 carriers</td>
<td>59</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[97]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted Cmax for CYP3A5*1 carriers</td>
<td>206</td>
<td>Kidney transplant patients</td>
<td>Mixed</td>
<td>[98]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted C0 for CYP3A5*1 carriers</td>
<td>30</td>
<td>Kidney transplant patients</td>
<td>Chinese</td>
<td>[33]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted C0 for CYP3A5*1 carriers</td>
<td>39</td>
<td>Kidney transplant patients</td>
<td>Japanese</td>
<td>[99]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted AUC for CYP3A5*1 carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CL/F for CYP3A5*1 carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CL for CYP3A5*1 carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Decrease in CL/F for CYP3A5*1 carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose adjusted Cmax for CYP3A5*1 carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted C0 for CYP3A5*1 carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Time to achieve therapeutic C0 for CYP3A5*1 carriers</td>
<td>24</td>
<td>Kidney transplant patients</td>
<td>Mostly Caucasian</td>
<td>[100]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted C0 for CYP3A5*1 carriers</td>
<td>64</td>
<td>Kidney transplant patients</td>
<td>Mixed</td>
<td>[18]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted C0 for CYP3A5*1 carriers</td>
<td>103</td>
<td>Kidney transplant patients</td>
<td>Chinese</td>
<td>[101]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose adjusted Cmax for CYP3A5*1 carriers</td>
<td>50</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted AUC for CYP3A5*1 carriers</td>
<td>30</td>
<td>Kidney transplant patients</td>
<td>Japanese</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Risk of supra-therapeutic exposure for CYP3A5*1 carriers</td>
<td>80</td>
<td>Kidney transplant patients</td>
<td>Mixed (mostly Caucasian)</td>
<td>[67]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose requirement for CYP3A5*1 carriers</td>
<td>1443</td>
<td>Kidney transplant patients</td>
<td>Mixed</td>
<td>[76]</td>
</tr>
<tr>
<td><strong>POR</strong></td>
<td>POR*28</td>
<td>Rs1057868</td>
<td>Dose-adjusted C0 for POR*28 carriers expressing CYP3A5</td>
<td>298</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[89]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose-adjusted C0 for POR*28 carriers expressing CYP3A5</td>
<td>184</td>
<td>Kidney transplant patients</td>
<td>Mixed</td>
<td>[59]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose adjusted AUC(0,12 h) for POR*28 carriers not expressing CYP3A5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ABCB1</strong></td>
<td>1236C&gt;T</td>
<td>Rs1128503</td>
<td>Intra-lymphocytic concentrations for 3435T or 22677T/A variant allele carriers (recipient)</td>
<td>96</td>
<td>Kidney transplant patients</td>
<td>Japanese</td>
<td>[87]</td>
</tr>
<tr>
<td></td>
<td>2677G&gt;T/A</td>
<td>Rs2032352</td>
<td>Intra-lymphocytic concentrations for 3435T donor and recipient haplotype carriers</td>
<td>252</td>
<td>Kidney transplant patients</td>
<td></td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>3435C&gt;T</td>
<td>Rs1045642</td>
<td>Intra-lymphocytic concentrations 1199A variant allele carriers (recipient)</td>
<td>96</td>
<td>Kidney transplant patients</td>
<td></td>
<td>[87]</td>
</tr>
<tr>
<td>1199G&gt;A</td>
<td>Rs2229109</td>
<td></td>
<td>Creatinine clearance for 1199A allele donor carriers</td>
<td>97</td>
<td>Kidney transplant patients</td>
<td>Caucasian</td>
<td>[88]</td>
</tr>
</tbody>
</table>
with the universal 0.1 mg kg\(^{-1}\) twice daily dosage. However, the advantage of reaching Tac therapeutic concentrations did not turn into a clinical response improvement assessed by the incidence of AR and DGF and still, a substantial percentage (56.8%) of patients did not reach the target level window at day 3. This observation suggests that factors other than CYP3A5 genotype also explain part of the between patients variability in Tac pharmacokinetics, and taking those into account might allow the establishment of a dosing algorithm with high predictive performance. In that respect, Passey et al. defined an equation aiming to calculate a Tac starting dose [81]. This equation includes the CYP3A5*3 genotype along with four clinical factors (time post-transplant, age, steroid sparing centre or not and calcium channel blocker use) and was based on data from 681 kidney transplant recipients participating in a multicentre observational study. The predictive value of this algorithm in an alternative population has been tested, but results suggest that this equation is not accurate enough to predict Tac clearance [82]. Introducing the CYP3A4*22 allelic status of the recipient into the equation significantly improved the accuracy of the Tac initial dose requirement [83]. The characterization of the CYP3A4*22 effect on Tac treatment is still in its early stages but some recent data suggest that it is a good candidate to consider in Tac PK for kidney transplantation [14]. We have shown in two independent studies that the Tac dose requirement was significantly lower for CYP3A4*22 carriers when compared with CYP3A4*1/*1 homozygous [40, 41], which is in accordance with the reduced activity related to this SNP [39]. This effect was proved to be independent from the CYP3A5*3 allelic status which allowed defining four groups of patients presenting different dose requirements. This influence was confirmed in a recent study considering the early period after transplantation [84], where it was demonstrated that CYP3A4*22 carriers were more at risk of experiencing supra-therapeutic Tac exposure during the first week after transplantation and that the time of overexposure was longer for CYP3A4*22 carriers when compared with CYP3A4*1/*1 transplanted patients. In addition, it was shown that Tac overexposure in CYP3A4*22 carriers might provide a renal function benefit as it was observed in that longitudinal study that creatinine clearance was on average 21.4% higher for CYP3A4*22 carriers compared with CYP3A4*1/*1 patients, corresponding to a mean difference of +9.5 ml min\(^{-1}\) for CYP3A4*22 carriers [84]. In a population of paediatric heart transplant recipients, it was confirmed that CYP3A4*22, either alone or in combination with CYP3A5*3, may help towards individualization of Tac therapy as differences were found in dose requirements among groups of CYP3A genotype clusters [85]. All in all, this recent investigation provided arguments to increase further the daily dose for CYP3A4*22 non-carriers and to decrease it for CYP3A4*22 carriers among CYP3A5 non-expressers. It was estimated to apply a difference of 20% in the daily dose between both groups. Starting doses of 0.150, 0.080 and 0.070 mg kg\(^{-1}\) body weight twice daily are thus proposed for extensive (CYP3A5 expressers-CYP3A4*1/*1), intermediate (CYP3A5 non-expressers-CYP3A4*1/*1) and poor (CYP3A5 non-expressers-CYP3A4*22 carriers) CYP3A-metabolizer clusters, respectively, in order to reach more rapidly the target concentrations (ranging from 10 to 15 ng ml\(^{-1}\)). At this stage, those recommendations are limited to those three groups and cannot be extended for the group of very uncommon patients expressing CYP3A5 and carrying a CYP3A4*22 allele. Before consideration of these dose recommendations for clinical implementation, these new proposed guidelines should be validated in a prospective study.

### ABCB1

Like CsA, Tac is also a substrate for ABCB1 and many studies have investigated the influence of ABCB1 SNPs on Tac PK. Again, results are conflicting and suggest no or at best a limited impact of ABCB1 SNPs on Tac C\(_{0}\) [45]. The hypothesis of a more pronounced effect of ABCB1 activity on intracellular accumulation is attractive and some data support this theory [60, 86, 87]. Indeed, Capron et al. demonstrated that patients heterozygous and variant homozygotes for the ABCB1 3435C>T or the 2677G>T/A SNPs showed 1.3- fold higher Tac concentrations within circulating lymphocytes compared with wild-type homozygotes [87]. Also, it was shown that carriers of an 1199A variant allele presented an increased Tac intralymphocytic accumulation when compared with homozygous 1199GG patients, suggesting a decrease in ABCB1 activity. Both SNPs effects, i.e. 3435C>T and 1199G>A, were synergistic with mutated alleles exerting a protective action against low Tac concentrations within lymphocytes, the drug target. As stated above, ABCB1 is also expressed in renal tubular cells where it can play a protective role against Tac accumulation within the kidney and hence save the graft from toxicity. Up to now, no study has explored the potential influence of ABCB1 activity on intra-graft Tac concentrations. Alternatively, some reports have assessed the impact of the kidney ABCB1 genotypes on histological signs of CNI-associated renal toxicity. Naensens et al. reported that both donor and recipient homozygosity for the ABCB1 3435T variant allele were correlated with a higher rate of Tac-associated histological damage to the kidney [46]. It was also found that the combined donor-recipient ABCB1 3435TT genotype constituted a risk factor for worse graft function (i.e. lower glomerular filtration rate) after the first year post-transplantation. The authors speculated that the relevance of the recipient genotype could possibly be explained by epithelial chimerism after renal transplantation as it was reported that up to 88% of allografts contain epithelial cells from recipient origin. However, the absolute number of epithelial cells from recipient origin in the allograft was very low and is not likely to elucidate fully this association.
As stated above, Moore et al. reported contradictory results as in their cohorts, the 3435CC donor genotype was associated with a worse death-censored graft survival outcome [51]. In a very recent study, the mutated ABCB1 1199A allele was found to have a protective effect on renal function [88]. All these observations need to be confirmed but in total, results related to the influence of the donor ABCB1 genotype on Tac-related renal toxicity suggest that ABCB1 activity within renal parenchyma is important to prevent local drug accumulation and the related increased susceptibility to side effects.

P450 oxidoreductase

De Jonge et al. observed that CYP3A5 expressers with at least one POR*28 variant allele showed higher Tac dose requirements when compared with POR*1/*1 homozygous patients expressing CYP3A5 and that this difference persisted throughout the first year post-transplantation [89]. This was recently confirmed in a longitudinal multicentre study [59]. These observations suggest an increased in vivo Tac CYP3A5-mediated metabolism. The effect was very weak in CYP3A5 non-expressers probably reflecting that POR*28 carrierness has a limited impact on CYP3A4 activity towards Tac, as it was shown for CsA. We do feel that this hypothesis is worth verifying in vitro by recombinant expression system as has been performed previously for other CYPs substrates.

Conclusions

There are several lines of evidence that support a potential benefit for pharmacogenetic testing before starting CNI-based immunosuppressive treatment in kidney transplantation. In view of the reported data, pharmacogenetic screening of the CYP3A5*3 allelic status, either or not in combination with CYP3A4*22 and/or POR*28 screening, may help the clinician to define a better dosage plan for Tac compared with the universal dosage that is currently prescribed. For CsA, pharmacogenetic analysis of the recipient genotype seems of limited value.

Knowledge of the donor genotype can possibly help to identify patients at risk of intra-renal drug accumulation either for CsA or for Tac. Given the inconvenience of intra-cellular drug quantification in daily clinical practice and the fact that reported associations are still premature, these might highlight the possibility of identifying patients at increased risk of CNI-related nephrotoxicity and consequently consider a different immunosuppressive regimen, with, for example, reduced CNI dosing and/or quick withdrawal of these drugs from their medication scheme. This might also warn the clinician to be more vigilant to any signs of nephrotoxicity. Other studies suggest that ABCB1 SNPs might be important for drug efficacy as they have been correlated with the drug accumulation within the therapeutic target, i.e. the lymphocyte. However, the pharmacogenetic perspectives regarding the utility of ABCB1 and CYP3A5 (donor) genotype screening are still obscured by the countless discrepancies observed in the genetic associations.

For Tac, it is not unlikely that in the near future the recipient CYP3A5*3 allele will be screened in every patient. However, some points need to be clarified first. TDM clearly moderates the potential benefit of a genotype-based adjustment as the routine use of TDM allows the clinician to bring the majority of the patients within the targeted Cs window quite rapidly after the first Tac administration, i.e. within 10 days [64]. However, in the only RCT performed to date [80], it has been shown that this delay can be significantly reduced when increasing or decreasing the dose for CYP3A5 expressers and non-expressers, respectively. As mentioned earlier in this review, decreasing the time of sub-therapeutic drug exposure can be beneficial for the clinical outcome [72]. However, in the RCT, the reduced time to achieve adequate concentrations was not translated into a better clinical response [80]. As already outlined in previous reviews and/or commentaries [69], the population considered for this RCT included patients at low immunological risk for AR and a large proportion of patients received induction therapy in addition to high mycophenolate mofetil doses and might thus not reflect a usual kidney transplant population. This allowed the investigators to delay the introduction of Tac in the immunosuppressive regimen for 1 week and can possibly explain the lack of clinical benefit of a CYP3A5 genotype-adjusted Tac dose. To get definitive evidence of the benefit of a routine implementation of a CYP3A genotype-based Tac starting dose, studies analogous to this RCT should be implemented in high risk patients, without induction therapy and when Tac therapy is started on the day of transplantation. Alternatively, in the trial conducted by Thervet et al. [80], even in the group that received a genotype-guided dose, there was still a large proportion of patients outside the therapeutic range after 3 days. A potential additional value can thus be credited to other genetic variants that explain the remaining PK variability. We do recommend to put special focus on the CYP3A4*22 DOF allele. In light of the preliminary observations, the presence of this allele might indicate a reduced Tac metabolism. This information can be of potential value, especially in patients with no CYP3A5 expression. Lowering the dose for those patients might avoid early overexposure to the drug and limit renal toxicity.

In conclusion, pharmacogenetic testing has certainly a future to optimize CNI-based therapy for kidney transplant patients but the lack of robust studies limits the clinical application of the current discoveries. On the same line, it is obvious that pharmacogenetics testing will not solve all the complications observed with the use of these immunosuppressants and will certainly not substitute the current TDM strategy. For instance, kidney transplant
patients are most of the time polymedicated and subjected to high risk of drug interactions that cannot be predicted by the patient genotype. We do feel that careful attention must be put on these possible drug–drug interactions as well as the genotype impact on metabolism induction and/or inhibition. At best, if we can provide clear evidence that adequate concentrations are achieved based on the genotype-defined dosing and that it provides a clinical improvement, the use of TDM will possibly be limited as a control and/or complementary tool and, eventually, be less intensive than it is at present. For pharmacogenetics, the road to the clinic is thus still long and not free from difficulties as the majority of the discoveries still await validation, confirmation and proper interpretation.

**Competing Interests**

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: DAH has received fees and grant support from Astellas Pharma Inc and T van G had support from Astellas and Pfizer and is a member of the Dutch Novartis Transplantation Advisory Board, no financial relationships with and Pfizer and is a member of the Dutch Novartis Transplantation Advisory Board, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

Laure Elens is a post-doctoral researcher with the Fonds National de la Recherche Scientifique (FRS-FNRS), Belgium.

**REFERENCES**


Pharmacogenetics of calcineurin inhibitors in kidney transplantation


41 Elens L, Bouamar R, Hesselink DA, Haufoird V, van der Heiden IP, van Gelder T, van Schaik RH. A new functional...


64 Hesselink DA, van Schaik RH, van Agteren M, de Fijter JW, Hartmann A, Zieer M, Budde K, Kuypers DR, Pisarski P, Le Meur Y, Mamelow KD, van Gelder T. CYP3A5 genotype is not associated with a higher risk of acute rejection in


