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REVIEW

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Pharmacogenetic aspects of the use of tacrolimus in renal transplantation: recent developments and ethnic considerations

J.T. Tang[a,b], L.M. Andrews[b], T. van Gelder[b,c], Y.Y. Shi[d], R.H.N. van Schaik[e], L.L. Wang[a] and D.A. Hesselink[c]

[a]Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, China; [b]Department of Hospital Pharmacy, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands; [c]Department of Internal Medicine, Division of Nephrology and Renal Transplantation, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands; [d]Department of Nephrology, West China Hospital of Sichuan University, Chengdu, China; [e]Department of Clinical Chemistry, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

ABSTRACT

Introduction: Tacrolimus (Tac) is effective in preventing acute rejection but has considerable toxicity and inter-individual variability in pharmacokinetics and pharmacodynamics. Part of this is explained by polymorphisms in genes encoding Tac-metabolizing enzymes and transporters. A better understanding of Tac pharmacokinetics and pharmacodynamics may help to minimize different outcomes amongst transplant recipients by personalizing immunosuppression.

Areas covered: The pharmacogenetic contribution of Tac metabolism will be examined, with a focus on recent discoveries, new developments and ethnic considerations.

Expert opinion: The strongest and most consistent association in pharmacogenetics is between the *CYP3A5* genotype and Tac dose requirement, with CYP3A5 expressers having a ~ 40–50% higher dose requirement compared to non-expressers. Two recent randomized-controlled clinical trials using *CYP3A5* genotype, however, did not show a decrease in acute rejections nor reduced toxicity. *CYP3A4*22*, *CYP3A4*26*, and *POR*28* are also associated with Tac dose requirements and may be included to provide the expected improvement of Tac therapy. Studies focusing on the intracellular drug concentrations and on calcineurin inhibitor-induced nephrotoxicity also seem promising. For all studies, however, the ethnic prevalence of genotypes should be taken into account, as this may significantly impact the effect of pre-emptive genotyping.

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1. Introduction

The calcineurin inhibitor (CNI) tacrolimus (Tac) is used to prevent acute rejection after solid organ transplantation (SOT).[1] Unfortunately, the clinical use of Tac is complicated by its considerable toxicity, narrow therapeutic window, and high interindividual pharmacokinetic variability.[2] Therapeutic drug monitoring (TDM) is universally applied to individualize Tac therapy in SOT recipients. However, despite TDM, many SOT recipients experience significant over- or underexposure to Tac. Part of the interindividual variability in Tac pharmacokinetics is explained by genetic polymorphisms in genes encoding for Tac metabolizing enzymes and transporter proteins.[3–5] Genetic variation may also explain interindividual differences in Tac's pharmacodynamics. In this article, the relevance of a pharmacogenetic approach to Tac therapy is discussed. The focus is on recent discoveries, new developments, and ethnic considerations.

2. Genetic variation and Tac pharmacokinetics

Tac is a substrate of the drug-efflux pump ABCB1 (encoded by the *ABCB1* gene), which is expressed in the intestine and thought to limit the absorption of Tac. Interindividual differences in the expression and/or function of ABCB1 determines the variability in the bioavailability of Tac.[6] Following absorption, Tac is metabolized in the intestine, liver, and to a limited degree in the kidney by cytochrome P450 (CYP) 3A4 and 3A5.[7] Interindividual differences in CYP3A activity are the most important determinants of the variability in Tac clearance. Other enzymes/receptors including P450 oxidoreductase (POR), the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-α), and CYP2C8 play minor roles in the disposition of Tac. Nonetheless, polymorphisms in *PPARA, POR*, and *CYP2C8* may explain residual variability in the response to Tac.

2.1. *CYP3A5*

Polymorphisms in the *CYP3A5* gene explain 40–50% of the variability in Tac dose requirement.[8,9] The best studied single-nucleotide polymorphism (SNP) in *CYP3A5* is *CYP3A5*3*, which is an A to G transition at position 6986 within intron 3 (rs776746). The *CYP3A5*3* allele causes alternative splicing, which results in protein truncation and a severe decrease of functional CYP3A5 enzyme.[10] Other *CYP3A5* SNPs are

CONTACT D.A. Hesselink d.a.hesselink@erasmusmc.nl Erasmus MC, University Medical Center Rotterdam, Dept. of Internal Medicine, Division of Nephrology and Renal Transplantation, Room D-427, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands

Article highlights

- The *CYP3A5* and *CYP3A4* genotypes of the transplant recipient have an impact on Tac dose requirement in SOT recipients. Other variants, such as *CYP3A4*22, CYP3A4*26*, and *POR*28* are also associated with Tac dose requirement.
- The evidence that implementing genotype-based Tac dosing will improve clinical outcome is missing.
- Dosing algorithms which incorporate genetics with demographic and clinical factors may allow for more precise Tac dosing.
- ABCB1 and CYP3A5 expression within the kidney transplant is associated with Tac-induced nephrotoxicity. The results about the relationship between other toxic effects of Tac (hypertension, neurotoxicity and PTDM) and pharmacogenetics are conflicting and seem to be of little value for the clinician.
- Ethnicity plays an important role in interindividual variability in Tac metabolism. Preemptive genotyping for *CYP3A5* or other relevant Tac metabolizing enzymes may be more promising in ethnic populations containing higher proportions of expressers.

This box summarizes key points contained in the article.

*CYP3A5**6 (rs10264272) and *CYP3A5**7 (rs41303343): *CYP3A5**6 encodes a 14690G>A transition, causing a splice variant mRNA and deletion of exon 7, resulting in nonfunctional CYP3A5 protein. [10,11] *CYP3A5**7 denotes a single base insertion at codon 346 causing a frameshift, resulting in a truncated mRNA and nonfunctional CYP3A5.[12]

Individuals homozygous for the *CYP3A5**3 allele are referred to as CYP3A5 non-expressers, whereas individuals carrying at least one *CYP3A5**1 allele are known as CYP3A5 expressers. The reduced enzymatic activity associated with the *CYP3A5**3 allele has been associated with a reduced Tac dose requirement (for a review, see reference [13]). CYP3A5 expressers require a Tac dose that is about 50% higher than that of CYP3A5 non-expressers to reach the same exposure. This is a consistent finding and has been observed in both adults and children, and among recipients of either a kidney, liver, heart, or lung transplant.[14–16]

Following standard, bodyweight-based dosing, CYP3A5 expressers are prone to have subtherapeutic Tac concentrations in the early phase after surgery and may therefore be at an increased risk for acute rejection. MacPhee et al.[17] demonstrated that CYP3A5 expressers did indeed have a delay in achieving the target Tac exposure, in spite of TDM. However, CYP3A5 expressers did not experience more biopsy-proven acute rejection, although rejection did occur earlier in CYP3A5 expressers compared with non-expressers with a median of 7 versus 13 days.[17] Other investigators have also reported that CYP3A5 expressers do not have a higher risk of developing acute rejection.[18–26]

Although numerous studies have reported the higher Tac dose requirement of CYP3A5 expressers compared to non-expressers, the clinical relevance of this association is unclear and has so far only been investigated in two randomized-controlled clinical trials (RCT). The Tactique study [27] was a multicenter RCT, including 280 renal transplant recipients. Patients were randomized 1:1 to receive either a standard starting dose of Tac (0.1 mg/kg twice daily) or a starting dose based on an individual patient's *CYP3A5* genotype (0.075 or 0.15 mg/kg twice daily for CYP3A5 non-expressers and expressers, respectively). The primary efficacy endpoint was the proportion of patients for whom the Tac predose concentration (C_0) was within the target range (10–15 ng/mL) after six unchanged doses of Tac. In the Tactique study, this was day 10 because Tac was started on day 7 after transplantation. Throughout the first post-transplant week, all patients were Tac-free to allow for *CYP3A5* genotyping, and received high-dose mycophenolate mofetil (MMF; 3 g/day), glucocorticoids, and induction therapy with rabbit antithymocyte globulin (rATG; in 82.2% of patients) or IL-2 receptor antibodies (in 17.8% of patients). In the Tactique study, *CYP3A5* genotype-based Tac (start)dosing led to significantly more patients reaching the target range 3 days after the start of Tac treatment as compared with standard, bodyweight-based Tac dosing: 43.2% versus 29.1%.[27] Also, the group that received a *CYP3A5* genotype-based Tac dose needed significantly less time and fewer dose adaptations to reach target. However, there were no differences between the two groups with regard to graft survival, acute rejection, delayed graft function, or Tac toxicity.[27]

Recently, the long-term follow-up results of Tactique were published.[28] Pallet et al. reported that the incidence of biopsy-proven acute rejection and graft survival were similar between the control and the *CYP3A5* genotype-adapted Tac dose groups. There were also no differences between the two groups in terms of patient survival, the incidence of cancer, cardiovascular events, infections, and kidney function. The authors concluded that optimization of initial Tac dosing using *CYP3A5* pharmacogenetic testing does not improve clinical outcomes.[28]

In a second RCT, 240 renal transplant recipients were randomized to receive a standard, bodyweight-based Tac starting dose (0.1 mg/kg twice daily) or a *CYP3A5* genotype-based starting dose (0.075 or 0.15 mg/kg twice daily for CYP3A5 non-expressers and expressers, respectively).[29] Unlike the Tactique study, this trial only included recipients of a living kidney donor (who were genotyped for *CYP3A5* during the workup for transplantation) and Tac was started on the day of transplantation rather than at day 7 post-transplant. All patients received basiliximab induction therapy and a standard MMF starting dose of 2 g/day followed by TDM. The primary endpoint of this trial was again the proportion of patients within the Tac therapeutic range on day 3 after transplantation (i.e. at first steady state). Unlike in Tactique, there was no difference in the proportion of patients 'on target' at day 3 after transplantation: 37.4% versus 35.6% for the standard-dose and the genotype-based groups, respectively. In addition, there was no difference in the time-to-reach target concentration or the number of Tac dose modifications required to reach the target concentration. In line with the French trial, there were no differences in any of the clinical endpoints, including the incidence of acute rejection.

It is unknown why the *CYP3A5*-based Tac dosing approach was beneficial in terms of early Tac exposure in the Tactique study, whereas this was not the case in the second. The main difference between these studies was the day on which Tac was initiated (day 0 vs. 7). Changes in glucocorticoid dosing or gastrointestinal motility during the first postoperative week may have had a greater effect on Tac exposure than *CYP3A5* genotype. Of note, in both studies, the percentage of patients 'on target' 3 days after initiation of Tac was low in spite of

CYP3A5 genotype adaptation. This observation demonstrates that there exists considerable residual variability in Tac pharmacokinetics that is not explained by *CYP3A5* genotype. The fact that neither study demonstrated a clinical benefit, does not support routinely genotyping kidney transplant recipients for *CYP3A5*. Possibly, donor genotype (including that of *CYP3A5*) may be more relevant for long-term clinical outcome than the genotype of the recipient.[30]

2.2. CYP3A4

The *CYP3A4* SNPs *CYP3A4**1B (rs2740574) and *CYP3A4**22 (rs35599367) have both been associated with altered Tac dose requirements. Individuals carrying the *CYP3A4**1B allele were reported to have a 35% lower Tac dose-adjusted C_0 compared to individuals having the *CYP3A4* wild-type allele.[31–33] However, whether the *CYP3A4**1B allele is truly itself responsible for the altered Tac dose requirement remains a matter of debate as this SNP is in linkage disequilibrium with the *CYP3A5**1 allele.[34]

*CYP3A4**22 (rs35599367) is located in intron 6 of *CYP3A4* and is a C to T substitution at g.15389. Wang and Sadee [35] demonstrated that *CYP3A4*22* increases the formation of the nonfunctional CYP3A4 splice variant with partial intron 6 retention, thereby reducing the production of functional full-length CYP3A4 mRNA and reduced CYP3A4 enzymatic activity. Elens et al. [36] were the first to find that the *CYP3A4**22 variant is associated with lower Tac dose requirements after renal transplantation. When *CYP3A4* and *CYP3A5* genotypes of individual patients were combined, Elens et al. were able to predict Tac dose requirements better compared with the *CYP3A4* or *CYP3A5* genotype alone. Based on these observations, it has been proposed to prescribe different Tac doses for ultrarapid (CYP3A5 expressers and *CYP3A4**1/*1), intermediate (CYP3A5 non-expressers and *CYP3A4**1/*1) and poor (CYP3A5 non-expressers and *CYP3A4**22 carriers) CYP3A metabolizers, respectively.[36–38] In pediatric heart transplantation, an association between *CYP3A4*22* and Tac dose requirement has also been observed.[39] *CYP3A4*22* carriers needed 30% less Tac to reach similar target concentrations compared with *CYP3A4**1/*1 carriers.

Recently a new and rare *CYP3A4* variant was described, which is now designated as *CYP3A4**26.[40] This variant is a c.802C>T transition and results in a premature stop codon at position 268 in exon 9 (R268*).[40] The resulting truncated CYP3A4 protein is nonfunctional. Werk et al. [41] first identified this mutation when they observed an unusually low Tac dose requirement in a kidney transplant recipient. This patient had very high Tac exposure following standard Tac dosing and only reached the therapeutic window once the Tac dose was reduced to 0.5 mg thrice weekly. This patient was a *CYP3A5**3 homozygote and was also homozygous for *CYP3A4**26, and therefore experienced complete failure of CYP3A enzyme activity.

2.3. POR

POR is a protein that functions as an electron donor for CYP enzymes (including CYP3A) and is essential for CYP-mediated drug oxidation.[42] More than 100 SNPs have been identified in the human *POR* gene and these may influence POR–CYP interaction and CYP activity.[42,43] The *POR*28* SNP (rs1057868; C>T) induces an amino acid substitution (p.Ala503Val) at position 503 which influences the electron binding moiety of POR and likely modifies its interaction with CYP enzymes.[42–44] Individuals homozygous for *POR*28* have an increased *in vivo* CYP3A activity with regard to midazolam compared with wildtype *POR*.[45]

In a study in 71 healthy Chinese volunteers, Zhang et al. [46] demonstrated that CYP3A5 expressers carrying the *POR*28* variant allele had a Tac exposure that was about 40% lower than CYP3A5 expressers with wildtype *POR*. The increased Tac dose requirement of CYP3A5-expressing kidney transplant recipients carrying the *POR*28* (T) variant allele was recently confirmed by Elens and Lunde et al. [47,48] Taken together, these studies suggest that the *POR*28* SNP leads to increased CYP3A5-mediated Tac metabolism, possibly resulting from a facilitated interaction between POR, CYP3A5, and Tac. In CYP3A5 non-expressers, Tac metabolism depends entirely on CYP3A4 and *POR*28* apparently does not influence CYP3A4 activity to a clinically relevant degree.

2.4. ABCB1

ABCB1 is thought to be responsible for the low oral bioavailability of Tac and is also considered important for the distribution of Tac throughout the body and its excretion into bile and urine.[49] The *ABCB1* gene contains more than 50 SNPs of which the 3435C>T (rs1045642), 1236C>T (rs1128503), and 2677G>T/A (rs2032582) SNPs, which are in linkage disequilibrium, have received the most attention. The functional significance of these SNPs on *ABCB1* expression and function remains unclear. It has been suggested that the synonymous *ABCB1* 3435C>T SNP affects the timing of co-translational folding and insertion of ABCB1 into the membrane, thereby altering the structure of substrate and inhibitor sites.[50]

Many studies have investigated the influence of *ABCB1* SNPs on Tac pharmacokinetics but results are conflicting and suggest no or at best a limited impact of *ABCB1* SNPs on Tac exposure. For an extensive review of these studies, the reader is referred to the literature.[51]

Because ABCB1 is also expressed in the membrane of lymphocytes, its activity may also impact the intracellular accumulation of Tac where the drug exerts its biologic effect. Vafadari et al. [52] found that patients with the *ABCB1* 3435CC genotype need more Tac for inhibition of IL-2 production in T cells compared with 3435TT genotype patients. Capron et al. demonstrated that patients carrying the *ABCB1* 3435T or the 2677T/A allele had 1.3-fold higher Tac concentrations within circulating lymphocytes compared with wildtype homozygotes.[53] These studies provide evidence that *ABCB1* 3435C>T and 2677G>T/A affect Tac distribution into lymphocytes with the variant alleles being associated with an increased pharmacodynamic effect of Tac. In line with the above, *ABCB1* SNPs also may be relevant with regard to its nephrotoxicity, because tissue concentrations of Tac are believed to be related to its renal side effects (see Section 3.1).

2.5. PPAR-α and PXR

The nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-α) has recently been recognized as potential contributor to intra- and interindividual variability in CYP3A expression and activity. Two sequence variants in the PPAR-α gene (*PPARA*) can affect PPAR-α expression. *In vitro, PPARA* c.209-1003G>A and c.208+3819A>G were associated with reduced expression of PPAR-α, and consistently related to lower CYP3A4 mRNA levels, protein expression, and enzymatic activity.[54] Recently, Lunde et al. found that expression of at least one *PPARA* variant allele was significantly associated with a higher Tac C_0/D ratio, when adjusting for *POR*28, CYP3A5**3, and *CYP3A4**22 among 229 kidney transplant recipients.[48] A detailed analysis of the two *PPARA* sequence variants showed significantly increased Tac exposure in patients homozygous for *PPARA-α* c.209-1003G>A. These results are in concordance with the reduced CYP3A4 protein/activity levels previously observed *in vitro*.[54] At present, *PPARA* c.208+3819A>G appears to be the *PPARA* sequence variant with the strongest influence on Tac pharmacokinetics but this observation requires confirmation.

The human pregnane X receptor (PXR; encoded by *NR1I2*), is a nuclear transcription factor that regulates the expression of CYP3A and ABCB1. Several SNPs in *NR1I2* have been identified but conflicting results regarding their association with Tac dose requirement have been reported.[8,55,56]

3. Genetic variation and clinical outcomes

Tac treatment is accompanied by adverse effects, including nephrotoxicity (both acute and chronic), posttransplant diabetes mellitus (PTDM), neurotoxicity, and hypertension. With TDM the majority of patients can be brought within the targeted window quite rapidly after transplantation. Nevertheless, some patients will experience acute rejection or Tac toxicity, despite being within the target range, reflecting differences in Tac pharmacodynamics. The relationship between genetic variation and Tac pharmacodynamics is the subject of the second part of this review.

3.1. Nephrotoxicity

Acute CNI-induced nephrotoxicity is caused by constriction of the afferent glomerular arteriole leading to a reduced renal blood flow and glomerular filtration rate (GFR). Chronic CNI-induced nephrotoxicity appears to be the result of structural changes in the kidney caused by chronic changes in renal hemodynamics. CNI-induced nephrotoxicity is likely to be related to intra-renal concentrations of CNIs which may not be properly reflected by whole-blood CNI concentrations.[57–60] CYP3A5 is the only CYP3A isozyme expressed in the kidney and may limit local exposure to CNIs by intra-renal metabolism.[61,62] Zheng et al. [63] demonstrated that Tac concentrations in the renal epithelium of CYP3A5 expressers are 53% lower compared with CYP3A5 non-expressers.

Studies on the relationship between *CYP3A5* genotype and the risk of Tac-induced nephrotoxicity have reported contradictory results. Kuypers et al.[32] observed a higher incidence of biopsy-proven Tac-nephrotoxicity (defined as *de novo* arteriolar hyalinization) in CYP3A5-expressing kidney transplant recipients. In a follow-up study, which included more patients (n = 304), this group confirmed that CYP3A5 expressers have an increased risk for biopsy-proven Tac-induced nephrotoxicity.[64] These counter-intuitive findings may be explained by the fact that it is not Tac itself but its metabolites that are responsible for its nephrotoxicity. These metabolites might be formed at an increased rate in the renal parenchyma of CYP3A5 expressers. However, there is at present little evidence to support this hypothesis.

In contrast to the studies by the Leuven group, a Chinese study including 67 kidney recipients showed a higher incidence of nephrotoxicity in CYP3A5 non-expressers at 1-month posttransplant.[65] In patients with the *CYP3A5**3/*3 genotype, interstitial fibrosis and proximal tubular vacuolization were more severe than in patients with the *CYP3A5**1/*3 genotype. Similarly, in a study with 136 renal transplant recipients (121 Caucasians, 12 Africans, and 3 Asians), those with the *CYP3A5**3/*3 genotype tended to have a higher incidence of biopsy-proven nephrotoxicity compared to *CYP3A5**1 allele carriers, although the difference was nonsignificant.[66] There are many reasons for these discrepancies, including differences in ethnicity, sample size, and the definition of nephrotoxicity.

ABCB1 is expressed in the apical membrane of renal tubular epithelial cells, where it may facilitate excretion of CNIs (and their metabolites) in urine and thus protect the kidney against intra-renal CNI accumulation. Studies on the relationship between *ABCB1* genotype and the risk of Tac-induced nephrotoxicity are more consistent compared with those on *CYP3A5*. In a prospective cohort study of 252 renal transplant recipients Naesens et al.[57] observed a progressive increase in glomerulosclerosis, vascular intimal thickening and IF/TA over the first 3 years posttransplantation. A lower ABCB1 expression in kidney transplant biopsies was a risk factor for such chronic histologic damage in patients receiving Tac. In another study they reported that both donor and recipient homozygosity for *ABCB1* 3435TT was associated with a higher risk of Tac-associated kidney damage.[67] Combined donor-recipient *ABCB1* 3435TT homozygosity was also a risk factor for worse graft function after the first posttransplant year. The authors speculated that the relevance of the recipient genotype could possibly be explained by renal epithelial chimerism in the allograft. Recently, in a study including 368 African-American and 314 European American deceased donors, Ma et al. found that the T allele at *ABCB1* 3435 of the kidney donor is associated with shorter renal allograft survival compared with 3435C for kidneys from European American donors. [68] A poorer renal function (i.e. a lower estimated GFR) was also observed among patients who received kidneys from donors with the *ABCB1* 3435TT genotype as compared with patients receiving *ABCB1* 3435CC kidneys.[69]

In contrast, Moore et al. reported contradictory results. In a very large cohort (n = 4471 white kidney transplant recipients), it was the 3435CC donor genotype (not 3435TT as in the study by Ma et al.) that was associated with a worse death-censored graft survival.[70]

The discrepancies between these studies are unexplained. Again differences in sample size, patient characteristics, and duration of follow-up may form an explanation. Perhaps more importantly, loss of renal function may have causes other than chronic CNI nephrotoxicity and in many of the larger genetic association studies, 'pure' chronic CNI nephrotoxicity was not distinguished from, for example, recurrent primary kidney disease, chronic rejection, or polyomavirus-associated nephropathy.[13] Finally, and perhaps most importantly, there is no 'gold standard' to diagnose chronic CNI nephrotoxicity. Even renal histology has its shortcomings and is not specific enough for making a definitive diagnosis.[71] Possibly, in the future, we will see an increasing use in genetic association studies of surrogate markers for the chronic nephrotoxic effects of CNIs. Preliminary data indicate that markers of epithelial-to-mesenchyme transition may serve as such.[72]

Some studies have investigated the association between CNI-nephrotoxicity and genetic variation in genes other than *ABCB1* and *CYP3A*. One such gene is *CYP2C8*, which is a member of the P450 superfamily and is expressed in the kidney where it is involved in the metabolism of arachidonic acid to biologically active epoxyeicosatrienoic acids (EETs). EETs help to maintain blood pressure, are involved in tubular reabsorption of water and sodium transport, protect against inflammation, and the maintenance of vascular smooth muscle tone.[73–75] Smith et al. [76] found that patients carrying one or more *CY2C8**3 variant alleles have a higher risk of developing CNI-induced nephrotoxicity after liver transplantation. Possibly, decreased production of EETs in patients with the variant *CYP2C8**3 allele may reduce the capacity of their kidneys to counter the vasoconstrictive effects of CNIs. Gervasini et al. observed that the rs1042032A>G SNP in *EPHX2*, the gene that encodes soluble epoxy hydrolase, the enzyme which metabolizes EETs to less active compounds, was associated with renal allograft function and the risk of acute rejection.[77]

3.2. Delayed graft function

Delayed graft function (DGF) is most commonly defined as the need for dialysis within the first week after transplantation.[78,79] It is associated with reduced long-term allograft survival and is closely related to ischemia/reperfusion injury.[78,80]

Hauser et al. [81] investigated the impact of *ABCB1, ABCC2*, and *PXR* polymorphisms of the donor and recipient on the development of DGF after renal transplantation. The *PXR* 8055TT genotype of the donor (but not the recipient) was significantly associated with an increased risk of DGF. Another study, including 304 kidney transplant recipients found that DGF was associated with higher initial Tac exposure which occurred more frequently in CYP3A5 non-expressers.[82] A recent study in renal transplant patients found that the *CYP3A4**22 allele was associated with a higher risk of DGF compared with *CYP3A4**1 homozygotes in cyclosporine (CsA)-treated patients.[83] There are no reports on the association between *CYP3A4**22 and DGF in patients treated with Tac. More recently, Gervasini et al.[84] investigated the association between DGF and the *CYP2C8**3 variant allele. They observed that subjects carrying one or two *CYP2C8**3 variant alleles had a higher risk of developing DGF and had a lower creatinine clearance 1 year after transplantation than *CYP2C8**1/*1 homozygotes.[84]

3.3. Acute rejection

The incidence of acute rejection may be related to genetic variation in the genes encoding the proteins involved in the absorption and elimination of immunosuppressive drugs (reviewed in references [3,51]). However, at present, no consistent association between *CYP3A* and *ABCB1* SNPs and an individual's risk for rejection has been demonstrated and the additional risk posed by certain genetic variants, if any, appears to be small and is unlikely to be clinically relevant.[85,86] That these studies did not find *CYP3A5* genotype to be associated with the risk of acute rejection may be perceived as a surprise, given the strong influence of *CYP3A5* genotype on Tac dose requirement. It thus seems that the very efficient process of TDM results in rapid correction of Tac concentrations outside the target range. As a result the genotype-induced underexposure only lasts for a few days, which is not sufficient to cause a clinically important increased incidence of acute rejection episodes.[87]

3.4. PTDM

PTDM is a frequent complication of Tac therapy.[88] Tac is directly toxic to islets of Langerhans and impairs insulin secretion and insulin gene expression.[88] A patient's genetic background may contribute to the risk of PTDM. Among 101 renal transplant recipients receiving Tac-based immunosuppressive therapy, Elens et al. found that the *PPARA* rs4253728A>G and *POR**28 variant alleles were both independently associated with an increased risk of developing PTDM with respective odds ratios of 8.6 (95%CI 1.4–54.2) and 8.1 (95%CI 1.1–58.3).[89] Several other investigators have reported associations between the risk of PTDM and polymorphisms in the vitamin D receptor gene, promoter region of the IL-6 gene, transcription factor 7-like 2 gene (rs7903146), and the zinc transporter-8 gene (*SLC30A8*; rs13266634).[90–94]

The role of pharmacogenetics in PTDM is complex. CNIs, glucocorticoids, and mTOR inhibitors are all diabetogenic but alternative immunosuppressive regimens (including anti-proliferative agents and the novel immunosuppressant belatacept) have been associated with higher rejection rates.[95] Possibly, genetic risk factors may be used together with non-genetic variables to estimate an individual's risk of developing PTDM. However, even if this becomes possible in the future, the current literature does not provide guidance on what the best immunosuppressive regimen would be for such patients.

4. Other Tac-related adverse events

Tac can cause hypertension and is neurotoxic. Tac causes hypertension by activating the renal sodium chloride co-transporter, which is under the control of the 'with-no-lysine'(WNK) kinase network. Ferrarresso et al. genotyped 92 Caucasian kidney transplant recipients receiving CsA or Tac and found that *CYP3A5**1 carriers had a higher blood pressure 1 week and 6 months after transplantation.[96] Torio et al. also found

a trend toward higher blood pressure in *CYP3A5**1 carriers treated with a CNI, 6 and 24 months after kidney transplantation.[97] At present, it appears that *CYP3A5* genotype may relate to an individual's risk of developing hypertension but there is no convincing evidence that SNPs in *ABCB1, WNK4,* or *SPAK* do the same (see reference [98] for an extensive review on the genetic basis of hypertension).

Using pharmacogenetics to guide antihypertensive therapy in Tac-treated patients appears to be more readily clinically applicable. Diltiazem is a calcium channel antagonist that interacts with Tac by inhibiting CYP3A-mediated Tac metabolism. Kidney transplant patients expressing CYP3A5 were much more susceptible to the inhibitory effects of diltiazem than non-expressers.[99]

Neurotoxic effects of Tac include tremor, headache, insomnia, and peripheral neuropathy.[100] Although the exact pathophysiology of Tac-induced neurotoxicity is unclear, penetration of Tac into the central nervous system (CNS) is considered important. ABCB1 is an important component of the blood brain barrier and loss of its function leads to accumulation of Tac in the CNS, at least in mice.[101,102] However, no clinically meaningful associations between *ABCB1* genotype and the risk of developing Tac-induced neurotoxicity have been identified. Yamauchi et al.[103] found that transplant recipients carrying the *ABCB1* 2677T/A allele had an increased risk of neurotoxicity, whereas carriers of an *ABCB1* 3435T allele had a decreased risk. Yanagimachi et al. [104] reported that among 30 pediatric patients who received CsA for the prevention of graft-versus-host disease the *ABCB1* 1236CC genotype tended to be associated with neurotoxicity after adjustment for age, hypertension, and renal dysfunction ($P = 0.07$). In the same study, the *CYP3A5**1 allele was found to be associated with an increased risk for neurotoxicity. Yanagimachi et al. suggested that it may not be Tac itself but its metabolites that cause neurotoxicity. Such metabolites might be formed locally at an increased rate in CYP3A5 expressers. An increased risk for neurotoxicity in association with the *ABCB1* 1236C or 2677G alleles was also observed by a Spanish research group.[24] Prospective studies that measure Tac concentrations in the cerebrospinal fluid of affected patients may shed more light on the pathophysiology of Tac-induced neurotoxicity and the role of genetic variation therein.

5. Ethnic considerations

Ethnicity may play an important role in interindividual variability of drug metabolism and response. Genetic variations of drug-metabolizing enzymes show pronounced differences between populations. Allelic frequencies of the most common SNPs in *CYP3A5, CYP3A4, ABCB1*, and *POR*28* in various ethnic groups are presented in Table 1. The most striking difference is the marked variation in the allelic frequency of the *CYP3A5*3* allele which is common among Caucasian patients but less frequently seen in patients of Asian or African descent. Patients of African descent require higher doses of Tac to reach the target concentration range. Vadivel et al. found that for patients of African descent a Tac starting dose of 0.3 mg/kg per day is probably more effective than the currently recommended starting dose of 0.2 mg/kg per day.[105] This higher dose requirement appears to result in part from the high number of CYP3A5 expressers among patients of African descent.[106]

The lower exposure to Tac following standard dosing may be responsible for the higher acute rejection risk after kidney transplantation in recipients of African descent.[112] By contrast, *CYP3A5* genotype appears not to be a risk factor for the poorer long-term kidney allograft survival observed in patients of African descent, despite its well-characterized influence on Tac dose requirement.[113]

In addition to *CYP3A5**3, the *CYP3A5**6 and *CYP3A5**7 variant alleles can also lead to the absence of functional CYP3A5 protein. These SNPs are rare or absent in Asian or Caucasian populations, but are found commonly in African populations. The presence of *CYP3A5**6 and *CYP3A5**7 in African populations may compensate for the relatively low frequency of the *CYP3A5**3 allele, resulting in a metabolic phenotype similar to that of Caucasians. The guidelines recommend increasing the starting dose by 1.5–2 times in extensive metabolizers (*CYP3A5**1/*1) and intermediate metabolizers (*CYP3A5**1/*3, *1/*6, or *1/*7) and to prescribe a standard dose in poor metabolizers (*CYP3A5**3/*3, *6/*6, *7/*7, *3/*6, *3/*7, or *6/*7).[114] This recommendation is supported by the findings of a study in African-American kidney transplant recipients (n = 354). In this study, it was observed that *6 and *7 allele carriers required lower Tac doses. In this African-American population, one or more nonfunctional *CYP3A5* alleles (*3, *6, or *7) were identified in 74.5%.[115] This study demonstrated that there are considerably more CYP3A5 non-expressers in African populations than was previously presumed. In 197 adult African kidney transplant recipients, Oetting et al.[116] also found that the variants *CYP3A5**3, *CYP3A5**6, and *CYP3A5**7 explained a great proportion of the observed Tac C_0 variability in African recipients. Taken together, these studies illustrate the importance ethnicity-specific genotypes (*CYP3A5**6 and *CYP3A5**7) for Tac clearance. Using dosing models that account for these genotypes may lead to a more precise dosing of Tac.

Table 1. Allele frequencies (by ethnic group) of relevant Tac metabolizing enzymes and transporters.

	Caucasians (%)	Africans (%)	Indians (%)	Asians (%)	References
*CYP3A5**3	90–93	32	66, 68	60–73	[107,108]
*CYP3A5**6	0–4.3	8.6–15	ND	0	[107]
*CYP3A5**7	0	5–12	ND	0	[107]
*CYP3A4**1B	2–9.6	35–67	3.5	0	[109]
*CYP3A4**22	8.3	4.3	ND	4.3	[37,110]
ABCB1 3435C	48–62	68–83	38	51–62	[111]
ABCB1 1236C	55–59	85	ND	35–41	[111]
*POR*28*	26	19	30	37	[43]

ABCB1: ATP-binding cassette subfamily B member 1; CYP: Cytochrome P450; POR: Cytochrome P450 oxidoreductase.

Given the size and ethnic diversity of the Chinese population, it is very important to investigate the interethnic variability in this particular group. In a study of six different Chinese ethnic groups, Lai et al. [117] found that significantly higher frequencies of *CYP3A5**3 variant alleles were observed in Uygur Chinese (88.1%), Kazakh Chinese (84.5%), and Tibetan Chinese (80.3%) than in Han (67.3%) and Bai Chinese (70.2%). The lowest frequency of the *CYP3A5**3 variant alleles was observed in Wa Chinese (56.3%). This result was consistent with what was reported previously by Li et al. [118] (Uygur Chinese 84.8%, Kazakh Chinese 86.6%, and Han Chinese 72.7%). The frequency of the *CYP3A5**3 variant allele in Uygur, Kazakh, and Tibetan Chinese appears to be more similar to Caucasians as compared with Han Chinese. Other studies, however, did not report significant differences in the *CYP3A5**3 allelic frequency among Uygur and Kazakh Chinese and Caucasians.[10,119,120]

The frequency of *CYP3A4*1*B in African-Americans (35–67%) is the highest amongst all ethnic groups.[109] The frequencies of the *ABCB1* 3435C and the 1236C alleles are also much higher in individuals of African descent than in populations of other ethnicity.[111] Recently, a novel *CYP3A4* loss-of-function allele (*CYP3A4*20*) was identified and was shown to be present in 1.2% of the Spanish population. This polymorphism has, however, not been investigated in relation to Tac dose requirement nor toxicity.[121,122]

In conclusion, ethnic variation in the prevalence of *CYP3A5, CYP3A4*, and *ABCB1* genotypes is high and clinically relevant. Given the fact that *CYP3A5* genotype has the strongest and most consistent association with Tac dose requirement, a *CYP3A5* genotype-based Tac dosing approach may be especially relevant for patients of African descent who are more often CYP3A5 expressers than Caucasians. How this genetic variability affects the metabolizing phenotype in the non-Caucasian and non-African population is incompletely understood and should be the subject for future studies.

6. Dosing algorithms

Dosing algorithms have only fairly recently been proposed to better individualize the Tac starting dose.[123] In 2011, Passey et al. created the first dosing algorithm using a combination of genetic information and clinical factors in adult kidney transplant recipients. The algorithm included *CYP3A5* genotype, days post-transplant, age, steroid, and calcium channel blocker use. Interestingly, other factors such as sex, ethnicity, and bodyweight did not have a statistically significant influence on Tac clearance.[124] The dosing algorithm was later successfully validated in an independent cohort of 795 kidney transplant recipients.[125] In 2013, the developed dosing algorithm was prospectively tested by an independent research group in the United Kingdom. Unfortunately, the dosing algorithm was not able to predict estimated Tac clearance accurately.[126] As mentioned before, not all pharmacokinetic variability is explained by the *CYP3A5* genotype. It was recently shown that the algorithm designed by Passey was improved by incorporating the *CYP3A4*22* allele.[127]

More recently, Størset et al. [128] used the dosing software BestDose, including fat-free mass, hematocrit, time after transplantation, Tac-dosing history and the patient's previously measured Tac concentrations, but not *CYP3A5* genotype, to determine the Tac starting dose in renal transplant recipients. They found that computerized dose individualization improved target achievement of Tac compared with conventional dosing early after renal transplantation and that the computer software may also potentially improve long-term outcome.[128] One advantage of not basing the dose predictions on genotype in this study is that it is useful also for centers without the opportunity to perform pretransplant genotyping. To our knowledge this dosing algorithm has not been further tested or improved. This Norwegian study mainly included Caucasian patients. If algorithms such as these are to gain widespread (clinical) acceptance their performance should not only be validated but this should also be done in populations of different ethnicity.[128]

7. Conclusions

In conclusion, the *CYP3A5* genotype of the transplant recipient has an impact on Tac dose requirement in SOT recipients. Other variants, such as *CYP3A4*22, CYP3A4*26*, and *POR*28* are also associated with Tac dose requirement. Besides these pharmacokinetic considerations, ethnicity plays an important role in interindividual variability in Tac metabolism. Unfortunately, the evidence that implementing genotype-based Tac dosing will improve clinical outcome is missing. Recent studies have shown that dosing algorithms which incorporate genetics with demographic and clinical factors may allow for more precise Tac dosing. However, further research is necessary to elucidate the role of pharmacogenetics in the pharmacodynamic effects of Tac.

8. Expert opinion

Immunosuppressive drug therapy is necessary to prevent acute rejection after SOT. Tac is the preferred CNI and it is to be expected that in the next 10 years many SOT recipients will continue to receive Tac as part of their immunosuppressive maintenance regimen. There is convincing evidence that the *CYP3A5* and the *CYP3A4* genotype of the recipient have a significant impact on Tac dose requirement. However, despite the strong genetic effect on Tac dose requirement, the evidence that implementing genotype-based dosing will improve clinical outcome is missing. Two RCTs using *CYP3A5* genetic information to guide Tac dosing have been performed. The first of these showing a small increase in the proportion of patients reaching the target Tac concentration, but without reduction in the incidence of acute rejection, and a second study, which even failed to show an improvement of the achievement of the pharmacokinetic outcome parameters.

CYP3A5 is currently the strongest known genetic predictor of Tac dose requirement, but it does not explain all variability. Other variants, including *CYP3A4*22, CYP3A4*26*, and *POR*28* are also associated with Tac dose requirements. These other variants may need to be taken into consideration. Given its proactive nature, pharmacogenetics may still be a potential complimentary tool to TDM for optimizing immunosuppressive therapy.

A more precise and rational strategy to optimize early Tac exposure is to use a dosing algorithm that incorporates more than just the bodyweight and *CYP3A5* genotype. Such dosing algorithms may include genetics and demographic and clinical factors. Although the implementation of dosing algorithms is appealing, they do have some limitations. The developed dosing algorithms are all very different and many have not been validated in independent data sets. Most algorithms are published in pharmacokinetic journals, which make them less accessible to clinicians. Refining of Tac dose prediction is possible using dosing algorithms. We propose that newly developed dosing algorithms for the starting dose need to be validated and subsequently tested in an independent cohort of patients. If successful, a clinical trial should be conducted with the amount of patients on target on day 3 after initiation as primary endpoint. Clearly, the rapid adjustment of Tac dose based on TDM can correct for any variability in Tac exposure resulting from genetic differences within a matter of days. Therefore, it is questionable if the transplant community will adopt the strategy of genotyping recipients prior to transplantation for these metabolizing enzymes and transporters.

To get the most out of the efficacy and safety of Tac, more effort has to be put into a better understanding of pharmacogenetics of the pharmacodynamic effects of the drug. It has been demonstrated that ABCB1 and CYP3A5 expression within the kidney transplant is associated with CNI-induced nephrotoxicity. However, with regard to the other toxic effects of Tac, like hypertension, neurotoxicity, and PTDM, the results about the relationship between these side effects and pharmacogenetics are conflicting and seem to be of little value for the clinician. Prospective studies using novel techniques such as mass spectrometry to detect Tac metabolites and tissue drug concentrations should be developed to elucidate the role of pharmacogenetics in Tac nephrotoxicity. Possibly, genetic information may predict the occurrence of drug toxicity and provide guidance to clinicians to choose for Tac-free or reduced-dose Tac immunosuppressive protocols in those at high risk.[129,130]

Ethnicity plays an important role in interindividual variability of drug metabolism and response. Where Caucasian patients are mostly CYP3A5 non-expressers, patients from Asian descent are CYP3A5 expressers in about one-third of cases, and those from African descent in two-thirds of cases. Preemptive genotyping for *CYP3A5* or other relevant Tac metabolizing enzymes may be more promising in ethnic populations containing higher proportions of expressers. The two prospective randomized trials were both performed in populations containing largely Caucasian patients.

Declaration of interest

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