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**Institutions:** University of Queensland, Stanford University

**Published on:** 01 Oct 2013 - Pharmacogenetics and Genomics (NIH Public Access)

**Topics:** Tacrolimus, Organ transplantation, Cyclosporin a, Calcineurin and Transplantation

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Published in final edited form as:

*Pharmacogenet Genomics*. 2013 October ; 23(10): 563–585. doi:10.1097/FPC.0b013e328364db84.

## PharmGKB summary: cyclosporine and tacrolimus pathways

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### Keywords

*ABCB1*; calcineurin; cyclosporine; *CYP3A4*; *CYP3A5*; pharmacodynamics; pharmacogenetics; pharmacokinetics; tacrolimus; transplantation

### Introduction

Tacrolimus (FK506) and cyclosporine (cyclosporin A, CsA) are cornerstone immunosuppressive agents administered to solid organ transplant recipients to prevent and treat allograft rejection. The discovery of cyclosporine in the 1970s, and its entry into the collection of immunosuppressants in the early 1980s, was a major breakthrough in medicine. Cyclosporine was the most successful antirejection drug to date, and it radically improved the chance of survival for transplant recipients. In 1994, the Food and Drug Administration (FDA) approved tacrolimus, an effective alternative to cyclosporine [1]. Since then, tacrolimus and cyclosporine have become the principal immunosuppressive drugs for solid organ transplantation. The United States Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients showed that in 2011, 86% of the 16 055 patients who received a kidney transplant were prescribed tacrolimus upon discharge, and 2.4% were prescribed cyclosporine. One year after transplant, 84 and 4% of patients received tacrolimus and cyclosporine therapy, respectively [2]. Global differences exist in the usage of tacrolimus and cyclosporine: 2008 figures from the Australia and New Zealand Dialysis and Transplant Registry show that 61% of the 391 Australian patients who received a deceased kidney donor graft were prescribed tacrolimus, and 35% were prescribed

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### Conflicts of interest

There are no conflicts of interest.

cyclosporine. At 1-year post-transplant, these numbers changed to 55 and 33% for tacrolimus and cyclosporine, respectively [3]. Both drugs are also prescribed for liver, intestinal, lung, and heart transplant recipients [2], and can be used to manage severe autoimmune conditions, such as atopic dermatitis [4,5] and rheumatoid arthritis [6,7].

Tacrolimus and cyclosporine differ in their chemical structure: cyclosporine is a cyclic endecapeptide [8], whereas tacrolimus is a macrocyclic lactone [9]. However, they act in a similar manner. Both are calcineurin inhibitors; their main mechanism of action involves inhibition of this important phosphatase [1]. Tacrolimus exhibits similar effects to cyclosporine, but at concentrations 100 times lower [10]. Despite these differences in potency, tacrolimus and cyclosporine both show excellent survival rates for grafts across many comparative studies (summarized in Maes and Vanrenterghem [11]). However, several studies have shown that use of tacrolimus is associated with a lower allograft rejection rate compared with cyclosporine [12-14].

The principal adverse effects associated with tacrolimus and cyclosporine treatment are neurotoxicity, nephrotoxicity, hypertension, hyperglycemia, gastrointestinal disturbances, infections, and malignancy [15]. Although the two drugs have similar side-effect profiles, they may differ in the frequency of effects. For example, tacrolimus is more likely to cause alopecia [16], tremors [17], and new-onset diabetes mellitus [12], whereas cyclosporine is associated with hyperlipidemia [18], hypertrichosis, and gingival hyperplasia [19]. The idea that tacrolimus is less nephrotoxic than cyclosporine remains controversial [20], particularly as most studies of renal injury are based on evaluations in renal transplant patients, making it difficult to discriminate between drug-induced organ damage and other causes of organ dysfunction [21]. A recent study in pancreatic transplant recipients examined baseline kidney biopsies and 5-year post-transplant biopsies, and reported that the chronic nephrotoxic effects of tacrolimus and cyclosporine were similar [20].

Despite the success of both drugs, treatment is complicated by narrow therapeutic indices and large inpatient and outpatient pharmacokinetic variability [22,23]. Although adequate exposure is essential to prevent rejection, overexposure can lead to toxicities that reduce tolerability and affect long-term allograft and patient survival [24]. Therapeutic drug monitoring (TDM), therefore, is mandatory for both drugs. However, because individual transplant recipients respond differently to similar immunosuppressant concentrations, achieving the recommended therapeutic target range does not guarantee absence of drug toxicity or complete immunosuppressant efficacy. A mechanistic understanding of the underlying factors affecting the pharmacokinetics and pharmacodynamics of calcineurin inhibitors may prove useful in being able to further personalize these therapies.

This review aims to provide a broad overview of recently published literature on the pharmacokinetics, pharmacodynamics, and pharmacogenetics of tacrolimus and cyclosporine in transplant patients, with the goals of clarifying current understanding and identifying areas of future research. In doing so, this review builds on the work of others in this field [1,8,24-27]. A particular emphasis is given to pharmacogenetics, as developments in this area may provide a way to optimize treatment with these drugs, potentially avoiding negative side effects while still maintaining efficacy.

## Pharmacokinetics

A schematic representation of tacrolimus and cyclosporine disposition within the body is provided in Fig. 1. Tacrolimus and cyclosporine are usually administered orally [8,24], and various formulations of the drugs are available for use. Both are Biopharmaceutics Classification System (BCS) Class II drugs [28,29], indicating they are poorly soluble and also highly lipophilic, and therefore readily absorbed through cell membranes [27,30]. Bioavailability of both drugs is generally poor with mean values around 25% [31,32]. However, it is important to note that wide variation in bioavailability is seen between individuals using the two drugs [32,33]. Significant firstpass metabolism in the small intestine and liver, as well as efflux from the intestine, both contribute to drugs' low overall bioavailability [34].

Upon entering enterocytes, both drugs are metabolized by gastrointestinal CYP3A isozymes, predominantly CYP3A4 and CYP3A5 [35,36]. Studies have shown that CYP3A5 is the predominant enzyme for metabolism of tacrolimus, with CYP3A4 contributing, but having a lower efficiency for catalysis [37,38]. In contrast, cyclosporine is primarily metabolized by CYP3A4 [39]. The CYP3A family of enzymes also includes CYP3A7 and CYP3A43 [40]. However, the involvement of CYP3A7 in cyclosporine metabolism is unclear [35], and it has a low affinity and capacity toward tacrolimus, suggesting that it likely plays a minimal role in tacrolimus metabolism [38]. Similarly, there is no evidence to support the involvement of CYP3A43 in cyclosporine metabolism [35], and its role in tacrolimus metabolism, if there is one, has yet to be elucidated [24]. Parent drug that escapes intestinal metabolism enters the hepatic portal system and the liver, where CYP3A4 and CYP3A5 metabolize tacrolimus and cyclosporine [41,42]. Upon entering systemic circulation, both drugs bind extensively to erythrocytes [1,43], and only unbound drug is capable of entering lymphocytes and exerting its main immunosuppressive effects.

Up to 15 metabolites of tacrolimus may be formed [44], with the most prevalent being 13-*O*-demethyl-tacrolimus [24,45]. This metabolite is approximately one-tenth as active as tacrolimus, whereas a minor metabolite, 31-*O*-demethyl-tacrolimus, has been found to have immunosuppressive activity comparable with tacrolimus [45,46]. The remaining metabolites have weak or negligible pharmacological activity [46,47]. For cyclosporine, ~25 metabolites are formed [48]. The major metabolites found in blood are AM1 and AM9, which are hydroxylated products, and AM4N, which is *N*-demethylated [48]. CYP3A4 is capable of transforming cyclosporine into AM1, AM9, and AM4N, whereas CYP3A5 only transforms the drug into AM9 [39]. Reported immunosuppressive activity of these metabolites varies between studies, but all metabolites studied so far have reduced activity compared with cyclosporine [49,50]. AM1 has the highest immunosuppressive activity: one study reported its activity to be close to 20% of native cyclosporine [50], whereas another found it to be as high as 80% [49].

Both tacrolimus and cyclosporine are extensively metabolized, with less than 0.5 and 1%, respectively, of the parent drug appearing unchanged in urine and feces [51,52]. Approximately 95% of tacrolimus metabolites are eliminated through the biliary route with urinary excretion accounting for around 2% [51]. Similarly, cyclosporine metabolites are

mainly excreted in the bile, with only around 3% of the drug undergoing renal elimination [52,53].

In addition to CYP3A4 and CYP3A5, the efflux transporter P-glycoprotein also plays a major role in the pharmacokinetics of tacrolimus and cyclosporine [34]. Encoded by the *ABCB1* gene, it pumps xenobiotics from the cytoplasm to the exterior of the cell [54]. It is present on the apical surface of cells, and transports both tacrolimus and cyclosporine [55]. P-glycoprotein is present at high concentrations in the villus tip of enterocytes of the small intestine [56,57] and lowers intracellular concentrations of both drugs by pumping them out of enterocytes into the intestinal lumen [54]. Variation in intestinal P-glycoprotein was found to account for ~ 17% of the variability in oral clearance of cyclosporine, where higher levels of P-glycoprotein indicated higher observed clearance of the drug. Indeed, the same study concluded that 75% of interpatient variability in cyclosporine clearance could be explained by variation of both CYP3A4 activity in the liver, and expression of P-glycoprotein in enterocytes [54]. For tacrolimus, a strong inverse correlation was seen between the concentration/dose ratio of tacrolimus and the intestinal mRNA level of *ABCB1* for the first 7 days after liver transplant in one study [58], and for the first 4 days after liver transplant in another [59]. In addition to enterocytes, P-glycoprotein also transports drugs across membranes within hepatocytes [60] and kidney cells [61,62]. It is also involved in drug transport within lymphocytes [57,63], so the actual concentration of cyclosporine and tacrolimus available for immunosuppression within these cells may be influenced by their P-glycoprotein content. However, as P-glycoprotein's role within enterocytes is better characterized, Fig. 1 shows its involvement only in intestinal drug transport.

TDM of cyclosporine and tacrolimus is performed by adjusting drug dosage according to concentrations within the blood. Evidence of an advantage for tacrolimus and cyclosporine TDM over no monitoring has not been formally established in a randomized control trial. However, given the narrow therapeutic indices of these agents, and their large interindividual pharmacokinetic variability, it is widely accepted that TDM is beneficial [64]. Although full dose interval area under the concentration–time curve from 0 to 12 h ( $AUC_{0-12}$ ) is generally considered the best marker of overall drug exposure, the requirement for collection of multiple samples over a 12-h period makes this approach infeasible within a clinical setting, both financially and practically [64,65]. Subsequently, for reasons of convenience, most transplant centers use trough blood concentration ( $C_0$ ) to guide tacrolimus dosing [64], and  $C_0$  or 2-h postdose blood concentration ( $C_2$ ) to guide cyclosporine dosing [66]. The strength of correlation between tacrolimus AUC and  $C_0$  is still a matter of debate, with some studies finding better relationships between  $C_3$  [67],  $C_4$  [68], and  $C_5$  [69] and AUC. For cyclosporine,  $C_0$  monitoring was initially used, though  $C_2$  was later found to correlate better with cyclosporine  $AUC_{0-4}$ , the time period in which cyclosporine shows the greatest pharmacokinetic variability [66].  $C_2$  was also shown to have stronger associations with clinical outcomes compared with  $C_0$  [66]. However, limited sample methods such as multiple linear regression or maximum a-posteriori (MAP) Bayesian analyses may provide more accurate estimates of tacrolimus exposure than single time points [65]. Large prospective trials are necessary to determine which of these monitoring strategies is most expedient. Guideline targets for cyclosporine  $C_2$  levels have

been suggested by the Consensus on Neoral C2: Expert Review in Transplantation (CONCERT) committee [66]; target concentrations for liver and renal transplant patients can be seen in Table 1. Targets for tacrolimus  $C_0$  in kidney, heart, and liver transplant patients have also been proposed by a recent expert consensus document [64]. Information from this report as well as from a separate report on lung transplant patients [70] can be found in Table 1. Despite the consensus on target concentrations, the expert statement on tacrolimus TDM noted the absence of multicenter, prospective, and concentration-controlled trials that assess relationships between tacrolimus concentrations and clinical outcome, as well as a lack of high quality studies that compare different TDM strategies to determine which might be most advantageous [64]. In addition, a systematic review of cyclosporine  $C_2$  monitoring studies found that there was a lack of good quality studies comparing  $C_0$  and  $C_2$  monitoring, with the majority being observational or nonrandomized with highly heterogeneous results. Furthermore, there is very limited evidence from prospective studies that support the short-term clinical benefits of  $C_2$  monitoring, and the authors suggest that better evidence from randomized and high quality trials is necessary to assess the advantages of using  $C_2$  as a timepoint [72]. Although TDM is widely accepted as critical for patient management, further studies are needed to clarify which clinically feasible time points give the most accurate assessment of drug exposure.

Although rejection rates are low with modern immunosuppressive regimens, graft rejection can still occur, even when whole blood tacrolimus and cyclosporine concentrations are inside proposed therapeutic ranges [72]. It may therefore be more beneficial to monitor drug levels at the site of action, either in the allograft tissue or within the lymphocyte itself, to better predict drug efficacy. Several studies in liver transplant recipients have found that hepatic tissue concentrations of cyclosporine [73] and tacrolimus [74,75] are significantly lower in patients who experience rejection. Associations have also been seen between variations in *ABCB1* and intracellular peripheral blood mononuclear cell (PBMC) concentrations of tacrolimus [76] and cyclosporine [77], as well as between intracellular lymphocyte or PBMC concentrations and risk of acute rejection [75,78]; PBMCs represent a blood compartment enriched with lymphocytes [75]. Further discussion of the relationship between *ABCB1* variants and lymphocyte intracellular concentrations can be found in the Pharmacogenetics section. One study in 9 kidney transplant patients prescribed cyclosporine found that intracellular T-lymphocyte  $AUC_{0-12}$  was 182% higher in the 5 patients who were rejection free, compared with those patients who experienced rejection [78]. When considering a larger group of 20 patients, the authors saw a significant decrease in T-lymphocyte intracellular concentrations 3 days before the rejection episodes occurred in the 7 patients who experienced biopsy-proven acute rejection. In contrast, no significant results were seen when considering any whole blood pharmacokinetic parameters, including dose,  $C_0$  or  $C_2$  values [78]. As cyclosporine uses these parameters in TDM [66], this finding is important in the context of drug monitoring. A study involving 90 liver transplant patients taking tacrolimus found concordant results: patients who experienced clinical rejection had intracellular tacrolimus trough concentrations that were 73, 77 and 76% lower on days 3, 5, and 7 post-transplant, respectively, compared with patients with no or mild rejection. As with cyclosporine, no association was seen between incidence of rejection and mean whole blood  $C_0$  over days 5–7 [75]. This is important to consider, as tacrolimus TDM uses  $C_0$  to

adjust dosage regimen [64]. Larger prospective trials are needed to further assess the clinical relevance of using intracellular lymphocyte concentrations as part of TDM [78].

A number of drugs have been reported to interact with tacrolimus and cyclosporine. Comprehensive lists can be found in reviews by Van Gelder [79], Christians *et al.* [80], and Campana *et al.* [81]. Drug interactions mainly occur when tacrolimus or cyclosporine is coadministered with either inhibitors or inducers of cytochrome P450 3A (CYP3A) or P-glycoprotein [80], two proteins that have significant overlap in substrate specificities [82].

In addition to genetic polymorphisms, a large variety of nongenetic factors have reported to influence the pharmacokinetics of tacrolimus and cyclosporine. These include transplant population studied, time since transplant, drug formulation given, patient hepatic function and liver allograft size, patient age, race and weight, patient hematocrit, albumin and lipoprotein levels, circadian rhythm, coadministration with food, diarrhea, and concomitant medications that induce or inhibit CYP3A or P-glycoprotein. Reviews by Staatz and Tett [24] and Lindholm [83] provide a comprehensive discussion of factors associated with variability in calcineurin inhibitor pharmacokinetics.

## Pharmacodynamics

A stylized depiction of the mechanism of action of tacrolimus and cyclosporine in lymphocytes as well as the candidate genes believed to interact with the two drugs is provided in Fig. 2. Tacrolimus and cyclosporine exert immunosuppression by several pathways, including inhibiting the calcineurin and the c-Jun N-terminal kinase (JNK) and p38 pathways, and inducing the increased expression of transforming growth factor  $\beta$  1 (TGF- $\beta$ 1). The majority of studies on the pharmacodynamic effects of tacrolimus and cyclosporine have focused on their effect within T cells. The involvement of natural killer (NK) cells in transplant rejection is not very well defined, however, both drugs have been found to inhibit natural killer cell degranulation in a dose-dependent manner [84].

### Action on calcineurin and NFAT

Upon entering T cells, both cyclosporine and tacrolimus bind with high affinity to proteins known as immunophilins. Cyclosporine binds mainly to cyclophilin A (encoded by the *PPIA* gene), the predominant cyclophilin found within T cells, whereas tacrolimus binds to FK-binding proteins, in particular FKBP12 (encoded by the *FKBP1A* gene). Both immunophilins interact with calcineurin in the absence of any ligands. However, the affinity of the immunophilin for calcineurin is enhanced upon binding of the drugs, resulting in the inhibition of the protein's activity [85]. Calcineurin is a calmodulin-independent phosphatase, which is stimulated during T cell activation by a chain of events involving calcium and calmodulin [86,87]. Once activated, it associates with and dephosphorylates members of the nuclear factor of activated T cells (NFAT) family, thereby activating these proteins [88,89]. Upon activation, NFAT proteins translocate to the nucleus [89], where they associate with other transcription factors, such as members of the activator protein-1 (AP-1) family, and bind to DNA to promote the transcription of interleukin (IL)-2 [90]. They also bind to promoter sites on a large variety of other cytokine genes, including those coding for IL-4, IL-10, and IL-17 [91]. Inhibition of calcineurin, therefore, prevents its ability to



dephosphorylate and activate NFAT, affecting the transcription of cytokines important in the immune response. The impact of the drugs on the transcription of IL-2 is probably the best addressed mechanism, and this particular cytokine plays a major role in the immune response, including the maintenance of regulatory T cells and the differentiation and survival of CD4+ and CD8+ T cells [92].

In addition to NFAT and AP-1 family members, nuclear factor  $\kappa$  light-chain enhancer of activated B cells (NF- $\kappa$ B) is also involved in the induction of IL-2 transcription [91,93,94]. NF- $\kappa$ B is the name given to a group of dimeric transcription factors that bind as homodimers or heterodimers, and exert both positive and negative effects on gene transcription [95]. In general, NF- $\kappa$ B has a large impact on the development, homeostasis, survival, and function of T cells [96]. It has a huge variety of target genes within T cells, and in addition to IL-2 is also involved in the regulation of cytokines such as tumor necrosis factor- $\beta$  [97] and interferon- $\gamma$  [98]. Calcineurin is also involved in the activation of NF- $\kappa$ B. It indirectly induces the degradation of a compound known as I $\kappa$ B, which is bound to inactive NF- $\kappa$ B and acts as an inhibitory protein, preventing NF- $\kappa$ B from associating with its nuclear target genes. The blockade of calcineurin activity by both drugs thereby affects the ability of NF- $\kappa$ B to exert its action on the genes of the immune system [96,99].

### Action on the JNK and p38 pathways

Though the effect of tacrolimus and cyclosporine on calcineurin is probably the best-studied mechanism, both drugs are also thought to be involved in the inhibition of the mitogen-activated protein kinase (MAPK) pathway. The MAPK pathway is a signaling cascade involved in a wide variety of processes, particularly in cells within the immune system [100]. It consists of three protein kinases: MAPK, MAPKK, and MAPKK-K. MAPKK-K phosphorylates and activates MAPKK, which in turn phosphorylates and activates MAPK [101]. There are three distinct MAPK subgroups – ERK, JNK, and p38 [100]. Both cyclosporine and tacrolimus (in complex with their immunophilins) have been shown to inhibit the JNK (MAPK8) and p38 (MAPK14) pathways, but not the ERK pathway. A study in Jurkat T lymphocytes showed reduced levels of both the JNK and p38 proteins under the administration of cyclosporine or tacrolimus [102]. JNK and p38 are activated through the MAPK signaling cascade by T cell and CD28 costimulatory receptors [103], and upon activation, translocate to the nucleus where they can fulfill their various roles [104]. This includes regulating the activity of AP-1 members [105], which are involved in promoting the transcription of IL-2 [90] and other cytokines [106]. Indeed, the blockade of the p38 and JNK pathways was shown to prevent the expression of the IL-2 gene [103]. The pathway of JNK and p38 activation through various kinases can be seen in Fig. 2, and the two drugs are thought to inhibit the pathways upstream of the MAPKKK level, as cyclosporine and tacrolimus have both been shown to inhibit the activation of an MAPKK-K known as MAP3K1 [102]. It is not believed that calcineurin is involved in this mechanism, as inhibitors of calcineurin have been seen to block the activation of NFAT, but not JNK or p38 pathways within T cells [102].



## Action on TGF- $\beta$ 1

TGF- $\beta$ 1 is a cytokine critical for the regulation of cells in the immune system. It is a member of the TGF- $\beta$  family, which also includes TGF- $\beta$ 2 and TGF- $\beta$ 3. TGF- $\beta$  has been shown to inhibit IL-2-dependent T cell proliferation [107], as well as exerting a variety of other immunosuppressive effects within T cells [108]. In-vivo studies in patients with end-stage renal disease undergoing transplantation have shown an increase in TGF- $\beta$ 1 protein and mRNA expression after treatment with cyclosporine [109], and in-vitro studies of tacrolimus in T cells also showed a significant increase in mRNA and protein levels after administration of the drug [110]. However, the mechanism by which these drugs affect levels of TGF- $\beta$ 1 remains to be elucidated. It is also important to note that some studies have found evidence showing that neither tacrolimus nor cyclosporine are capable of affecting TGF- $\beta$ 1 protein or mRNA levels at concentrations where IL-2 production is successfully inhibited [111,112]. Therefore, at this stage, it is not possible to state definitively whether the two drugs affect TGF- $\beta$ 1 levels at clinically relevant concentrations. However, it is important to note that along with being involved in the immune system, TGF- $\beta$ 1 also has fibrogenic properties that can lead to the development of nephrotoxicity [113]. A study in renal transplant patients found that expression of TGF- $\beta$ 1 (*TGFB1*) mRNA within kidney biopsies was increased in patients with either tacrolimus or cyclosporine nephrotoxicity, compared with those who exhibited acute rejection. This suggests that increased levels of the protein may lead to the nephrotoxicity often associated with these drugs [114].

## Pharmacogenetics

A summary of genetic variants that show associations with tacrolimus or cyclosporine pharmacogenetics, as well as their corresponding rsIDs and effects on the protein are shown in Table 2. The majority of pharmacogenetic studies on tacrolimus and cyclosporine have focused on the effects of variants in the *CYP3A4*, *CYP3A5*, and *ABCB1* genes because of the central role the enzymes and transporters they code for play in tacrolimus and cyclosporine disposition. However, a few studies have examined the influence of single nucleotide polymorphisms (SNPs) within the gene encoding the pregnane X receptor (*NR1I2*), which regulates the expression of multiple genes including *CYP3A* and *ABCB1* [127]. In addition, a couple of studies have examined SNPs in the *POR* gene, which encodes for CYP450 oxidoreductase, a protein responsible for transferring electrons from NADPH to CYP450 enzymes, enabling their activity [128]. Finally, several studies have looked at variations in the *TGFB1* gene and their associations with renal dysfunction.

Within this section, we highlight only genes coding for metabolic enzymes or direct drug targets. Two exceptions are the proteins encoded by cyclophilin A gene (*PPIA*), important in that it allows cyclosporine to inhibit calcineurin, and *CYP2C8*, whose importance will be explained further on. However, variations within cytokine genes also show associations with side effects and outcomes. These include *IL-6*, where GG homozygotes at position – 174 had an increased risk of new-onset diabetes after transplantation [129], *IL-12B*, where the C allele in rs3212227 conferred increased risk for cytomegalovirus infection with an odds ratio (OR) of 1.52 [130], and *IL-2*, where TT homozygotes at position 330 had a higher risk of developing chronic allograft nephropathy [131]. Patients in these studies were treated with

either tacrolimus or cyclosporine as part of their immunosuppressive regime. However, studies analyzing polymorphisms in cytokine genes and other innate immune response molecules have been conflicted. For a review of these studies, please see Goldfarb-Rumyantzev and Naiman [132].

## Influence on tacrolimus pharmacokinetics and pharmacodynamics

An overview of tacrolimus pharmacogenetic studies can be found in Table 3. This table provides information on the pharmacogenetic studies as they pertain to both pharmacokinetic and pharmacodynamic parameters. A detailed discussion of tacrolimus pharmacogenetics can be found below, segregated by gene.

### **CYP3A5 gene**

Variations in the *CYP3A5* gene have shown some of the firmest associations with tacrolimus pharmacokinetics. In particular, the rs776746 SNP in intron 3 of the gene has been found to be the strongest predictor of tacrolimus dosing requirements [133], and to explain up to 45% of the variability in dose [154] and 30% of the variability in oral clearance of tacrolimus [134]. Homozygosity for the G allele of this SNP (also referred to as *CYP3A5*\*3) is associated with a range of responses to tacrolimus, including increased dose-adjusted trough concentrations ( $C_0/D$ ), decreased dose requirements, and decreased oral clearance [135,136]. The \*3 allele affects CYP3A5 protein levels by creating a cryptic splice site within the intron, resulting in altered mRNA splicing and eventually leading to a premature stop codon and a nonfunctional protein. Patients homozygous for the \*3 allele are known as CYP3A5 nonexpressers [115]. This reduction in enzymatic activity leads to the reduced dose requirement – renal transplant patients who are CYP3A5 nonexpressers require lower mean daily doses by ~ 0.05 mg/kg [135]. This association between *CYP3A5* genotype and tacrolimus metabolism has been replicated many times in a variety of different studies: a recent systematic review of literature by the Royal Dutch Association for the Advancement of Pharmacy found a very high level of evidence to support the gene–drug interaction. However, they did not advise making dosing recommendations based on this genotype, as tacrolimus dose is changed based on TDM [155].

As tacrolimus is metabolized by both intestinal and hepatic CYP3A5 enzymes, the combined contribution of CYP3A5 expression in the native intestine and liver allograft is likely to influence the pharmacokinetics of tacrolimus in liver transplant recipients [34]. Studies to date examining the relative influence of donor and recipient *CYP3A5* rs776746 genotype in liver transplant patients have involved only small numbers of patients, and hence have been somewhat inconclusive. Several studies have considered the combination of donor and recipient *CYP3A5* genotype on the  $C_0/D$  of tacrolimus [156-158]. These studies segregated patients into four groups based on whether they were a CYP3A5 expresser (*CYP3A5*\*1/\*1 or \*1/\*3) or nonexpresser (*CYP3A5*\*3/\*3): recipient expresser and donor expresser ( $R_E/D_E$ ), recipient expresser and donor nonexpresser ( $R_E/D_N$ ), recipient nonexpresser and donor expresser ( $R_N/D_E$ ), or recipient nonexpresser and donor nonexpresser ( $R_N/D_N$ ). One study in 58 living Korean donors and liver transplant recipients showed that, shortly after transplant, the tacrolimus  $C_0/D$  ratio was significantly higher in  $R_N/D_E$  group members than  $R_E/D_N$  group members. However, over time, the tacrolimus

$C_0/D$  ratio in the  $R_N/D_E$  group decreased until, after 1 month post-transplant, the  $R_N/D_E$  and  $R_E/D_N$  groups had similar  $C_0/D$  values. The authors concluded that native intestinal genotype had the most significant influence on tacrolimus metabolism in the early stages after transplantation, but after enough time had passed for the transplanted liver to enlarge in mass and regenerate from ischemia reperfusion injury, donor liver genotype became increasingly important [156]. A second study involving 51 Japanese living liver donors and recipients reported supporting results – patients with the  $R_N/D_N$  and  $R_N/D_E$  combination genotypes displayed similar  $C_0/D$  values at weeks 2–4 and months 2–6 after transplant. However, at months 8, 10, and 12 posttransplant, values across the groups began to significantly differ, with  $R_N/D_N$  patients showing a  $C_0/D$  ratio 125% higher than that of  $R_N/D_E$  patients. In contrast, differences between  $R_N/D_N$  and  $R_E/D_N$  genotype groups were significant in the early post-transplant period, but only up to 6 months after transplant. These two sets of results suggest that the donor expresser genotype has a greater influence on the metabolism of tacrolimus, but only after 6 months. Before this time, the impact of recipient genotype is more significant [157]. In contrast, Yu *et al.* [158], in their study involving 53 cadaveric transplant patients, reported that only  $R_N/D_N$  and  $R_E/D_E$  genotype groups displayed significant differences in tacrolimus  $C_0/D$  ratio, and only at 2 weeks and 1 month post-transplant. Results at 1 week post-transplant were nonsignificant, and no other time points were measured.

A number of studies have also examined the effect of donor and recipient genotype without considering combinational influence. A study involving 50 Chinese cadaveric liver recipients reported that donor *CYP3A5* nonexpressers had significantly higher  $C_0/D$  ratios at 2 weeks and 1 month post-transplant, as compared with expressers. However, no significant difference was seen at 1 week. In contrast, recipient *CYP3A5* genotype had no effect on  $C_0/D$  at any of the time points studied [159]. The previously mentioned study by Yu *et al.* [158] also found the same results in their cohort of cadaveric liver transplant recipients, when donor and recipient were considered separately. Two other studies in 60 living donor [160] and 70 unspecified living or cadaveric donor [161] transplant patients, however, found that donor genotype did significantly affect  $C_0/D$  at 1 week, as well as weeks 2 and 3 for one study [161], and months 1–12 for another study [160]. In addition, while the study in 60 patients found that recipient genotype was only significant at weeks 1 and 2 [160], the study in 70 patients saw that recipient genotype had no effect on  $C_0/D$  at any time [161].

Although it appears that donor genotype does play a significant role in tacrolimus pharmacokinetics, it is unclear at what point it becomes relevant. Although several studies show that donor genotype significantly alters  $C_0/D$  values from the first week, others show that it doesn't begin playing a role until the second week or even the sixth month post-transplant. In addition, evidence is conflicted for the role of the intestinal, or recipient, genotype: a few studies show it is never significant, whereas others show it is significant only up to the timepoint at which donor genotype becomes significant. Studies considering donor genotype in liver transplant patients are not included in Table 3. This is because of their limited number relative to those which included renal transplant patients, and the complex combinatory effects of donor liver and recipient intestinal genotypes on pharmacokinetic parameters.

Though the Royal Dutch Association for the Advancement of Pharmacy does not recommend adjusting dosing based on genotype [155], recipient or donor, a 2011 multicenter study on renal transplant patients found that dosing based on recipient *CYP3A5*\*3 genotype was beneficial: patients whose dosage was adapted based on genotype had tacrolimus trough blood concentrations in the target range more frequently than those on the standard regimen. In addition, patients on the genotype-adapted dosage required less time to reach the target range and had a lower number of dose modifications. Nevertheless, the improved dosing accuracy did not result in any positive clinical endpoints; there was no difference in occurrences of acute rejection, nephrotoxicity or delayed graft function between those on the adapted dose and those on the standard regimen. Therefore, it is still uncertain whether taking tacrolimus pharmacogenetics into account when dosing would be clinically relevant [162]. Indeed, there is a lack of consistent evidence for organ rejection as a result of genotype-related under-immunosuppression, likely because careful tacrolimus dose adjustments are performed in the early phase after transplantation in response to measured trough concentrations [26]. However, several studies in renal transplant patients, including one relatively large study with 304 participants, have shown an association between the *CYP3A5*\*3 allele and a decreased risk of nephrotoxicity [137,141,163]. One of these studies, by Glowacki *et al.* [137], considered donor kidney genotype, but only found significant results for nephrotoxicity when considering recipient genotype. The mechanism behind this pharmacodynamic effect is unclear, but could stem from a lower systemic exposure to the drug because of lower dose requirements [141], as well as potentially a reduction in renal metabolite formation [163]. *CYP3A5* is primarily responsible for transforming tacrolimus into its metabolites, and in-vitro experiments have shown that formation of the 13-O-demethyl-tacrolimus metabolite was 13.5-fold higher in human kidney microsomes with the *CYP3A5*\*1/\*3 genotype, than those with the *CYP3A5*\*3/\*3 genotype [37]. It is possible that an increased amount of these metabolites in renal cells could induce or aggravate nephrotoxicity [163]. Decreased levels of the fibrogenic TGF- $\beta$ 1 resulting from lower drug doses could also lead to a reduced incidence of nephrotoxicity. In contrast, a smaller study in renal transplant patients found that *CYP3A5*\*3 homozygotes have an increased incidence of nephrotoxicity [137]. In addition, a study within liver transplant patients also found that recipient *CYP3A5* \*3/\*3 genotype was associated with an increased risk of nephrotoxicity. No significant results were seen when considering donor genotype. The authors suggested active *CYP3A5* in the kidney may help reduce exposure of renal cells to tacrolimus, thereby exerting a protective role [160]. Given these conflicting results, at this time it is difficult to pinpoint any particular pharmacodynamic role for *CYP3A5*.

Other *CYP3A5* SNPs have also been found to affect tacrolimus pharmacokinetics, including rs10264272 (*CYP3A5*\*6) and rs41303343 (*CYP3A5*\*7), which both lead to nonfunctional proteins [116]. These are rare or absent in Asian or Caucasian populations, but are found commonly in those of African descent. A study in Brazilian individuals (who tend to have a significant amount of African ancestry) found that genotyping both of these alleles, along with *CYP3A5*\*3, is critical for determining the activity level of the *CYP3A5* enzyme, and therefore the appropriate dose of tacrolimus for those of African descent. Results showed that as the number of defective alleles decreased from two to one to zero, the tacrolimus

dose-adjusted trough concentration also decreased, which is consistent with the inferred activity phenotype of these alleles. No such association was seen for cyclosporine [116], and no associations have been seen between these SNPs and any pharmacodynamic parameters.

### **CYP3A4 gene**

Within *CYP3A4*, rs2740574 (*CYP3A4\*1B*), and rs35599367 (*CYP3A4\*22*) have both shown associations with tacrolimus dose requirements. rs2740574 is a promoter variant, and is known to increase gene transcription [117]. Carriers of the *\*1B* allele have been seen to have 35% lower tacrolimus dose-adjusted trough concentrations compared with those homozygous for the normal *\*1* allele [164]. However, rs2740574 was shown to be in linkage disequilibrium (LD) with rs776746 within the *CYP3A5* gene, so its effect on tacrolimus dosage requirements (as well as any pharmacodynamic parameters) is likely mediated by this SNP [165]. A small number of haplotype studies looking at combinations of the *CYP3A4\*1B* and *CYP3A5\*3* alleles have found significant associations with tacrolimus pharmacokinetics [163,166]. Though given the strong evidence supporting the role of *CYP3A5\*3* in tacrolimus pharmacokinetics [155], and the LD between these two alleles [165], any effect of this haplotype may be due to the *CYP3A5\*3* allele. rs35599367 is present in intron 6 of the *CYP3A4* gene, and results from a C to T substitution. *\*22* allele carriers require a mean daily dose of tacrolimus 33% lower than wild-type homozygotes to reach the same predose tacrolimus blood concentration [143]. *\*22* carriers also have reduced mRNA and enzyme activity levels compared with wildtype homozygotes [119]. Unlike *CYP3A4\*1B*, rs35599367 is not in LD with rs776746, and it partially contributes to the variation in tacrolimus dose requirement independently of the *CYP3A5\*3* allele [144]. *CYP3A4\*18* is another allele recently discovered to affect tacrolimus pharmacokinetics. Also known as rs28371759, the SNP is found in intron 10 of the *CYP3A4* gene, is characterized by a G to A substitution, and is suggested to increase CYP3A4 activity [118]. Wild-type homozygotes for this allele had a reduced apparent clearance of tacrolimus compared with carriers of the mutant allele [138]. However, like rs2740574, this allele is also in LD with rs776746 [167]. There is very little evidence to support an influence of *CYP3A4* gene SNPs on pharmacodynamic outcomes relevant to tacrolimus – associations between *CYP3A4\*1B* have so far been exclusively negative [26]. To the best of our knowledge, no association studies between the *CYP3A4\*18* or the *\*22* alleles, and the pharmacodynamics of tacrolimus, have been completed at this time.

### **ABCB1 gene**

Associations between tacrolimus pharmacokinetics and variations in *ABCB1* have been variable. A recent systematic review of available literature found no consistent evidence for an association between tacrolimus dose-adjusted trough concentrations and rs1045642 (3435C>T), a well-studied SNP within the gene [139]. rs1045642 had been of particular interest, as it was shown to reduce intestinal P-glycoprotein expression and function, and therefore had the potential to affect drug bioavailability [120]. Evidence supporting the involvement of other *ABCB1* SNPs in tacrolimus dose-adjusted exposure has also been inconsistent. A large number of studies have also failed to find any association between rs2032582 (2677G>T/A) and rs1128503 (1236C>T), two other commonly studied SNPs, and tacrolimus pharmacokinetics [25]. However, one retrospective study of 81 renal

transplant patients found that T allele homozygotes in both SNPs had higher dose-adjusted trough concentrations compared with wild-type homozygotes 1 month after tacrolimus introduction. T allele homozygotes for rs2032582 had 55% higher dose-adjusted trough concentrations; T allele homozygotes for rs1128503 had 45% higher dose-adjusted trough concentrations [168]. Another study in 83 lung transplant patients found that T allele carriers for rs2032582 also had higher dose-adjusted trough concentrations as compared with GG homozygotes, but only in the first month after transplant [169]. Haplotype analyses using these three *ABCB1* alleles have also been conducted: the same study of 81 renal transplant patients found that those with the C-G-C haplotype for the SNPs rs1045642, rs2032582 and rs1128503, respectively, required higher daily doses of tacrolimus compared with those with the T-T-T haplotype [168]. Studies suggest that these three alleles are in LD to some extent [61,170,171], hence it is uncertain whether only one of these three alleles is driving associations with pharmacokinetic or pharmacodynamics parameters for tacrolimus or cyclosporine. rs2032582 and rs1045642 in particular have shown strong LD, with  $r^2$  [170] or  $D'$  [61] values above 0.8. In addition, rs2032582 is a nonsynonymous SNP, whereas the remaining two SNPs are synonymous, suggesting that it may be the main effector behind any influence on the gene [171]. Indeed, a pharmacodynamic study, discussed later in this paper, found that rs2032582 was the driving force behind an association of an *ABCB1* haplotype with acute rejection in cyclosporine-treated patients [170]. Despite this, only rs1045642 has been shown to affect *ABCB1* expression [120]. A number of other studies have found no relationship with *ABCB1* haplotype combinations, including one with 206 renal recipients, in which haplotype associations were not significant after *CYP3A5* status was taken into account [172]. This suggests that, with or without LD, these alleles likely play a minor role in affecting tacrolimus pharmacokinetics compared with the effect of *CYP3A5* alleles [172].

However, it is possible that *ABCB1* alleles may play a stronger role in affecting intracellular concentrations of tacrolimus within lymphocytes. A 2010 study by Capron *et al.* [76] in 96 renal transplant patients found that carriers of the rs1045642 T allele had a 1.3-fold increase in intracellular PBMC tacrolimus trough concentrations as compared with noncarriers. This finding agrees with the assumed effect of the T allele, which is a reduction in P-glycoprotein expression and function [120]. The authors also found that carriers of the *ABCB1* rs2032582 T/A allele or the rs2229109 A allele showed significantly increased PBMC concentrations compared with noncarriers, by 1.3- and 1.4-fold, respectively [76]. rs2229109 represents a G to A change at position 1199 in the *ABCB1* gene. It is not as well studied as rs1045642 or rs2032582, and has varied and drug-specific effects on efflux [173-175]. In this case, as with rs1045642 and rs2032582, it appears to reduce activity toward tacrolimus. It should be noted that only rs1045642 and rs2229109 remained significantly associated with intracellular concentrations once multivariate analyses were used; the initial association of rs2032582 was suggested to be because of its LD with rs1045642. None of these three alleles was associated with tacrolimus blood concentrations, leading the authors to conclude that *ABCB1* polymorphisms may have a greater influence on intracellular concentrations than on blood concentrations. Indeed, the authors also noted that intracellular PBMC concentrations did not correlate significantly with blood concentrations, demonstrating that blood concentration may not accurately reflect the level of tacrolimus capable of



immunosuppression within lymphocytes. Unfortunately, though links have been shown between intracellular concentrations and rejection, the authors could find no association between these particular *ABCB1* polymorphisms and rejection in the population. This may be because of the low incidence of rejection episodes in the group, only 6% [76]. As intracellular concentrations have been shown to be significant predictors of acute rejection, understanding more about the role of *ABCB1* variants in affecting lymphocyte concentrations could potentially help reduce risk of allograft rejection in the future. This study is not included within Table 3.

Though the study by Capron *et al.* [76] found no association between these three *ABCB1* variants and rejection, the TT genotypes for SNPs rs1045642, rs2032582, and rs1128503 have all been associated with higher success rates in achieving short-term remission of ulcerative colitis (UC) as compared with the other genotypes [145]. Along with drugs such as infliximab, calcineurin inhibitors are also used to treat steroidrefractory UC [176]. A total of 84 patients prescribed tacrolimus participated in this study, and the ORs for achieving short-term remission with the TT genotype were 2.16, 1.59, and 1.74, respectively, for each *ABCB1* SNP. In addition, these effects were seen after correction for age, sex and tacrolimus dose-adjusted trough levels, among several other nongenetic factors [145]. As rs1045642 is thought to affect intestinal P-glycoprotein expression [120], it was suggested that the local intestinal action of tacrolimus on UC might be why an association existed in this particular study, but not in pharmacokinetic or clinical response studies in transplant patients [145]. Associations between *ABCB1* alleles and other pharmacodynamic effects have been inconsistent, and a large number of studies have failed to show any relationship between rs1045642, rs2032582, and rs1128503 and pharmacodynamic parameters such as graft loss or acute rejection (summarized in Staatz *et al.* [26]). Nevertheless, a few studies have found associations between these SNPs and clinical outcomes. A study in 117 lung transplant recipients found that rs1045642 TT homozygotes had a lower incidence of acute rejection by 25% [177]. In addition, a study with 120 liver recipients found that the rs2032582 T allele was associated with a lower risk of chronic renal dysfunction – TT homozygotes had an OR of 0.26 when compared against all other genotype combinations for this SNP (i.e. GG, GT, AG, AT) [178]. The T-G-C haplotype (in comparison with the wild-type C-G-C haplotype) for the three main SNPs (respectively, rs1045642, rs2032582, and rs1128503) was found to significantly increase the risk for chronic irreversible drug-induced nephrotoxicity (CNIT) by an OR of 4.7 in 103 renal transplant patients. It was suggested that, as the presence of the rs1045642 T allele reduces the efflux capacity of P-glycoprotein, it might lead to an accumulation of the drug inside the cells [146]. This same haplotype combination was also associated with a 1.4-fold increased risk for acute rejection, as compared with the wild type or T-T-T variant haplotypes in a study of 832 renal transplant patients taking either tacrolimus or cyclosporine [166]. This result is inconsistent with the previously mentioned study, in which the T allele from rs1045642 was associated with a lower rate of acute rejection [177]. Results for neurotoxic associations also remain conflicted: wild-type homozygotes for both rs2032582 and rs1128503 were found to have an increased risk for neurotoxic events when taking tacrolimus, with an OR of ~ 3 in both cases [146]. However, two other earlier studies found the opposite effect, in that the presence of the mutant T allele in rs2032582 [179] or rs1128503 [180] was associated with a higher risk of neurotoxicity. It



is important to note though, that within the neurotoxicity studies, the first study included 103 patients, whereas the latter two studies only included 17 and 63 patients, respectively. In addition, each study differed in type of transplant patients, with patients undergoing renal, liver, and hematopoietic stem cell transplants, respectively, making it hard to fairly compare the results. Overall, however, studies relating *ABCB1* alleles with tacrolimus pharmacokinetics are generally inconsistent, and as there is a lack of mechanistic evidence to back up any associations, it is difficult to ascertain which sets of results are valid.

### ***POR* and *NR1I2* genes**

Variants in *POR* and *NR1I2* have also been found to affect tacrolimus pharmacokinetics. A recent study in 158 adult kidney transplant patients showed that carriers of the 8055T allele in the *NR1I2* gene had a 33% higher dose-adjusted exposure ( $AUC_{12}/\text{dose}$ ) to tacrolimus compared with wild-type individuals [127]. *NR1I2* is responsible for the upstream regulation of drug-metabolizing enzymes and transporters, including the CYP3A family and *ABCB1*. However, the mechanism behind the influence of the 8055T allele is still unclear [127]. P450 oxidoreductase (*POR*) is responsible for enabling the activity of cytochrome p450 enzymes [128]. Patients carrying at least one T allele of the rs1057868 SNP (also known as *POR*\*28) were found to have significantly lower tacrolimus trough concentrations within the first few days after transplant, and require higher doses to maintain target concentrations over the first year after surgery, as compared with wild-type homozygotes. However, this effect was only seen in patients who were CYP3A5 expressers (i.e. *CYP3A5*\*1 allele carriers). It is thought that this SNP might modify the *POR*–cytochrome interaction, affecting the activity of CYP enzymes [121]. Indeed, in-vivo studies with the drug midazolam have shown that the \*28/\*28 genotype is associated with increased CYP3A activity [181]. It is possible that the same effect may occur with tacrolimus, which would lead to an increase in daily dose requirement for patients with that genotype [121]. Another study showed supporting results, finding that patients heterozygous for the \*28 allele had 35% lower levels of tacrolimus exposure ( $AUC_{24}$ ) compared with wild-type homozygotes. This effect was also only significant in CYP3A5 expressers [147]. As of yet, no associations have been found between variants in *POR* and pharmacodynamic effects. However, some associations have been seen for the *NR1I2* 8055T variant. In the study of 158 adult renal transplant patients (discussed above in regard to pharmacokinetic parameters), those in possession of the T allele had a greater incidence of BK viremia during the first year of treatment with tacrolimus compared with wild-type individuals, suggesting possible over-immunosuppression. These T allele patients also carried a higher risk of BK viremia, with an OR of 2.76, suggesting possible clinical relevance for the increase in dose-adjusted exposure seen with this variant [127]. However, it is possible that the influence of the T allele on BK viremia incidence occurred through a different mechanism than altered immunosuppressant levels within the body, given that tacrolimus exposure in transplant patients is controlled by TDM [127]. One possible mechanistic explanation is that, as *NR1I2* regulates P-glycoprotein [182], it could alter the P-glycoprotein-mediated export of tacrolimus out of lymphocytes [127].

### ***TGFB1* gene**

Studies on *TGFB1* have focused exclusively on renal side effects and outcomes. Patients receiving tacrolimus and cyclosporine were combined in all but one of these studies examined, so the discussion here will pertain to both tacrolimus and cyclosporine, and will not be repeated in the cyclosporine section. The summary of tacrolimus pharmacogenetic studies in Table 3 does not include data from the study with patients exclusively taking cyclosporine [148]. The TGF- $\beta$ 1 protein is known to be associated with the development of calcineurin-inhibitor nephrotoxicity [113], and is a potential target gene for tacrolimus and cyclosporine [109,110]. All studies analyzing the relationship between renal dysfunction and this gene have focused on two alleles: rs1800470 and rs1800471, and studies are inconsistent about the effect of these alleles on clinical outcomes. CC homozygotes of the former have increased serum levels of the protein [125], but the effect of the latter allele is still unknown. Several studies, including one in 53 and another in 158 heart transplant patients, have seen no associations between these alleles and renal dysfunction [151,152]. Another in 168 heart transplant patients taking only cyclosporine saw an association only for rs1800470, where C allele carriers had a higher incidence of renal dysfunction [148]. The largest study done so far in heart patients, with 237 members, saw concordant results: C allele carriers for both variants had a higher risk of end-stage renal failure, with relative risks of 2.9 and 2.6, respectively, for rs1800470 and rs1800471 [149]. However, a slightly smaller study in 175 heart transplant patients found inconsistent results: CC homozygotes for rs1800470 and C allele carriers for rs1800471, had improved progression of renal insufficiency compared with the other genotypes [150]. Finally, one very large study with 4199 renal transplant patients found that neither *TGFB1* variant was associated with graft survival [153]. It is important to note that within this study peripheral blood lymphocytes from the recipients were used for genotyping [153], therefore the negative result could be explained by the fact that the donor genotype is more important than the recipient genotype. However, this theory does not explain the discrepancies seen in the heart transplant patient studies.

### **Other potentially important genes**

Two other cytochrome p450 family members, CYP2C8 and CYP2J2, have also been analyzed for relationships with tacrolimus-related clinical outcomes. Alleles in *CYP2J2* have not been associated with any pharmacodynamic factors to date [122,146]. However, the *CYP2C8*\*3 allele was associated with a higher risk of kidney dysfunction post-transplantation, with an OR of almost 2.5 for those carrying at least one \*3 allele. Patients in this study had been treated with tacrolimus for at least 3 years, and this relationship was not seen for cyclosporine [122]. A later study in renal transplant patients receiving tacrolimus found that \*3 homozygotes also had a higher incidence of delayed graft function compared with \*1 allele carriers. No relationship was seen between these alleles and acute rejection or neurotoxicity [146]. Both of these enzymes are involved in the metabolism of arachidonic acids (AAs) into epoxyeicosatrienoic acids (EETs), metabolites implicated in preventing hypertension and maintaining normal renal function. Calcineurin inhibitors are believed to influence the production of these AAs, indicating the potential importance of pharmacogenetic studies on this gene. In-vitro studies showed that the enzyme products

from the *CYP2C8*\*3 genes were deficient in the epoxidation of AAs into EETs [122]. This reduced transformation would lead to lower amounts of the protective EETs, which might explain the higher incidence of delayed graft function and kidney dysfunction in the \*3 carriers [146].

## Influence on cyclosporine pharmacokinetics and pharmacodynamics

An overview of cyclosporine pharmacogenetic studies can be found in Table 4. This table provides information on pharmacogenetic studies as they pertain to both pharmacokinetic and pharmacodynamic parameters. A detailed discussion of cyclosporine pharmacogenetics can be found below, segregated into pharmacokinetic and pharmacodynamic sections.

### *CYP3A5*, *CYP3A4*, *ABCB1*, and pharmacokinetics

The impact of variants within *CYP3A5*, *CYP3A4*, and *ABCB1* on cyclosporine pharmacokinetics is controversial [25,186]. Studies have shown highly mixed results in finding an association between the *CYP3A5*\*3 and cyclosporine pharmacokinetics. Large studies, such as one in 171 renal transplant patients [186] and another in 151 heart and renal patients [193], have found no associations between this allele and cyclosporine pharmacokinetics. However, a study in 110 renal recipients found that *CYP3A5*\*3 homozygotes have higher dose-adjusted trough concentrations than heterozygotes [164], and another a study in 91 bone marrow recipients reported that \*3 homozygotes had greater dose-adjusted trough concentrations on days 1–10 of treatment, and greater dose requirements on days 16–30, as compared with \*1 homozygotes [183]. This type of inconsistency is common, and does not appear to be influenced by race. Indeed, three different studies, all with around 100 Chinese renal recipients, found that, respectively, the \*3 allele was associated with higher dose-adjusted trough concentrations, lower dose-adjusted trough concentrations, and not associated with trough concentrations at all [194–196], exemplifying the type of conflicting evidence seen in these pharmacokinetic studies. Indeed, the study finding lower dose-adjusted trough concentrations [196] is particularly confusing, as the *CYP3A5*\*3 allele results in a nonfunctional protein [115], implying that dose-adjusted trough concentrations should be higher in \*3 allele carriers.

Similar to tacrolimus, the influence of SNPs rs1045642, rs2032582, and rs1128503 within *ABCB1* are also unclear, and the majority of studies show no significant associations with pharmacokinetic parameters [25]. Indeed, a meta-analysis of 1036 individuals showed no influence of the rs1045642 SNP on any cyclosporine pharmacokinetic parameters except increased AUC<sub>12</sub> for T allele carriers [197]. A study in 106 renal transplant patients found that the rs1128503 T allele was associated with increased dose-adjusted maximum blood concentrations [198], but most of the other studies on rs1128503, as well as rs2032582, have found no relationships [25]. Most studies, including one with 407 renal transplant patients [166], also do not find any significant associations between *ABCB1* haplotypes and cyclosporine pharmacokinetics. Given this large number of negative results, it is probable that SNPs within *ABCB1* explain only a small amount of the variation in cyclosporine pharmacokinetics, if any.

However, as with tacrolimus and *ABCB1* SNPs, a stronger association may be seen when intracellular, as opposed to whole blood, concentrations of cyclosporine are considered. A study by Crettol *et al.* [77] in 64 renal, liver, and lung transplant patients found that carriers of the rs1045642 T allele had 1.7-fold higher intracellular PBMC cyclosporine trough concentrations compared with noncarriers. These results are in concordance with the study by Capron *et al.* [76], who found a 1.3-fold increase in intracellular PBMC tacrolimus concentrations for carriers of the T allele. However, unlike the study in tacrolimus, Crettol *et al.* [77] found that the T allele was also associated with trough blood concentrations, with a 1.2-fold increase for carriers compared with noncarriers. No significant results were seen for rs2032582 or rs1128503, the other two commonly studied *ABCB1* SNPs [77]. Capron *et al.* [76] did not study rs1128503, but found that rs2032582 was not significant in multivariate analyses. However, results were different when considering the rs2229109 A allele: while Crettol *et al.* [77] saw no association when considering blood concentrations, which was the same result seen by Capron *et al.* [76] the authors also found that carriers of this allele had 1.8-fold decreased intracellular concentrations compared with noncarriers. This suggests that the A allele results in increased P-glycoprotein activity toward cyclosporine, thereby reducing intracellular concentrations compared with noncarriers [77]. Capron *et al.* [76], in contrast, found a 1.4-fold increase in PBMC concentrations for A allele carriers. Capron *et al.* [76] suggested that this difference was due the structural dissimilarities between the two drugs, which may affect the way they interact with the P-glycoprotein binding site. As rs2229109 is situated close to the domain involved in substrate binding, changes in this SNP could affect binding affinities for tacrolimus and cyclosporine differently. All patients that participated in the study by Crettol *et al.* [77] were stable, and as no evidence of rejection was reported, no links between *ABCB1* variants and rejection were established. Similarly to Capron *et al.* [76] the authors reported that cyclosporine blood concentrations correlated only moderately with intracellular concentrations ( $r^2=0.30$ ) [77], again showing the potential importance of considering intracellular lymphocyte concentrations in addition to blood concentrations. The study by Crettol *et al.* [77] is not included within Table 4.

As CYP3A4 is the predominant enzyme involved in metabolizing cyclosporine [39], it would be expected to show strong associations with cyclosporine pharmacokinetics, particularly in comparison with CYP3A5. Despite this, evidence for an association between *CYP3A4\*1B* alleles and cyclosporine pharmacokinetics has been inconsistent, with several studies showing no significant relationships [164,186,199,200]. Studies that have seen associations include one with 151 heart and renal transplant patients [193], which reported an increased clearance for *\*1B* allele carriers, and one in 100 renal recipients, which showed an increased mean dose requirement for *\*1B/\*1* patients as compared with *\*1* homozygotes by ~ 200mg/day [184]. These results are both consistent with the theory that the *\*1B* allele may increase gene transcription [117]. A very limited amount of work has been done analyzing the relationship between *CYP3A5\*3* and *CYP3A4\*1B* haplotypes and cyclosporine pharmacokinetics, and only nonsignificant results have been seen [25]. Though studies of the *CYP3A4\*1B* allele have had conflicting results, several studies have recently reported associations between the *CYP3A4\*18* allele and cyclosporine pharmacokinetics. Patients homozygous for the wild-type *\*1* allele had 40% higher dose-adjusted trough

concentrations ( $C_0$ /dose) of cyclosporine on days 16–30 of treatment compared with  $*18$  homozygotes. For 2-h postdose-adjusted concentrations ( $C_2$ /dose), the wild-type homozygotes had concentrations 35% higher on days 16–30, and 19% higher on days 8–15 [167]. A different study found supporting results:  $*18$  homozygotes had a 50% reduction in 2-h postdose concentrations compared with the other genotypes [189]. These concordant results suggest that, indeed, the  $*18$  allele may increase enzymatic activity, thereby reducing levels of the drug in the body [189]. However, it is important to note that a strong LD ( $D' = 0.88$ ) between this allele and the  $CYP3A5*3$  allele was observed [167]. Associations have also been seen between rs35599367 ( $CYP3A4*22$ ) and cyclosporine pharmacokinetics –  $*22$  carriers had dose-adjusted trough concentrations 1.6-fold higher than  $*1/*1$  homozygotes [144], a result that is consistent with the finding that  $*22$  carriers have reduced mRNA levels and enzyme activity [119].

### ***CYP3A5*, *CYP3A4*, *ABCB1*, and cyclosporine pharmacodynamics**

Studies analyzing the association between  $CYP3A5*3$ ,  $CYP3A4*1B$ , and *ABCB1* alleles and cyclosporine pharmacodynamics have shown mixed results. Only one study, albeit exceptional in its relative size (399 German renal recipients), found a significant association between the  $CYP3A5*3/*3$  genotype and a pharmacodynamic parameter: decreased patient survival compared with  $*1$  allele carriers [185]. The remaining studies have all seen negative results [26,186].

A very limited number of studies have analyzed the effect of the  $CYP3A4*1B$  alleles on pharmacodynamic parameters, and no relationships have been found at this point [26,186]. In addition, only one study has analyzed the combined haplotypic influence of  $CYP3A4*1B$  with *ABCB1* rs1045642, and saw no significant results [199]. No studies have looked at the combined haplotype influence of  $CYP3A4*1B$  and  $CYP3A5*3$  alleles on cyclosporine pharmacodynamics. A recent study on the effect of rs35599367 ( $CYP3A4*22$ ) on renal transplant patients found that the  $*22$  allele was associated with a higher risk of delayed graft function compared with  $*1$  homozygotes. Indeed, the OR was 6.3 after adjustment for other factors such as age, sex or primary kidney disease [190].

A relatively large study that included 237 renal transplant patients found that the rs2032582 TT genotype in *ABCB1* was associated with a three-fold higher risk of biopsy-proven acute rejection compared with the other genotypes [170]. This SNP was found to be in high LD with rs1045642 and rs1128503, and these two SNPs also showed association with acute rejection, but only before adjustment for the rs2032582 SNP [170]. In addition, the same authors found that the T-T-T haplotype for these SNPs (rs1045642, rs2032582, and rs1128503, respectively) was associated with a two-fold increased risk for rejection compared with the wild-type C-G-C haplotype [170]. Confusingly, the study in 832 renal transplant patients treated with tacrolimus or cyclosporine found that the T-T-T haplotype is associated with a lower risk of acute rejection, except in this case it was compared against the T-G-C haplotype [166]. A different study in 68 renal transplant recipients also found an association between the TT genotype and an increased risk of nephrotoxicity [191]. Various other smaller studies have found no association between *ABCB1* variants and clinical outcomes (summarized in Staatz *et al.* [26]).

However, several studies have shown the importance of considering donor *ABCB1* genotype when considering pharmacodynamic outcomes such as nephrotoxicity or rejection within renal transplant patients. The study of *ABCB1* variants in particular is important as P-glycoprotein is highly expressed in renal proximal tubule epithelial cells, so activity in the donor kidney could affect the development of cyclosporine-related adverse events [201]. Although variations in *CYP3A5* or *CYP3A4* genotypes in donor kidneys could also conceivably affect levels of cyclosporine within renal cells, neither *CYP3A5\*3* or *CYP3A4\*1B* variants have shown significant associations with graft loss [187,201], or any other adverse events of which we are aware at this time. However, a study of 97 renal transplant donors and recipients of White ethnicity found that donor *ABCB1* rs1045642 TT genotype was highly predictive of nephrotoxicity – 40% of patients with this donor genotype developed cyclosporine nephrotoxicity within two and a half years post-transplant, compared with only 10% of those with the CT or CC donor genotypes. In multivariate analysis, the TT genotype gave an OR of 13.4 for development of nephrotoxicity. No significant association was seen for the rs2032582 SNP and nephrotoxicity [188]. Another study in 259 renal transplant patients of unspecified ethnicity found similar results – the homozygotes for the *ABCB1* T-T-T haplotype (rs1045642, rs2032582, and rs1128503) had a hazard ratio of 9.4 for development of graft loss, as compared with all other haplotype combinations for these SNPs. Each TT genotype for rs1045642, rs2032582, rs1128503 was also associated with graft loss as compared with the wild-type homozygotes, but in multivariate analysis, only rs2032582 remained significant; patients with the TT genotype had a hazard ratio of 12.1 for graft loss, as compared with the wild-type homozygotes [201]. These two findings further exemplify the conflict as to whether rs1045642 or rs2032582 is driving associations between *ABCB1* and various drug-related pharmacokinetic and pharmacodynamic parameters. Despite these two sets of strong, albeit somewhat conflicted, results, a recent study by Moore *et al.* [187] using a larger number of patients (670 in the discovery cohort, 675 in a validation cohort) found entirely opposing results: patients with a donor kidney rs1045642 CC genotype had an increased risk of long-term graft failure, as compared against the CT or TT genotypes, with a hazard ratio of 1.7. One hundred percent of patients were given cyclosporine in the discovery cohort, whereas 82.5% were given cyclosporine in the validation cohort (the remaining were given tacrolimus). They were also uniformly of White ethnicity. Another validation cohort in 2985 patients found no significant association with donor genotype, which Moore *et al.* [187] suggested may be due to the heterogeneity of the population – though patients were again all of White background, they came from the Collaborative Transplant Study population, which includes participants from multiple countries and transplant centers, in contrast to the single-center populations used for the discovery and first replication cohort. These varied countries and centers may all use different treatment approaches and algorithms, which could affect the data analysis. No significant association was seen between the other two commonly studied SNPs, rs2032582 or rs1128503. Moore *et al.* [187] suggested that the conflicting results seen with previous studies of adverse outcomes could be because of population size or patient characteristics, as well as the endpoints evaluated. The authors also evaluated donor and recipient genotypes for *CYP3A4*, *CYP3A5*, *NR1I2*, and *PPIA* in the discovery cohort only, but found no significant associations with allograft survival. Neither this study by Moore and colleagues nor the study with 97 patients saw significant associations with recipient



genotypes [187,188]; the study with 259 patients did not analyze recipient genotype [201]. Though significant results have been seen when considering *ABCB1* donor genotype, studies in this area are currently limited and highly conflicted. Information on donor genotype results for these three studies is not included in Table 4. However, given that a number of studies have analyzed pharmacodynamic outcomes in kidney transplant patients and considered only recipient genotype [126,153,186,190,191] information on recipient genotype from these donor-focused studies is included in Table 4. Very limited research has been done investigating whether patients on tacrolimus therapy show the same relationship between donor kidney genotype and renal-related adverse events: one study showed that combined donor and recipient *ABCB1* rs1045642 TT genotypes was associated with an increased risk of chronic allograft damage, with an OR of 3.9 when compared against all other genotypes. The authors suggested that this could be caused by an accumulation of tacrolimus within renal cells because of reduced function of this particular form of P-glycoprotein. Recipient and donor rs1045642 TT genotypes were also associated with chronic allograft damage individually, but only in univariate analyses. No association was seen between *ABCB1* genotypes and delayed graft function or graft survival [202].

It is possible that nongenetic factors play a larger role in determining patient response to cyclosporine than do genetic ones. A recent genetic epidemiology study estimated the heritability of induced CYP3A4 activity at 66%, implying that genetic factors do play a large role, but environmental factors such as smoking and BMI may also significantly influence enzymatic activity [203]. Indeed, a different study found that patient weight explained 35% of the variability in cyclosporine oral clearance, and concomitant use of prednisolone at doses 20 mg/day or higher was also associated with higher clearance of the drug. However, no genotype effects of clinical relevance were seen, including SNPs in the *CYP3A4*, *CYP3A5*, and *ABCB1* genes, as well as *NR1I2* [204].

### Other potentially important genes

CYP3A7, another member of the CYP3A family along with CYP3A4 and CYP3A5, has also shown some associations with cyclosporine pharmacokinetics. However, no association with tacrolimus pharmacokinetics has been seen, likely because the enzyme has a low affinity and capacity for the drug [38]. Carriers of the *CYP3A7*\**1C* allele required 1.4- to 1.6-fold higher cyclosporine daily doses than noncarriers during the first year after transplantation [35]. Initially, it was believed that CYP3A7 was expressed exclusively in fetuses, but later studies showed that it is expressed at significant levels within some adult livers [205]. This allele was also demonstrated to be a marker of increased *CYP3A7* mRNA expression in both the adult liver [123,124] and the intestine [124], which supports the data showing that carriers of the \**1C* allele require higher cyclosporine doses. However, its mechanistic involvement in cyclosporine metabolism is still unknown [35].

Theoretically, polymorphisms in FK-binding protein, cyclophilin A and calcineurin genes may affect the immunosuppressive potential of cyclosporine and tacrolimus. However, limited studies have been done in this area. One study found that a promoter variant, rs8177826 (– 11 C>G) in the cyclophilin A gene (*PPIA*), affected gene expression and nephrotoxicity. Indeed, in the group of 290 kidney transplant patients taking cyclosporine,



the strongest predictors of nephrotoxicity were a renal donor age of above 55 years, and GG or GC genotypes in the promoter polymorphism. The C allele was associated with lower gene expression, implying that the effect on nephrotoxicity could be mediated by higher cyclophilin A expression in G allele carriers. No association with cyclosporine pharmacokinetics was seen [126]. However, Moore *et al.* [187], as part of their study on the effect of kidney donor genotype on development of allograft failure, analyzed several SNPs within *PPIA*, including rs8177826, and found no associations with graft failure when considering donor or recipient *PPIA* genotype. The study population consisted of 670 kidney transplant patients prescribed cyclosporine.

## Conclusion

Both tacrolimus and cyclosporine are invaluable drugs for the prevention of transplant rejection. However, a great deal of their pharmacokinetic and pharmacodynamic variability remains to be explained. Though the *CYP3A5*\*3 allele has shown strong associations with tacrolimus pharmacokinetics, very little consistent evidence has emerged for factors affecting tacrolimus pharmacodynamics or cyclosporine pharmacokinetics and pharmacodynamics. The overall inconsistency of these studies may be related to ethnic variability, small numbers of patients, nonspecific pharmacokinetic assays, variation in when outcomes are measured, and the impact of donor genotype – particularly in nephrotoxicity studies in kidney transplant patients or pharmacokinetic studies in liver transplant patients. Larger studies and metaanalyses that take ethnicity and donor genotype into account may help resolve some of this variability. The vast majority of studies have focused on single SNPs, and the potential role of haplotypes, both within and between multiple genes, needs investigation. It is possible that combinations of SNPs have synergistic effects on tacrolimus and cyclosporine pharmacokinetics or pharmacodynamics. In addition, the exact and comprehensive mechanisms of the drugs' immunosuppressive actions are still being discovered. In particular, the contribution of altered expression of P-glycoprotein within lymphocytes to the immunosuppressive effects of the drugs is unclear. Further study of the pharmacokinetic, dynamic, and genetic aspects of these drugs should help clinicians avoid the severe side effects associated with both of these drugs. Genotyping before treatment for these drugs has potential for preventing side effects such as nephrotoxicity, rejection, or neurotoxicity. However, currently no genes (or variations within these genes) show consistent associations with pharmacodynamic parameters. Indeed, only *CYP3A5*\*3 shows reliably positive associations with pharmacokinetic parameters for tacrolimus. As both tacrolimus and cyclosporine are subjected to careful dose-monitoring, genotyping *CYP3A5* to accurately predict dosage may not be necessary. Indeed, Thervet *et al.* [162] found that, though patients given genotype-adapted tacrolimus dosing had trough blood concentrations in the target range more often than those on the standard regimen, this did not result in any positive clinical endpoints such as decreased incidence of rejection or nephrotoxicity. To make genetic testing relevant for these drugs, further large-scale studies should focus on whether testing for *CYP3A5* nonexpressers before treatment with tacrolimus improves clinical outcome. Further investigation should also be conducted on genes such as *TGFB1* and *PPIA*, which are potential or known gene targets of either tacrolimus or cyclosporine. Variations in these genes could have a large impact on the

development of side effects, but there is currently not enough research to make any strong conclusions about their involvement. The effect of upstream CYP3A regulators such as POR and NR1I2 should also undergo further research. The pharmacogenetics of tacrolimus and cyclosporine is complex, and a great number of factors likely contribute to its variability. However, improving our understanding in this area will have a significant impact on the health and well-being of patients treated with these drugs.

## Acknowledgments

The authors thank Feng Liu for assistance with the graphics. PharmGKB is supported by the National Institutes of Health/National Institute of General Medical Sciences (R24 GM61374).

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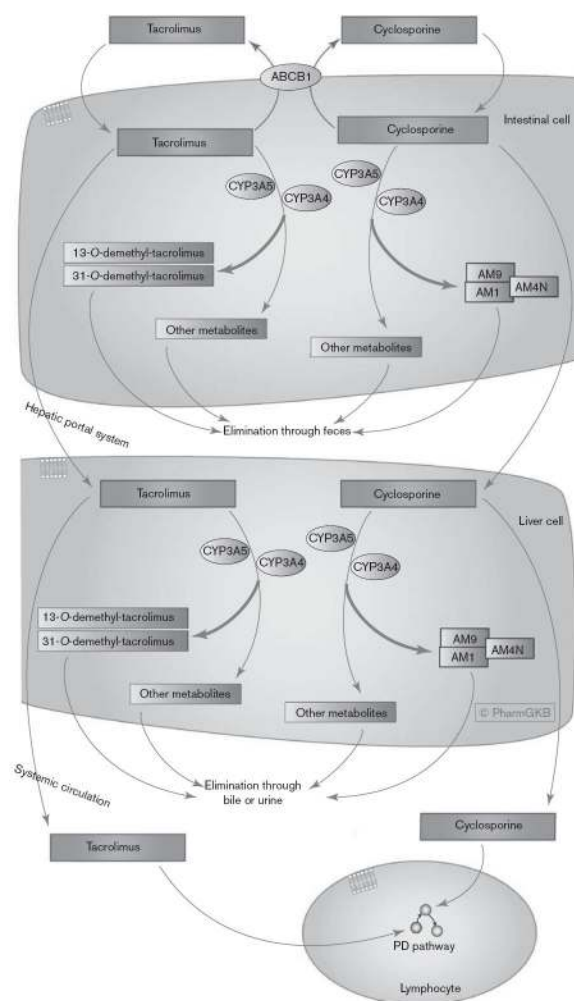
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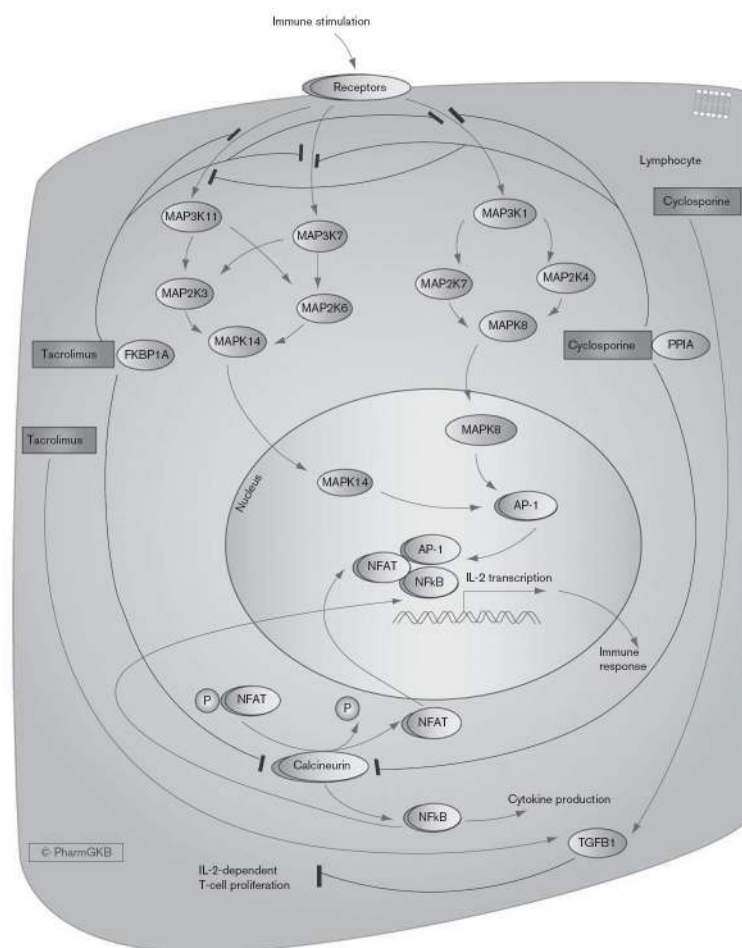
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**Fig. 1.**  
Schematic representation of tacrolimus and cyclosporine metabolism. A fully interactive version is available at: <http://www.pharmgkb.org/pathway/PA165986114>.

**Fig. 2.**

Stylized depiction of the mechanism of action of tacrolimus and cyclosporine in lymphocytes, as well as the candidate genes believed to interact with the two drugs. A fully interactive version is available at: <http://www.pharmgkb.org/pathway/PA165985892>.

**Table 1**

Summary of suggested therapeutic target ranges for cyclosporine and tacrolimus

Organ	Cyclosporine (Neoral) <sup>a</sup>		Tacrolimus <sup>b</sup>	
	Months post-transplant	C <sub>2</sub> target (μg/ml)	Months post-transplant	C <sub>0</sub> target (ng/ml)
Kidney	2	1.5	0–3	10–15 <sup>c</sup>
	3	1.3	3–12	5–15 <sup>c</sup>
	4–6	1.1	> 12	5–10 <sup>c</sup>
	7–12	0.9	–	–
	12 +	0.8	–	–
Liver	0–6	1.0	0–1	10–20
	6–12	0.8	1–3	5–15
	> 12	0.6	> 3	5–10
Heart	–	–	0–2	15–20
	–	–	2–6	10–15
	–	–	> 6	8–10
	–	–	> 6–9 (stable patients)	5–10
Lung	–	–	0–2 weeks	10–25
	–	–	6–10 weeks	10–20
	–	–	> 10 weeks	10–15

Targets are separated by months post-transplant and type of organ. Only information for adult patients is included.

<sup>a</sup>Data taken from Levy *et al.* [66].<sup>b</sup>Data for kidney, liver, and heart taken from Wallemacq *et al.* [64]; data for lung taken from Garrity *et al.* [70].<sup>c</sup>Target concentrations for renal transplant patients taking tacrolimus without induction therapy. Target values for patients taking tacrolimus with IL-2 receptor antibody therapy, induction with thymoglobulin or with an mTOR inhibitor will vary. Please refer to Wallemacq *et al.* [64] or Schiff *et al.* [71] for more information.

**Table 2**

Summary of genetic variants that show associations with tacrolimus or cyclosporine pharmacogenetics

Gene name	Variant	rsID	Effect on gene or protein	References
<i>CYP3A5</i>	<i>CYP3A5</i> *3	rs776746	Nonfunctional protein	Kuehl <i>et al.</i> [115]
	<i>CYP3A5</i> *6	rs10264272	Nonfunctional protein	Santoro <i>et al.</i> [116]
	<i>CYP3A5</i> *7	rs41303343	Nonfunctional protein	Santoro <i>et al.</i> [116]
<i>CYP3A4</i>	<i>CYP3A4</i> *1B <sup>a</sup>	rs2740574	Increase gene transcription	Amirmani <i>et al.</i> [117]
	<i>CYP3A4</i> *18 <sup>b</sup>	rs28371759	May increase enzyme activity	Fukushima-Uesaka <i>et al.</i> [118]
	<i>CYP3A4</i> *22	rs35599367	Reduced mRNA levels, reduced enzyme activity	Wang <i>et al.</i> [119]
<i>ABCB1</i>	3435C > T	rs1045642	Reduced protein expression and function	Hoffmeyer <i>et al.</i> [120]
	2677G > T/A	rs2032582	Currently unknown	
	1236C > T	rs1128503	Currently unknown	
<i>NR1I2</i>	8055C > T	rs2276707	Currently unknown	
<i>POR</i>	<i>POR</i> *28	rs1057868	May modify the POR-cytochrome interaction	De Jonge <i>et al.</i> [121]
<i>CYP2C8</i>	<i>CYP2C8</i> *3	rs11572080	Reduced enzyme activity	Smith <i>et al.</i> [122]
<i>CYP3A7</i>	<i>CYP3A7</i> *1C		Increased mRNA expression	Sim and colleagues [123,124]
<i>TGFB1</i>	29 T > C	rs1800470	Increased serum concentration of protein	Yokota <i>et al.</i> [125]
	74 G > C	rs1800471	Currently unknown	
<i>PPIA</i> (cyclophilin A)	– 11C > G	rs8177826	Increased gene expression	Moscoso-Solorzano <i>et al.</i> [126]

rsIDs (if known) and effects on the gene or protein are also listed. References pertain to the effect on the gene or protein.

<sup>a,b</sup> In linkage disequilibrium with *CYP3A5*\*3.

Table 3

Summary of tacrolimus pharmacogenetic studies

Variant	Associated genotype	Pharmacokinetic phenotype	References	Associated genotype	Pharmacodynamic phenotype	References
<i>CYP3A5</i> *3	*3/*3	$\downarrow D^{a,b}$ , $\downarrow CL/F$ , $\uparrow C_0/D$ , $\uparrow C_{max}/D$ , $\uparrow C_0$ , $\uparrow AUC_{12/D}$	Staatz and colleagues [25,133-140]	*3/*3	304 renal recipients: $\downarrow CNIT$ Smaller studies: $\downarrow$ and $\uparrow$ nephrotoxicity, $\downarrow$ rejection (renal), $\downarrow$ hypertension	Staatz and colleagues [26,137,141]
<i>CYP3A5</i> *6	*6/*1 *6/*6	One study: NS $\uparrow C_0/D$	Mai <i>et al.</i> [142]			
<i>CYP3A5</i> *7	*7/*1 *7/*7	$\uparrow C_0/D$	Santoro <i>et al.</i> [116] Santoro <i>et al.</i> [116]			
<i>CYP3A4</i> *1B <sup>c</sup>	*1B/*1 *1B/*1B	$\uparrow D$ , $\downarrow C_0/D$	Staatz <i>et al.</i> [25]	*1B/*1 *1B/*1B		
<i>CYP3A4</i> *18 <sup>c</sup>	*18/*1 *18/*18	Other studies: NS $\uparrow CL/F$ , $\downarrow C_0/D$ , $\downarrow C_2/D$	Staatz <i>et al.</i> [25] Staatz and colleagues [25,138]	Limited studies: NS		Staatz <i>et al.</i> [26]
<i>CYP3A4</i> *22	*22/*1 *22/*22	$\downarrow D$ , $\uparrow C_0/D$	Elens <i>et al.</i> [143,144]			
<i>ABCB1</i> 3435C > T	TT	$\uparrow D$ , $\downarrow D$ , $\uparrow C_0/D$	Staatz and colleagues [25,145] Staatz and colleagues [25,139]	TT	$\uparrow UC$ remission success, $\downarrow$ rejection (lung) Majority: NS	Staatz and colleagues [26,145] Staatz <i>et al.</i> [26]
<i>ABCB1</i> 2677G > T/A	GT TT	$\uparrow C_0/D$ , $\downarrow D$ , $\uparrow D$	Staatz and colleagues [25,146]	TT	$\uparrow UC$ remission success, $\downarrow$ renal dysfunction, $\uparrow$ and $\downarrow$ neurotoxicity	Staatz and colleagues [26,145,146]
<i>ABCB1</i> 1236C > T	CT TT	Majority: NS $\uparrow C_0/D$	Staatz <i>et al.</i> [25]	TT	Majority: NS $\uparrow UC$ remission success, $\uparrow$ and $\downarrow$ neurotoxicity	Staatz <i>et al.</i> [26] Herrlinger and colleagues [145,146]
Haplotype: 3435C > T, 2677G > T/A, 1236C > T	TTT	Majority: NS $\downarrow D$ , $\uparrow C_0/D$ (vs. CGC)	Staatz <i>et al.</i> [25] Staatz <i>et al.</i> [25]	TGC	Majority: NS $\uparrow CNIT$ (vs. CGC), $\uparrow$ rejection (renal) (vs. TTT)	Staatz <i>et al.</i> [26] Staatz and colleagues [26,146]
<i>NR1H2</i> 8055C > T	CT TT	Majority: NS $\uparrow AUC_{12/D}$	Staatz <i>et al.</i> [25] Barracough <i>et al.</i> [127]	CT TT	Other studies: NS $\uparrow BK$ viremia	Staatz <i>et al.</i> [26] Barracough <i>et al.</i> [127]
<i>POR</i> *28	*28/*1 *28/*28	$\downarrow C_0^d$ , $\uparrow D^d$ , $\downarrow AUC_{24}^d$ , $\downarrow C_{max}^d$	De Jonge and colleagues [121,147]	*3/*1 *3/*3	$\uparrow$ renal dysfunction, $\uparrow$ DGF	Smith and colleagues [122,146]
<i>CYP2C8</i> *3						

Variant	Associated genotype	Pharmacokinetic phenotype	References	Associated genotype	Pharmacodynamic phenotype	References
<i>TGFB1</i> <sup>e</sup> 29T > C				CT CC	↑ ESRF, ↑ renal dysfunction  Majority: NS	Baan and colleagues [148-150]  ↓lauLe and colleagues [151-153]
<i>TGFB1</i> <sup>e</sup> 74G > C				GC CC	↑ ESRF, ↓ renal dysfunction  Majority: NS	Van de Wetering and colleagues [149,150]  Klauke and colleagues [151-153]

Information on significant results can be found in the first row for each variant; information on nonsignificant results can be found in the second row. Empty rows indicate that no studies were available. Comprehensive tables of studies before 2010 showing these associations can be found in the reviews by Staatz *et al.* [25,26] referenced throughout this table. Additional studies not mentioned in these tables are referenced individually.

AUC<sub>x</sub>, area under the blood concentration–time curve from 0 to x hours; AUC<sub>0–D</sub>, dose-adjusted AUC<sub>x</sub>; C<sub>0</sub>, trough blood concentration; C<sub>0</sub>/D, dose-adjusted C<sub>0</sub>; C<sub>2</sub>, 2-h postdose blood concentration; C<sub>2</sub>/D, dose-adjusted C<sub>2</sub>; CL/F, apparent oral clearance; C<sub>max</sub>, maximum blood concentration; C<sub>max</sub>/D, dose-adjusted C<sub>max</sub>; CNIT, chronic irreversible drug-induced nephrotoxicity; D, dose requirements; DGF, delayed graft function; ESRF, end-stage renal failure; Majority, majority of studies; UC, ulcerative colitis; ↓, decreased; ↑, increased.

<sup>a</sup> An example of how to read this table: \*3/\*3 is associated with decreased D as compared with remaining genotypes – in this case \*3/\*1 and \*1/\*1.

<sup>b</sup> Only PK parameters analyzed in multiple studies were included in this table for the purposes of brevity.

<sup>c</sup> In linkage disequilibrium with *CYP3A5*\*3.

<sup>d</sup> These effects only seen in *CYP3A5*\*1 carriers.

<sup>e</sup> Studies included patients on tacrolimus and cyclosporine.



Table 4

## Summary of cyclosporine pharmacogenetic studies

Variant	Associated genotype	Pharmacokinetic phenotype	References	Associated genotype	Pharmacodynamic phenotype	References
<i>CYP3A5</i> *3	*3/*3	↓ <i>C<sub>0</sub></i> / <i>D<sup>a,b</sup></i> , ↑ <i>C<sub>0</sub></i> / <i>D</i> , ↑ <i>C<sub>2</sub></i> / <i>D</i> , ↓ <i>CL/F</i> , ↓ <i>D</i> Other studies: NS	Staatz and colleagues [25,183,184] Staatz and colleagues [25,186]	*3/*3	One study: ↓ survival Majority: NS	Kreutz <i>et al.</i> [185] Staatz and colleagues [26,186-188]
<i>CYP3A4</i> *1B <sup>c</sup>	*1B/*1 *1B/*1B	151 heart and renal recipients: ↑ <i>CL/F</i> Smaller studies: ↑ <i>D</i> , ↓ <i>AUC<sub>12</sub></i> / <i>D</i> , ↓ <i>C<sub>0</sub></i> / <i>D</i> Majority: NS	Staatz and colleagues [25,184]	*1B/*1 *1B/*1B		
<i>CYP3A4</i> *18 <sup>c</sup>	*18/*18	↓ <i>C<sub>0</sub></i> / <i>D</i> , ↓ <i>C<sub>2</sub></i> / <i>D</i>	Staatz and colleagues [25,186] Qiu and colleagues [167,189]		Limited studies: NS	Staatz and colleagues [26,186,187]
<i>CYP3A4</i> *22	*22/*1 *22/*22	↑ <i>C<sub>0</sub></i> / <i>D</i> , ↑ <i>C<sub>0</sub></i>	Elens <i>et al.</i> [144]	*22/*1 *22/*22	↑DGF	Elens [190]
<i>ABCB1</i> 3435>T	CT TT	Meta-analysis: ↑ <i>AUC<sub>12</sub></i> only Smaller studies: ↑ <i>C<sub>0</sub></i> / <i>D</i> , ↑ <i>C<sub>2</sub></i> / <i>D</i> , ↓ <i>D</i> Majority: NS	Staatz and colleagues [25,183]	TT	↑ rejection (renal), ↑ nephrotoxicity	Staatz and colleagues [26,191]
<i>ABCB1</i> 2677G>T/A	CT TT	↑ <i>C<sub>0</sub></i> / <i>D</i>	Staatz and colleagues [25,186] Staatz <i>et al.</i> [25]	TT	237 renal recipients: ↑ rejection, 120 liver recipients: ↓ renal dysfunction Majority: NS	Staatz and colleagues [26,187,188] Staatz <i>et al.</i> [26]
<i>ABCB1</i> 1236C>T	CT TT	↓ <i>C<sub>0</sub></i> / <i>D</i> , ↑ <i>C<sub>max</sub></i> / <i>D</i> Majority: NS	Staatz and colleagues [25,186] Staatz and colleagues [25,186]	TT	↑ rejection (renal) Majority: NS	Staatz <i>et al.</i> [26] Bouamar and colleagues [186,187]
Haplotype: 3435C>T, 2677G>T/A, 1236C>T	TTT	One study (vs. CGC): ↑ <i>C<sub>0</sub></i> , ↑ <i>C<sub>max</sub></i> , ↑ <i>AUC<sub>12</sub></i> Majority: NS	Chowbay <i>et al.</i> [192] Staatz <i>et al.</i> [25]	TTT	↓ risk rejection (renal) (vs. TGC), ↑ risk rejection (renal) (vs. CGC)	Staatz <i>et al.</i> [26]

Variant	Associated genotype	Pharmacokinetic phenotype	References	Associated genotype	Pharmacodynamic phenotype	References
<i>CYP3A7</i> *1C	*1C/*1 *1C/*1C	↑ D	Crettol <i>et al.</i> [35]	*1C/*1 *1C/*1C	↑ ESRF, ↑ and ↓ renal dysfunction	Baan and colleagues [148-150]
<i>TGFB1</i> <sup>d</sup> 29T>C				CT CC	Other studies: NS	Klauke and colleagues [151-153]
<i>TGFB1</i> <sup>d</sup> 74G>C				GC CC	↑ ESRF, ↓ renal dysfunction	Van de Wetering and colleagues [149,150]
<i>PPIA</i> -11C >G				CG GG	Majority: NS ↑ nephrotoxicity	Klauke and colleagues [151-153] MosC <sub>950</sub> -Solorzano <i>et al.</i> [126]
					One study: NS	Moore <i>et al.</i> [187]

Information on significant results can be found in the first row for each variant; information on nonsignificant results can be found in the second row. Empty rows indicate that no studies were available. Comprehensive tables of studies before 2010 showing these associations can be found in reviews by Staatz *et al.* [25,26] referenced throughout this table. Any additional studies not mentioned in this review are referenced individually.

AUC<sub>12</sub>, area under the blood Concentration–time curve from time zero to 12 h; AUC<sub>12</sub>/D, dose-adjusted AUC<sub>12</sub>; C<sub>0</sub>, trough blood concentration; C<sub>0</sub>/D, dose-adjusted C<sub>0</sub>; C<sub>2</sub>, 2-h postdose blood concentration; C<sub>2</sub>/D, dose-adjusted C<sub>2</sub>; CL/F, apparent oral clearance; C<sub>max</sub>, maximum blood concentration; C<sub>max</sub>/D, dose-adjusted C<sub>max</sub>; D, dose requirements; DGF, delayed graft function; ESRF, end-stage renal dysfunction; Majority, majority of studies; ↓, decreased; ↑, increased.

<sup>a</sup> An example of how to read this table: \*3/\*3 is associated with decreased C<sub>0</sub>/D as compared with remaining genotypes – in this case \*3/\*1 and \*1/\*1.

<sup>b</sup> Only PK parameters analyzed in multiple studies were included in this table for the purposes of brevity.

<sup>c</sup> In linkage disequilibrium with CYP3A5\*3.

<sup>d</sup> Studies included patients on tacrolimus and cyclosporine.