

WJG 20<sup>th</sup> Anniversary Special Issues (7): Liver transplant**Pharmacogenetic considerations for optimizing tacrolimus dosing in liver and kidney transplant patients**

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**Abstract**

The introduction of tacrolimus in clinical practice has improved patient survival after organ transplant. However, despite the long use of tacrolimus in clinical practice, the best way to use this agent is still a matter of intense debate. The start of the genomic era has generated new research areas, such as pharmacogenetics, which

studies the variability of drug response in relation to the genetic factors involved in the processes responsible for the pharmacokinetics and/or the action mechanism of a drug in the body. This variability seems to be correlated with the presence of genetic polymorphisms. Genotyping is an attractive option especially for the initiation of the dosing of tacrolimus; also, unlike phenotypic tests, the genotype is a stable characteristic that needs to be determined only once for any given gene. However, prospective clinical studies must show that genotype determination before transplantation allows for better use of a given drug and improves the safety and clinical efficacy of that medication. At present, research has been able to reliably show that the *CYP3A5* genotype, but not the *CYP3A4* or *ABCB1* ones, can modify the pharmacokinetics of tacrolimus. However, it has not been possible to incontrovertibly show that the corresponding changes in the pharmacokinetic profile are linked with different patient outcomes regarding tacrolimus efficacy and toxicity. For these reasons, pharmacogenetics and individualized medicine remain a fascinating area for further study and may ultimately become the face of future medical practice and drug dosing.

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**Key words:** Pharmacogenetics; Calcineurin inhibitors; Tacrolimus; Liver transplant; Kidney transplant; Single nucleotide polymorphisms; *CYP3A4*; *CYP3A5*; *ABCB1*

**Core tip:** As researchers continue to evaluate the influence of single nucleotide polymorphisms on tacrolimus dosing and on the response to the drug, the challenge now becomes to assess the potential clinical implications of this research for medical practice. Sufficient data have been accumulated to be certain that the liver donor and kidney recipient *CYP3A5* genotype has an important influence on tacrolimus dosing and on the

observed blood trough levels of the drug. However, the question remains, should genotyping become a standard of practice in transplantation?

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## INTRODUCTION

Transplantation is typically the standard of therapy for all patients with end-stage liver or kidney disease. Almost sixty years have passed since the first kidney transplant between identical twins was successfully performed, in 1954<sup>[1]</sup>. The first human liver transplant was performed almost 10 years later, in 1963. Another decade would pass before it was performed again. Since then, a significant effort has been made to improve the graft survival, as well as the patient outcome.

Despite the significant advances in terms of surgical techniques, tissue typing and patient care, most of the progress in organ transplantation is largely attributable to the recognized importance of immunosuppressive therapy<sup>[2,3]</sup>.

Since the success of the transplant depends on a delicate balance between immunosuppression and rejection, reaching and maintaining an adequate therapeutic level by giving appropriate doses of immunosuppressive drugs is extremely important, especially in the first phases after the transplant.

The introduction of tacrolimus into clinical practice has undoubtedly improved patient survival after organ transplant. However, this drug is characterized by a restricted therapeutic index, a high inter- and intra-individual pharmacokinetic variability, including irregular oral bioavailability, and a series of severe adverse effects<sup>[4,5]</sup>.

Given the high variability in blood levels and clinical response after administering fixed doses of tacrolimus, several studies have recently been conducted to find the optimal dosage of tacrolimus and thus to minimize its toxicity and to improve its risk/benefit ratio<sup>[6]</sup>.

A number of studies have found a close correlation between the pharmacokinetic parameters of tacrolimus and the clinical outcome<sup>[7,8]</sup>. However, despite the long use of the drug in clinical practice, the best way to use tacrolimus is still a matter of intense debate<sup>[9,10]</sup>.

The start of the genomic era has generated new research areas, such as pharmacogenetics, which studies the variability of drug response in relation to the genetic factors involved in the processes responsible for the pharmacokinetics and/or action mechanisms of a drug in the body<sup>[11,12]</sup>.

This variability seems to be correlated with the presence

of genetic polymorphisms, where, for example, some of the genes of the enzymes of phase I and II drug metabolic processes present, in at least 1%-2% of the population, allelic variants<sup>[13,14]</sup>.

These variants can encode for different molecular isoforms of the same protein and, in most cases, consist of single nucleotide polymorphisms (SNPs), which may determine the production of isoforms differing by a single amino acid<sup>[14]</sup>.

The variations in the DNA sequence of genes encoding for drug metabolizing enzymes can cause significant phenotypic differences in their expressivity and activity<sup>[15-17]</sup>.

The clinical implications of genetic polymorphisms can include other aspects of drug bioavailability and elimination, as well as the pharmacodynamics of the drug and/or its metabolites and the therapeutic index. Despite the many genetic polymorphisms, only a small number of them have clinically significant consequences in terms of drug metabolism. This occurs mainly when two conditions coexist: the concerned metabolic pathway is the only pathway for the biotransformation of the drug and the drug has a low therapeutic index.

For all these reasons, pharmacogenetics research has begun to delve into genotyping approaches which may help to optimize the initiation and maintenance dosing of tacrolimus, to attain faster its target concentrations and to limit its dose-related adverse reactions<sup>[18]</sup>. This paper will review various studies that highlight the current genetic considerations in the dosing of tacrolimus and the future implications that this data may have for best individualizing the treatment with this drug.

## TACROLIMUS PHARMACODYNAMICS AND PHARMACOKINETIC CHARACTERISTICS AND CONSIDERATIONS

The two calcineurin inhibitors utilized in transplantation are cyclosporine and tacrolimus.

Tacrolimus is a macrolide containing a 23-membered lactone ring produced by the *Streptomyces tsukubaensis* fungus (Figure 1). Its molecular weight is 822.05 Da<sup>[19]</sup>. Tacrolimus is now preferred to cyclosporine for its potency (10-100 times higher in *in vitro* and *in vivo* immunosuppression models) and for the reduction in episodes of rejection; it allows the use of lower doses of combination corticosteroids, thus reducing the possibility of adverse effects associated with such drugs<sup>[20,21]</sup>.

Tacrolimus becomes biologically active only when it forms a complex with the immunophilin FK binding protein 12 (FKBP-12), that is different from the immunophilin (cyclophilin) to which cyclosporine binds. The complex FKBP-12-tacrolimus interferes with the transduction pathway of the intracellular calcium-dependent signal, which is a fundamental processes for the activation of T lymphocytes. The biological target of the complex

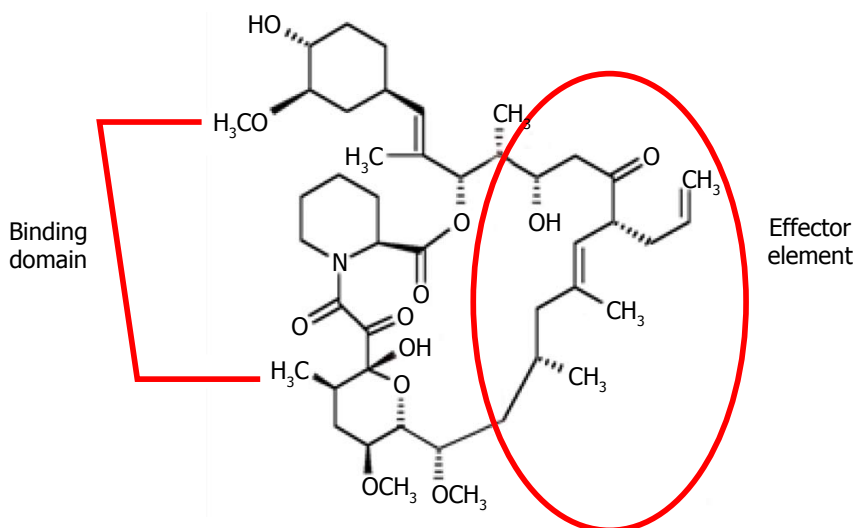


Figure 1 Functional active groups of tacrolimus.

is the calcium/calmodulin-dependent protein phosphatase calcineurin, a fundamental molecule for the reactions necessary to the synthesis of various cytokines, including IL-2.

Tacrolimus acts as a molecular linker between the calcineurin/calmodulin complex and immunophilin, which are molecules that in normal conditions would not interact. The tacrolimus-FKBP-12 complex has a strong inhibitory dose-related effect on calcineurin phosphatase activity and consequently on IL-2 expression. The passage of the signal from the cytoplasm to the nucleus to activate the transcription of the IL-2 gene involves in fact a protein named nuclear factor of activated T-cell (NF-ATc). This protein is a T lymphocyte-specific transcription factor, the activity of which is correlated with the level of transcription of IL-2 after the T-cell receptor is activated<sup>[20,22]</sup>. The NF-ATc has two subunits, one of which is confined to the cytoplasm, while the other is mostly nuclear. An increase in intracellular calcium allows the cytoplasmatic unit to move into the nucleus, where it combines with the nuclear component and allows the formation of the IL-2 transcription factor. The immunophilin/drug complexes would inhibit NF-ATc transcription activities, hindering the formation of the functional transcription factor. The signal transduction cascade starts at the presentation of the antigen to the T-cell receptor, which induces an increase in intracellular calcium, the activation of the calcium/calmodulin complex and the formation of the competent T-cell transcription factor (NF-ATc). The specific role of calcineurin is not entirely clear, but it is widely accepted that dephosphorylation induces the translocation into the nucleus of cytoplasmatic NF-ATc, a process that can be blocked by the immunophilin/drug complexes. The liaison of DNA and the genetic transcription of IL-2 require both nucleic and cytoplasmatic subunits of NF-ATc. Tacrolimus stops transduction pathways of the signal and therefore hinders the IL-2 production by means

of the intracellular action of the drug-FKBP-12 complex. The tacrolimus molecule can therefore be divided into two separate functional groups<sup>[22]</sup>: a binding group for the drug-FKBP-12 complex, and an effector group to bind to calcineurin (Figure 1).

Tacrolimus, a lipophilic drug, exhibits variable absorption and first pass metabolism when administered orally and this can influence its efficacy and toxicity. P-glycoprotein (P-gp, also known as ABCB1), an ATP-dependent membranous transporter which helps to protect the body against toxic xenobiotics by extruding these compounds out of cells and into the intestinal lumen and bile<sup>[23]</sup>, can limit the oral bioavailability and influence the disposition of the calcineurin inhibitors<sup>[24-28]</sup>. In particular, the presence of P-gp in the intestine can limit tacrolimus absorption. Also, its presence in liver and kidney promotes tacrolimus efflux into bile and urine, respectively.

However, the conclusions drawn so far on the actual influence of P-gp SNPs on tacrolimus pharmacokinetics are highly controversial<sup>[29-31]</sup>.

Additionally, CYP3A4 and CYP3A5, which exhibit variable levels of activity among transplant patients, are the primary enzymes responsible for the metabolism of the calcineurin inhibitors. The same drugs are also known inhibitors of P-gp and of the CYP enzyme system, so that they inhibit their own metabolism and excretion<sup>[32]</sup>. Other factors that can influence the pharmacokinetics of the calcineurin inhibitors include, but are not limited to, transplant type, baseline renal and hepatic function, concomitant use of corticosteroids, which induce both CYP3A and P-gp activity, patient age and race, time after transplantation, albumin and hematocrit concentration, trauma and food administration<sup>[33-40]</sup>.

As a result, therapeutic drug monitoring is typically initiated after transplantation to facilitate the choice of the dosage of the calcineurin inhibitors and ensure appropriate levels of exposure to these medications. To date the most widely used parameter for the therapeutic monitor-

ing of tacrolimus is its trough whole blood concentration ( $C_0$ ), which is measured 12 h after the dose administration and correlates well with the area under the concentration-time curve ( $AUC_{0-12}$ )<sup>[41,42]</sup>.

In practice, target trough levels for tacrolimus are typically set at around 10 ng/mL, but this can vary depending on individual patient characteristics, type of transplant and time after transplantation. Pharmacogenetics is estimated to account for between 20%-95% of drug variability in patients and this has prompted research to assess the feasibility of genotyping as a mean to more rapidly and accurately determine the appropriate starting and maintenance dosages of the immunosuppressant<sup>[43]</sup>.

Also to assess the economic advantage of the genotypic determinations, a number of pharmacodynamics studies have been undertaken to define the overall impact of a more delayed optimization of the drug dosage on patient outcomes. Typically these studies evaluate the effects of sub- or supra-therapeutic calcineurin inhibitor drug levels on graft life, patient mortality and development of various drug toxicities. The calcineurin inhibitors are known to be endowed with a number of possible deleterious effects including seizures, tremors, nephrotoxicity, malignancy, hyperglycemia, hypertension, insomnia, hyperesthesia and hyperlipidemia<sup>[4]</sup>. They are also expensive and potentially life-long medications that can impose a heavy economic burden on patients and on the health care system in general. As a result, it would be beneficial to rapidly attain target blood trough drug levels in order to avoid side-effects, limit costs and assure appropriate level of immunosuppression.

## GENETIC POLYMORPHISMS

To date, a number of SNPs have been studied in relation to the dosing of tacrolimus. However, alleles relating to the following three genes have been the most frequently studied and shown to be the most promising.

### CYP3A4

CYP3A4, located in the liver, jejunum, colon, and pancreas, is polymorphically expressed, with at least 42 SNPs identified to date<sup>[44]</sup>. The most known CYP3A4 polymorphisms are CYP3A4\*1B (A392G)<sup>[45]</sup>, CYP3A4\*2 (Ser 222 Pro), and CYP3A4\*3 (Met 445 Thr)<sup>[46]</sup>.

The primary polymorphism implicated and studied in the metabolism of the calcineurin inhibitors occurs at position 392 and is an A>G substitution that produces a variant allele with diminished enzymatic activity, referred to as CYP3A4\*1B<sup>[47-51]</sup>. On the other hand, researchers have demonstrated that CYP3A4 expression is higher in carriers of the mutant allele due to reduced binding of a transcriptional repressor<sup>[52,53]</sup>. Consequently, the functional significance of this SNP is controversial and *in vivo* studies have generally failed to evidence an association between this polymorphism and the metabolism of various drugs<sup>[54-56]</sup>. This allele has been shown to occur in 2%-10% of Caucasians, 4.2%-11% of Hispanics, 35%-67% of

African-Americans, and about 0% of Asians<sup>[57-60]</sup>.

### CYP3A5

CYP3A5 in the liver, small intestine, stomach and kidney shows polymorphic expression, which is currently known to occur with at least 11 different SNPs. The most important polymorphism is that of the CYP3A5\*3, which, in homozygous condition, determines the absence of the enzyme, since the variant sequences A→G at nucleotide 6986 in intron 3 of the CYP3A5 gene cause alternative splicing and the formation of a truncated protein that is not functional<sup>[61,62]</sup>. On the contrary, the G6986A (CYP3A5\*1) allele is correlated with a high expression of the protein<sup>[63]</sup>. Consequently, individuals that exhibit homozygous expression of the variant allele CYP3A5\*3 are often referred to as “CYP3A5 non-expressers”. Patients with at least one CYP3A5\*1 wild type allele are able to produce functional CYP3A5 enzymes and are known as “CYP3A5 expressers”; they have a different pattern of metabolite formation compared with the non-expressers, resulting also in the belief that CYP3A5 expression in the kidney may play a protective role against the development of nephrotoxicity by limiting the exposure of the organ to toxic metabolites<sup>[64-66]</sup>. Several studies have also suggested a link between CYP3A4\*1B and the CYP3A5\*1 wild type allele, as these two allelic variants appear generally to be inherited together<sup>[59,61,67-69]</sup>. Again the CYP3A5\*1 wild type allele is differently distributed among the races and occurs in 5%-15% of Caucasians, 15%-35% of Asians, 25% of Mexicans, and 45%-73% of African-Americans<sup>[57]</sup>.

### ABCB-1 (MDR-1, P-gp)

The multidrug resistance-1 (MDR-1) gene, which encodes for the P-gp (ABCB-1) efflux pump in many organs and tissues (*e.g.*, liver, kidney, hematoencephalic barrier, blood testis barrier, maternal side of the placenta, adrenal glands and small intestines), is also polymorphically expressed, with at least 50 currently known SNPs. Its name derives from the fact that it was first found in tumor cell lines where it enhanced the resistance to anti-neoplastic drugs<sup>[23,70-74]</sup>.

The most commonly studied ABCB1 polymorphisms include a C to T substitution at position 3435 on exon 26, a C to T substitution at position 1236 on exon 12, and a G to T/A substitution at position 2677 on exon 21<sup>[24]</sup>.

These three variant alleles have been shown to typically occur together, exhibiting a linkage disequilibrium that suggests that they may be further genetically linked<sup>[74-78]</sup>.

Several studies have also suggested that this haplotype results in diminished P-gp expression *in vivo* and, in turn, in lower drug efflux activity. Theoretically this could result in tacrolimus accumulation in the blood stream and nervous system and, as a result, in symptoms of neurotoxicity<sup>[79,80]</sup>.

In addition, recent data have suggested that 3435C>T may reduce MDR-1 mRNA stability in the liver<sup>[81]</sup> or affect the insertion and folding of P-gp into the membrane, resulting in an altered substrate specificity of the transporter<sup>[82]</sup>.

This haplotype occurs in 5% of African Americans, 27%

**Table 1** Effect of *CYP3A4\*1B* single nucleotide polymorphism on tacrolimus pharmacokinetics

Authors	Study population	Transplant type/analysis of recipients, donors or both	Findings
Cho <i>et al</i> <sup>[84]</sup>	70 Korean	Kidney recipients	No association between <i>CYP3A4*1B</i> genotype and tacrolimus dose requirements up to 6 mo after transplantation
Roy <i>et al</i> <sup>[85]</sup>	38 Caucasian, 4 Black, 2 Asian	Kidney recipients	No correlation between the <i>CYP3A4*1B</i> SNP and tacrolimus pharmacokinetic at first week and third month after transplantation
Hesselink <i>et al</i> <sup>[67]</sup>	37 Caucasian, 9 Black, 18 Asian	Kidney recipients	<i>CYP3A4*1B</i> allele carriers had lower tacrolimus dose-adjusted trough levels with respect to patients carrying the wild-type (*1/*1) genotype at third and 12th month after transplantation This effect was not observed when analyzing only the Caucasian population.
Hesselink <i>et al</i> <sup>[87]</sup>	120 Caucasian, 7 Black, 8 Asian, 1 other	Kidney recipients	No significant correlation observed between <i>CYP3A4*1B</i> SNP and tacrolimus pharmacokinetics when <i>CYP3A5</i> and <i>ABCB1</i> SNPs were taken into account
Gervasini <i>et al</i> <sup>[33]</sup>	103 Spanish	Kidney recipients	Carriers of the <i>CYP3A4*1B</i> variant allele had 59% lower tacrolimus concentrations than those with <i>CYP3A4*1/*1</i> wild type genotype All <i>CYP3A4*1B</i> carriers were also carriers of <i>CYP3A5*1</i> allele (linkage disequilibrium)

of Asians, 32% of Caucasians and 35% of Mexicans<sup>[77]</sup>.

## GENETIC INFLUENCE ON TACROLIMUS PHARMACOKINETICS

A number of clinical studies have begun to evaluate the actual impact of the previously described polymorphisms on tacrolimus dosing, efficacy and toxicity. We will now review a number of these studies and summarize their findings before analyzing the potential clinical implications of their data.

### *CYP3A4\*1B*

Data regarding the influence of *CYP3A4* polymorphisms on tacrolimus pharmacokinetics are often inconsistent and confounded by the highly frequent linkage disequilibrium found between the *CYP3A4\*1B* variant allele and the *CYP3A5\*1* wild-type allele<sup>[59,61,67-69,83]</sup>. The overall impact of the *CYP3A4* genotype on tacrolimus dose requirements appears uncertain and should be further studied.

A study by Cho *et al*<sup>[84]</sup> on 70 Korean renal transplant patients found no association between *CYP3A4* genotype and tacrolimus dose requirements up to 6 mo after transplantation] (Table 1).

Another study, by Roy *et al*<sup>[85]</sup>, confirmed these results, showing no correlation between the *CYP3A4\*1B* (392A>G) SNP and tacrolimus pharmacokinetics (Table 1). However, as other authors have pointed out, due to the limited data available it is not possible to understand if these results were influenced by the ethnicity or by a genetic linkage with the *CYP3A5* 6986A>G SNP<sup>[86]</sup>.

In a study on 64 kidney transplant patients, Hesselink *et al*<sup>[67]</sup> showed that patients carrying the *CYP3A4\*1B* allele had lower tacrolimus dose-adjusted trough levels with respect to patients carrying two copies of the wild-type \*1 allele. This effect was not observed when the analysis was made only in the Caucasian population (Table 1).

However, in a further study carried out in a more con-

sistent population composed of 136 renal transplant patients the same authors found that there was no significant correlation between the *CYP3A4\*1B* (392A>G) SNP and tacrolimus pharmacokinetics (dose and C<sub>0</sub>/Dose) when the influences of the *CYP3A5* 6986A>G SNP and *ABCB1* polymorphisms were taken into account<sup>[87]</sup> (Table 1).

In another study on 103 Spanish renal transplant patients, Gervasini *et al*<sup>[33]</sup> found that carriers of the *CYP3A4\*1B* variant allele displayed tacrolimus concentrations that were on average 59% lower than those of patients with the *CYP3A4\*1/\*1* genotype. The dose-adjusted trough levels observed were 145.59, 86.89, and 58.21 ng/mL per mg/kg per day for the 3A4\*1-3A5\*3, 3A4\*1-3A5\*1 and 3A4\*1B-3A5\*1 haplotypes, respectively, suggesting that the *CYP3A4\*1B*-*CYP3A5\*1* haplotype may have a more profound impact on tacrolimus pharmacokinetics than the *CYP3A5\*1* allele alone (Table 1). However, because of the linkage disequilibrium between the *CYP3A4* and *CYP3A5* polymorphisms, all *CYP3A4\*1B* carriers were also carriers of the *CYP3A5\*1* allele.

### *CYP3A5\*3*

Many studies have confirmed that *CYP3A5* polymorphisms have a major influence on the pharmacokinetics of tacrolimus. Consistently, patients homozygous for the *CYP3A5\*3* allele have shown lower dose requirements and higher whole blood trough levels of tacrolimus after transplantation, as well as clearances of the drug 25%-45% lower than patients expressing the *CYP3A5\*1* allele. In liver transplant patients, donor genotype has also generally been shown to have more important consequences on tacrolimus pharmacokinetics and dose requirements than recipient genetics<sup>[38,63,88-93]</sup>. However, it still remains to be seen whether these alterations in the drug pharmacokinetics correlate or not to the patient clinical outcomes.

A study by Barrera-Pulido *et al*<sup>[94]</sup> on 53 liver transplant recipients found that recipients with the *CYP3A5\*1/\*3* genotype receiving organs from \*1/\*3 donors failed to

achieve minimum blood tacrolimus levels at one month post-transplant (Table 2). Between days 30 and 60 post-transplant  $*3/*3$  recipients from  $*1/*3$  donors also had significantly greater tacrolimus dose requirements than recipients from  $*3/*3$  donors.

These results also occurred in a study on 24 Native American kidney transplant recipients where it was observed that after 1 mo from transplant the patients required a significantly lower daily tacrolimus dose than a control group of Caucasian kidney transplant patients (0.03 mg/kg per day *vs* 0.5 mg/kg per day). To explain these data, many of these Native Americans, but not the Caucasians, were found to express the *CYP3A5\*3/\*3* genotype, associated with diminished CYP3A5 enzymatic activity (Table 2). However, despite the differences in tacrolimus dose requirements, there were no differences in the drug trough levels or the incidence of nephropathy between the two study groups<sup>[95]</sup>.

A study on 32 Caucasian liver transplant patients by Provenzani *et al*<sup>[91]</sup> found that dose requirements were significantly higher in patients receiving a liver with the *CYP3A5\*1* allele compared with donors who were homozygous for the  $*3$  polymorphism (0.111 mg/kg per day *vs* 0.057 mg/kg per day). In the organ recipients, the *CYP3A5\*1* genotype tended to increase tacrolimus doses, though not to a statistically significant degree (Table 2).

In a case report, the same research group found that a 53-year-old Caucasian male who was homozygous for the *CYP3A5\*3* allele and had received a liver from a donor expressing the *CYP3A5\*1/\*1* genotype required a dose two-fold higher than that reported in the literature for adult liver transplant patients. During the first, second and third week of therapy the patient received tacrolimus doses of 0.219, 0.287, and 0.273 mg/kg per day, respectively, while the trough drug levels obtained remained below the target of 10-12 ng/mL (4.6, 5.6 and 6.1 ng/mL at the first, second and third week of therapy, respectively). The patient reached a target level of 10.4 ng/mL only after one month of therapy. This corroborates that the *CYP3A5\*1* allele may be associated with increased hepatic metabolic capacity for tacrolimus and, consequently, delayed response to drug therapy<sup>[93]</sup>.

The authors further confirmed these results when they looked at 51 Caucasian liver and 50 Caucasian kidney transplant recipients at 1, 3, and 6 mo post-transplant and again found that the presence of the *CYP3A5\*1* allele in liver donors, but not in recipients, had a statistically significant effect of decrease on the tacrolimus dose-adjusted trough levels. A similar result was also observed in the kidney transplant recipients, where the dose required to achieve and maintain target trough blood levels at 1, 3, and 6 mo was statistically lower in patients homozygous for the *CYP3A5\*3* allele compared with the patients expressing at least one copy of the wild type allele *CYP3A5\*1*<sup>[92]</sup> (Table 2).

Another study by Cho *et al*<sup>[84]</sup> on 70 Korean renal transplant patients found that patients expressing either the

*CYP3A5\*1/\*3* or *CYP3A5\*1/\*1* genotype, and thus a functional CYP3A5 protein, had tacrolimus dose requirements up to 80% greater than patients homozygous for the  $*3$  allele up to 6 mo post-transplant (Table 2).

Glowacki *et al*<sup>[96]</sup> in a study on 209 French kidney transplant patients, also found that patients with at least one *CYP3A5\*1* allele had significantly higher tacrolimus dose requirements and lower trough drug levels than  $*3$  homozygotes. However, these pharmacokinetic findings appeared to have no influence on the incidence of biopsy-proven acute rejection or on delayed graft function (Table 2). Patients were followed for a mean period of 21.8 mo, with no data suggesting that alterations in tacrolimus pharmacokinetics might have any significant impact on long-term clinical outcomes.

Another study, in 181 Japanese liver transplant recipients and 114 donors, showed that the level of CYP3A5 mRNA was significantly reduced in patients with livers carrying the *CYP3A5\*3/\*3* genotype (0.41 amol/ $\mu$ g total RNA) *vs* the  $*1/*1$  and  $*1/*3$  genotypes (4.85 and 2.99 amol/ $\mu$ g total RNA, respectively). As a result, the dose-adjusted tacrolimus trough levels were significantly decreased, due to increased metabolism, in patients receiving a liver carrying the *CYP3A5\*1/\*1* genotype<sup>[63]</sup> (Table 2).

Wei-lin *et al*<sup>[88]</sup>, in a study on 50 Chinese liver transplant donors as well as recipients, found again that at one month after transplantation, recipients who received organs from *CYP3A5\*3/\*3* donors had significantly higher dose-adjusted tacrolimus trough levels than the patients receiving livers from *CYP3A5\*1* expressers (Table 2). However, neither the donors' ABCB1 genotype nor the recipients' CYP3A5 genotype had any impact on the recipients' tacrolimus pharmacokinetic profile, suggesting once more that in liver transplantation the donors' CYP3A5 genetics, rather than that of the recipient, has a more important effect on tacrolimus dosing.

López-Montenegro Soria *et al*<sup>[97]</sup> studied 35 kidney transplant patients and found that during the first six weeks after transplant the tacrolimus concentration/dose ratios were remarkably lower for patients expressing at least one *CYP3A5\*1* allele compared with those homozygous for the *CYP3A5\*3* genotype (0.65 *vs* 1.45), due to higher drug clearances in *CYP3A5\*1* expressers (Table 2).

Another trial, by Shi *et al*<sup>[66]</sup>, involving 216 Chinese liver transplant recipients concluded that daily tacrolimus dose requirements were higher for recipients with the *CYP3A5\*1/\*1* genotype than patients expressing the  $*3/*3$  genotype (3.0 mg *vs* 2.0 mg per day). Dose-adjusted tacrolimus trough levels were also lower in the  $*1/*1$  genotype than  $*1/*3$  expressers and in the  $*3$  homozygotes (97.5, 124.8, and 144.4, respectively), suggesting in particular that CYP3A5 enzymatic activity is increased proportionally by the presence of one or two copies of the  $*1$  allele (Table 2).

These results were supported by Jun *et al*<sup>[98]</sup> in a study of 506 Korean solid organ transplant recipients and 62 corresponding liver transplant donors, which concluded that the blood tacrolimus concentrations per adjusted

**Table 2 Effect of CYP3A5\*3 single nucleotide polymorphism on tacrolimus pharmacokinetics**

Authors	Study population	Transplant type/analysis of recipients, donors or both	Findings
Barrera-Pulido <i>et al</i> <sup>[94]</sup>	53 Caucasian	Liver recipients and donors	CYP3A5*1/*3 recipients with *1/*3 donor livers had lower than minimum required blood tacrolimus levels at 1 mo after transplantation *3/*3 recipients with *1/*3 donors had significantly greater tacrolimus dose requirements at 1 and 2 mo after transplantation
Chakkerla <i>et al</i> <sup>[95]</sup>	24 native American and Caucasian control group	Kidney recipients	Native Americans had lower tacrolimus dose requirements than Caucasians at 1 mo after transplantation Native Americans more commonly expressed CYP3A5*3/*3 No difference in blood trough levels or nephropathy between the two groups
Provenzani <i>et al</i> <sup>[91]</sup>	32 Caucasian	Liver recipients and donors	Dose requirements significantly higher in the case of donors with the CYP3A5*1 allele at 1, 3 and 6 mo after transplantation No statistically significant difference in dose requirements considering recipient's genotypes
Provenzani <i>et al</i> <sup>[92]</sup>	101 Caucasian	Kidney (n = 50, recipients) and liver (n = 51, recipients and donors)	CYP3A5*1 allele in liver donors (n = 51) had a significant effect of decrease on tacrolimus dose-adjusted trough levels at 1, 3 and 6 mo after transplantation. No statistically significant difference in dose requirements considering recipient's genotype Tacrolimus dose in kidney recipients (n = 50) with CYP3A5*3/*3 genotype was significantly lower than in patients with at least one copy of the wild type allele
Cho <i>et al</i> <sup>[84]</sup>	70 Korean	Kidney recipients	Those patients who had CYP3A5*1/*3 or *1/*1 genotypes had 80% higher tacrolimus dose requirements than patients homozygotes for *3 allele (up to 6 mo after transplantation)
Glowacki <i>et al</i> <sup>[96]</sup>	209 French	Kidney recipients	Patients with at least one copy of the CYP3A5*1 allele had significantly higher dose requirements and lower blood trough levels than patients homozygous for the *3 allele No influence of this SNP on rejection or graft dysfunction rates.
Goto <i>et al</i> <sup>[63]</sup>	181 Japanese	Liver recipients and donors	Patients with the CYP3A5*3/*3 genotype had reduced levels of CYP3A5 mRNA Dose-adjusted tacrolimus trough levels decreased in patients receiving a liver with the *1/*1 genotype
Wei-Lin <i>et al</i> <sup>[88]</sup>	50 Chinese	Liver recipients and donors	Those patients receiving a liver with the *3/*3 genotype had, at first month after transplantation, significantly higher tacrolimus dose-adjusted trough levels than those with at least one copy of the *1 allele
López-Montenegro Soria <i>et al</i> <sup>[97]</sup>	35 Spanish	Kidney recipients	Concentration/dose ratios were remarkably lower in patients with at least one copy of the *1 allele than in patients homozygous for the *3 allele
Shi <i>et al</i> <sup>[66]</sup>	216 Chinese	Liver recipients	Recipients with *1/*1 genotype had higher dosage requirements than those with *3/*3 genotype The study suggested also that CYP3A5 enzymatic activity is increased proportionally by the presence of the *1 allele
Jun <i>et al</i> <sup>[98]</sup>	568 Korean	Kidney and liver recipients (n = 506), and liver donors (n = 62)	Patients with the *3 alleles had higher tacrolimus dose-adjusted trough levels than patients with the *1 allele *1/*1 patients may be more rapid metabolizers than *1 heterozygous patients
Elens <i>et al</i> <sup>[99]</sup>	150 Belgian	Liver donors	Those patients with at least one *1 allele had at least 67% higher tacrolimus dose requirements No influence of CYP3A5 expression on tacrolimus hepatic concentrations
Macphee <i>et al</i> <sup>[100]</sup>	119 White, 23 Black, 26 South Asian, 12 Middle Eastern	Kidney recipients	Patients with at least one copy of the wild-type *1 allele achieved twofold lower dose-normalized tacrolimus blood concentrations compared with CYP3A5*3/*3 homozygote patients
Thervet <i>et al</i> <sup>[101]</sup>	168 Caucasian, 8 Black, 12 other	Kidney recipients	Pre-transplant dose adaptation, according to CYP3A5 genotype, is associated with improved achievement of the target blood trough levels
Spierings <i>et al</i> <sup>[102]</sup>	81 Caucasian, 12 Black, 20 South Asian, 5 other	Kidney recipients	Tacrolimus dose requirements were significantly higher in patients expressing the wild type CYP3A5 genotype Intra-patient variability of tacrolimus clearance was not associated with the same genotype
Chen <i>et al</i> <sup>[103]</sup>	120 Chinese	Kidney recipients	CYP3A5 expressers not receiving diltiazem required significantly higher tacrolimus doses than those who received the CYP inhibitor. In non-expressers, no significant difference in tacrolimus dose requirements was observed between the subjects treated with diltiazem and those who were not

dose ratio was significantly higher in recipients with the \*1/\*3 genotype than in those with the \*1/\*1 one, and again higher in \*3/\*3 patients rather than in heterozygous \*1/\*3 recipients, suggesting that \*1 homozygous patients may be even more rapid metabolizers than heterozygous patients expressing only one \*1 allele (Table 2).

A study by Elens *et al*<sup>[99]</sup> on 150 liver donors found that tacrolimus dose requirements were at least 67% higher among patients with at least one *CYP3A5*\*1 allele and expressing hepatic CYP3A5 (Table 2). However, though hepatic CYP3A5 expression reduced blood tacrolimus levels and increased dose requirements, it failed to influence hepatic tacrolimus concentrations, which may be better related to liver graft outcome<sup>[99]</sup>.

Another study, by Macphee *et al*<sup>[100]</sup>, in white and South Asian renal transplant patients, suggested that patients with at least one copy of the wild-type \*1 allele achieved twofold lower dose-normalized tacrolimus blood concentrations compared with *CYP3A5*\*3/\*3 homozygote patients (Table 2).

In a prospective study involving 280 kidney transplant patients, Thervet *et al*<sup>[101]</sup> found that a pre-transplant tacrolimus dose adaptation according to the CYP3A5 genotype is associated with fewer successive dose modifications and with a rapid achievement of target trough levels (Table 2).

In a more recent study by the Macphee's group on 118 renal transplant patients, Spierings *et al*<sup>[102]</sup> confirmed that the tacrolimus dose requirements were significantly higher in patients with the wild type *CYP3A5* genotype (Table 2). However, they also found that intra-patient variability of tacrolimus clearance was not associated with the wild type *CYP3A5* genotype.

Finally, in a 42-mo, prospective, randomized, parallel-controlled, open-label, single-center study, 62 Chinese CYP3A5 expressers and 58 non-expressers who had received kidney transplants were randomized to receive 30 mg of diltiazem (a known CYP inhibitor) three times daily in order to assess the efficacy of the drug as a calcineurin sparing agent. Patients who were known to be CYP3A5 expressers and did not receive diltiazem required significantly higher tacrolimus doses than the other groups ( $P = 0.017$ ). Among the CYP3A5 non-expressers, there was not a significant difference in tacrolimus dose requirements between the subjects treated with diltiazem and those who were not. This was expected, as the proposed mechanism for diltiazem as a calcineurin sparing agent involves the inhibition of the metabolism of tacrolimus through the CYP3A5 pathway (Table 2). This suggests that CYP3A5 expressers are more susceptible to diltiazem-induced tacrolimus dose reductions and may possibly provide the prescribers with a mechanism able to limit the cost of immunosuppressive therapy as well as to treat concomitant hypertension in transplant patients<sup>[103]</sup>.

### ABCB1

Data showing a link between a patient's ABCB1 genotype and tacrolimus pharmacokinetics have been inconsistent. Though most studies have failed to find any

association, some clinical trials have found a significant relation between the *ABCB1* genotype and tacrolimus dosing. These results are often confounded by the linkage disequilibrium expressed among genetic variants, underscoring the need for further research on *ABCB1* genetics before a definitive conclusion can be reached.

Provenzani *et al*<sup>[91]</sup>, in a study on 32 Caucasian liver transplant patients, found no influence of the 3435C>T and 2677G>T SNPs on tacrolimus dose requirements (Table 3). A study by Cho *et al*<sup>[84]</sup> on 70 Korean renal transplant patients also found no association between the ABCB1 genotype and tacrolimus dose requirements up to 6 mo after transplantation (Table 3).

Further supporting these results, Shi *et al*<sup>[66]</sup> found that in 216 Chinese liver transplant patients, there was no significant association between any of the *ABCB1* polymorphisms and daily tacrolimus dose requirements or trough levels (Table 3).

This was again confirmed by Jun *et al*<sup>[98]</sup>, who studied 506 Korean solid organ transplant recipients and 62 corresponding liver transplant donors. They found no correlation between the *ABCB1* patient genotype and tacrolimus concentration to adjusted dose ratios (Table 3).

Gervasini *et al*<sup>[33]</sup> also found that, in 103 renal transplant patients, none of the *ABCB1* polymorphisms were associated with altered dose-adjusted trough levels or increased dose requirements. This study also found no association between the ABCB1 genotype and tacrolimus-induced toxicity (Table 3).

Another study by Kuypers *et al*<sup>[104]</sup> found that in 304 kidney transplant patients the ABCB1 genotype had no significant impact on tacrolimus exposure parameters or dosing requirements (Table 3).

A study by Provenzani *et al*<sup>[92]</sup> on 51 liver and 50 kidney transplant patients found no association between the *ABCB1* polymorphisms and tacrolimus dosing among liver transplant patients, but did observe that kidney transplant patients carrying the 2677T/A allele required a significantly higher daily tacrolimus dose than patients homozygous for the wild type allele (Table 3).

Another study on 181 liver transplant recipients and 114 donors found that, in the first week post-transplantation, the recipients who displayed the wild type *MDR-1* allele and thus high *ABCB-1* activity in the intestine, had lower dose-adjusted tacrolimus trough levels than patients who displayed *MDR-1* variant alleles and were low ABCB-1 expressers, even among patients with the same liver CYP3A5 genotype. However, this difference was not observed after two weeks, suggesting that *MDR-1* expression in the intestine may contribute to tacrolimus trough levels in the first week post-transplantation; afterwards the transplanted liver would achieve a greater metabolic capacity and becomes the main organ that influences tacrolimus pharmacokinetics<sup>[63]</sup> (Table 3).

This was supported by Herrero *et al*<sup>[43]</sup> in a study on 71 renal transplant patients, in which it was found that patients with the wild type *ABCB1* genotype tended to have more stable tacrolimus concentrations within the



**Table 3** Effect of *ABCB1* single nucleotide polymorphism on tacrolimus pharmacokinetics

Authors	Study Population	Transplant type/analysis of recipients, donors or both	Findings
Provenzani <i>et al</i> <sup>[91]</sup>	32 Caucasian	Liver recipients and donors	No influence of 3435C>T and 2677G>T SNPs on tacrolimus dose requirements
Cho <i>et al</i> <sup>[84]</sup>	70 Korean	Kidney recipients	No association between <i>ABCB1</i> genotype and tacrolimus dose requirements
Shi <i>et al</i> <sup>[66]</sup>	216 Chinese	Liver recipients	No association between any <i>ABCB1</i> SNPs and tacrolimus dose requirements or blood trough levels
Jun <i>et al</i> <sup>[98]</sup>	568 Korean	Kidney and liver recipients ( <i>n</i> = 506), and liver donors ( <i>n</i> = 62)	No correlation between <i>ABCB1</i> genotype and tacrolimus dose-adjusted blood trough levels
Gervasini <i>et al</i> <sup>[33]</sup>	103 Spanish	Kidney recipients	None of the <i>ABCB1</i> polymorphisms were associated with changes in dose-adjusted blood trough levels and in dose requirements No association between <i>ABCB1</i> genotype and tacrolimus-induced toxicity
Kuypers <i>et al</i> <sup>[104]</sup>	304 Belgian	Kidney recipients	No significant impact of <i>ABCB1</i> genotype on tacrolimus exposure parameters or dosing requirements
Provenzani <i>et al</i> <sup>[92]</sup>	101 Caucasian	Kidney ( <i>n</i> = 50) and liver ( <i>n</i> = 51, recipients and donors)	No <i>ABCB1</i> influence on dosing in liver transplant patients Those patients receiving kidney transplant carrying the 2677T/A allele required significantly higher doses than those patients with the wild type allele
Goto <i>et al</i> <sup>[63]</sup>	181 Japanese	Liver recipients and donors	In the first week after transplantation, the recipients with wild type <i>ABCB1</i> allele had lower tacrolimus dose-adjusted blood trough levels No difference observed after 2 wk
Herrero <i>et al</i> <sup>[43]</sup>	71 Spanish	Kidney recipients	Patients with wild type <i>ABCB1</i> alleles had more stable tacrolimus concentrations within the therapeutic range during the first 3 mo On the contrary, patients carrying the polymorphic <i>ABCB1</i> alleles showed a mean increase in tacrolimus blood concentration of more than 60%
Wei-Lin <i>et al</i> <sup>[88]</sup>	50 Chinese	Liver recipients and donors	Recipients with the wild type <i>ABCB1</i> -3435CC allele had significantly higher tacrolimus dose requirements than those with C3435T at 1 and 2 wk and 1 mo after transplantation
López-Montenegro Soria <i>et al</i> <sup>[97]</sup>	35 Spanish	Kidney recipients	Wild type <i>ABCB1</i> 3435CC patients had 40% lower concentration/dose ratios than those patients with variant alleles
Elens <i>et al</i> <sup>[99]</sup>	150 Belgian	Liver donors	<i>ABCB1</i> genetic polymorphisms significantly influence tacrolimus hepatic concentrations, but have no effect on tacrolimus blood levels Patients with <i>ABCB1</i> 1236C>T polymorphism showed significantly better liver functions and lower Banff scores with respect to patients with the wild-type allele

therapeutic range during the first 3 mo after transplantation, while patients expressing polymorphic *ABCB1* alleles showed a mean increase in the drug blood concentrations greater than 60% due to a diminished elimination capacity by the body (Table 3).

A study on 50 Chinese liver transplant donors and recipients also evidenced that daily tacrolimus dose requirements were significantly higher in recipients carrying the wild type *ABCB1*-3435CC rather than the C3435T allele at the weeks 1 and 2 and at 1 mo post-transplantation (Table 3). These data suggested that in Chinese people the *ABCB1* genotype plays a dominant role in the intestinal tacrolimus pharmacokinetics<sup>[88]</sup>: in fact patients with the wild type *MDR-1* genotype are more likely to extrude tacrolimus from enterocytes and therefore need a higher daily dose to achieve adequate blood tacrolimus levels.

López-Montenegro Soria *et al*<sup>[97]</sup>, in a study on 35 renal transplant patients, also found that patients expressing the wild type *ABCB1*-3435CC genotype showed up to 40% lower concentration/dose ratios compared with patients carrying variant alleles (Table 3).

Finally, a study by Elens *et al*<sup>[99]</sup> on 150 liver transplant patients found that *ABCB1* genetic polymorphisms in the donors significantly influenced tacrolimus concentrations in the liver, but failed to influence the drug mean blood levels. The *ABCB1*-1236C>T polymorphism was also associated with improved liver function and significantly lower Banff scores compared with the situation of patients with the wild type allele (Table 3). These data suggest that *ABCB1* polymorphisms may be important in liver transplant patients due to their effects on tacrolimus levels in the liver, which, as already said, may be a good marker to predict the liver graft rejection.

## INFLUENCE OF GENETICS ON TACROLIMUS PHARMACODYNAMICS

Despite many studies have demonstrated a strong association between *CYP3A5* genotype and alterations in tacrolimus pharmacokinetics, the results do not provide consistent evidence of organ rejection or drug-related toxicity as a consequence of genotype-related sub- or supra-therapeutic immunosuppression. This is likely due to the fact that the

patients are closely monitored in the first period following transplantation and undergo dose adjustments to more rapidly achieve target trough drug levels. However, different clinical trials have begun to explore the practical pharmacodynamics implications of genetic alterations in tacrolimus pharmacokinetics, and some of them have found clinically significant results.

Jun *et al.*<sup>[98]</sup> found no significant difference in the incidence of organ rejection in 506 Korean solid organ transplant recipients and 62 liver transplant donors after comparing both patients' genotypes and mean tacrolimus concentration per an adjusted dose ratios (Table 4).

Another study by Chen *et al.*<sup>[103]</sup> on 120 Chinese kidney transplant patients who were a mix of CYP3A5 expressers and non-expressers, found that patients who received genotype-guided initial tacrolimus dosing achieved target drug levels more rapidly than the patients who received a standard protocol dose of tacrolimus (90.9% *vs* 27.3% of patients in target range, respectively). However, no differences were observed between the two groups with respect to the incidence of leukocytopenia, nephropathy, abnormal liver function, hyperlipidemia, diarrhea or hyperglycemia (Table 4).

Jacobson *et al.*<sup>[105]</sup>, in a prospective study on 945 kidney transplant patients, found that every increase in tacrolimus trough level of 1 ng/mL increased the hazard of early calcineurin-inhibitor-associated nephrotoxicity by 22%, even after adjusting for clinical factors. Nine SNPs of the *XPC*, *CYP2C9*, *PAX4*, *MTRR* and *GAN* genes exhibited an association with cyclosporine, but not with tacrolimus, nephrotoxicity (Table 4).

In a prospective, open-label, observational cohort study, Kuypers *et al.*<sup>[106]</sup> found that among 304 kidney transplant patients, the proportion of patients who developed new-onset diabetes after transplant (NODAT) was significantly higher in patients with delayed graft function and who displayed trough tacrolimus levels greater than 15 ng/mL on the first day post-transplantation. In this study, the presence of the *CYP3A5\*1* allele and a functional *CYP3A5* enzyme appeared to attenuate the effects of delayed graft function on initial tacrolimus exposure and dose requirements, suggesting that CYP3A5 expressers may be at lower risk of NODAT following kidney transplantation due to diminished exposure to potentially toxic levels of tacrolimus (Table 4).

In a separate study, but in the same population of 304 kidney transplant patients, Kuypers *et al.*<sup>[104]</sup> found that calcineurin-inhibitor-associated nephrotoxicity (CNIT) was more common in patients carrying the *CYP3A5\*1* allele than in patients who did not (32.4% *vs* 15.2%). Additionally, these researchers observed that CNIT developed in 25% of patients with dose requirements exceeding 0.2 mg/kg per day, 16.2% of patients with doses between 0.1-0.2 mg/kg per day and 4.5% of patients needing less than 0.1 mg/kg per day; the carriers of the *CYP3A5\*1* allele predominantly comprised the higher tacrolimus dose ranges. These results suggest that patients expressing the *CYP3A5\*1* allele and a functional CYP3A5 enzyme may

be predisposed to developing CNIT following transplantation due to greater daily tacrolimus dose requirements. This was observed especially in patients who continued corticosteroid therapy (Table 4). However, the incidence of delayed graft function and post-transplant diabetes mellitus was not different between CYP3A5 expressers and non-expressers.

In a more recent study, on 319 Hispanic kidney transplant patients, other authors found that the SNPs in the cytoplasmic nuclear factor of activated T cells 4 (NFATc4) gene, which is expressed in pancreatic islets, may confer a certain protection or also a predisposition with regard to NODAT; in particular, the patients carrying the SNP (rs10141896) T allele (T-T-T-T-G haplotype) showed a protection from NODAT, while patients homozygous for the C-C-C-G-G haplotype were associated with increased risk of NODAT. Furthermore, the authors found that the use of sirolimus and tacrolimus and a more advanced age (> 45 years) were also possibly correlated to the development of NODAT<sup>[107]</sup> (Table 4).

Cho *et al.*<sup>[84]</sup> found that in 70 Korean renal transplant patients tacrolimus toxicity was more frequent in the subjects with *CYP3A5\*1* alleles, who had significantly higher dose requirements of the drug than patients expressing the \*3 polymorphism (Table 4). Despite these findings, the study found no difference in the rate of graft survival between the various genotype-differentiated study groups.

A study by Barrera-Pulido *et al.*<sup>[94]</sup> on 53 liver transplant recipients found that patients with the *CYP3A5\*3*/*\*3* genotype receiving the organs from donors with an *ABCB1* polymorphism had a lower frequency of renal dysfunction, the same rejection rate and a higher rate of diabetes than the other groups studied (Table 4).

However, Shi *et al.*<sup>[66]</sup> found that in 216 Chinese liver transplant patients, carriers of the *CYP3A5\*3* allele had an increased risk of early renal injury compared with expressers of the *CYP3A5\*1* allele, possibly due to decreased enzymatic activity and higher dose-adjusted trough concentrations (Table 4).

## CONSIDERATIONS FOR FURTHER GENETIC RESEARCH

In addition to the previously discussed genetic polymorphisms, a number of other variants that may potentially influence the pharmacokinetics and pharmacodynamics of tacrolimus and transplant outcomes have been proposed for further study.

### P450 oxidoreductase\*28

Cytochrome P450 oxidoreductase (POR) is essential for the electron donation in the microsomal-CYP450-mediated mono-oxygenation that catalyzes the metabolism of approximately 85%-90% of therapeutic drugs. More than 40 SNPs have been identified in the *POR* gene, and it has been suggested that several of these mutations, specifically the *POR\*28-C>T* polymorphism, can increase this

**Table 4 Effect of various single nucleotide polymorphisms on tacrolimus pharmacodynamics**

Authors	Study Population	Transplant type/analysis of recipients, donors or both	Findings
Jun <i>et al</i> <sup>[98]</sup>	568 Korean	Kidney and liver recipients (n = 506) , and liver donors (n = 62)	No difference in incidence of organ rejection between different genotypes
Chen <i>et al</i> <sup>[103]</sup>	120 Chinese	Kidney recipients	Patients that received genotype-guided initial tacrolimus dosing vs standard protocol dose were more likely to achieve target drug levels No influence on incidence of adverse effects between CYP3A5 expressers and non-expressers
Jacobson <i>et al</i> <sup>[105]</sup>	945 (different ethnicities)	Kidney recipients	Every increase in tacrolimus blood trough level of 1 ng/mL increased the risk of early tacrolimus nephrotoxicity by 22% Polymorphism was not associated with an increased or decreased risk of tacrolimus-related nephrotoxicity
Kuypers <i>et al</i> <sup>[106]</sup>	273 White, 3 Hispanic, 24 North African, 2 African, 2 Asian	Kidney recipients	Delayed graft function was associated with higher initial mean tacrolimus blood trough levels and lower tacrolimus daily dose requirements, especially in CYP3A5 non-expressers CYP3A5 expressers may be at lower risk of new-onset diabetes after transplant (NODAT) due to diminished exposure to potentially toxic tacrolimus levels
Kuypers <i>et al</i> <sup>[104]</sup>	273 White, 3 Hispanic, 24 North African, 2 African, 2 Asian	Kidney recipients	Patients expressing the CYP3A5*1 allele and a functional CYP3A5 enzyme may be predisposed to developing calcineurin-inhibitor-associated nephrotoxicity (CNI) following transplantation due to greater daily tacrolimus dose requirements This was observed especially in patients continuing corticosteroid therapy The incidence of delayed graft function and post-transplant diabetes mellitus was not different between CYP3A5 expressers and non-expressers
Chen <i>et al</i> <sup>[107]</sup>	319 Hispanic	Kidney recipients	SNPs in the cytoplasmic NFATc4 gene may confer a certain protection or also predisposition for NODAT. Patients carrying the T allele and the T-T-T-T-G haplotype showed a trend of protection from NODAT while patients with the C-C-C-G-G haplotype were associated with an increased risk of NODAT The use of sirolimus and tacrolimus and advanced age were also possibly correlated in development of NODAT
Cho <i>et al</i> <sup>[84]</sup>	70 Korean	Kidney recipients	Higher drug-related toxicity in patients with the CYP3A5*1 allele than in those with the CYP3A5*3 SNP No difference in graft survival between the two genotypes
Barrera-Pulido <i>et al</i> <sup>[94]</sup>	53 Spanish	Liver recipients and donors	Patients with CYP3A5*3/*3 allele receiving livers with an ABCB1 SNP had lower frequency of renal dysfunction, same rejection rate and higher diabetes rate
Shi <i>et al</i> <sup>[66]</sup>	216 Chinese	Liver recipients	Patients with the CPY3A5*3 allele had greater risk of early renal injury than the patients with the *1 allele

activity and alter the baseline metabolic capacity of several CYP isoforms. The \*28 allelic variant has been found to be expressed in 19.1% of African-Americans, 26.4% of Caucasian Americans, 31.0% of Mexican Americans, and 36.7% of Chinese Americans<sup>[108]</sup>.

A study by Zhang *et al*<sup>[109]</sup> on 71 healthy Chinese volunteers found that the mean tacrolimus AUC<sub>(0-24)</sub> and C<sub>max</sub> (71.5 and 17.6 ng/mL, respectively) for patients who were CYP3A5 expressers as well as carriers of the wild type CC POR genotype were 1.53 and 1.57 fold higher than those (46.7 and 11.2 ng/mL) observed in patients carrying POR allelic variants. No significant differences were observed between POR\*28-CC homozygotes and POR\*28-T carriers in CYP3A5 non-expressers, suggesting that the POR genotype is important in altering tacrolimus metabolism only in CYP3A5 expressing patients.

These results were supported by a cohort study of de Jonge *et al*<sup>[110]</sup> on 298 renal transplant recipients, which it was found that in CYP3A5 expressers, POR\*28T allele carriers had lower trough tacrolimus levels in the first three

days post-transplant and took longer to reach the target trough levels when compared with POR\*28CC homozygous patients. These patients with the variant POR genotype ultimately had 25% higher tacrolimus dose requirements than patients expressing the wild type allele. Again, POR\*28 polymorphisms were found to have no influence on tacrolimus pharmacokinetics in CYP3A5 non-expressers, and no differences in transplant outcomes were observed between the study groups.

### CYP3A7

Previously thought to be confined to the fetal liver, CYP3A7 has been found to be expressed in up to 54-88% of adult livers, but with a diminished metabolic capacity compared with that observed in children<sup>[111,112]</sup>. The role of CYP3A7 in the biotransformation of the CYP3A substrates in the adult liver and intestine is unknown. However, it was observed that CYP3A7 expression in the adult liver and intestine is increased in the carriers of the CYP3A7\*1C allele<sup>[112,113]</sup>. This allele has a very low frequency (3%) both

in Caucasians and African-Americans<sup>[61]</sup>.

Although tacrolimus is believed to be a substrate for the CYP3A7 enzyme, the influence of CYP3A7 metabolism on the pharmacokinetics of tacrolimus requires further study, especially in pediatric patients<sup>[99]</sup>.

### **CYP3A4\*18B**

This *CYP3A4* polymorphism appears only in Asian (25%-30%)<sup>[114]</sup>, primarily Korean, populations, but has been linked with a potentially increased metabolic capacity of the CYP3A4 enzyme. It has also been shown that carriers of the *CYP3A5\*1* allele are more likely to possess the *CYP3A4\*18B* allele. As a result, further study is required to determine whether linkage disequilibrium with the *CYP3A5\*1* allele may confound the observed metabolic effects of the *CYP3A4\*18B* polymorphism. One study, by Jun *et al.*<sup>[98]</sup>, found no correlation between the *CYP3A4\*18B* allele and tacrolimus concentration to adjusted dose ratios in 506 Korean solid organ transplant recipients. A study of 22 healthy Chinese people showed a higher tacrolimus clearance in patients carrying the *CYP3A4\*18B* allele with respect to those carrying the *CYP3A4\*1* allele<sup>[115]</sup>. A more recent study, by Li *et al.*<sup>[116]</sup>, on 83 Chinese renal transplant recipients confirmed the results of the previous study. It found that the tacrolimus-dose-adjusted trough concentration was significantly lower in patients carrying the *CYP3A4\*18B* allele compared with patients with the *CYP3A4\*1* allele.

### **CYP3A4\*22**

A new *CYP3A4* allele (*CYP3A4\*22*; rs35599367 C>T in intron 6) was recently discovered and also investigated in transplant patients<sup>[117,118]</sup>.

In particular, a study on 185 renal transplant patients, mostly Caucasians, evaluated the impact of this new SNP on tacrolimus pharmacokinetics. It showed that in the first year after transplantation, patients carrying one or two T alleles required significantly lower tacrolimus doses (33%) compared with patients homozygous for the wild-type C allele<sup>[118]</sup>. The authors attributed the result to the fact that this *CYP3A4\*22* SNP is significantly linked to reductions in *CYP3A4* mRNA production and enzyme activity in human livers<sup>[118-120]</sup>. This SNP is relatively frequent in Caucasians (2.5%-6.9%). The authors also suggested that, though further studies are necessary, that pre-transplant genotyping of the *CYP3A4* C>T could reduce the risk of achieving supra-therapeutic tacrolimus levels<sup>[118]</sup>.

However, in a study done on Brazilian renal transplant patients, *CYP3A4\*22* was not associated with changes in tacrolimus dose requirements<sup>[121]</sup>.

### **CYP2C8 and CYP2J2**

These enzymes, which are polymorphically expressed in the kidney, are involved in the synthesis of epoxyeicosatrienoic acids that play a protective role against acute rejection and toxicity by acting as vasodilators to maintain adequate renal perfusion and limit hypertension.

In a study on 163 liver transplant patients the authors

found that patients with the *CYP2C8\*3* variant genotype appeared to be at higher risk of tacrolimus-induced kidney disease, possibly because of reduced formation of the kidney protecting epoxyeicosatrienoic acids<sup>[122]</sup>.

In another study, on 103 renal transplant patients, the authors found a higher incidence of delayed graft function and nephrotoxicity in patients homozygous for the *CYP2C8\*3* genotype, associated with reduced epoxyeicosatrienoic acid production and, consequently, less vasodilator activity<sup>[33]</sup>.

In a more recent study the same research group could associate both *CYP2C8\*3* and donor age (> 48 years) with a higher incidence of delayed graft function and poorer creatinine clearance<sup>[123]</sup>.

### **SLC01B1**

This gene is responsible for expressing the organic anion transporting polypeptides *OATP1B1* and *OATP1B3*. These transporters play a role in the transport of multiple compounds from the portal vein to hepatocytes and in the biliary excretion of many drugs. Recently, Elens *et al.*<sup>[99]</sup> found that the 388A>G and 521T>C polymorphisms in the *SLC01B1* gene influenced tacrolimus trough blood concentrations after the administration of the first dose in 150 liver transplant patients. In this study, patients expressing the 388 polymorphism showed a lower mean tacrolimus blood level, while alterations of the 521 allele resulted in significantly greater trough drug levels. It was also recently demonstrated that cyclosporine and tacrolimus are inhibitors of the organic anion transporters, so that one cannot exclude the possibility that these drugs may be substrates of *OATP1B1* and *OATP1B3* as well.

### **Angiotensinogen C3889T (rs4762) gene polymorphism**

It is well known that tacrolimus has a negative effect on pancreatic beta islet cells and can cause glucose intolerance and diabetes mellitus<sup>[124]</sup>. However, new studies have suggested that post-transplant diabetes mellitus can also be related to other factors and, consequently, not only to tacrolimus administration<sup>[124,125]</sup>. Angiotensinogen (AGT) is the initial component of the renin-angiotensin system (RAS) and a precursor of both angiotensin I and II. In a study on 302 subjects, the authors found that the AGT gene polymorphism (rs4762) is associated with post-transplant diabetes mellitus, due to insulin resistance, in Korean renal transplant patients<sup>[126]</sup>. Molecular and genetic studies demonstrate a relationship between variants of the AGT gene, AGT gene expression and plasma AGT levels<sup>[127,128]</sup>. However, the association between this gene and glucose metabolism remain controversial.

## **DISCUSSION**

As clinical trials continue to evaluate the influence of genetics on drug dosing and response, the challenge now becomes to assess the potential clinical implications of this research for medical practice. Sufficient data has been accumulated to be certain that the liver donors and renal re-

ipients CYP3A5 genotype has important influences on tacrolimus dosing and on its blood through levels. However, it remains the question whether genotyping should become a standard practice in transplantation.

This question is difficult to answer because of the multi-factorial approach needed to assess the pharmacokinetic profile of a drug. Wide variability of tacrolimus dosing requirements to reach target blood levels has been observed even among patients carrying the same genotype. This underlies the fact that genetic polymorphisms are only one of the possible factors that can influence tacrolimus pharmacokinetics. Patient age, race, metabolic level, concomitant medications and a variety of other environmental factors appear to play an even more significant role than genotype in altering drug pharmacokinetics. Specifically in liver transplant patients, time after transplantation also plays a critical role in altering drug metabolism and distribution. The intestine may play a more important role soon after liver transplantation, before the liver recovers from the trauma of surgery and resumes a higher level of metabolic capacity. As liver function improves, hepatic synthesis of albumin also increases, which, in turn, decreases the unbound fraction of tacrolimus and lowers drug clearance. This is just one example of the many considerations that can ultimately impact the pharmacokinetics of an agent and highlights the difficulties in basing drug dosing on just one parameter.

To further complicate the issue, studies have yet to demonstrate a clear association between tacrolimus blood trough levels, genotype and transplant outcomes. Organ rejection and drug toxicities have been seen to develop in patients without any notable difference in tacrolimus blood concentration, making difficult to predict the optimal trough drug targets in relationship to the characteristics of the individual patient. Toxicities associated with tacrolimus are also often difficult to study because of their insidious onset. Hypertension, hyperlipidemia and NO-DAT develop slowly over a period of many years, making the length of a trial an issue when one wants to monitor these chronic medication effects. The mechanisms of such adverse effects are, again, not fully understood and require further research to determine the need to genotype patients, not only as a way of lowering the incidence of organ rejection, but also of preventing drug toxicity after transplantation.

Studies in transplantation are also often difficult to conduct because of the limited patient population. Many studies involve fewer than 100 patients, which may help explain some of the variable results. A number of these studies also differ in their pharmacokinetic methods, dosing strategies, times when blood drug concentrations are assessed and patient's characteristics. Differences between donor and recipient organ genotypes may also have confounded the results of some studies, as the genetics of both the recipient and of the donor were not always taken into account.

Genotyping is an attractive option for starting the dosing of tacrolimus; also, unlike phenotypic tests, the results

of which may vary with environmental factors, the genotype is a stable characteristic that needs to be determined only once for any given gene. However, to ultimately prove the usefulness of genotyping, prospective clinical studies must show that genotype determination before transplantation allows the better use of a given drug and improves the safety and clinical efficacy of that medication. Currently Amplichip, a genetic test manufactured by Roche Pharmaceuticals, can determine a patient's *CYP2D6* and *CYP2C19* polymorphisms for between United States \$350 and \$400, not including the mark up and other costs associated with the test. As a result, to offset the cost of genetic testing, genotypic analyses must demonstrate the ability to significantly improve transplant patient outcomes, in particular, graft life and patient survival, and show a cost saving for patients and for the health care system as a whole.

## CONCLUSION

At present, research has been able to reliably show that the CYP3A5, but not the CYP3A4 or ABCB1, genotype modifies the pharmacokinetics of tacrolimus. However, it has not been possible to incontrovertibly show that the corresponding changes in the pharmacokinetic profile are linked with different patient outcomes regarding tacrolimus efficacy and toxicity. Additionally, given the high cost of genotypic tests and the wide availability and utility of therapeutic drug monitoring, genotyping all transplant patients is not convenient for many individuals or Institutions. This may change in the near future as further studies on pharmacogenetics will produce new data and the improvements in the genotyping analyses will drive down the costs associated with this type of tests. For these reasons, pharmacogenetics and individualized medicine remain a fascinating area for further study and may ultimately become the face of future medical practice and drug dosing.

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