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Review Article

Pharmacogenetics of Tacrolimus in Solid Organ Transplant Patients

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Abstract

Tacrolimus (Tac) is one of the most important immunosuppressant drugs used in organ transplants. The primary enzymes responsible for the metabolism of Tac are CYP450, CYP3A4, and CYP3A5. Moreover, Tac has a narrow therapeutic index, and dose monitoring and adjustment are required. Single nucleotide polymorphisms (SNPs), particularly in the *CYP3A4*, *CYP3A5*, and *ABCB1* genes, have been connected to changes in Tac metabolism and clearance, especially *CYP3A5* genotypes wield a substantial influence on the variation in Tac metabolism and clearance. Its pharmacokinetics has also been widely studied in relation to *ABCB1* polymorphisms, and some studies have demonstrated a notable impact on Tac hepatic concentrations. However, contradictory findings also have been documented, highlighting the necessity for more research in this field. In this manuscript, we compiled, reviewed, and discussed pharmacogenetic studies showing associations of genetic polymorphism with the efficacy and/or adverse effects of Tac in solid organ transplant patients. We argue that knowledge of the function of genetic markers—specifically, SNPs in the *CYP3A4*, *CYP3A5*, and *ABCB1* genes—is essential to maximizing the use of Tac in clinical settings. This knowledge makes it possible to create customized treatment plans that will improve the effectiveness and safety of the drug for organ transplant recipients. Tac medication tailored to each patient based on genetic markers presents a viable way to enhance outcomes for organ transplant recipients. We recommend that Tac dosage be customized to each patient's distinct genetic profile by identifying particular SNPs in the *CYP3A4*, *CYP3A5*, and *ABCB1* genes. The danger of side effects can be thus reduced and the effectiveness of the drug can be maximized.

Keywords: Solid organ transplant, tacrolimus, single nucleotide polymorphism, genetic variation, efficacy, adverse effects

1. Introduction

There The liver is one of the vital organs of the body. It is responsible for the detoxification of various drugs in the body (Abdel-Misih and Bloomston 2010). Morphologically, it is the largest gland of the body is the liver, weighing approximately 1.3 to 1.5 grams (Ghallab 2013, Gijsen et al. 2013). A thin layer of connective tissue called the 'Glissons capsule' surrounds the liver. It lies in the abdominal cavity which is related caudal (posterior) to the diaphragm. It consists of four lobes, namely the right, left, median, and caudate lobes, and a small lobe known as the papillary lobe. The falciform ligament is attached to the median lobe, while the left lobe faces the peritoneal cavity. The caudate lobe has a visceral surface and the right lobe has the anterior and the posterior surfaces. The gall bladder lies between the median lobes at the site of the attachment of the falciform ligament (Malarkey et al. 2005). In human beings, the liver has superior, inferior, right, left, anterior, and posterior surfaces, with a distinct inferior border. The anterior, superior, and right surfaces are continuous with each other, having no separate borders. It is collectively called the

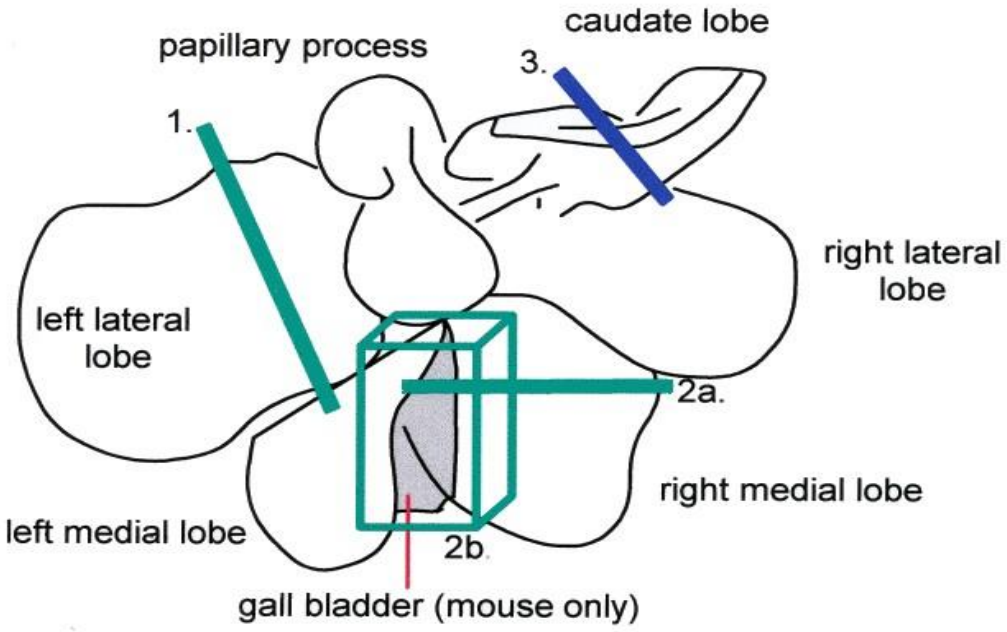

Figure 1: Anatomy of the liver (Abdel-Misih and Bloomston 2010).

diaphragmatic surface. The inferior surface is also the visceral surface because of its relation to the viscera (Favelier et al. 2015).

2. Causes of Liver Transplant

The prevalence of liver transplant (LT) is steadily increasing, driven by a range of factors such as viral, immunological, and bacterial diseases that significantly impact the health of liver cells. The liver's susceptibility to damage is attributed to various causes, with cirrhosis being a predominant factor. This condition often arises from infections, particularly those caused by Hepatitis B or Hepatitis C viruses. The subsequent list outlines the primary contributors to liver failure, necessitating the requirement for LT:

2.1. End-stage liver disease

The most common reason for LT is end-stage liver disease (ESLD). The most common cause for this is cirrhosis of the liver. It involves viral hepatitis, biliary cirrhosis, and chronic cholangitis (Gijsen et al. 2013)

2.2. Liver failure

The last resort after liver failure is LT. Many factors such as metabolic diseases, acute liver shutdown, and hepatocytic injury due to ingestion of certain chemicals may also lead to liver failure (Wiegand and Berg 2013).

2.3. Hepatocellular Carcinoma (HCC)

Another disease requiring LT is HCC. Alpha-feto protein is the diagnostic marker for the detection of HCC.(Tsochatzis, Bosch, and Burroughs 2014)

2.4. Biliary Atresia

This is a rare but congenital disease where either the bile duct is blocked or absent. LT is the only treatment of choice for this disease (Hartley, Davenport, and Kelly 2009).

3. Drugs Used in Liver Transplant

Many immunosuppressants play vital roles in the acute rejection of transplants (Addolorato et al. 2016). Tac is the most important and commonly used drug in transplant patients. It helps to combat the immune system towards new organs and it helps reduce the chances of acute rejection. Cyclosporine is another immunosuppressant agent which is also used in transplant patients. Similarly, mycophenolate mofetil, an immunosuppressant drug, also plays a critical role in transplant patients. It modulates

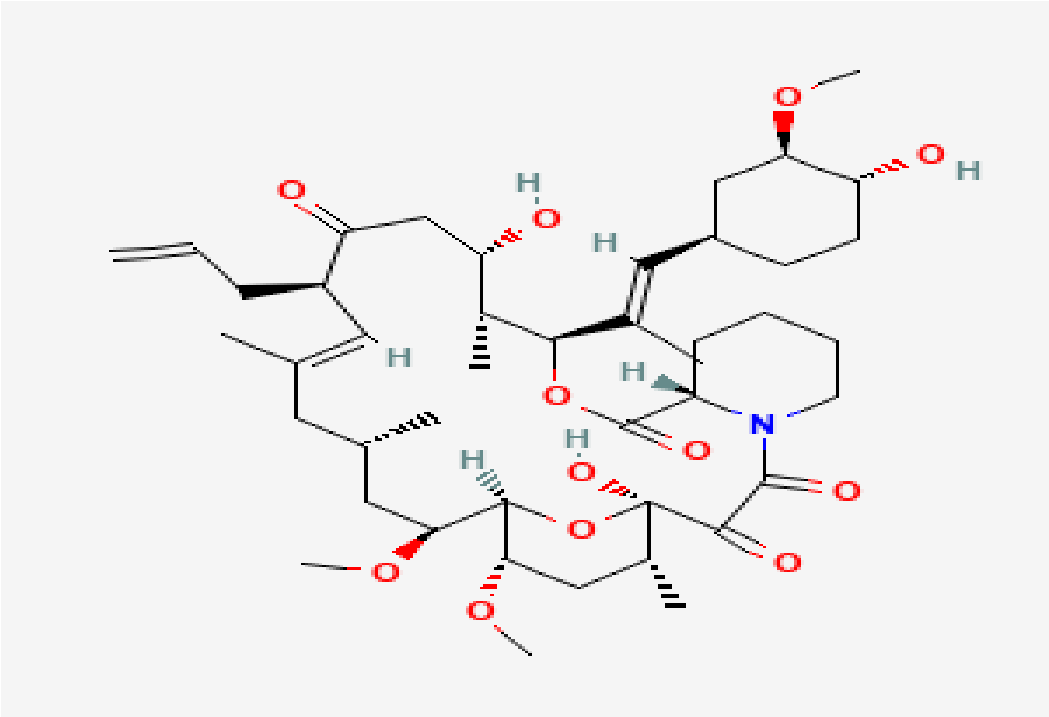

Figure 2: Chemical structure of Tac (Sehgal, Srivastava, and Dogra 2008).

the function of proliferating T cells and B cells. Furthermore, steroids also play an important role in suppressing the immune response (Keeffe 2001, Mukherjee and Mukherjee 2009).

The most important calcineurin inhibitor (CNI) is Tac, which is used to prevent acute rejection after transplantation. Tac has a low therapeutic index, therefore, its use has been complicated by its toxicity potentials and high interindividual pharmacokinetic variability (Abdel-Misih and Bloomston 2010). Hence, therapeutic drug monitoring (TDM) becomes pivotal for treatment success for those patients receiving Tac therapy. Various recipients of solid organ transplants (SOT) experience the toxic effect of Tac despite TDM. In light of the above-mentioned facts, it is high time that pharmacogenomics studies on Tac metabolism and its biotransformation were conducted. Various proteins play an important role in Tac pharmacokinetics, including genetic variations among individuals (Azzi, Sayegh, and Mallat 2013).

Tac was initially discovered in the early 1980s. It was isolated from the fungus *Streptomyces tsukubaensis*, found in soil samples collected in Japan. Scientists working on the sample named this compound FK-506 (Hamawy 2003). Various trials were conducted on this sample, and finally, in 1994 it got approval from The Food and Drug Authority (FDA) and was named Tacrolimus (Tac).

Tac bonds to an immunophilin, FK506 binding protein (FKBP). This complex inhibits calcineurin phosphatase. The drug inhibits calcium-dependent events, such as interleukin-2 gene transcription, nitric oxide synthase activation, cell degranulation, and apoptosis (Thomson, Bonham, and Zeevi 1995).

4. Pharmacogenetics of Tacrolimus

4.1. ABCB1

ABCB1 is a drug-efflux pump (encoded by the *ABCB1* gene), and Tac is a substrate for this pump. (Tang et al. 2016) This *ABCB1* gene is expressed in the intestine and is thought to limit the absorption of Tac (Matas et al. 2013).

Interindividual differences in the expression and/or function of *ABCB1* determine the variability in the bioavailability of the drug. Tac after oral intake is metabolized in the intestine, biotransformation takes place in the liver, and then to a limited degree in the kidney by cytochrome (CYP) P450, CYP3A4, and CYP3A5. However, CYP450 is the most important enzyme for Tac metabolization (Staatz, Goodman, and Tett 2010, Gijsen et al. 2013).

ABCB1 is considered to be accountable for the low oral bioavailability of Tac and is also considered important for the pharmacokinetics of it throughout the body, and its elimination through kidney and bile. *ABCB1* gene has more than 55 SNPs. Among them, 3435C>T (rs1045642), 1236C>T (rs1128503), and 2677G>T/A (rs2032582) SNPs, which are in linkage disequilibrium, are the most notable (Shuker et al. 2012).

The significance of these SNPs on *ABCB1* expression and function is still not well understood. It has been evaluated that the synonymous *ABCB1* 3435C>T SNP affects co-translational folding duration and insertion of ABCB1 into the membrane, thereby changing the structure of substrate and attachment sites. Although numerous studies have investigated the impact of *ABCB1* SNPs on Tac pharmacokinetics, the results are still conflicting and suggest a limited impact of *ABCB1* SNPs on Tac efficacy (Vafadari et al. 2013).

Vafadari et al. conducted another study and found that patients with the *ABCB1* 3435CC genotype needed a higher concentration of Tac for IL-2 inhibition in T cells, as compared to 3435TT genotype patients. Capron et al. found that patients with *ABCB1* 3435T or the 2677T/A allele had 1.3-fold higher Tac concentrations within circulating lymphocytes compared with wild-type homozygotes (Capron et al. 2010, Klein et al. 2012)

4.2. CYP3A5

Polymorphisms in the *CYP3A5* gene explain 40–50% of the variability in Tac dose requirement (Press et al. 2009). One of the notably studied SNP in *CYP3A5* is *CYP3A5**3 (rs776746), whose position is on A to G transition at position 6986 within intron 3 (Haufroid et al. 2004).

The *CYP3A5**3 allele is responsible for alternative splicing, which leads to protein truncation and a severe decrease of functional CYP3A5 enzyme (Kuehl et al. 2001). Other *CYP3A5* SNPs include *CYP3A5**6 (rs10264272), and CYP3A5*7 (rs41303343). *CYP3A5**6 encodes a 14690G>A transition, causing a splice variant and deletion of exon 7, resulting in a nonfunctional CYP3A5 protein. CYP3A5*7 denotes a single base insertion at codon 346, causing a frameshift mutation, which results in truncation of mRNA and nonfunctional CYP3A5 (Hustert et al. 2001).

People who are homozygous for the *CYP3A5**3 allele are referred to as *CYP3A5* non-expressers, whereas individuals carrying at least one *CYP3A5**1 allele are known as *CYP3A5* expressers. Further observations revealed that if there is a reduced enzymatic activity associated with the *CYP3A5**3 allele, leading to Tac dose reduction (Picard et al. 2016). *CYP3A5* expressers require almost 50% higher doses than those for *CYP3A5* non-expressers (Ruiz et al. 2015). This is one of the most predominant findings seen in adults, children, and among organ recipients (kidney, liver, heart, or lung transplant) (Yang et al. 2015).

Furthermore, body weight-based Tac dosing in *CYP3A5* expressers often leads to sub-therapeutic Tac concentrations during the initial post-surgery phase; therefore, they may be vulnerable to an acute rejection of the transplant (MacPhee et al. 2004, Zhang et al. 2005). In *CYP3A5* expressers, it was observed that they faced a delay in achieving the target Tac exposure, despite TDM (MacPhee et al. 2004). However, *CYP3A5* expressers did not experience more biopsy-proven acute rejection than non-expressers with a median of 7 versus 13 days. Although rejection did occur earlier among *CYP3A5* expressers. Other investigators

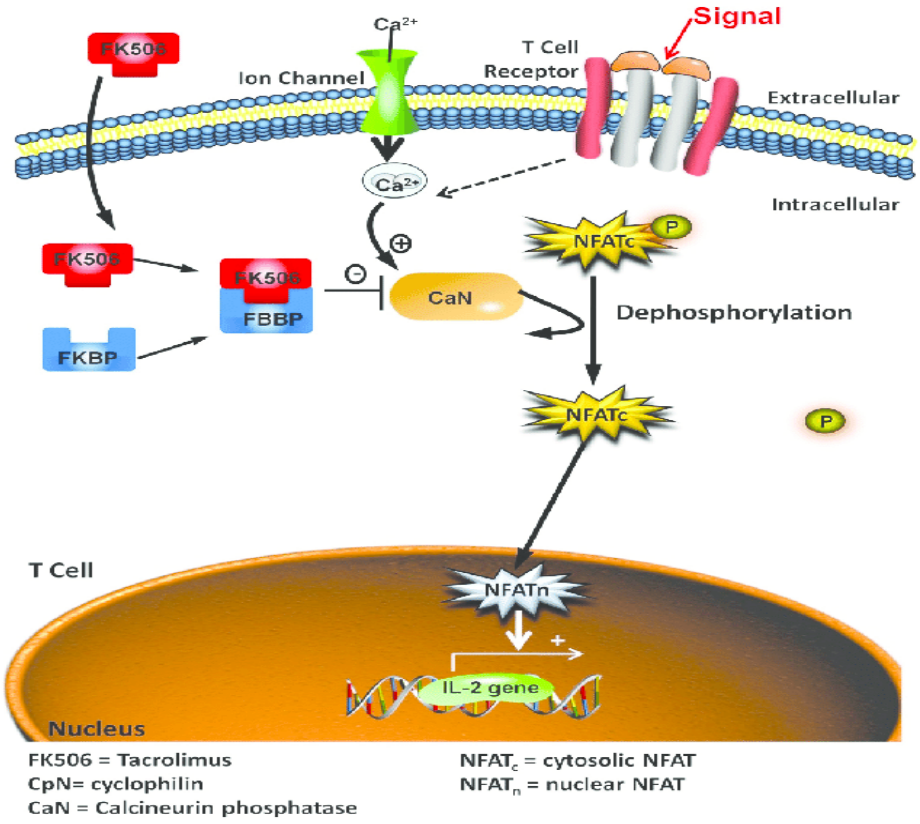

Figure 3: Mechanism of action of Tac (Letko et al. 1999)

have also reported that *CYP3A5* expressers do not have a higher risk of developing acute rejection (Roy et al. 2006).

Various studies have been published pertaining to the higher Tac dose requirement in *CYP3A5* expressers compared with the non-expressers. The clinical features of the association are still not thoroughly understood and only two randomized-controlled clinical trials (RCT) have shown the association between expressers and non-expressers. In a study involving 280 renal transplant patients, the impact of Tac doses was assessed. The participants were randomly assigned in a 1:1 ratio to either receive the standard Tac dose (0.1 mg/kg twice daily) or an initial dose tailored to the individual patient's CYP3A5 genotype (0.075 or 0.15 mg/kg twice daily for *CYP3A5* non-expressers and expressers) (Thervet et al. 2010).

The primary efficacy endpoint of this study focused on ensuring that Tac pre-dose concentration (C_0) remained within the target range of 10–15 ng/mL. Tac administration commenced on day 7 post-transplant, and the pre-dose concentration was assessed on day 10. During a 6-day period, patients were intentionally kept Tac-free to facilitate *CYP3A5* genotyping. In addition to genotyping, patients in this study received high-dose mycophenolate mofetil (MMF; 3 g/day), glucocorticoids, and induction therapy with rabbit anti-thymocyte globulin (rATG; administered in 82.2% of patients) or IL-2 receptor antibodies (administered in 17.8% of patients). The study observed that *CYP3A5* genotype-based Tac dosing resulted in a significantly higher percentage of patients achieving the target Tac concentration three days after initiating treatment compared to bodyweight-based

Tacrolimus dosing: 43.2% versus 29.1% (Pallet et al. 2016).

Interestingly, the group that received a *CYP3A5* genotype-based Tac dose needed significantly less time and fewer dose adaptations to reach the target (Shuker et al. 2016, Brunet et al. 2016).

4.3. CYP3A4

Another gene which also plays a significant role in Tac metabolism is *CYP3A4*. The SNPs for CYP3A4*1B (rs2740574), and CYP3A4*22 (rs35599367) are very commonly associated with variations in Tac dose. People carrying the *CYP3A4**1B allele were reported to have a 35% lower Tac dose-adjusted C_0 compared to individuals having the *CYP3A4* wild-type allele. Nevertheless, the question of whether *CYP3A4**1B is responsible for the modified Tac dose requirement remains a matter of debate (Birdwell et al. 2012).

The variant *CYP3A4**22 (rs35599367) is located in intron 6 of the *CYP3A4* gene, involving a C to T substitution at position g.15389. It was studied that *CYP3A4**22 increases the formation of the non-functional *CYP3A4* splice variant with partial intron 6 retention, thereby reducing the production of functional full-length CYP3A4 mRNA and decreasing CYP3A4 enzymatic activity. (Wang and Sadee 2016, Elens, Van Schaik, et al. 2011)

One of the studies, for the first time, demonstrated *CYP3A4**22 variant association with lower Tac dose requirements, post-renal transplantation. Additionally, they compared the combined genotyping of *CYP3A4* and *CYP3A5* in individuals and determined the Tac dose requirements. Association between CYP3A4*22 and Tac dose requirement has also been observed in pediatric heart transplant patients. The assessment revealed that individuals carrying CYP3A4*22 required 30% less Tac to attain comparable target concentrations compared to those carrying CYP3A4*1/*1 (Gijsen et al. 2013, Werk and Cascorbi 2014).

4.4. CYP450 Oxidoreductase

One of the other crucial proteins is P450 oxidoreductase (POR), which serves as an electron donor for various CYP enzymes, primarily CYP3A, playing a significant role in the oxidation of a wide range of drugs (Werk et al. 2014).

Several SNPs have been discovered in the human *POR* gene. This gene may affect the POR-CYP interaction and the activity of various enzymes. Various studies indicated that POR*28 (rs1057868; C>T) is a critical SNP, which induces an amino acid substitution (p.Ala503Val) at position 503. This may affect the electron binding moiety of POR and likely influence its interaction with CYP enzymes (Oneda et al. 2009).

Individuals homozygous for *POR**28 exhibit a significant decrease in in-vivo CYP3A activity concerning benzodiazepines (midazolam) when compared to those with wild-type *POR*. In a study conducted in China involving 71 healthy Chinese volunteers, led by Zhang et al., it was demonstrated that *CYP3A5* expressers carrying the POR*28 variant allele experienced a 40% reduction in Tac exposure compared to *CYP3A5* expressers with the wild-type *POR* (Zhang et al. 2013).

Now both of these studies suggest that POR*28 leads to greater *CYP3A5*-mediated Tac metabolism, possibly resulting from an interaction among *POR*, *CYP3A5*, and Tac. For those individual who are *CYP3A5* non-expressers, Tac metabolism depends totally on *CYP3A4.* Ostensibly, POR*28 does not have any role in CYP3A4 activity to a clinically relevant degree (Oneda et al. 2009).

4.5. Peroxisome Proliferator-Activated Receptor-Alpha (PPAR)-α

Another crucial gene influencing drug metabolism and impacting the activity of CYP450 is Peroxisome proliferator-activated receptor (PPAR)-α. This ligand-activated transcriptional factor belongs to the family of nuclear receptors and serves as a significant regulator of genes associated with fatty acid

able 1. Frequencies of important SNPs in various populations

Gene	Caucasians	Africans	Indians	Asians	Reference
*CYP3A5*3*	90-93%	32%	66%	60-73	(Liu et al. 2007)
*CYP3A5*6*	0-4.3%	8.6-15	ND	0	(Xie et al. 2004)
*CYP3A*7*	0	5-12	ND	0	(Xie et al. 2004)
*CYP3A4*1B*	2-9.6	35-67	3.5	0	(McGraw and Waller 2012)
*CYP3A4*22*	8.3	4.3	ND	4.3	(Elens, Bouamar, et al. 2011)
ABCB1 3435C	48-62	68-83	38	51-62	(Scheiner, Damasceno, and Maia 2010)
*POR*28*	26	19	30	37	(Scheiner, Damasceno, and Maia 2010)

beta-oxidation, playing a key role in maintaining energy homeostasis (Lunde et al. 2014b).

Transcription factor PPAR-α has recently emerged as a potential contributor to intra- and inter-individual variability in *CYP3A* expression and subsequent activity. Two sequence variants within the PPAR-α gene (*PPARA*) have been identified to impact the expression of *PPAR-α*.

In-vitro studies have identified that *PPARA* c.209-1003G>A, and c.208+3819A>G were responsible for the down-regulation of *PPAR-α*, consistently correlating to reduced CYP3A4 mRNA levels, protein expression, and enzymatic activity (Lunde et al. 2014b).

Another recent study, conducted in 229 kidney transplant recipients by Lunde et al., observed that expression of at least one *PPARA* variant allele was significantly associated with an increased Tac C_0/D ratio when adjusting for POR*28, CYP3A5*3, and CYP3A4*22.

Analysis conducted on two *PPARA* sequence variants revealed considerably higher Tac exposure in patients homozygous for *PPARA-α* c.209-1003G>A. These results are similar to the reduced CYP3A4 protein/activity levels as noted in previously in-vitro studies.

Furthermore, it is suggested that sequence variants in the *PPARA* gene, particularly the c.208+3819A>G variant, may significantly affect the pharmacokinetics of Tac. However, these observations require confirmation through further clinical trials to validate these findings and comprehend the underlying mechanism (Lunde et al. 2014b).

4.6. PXR

An additional important transcription factor is the human pregnane factor, encoded by *NR1I2*, which plays a pivotal role in enhancing the activity of *CYP3A5* and the *ABCB1* gene. Given its influence on these genes, it is recognized that this factor also holds significance in the dose adjustment of Tac (Israni et al. 2010).

Moreover, variation has been noticed in how each person reacts to Tac. These variations may impact the therapeutic effectiveness of Tac, as well as the probability of side effects. It has been determined that genetic variation, particularly SNPs, may play a role in the inter-individual variations in Tac pharmacokinetics and responsiveness. Through genetic screening, clinicians can enhance their understanding of individual reactions to Tac by investigating genetic variations related to drug metabolism. This includes examining variations in CYP450 enzymes to calibrate doses and achieve an optimum therapeutic response (Brazelton 1996, Fukudo et al. 2006).

5. Literature Review

A genetic polymorphism modifies the structure of protein molecules which plays a key role in metabolizing many drugs and also alters the pharmacokinetic properties of calcineurin inhibitors. This causes variation in the time required for dose titration to attain optimum drug levels in blood.(Kelly, Burckart, and

Venkataramanan 1995). Due to this, genotyping of relevant polymorphism may contribute vitally to determining the response of a drug, its dosage, and evading adverse effects (Emre et al. 2000).

Polymorphisms in *CYP3A4* and interrelated *PPARA* genes have a critical role in modifying the clinical response of Tac. Various studies conducted on Tac-SNP association noted that the Tac dosing in LT is affected by *CYP3A4* (rs35599367), *PPARA* (rs4253728, rs4823613) polymorphism (Martin 2008).

Moreover, the *ABCB1* (rs104564) CC genotype showed a lower Tac concentration to dose ratio (CDR) in the first-week post-LT (Azam et al. 2021). Therefore, a higher Tac dose was required in recipients with CC genotype than those with CT and TT genotype during the first, third, and 3 months follow-up post-transplantation period (Korkor et al. 2023). The observed main factor influencing the bioavailability of Tac appeared to be the homozygous 3435CC variant of *ABCB1* genes, which demonstrates significantly reduced expression of intestinal P-gp compared to individuals with the *ABCB1* 3435TT genotype (Azam et al. 2021). One study found that those with *ABCB1* 2677G>T/A (rs2032582), and *ABCB1* C1236T (rs1128503) exhibited higher levels of CDR throughout (1st to 4th week). In another study, it was observed that a high dose of Tac was required in kidney transplant recipients with *ABCB1* 2677GG wild-type.

It is reported that high serum concentrations of Tac damage various organs of the body. The most notable adverse effects were neurotoxicity, nephrotoxicity, sepsis, cardiotoxicity, and gastrointestinal effects in LT recipients. Furthermore, one study associated *ABCB1* C3435T (rs1045642) with psychosis (Papazisis et al. 2018). Another study correlated the presence of T alles *ABCB1* C3435T (rs1045642) with a higher dose requirement of Tac, and neurotoxicity. Additionally, the recipients with *ABCB1* G2677T/A (rs2032582) TT polymorphism had a predisposition for sepsis (Papazisis et al. 2018).

SNP in *CYP3A5* may potentially change the enzyme expression, with higher expression leading to more extensive metabolism of the drug. One of the most influential studies on *CYP3A5* SNP is the A6986G transition within intron 3, which is mostly associated with reduced function as a result of alternative splicing. In homozygous carriers of the *CYP3A5**3 gene (3*/3*), it was noted that they exhibited negligible CYP3A5 activity. Consequently, they required a higher dose-normalized trough Tac concentration compared to *CYP3A1* carriers. Research has indicated that in LT cases, polymorphisms in *CYP3A7*, *CYP3A4*, and *CYP3A53* in the recipients, rather than the donors, were correlated with Tac C_0/D in the early post-transplant period (Fung 2023).

In a separate study, the impact of SNPs for *CYP3A5, MDR1, ABCC2*, and *POR**28 in both donors and recipients on Tacrolimus dose-adjusted trough levels was investigated in liver transplant recipients after transitioning from immediate-release Tacrolimus (IR-TAC) to once-daily prolonged-release Tac (PR-Tac). Donors were also considered for each recipient. The study categorized patients into two groups: those with a <30% decrease in C_0/dose (group 1) and those with a ≥30% decrease in C0/dose (group 2) following the switch to PR-Tac. Results revealed that recipient CYP3A5 *1/*3 and *3/*3 were more prevalent in group 1 compared to group 2, while *CYP3A5* *1/*1 was more frequent in group 2 ($p = 0.016$). These findings align with existing knowledge regarding *CYP3A5* genotypes. Notably, no significant difference in donor *CYP3A5* variants was observed between the selected groups. The disparity in recipient and donor findings may underscore differences in hepatic and intestinal *CYP3A* influences, as well as the impact of the time elapsed post-liver transplant on the function and relative influence of these two enzymes. Those in group 2, who switched earlier ($p = 0.025$), exhibited this

discrepancy (Fung 2023). However, this is a small retrospective study, and the time period for Tac measurements, following the switching, widely varied from 5 to 102 days. Furthermore, the switching time was significantly different between the two groups and not controlled, and after adjusting for switching time, no other factors were found to be associated with Tac C_0/dose reduction. The significantly higher Tac-trough levels before switching in group 2 may be due to the shorter switching time, further casting a shadow of doubt on the findings (Park et al. 2023, Fung 2023, Pulk et al. 2015). Therefore, further prospective studies, with improved control over the timing of drug intake, time of switching from LT, and fixed time points of Tac measurement are needed to validate and consolidate these results.

It has also been observed in one of the studies, that the *PPARA* variant alleles and the *POR**28 allele were associated with higher and lower Tac C_0/D ratios, respectively, whereas the *CYP3A4**22 allele influenced cyclosporin ratios. Carriers of functional *CYP3A5**1 alleles demonstrated a 58 % lower Tac C0/D ratio ($P<0.001$). This confirms the *CYP3A5* genetic polymorphism significance on Tac metabolism, as previously observed, where carriers of *CYP3A5**1 alleles (*CYP3A5* expressers) needed twice as much dose of Tac as *CYP3A5**3/*3 individuals. Thus, pre-transplant *CYP3A5* genotyping may be a useful approach for better prediction of individual Tac starting doses. Even though the effect of this genotype in a Caucasian population is limited, the influence on those carrying this variant allele is substantial (Lunde et al. 2014a, Wang et al. 2011b, Pulk et al. 2015)

In an investigation by (Yu et al. 2018), it was observed that the recipients with one or two *CYP3A4**22 alleles need half the dose of cyclosporin to reach the therapeutic target. Surprisingly, we did not observe any association between the *CYP3A4**22 genotype and Tac C_0/D ratios. This difference in the *CYP3A4**22 genotype on Tac and cyclosporin pharmacokinetics could be attributed to a difference in the metabolic pathways. Additionally, there were no recipients with homozygous *CYP3A4**22 variant allele among the 123 patients treated with Tac, which may have confounded the results

In contrast, Elens et al. reported a significant association between *CYP3A4**22 with Tac and cyclosporin pharmacokinetics, reporting higher dose-adjusted cyclosporin and Tac concentrations in kidney transplant recipients. However, these authors failed to confirm the association between cyclosporin C/D ratio and *CYP3A4**22 in an independent cohort. Although there seems to be a broader consensus on the reduced *CYP3A4* metabolic capacity among *CYP3A4**22 carriers, further investigations are needed to clarify the clinical relevance of this sequence variant. Purportedly, this is the first study showing the potential impact of the *PPARA* genetic variations on Tac exposure in kidney transplant recipients. Owing to the strong correlation between *PPARA* c.209-1003G>A and *PPARA* c.208+ 3819A>G, the combined effect of these two sequence variants was analyzed.

The independent effect of expressing at least one *PPARA* variant allele was significantly associated with a higher Tac C_0/D ratio ($P=0.01$), when adjusting for the other sequence variants (*POR**28, *CYP3A5**3, and *CYP3A4**22). A detailed analysis of the two *PPARA* sequence variants revealed a significantly increased Tac exposure in homozygote *PPARA* c.209-1003G>A carriers. These results are in concordance with the reduced CYP3A4 protein/activity levels, as previously presented. However, inclusion of the other sequence variants assessed in the present study reduced the effect of *PPARA* c.209-1003G>A on Tac C_0/D ratios, indicating other possible explanatory variables, in addition to the difference observed in Tac C_0/D ratios between homozygote *PPARA* c.209-1003G>A carriers and homozygote *PPARA* c.209-1003G carriers. On the other hand, the

expression of at least one *PPARA* c.208+3819G allele was an independent explanatory factor for higher Tac exposure. This suggests that *PPARA* c.208+3819A>G is the variant with the strongest influence on Tac pharmacokinetics.(Elens, Van Schaik, et al. 2011, Lunde et al. 2014c)

Despite the statistically significant effect of *PPARA* sequence variants on Tac exposure, no notable effect was shown on cyclosporin C_2/D ratios. Although the mechanism is not fully understood, activation of PPAR-α has been shown to increase the expression of *CYP3A4*.

Consequently, PPAR-α activity should theoretically also have influenced cyclosporin pharmacokinetics. However, there are inconsistent reports on whether the regulation of *CYP3A4* occurs directly or indirectly by PPAR-α. Recently, the sequence variants *PPARA* c.209-1003G>A, and *PPARA* c.208+3819A>G were associated with reduced expression of PPAR-α, and consistently related to lower CYP3A4 mRNA levels, protein expression, and enzymatic activity. PPAR-α has been linked to *CYP3A4* expression, but an association between PPAR-α and *CYP3A5* expression and activity has not yet been reported (Lunde et al. 2014c).

The significantly lower Tac C_0/D ratio observed among *POR**28 allele carriers, after correction for *CYP3A5**3, *CYP3A4**22, and *PPARA* genotype, supports the previous findings of De Jonge et al. and Oneda et al, reporting a lower Tac C_0/D ratio among *POR**28 allele carriers expressing functional CYP3A5. However, this study did not report any significant impact of the *POR**28 allele on the Tac C_0/D ratio in the sub-group of patients expressing functional *CYP3A5*. The *POR**28 allele has the potential to explain interindividual variability in CYP3A capacity. However, the proposed link between *CYP3A5* and the *POR**28 allele needs further elucidation.

6. Adverse Effects

It has been found that patients who received a liver transplant, on Tac showed various cardiovascular issues on different doses of the drug. It mostly includes hypertension, various types of arrhythmias, and heart failure. *SLC12A8*, *SLC12A6*, *SLC12A7* (members of the cation-coupled chloride ion co-transporter family), and *GRIK* are responsible for transport activity in potassium-ion efflux and might play a critical role in the genesis of arrhythmias, following myocardial ischemia

Although all the substrates transported by *SLC12A8* are not known, data suggested that polymorphism in this gene may be an important determinant of risk for cardiovascular toxicity with anticancer therapy. (Mushiroda et al. 2005, Seino, Hori, and Sonoda 2003)

Nephrotoxicity is another important adverse effect observed in LT patients taking Tac. It is reported that the carriers of *CYP3A4**1/*CYP3A5**1 expresser genotype were significantly more susceptible to the development of biopsy-confirmed Tac-mediated nephrotoxicity than non-carriers of the alleles (Bentata 2020).

A higher incidence of nephrotoxicity has been reported for the *CYP3A5**3/*3 genotype recipients treated with Tac (Quteineh et al. 2008). It's suggested that 3435C>T Homozygous TT genotype in organ recipients is associated with enhanced incidence of acute rejection and nephrotoxicity *(Pratschke et al. 1997, Ekberg et al. 2007)*

7. Discussion

Ethnicity is an important factor in comprehending the pharmacogenomics aspects of Tac (Yanagimachi et al. 2010). There are pronounced differences in drug metabolism across various ethicalities. Allelic frequencies of the most common SNPs in *CYP3A5*, *CYP3A4*, *ABCB1*, and *POR**28 in various ethnic groups are presented in below mentioned **Table 1.**

An immunosuppressive medication called Tac is frequently used in solid organ transplantation to prevent organ rejection. However, due to genetic variations, each person's reaction to Tac may differ significantly.

The *CYP3A5* genotype is one of the crucial and thoroughly investigated genetic variants. Tac is metabolized more quickly by those with at least one functioning *CYP3A5**1 allele, necessitating greater dosages to reach therapeutic concentrations. The significance of taking pharmacogenetics into account for optimum Tac dose determination.

Tac dosage for kidney transplant recipients may be aided by developing a *CYP3A4*, and *CYP3A5* genotype testing protocol. In addition to *CYP3A5*, other genetic variations may also impact the response to Tac dosage; SNPs like rs776746, rs10264272, and rs41303343 are among them.

There is a significant difference in the allelic frequency of *CYP3A**3 allele among different populations. This allele is associated with variation in the *CYP3A5* gene, which encodes P450, and the 3A5 enzyme involved in drug metabolism. *CYP3A5**3 is most commonly present in Caucasian populations but absent in Asian populations. Similarly, it is found in 66% of the Indian population and probably affects dosing (Vadivel et al. 2007).

Furthermore, the lower doses of Tac lead to acute rejection in African populations. There are other *CYP3A* alleles like *CYP3A5**6, and *CYP3A5**7, resulting in the absence of functional CYP3A5 protein. Unlike the African population, these SNPs are absent in Caucasian and Asian populations. The presence of *CYP3A5**6 and *CYP3A5**7 in African populations may compensate for the relatively low frequency of the *CYP3A5**3 allele, resulting in a metabolic phenotype similar to that of Caucasians. However, the difference of *CYP3A5**6 and *CYP3A5**7 distribution in the African population may be responsible for the relatively low frequency of the *CYP3A5**3 allele, resulting in a metabolic phenotype similar to that of Caucasians. The protocols recommend increasing the starting dose by 1.5–2 times in extensive metabolizers (*CYP3A5**1/*1), and intermediate metabolizers (*CYP3A5**1/*3, *1/*6, or *1/*7), and a standard dose in poor metabolizers (*CYP3A5**3/*3, *6/*6,*7/*7, *3/*6, *3/*7, or *6/*7) (Jacobson et al. 2011, Xie et al. 2004, Wang et al. 2011a). It has been noted that *6 and *7 required lower Tac doses as compared to other alleles. The non-functional alleles found in the African and American populations are *3,*6, and *7 were identified in 74.5% of individuals. It has also been observed that there were more *CYP3A* non-expressers in African population. It has been found that in one study about 197 adult African SOT recipients, the variants *CYP3A5**3, *CYP3A5**6, and *CYP3A5**7 explained a great proportion of the observed Tac C_0 variability in African recipients. Through understanding these differences, we came to know the importance of ethnicity-specific genotypes (*CYP3A5**6 and *CYP3A5**7) for Tac clearance. Using dosing models with these genotypes may lead to a more precise dosing of Tac (Wang et al. 2011a).

The frequency of *CYP3A4**1B in African Americans (35–67%) is the highest amongst all ethnic groups. The frequencies of the *ABCB1* 3435C and 1236C alleles are also much higher in individuals of African descent than in populations of other ethnicity. Recently, a novel *CYP3A4* loss-of-function allele (*CYP3A4**20) was identified, present in 1.2% of the Spanish population. However, this polymorphism has not been investigated in relation to Tac dose requirement or toxicity (Sanghavi et al. 2017).

It has been clearly understood that Immunosuppressive agents play a critical role in preventing acute rejection after SOT. The most important calcineurin inhibitor that should be given and most frequently prescribed is Tac. It is further narrated that that Tac is preferred over other agents and expected to be given for the next 10 years to the SOT recipients as a maintenance therapy. There is strong evidence that *CYP3A4* and *CYP3A5* significantly influence Tac dose requirements. However, despite the strong genetic effect on Tac dose requirement, the evidence that implementing genotype-based

dosing will improve clinical outcomes is missing (Lai et al. 2011).

8. Conclusions

Tac medication tailored to each patient based on genetic markers presents a viable way to enhance outcomes for organ transplant recipients. We recommend that Tac dosage be customized to each patient's distinct genetic profile by identifying particular SNPs, especially in the *CYP3A4*, *CYP3A5*, and *ABCB1* genes. The danger of side effects can be thus reduced and the effectiveness of the drug can be maximized. Specifically, it has been observed that the oral clearance at the beginning and the oral clearance that recovers over time following surgery are affected by the *CYP3A51* allele and the *MDR1* mRNA level, respectively. To assess the clinical utility of the genome-based population pharmacokinetic model, more prospective analyses in a sizable population are needed. This will require additional studies on *CYP3A5, MDR1,* and other important genes to individually tailor the Tac dosage regimen for liver transplant recipients who are living donors.

Conflict of Interest

The authors declare that they have no competing interests.

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Authors Contribution

SFAS conceptualized the study, AA, ZB, and GH did the literature review and analysis, and SFAS led the project and wrote the final manuscript.

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