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Review Article

Genetic Polymorphism in *CYP3A4* and *CYP3A5* Genes and their Association with the Clinical Response to Calcineurin Inhibitors; A Narrative Review

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Abstract

Calcineurin inhibitors, including cyclosporine and tacrolimus, are the fundamental pillars of immunosuppressant therapy in patients with allogeneic transplants. Calcineurin inhibitors are mainly the substrates for the enzymes *CYP3A4* and *CYP3A5*. The literature search in this review has affirmed that many SNPs located in the genes responsible for *CYP3A4* and *CYP3A5* expression are associated with influencing the pharmacokinetics of calcineurin inhibitors and eventually maneuvering their efficacy and toxicity profiles. *CYP3A5 A6986G* (rs776746) had an association with the pharmacokinetics of cyclosporine and tacrolimus in transplant patients. The patients with the *CYP3A5*3* (rs776746) allele had higher initial plasma concentrations of cyclosporine and tacrolimus, while those with the *CYP3A5*1* (rs776746) allele had higher oral clearance of both drugs, resulting in lower initial plasma concentrations. More patient survival was also associated with the *CYP3A5*1* genotype in the Caucasian population. Higher doses of cyclosporine were required in patients with allogeneic grafts carrying the *CYP3A4*18B* (rs28371759) allele in the Chinese population. In various other populations, it was revealed that therapeutic drug monitoring with calcineurin inhibitors was necessary in patients carrying the *CYP3A4*1B* (rs2740574) allele. *CYP3A4*22* (rs35599367) carrying individuals were more prone to tacrolimus toxicity. *CYP3A5A44G* also affected the pharmacokinetics and efficacy of tacrolimus in Asian, white, and other populations. Many of these SNPs manifested no associations in numerous populations. Many confounding factors may have canceled out these associations. More studies are required to explore the effects of these SNPs on larger samples in order to validate these findings. Attempts should also be made to discover novel SNPs in these genes to find out more associations that can influence the overall clinical response to calcineurin inhibitors in transplant patients.

Keywords: Calcineurin inhibitors, cyclosporine, tacrolimus, genetic polymorphism; *CYP3A4, CYP3A5,* allogeneic transplant

1. Introduction

Solid organ transplants are the treatment of choice in many cases especially renal and hepatic failure (Karolin, Genitsch, and Sidler 2021). Organ graft rejection, in these scenarios, is a major concern that may affect the outcomes of the treatment process (Oweira et al. 2022). Since the 1980s, calcineurin inhibitors have been a breakthrough in preventing the recipient's immune response against the transplanted tissue. Calcineurin is a serine-threonine phosphatase that is activated by the calcium-calmodulin complex (Li, Rao, and Hogan 2011). It is a cytosolic protein that is located near the calcium stores in the smooth endoplasmic reticulum, bound by A-kinase anchoring protein-79 (Li et al. 2012). Calcineurin is involved in the regulation of Na+/H+ exchanger 1 (NHE1) (Wakabayashi, Hisamitsu, and Nakamura 2013). Its function has also been discovered as a modulator of

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) and regulates the glutamatergic neurotransmission (Woolfrey and Dell'Acqua 2015). Most importantly, calcineurin dephosphorylates nuclear factor for activated T-cell (NFAT) hence activating translocation of NFAT into the nucleus of T-cell, transcribes the interleukin-2 (IL-2) genes and activation of T-cell by IL-2 in autocrine fashion (Otsuka et al. 2021). The inhibition of calcineurin causes suppression of the immune response that may help in avoiding graft rejection in renal, liver, heart, and other allogeneic transplants (Cajanding 2018). Cyclosporine and tacrolimus are promising calcineurin inhibitors that provide a marked immunosuppressant effect to prevent transplant rejection (Roy and Cyert 2020).

Various studies have established the efficacy of calcineurin inhibitors in treating autoimmunity, transplant rejection, and other immune disorders. A study revealed that cyclosporine provides a therapeutic activity in suppressing the helper T-cell 17 (Th17 cell) immune response leading to autoimmune disorders. Transgenic mice having hen egg lysozyme (HEL)-specific T-cell receptor (TCR) were used to acquire T-cells exhibiting rich expression of Interleukin (IL) - 17 and interferon-gamma (INF-γ). During the *in-vitro* experiment, these cells were cultured and it was established that they were resistant to dexamethasone. The dexamethasone-resistant growing Th17 cells were treated with cyclosporine and showed a marked reduction in cell division. This experiment was replicated in another group of transgenic mice i.e. ovalbumin specific OT-II mice. The Th17 cells obtained from these mice were treated with tacrolimus which successfully suppressed the growth of these cells *in vitro* (Schewitz-Bowers et al. 2015).

Many randomized clinical trials have provided data that has reflected the efficacy of both calcineurin inhibitors including cyclosporine and tacrolimus in preventing renal transplant rejection. Many clinical trials have reported tacrolimus to be more efficacious in preventing transplant rejection and reducing adverse drug reactions while some clinical studies have hypothesized that cyclosporine is far superior in clinical efficacy as compared to tacrolimus in reducing the events of graft loss (Ravanshad et al. 2020, Azarfar et al. 2018). Another randomized trial noted that tacrolimus daily doses of up to seven days in patients who underwent recent kidney transplants, were quite safe and no graft loss was reported throughout the study (Polatkan et al. 2020).

The pharmacokinetics of a drug play a very important role in prompting the efficacy and toxicity of the drugs (Li, Meng, et al. 2019, Fatima et al. 2023, Ibrahim, Fatima, and Babar 2023). Calcineurin inhibitors have a narrow therapeutic index and there may exist variations among the patients regarding the pharmacokinetics of these drugs (Ensor et al. 2018). Many serious adverse effects such as hyperglycemia, nephrotoxicity, and neurotoxicity are associated with the use of calcineurin inhibitors (Montero and Pascual 2015, Peng et al. 2016, Arnold et al. 2013).

The metabolism of cyclosporine and tacrolimus is controlled by many genes i.e. *CYP3A4*, *CYP3A5*, P-glycoprotein, and *ABCB1* genes, encoding several important proteins that regulate the pharmacokinetics of calcineurin inhibitors (Vanhove, Annaert, and Kuypers 2016). The pharmacogenomics of calcineurin inhibitors has been vastly studied in recent decades. *CYP3A4* and

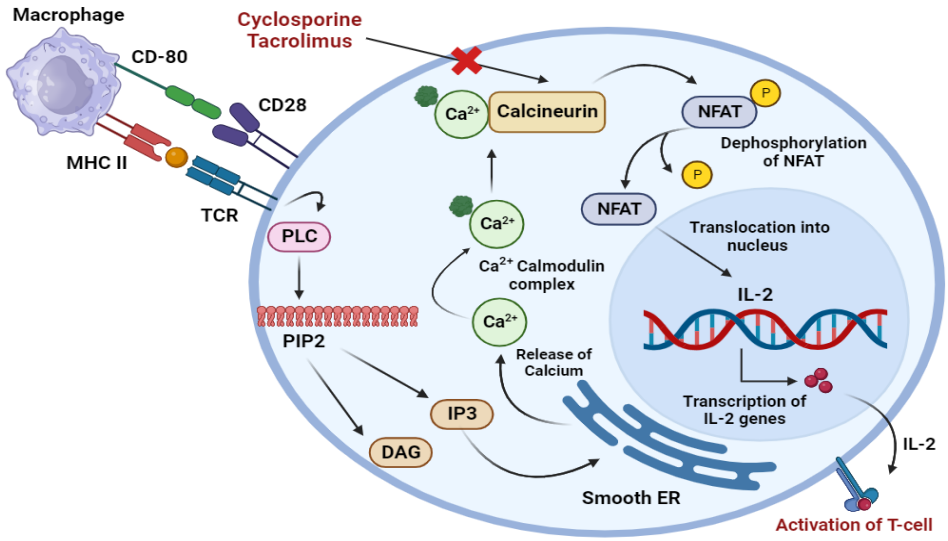

Figure 1: The figure shows the mechanism of T-lymphocyte activation by calcineurin and inhibition of calcineurin by cyclosporine and tacrolimus; TCR (T-cell receptor), MHC II (Major Histocompatibility Complex), PLC (Phospholipase C), PIP2 (Phosphatidylinositol di-phosphate), IP3 (Phosphoinositol 3 phosphate), DAG (Diacylglycerol), NFAT (Nuclear factor for activated T- cell), IL-2 (Interleukin-2).

CYP3A5 polymorphisms have been found to be closely associated with varying pharmacokinetics from person to person (Tamashiro et al. 2017, Zhu and Ge 2011). The main challenge linked with immunosuppressant therapy with calcineurin inhibitors is the variable pharmacokinetics in several populations due to genetic diversity. These variations lead to altered clinical effects manipulating the overall efficacy and toxicity of calcineurin inhibitors. One of the major determinants of this phenomenon is the genetic polymorphism of *CYP3A4* and *CYP3A5* genes. Many single nucleotide polymorphisms (SNPs) have been detected and their effects on the clinical outcomes of therapy with calcineurin inhibitors have been inquired. The varying expression of intestinal *CYP3A4* and *CYP3A5* enzymes among individuals also affects the disposition of cyclosporine and tacrolimus (Staatz, Goodman, and Tett 2010). The pharmacokinetics of calcineurin inhibitors in liver transplant patients are also affected by the genotype of the donors as well as the recipients (Muraki et al. 2011). All these factors lead to the alterable clinical response towards calcineurin inhibitors in several populations (Ain et al. 2023, Farouk and Rein 2020c, Ibrahim et al. 2023). It has been suggested that more pharmacogenetic studies are required to understand and cope with these challenges that can cause optimization of the current therapy with calcineurin inhibitors.

This review aims to summarize the effects of genetic polymorphism of *CYP3A4* and *CYP3A5* in varying efficacy and adverse effects of calcineurin inhibitors. It also focuses on the patterns influencing the pharmacokinetics of calcineurin inhibitors in various populations due to the genetic polymorphism of *CYP3A4* and *CYP3A5*. Figure 01 demonstrates the mechanism of T-cell activation by calcineurin and its inhibition by cyclosporine and tacrolimus.

2. Role of *CYP3A4* and *CYP3A5* in Calcineurin Inhibitor's Metabolism

Calcineurin inhibitors such as cyclosporine and tacrolimus are the most prominent agents employed in immunosuppressant therapies, especially organ transplants (Malvezzi and Rostaing 2015). Most of the drugs used in clinical practice including calcineurin inhibitors are the substrates of *CYP3A4* enzymes (Prytuła, Cransberg, and Raes 2019). *CYP3A4* enzymes reside in the hepatic tissue and the gut. The expression of *CYP3A4* genes among people is important regarding the metabolism of calcineurin inhibitors. The dissimilarities in the genetic expression of *CYP3A4* genes lead to subsequent inter-individual variations in metabolizing calcineurin inhibitors (Hesselink et al. 2014, Temesvári et al. 2012). The presence of the *CYP3A4*1B* (rs2740574) allele is associated with increased transcription of *CYP3A4* enzymes while the possession of the *CYP3A4*22* (rs35599367) allele may be linked with the decreased expression of the *CYP3A4* enzymes (Okubo et al. 2013).

CYP3A5 enzymes are also significantly involved in the metabolism of calcineurin inhibitors, especially tacrolimus. Their presence in the hepatic tissue contributes to the first-pass metabolism of calcineurin inhibitors (Farouk and Rein 2020b). The individuals with *CYP3A5*1* (rs776746) genotype are expressers while the *CYP3A5*3* (rs776746) genotype are non-expressers. The most famous SNP studied extensively in *CYP3A5* is at position 6986 (*6986A>G*) (Buendía et al. 2020). The *CYP3A5*1* allele can be associated with enhanced expression of *CYP3A5* enzymes. The individuals with the *CYP3A5*1*1* allele are high metabolizers of tacrolimus. Individuals possessing *CYP3A5*1*3* exhibit an intermediate metabolism while those with *CYP3A5*3*3* allele have a lower *CYP3A5* activity for metabolizing tacrolimus and subsequent low clearance (Chen and Prasad 2018). A study reported that the genetic variability can affect the tacrolimus dosing. In order to achieve 4 to 8 μg per ml serum concentrations of tacrolimus, a dose of 0.1 mg/kg was administered in 194 Chinese individuals orally. After genotyping and measurement of serum tacrolimus levels, it was revealed that approximately 64 % of individuals with *CYP3A5*1*1* or**1*3* alleles achieved therapeutic (trough) concentrations on the 7th day, while about 55% of individuals carrying *CYP3A5*3*3* allele reached therapeutic levels on day 7 (Chen et al. 2017).

The genotypes of *CYP3A4* and *CYP3A5* in the donors also affect the metabolism of calcineurin inhibitors. A study has reported this association using *in vitro* testing. A bank of human liver microsomes was screened *in vitro* for their activity to convert tacrolimus into its metabolite i.e. 13-O-demethyltacrolimus. The presence of the dominant *CYP3A5* genotype in the microsomes was associated with a higher conversion of tacrolimus into its metabolite as compared to the *CYP3A4* genotype (Kamdem et al. 2005). Besides *CYP3A4* and *CYP3A5*, efflux pumps such as P-glycoprotein encoded by the *ABCB1* gene are

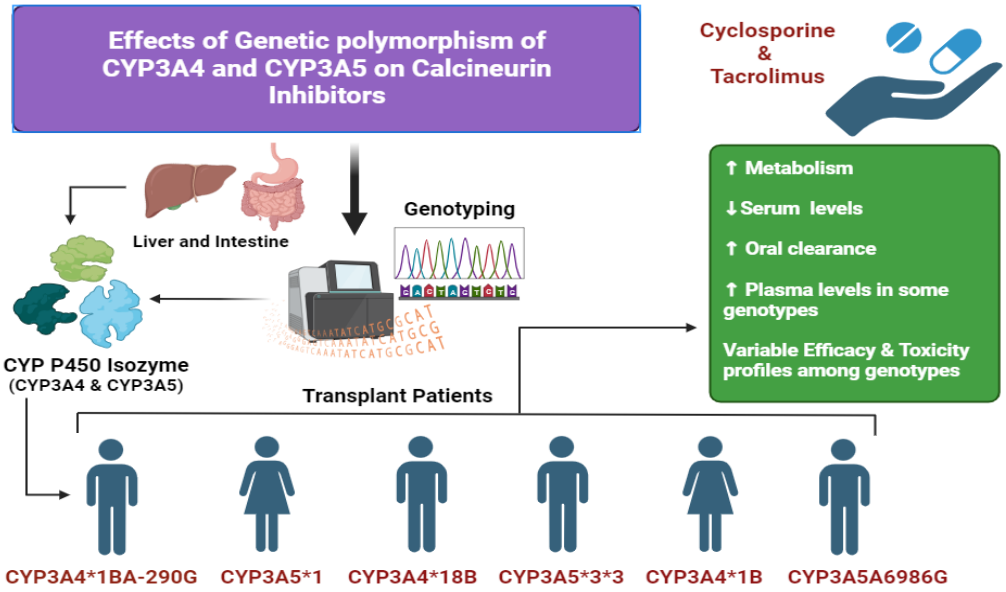

Figure 2: The figure illustrates the general effects of genetic polymorphism of CYP3A4 and CYP3A5 in the metabolism and disposition of calcineurin inhibitors leading to variable clinical responses in transplant patients.

also involved in the predisposition of calcineurin inhibitors by expulsion of these agents out of the gut cells. This decreases the absorption of calcineurin inhibitors (Knops et al. 2023). Figure 02 presents a general overview of genetic variability resulting in diverse clinical responses due to genetic polymorphism of *CYP3A4* and *CYP3A5*.

3. Studies on *CYP3A4* and *CYP3A5* Genetic Polymorphisms Associated with Efficacy of Calcineurin Inhibitors

The patients on immunosuppressive therapy with calcineurin inhibitors may show their plasma concentrations beyond the therapeutic window of these drugs due to pharmacogenetic differences (Islam et al. 2023). A group of 299 Indian renal transplant patients were included in a study to assess the effects of *CYP3A4* and *CYP3A5* polymorphism on the efficacy of therapy with calcineurin inhibitors. All of the patients were receiving cyclosporine and tacrolimus along with other immunosuppressive agents i.e. prednisolone and mycophenolate mofetil. The doses were adjusted for both cyclosporine and tacrolimus in all individuals at the interval of month 1 and month 3. The enzyme multiplied immunologic technique (EMIT) was used to determine the plasma levels of cyclosporine in the blood samples of the subjects. The principle of this technique includes the measurement of cyclosporine concentrations on the basis of binding with cyclosporine antibodies. On the other hand, the plasma concentrations of tacrolimus were determined using microparticle enzyme immunoassay (MEIA). It was revealed that *CYP3A5* expressers (*CYP3A5*3 *1/*1* and **1/*3* genotypes; rs776746) had lower plasma levels of cyclosporine and tacrolimus and manifested higher dose requirements at both month 1 and month 3. An increased risk of transplant rejection was associated with the *CYP3A5* polymorphism. The combination of *CYP3A5*3* and *CYP3A4*1B* genotype (rs2740574) was also

evaluated. Significantly lower concentrations of cyclosporine and tacrolimus were observed in these genotypes as compared to the wild-type, hence reinforcing the requirement for higher doses of calcineurin inhibitors in these genotypes (Singh, Srivastava, Kapoor, K. Sharma, et al. 2009).

In another pharmacogenetic study, the pharmacokinetics of cyclosporine in *CYP3A4* variants were studied. The study was performed on approximately 140 cardiac and renal transplant patients in the Netherlands. Cyclosporine was being administered as immunosuppressive therapy in these transplant patients. EMIT assay technique was employed to measure the plasma concentration of cyclosporine in the participants of the study. The study revealed that the patients carrying *CYP3A4*1/*1B* genotype had high oral clearance of cyclosporine and they will need higher doses of cyclosporine for better pharmacodynamics performance to sustain the immunosuppressive effects to prevent graft rejection. Patients carrying *CYP3A5*3* genotype also demonstrated slightly lower plasma concentrations of cyclosporine but no statistically significant change in pharmacokinetic parameters was noticed (Hesselink et al. 2004b). Another study was undertaken in 53 Chinese hepatic transplant donors to evaluate the association of *CYP3A5*1/*1*, **1/*3* (expressers) and **3/*3* (non-expresser) genotypes polymorphism with pharmacodynamics of tacrolimus. After genotyping using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), the concentration dose ratio was calculated after week 2 and month 1 after the transplant procedure. This ratio was lower in the expresser group up to a significant extent as compared to the non-expresser group. Hence, the low levels of tacrolimus in plasma seem to be associated with *CYP3A5*1/*1* and **1/*3* genotype carriers. In order to maintain therapeutic concentrations of tacrolimus in recipients, higher doses of tacrolimus may be required in these patients who received a graft from donors possessing *CYP3A5*1/*1* and **1/*3* genotype. The study on genotypes of recipients also provided similar trends observed in recipients (Yu et al. 2006).

In a research study conducted in Japan, the genetic polymorphisms in individuals who underwent allogeneic stem cell transplants were studied to relate their effects on the bioavailability of calcineurin inhibitors. Calcineurin inhibitors were being administered as a prophylaxis of graft versus host disease (GVHD). Many SNPs were investigated including *CYP3A5* rs4646450 *C/C* and rs776746 *G/G*. The patients having *CYP3A5* rs4646450 *C/C* genotype developed higher concentrations of tacrolimus as compared to *T/T* or *T/C* genotype. Similar trends were observed for the rs776746 *G/G* genotype for *CYP3A5*. It was also reported that in order to maintain the plasma concentrations of tacrolimus within the therapeutic window, the dose was reduced from day 1 to day 28 of the study for patients with genotype *CYP3A5* rs4646450 *C/C* to uphold the therapeutic immunosuppressive effect.

In another study, 67 patients with newly performed renal transplants were studied to assess the association of *CYP3A5 A6986G* genotype (rs776746) on the plasma levels of cyclosporine A. Patients with *CYP3A5*1 A6986G* genotype (rs776746) had fewer levels of cyclosporine in their serum and require higher doses for adequate pharmacological effect. Such a phenomenon

was not seen in patients with the *CYP3A5*3* genotype (Eng et al. 2006).

In a study conducted on the Caucasian population in Belgium, about 100 renal transplant patients were selected on the basis of inclusion criteria. Half of these patients were administered cyclosporine and the other half received tacrolimus. After genotyping, the results manifested that the patients having *CYP3A5*3*3* and *CYP3A5*1*3* allele have lower plasma trough levels for both cyclosporine and tacrolimus. Very low plasma trough levels for both of these drugs were found in patients with the *CYP3A5*1*1* genotype. This may indicate a difficulty in achieving the therapeutic concentrations of tacrolimus and cyclosporine in patients who are rapid metabolizers resulting in sub-therapeutic levels and impaired efficacy (Haufroid et al. 2004). Similar findings were obtained in a study in Thailand. The retrospective study involved the analysis of clinical data of sixty-eight patients with renal transplants treated with tacrolimus. Genotypic analysis showed that the induction phase required much higher doses in patients with the *CYP3A5*1*1* genotype, while the doses required in the maintenance phase of therapy in the *CYP3A5*1*1* carrier were 1.3 folds higher than the patients with *CYP3A5*3*3* AND *CYP3A5*1*3* genotypes. The results show that the therapeutic concentrations of tacrolimus were hard to achieve in the induction and maintenance phases of therapy in patients with the *CYP3A5*1*1* genotype (Vannaprasaht et al. 2013).

In a Chinese study, 106 renal transplant patients, receiving cyclosporine, were subjected to genotyping. It was revealed that the *CYP3A5*1* genotype carriers needed higher doses of cyclosporine to maintain steady-state concentrations for acquiring optimal therapeutic efficacy, while such a requirement was not seen in patients with the *CYP3A5*3*3* genotype (Hu et al. 2006). Another study was conducted on 38 renal transplant patients in the Japanese population. Tacrolimus was administered to the patients. Genotypic findings reported that patients carrying the *CYP3A5*1* genotype have more amount of drug absorbed (higher area under the curve; AUC) while the patients with the *CYP3A5*3*3* genotype were discovered to have higher blood concentrations of tacrolimus. Hence, it could be hypothesized that the genetic polymorphism of *CYP3A5* genes may have an association with a variable efficacy of tacrolimus in the Japanese population (Soda et al. 2017).

A study was conducted in 30 Italian transplant patients. They were receiving tacrolimus as post-transplant therapy. The findings of the study describe the effects of genetic polymorphism of the *CYP3A5* gene. The individuals with *CYP3A5*1*1* homozygous genotype were receiving higher doses to avoid transplant rejection, while no need for higher doses was observed in patients with *CYP3A5*3*3* genotype. Better survival and less incidence of graft rejection were recorded in patients with the *CYP3A5*3*3* genotype.

In another study, 56 healthy Chinese individuals were administered cyclosporine. The genotypes of interest in this study were *CYP3A4*18B* (rs28371759) and *CYP3A5*3* (rs776746). The outcomes of the study demonstrated that the initial maximum concentration after oral administration of cyclosporine was lower in individuals with the *CYP3A4*18B* genotype. These levels were higher in *CYP3A5*1*3* genotype carriers as compared to individuals with *CYP3A5*3*

genotype. Hence, it could be interpreted that *CYP3A4*18B* and *CYP3A5*3* genotypes influence the efficacy of cyclosporine and close monitoring is necessary to avoid transplant rejection.

In another study, the effect of genetic polymorphism of *CYP3A4* and *CYP3A5* genes was examined in Chinese children with allogeneic hematopoietic stem cell transplants. Increased clearance of intravenous cyclosporine was recorded for Chinese children carrying the *CYP3A4*1 G* (rs2242480) genotype, hence maintaining therapeutic levels of cyclosporine in the systemic circulation is a challenge, requiring monitoring (Li, Hu, et al. 2019).

4. Studies on *CYP3A4* and *CYP3A5* Genetic Polymorphisms Associated with Toxicity of Calcineurin Inhibitors

Calcineurin inhibitors have variable pharmacokinetics so toxicity profile among the individuals may vary. Calcineurin inhibitors may precipitate adverse drug reactions like hypertension, acute nephropathy, and metabolic abnormalities (Karolin, Genitsch, and Sidler 2021, Jouve et al. 2019). Mechanistically, the kidney injury associated with the use of calcineurin inhibitors is associated with the damage of endothelial cells of the renal vasculature due to impaired production of vasodilators such as prostaglandins, leading to severe vasoconstriction and reduced renal perfusion (Farouk and Rein 2020a). A study revealed that the alternative splicing phenomenon in *CYP3A5*3* and *CYP3A5*6* (rs10264272) SNPs leads to the complete absence of *CYP3A5* from the liver tissue in American and Caucasian populations and are more prone to the toxic effects of calcineurin inhibitors, while those with at least a single *CYP3A4*1*(rs2740574) allele have high hepatic *CYP3A5* content (Kuehl et al. 2001). A study was conducted in Italy on Caucasian subjects to explore the association of *CYP3A5* polymorphism with the development of hypertension in patients on calcineurin inhibitors therapy after renal transplantation. A total of 92 patients were recruited and the subsequent genotyping was performed. It was discovered that the patients carrying *CYP3A5*1* (rs776746) allele had higher systolic as well as diastolic pressures post-transplant as compared to those having *2 or *3 alleles (Ferraresso et al. 2011).

Another study was undertaken in Korea to assess the association of tacrolimus toxicity with *CYP3A4* and *CYP3A5* polymorphism. A total of 70 renal transplant patients were employed in this study. After genotyping, it was established that the individuals having *CYP3A4*1*, *CYP3A5*1*, or *CYP3A4*1B*/*CYP3A5*1* genotypes require higher doses for tacrolimus and subsequently show higher toxicity events especially nephropathy as compared to other genotypes (Cho et al. 2012). In another study in Switzerland, 73 subjects from the local population with either kidney or lung transplants at least 6 months before were included in the study. All of them were using cyclosporine A after the procedure. Genotyping was done for all these patients and the plasma trough levels of cyclosporine A were measured before every dose. It was discovered that the subjects with *CYP3A5*1* genotype were receiving higher doses for achieving a clinically meaningful response, while similar trends were witnessed for *CYP3A4*1B* allele and *CYP3A4* rs4646437-*T* carriers, even after 12-month post-transplant. In this study, ten patients developed cyclosporine A-induced nephrotoxicity but the genetic associations

with *CYP3A4* and *CYP3A5* were not found in this study, rather polymorphism of the *ABCB1* gene of the donors was found to be associated with nephrotoxicity (Crettol et al. 2008).

A study with a cohort of 170 patients, conducted in France evaluated the association of *CYP3A4* and *CYP3A5* polymorphism of both the donors and recipients of liver transplant, with patient survival and calcineurin inhibitors associated nephrotoxicity. The patients were divided into two groups with the first group being administered with cyclosporine A and the other group receiving tacrolimus. The results of the study disclosed that more graft losses were observed in the individuals who were *CYP3A5*1* expressers. Individuals with *CYP3A4*22* (rs35599367) allele also exhibited more graft losses with cyclosporine A use. No short-term nephropathy was reported in this population during the study (Debette-Gratien et al. 2016). In a retrospective study, a total of 63 white individuals were recruited in the study who had undergone allogeneic stem cell transplants. The individuals had doses of intravenous tacrolimus for up to 14 days. This study analyzed the association between the *CYP3A4* polymorphism with tacrolimus-induced toxicities in the population of North Carolina. The results displayed the firm association of this polymorphism with toxicities such as hypertension, acute kidney injury, hyperglycemia, etc. Hypertension occurred in more than 25% of individuals while acute kidney damage was found in more than 11% of individuals. The genotypes i.e. *CYP3A4*1*, *2, and *CYP3A4*22* were not statistically associated with these ADRs (Hamadeh et al. 2019).

In a retrospective study in Thailand, the association of tacrolimus-induced nephropathy with *CYP3A5* polymorphism was examined. A total of 50 patients were enrolled including 21 donors and 29 recipients of allogeneic kidney transplantation. *CYP3A5*1*1*, *CYP3A5*1*3* (expresser genotypes), and *CYP3A5*3*3* (non-expresser genotype) were compared for both donors and recipients. The results displayed a firm association of the *CYP3A5*3*3* genotype with an incidence of tacrolimus-induced nephrotoxicity. These trends were more prominent in donors as compared to recipients. No such association was found in the expresser genotype (Udomkarnjananun et al. 2018).

In a study, 67 Chinese renal transplant patients were administered tacrolimus along with other immunosuppressants as post-transplant therapy. Genotyping promulgated the occurrence of nephrotoxicity in patients with genotype *CYP3A5*3*3*, while no kidney-associated adverse events were recorded in all other genotypes (Chen et al. 2009).

Another pharmacogenetic study was conducted in the Jordanian population. About 109 kidney transplant patients were administered cyclosporine and genotyping was done for *CYP3A4*22*, *CYP3A5*3*, and *CYP3A4*1B*. The results showed increased plasma levels of cyclosporine after the second dose in the patients with the *CYP3A4*22* genotype posing a risk for cyclosporine-associated toxicities and hence creating an urge for dose adjustment in these patients (El-Shair et al. 2019).

Table 01 demonstrates the effects of genetic polymorphism of *CYP3A4* and *CYP3A5* in maneuvering the pharmacokinetics and subsequent drug response in different populations.

Table 1: The table depicts the effects of CYP3A4 and CYP3A5 genetic polymorphism in altering the pharmacokinetics and pharmacodynamics in various populations receiving calcineurin inhibitors as post-transplant therapy

Gene	SNPs	Sample Size	Population	Transplant	Association/effect	Reference
*CYP3A4*18B*, *CYP3A5*1*	rs28371759, rs776746	91	Chinese	Bone marrow	Associated with lower initial concentrations of cyclosporin A.	(Qiu et al. 2011)
*CYP3A4*18B*	rs28371759	103	Chinese	Renal	Associated with the need for higher doses of cyclosporine	(Qiu et al. 2008)
CYP3AP1 (connected with *CYP3A5* expression)	rs776746	67	Asian	Renal	Associated with higher doses of cyclosporine.	(Eng et al. 2006)
		178	Caucasian	Renal	Associated with more time for achieving the therapeutic concentration and lower serum concentration post-administration	(MacPhee et al. 2004)
*CYP3A4*1B A290G/CYP3A5*1*	rs2740574, rs776746	95	Caucasian	Renal	Associated with tacrolimus-induced nephrotoxicity	(Kuypers et al. 2007)
*CYP3A4*1B*	rs2740574	64	White, Black, Asian	Renal	Associated with higher doses of cyclosporine and tacrolimus.	(Hesselink et al. 2003)
*CYP3A4*1B*	rs2740574	151	Caucasian, Black	Renal	Associated with Increased oral clearance of cyclosporine	(Hesselink et al. 2004a)
*CYP3A5*3 A6986G*	rs776746	399	Caucasian	Renal	Associated with the maintenance of steady-state levels of cyclosporine	(Kreutz et al. 2008)
*CYP3A5*3*, *CYP3A5*6*	rs776746, rs10264272	167	Chinese	Renal	Associated with the need for higher doses of tacrolimus	(Zhao et al. 2005)
*CYP3A4*1B*	rs2740574	293	North Indian	Renal	Not associated with the pharmacokinetics of calcineurin inhibitors	(Singh, Srivastava, Kapoor, R, et al. 2009)
		124	Caucasian	Renal	Not associated with the variability in efficacy and toxicity of cyclosporine.	(von Ahsen et al. 2001)
*CYP3A4*22*	rs35599367	185	Caucasian	Renal	Associated with supratherapeutic levels of tacrolimus.	(Elens et al. 2011)
*CYP3A5*3 A44G*	rs776746	180	White, South Asian	Renal	Associated with higher doses in transplant patients	(Macphee et al. 2005)
CYP3A5 A6986G	rs776746	134	German Caucasians	Renal	Associated with higher oral clearance of tacrolimus.	(Renders et al. 2007)
CYP3A5 A6986G	rs776746	237	Caucasian	Renal	Not associated with the pharmacokinetics of cyclosporine	(Grinyó et al. 2008)
CYP3A5 A6986G	rs776746	136	Caucasian, Black, Asian	Renal	Associated with the lower plasma levels of tacrolimus	(Hesselink et al. 2008)
*CYP3A5*3*	rs776746	60	Japanese	Liver	Associated with lower oral clearance of tacrolimus	(Fukudo et al. 2008)
CYP3A4 A-290G, *CYP3A5 G6986A*	rs2740574, rs776746	252	Belgian	Renal	Associated with tacrolimus-associated toxicity	(Naesens et al. 2009)
*CYP3A5*1*	rs776746	143	Japanese	Liver	Associated with higher doses in transplant recipients.	(Goto et al. 2004)
CYP3A4 A-392G, *CYP3A5*	rs2740574, rs776746	44	Caucasian	Renal	Associated with higher plasma levels of tacrolimus	(Roy et al. 2006)

5. Discussion

The study of genetic polymorphisms and their association with the absorption, distribution, metabolism, and elimination of drugs provides valuable knowledge and understanding regarding the efficacy and toxicity of the drugs. Calcineurin inhibitors are an important class of drugs used every day to save millions of lives. There is a lot of variability in individual responses to calcineurin inhibitors from an efficacy and toxicity point of view. Pharmacogenomics provides assistance in the provision of individualized pharmacotherapy with calcineurin inhibitors and making key clinical decisions keeping in view the genetic variability among populations and ethnicities.

The literature review has furnished precious insights regarding the effects of genetic polymorphisms of *CYP3A4* and *CYP3A5* genes in many populations. The transplant recipients of numerous populations i.e. Caucasians, Chinese, White, etc. with *CYP3A5*1*1* genotype indicated an increased metabolism and subsequent low plasma concentrations of cyclosporine and tacrolimus, precipitating a requisition for higher doses to maintain the therapeutic levels for marked immunosuppressant effects with calcineurin inhibitors. In a study known as "Cyclosporine Avoidance Eliminates Serious Adverse Renal-toxicity (CAESAR)", a worldwide investigation of genetic polymorphism of several important genes and their association with pharmacokinetics and toxic effects of cyclosporine were studied. The study reported no significant association of *CYP3A5* polymorphisms in the Caucasian population (Grinyó et al. 2008). The *CYP3A5 A6986G* has well-highlighted effects on the pharmacokinetics of tacrolimus, reported in many studies. The transplant patients with *CYP3A5*3* genotypes have proclaimed higher serum concentrations for tacrolimus post-administration in Chinese and Caucasian populations, while lower oral clearance has been witnessed in the Japanese population. In some studies, the genetic polymorphism of the *CYP3A5 A6986G* in Caucasian ethnicity indicated no association with the pharmacokinetics and efficacy of calcineurin inhibitors.

The individuals undergone recent transplants possessing the *CYP3A5*1*1* genotype in the Caucasian, South Asian, Japanese, White, and German populations have shown the necessity for higher doses to reach the plasma levels of cyclosporine and tacrolimus within the therapeutic range for optimum immunosuppressant effect to avoid graft rejection. The association of *CYP3A5*1* allele was also found with better treatment outcomes in kidney transplant patients (Caucasians) being treated with calcineurin inhibitors. An increased overall survival was observed (Kreutz et al. 2008).

The association of the *CYP3A4*1B* allele with variable clinical effects of calcineurin inhibitors is also well pronounced. In the Asian, Black, and White populations, the need for high doses following the induction and maintenance phase of immunosuppressant therapy with calcineurin inhibitors is established in several studies. The *CYP3A4*1B* genotype has been associated with enhanced oral clearance of calcineurin inhibitors in the Caucasian population, while some studies report no significant associations of this allele with the clinical profile of calcineurin inhibitors. The therapeutic plasma concentrations of cyclosporine and tacrolimus took longer to reach target ranges in transplant patients having the *CYP3A4*18B C1236T* genotype, creating a need for therapeutic drug monitoring in these patients to avoid transplant rejection. Some research studies have also investigated the combinations of many genotypes and their effects on the clinical aspects of the treatment with calcineurin inhibitors. The risk of tacrolimus-associated nephrotoxicity was higher in *CYP3A4*1*B/*CYP3A5*1* and *CYP3A4*1*/*CYP3A5*1* genotype as compared to

*CYP3A4*1/ CYP3A5*3* genotype in the Caucasian populations. Some investigations provided no association of these genotypes with the toxic effects of calcineurin inhibitors. Many pharmacogenetic studies have associated *CYP3A4*22* rs35599367*C>T* genotype with higher serum levels of tacrolimus and cyclosporine and subsequent dose adjustment. The variation of clinical response towards calcineurin inhibitors was less associated with *CYP3A4* and *CYP3A5* while the MDR1 gene had vast effects on the pharmacokinetics of calcineurin inhibitors in the Belgian population. In the case of hepatic transplant, the pharmacokinetics of calcineurin inhibitors may be affected by the genetic polymorphism of *CYP3A4* and *CYP3A5* in both donors and recipients. Many studies have also found the association of expression of *CYP3A4* and *CYP3A5* enzymes by intestinal tissue. In some studies, the absence of associations may result from a small sample size and some other confounding factors, such as the lesser frequency of a particular SNP or a genotype in a specific population. Further studies should be done to unveil novel SNPs in all ethnicities in the world to provide a robust foundation for personalized medicine and better treatment outcomes with calcineurin inhibitors.

6. Conclusion and Future Prospects

The study of genetic polymorphism of genes linked with the pharmacokinetics of drugs is essential to develop an understanding and development of an optimized pharmacotherapeutic plan. Such a refined therapeutic regimen is indispensable for a patient with an allogeneic transplant. The pharmacogenetics of calcineurin inhibitors has been extensively studied. Polymorphism of numerous genes including majorly *CYP3A4* and *CYP3A5*, is considerably associated with the metabolism and disposition of calcineurin inhibitors. These associations result in an impact on the efficacy and toxicity of cyclosporine and tacrolimus. Various genotypes and SNPs of *CYP3A4* and *CYP3A5* influence the kinetics of cyclosporine and tacrolimus in the body leading to clinical implications such as increased or decreased levels of these drugs leading to increase or decrease in dose. The process of diminished efficacy may also occur in transplant patients accelerating graft rejection. The present pharmacogenetic data on genotypes such as *CYP3A4*1B* and *CYP3A5 A6986G* can serve as a guide in designing therapeutic regimens for cyclosporine and tacrolimus. Further research is required to locate the novel SNPs in these genes and their association with the pharmacokinetics of calcineurin inhibitors in most of the populations around the globe. This study also emphasizes the need for pharmacogenetic testing of *CYP3A4* and *CYP3A5* polymorphism. Vast pharmacogenetic testing of the donors and the recipients can assist in identifying the patients at risk of drug accumulation or dose adjustments to maintain the efficacy of calcineurin inhibitors. These efforts can be a step up towards individualized medicine in post-transplant therapy hence improving the clinical outcomes of the transplant patients.

Conflict of Interest

The author declare no competing interests.

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