Effect of Genetic Polymorphisms on Tacrolimus Dosage Among Kidney Transplant Recipients

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Abstract:

Background: Kidney transplantation is the gold standard treatment for end-stage renal disease patients. Tacrolimus is the backbone of most of maintenance immunosuppressive protocols nowadays. Achieving accepted Tacrolimus trough level is multifactorial. Previous research suggested presence of genetic varieties responsible for the heterogenicity of tacrolimus doses required to achieve satisfactory trough level.
Purpose: Revise the already published data about effect of genetic polymorphisms on tacrolimus doses in kidney transplant recipients.
Methods: Published research from google scholar, MIDLINE, Web of Science and PubMed were reviewed for valid articles and studies which evaluated certain types of genetic polymorphisms which affected tacrolimus doses among kidney transplant recipients in particular, cytochrome 3A4 and 3A5 genetic polymorphisms.
Conclusion: CYP 3A4 and CYP 3A5 plays crucial role in determining tacrolimus doses required to achieve accepted trough level among kidney transplant recipients.

Keywords: Genetic Polymorphisms, Tacrolimus, Kidney transplantation.

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INTRODUCTION:

Kidney transplantation presents the best way of treating patients with end-stage renal disease. The success of kidney transplantation depends on a delicate balance between the level of immunosuppression, graft rejection and occurrence of adverse effects of immunosuppressive drugs **(Provenzani et al., 2013)**.

Despite the recent advances in immunosuppressive therapies, acute rejection episodes (ARE) following organ transplantation may happen even in the presence of the maximal anti-rejection treatment. Acute rejection rates have fallen from over 50 % in (1987–1991) to close to 10 % (2007–2013) at 1 year after kidney transplantation due to fine tailoring of immunosuppressive treatment and minimizing infections**(Van Arendonk et al., 2014).**

Acute renal rejection is a sudden decline in the function of the transplanted kidney that gives rise to the creatinine levels. Rapid deterioration in the allograft function is associated with specific pathologic changes in the graft. The acute cellular rejection is characterized by infiltration of the allograft by lymphocytes and other inflammatory cells. In acute antibody-mediated rejection, the diagnosis requires morphologic evidence of acute tissue injury, circulating donor-specific alloantibodies, and immunologic proof of an antibody mediated process (such as C4d deposition in the allograft) **(Haas et al., 2014).**

Tacrolimus (Tac) is an immunosuppressant that has been used on a large scale since 1994 for the prevention of graft rejection in kidney transplant patient **(Pirsch et al., 1997).** Tacrolimus has a narrow therapeutic index. Tacrolimus toxicity occurs at concentrations slightly above or even within its therapeutic range. Toxic effects include nephrotoxicity, infection, hypertension, hyperkalemia, hypomagnesemia, hyperglycemia, diabetes, tremor, and other neurotoxic effects. On

the other hand, under dosing can lead to under immunosuppression and graft rejection. Therefore, therapeutic drug monitoring of tacrolimus is used regularly in clinical practice with the goal of optimizing the fine balance between graft rejection and drug toxicity **(Schiff et al., 2007)**|.
However, several factors influence the pharmacokinetics of tacrolimus, including hepatic dysfunction, post transplantation time, hematocrit, serum albumin, age, race and drug interactions, especially gene polymorphism **(Rao et al., 2010)**.

Genetic polymorphisms are responsible for synthesis of the enzymes and transporter P glycoprotein involved in the pharmacokinetics of the drug. It is believed that in the general population genetic is responsible for 20−95% of variability of Tac bioavailability **(Yagil and Yagil, 2002)** . Recently, there has been a great interest in determination of genetic polymorphisms which could predict a degree of Tac bioavailability in each patient individually **(Danesi et al., 2000).**
Cytochrome P450 3A (CYP3A) is the most abundant drug-metabolizing enzyme expressed in human liver, intestine, and kidney. Different expression of CYP3A4 and CYP3A5 causes individual pharmacokinetic variability and may lead to differing drug efficacy or drug toxicity **(Staatz et al., 2010).**
Significant interracial/interethnic variation in the expression and function of CYP3A5 and CYP3A4 is caused by SNPs of genes encoding these proteins. To date, more than ten CYP3A5 alleles have been identified. Among them, CYP3A5* 3 resulting from the substitution of adenosine (A) by guanine (G) at position 6986 (6986A>G) in intron 3, the presence of the allele is associated with a truncated enzyme leading to a decrease activity and lower Tac dose requirement**(Kuehl et al., 2001)** . However, the involvement of CYP3A4 in the metabolism of Tac is currently controversial. No clear relationship between the CYP3A4*1B (-392A>G) variant allele, associated with increased expression of the protein, and Tac dose requirements was demonstrated in several studies, possibly because of differences in ethnicity **(Hesselink et al., 2003).**Meta-analyses provided equivocal results on the impact of the CYP3A5*3 genotype and the risk of nephrotoxicity and acute rejection.The prevalence of acute rejection episodes was similar over the 5 years after transplantation, and rejection free survival was comparable for all genotype groups. CYP3A5 genotype does not significantly impact graft function before and after adjustment to time, donor and recipient age. The CYP3A5 6986A>G variant, which is critical for tacrolimus metabolism and exposure, has no effect on the occurrence of adverse outcomes associated with transplantation**(Flahault et al., 2017).**

REVIEW:

Although successful renal transplantation is the best option for treating chronic kidney disease in children, this was not always the case. Children have had lower patient and graft survival rates than adults since the first kidney transplants in the 1950s. Yet, over the past 60 years, advances in immune system knowledge have guided pediatric multi-center clinical/pharmacokinetic and mechanistic investigations that have shaped modern immunosuppression with noticeably better patient and graft survival rates. Additionally, these kids are now diagnosed with and treated efficiently for pediatric-specific problems with growth, development, neurocognitive maturation, increased complications from primary viral infections, and concomitant congenital/inherited illnesses. enhanced donor selection, stronger immunosuppressive, attention to cognitive delays during dialysis, and refined pretransplant preparation (vaccinations for avoidable infections) ***(Verghese et al. 2017)*.**

SURGICAL AND MEDICAL CONSIDERATIONS BEFORE AND DURING TRANSPLANT

The first successful kidney transplant was carried out by plastic surgeon Joseph Murray on the adult identical twins Herrick brothers in 1954, launching the area of transplant surgery. The first non-twin sibling transplant was performed successfully by Gordon Murray in Toronto in 1959. A few years later, an organ transplant from a brain-dead donor with a beating heart was accomplished***(Squifflet. 2003)***. While some features of kidney transplantation were the same for adults and children, the surgical method was different and there were several technical difficulties with young children **(Cochat et al. 1994).** Long-term paediatric dialysis was complicated by problems with access to dialysis, nutrition, growth, bone disease, and delays in development and cognition **(Davis et al. 1990).** But because of the irrational notion that paediatric receivers needed paediatric donors, transplantation frequently remained an unworkable option with worse outcomes than dialysis **(Fine et al. 1969)**. Children waiting for kidney transplants are at a disadvantage because there are few paediatric deceased donors and almost no paediatric living donors. Problems were complicated by the high rate of graft loss that was reported when matching very young donors with very young recipients, which was frequently related to thrombosis **(Harmon et al. 1992).**

PEDIATRIC IMMUNOSUPPRESSION: THEN AND NOW

Our initial transplant failures were mostly the result of ineffective immunosuppression. While there were preliminary safety and efficacy data from adult studies, new immunosuppression in children was most frequently examined. The area of paediatric kidney transplant has followed but

lagged behind that of adult kidney transplant due to smaller numbers of children recipients and decreased ability for adequately powered randomised controlled studies. ***(Moudgil et al. 2013)*.**

As knowledge of the immune system has evolved, therapy targeted to specific immune-regulatory sites has become possible. Cyclosporine, introduced in the 1980s, a calcineurin inhibitor, was used in combination with azathioprine and steroids and was credited with a dramatic improvement in graft survival ***(Bökenkamp et al. 1995)***. In 1994, mycophenolate mofetil ***(Bunchman et al. 2001)*** was introduced and over the past two decades has almost universally replaced azathioprine. After tacrolimus (another calcineurin inhibitor) became available in 1994, debate followed regarding which calcineurin inhibitor was superior; and it has gradually supplanted cyclosporine in many pediatric centers. To expand the armamentarium further, sirolimus (Rapamune), a macrolide antibiotic, was developed and released. Today, there are many other immunosuppressive agents available on the market including belatacept targeting the costimulatory pathway and tocilizumab a humanized monoclonal antibody that binds the IL-6 receptor. Nevertheless, many of these newer agents are yet to be used with confidence in children ***(Verghese. 2017)*.**

Access to Pediatric Kidney Transplantation

Although children make up only a small fraction of persons awaiting kidney transplantation, today they have been afforded exceptional societal benefits in many countries while in others despite excellent graft outcomes, they remain disadvantaged and neglected due to socio-economic and cultural issues ***(Sibal et al. 2014)***. The current kidney allocation scheme in the United States preferentially allocates higher-quality kidneys from deceased donors to children in relatively prompt fashion ***(Organ Procurement and Transplantation Network. 2016)***with an unintended decline in the donation of kidneys from living donors; a greater proportion of ***Acute cell-mediated rejection (Haas et al. 2018)*** poorly HLA-matched kidney transplants from deceased donors in children; a reduction in racial disparities in access to pediatric kidney transplantation ***(Amaral et al. 2012)*.**

Incidence of acute rejection:

Once as high as 80–90%, rejection rates are now 10% in most pediatric transplant institutions. Due to the various diagnostic definitions of rejection, it is impossible to compare rejection exactly between then and now, but there has been a clear decrease in the incidence of rejection. The yield and quantity of biopsies per transplant patient have grown while morbidity has decreased thanks to safer ultrasonography guided transplant biopsy procedures, in addition to the advancements already discussed. The field has also tremendously benefited from surveillance biopsies, the Banff diagnostic pathology guidelines for the diagnosis and staging of rejection, and the constantly expanding selection of cutting-edge treatments for acute cellular and antibody-mediated rejection. To replace transplant biopsy, research is being done to develop noninvasive indicators of acute rejection **(Halloran et al.2015).**

Types of acute rejection

Cell-mediated rejection

Effector T cells from lymphoid organs carry out cell-mediated rejection (CMR), invading the allograft and triggering an inflammatory reaction. T-cell-mediated rejection is what is meant when the term "cell-mediated rejection" is used (TCMR). It is believed that the T cells are sensitive to donor alloantigen **(Halloran et al. 2004).** This cellular infiltrate penetrates the interstitium and eventually results in tubulitis lesions (i.e., the infiltration of T cells between tubular epithelial cells). The majority of the interstitium's cells are CD4+ and CD8+ T cells as well as CD68+ monocytes and macrophages. Moreover, the presence of eosinophils could portend a worse result **(Emovon et al. 2003).**

Category 3: Borderline changes	
Suspicious (Borderline) for acute TCMR	
Foci of tubulitis (t > 0) with minor interstitial inflammation (i0 or i1), or moderate-severe interstitial inflammation (i2 or i3) with mild (t1) tubulitis; retaining the i1 threshold for borderline with t > 0 is permitted although this must be made transparent in reports and publications	
No intimal or transmural arteritis (v = 0)	
Category 4: TCMR	
Acute TCMR	
Grade IA	Interstitial inflammation involving >25% of nonsclerotic cortical parenchyma (i2 or i3) with moderate tubulitis (t2) involving 1 or more tubules, not including tubules that are severely atrophic[5]
Grade IB	Interstitial inflammation involving >25% of nonsclerotic cortical parenchyma (i2 or i3) with severe tubulitis (t3) involving 1 or more tubules, not including tubules that are severely atrophic[5]
Grade IIA[1]	Mild to moderate intimal arteritis (v1), with or without interstitial inflammation and/or tubulitis
Grade IIB[1]	Severe intimal arteritis (v2), with or without interstitial inflammation and/or tubulitis
Grade III[1]	Transmural arteritis and/or arterial fibrinoid necrosis of medial smooth muscle with accompanying mononuclear cell intimal arteritis (v3), with or without interstitial inflammation and/or tubulitis

Antibody-mediated rejection

Antibody-mediated rejection (AMR) has emerged as a major clinical challenge and has recently been identified as the most frequent cause for renal allograft failure in adults **(Gaston et al. 2010)**. Donor-specific anti HLA-antibodies (DSA) are identified in the vast majority of AMR and patients with DSA are at increased risk for allograft failure. The primary target of DSA is the endothelium of the microcirculation in the allograft. Clinical management of AMR differs significantly from TCMR. Therefore, accurate diagnosis of AMR is crucial. However, the morphological spectrum of AMR is heterogeneous and comprises a set of non-specific morphological lesions which are nevertheless an essential part of current diagnostic criteria for AMR along with C4d deposition on the endothelium, presence of DSA, and graft dysfunction **(Racusen et al. 2003)**. Chronic AMR has been widely recognized in kidney transplants, but needs yet to be defined in other organ transplants. Despite being a significant contributor to late graft loss, it is often missed due to limitations of current diagnostic criteria. In particular the lack of sensitivity of C4d and the limited specificity of DSA account for most missed AMR cases ***(Racusen et al. 2003).***

Genetics of acute rejection (AR) in kidney

Allograft rejection can be divided into three main categories: hyper acute rejection, which happens right away after the transplant, AR, which happens days to months later, and chronic rejection, which happens years later. AR can also be categorized as mixed, C4d-negative ABMR, T cell-mediated rejection (TCMR), antibody-mediated rejection (ABMR), and mixed. In this review, we concentrated on all kinds of AR. As the AR phenotype has become better understood, so has its definition. While having very distinct mechanisms, various forms of AR all result in the allograft being rejected. Hence, it has been challenging to identify and evaluate genetic association studies due to the variety of the AR phenotype ***(Loupy et al. 2017)***.

The approaches used to explore the genetics connected to AR have evolved throughout time, just like the notion of AR. Blood or biopsy samples are used to extract DNA and/or RNA, which is then used to genetically test for AR associations. These association tests have progressed from testing potential single nucleotide polymorphisms (SNPs) to testing multiple potential SNPs, panels of potential SNPs on gene chips, and genome-wide SNP chips. Currently, we are using whole genome and targeted next-generation sequencing to identify known and novel SNPs **(Dorr et al. 2018).**

Single nucleotide polymorphisms associated with acute rejection

Although solid organ transplant outcomes have improved recently, graft failure, chronic rejection, and AR continue to pose obstacles to success. Finding genetic markers linked to AR and AR risk is of tremendous interest because AR has consistently been proven to be one of the strongest predictors of allograft survival **(Matas et al. 1994).** Researchers have sought to link genetic variation with the incidence of AR in numerous papers over the past few decades. Human leukocyte antigens (HLA), also known as MHC antigens, have shown the strongest correlation between genetic variations and transplant outcomes **(Sheldon et al. 1994).** Because to this strong relationship, transplant centres work to minimise the immunogenicity of the allograft and lower the risk of AR by matching as many HLA alleles between the donor and recipient as possible. There have also been additional reports demonstrating a relationship with AR of genetic variations in other genes. The majority of genetic association studies seek out intriguing genes before attempting to comprehend the biological significance of those genes to AR. Due to the tiny impact sizes of individual SNPs and the variable character of AR, it is challenging to

predict AR susceptibility using genetic association studies. These genes would include, among others, those in tissue repair pathways, immunological pathways, innate and adaptive immune systems, stimulatory chemicals, and genes involved in systemic hypertension. Initial reports for numerous variations show a statistically significant correlation with AR for a particular SNP; however, validation for the majority of these SNPs has remained elusive **(Chen et al. 2014).** There are identified 76 variants in the literature which were associated with AR. This list was compiled from an extensive literature search of numerous genetic variants that have been associated with AR in kidney and some liver transplant studies. Nearly all the reports in Table 1 are from candidate gene analysis of recipient genomic DNA. Few studies have been conducted that investigate the genetic interactions between donor and recipient beyond HLA matching. Given the small cohort size of most transplant studies, donor and recipient genetics have not been thoroughly studied in combination. Note that there are several limitations of the reports in the literature. As stated in the Introduction, there are variations in the definition of AR between studies and that definition has changed over time. Additionally, population stratification was not done in the analysis for many of the reviewed studies. In most of these reports, the statistical power to detect a true association was low. It is common for small cohorts to be used in the analysis (N < 300) or to not take into account the testing of multiple SNPs. Additionally, validation cohorts are typically absent in the studies, or subsequent studies failed to validate the initial positive reports. Validation could fail because of the heterogeneity of the AR phenotype. Each type of AR has different mechanisms, so each type would likely have different genetics and thus be difficult to validate unless large sample sizes of each type of AR are obtained. However, some of these SNPs in Table 1 have been associated with AR by multiple studies, such as ACE (rs4340), CCR2 (rs1799864), CCR5 (rs1799987), CD28C (rs3116496), CTLA4 (rs5742909, rs231775), F5 (rs6025) and IL10 (rs1800896, rs1800872). Most of these studies have focused on recipient SNPs, but there have been a few studies analyzing donor-related SNPs. At present, there are no candidate SNPs that have been unambiguously shown to be associated with AR, through the use of large (1000+) discovery and validation cohorts***(Dorr et al. 2018)*.**

Pharmacogenomics and acute rejection

To reduce the risk of AR, research has focused on optimizing immune suppression drugs following transplantation. Tacrolimus (TAC) is the primary immune suppressant used in >90% of kidney transplants to prevent AR and has differing efficacy in different populations, in large part due to the diverse rates of TAC metabolism, and has been the predominate focus of pharmacogenomics research in transplantation ***(Matas et al. 2015)*.** SNPs in the CYP3A5 and CYP3A4 drug metabolism genes, as well as TAC transporters, can influence the metabolic rates of TAC. Some of these SNPs, which impact the pharmacokinetics of TAC, lead to higher or lower dose requirements to achieve the therapeutic target. There is also evidence that high metabolic rates of immune suppressants in the blood, such as TAC ***(Egeland et al. 2017)*** or cyclosporine ***(Lindholm et al. 1993),*** are associated with higher risk of AR. TAC and cyclosporine are transported by ABCB1 and metabolized by the CYP3A4 and CYP3A5 enzymes ***(Barbarino et al. 2017)*.** SNPs in the genes that express these, and related, enzymes have been the focus of most investigations of TAC trough blood concentrations and have been extensively reviewed elsewhere ***(van Gelder et al. 2014, Hesselink et al. 2014, Tang et al. 2016)***. The CYP3A5*3 SNP (rs776746) is the most commonly studied SNP and significantly decreases TAC metabolism ***(Rojas et al. 2015)***and the dose needed to reach therapeutic TAC blood concentrations. The CYP3A5*3 SNP has inconsistently been associated with rejection ***(Yaowakulpatana et al. 2016, Flahault et al. 2017)***. Further studies have shown that combinations of SNPs affect TAC metabolism **(Pulk et al. 2015)**. In a study of mainly Caucasian subjects, the effects of ABCB1 SNPs on TAC concentrations were strongly accentuated by CYP3A4 and CYP3A5 genotype **(Vanhove et al. 2016)** although this has been inconsistently shown. Some studies have shown that the *28 SNP in the cytochrome P450 oxidoreductase gene (POR) is associated with increased metabolic activity of CYP3A4 and CYP3A5 ***(Lunde et al. 2014)*.** Subjects with rapid TAC-metabolizing SNPs such as CYP3A5*1 and POR*28T when compared with slow-metabolizing CYP3A5*3 and CYP3A4*22 SNPs have dose requirements two to three times greater and a significantly longer time to reach therapeutic trough blood concentrations. However, no differences were observed in AR by SNP genotype ***(Kuypers et al. 2014)***. Another study suggested that SNPs in the POR, ABCB1 and CYP3A5 genes should all be considered when dosing TAC. Optimal dosing of immune suppressants may lead to lower rates of AR ***(Almeida-Paulo et al. 2016)*.**

Numerous recent articles have discussed the relationship between AR and interpatient variability in TAC concentration. Genetics, diarrhea, drug-drug interactions, non-adherence, and generic TAC substitution are a few of the multifactorial factors that contribute to interpatient TAC heterogeneity in trough concentrations **(Shuker et al. 2015).**

A higher risk of AR was linked to high interpatient variability in TAC blood level **(Huang et al. 2016)**. However, the TAC interpatient variability was not explained by the CYP3A5*3 genotype alone **(Ro et al. 2012)**, indicating that other genetic variations and clinical variables may also be involved. TAC interpatient variability was strongly linked with renal AR. According to other research, greater interpatient TAC variability is linked to biopsy histology post-transplant and worse short- and long-term results **(Borra et al. 2010, Shuker et al. 2016, Vanhove et al. 2016 Whalen et al. 2017).**

High TAC interpatient trough variability has been linked to higher rates of late rejection and graft loss in juvenile kidney allografts, according to studies **(Pollock-Barziv et al. 2010, Prytula et al. 2012)**. Moreover, the once-daily TAC formulation did not lower the high TAC interpatient trough variability compared to the twice-daily dose, while having the potential to increase adherence **(Shuker et al. 2015)**

We plan to implement genotype-based TAC dosing models in the future that take both clinical and genetic aspects into consideration. The purpose of the dosing models would be to maintain the ideal troughs after transplanting and to quickly reach therapeutic TAC concentration. According to one of these dosage equations, the CYP3A5*1 genotype and four clinical variables were crucial for TAC clearance **(Passey et al. 2011).**

A TAC dosing strategy was created in a different study for Black American kidney transplant recipients **(Sanghavi et al. 2017).** Future dosing models will need to take into account low-frequency variants found in next-generation sequencing research, as well as maybe add epigenetics and more clinical variables, as these studies were restricted to common genotypes. We envision genetic-based TAC dosing as a strategy to enhance transplant outcomes and potentially lower incidence of AR by maximising immune suppression **(Dorr et al. 2018).**

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