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ORIGINAL ARTICLE

ASSOCIATION BETWEEN TACROLIMUS VARIABILITY AND *ABCB1/CYP3A5* GENETIC POLYMORPHISMS IN KIDNEY TRANSPLANT RECIPIENTS

Canevska Taneska A[1]*, Trajceska L[1], Rambabova Bushljetik I[1], Severova Andreevska G[1], Gjorgjievski N[1], Staninova Stojoska M[2], Dejanov P[1], Spasovski G[1], Sterjev Z[2]

*Corresponding Author: Aleksandra Canevska Taneska, MD, University Clinic of Nephrology, Skopje, North Macedonia; Faculty of Medicine, University "St. Cyril and Methodius", Skopje, R. of North Macedonia, str. Vodnjanska 17, 1000 Skopje, Republic of North Macedonia; Tel. 0038971292705; E-mail:aleksandra.canevska.taneska@medf.ukim.edu.mk

ABSTRACT

The aim of this study was to assess tacrolimus variability in kidney transplanted patients and its associations with genetic polymorphisms in *CYP3A5*(rs776746) and *ABCB1*(rs1045642) in North Macedonia. We conducted an observational study including 84 kidney transplant recipients who met inclusion criteria. Variability of tacrolimus was evaluated using the concentration-to-dose ratio (C0/D) and coefficient of variation (CV%). Single-nucleotide polymorphisms (SNPs) were identified using real-time polymerase chain reaction. Among the 84 patients, males predominated (70%), with a mean age of 37.57years. Analysis for variability revealed C0/D<1.05ng/ml/mg in 15% and CV>30% in 17% examined population. The majority of patients (86%) had the *CYP3A5* GG genotype, whereas 14% carried at least one expresser allele (*CYP3A5* AA/AG). Regarding *ABCB1*, 26% of patients had the CC genotype, 46% CT, and 27% TT. Patients carrying the *CYP3A5* expresser genotype (AA/AG) demonstrated a significantly higher risk of low C0/D ratio (<1.05 ng/mL/mg) compared to non-expressers (58% vs. 8%; OR = 2.01; 95% CI: 1.114–3.624; p = 0.001). Additionally, expresser patients required significantly higher daily doses of tacrolimus to achieve target blood concentrations compared to non-expressers (5.11 ± 2.21 mg vs. 3.21 ± 1.78 mg, p = 0.014). No significant association was found between *ABCB1* polymorphisms and tacrolimus variability parameters. This is first study in our country that explores and verifies the association between variability of tacrolimus and genetic polymorphisms in kidney transplant recipients. Our work advances the effort to tailor more personalized treatment, minimizing negative outcomes and adverse effects to the lowest achievable level.

Key words: genetic polymorphisms, tacrolimus, transplanted kidney, variability,

INTRODUCTION

Transplantation remains the most favorable choice of renal replacement therapy in patients with end-stage renal disease, necessitating the obligatory use of immunosuppression. For decades, tacrolimus has been the foundation of standard immunosuppressive protocols and the preferred choice among calcineurin inhibitors, significantly improving allograft survival and reducing acute rejections [1]. Despite its well-defined safety and efficacy profile, tacrolimus exhibits narrow therapeutic index and substantially high variability, which can result in unpredictable drug exposure and thereby increase the risk for significant toxicity, adverse reactions or graft rejection [2]. The treatment challenges posed by tacrolimus variability are well recognized and have been extensively analyzed in numerous studies worldwide. The literature suggests that factors such as age, gender, anemia, albumin, drug interactions (azoles and Ca channel blockers), dietary factors and gastrointestinal conditions can influence variability [3,4]. However, many authors also emphasize the important role of genetic polymorphisms [3,4]. Polymorphisms in genes encoding cytochrome P450 3A enzymes and P-glycoprotein (*CYP3A5* and *ABCB1*), which are involved in the metabolism of tacrolimus, are among the most extensively

[1] University Clinic of Nephrology; Faculty of Medicine, University "St. Cyril and Methodius," Skopje, Republic of North Macedonia

[2] Faculty of Pharmacy, University "St. Cyril and Methodius", Skopje, Republic of North Macedonia

studied genetic factors related to this variability [4,5]. According to several studies, the involvement of *CYP3A5* A>G transition et position 6986 in intron 3 (also known as *CYP3A5*3* polymorphism) is recognized as a major factor influencing tacrolimus metabolism, contributing to differences in tacrolimus clearance and dose requirements among patients [5,6]. Consequently, based on the recommendation of Clinical Pharmacogenetics Implementation Consortium, genotyping of *CYP3A5* has become important tool for optimizing dosing and enabling more personalized immunosuppressive treatment for these patients, even prior the act of transplantation [6,7]. The role of *ABCB1* in tacrolimus pharmacokinetics remains controversial and appears to vary across different ethnicities and regions. While some studies suggest that *ABCB1* affects drug absorption, distribution and contributes to inter-individual variability in treatment response, others conclude that its influence od tacrolimus metabolism is insignificant [8,9]. Two previous studies conducted in kidney transplanted patients in North Macedonia showed that approximately 12-15% of patients exhibited high variability for tacrolimus. These patients also presented with higher serum creatinine levels, indicating poorer kidney function. Despite age, both studies did not identify any clinical, laboratory factor, nor drug interactions as a causes of this variability. However, neither of these studies included genetic analysis [10,11].

The objective of this study was to assess tacrolimus variability in kidney transplanted recipients and its association with *CYP3A5* and *ABCB1*genetic polymorphisms in the Republic of North Macedonia.

MATERIALS AND METHODS

We conducted an observational, cohort, single-center study in the unique public kidney transplantation center in the Republic of North Macedonia. The inclusion criteria encountered age above 18 years, standard immunosuppressive protocol with calcineurin inhibitor-tacrolimus, mycophenolic acid, steroids, living and brain-death donor transplantation and signed informed consent-approved by the ethical committee. Patients with poor treatment adherence were excluded from the study. Only clinically stable patients after third month of transplantation were included. Patients that met the following criteria: stable graft function (with serum creatinine fluctuations <15% within the last 30 days), absence of biopsy-proven or suspected acute rejection episodes, no active viral (such as CMV or BK virus nephropathy) or systemic infectious requiring hospitalization, were considered clinically stable after the 3rd post-transplant month [12]. Participants in the study were analyzed for demographics, comorbidities, kidney disease etiology, pre-transplantation dialysis modality, laboratory and data associated with the act of transplantation. To assess intra- and inter-individual variability, whole blood tacrolimus trough concentrations and corresponding dosage prescriptions were sequentially analyzed for each individual patient beyond the third month post-transplantation at multiple consecutive time points. All patients included in the study were maintained on an oral, standard immediate-release formulation of tacrolimus. Blood samples for the determination of tacrolimus trough concentrations (C0) were collected in the morning under fasting conditions, immediately prior to the administration of the next scheduled dose. Therapeutic drug monitoring of tacrolimus was performed at the Institute of Clinical Biochemistry as part of routine clinical analysis using a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of tacrolimus in whole blood on the ARCHITECT iSystem (Abbott Laboratories, Abbott Park, IL, USA). The initial dosing regimen was based on the patient's actual body weight (0.1–0.2 mg/kg) divided into two doses administered every 12 hours. Subsequent dosages were titrated and adjusted until a target range of 5.0–8.0 ng/mL was reached [13]. Tacrolimus concentration values falling below 2.0 ng/mL or exceeding 10.0 ng/mL were either repeated or discarded as invalid.

The coefficient of variation was calculated as ratio between the standard deviation and arithmetic mean value of TAC (from at least 3 to 6 repeated measurements or samples) expressed in percentages, for the period between 6th and 12th month, after the date of transplantation [14]. The inter-patient variability, evaluated via the concentration-to-dose (C0/D) ratio, was analyzed at three specific time points for each patient (3-, 6-, and 12-months' post-transplantation), and the mean C0/D value was calculated for subsequent statistical analysis across the genotype groups [15]. Peripheral whole blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes from all participants for pharmacogenetic analysis, which was conducted anonymously. Single-nucleotide polymorphisms (SNPs) within the target genomic sequences were identified via Real-Time Polymerase Chain Reaction (PCR) based on allelic. All amplification reactions were performed using fluorescent Mx PRO 3005P thermal analyzer (Agilent technologies, Santa Clara, CA USA) utilizing specific TaqMan tests for genotyping of medication-metabolizing genes (TaqMan Drug Metabolism Genotyping Assay) and TaqMan SNP Genotyping Assay, according to manufacturer's protocols (Applied Biosystems, Life technologies; Grand Island, NY USA). Specifically, the PCR protocols for genotyping the *ABCB1* (rs1045642; assay ID: C__7586657_20) and *CYP3A5*3* (rs776746; assay ID: C__26201809_30) variant alleles consisted of an initial denaturation step at 95°C for 10 minutes, followed by 45 cycles of dena-

turation at 92°C and annealing/extension at 60°C for 60 seconds. All pharmacogenetic analyses were performed at the Center for Biomolecular and Pharmaceutical Analysis, Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, North Macedonia.

Statistical analysis was obtained with SPSS version 27. The data was presented as mean, median, standard deviation and compared by Mann-Whitney and Fishers Exact test. Detected genotype distributions were assessed for the Hardy-Weinberg equilibrium with a Chi-Square test (X^2). As cut off values for C0/D the value above 1.05ng/mL/mg indicated slow metabolizers and below 1.55ng/mL/mg - fast metabolizers [16]. For the tacrolimus variability, CV>30% was indicative for a high intra-patient variability [17].

RESULTS

Our study included 84 kidney transplant recipients. Table 1 presents the demographic, clinical, dialysis-related, tacrolimus variability, and transplantation-related characteristics of the study population. Male patients predominated (70%), and the mean age was 37.57 years. Regarding comorbidities, 10% of patients had diabetes, while 76% were diagnosed with hypertension prior to transplantation. According to the ERA registry classification for chronic kidney disease and underlying etiology, patients were classified into six groups (18). The most common cause of kidney disease was glomerulonephritis (40%), while 17% of patients had an undefined etiology. Most patients (88%) underwent dialysis prior to transplantation, with a mean dialysis vintage of 32 months. The number of patients who received a kidney from a living donor was slightly higher than those who underwent brain death donor transplantation ((49 (58%) vs 35 (42%)). Analysis of intra- and inter-individual variability showed low C_0/D (<1.05 ng/mL/mg) in 15% of patients and high intra-patient variability (IPV), defined as CV > 30%, in 17% of the study population (Table 1).

Table1. Demographic, clinical, dialysis, variability and transplantation-related parameters.

N=84	Mean value ± SD N (%)
Age (years)	37.57 ± 12.34
Male (%)	59 (70)
Hypertension (%)	64 (76)
Diabetes (%)	8 (9)
Preemtive transplantation (%)	10(12)
Dialysis treatment prior transplantation (%)	74(88)
Dialysis vintage (months)	32.26 ± 42.93
hemodialysis (%)	71 (95.94)
peritoneal dialysis (%)	6 (8.1)
Brain death donor BDD (%)	35 (42)
Living donor (%)	49(58)
primary kidney disease	
Glomerulonephritis	34 (40)
Hypertensive/vascular nephropathy	7(8)
Diabetes Mellitus	6(7)
Tubulointerstitial disease	16(19)
ADPKD	4(4)
Unknown/uncertain etiology	17(20)
CV>30%	14(17)
C0/D<1.05ng/mL/mg	13(15)

Abbreviations: BDD - brain death donor; ADPKD – adult polycystic kidney disease; CV – coefficient of variation; mean C0/D - concentration/dose ratio

The results of the genetic analysis for the detection of SNP polymorphisms in the DNA sequences of *CYP3A5* (6986A>G) and *ABCB1* (3435C>T), along with the corresponding genotypes, are presented in Table 2. The majority of patients (86%) carried the *CYP3A5* GG genotype (*CYP3A5*3/*3*), which is associated with a non-expresser phenotype. In contrast, 14% of the study population were carriers of at least one functional A allele, indicating active *CYP3A5* enzyme expression. Among these, 13% were

Table 2. Detected *ABCB1* and *CYP3A5** genotypes and functional phenotype.

Gene	Nucleotide change (SNP)	Genotype	N(%)	Functional phenotype
*CYP3A5** (rs776746)	6986A>G	AA (CYP*3A5*1/1**)	1(1.2)	Expressers
		AG (CYP*3A5*1/*3*)	11(13.09)	Expressers
		GG (*CYP3A5*3/*3*)	72(86)	Non-expressers
ABCB1 (rs1045642)	3435 C>T	CC	22(26)	High P-gp expression
		CT	39(46)	Intermedium expression
		TT	23(27)	Low P-gp expression

Abbreviations: CYP3A5 - Cytochrome P450 Family 3 Subfamily A Member 5; ABCB1 - ATP Binding Cassette Subfamily B Member 1; P-gp – P glycoprotein; SNP- single-nucleotide polymorphisms

heterozygotes with genotype *CYP3A5* AG (*CYP3A5**3/*1) and 1% was homozygote with *CYP3A5* AA genotype (*CYP3A5**1/*1). Both hetero and homozygotes carriers of allele A are classified as expressers, reflecting an active metabolic phenotype. Regarding *ABCB1* (3435 C>T), 26% of patients carried the CC (wild-type) genotype, associated with high P-gp expression, 46% CT genotype, corresponding to intermediate expression, while 27% were carriers of the TT genotype, linked with low P-gp expression.

A graphical distribution display of detected genotypes for both examined *CYP3A5* and *ABCB1* genes (expressed in percentages) is presented in Picture 1. A marked predominance of the *CYP3A5* GG genotype is evident (86%), indicative of absent or markedly reduced *CYP3A5* enzymatic activity, compared with 14% of cohort carriers of at least one functional *CYP3A5* AA/AG allele (Figure 1A).

In figure 1B a relatively balanced distribution of *ABCB1* genotypes is presented, whereas 27% of patients carried TT genotype.

The genotype distributions did not show deviation from the Hardy-Weinberg equilibrium (P>0.05), indicating a well-chosen representative pool of patients for the purpose of the study. (Figure 2-A and B)

The comparative analysis for both *CYP3A5** and *ABCB1* examined variant genes regarding detected genotypes, age and gender (Table 3). No statistical significance was found in respect for age (47.19 vs 45.75, p=0.618; 46.23 vs 41.02, p=0.364), or gender (8(67) vs 51(70), p=0.744; 16(68) vs 43(70); p=0.567), respectively.

When we analyzed the association between the observed genotypes for *CYP3A5* and parameters for tacrolimus variability, patients with *CYP3A5* AA an AG genotypes were more likely to present with C0/D<1.05ng/

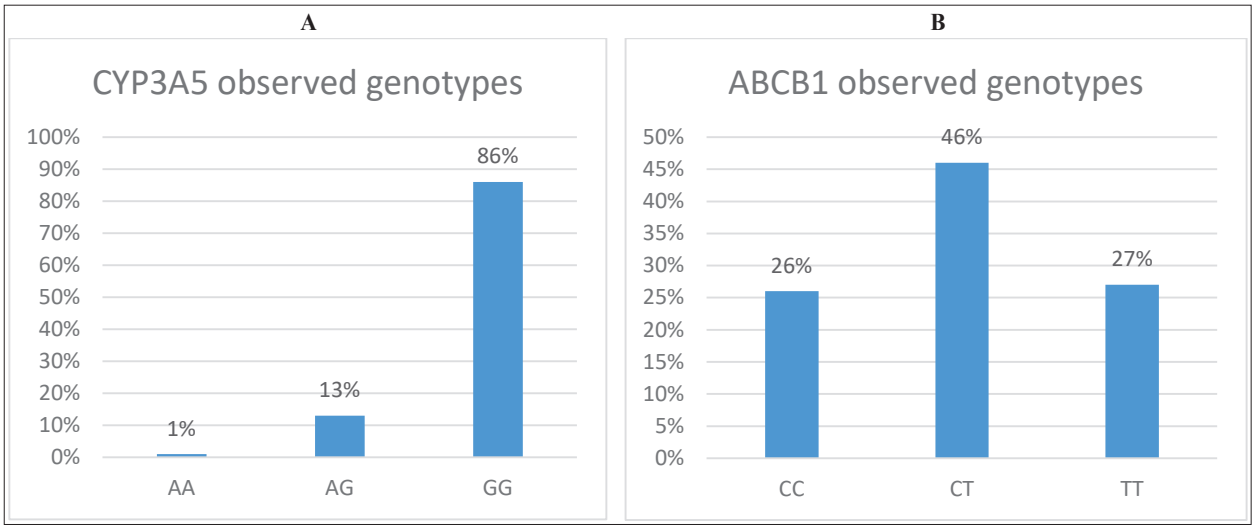


Figure 1. Genotype distribution of the tested variants for both genes: **A** - *CYP3A5*3* (rs 776746) 6986 A>G, frequencies of AA, AG and GG genotypes; **B** - *ABCB1*(rs 1045642) 3435C>T, frequencies of CC, CT and TT genotypes

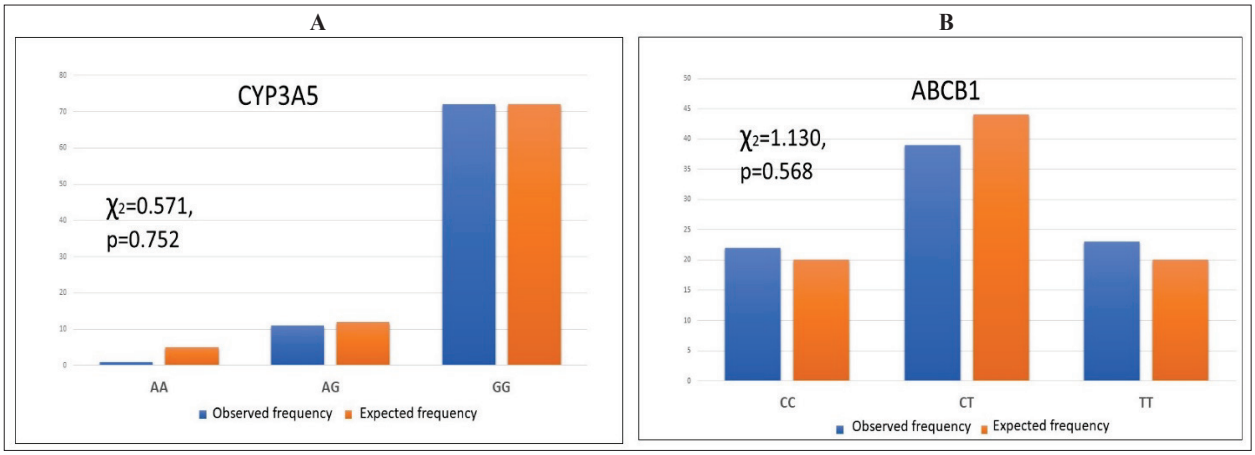


Figure 2. Hardy-Weinberg equilibrium for genotype distributions **A** - *CYP3A5* genotypes, **B** - *ABCB1* genotypes

Table 3. Comparative analysis of patients with each genotype for both genes regarding age and gender.

N=84	*CYP3A5**			*ABCB1*		
	AA + AG (*CYP3A5* *1/1*+*1/3*)	**GG** (*CYP3A5* *3/3*)	p	**TT+CT** (*ABCB1*)	**CC** (*ABCB1*)	p
	N=72 Medium rank N⁰ (%)	N=12 Medium rank N⁰ (%)		N=61 Medium rank N⁰ (%)	N=23 Medium rank N⁰ (%)	
Age (years)	47.19	45.75	0.618	41.02	46.23	0.364
Gender (male)	8(67)	51(70)	0.744	43(70)	16(68)	0.567

Abbreviations: CYP3A5 - Cytochrome P450 Family 3 Subfamily A Member 5; ABCB1 - ATP Binding Cassette Subfamily B Member 1

Table 4. Association between observed genotypes and tacrolimus variability.

CYP3A5	**AA/AG**	**GG**	**p**		
N (%)	12 (14%)	72 (86%)			
C0D <1.05ng/mL/mg	**7 (58%)**	**6 (8%)**	**0.001**	**2.01**	**1.114-3.624**
CV>30%	3 (25%)	11 (15%)	0.197		
***ABCB1* 3435C>T**	**TT**	**CT+CC**			
N (%)	23 (27%)	61 (73%)			
C0D <1.05 ng/mL/mg	6 (27.3%)	7 (11.5%)	0.096		
C0/D>1.55	13 (59.1%)	47 (77%)	0.163		
CV>30% ng/ml/mg	5 (22.7%)	9 (14.8%)	0.507		
ABCB1	**CC**	**CT+TT**			
N (%)	22 (26%)	62 (74%)			
C0D <1.05 ng/mL/mg	4 (18.2%)	9 (14.8%)	0.737		
C0/D>1.55ng/mL/mg	17 (77.3%)	43 (70.5%)	0.593		
CV>30%	3 (13.6%)	11 (18%)	0.751		
ABCB1	**CT**	**CC+TT**			
N (%)	39 (46%)	45 (54%)			
C0D <1.05 ng/mL/mg	3 (7.7%)	10 (22.7%)	0.075		
C0/D>1.55 ng/mL/mg	30 (76.9%)	30 (68.2%)	0.464		
CV>30%	6 (15.4%)	8 (18.2%)	0.483		

Abbreviations: CYP3A5 - Cytochrome P450 Family 3 Subfamily A Member 5; ABCB1 - ATP Binding Cassette Subfamily B Member 1;
CV – coefficient of variation; mean C0/D - concentration/dose ratio

mL/mg. An assessment for the observed risk in these patients, performed with Mantel Haenszel test estimated twice higher risk for C0/D<1.05ng/mL/mg in patients' carriers of A variant alleles (7(58%) vs (6(8%); OR=2.01; 95%CI (1.114-3.624) p=0.001). Although the percentage of patients with CV>30% was higher in those presented with AA/AG genotypes than those with GG, the difference did not reach statistical significance (3(25%) vs 11(15%), p=0.197). Regarding *ABCB1* no significant relation was found between indicators of variability and observed genotypes. (Table 4)

In Table 5 are presented results of the comparative analysis between patients with *CYP35A5* AA/AG and GG genotypes in respect of the tacrolimus blood concentration, the daily dose requirements for achieving therapeutic drug level and parameters for variability. In both groups patients reached the desired tacrolimus blood concentrations and in similar ranges (6.73±1.27 vs 6.166 ± 1.28, p=0.157), but the required daily doses of TAC for its achieving were remarkable higher in those with genotypes *CYP3A5* AA/AG compared to those without (5.107± 2.21 vs 3.2066 ± 1.78; p=0.014). Also, the mean value of C0/D in patients with *CYP3A5* AA/AG genotypes was noteworthy lower compared to others (1.505 ± 0.98 vs 2.627 ± 1.30, p=0.003), respectably, as shown on Figure 3.

Table 5. Tacrolimus daily dosage and variability parameters for *CYP3A5 AA* and AG genotypes.

CYP3A5 AA/AG	N=72	N=12	p
TAC (ng/mL)	6.73±1.27	6.166 ± 1.28	0.157
TAC SD	1.526 ± 0.63	1.369 ± 0.59	0.502
Dosage (mg)	**3.206 ± 1.78**	**5.107 ± 2.21**	**0.014**
Dosage SD	0.338 ± 0.33	0.572 ± 0.438	0.096
CV% TAC	22.74 ± 8.38	23.00 ± 9.39	0.931
C0/D (ng/mL/mg)	**2.627 ± 1.30**	**1.505 ± 0.98**	**0.003**

Abbreviations: CYP3A5 - Cytochrome P450 Family 3 Subfamily A Member 5; CV – coefficient of variation; mean C0/D – concentration/dose ratio; TAC – tacrolimus; SD – standard deviation

Table 6. Tacrolimus daily dosage and variability parameters for *ABCB1* TT genotype.

***ABCB1* TT**	N=61	N=23	p
TAC (ng/mL)	6.678±1.26	6.598 ± 1.35	0.803
TAC SD	1.509 ± 0.58	1.504 ± 0.71	0.976
Dosage (mg)	3.279 ± 1.74	3.904 ± 2.35	0.254
Dosage SD	0.402 ± 0.33	0.443 ± 0.402	0.654
CV% TAC	22.94 ± 8.55	22.41 ± 8.45	0.795
C0/D (ng/mL/mg)	2.49 ± 1.26	2.40 ± 1.46	0.788

Abbreviations: ABCB1 - ATP Binding Cassette Subfamily B Member 1; CV – coefficient of variation; mean C0/D - concentration/dose ratio; TAC – tacrolimus; SD – standard deviation

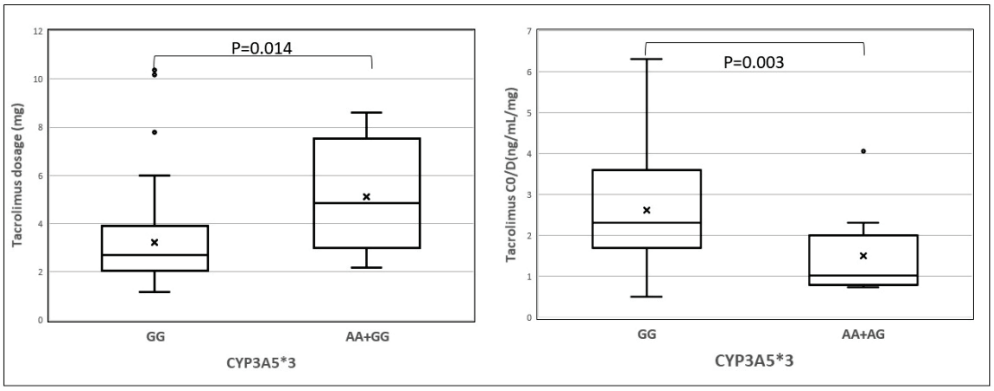


Figure 3 Illustrations of dose regimens for tacrolimus for *CYP3A5* genotypes

A visual representation of significant difference for tacrolimus daily dose requirements between observed genotypes for *CYP3A5* is exhibited in Figure 3.

The analysis for TAC blood concentration, daily dosing and variability for carriers of *ABCB1* TT genotype linked with functional phenotype for low P-gp expression did not show statistical significance for neither parameter of interest related to any metabolic profile. (Table 6)

DISCUSSION

Our study included 84 kidney transplant recipients, with a predominance of a mail patient (70%). This is consistent with the data from the latest European registry for patients with CKD requiring any kind of renal replacement therapy (ERA/EDTA 2022/published year 2024), which reports that 62% of patients with end-stage kidney disease

and 59% of kidney transplant recipients are male [18]. The number of living-donor transplantations in our study population was higher than that of deceased-donor transplantation. Although this distribution differs from overall European data (deceased 66% vs 33% living), it is comparable with data for countries in our region, where living-donor transplantation remains predominant [18]. Despite ongoing improvements in healthcare and the increasing upper age limit for eligible transplant recipients, the mean age of our study population was approximately 37 years. This is in correlation with reports indicating that the highest proportion of transplantations is still performed in the middle-age patients [19]. Notably, the proportion of patients with glomerulonephritis as the primary kidney disease in our cohort was higher than that reported in regional studies, which may partly explain the younger age of our population. For the pharmacogenomic analysis, participants were tested for the distribution frequencies of *CYP3A5* (rs776746) and *ABCB*1(rs1045642) genetic polymorphisms, which have been identified as key factors that influencing tacrolimus pharmacokinetics (5-9). The observed frequency of *CYP3A5* expressers genotypes (AA/AG) was around 15%, consistent with data obtained from regional studies and studies conducted in healthy population in our country [20,21]. Similarly, the distribution of *ABCB1*genotypes (CC, TT, CT) was comparable to that reported in Caucasians populations globally, regionally and in local healthy cohorts [5,9,20,22]. The Hardy-Weinberg Equilibrium (HWE) for *CYP3A5* and *ABCB1* genotype distributions confirmed the genetic stability of the study population. Regarding interrelations between tacrolimus variability and detected genetic polymorphisms, a firm, statistically significant association was observed between a C0/D ratio <1.05ng/m/mg and carriers of *CYP3A5* AA/AG genotypes, holding twofold higher risk of belonging to this group compared to non-expressers. This finding is in accordance with existing literature, which highlights the significant impact of *CYP3A5* polymorphisms on tacrolimus pharmacokinetics and their potential implications for graft function and outcomes [2,4,23]. The tacrolimus concentration-to-dose (C0/D) ratio is widely used as an accessible pharmacokinetic marker reflecting inter-individual variability in drug clearance and enabling the identification of fast and slow metabolizers [2,24]. Patients with a C0/D ratio <1.05ng/ml/mg are considered fast metabolizers and typically require higher daily tacrolimus doses to achieve target blood concentration [6,23,24]. In line with this, our study confirmed that carriers of *CYP3A5* expressers genotypes with C0/D ratio <1.05ng/mL/mg required higher daily tacrolimus doses compared to non-expressers to reach therapeutic levels. When assessing the connection between *CYP3A5* genotypes and intra-patient variability (IPV) expressed as CV>30%, a trend toward higher IPV in *CYP3A5* expressers genotypes was noted, this did not reach statistical significance. This may be partly explained by the relatively small sample size, as well as the influence of additional factors such as drug interactions, dietary habits and adherence to therapy, which were not included in the analysis [25]. The most common studied *ABCB1* C3435T (rs1045642) polymorphism, which may influence P-glycoprotein expression and by thereby affect tacrolimus absorption and tissues distribution, has not demonstrated consistent or strong proof of relevance for tacrolimus variability as *CYP3A5* [8,9,26]. Although some studies have reported association between the *ABCB1*(3435) TT genotype and higher TAC C0/D ratios especially in the early post-transplant period [26], our results did not demonstrate a significant association between *ABCB1* genotypes and TAC variability, dosing requirements, or blood concentration, consistent with findings from regional studies [8,20]. Nevertheless, the lack of an independent effect of *ABCB1* does not exclude its role in tacrolimus pharmacokinetics. On the contrary, several studies suggest that the combined effect of *CYP3A5* and *ABCB1* polymorphisms (including 3435C>T) may be more relevant in explaining tacrolimus variability than the effect of *ABCB1* alone [27,28].

A limitation of this study is the absence of a multivariate predictive analysis due to the relatively limited cohort size, which restricted our ability to fully control for potential confounding factors. Consequently, future studies with larger sample sizes should employ multivariate modeling to effectively adjust for confounders and establish more robust predictive frameworks.

CONCLUSION

This is the first study in Republic of North Macedonia that explored and verified the association between tacrolimus variability and genetic polymorphisms in kidney transplant recipients. It's consistency with data from the region and international studies emphasizes its importance for both patients and clinicians involved in the transplantation process. This work contributes to ongoing efforts to tailor more personalized and patient-centered treatment strategies, with aim of minimizing adverse effects and improving clinical outcomes. Further studies are warranted to evaluate the impact of combined genetic polymorphisms on allograft function and long-term survival.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES:

1. Shrestha BM. Two Decades of Tacrolimus in Renal Transplant: Basic Science and Clinical Evidences. Exp Clin Transplant. 2017 Feb;15(1):1-9. doi: 10.6002/ect.2016.0157.
2. Thongprayoon C; Hansrivijit P; Kovvuru K et al, W. Impacts of High Intra- and Inter-Individual Variability in Tacrolimus Pharmacokinetics and Fast Tacrolimus Metabolism on Outcomes of Solid Organ Transplant Recipients. J. Clin. Med. 2020, *9*, 2193. doi.org/10.3390/jcm9072193
3. Chen Sh, Liu S, Yu L, Hou X, Zhao R, Factors and interventions affecting tacrolimus intrapatient variability: A systematic review and meta-analysis,Transplantation Rev (Orlando),2024,38(4):100878,doi.org/10.1016/j.trre.2024.100878
4. Degraeve AL, Moudio S, Haufroid V, et al Predictors of tacrolimus pharmacokinnetik variability: current evidences and future perspectives. Expert Opinion on Drug Metabolism & Toxicology, 2020;16(9), 769-782. doi.org/10.1080/17425255.2020.1803277
5. Cusinato, DAC, Lacchini R, Romao EA, Moysés-Neto M and Coelho EB. Relationship of CYP3A5 genotype and ABCB1 diplotypes to tacrolimus disposition in Brazilian kidney transplant patients. Br J Clin Pharmacol, 2014;78(2): 364-372. doi.org/10.1111/bcp.12345
6. Pratt VM, Cavallari LH, Fulmer ML, et al. CYP3A4 and CYP3A5 Genotyping Recommendations: A Joint Consensus Recommendation of the Association for Molecular Pathology, Clinical Pharmacogenetics Implementation Consortium, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, European Society for Pharmacogenomics and Personalized Therapy, and Pharmacogenomics Knowledgebase. J Mol Diagn. 2023 Sep;25(9):619-629. doi: 10.1016/j.jmoldx.2023.06.008
7. Birdwell KA, Decker B, Barbarino JM, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP3A5 Genotype and Tacrolimus Dosing. Clin Pharmacol Ther. 2015 Jul;98(1):19-24. doi: 10.1002/cpt.113
8. Tsironi A, Lazaros K, Mendrinou E, et; Impact of CYP3A4 and ABCB1 genetic variants on tacrolimus dosing in Greek kidney transplant recipients. Front. Pharmacol. 2025; 16:1538432. doi: 10.3389/fphar.2025.1538432
9. Rotarescu CA, Maruntelu I, Rotarescu I, Constantinescu AE, Constantinescu I. Analysis of ABCB1 Gene Polymorphisms and Their Impact on Tacrolimus Blood Levels in Kidney Transplant Recipients. Int J Mol Sci. 2024;25(20):10999. doi: 10.3390/ijms252010999.
10. Taneska-Canevska A,Trajceska L, Severova G, et al. Tacrolimus variability factors and interplay with kidney graft function. Journal of Morphological Sciences, 2025;8(2):40-49. doi:1055302/JMS2582
11. Trajceska L, Rambabova-Busletik, I Nikolov, et al. Tacrolimus variability in transplantation, Bantao Journal 2019 17:26-28
12. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant. 2009;9(suppl 3): S1-S155. doi:10.1111/j.1600-6143.2009.02834.
13. Wojciechowski D, Wiseman A. Long-term immunosuppression management: Opportunities and uncertainties. Clin J Am Soc Nephrol. 2021;16(8):1264-1271. doi:10.2215/CJN.15040920
14. Xie W, Fan S, Liu R, et al;Tacrolimus intra-patient variability measures and its associations with allograft clinical outcomes in kidney transplantation. Transplant Rev (Orlando). 2024 38(3):100842. doi: 10.1016/j.trre.2024.100842.
15. Shenoy MT, Manavalan J, A H, K S, Mohanty PK. Tacrolimus Concentration/Dose Ratio: A Tool for Guiding Tacrolimus Dosage Post- renal transplantation. Cureus. 2024;16(2): e53421. doi: 10.7759/cureus.53421.
16. Park Y, Lee H, Eum SH, Ko EJ, Min JW, Yoon SH et al. Combined impact of the inter and intra-patient variability of tacrolimus blood level on allograft outcomes in kidney transplantation. Front Immunol. 2022 Nov16;13:1037566
17. Davit BM, Chen ML, Conner DP, Haidar SH, Kim S, Lee CH, et al Implementation of a reference-scaled average bioequivalence approach for highly variable generic drug products by the US Food and Drug Administration. AAPS J. 2012 Dec;14(4):915-24
18. Boenink R, Bonthuis M, Boerstra BA, et al, The ERA Registry Annual Report 2022: Epidemiology of Kidney Replacement Therapy in Europe, with a focus on sex comparisons, Clinical Kidney Journal, 2025;18(2): sfae405, doi.org/10.1093/ckj/

19. Boenink R, Kramer A, Masoud S, Rodríguez-Benot A, et al. International comparison and time trends of first kidney transplant recipient characteristics across Europe: an ERA Registry study. Nephrol Dial Transplant. 2024;39(4):648-658. doi: 10.1093/ndt/gfad189.
20. Stefanović NZ, Cvetković TP, Jevtović-Stoimenov TM, Ignjatović AM, Paunović GJ, Veličković RM. Investigation of CYP 3A5 and ABCB1 gene polymorphisms in the long-term following renal transplantation: Effects on tacrolimus exposure and kidney function. Exp Ther Med. 2015;10(3):1149-1156. doi: 10.3892/etm.2015.2598.
21. Jakovski K, Kapedanovska Nestorovska A, Labacevski N, Dimovski A. Frequency of the most common CYP3A5 polymorphisms in the healthy population of the Republic of Macedonia. Maced Pharm Bull. 2012;5(1-2):25-30. doi 10.33320/maced.pharm.bull.2012.58.003.
22. Naumovska Z, Nestorovska AK, Sterjev Z, Filipce A, Dimovski A, Suturkova L. Genotype variability and haplotype profile of ABCB1 (MDR1) gene polymorphisms in Macedonian population. Pril (Makedon Akad Nauk Umet Odd Med Nauki). 2014;35(3):121-33. doi: 10.1515/prilozi-2015-0016.
23. Chauhan PM, Hemani RJ, Solanki ND, et al. A systematic review and meta-analysis recite the efficacy of Tacrolimus treatment in renal transplant patients in association with genetic variants of *CYP3A5* gene. Am J Clin Exp Urol. 2023;11(4):275-292.
24. Noble J et al; Improved Tacrolimus Therapeutic Drug Monitoring in Kidney Transplantation: Optimizing an Old Drug Prescription; Kidney International Reports, 2025;10(5):1326 – 1328. doi:10.1016/ j.ekir.2025.02.019
25. Leino AD, King EC, Jiang W, et al. Assessment of tacrolimus intrapatient variability in stable adherent transplant recipients: Establishing baseline values. Am J Transplant. 2019;19(5):1410-1420. doi: 10.1111/ajt.15199.
26. Kurzawski M, Dąbrowska J, Dziewanowski K, Domański L, Perużyńska M, Droździk M. CYP3A5 and CYP3A4, but not ABCB1 polymorphisms affect tacrolimus dose-adjusted trough concentrations in kidney transplant recipients. Pharmacogenomics. 2014;15(2):179-188. doi: 10.2217/pgs.13.199.
27. Genvigir FDV, Salgado PC, Felipe CR, et al. Influence of the CYP3A4/5 genetic score and ABCB1 polymorphisms on tacrolimus exposure and renal function in Brazilian kidney transplant patients. Pharmacogenet Genomics.2016;26(10): 462-72.doi: 10.1097/FPC.0000000000000237.
28. Brazeau DA, Attwood K, Meaney CJ, et al. Beyond Single Nucleotide Polymorphisms: CYP3A536∗7 composite and ABCB1 haplotype associations to tacrolimus pharmacokinetics in black and white renal transplant recipients; Front Genetics.2020;11:889; doi:10.3389/fgene.2020.00889.