# Distribution of CYP3A4*1/*1B (rs2740574) and CYP3A5*1/*3 (rs776746) Genetic Variants in Renal Transplant Recipients Treated with Sirolimus in Urmia (Iran)

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**Keywords.** CYP3A4*1/*1B (−392A > G); CYP3A5*1/*3 (6986A > G); Renal transplantation; Sirolimus

Brief communication

**Introduction.** Rapamycin, also referred to as Sirolimus, is an immunosuppressive medication used in kidney transplantation to prevent organ rejection. Sirolimus is metabolized predominantly by the enzymes CYP3A4 and CYP3A5. The objective of this study was to evaluate the distribution of CYP3A4*1/*1B (rs2740574, –392A > G) and CYP3A5*1/*3 (rs776746, 6986A > G) genetic variants in renal transplant recipients treated with Sirolimus in Urmia (Iran).

**Methods.** This assessment involved thirty-nine renal transplant recipients in Urmia Imam Khomeini University Hospital, Urmia (Iran) who were treated with a daily dose of 1mg/day Sirolimus. The CYP3A4*1/*1B and CYP3A5*1/*3 allelic variants were identified using the Restriction Fragment Length Polymorphism-Polymerase Chain Reaction (RFLP-PCR) technique.

**Results.** The frequencies of CYP3A4 *1/*1 (-392AA), CYP3A4 *1/*1B (-392AG), and CYP3A4 *1B/*1B (-392GG) genotypes and CYP3A4 *1 (-392A) and CYP3A4 *1B (-392G) alleles were 37(94.87%), 2(5.13%), 0(0%), 76(97%), and 2(3%), respectively. The genotypic distribution for CYP3A5 was 0(0%) for CYP3A5*1/*1 (6986AA), 6(15.38%) for CYP3A5*1/*3 (6986AG), and 33(84.62%) for CYP3A5*3/*3 (6986GG). The allelic frequencies were 6(8%) for CYP3A5*1 (6986A) and 72(92%) for CYP3A5*3 (6986G). Only one patient with CYP3A4 *1B allele had CYP3A5 *3 allele simultaneously.

**Conclusion.** The results of this investigation indicated that most participants carried CYP3A5*3/*3 and CYP3A4*1/*1 genotypes. Additional clinical trials are necessary to elucidate the association between the pharmacokinetics of Sirolimus and the CYP3A gene polymorphisms in tested population.

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## INTRODUCTION

Rapamycin (Sirolimus) is a macrocyclic compound, which is used in kidney transplantation to prevent transplant rejection. It acts by blocking the function of an enzyme known as mammalian Target of Rapamycin (mTOR), which is responsible for enhancing the immune reaction.[1] The usual maintenance dose of Sirolimus in kidney transplant patients is 2 to 5 mg/day according to the literature.[1,2] Cytochrome P450 3A (CYP3A) is the most common cytochrome P450 enzyme, predominantly expressed in the liver and small intestine. Given that Sirolimus serves as a substrate for both CYP3As and P-glycoprotein(P-gp), the product of the multidrug resistance protein 1

(MDR1) or ATP-binding cassette sub-family B member 1 (ABCB1), its pharmacokinetics could be influenced by alterations in their activity due to genetic variations and/or pharmacokinetic interactions.[3] Patients treated with Sirolimus are at a higher risk of developing hypertriglyceridemia, lower-extremity edema, oral ulcerations, wound healing complications, thrombocytopenia and leukopenia. Regarding the side effects of Sirolimus, the systemic exposure needs close monitoring.[2,3] The subfamily of CYP3A enzymes, CYP3A4 and CYP3A5, metabolizes Sirolimus.[3] Although several single nucleotide polymorphisms (SNPs) have been reported in CYP3A genes, the role of few of them has been studied.[3] The polymorphism of CYP3A5, known as CYP3A5*3 (rs776746, 6986 A > G) results in alternative splicing of mRNA and truncation of the protein, which leads to the complete absence of CYP3A5 expression in individuals with the CYP3A5*3/*3 genotype. The CYP3A5∗1 allele, characterized by an A at position 6986, seems to be the primary allele linked to CYP3A5 expression and activity.[4] Individuals with at least one CYP3A5*1 allele (homozygous and heterozygous carries) express large amounts of CYP3A5 (CYP3A5-expressing subjects).[4,5] To attain adequate blood concentrations, individuals within the CYP3A5-expressing population (*1/*1 + *1/*3) are required to administer higher doses of Sirolimus.[4,5] In the case of CYP3A5*3 allele (6986G SNP), functional enzyme of CYP3A5 is not produced due to production of a truncated protein.[4,5] Variations in the coding regions of CYP3A4 are found at allele frequencies of less than 5% and are usually present as heterozygous for wild-type allele.[6] Most research are focused on CYP3A4*1B (rs2740574, 392 A > G). There is a correlation between the *1/*1B and *1B/*1B genotypes and the blood concentrations of Sirolimus. When receiving Sirolimus-based therapy, patients carrying the CYP3A4∗1B allele required a significantly higher doses of Sirolimus.[6-8] It has been demonstrated that the role of CYP3A5 in the metabolism of Sirolimus is significantly less than CYP3A4.[7] Patients carrying the CYP3A5*1 or CYP3A4*1B alleles requires a higher dose of cyclosporine to attain therapeutic blood concentration compared to patients carrying the CYP3A4*1 or CYP3A5*3 alleles.[8] The results of an Iranian study showed that the Sirolimus dosage to maintain a therapeutic blood level is 1.2 ± 0.44 mg/day.[9] Sirolimus is less nephrotoxic than the calcineurin inhibitors.[10] It may inhibit chronic allograft nephropathy in vasculopathy by inhibiting the transcription of several growth factors.[11] Evaluating the CYP3A4*1/*1B (rs2740574) and CYP3A5*1/*3 (rs776746) genetic variants could help explain inter-individual differences in dose requirements and support personalized Sirolimus therapy to maximize efficacy while minimizing the side effects. These variables have not been studied in Urmia (Iran) so far. Given the lack of similar studies, it is important to evaluate the issue. The purpose of the current study was to assess the distribution of CYP3A4*1/*1B (rs2740574) and CYP3A5*1/*3 (rs776746) genetic variants in renal transplant recipients treated with 1mg/day Sirolimus in Urmia (Iran).

## MATERIALS AND METHODS

This cross-sectional study was performed between January 2018 and December 2022 in Urmia Imam Khomeini University Hospital, affiliated to Urmia University of Medical Sciences, Urmia, Iran. The patients were selected through consecutive sampling from the Department of Internal Medicine, Nephrology and Kidney Transplant Research Center, and Clinical Research Institute. Thirty-nine patients with renal transplantation treated with 1 mg/day of Sirolimus for at least three months post-transplantation were included in this study. The inclusion criteria were as follows: 1) first- kidney transplant recipients who underwent kidney transplantation; 2) Patients who had not experienced any episodes of transplant rejection. All participants, received prednisolone and mycophenolate mofetil in addition to Sirolimus as immunosuppression therapy. Patients without informed consent were excluded from the study. After obtaining informed consent from all subjects and/or their legal guardian(s), 3-4 milliliters of whole blood were taken from the patients. Blood samples were collected in a 15 ml Falcon tube containing 500 µl EDTA. Genomic DNA was isolated from the blood samples by salting out method.[12] The CYP3A4*1/*1B and CYP3A5*1/*3 variants were determined via the Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP) method using two sets of specific primer pairs as mentioned in table 1. The PCR reactions were performed in 20 µl comprising

**Table 1.** SNPs in CYP3A4*1/*1B and CYP3A5*1/*3 underwent PCR followed by restriction enzyme digestion and restriction fragment length analysis on an 3% agarose gel.

| SNPs | Primers | Fragments (bp) | restriction enzymes | Wild-type allele | Mutant-type allele | Reference |
|---|---|---|---|---|---|---|
| CYP3A4*1/*1B | forward:5´-ggacagccatagagacaactgca-3´<br>reverse: 5´-ctttcctgccctgcacag-3´ | 334 | PstI | 220, 81, 33 | 199, 81, 33 | 8 |
| CYP3A5*1/*3 | forward:5´-atggagagtggcataggagata-3´<br>reverse: 5´-tgtggtccaaacagggaagaaata-3´ | 130 | SspI | 107,23 | 130 | 13 |

50 ng of DNA, 1x reaction buffer, 0.45 µl of each primer (10 pmol), 200 µmol of dNTPs, 0.25 unit of Taq DNA polymerase, and 1.5 mmol $MgCl_2$ (Genefanavaran, Tehran, Iran); with the following program: initial denaturation at 94 °C for 8 min, Denaturation at 94 °C for 30 secs, annealing at 55 °C for 30 secs, and extension at 72 °C for 30 secs (40 cycles); and a final 7 min at 72 °C. The PCR-amplified DNA fragment being cut with the restriction endonuclease PstI (Fermentas, USA, Catalogue number: ER0611) for CYP3A4*1/*1B and SspI (Fermentas, USA, Catalogue number: ER0771) for CYP3A5*1/*3. The PCR products of CYP3A4*1/*1B(334 bp) and CYP3A5*1/*3(130 bp) then were cut with the restriction enzymes in a reaction containing of 20 µl PCR product, 5 µl buffer, 1 µl restriction enzyme, and 5 µl distilled water. The reactions were incubated at 37 °C for two hours. Then, the reaction products were evaluated on a 3% agarose gel supplemented by DNA safe Stain (Cinna Gen, Tehran, Iran) and visualized using a UV-trans illuminator.

### Statistical analysis

The allele and genotype frequencies were determined by direct counting, considering the overall number of participants. The information is displayed as frequency (percentage) or percentage and mean ± standard deviation for quantitative variables. The deviation of genotype frequencies from the Hardy–Weinberg Equilibrium (HWE) was assessed using the Chi-Square ($\chi^2$) test. All statistical analyses were conducted using Excel 2016. The significance level was established at $P = .05$. A $P$-value of less than 0.05 was considered statistically significant.

## RESULTS

A total of thirty-nine patients with renal transplantation participated in the study, comprising 22 males (56.41%) and 17 females (43.59%). The average ages of the male patients were 46.24 ± 13.5 years, while the average ages for the female patients were 42.07 ± 10.15 years. The distribution of CYP3A4*1/*1B and CYP3A5*1/*3 genotypes was in Hardy-Weinberg equilibrium ($P > .05$) (Table 2). In this study, the frequencies of CYP3A4 *1/*1 (-392AA), CYP3A4 *1/*1B (-392AG), and CYP3A4 *1B/*1B (-392GG) genotypes and CYP3A4 *1 (-392A) and CYP3A4 *1B (-392G) alleles were 37(94.87%), 2(5.13%), 0(0%), 76(97%), and 2(3%), respectively. The genotypic distribution for CYP3A5 was 0(0%) for CYP3A5*1/*1 (6986AA), 6(15.38%) for CYP3A5*1/*3 (6986AG), and 33(84.62%) for CYP3A5*3/*3 (6986GG). The allelic frequencies were 6(8%) for CYP3A5*1 (6986A) and 72(92%) for CYP3A5*3 (6986G). Only one patient with CYP3A4 *1B allele had CYP3A5 *3 allele, simultaneously.

**Table 2.** Genotypic/Allelic frequency of CYP3A4 and CYP3A5 in the study group(n = 39)

| Gene | Genotype/Allele | Genotypic/Allelic frequency % |
|---|---|---|
| CYP3A4* | CYP3A4 *1/*1 (wt/wt) | 94.87 |
| | CYP3A4 *1/*1B (wt/mt) | 5.13 |
| | CYP3A4 *1B/*1B (mt/mt) | 0 |
| | CYP3A4 *1 (wt) | 97 |
| | CYP3A4 *1B (mt) | 3 |
| CYP3A5** | CYP3A5 *1/*1 (wt/wt) | 0 |
| | CYP3A5 *1/*3 (wt/mt) | 15.38 |
| | CYP3A5 *3/*3 (mt/mt) | 84.62 |
| | CYP3A5 *1 (wt) | 8 |
| | CYP3A5 *3 (mt) | 92 |

*: Variant Allele Frequency = 0.03, $\chi^2 = 0.027 < 3.84$, $P = 0.98 > 0.05$ (df = 2)
**: Variant Allele Frequency = 0.92, $\chi^2 = 0.27 < 3.84$, $P = 0.87 > 0.05$ (df = 2)

## DISCUSSION

In this study, the frequency of CYP3A4*1/*1B (rs2740574, –392A > G) and CYP3A5*1/*3 (rs776746, 6986A > G) variants were determined in renal transplant recipients treated with 1mg/day Sirolimus in Urmia, Iran. Sirolimus is considered to be safer than calcineurin inhibitors due to lacking

renal and neurologic toxicity. Additionally, it offers advantages in the treatment of tumors and preventing cytomegalovirus infection. However, incorrect dosing can lead to adverse effects such as acute allograft rejection. As ethnicity greatly influences the metabolism of Sirolimus, adjustment and monitoring of therapeutic dose of Sirolimus is essential. Sirolimus suppresses the activity of mTOR, that controls cellular metabolism, immune responses, and cell survival.[14] In this study, our results indicated that the most participants carried CYP3A5*3/*3 (6986GG) and CYP3A4*1/*1 -392AA genotypes in tested patients. Our findings were in agreement with Ghasemi *et al*.[9] and Shi *et al*.[15] Individuals with the *1B allele of the CYP3A4 gene and the *1 allele of the CYP3A5 gene are more likely to suffer from the side effects of Sirolimus.[8] Therefore, the blood concentration of Sirolimus should be monitored, exclusively in patients with CYP3A5*1 and CYP3A4*1B alleles, to guarantee precise dosing in clinical practice.[8] It is essential for healthcare professionals to be aware of these genetic variations and their implications during prescribing Sirolimus in the kidney transplantation. By identifying individuals with the *1/*1B or *1/*3 genotypes, nephrologists can adjust the Sirolimus dosage to minimize the risk of adverse effects while maintaining the desired therapeutic effect. The personalized approach can improve patient outcomes and reduce the risk of Sirolimus-related complications.

## THE STUDY LIMITATIONS

In this research, there were certain limitations such as the small sample size, single-center design, lack of pharmacokinetic and outcome data, and limited generalizability as well as interaction of genetic factors (like CYP2C8 *1/*3, CYP2J2 *1/*7, ABCB1 C3435T, ABCB1 G2677T/A, and ABCB1 C1236T).

## CONCLUSION

The results of this investigation indicated that the most participants carried CYP3A5*3/*3 and CYP3A4*1/*1 genotypes in Urmia, Iran. Additional clinical trials are necessary to elucidate the connection between the pharmacokinetics of Sirolimus and the CYP3A gene polymorphisms.

## AUTHORS' CONTRIBUTIONS

KM and MB: performed the investigation, designed and performed the study. ZG, AM, LJ and IAR: the principal investigator in the study and reviewed the article. KM and MB: collected samples of the patients. MB: performed genotyping analyses and wrote the article. All authors reviewed the article.

## ETHICAL STATEMENT

All stages of current study have been approved by the Research Ethics Committee of the Urmia University of Medical Sciences (IR.UMSU.REC.1398.429).

## CONSENT FOR PUBLICATION

Manuscript is approved by all authors for publication.

## AVAILABILITY OF DATA AND MATERIALS

The data and materials of this study are available upon a reasonable request from the corresponding author.

## CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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