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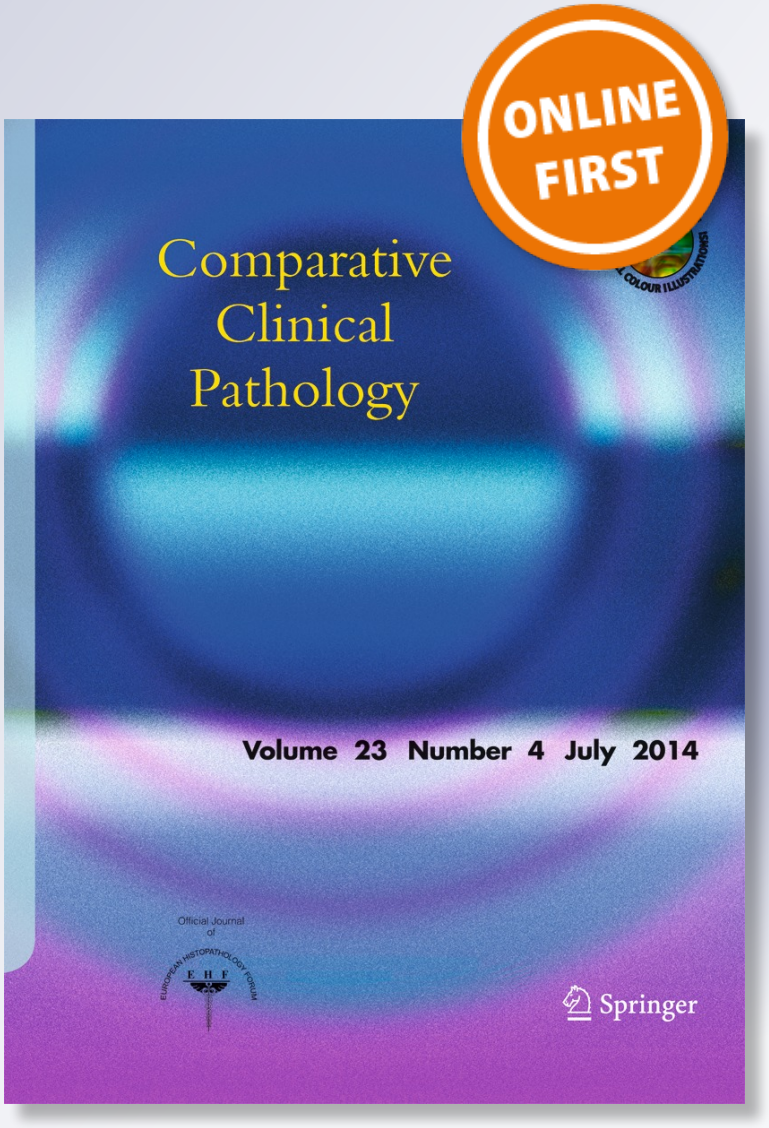

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ORIGINAL ARTICLE

The effect of CYP3A5 polymorphism on cyclosporine plasma level in Egyptian renal transplant recipients

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Abstract Cyclosporine (CsA) and tacrolimus are immunosuppressants used for the prevention of rejection of transplanted organs. The genes encoding cytochrome (CYP) P450 enzymes, CYP3A4, and CYP3A5 are the main ones involved in the pharmacokinetics of calcinurin inhibitors (CNI). Several single nucleotide polymorphisms were identified in these genes such as CYP3A5*3 (6986A>G). The association of the CYP3A5*3/*3 genotype with decreased clearance of its substrates was reported among different ethnic populations. This study aims to evaluate the effect of CYP3A5*3 polymorphism on CsA plasma levels in Egyptian renal transplant patients at the first week and first month of transplantation. A total of 44 renal transplant recipients receiving CsA were genotyped for CYP3A5*3 polymorphism. The C0 and C2 of CsA were measured and their relationships with CYP3A5*3 genotypes were investigated. CYP3A5*3 allele was present in six patients and the CsA level didn't differ significantly between the CYP3A5*3 the CYP3A5*1 allele carriers at the first week and the first month post transplantation. Large-scale studies with the involvement of multiple genetic markers claimed to affect the CsA pharmacokinetics are highly recommended to elucidate their pharmacogenetic role in renal transplant patients.

Keywords Immunosuppressants · Renal transplantation · Pharmacokinetics

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Abbreviations

CsA	Cyclosporine
CYP	Cytochrome
CNI	Calcinurin Inhibitors

Introduction

The cyclosporine (CsA) and tacrolimus are immunosuppressive drugs widely used to prevent the allograft rejection among renal transplant patients (Dunn et al. 2001; Mendes et al. 2009).

There is wide inter-individual difference in CsA pharmacokinetics especially in the critical early phase post transplantation; resulting in large number of patients exposed to drug levels that is outside the therapeutic window, leading to either toxicity or acute rejection (Mendes et al. 2009). This inter-individual variability are related to several factors (Rosso Felipe et al. 2009) including genetic polymorphisms in the common drug-metabolizing genes (Singh et al. 2009).

The human cytochrome P450 subfamily, CYP3A mainly represented by CYP3A4 and CYP3A5 are the major enzymes responsible for CsA metabolism (Sattler et al. 1992). Both enzymes are highly polymorphic, and substantial inter-individual differences in their expression might contribute to the variability of the oral bioavailability and systemic clearance of its substrates (Singh et al. 2009).

The CYP3A5*3 6986A>G SNP (rs776746), a transition within intron 3 of CYP3A5 gene, was reported to be associated with the polymorphic expression of CYP3A5 (Rosso Felipe et al. 2009; Staatz et al. 2010). It creates an alternative splice resulting in the absence of functional CYP3A5 from liver tissue (Qiu

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et al. 2008). Homozygote CYP3A5*3 carriers lack the CYP3A5 expression, while individuals with at least one wild-type allele CYP3A5*1 express CYP3A5 (Singh et al. 2009). Homozygous or heterozygous CYP3A5*1 allele carriers should theoretically have lower oral bioavailability and higher clearance of the drugs principally inactivated by CYP3A5, and show a lack of efficacy from the standard dosage (Staatz et al. 2010). Several studies reported that CYP3A5*3/*3 genotype was associated with reduced clearance of its substrates in different ethnic populations (Hustert et al. 2001).

The under/over immunosuppression might be one of the reasons of the early allograft rejection (Mendes et al. 2009). Therefore, the present study aims to analyze the influence of the CYP3A5*3 SNP on the plasma levels of CsA at 1 week and 1-month post transplantation in Egyptian renal transplant recipients.

Patients and methods

This study involved 44 adult renal transplant recipients attending the renal transplantation unit of the New Cairo University Hospital and receiving CsA immunosuppressive therapy. Patients were excluded from the study if CsA was switched to another immunosuppressant due to major side effects or if they were receiving drugs known to affect the CsA levels, such as diltiazem, phenytoin, verapamil, erythromycin, or clarithromycin.

Immunosuppressive treatment of the patients consisted of a combination of CsA (Neoral), mycophenolate mofetil (MMF), and prednisolone. The patients received a loading dose of methylprednisolone (500 mg) before transplantation and then daily cyclosporine (6 mg/kg/day) with dose adjustments to achieve C2 concentrations of 1,400 ng/mL during the first month, 800–1,000 ng/mL at 6 months, and 600–800 ng/mL at 12 months after transplantation. MMF (CellCept, Roche; 2 g/day) was administered on the day of operation as 1 g preoperatively and another 1 g 12 h after, with continuation of the same dose of MMF throughout the study 2 g/day. Corticosteroids were given intra operatively at the time of the anastomosis as a 500-mg IV bolus of methylprednisolone followed by 200 mg on day one. Steroids were tapered by 25 mg each day until 20-mg oral drug at day 10.

Clinical suspicion of an acute rejection episode was confirmed by renal biopsy according to the Banff 97 criteria (Racusen et al. 1999). First-line anti-rejection treatment in all patients consisted of intravenous methylprednisolone for five consecutive days followed by 40-mg oral prednisolone, tapered daily by 10 mg until achieving the baseline steroid maintenance dose. Steroid-resistant acute rejection episodes were treated with the polyclonal anti-T cell antibody antithymocyte globulin (ATG; ATG-Fresenius; 3 mg/kg/day) for a minimum of 7 and up to 10 days.

All subjects underwent the following assessment:

1. Clinical data collection involved the following: age, sex, creatinine level, etiology of end-stage renal disease and weight (kg). Written informed consent was obtained from all subjects.
2. Cyclosporine assay: whole blood samples withdrawn for CsA level measurement, Trough (C0) and 2-h post-oral dose (C_2) using the antibody-conjugated magnetic immunoassay (ACMIA) (Dimension RxL, Siemens) at 1 week and 1-month post transplantation.
3. Molecular genetic analysis:

   (a) Genomic DNA was extracted from the peripheral leukocytes using the salting out method (Lahiri and Schnabel 1993).

   (b) CYP3A5*3 genotyping was done using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method According to King et al. (2003). For amplification, the following primers were used: forward (5′CCTGCCTTCAAT TTTCACT-3′) and reverse (5′GGTCCAAACAGG GAAG AGGT3′). The PCR components were: 10× buffer without $MgCl_2$, 50 mM $MgCl_2$, 25 mM dNTPs, 5 U/μL Dream Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania), 50–100 ng DNA, and 0.5 mM of each of the primers. PCR reactions were performed using the thermal cycler PCR Express (Thermo Hybaid, Middlesex, UK). The final PCR volume was 50 μL. The amplification conditions were as follows: initial denaturation at 94 °C for 3 min (1 cycle) followed by 35 amplification cycles; denaturation at 94 °C for 1 min; annealing at 61 °C for 1 min; and extension at 70 °C for 1 min, with a final extension step at 72 °C for 7 min.

   The PCR product (196 bp) was digested with the RsaI enzyme (Fermentas, Vilnius, Lithuania). The homozygote CYP3A5*1 genotype produced 102-, 74-, and 20-bp fragments; the homozygote CYP3A5*3 genotype produced 102,94-bp fragments; and the heterozygote CYP3A5*3 genotype produced 102-, 96-, 74- , and 20-bp fragcments (Fig. 1). To identify the genotypes of CYP3A5 polymorphism, the digestion fragments were separated using 15 % polyacrylamide gels for 90 min (120 V) using the Bio-Rad Mini-PROTEAN Tetra gel system (Bio-Rad, Hercules, CA, USA). The separated fragments were stained with ethidium bromide and visualized along with 50-bp ladder (MBI Fermentas, Vilnius, Lithuania) as a size marker using transilluminator (Bio-Rad, USA).

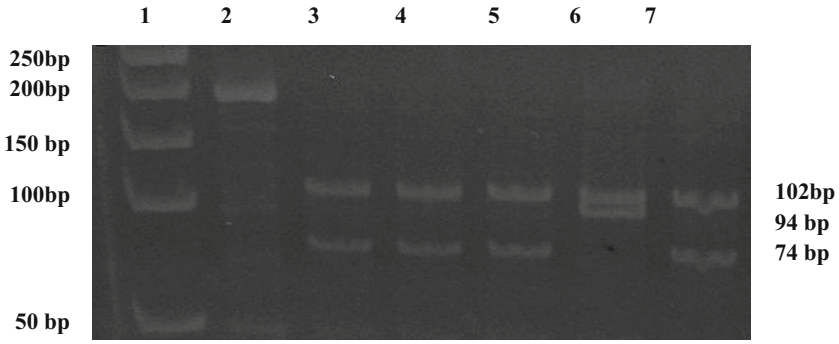

Fig. 1 RFLP analysis showing homozygote CYP3A5*3 mutation. *Lane 1* 50 base pair (bp) ladder, *lane 2* uncut PCR product, *lanes 3*,*4*,*5*,*7* wild type (Wt) and *lane 6* mutant type (Mt)

(c) The restriction enzyme analyses were confirmed by sequencing analysis. PCR amplifications were done using the same primers used before. The PCR products were recovered from agarose gels using MinElute Gel Extraction Kit (QIAGEN, Inc., CA, USA) according to the manufacturer's instructions. Sequencing with appropriate oligonucleotide primers was carried out by using a BigDye Terminator Version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and a 310 automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Data were analyzed using IBM SPSS Advanced Statistics version 20.0 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation or median and range, as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. The SNP genotyping was correlated with the concentrations of CsA using a one-way ANOVA test. A p value of <0.05 was considered significant.

Table 1 The demographic and clinical data of the studied population, n= 44

Age (years)	36.6±19.7
Sex (male/female)	34/10
Body weight (kg)	71.1±7.3
Donor age (years)	25.1±8.9
Donor sex (male/female)	38/6
Serum creatinine (mg/dL)	1.18±0.3
Cause of end stage renal disease *n* (%)	
Chronic glomerulonephritis	2 (4.5)
Chronic interstitial nephropathy	1 (2.5)
Diabetic nephropathy	4 (9)
Hypertension	37 (84)

Data are presented as mean ± SD or number (%)

Results

This study included 44 renal transplant patients. The mean (SD) age was 36.6 (19.7)years. The frequency of males and females were 77.2 % (n=34) and 22.8 % (n=10), respectively. The demographic and clinical data are summarized in Table 1.

The frequency of the CYP3A5 genotypes among the studied patients is presented in Table 2. The frequency of the CYP3A5 variant *3 allele was lower among the patients than *1 (11.4 vs. 88.6 %, p=0.01). The frequency of the combined genotypes (*1/*3+*3/*3) was lower among the patients (13.6 %) than the *1/*1 genotype (86.4 %) (p=0.02) (Table 3). The results of the present study showed no effect of the genotype on the C0 and C2 plasma levels at 1 week and 1-month post transplantation (Table 3).

Four patients developed an episode of biops-proven acute rejection, steroid-resistant acute rejection episodes requiring the use of ATG occurred in one recipient and all of them had the*1/*1 genotype.

Discussion

The narrow therapeutic window with large inter- and intra-individual differences in pharmacokinetics are two main characteristics of the calcinurin inhibitor, CsA (Ishikawa et al. 2004; Schiff et al. 2007).

Several studies reported that the patient genotype affects the metabolism of the immunosuppressants usually used to prevent allograft rejection (Press et al. 2009; Haufroid et al. 2006; MacPhee and Holt 2008; Turolo et al. 2010).

Results of the present study showed no effect of the genotype on the CsA plasma levels at 1 week and 1-month post-

Table 2 summarizes the frequency of CYP3A*3 genotypes among the renal transplant patients

Genotype	*n* (%)	*p* value	Allele	*n* (%)	*p* value
*1/*1	38 (86.4)	0.02	*1	39 (88.6)	0.01
*3/*3	1 (2.3)		*3	6 (11.4)	
*1/*3	5 (11.3)				

Table 3 Relationship between CYP3A5*1/3 SNP and Cyclosporin pharmacokinetics in renal transplant patients

	Genotype	*n*	C0, ng/mL	*p* value	C0, ng/mL	*p* value	C2, ng/mL	*p* value	C2, ng/mL	*p* value
Days			7		30		7		30	
CYP3A5*	*1/*1	38	266 (80)	0.1	256 (63)	0.2	1900 (97)	0.4	944 (88)	0.3
1/3	*3/*3+*1/*3	6	235 (98)		212 (86)		2100 (80)		855 (76)	

Data presented as mean (SD)

C0 cyclosporin trough concentration; *C2* cyclosporin 2-h post dose

transplantation, with no effect on the incidence of biopsy proven acute rejection. This finding was similar to the study of Turolo et al.(2010); who reported no significant difference in the CsA level among the different CYP3A5 genotypes in 87 Italian renal transplant recipients at 6, 30, and 90 days post transplantation, This finding was also observed in other studies suggesting that the role of pharmacogenetic profiling of calcineurin inhibitors as a useful clinical tool for personalizing immunosuppressant is not yet clear (Hesselink et al. 2003; Haufroid et al. 2004; Kreutz et al. 2004; Yates et al. 2003).

Contrary to the results of the present study, Hu et al. (2006) reported lower dose-adjusted CsA levels at 1-week post transplantation in CYP3A5 expressers. In another study by Meng et al. (2012), the CYP3A5*3 correlated with the dose-adjusted CsA on the day 7 post transplantation. Also different from the results of the present study, the dose-adjusted C2 concentrations showed significant differences among the CYP3A5 genotypes during the 15–21 days post transplantation in Chinese renal transplant patients (Li et al. 2013).

As variant genotypes are often more frequent in particular ethnic groups (Qiu et al. 2008; Staatz et al. 2010). The role of ethnicity should be considered as an explanation of our results. The frequency of the G allele was low among our studied population. This finding was similar to Tanzanians and Afro-Americans who showed greater presence of the A allele (Ferreira et al. 2008; Suarez-Kurtz et al. 2007). However, this was in contrast to other ethnicities like the European Caucasian populations (Fredericks et al. 2007; Gervasini et al. 2005; Haufroid et al. 2004) and Asian populations (Hu et al. 2006; Choi et al. 2007).

Finally, The inconsistency of findings among the different studies could be explained by several interacting factors such as limited number of the studied subjects that might limit the statistical power to detect any small differences, heterogeneity of the evaluated pharmacokinetic parameters, such as the trough concentration being a poor predictor of drug exposure, the complexity of drug metabolism, and the presence of multiple polymorphisms in the same subject involving more than one metabolic pathway (Rosso Felipe et al. 2009; Staatz et al. 2010). Our study has some limitations: we did not follow up our patients more than 1 month and the small sample size.

Conclusion

Large-scale studies with the involvement of multiple genetic markers claimed to affect the CsA pharmacokinetics are highly recommended to elucidate their possible pharmacogenetic effect among renal transplant patients.

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Conflict of interest Bahaaeldin Mostafa Zayed declares that he received Cairo University research fund. Dina Mehaney declares that she has no conflict of interest.

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