ORIGINAL ARTICLE

Distribution of CYP3A4 and CYP3A5 Polymorphisms and Genotype Combination Implicated in Tacrolimus Metabolism

Fréquences génotypiques et alléliques des CYP3A4 et CYPA5

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Abstract

Introduction: Human cytochrome P450 (CYP), particularly CYP3A4 and CYP3A5 is mainly responsible for the metabolism of several drugs including tacrolimus. Significant interracial/interethnic variation in the expression and function of CYP3A5 and CYP3A4 is caused by Single Nucleotide Polymorphisms (SNPs) of genes encoding these proteins.
Aim: The present study investigated the genetic polymorphisms CYP3A4*1B, CYP3A4*22, and CYP3A5*3 in the Tunisian population.
Methods: We included in this study, Tunisian healthy subjects and renal transplant recipients receiving tacrolimus. CYP3A4 and CYP3A5 genotyping were performed using polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP). According to the genotypic combination of the three CYP polymorphisms, we have identified for the first time four metabolizers statuses: slow metabolizers (SM), intermediate metabolizers (IM), high metabolizers (HM), and extensive metabolizers (EM).
Results: A total of 101 renal transplant patients and 102 healthy subjects were included. Our results showed that the predominant alleles in the Tunisian population are a wild type of CYP3A4*1B (0.87), likewise CYP3A4*22 (0.975) and CYP3A5*3 (0.82). The genotype frequencies of CYP3A4*1B, CYP3A4*22, and CYP3A5*3 were found to be 3.9%, 0.0%, and 69.5%, respectively. Also, we found a significant linkage disequilibrium between CYP3A4*1B and CYP3A5*3. We approved that the IM is the predominant phenotype in our population with 124 patients followed by and EM with 41 patients, HM in 29 patients and SM in 9 patients. These results showed that Tunisians are most similar to Caucasians.
Conclusion: The genetic background of these enzymes CYP3A4*1B, CYP3A4*22, and CYP3A5*3 in this study are important in the prescription of personalized medicine.

Keys words: CYP3A4*1B, CYP3A4*22, CYP3A5*3, tacrolimus, Pharmacogenetics, Tunisian Population

Résumé

Introduction: Le cytochrome humain P450 (CYP), en particulier le CYP3A4 et le CYP3A5, est principalement responsable du métabolisme de plusieurs médicaments, dont le tacrolimus. Une variation interraciale/interethnique significative dans l'expression et la fonction de CYP3A5 et CYP3A4 est causée par des polymorphismes nucléotidiques uniques (SNP) de gènes codant pour ces protéines.
Objectif: La présente étude a étudié dans la population tunisienne, les polymorphismes génétiques de CYP3A4*1B, CYP34*22 et CYP3A5*3.
Méthodes: Nous avons inclus dans cette étude les sujets sains tunisiens et les receveurs de transplantation rénale recevant tacrolimus. Le génotypage CYP3A4 et CYP3A5 a été réalisé en utilisant le polymorphisme de longueur de fragment de restriction de réaction en chaîne par polymérase (PCR-RFLP). Selon la combinaison génotypique des trois gènes CYP, nous avons identifié pour la première fois quatre statuts de métaboliseurs : métaboliseurs lents (SM), métaboliseurs intermédiaires (IM), métaboliseurs élevés (HM) et métaboliseurs étendus (EM).
Résultats: Nos résultats ont montré que les allèles prédominants dans la population tunisienne sont un type sauvage de CYP3A4*1B (0,87), de même que CYP3A4*22 (0,975) et CYP3A5*3 (0,82). Les fréquences génotypiques de CYP3A4*1B, CYP3A4*22 et CYP3A5*3 étaient respectivement de 3,9 %, 0,0 % et 69,5 %. En outre, nous avons trouvé un déséquilibre de liaison significatif entre CYP3A4*1B et CYP3A5*3. Ces résultats ont montré que les populations tunisiennes sont des métaboliseurs intermédiaires et les plus similaires aux caucasiens.
Conclusion: Le contexte génétique de ces enzymes CYP3A4*1B, CYP3A4*22 et CYP3A5*3 dans cette étude est important dans la prescription de la médecine personnalisée.

Mots clés: CYP3A4*1B; CYP3A4*22 CYP3A5*3; Tacrolimus, Pharmacogénétique; Population tunisienne.

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LA TUNISIE MEDICALE-2024; Vol 102 (09): 537-542 DOI: 10.62438/tunismed.v102i9.4969

Introduction

The human genome encodes at least 57 cytochrome P450 (CYP) genes and 59 pseudogenes organized into 18 families and 43 subfamilies [1]. The CYP3A subfamily is the most abundant group of CYP enzymes and is responsible for metabolizing 45–60% of all clinically prescribed drugs such as immunosuppressant drugs including tacrolimus (Tac) [2–5]. CYP3A4 and CYP3A5 isoforms are responsible for the hepatic and intestinal metabolism of tacrolimus [2]. This immunosuppressant is characterized by the high interindividual variability of its pharmacokinetics and its narrow therapeutic index. Genetic variation in the expression and function of the CYP3A4 and CYP3A5 enzymes is caused by single nucleotide polymorphisms (SNPs) of genes encoding these proteins.

Among these, SNPs, CYP3A4*1B (rs2740574) results from the substitution of A by G in promoter region at -392 position from the transcription start site. This variation is associated with increased expression of the protein, due to reduced binding of a transcriptional repressor [6–8]. Of the same kind, a novel functional SNP located at position C15389T in intron 6 of CYP3A4 (CYP3A4*22, rs35599367) was identified [9]. The T-variant allele was associated with lower levels of heteronuclear RNA (hnRNA) which affects the expression of CYP3A4 mRNA fragments and increases the formation of the nonfunctional CYP3A4 splice variant [5]. Also, the most common nonfunctional allele is CYP3A5*3 (rs776746) resulting from substitution 6986 A>G in intron 3 [7]. This transition creates an alternative splice site in the pre-mRNA, leading to the production of aberrant mRNA with a premature stop codon [9–11].

Many groups have studied the distribution of CYP3A4 and CYP3A5 genetic variation in many populations. However, to our knowledge, no study was interested in investigating the distribution of CYP3A4*1B, CYP3A4*22, and CYP3A5*3 SNPs, simultaneously. This study aimed to describe the distribution of these three polymorphisms in Tunisian healthy and renal transplant recipients and establish, accordingly, the metabolizer status of certain drugs such as tacrolimus in this population.

Methods

Subjects

Tunisian kidney transplant recipients and healthy subjects were examined in a single center analysis from 2016-2022. Healthy subjects were recruited from blood donors at Fattouma Bourguiba Hospital in Monastir; they were at least 18 years old, without chronic illnesses, and not using any medications that could interfere with the study. All participants provided informed consent. All patients were recruited consecutively from the Pharmacology Department of Fattouma Bourguiba Hospital in Monastir. They received daily oral treatment with tacrolimus (Prograf®, Hikma Pharmaceuticals, Tunisia), mycophenolate mofetil (MMF®, Medis, Tunisia), and prednisone. The inclusion criteria for recipients were: having undergone a new kidney transplant, experienced no rejection episodes, and not taken any interacting medications. Non-inclusion criteria only those who do not provide consent. Also; All the participants gave informed consent and the study protocol was approved by the Local Ethics committee of Fattouma Bourguiba Hospital of Monastir.

DNA extraction

The genomic DNA was extracted from peripheral blood mononuclear cells using a “salting-out” procedure [13].

Genotyping

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was used to identify CYP3A4*1B (rs2740574; -392A>G), CYP3A5*3 (rs776746, 6986A>G) and CYP3A4*22 (rs35599367, c.522-191 C>T) polymorphisms as previously described [7,14,15].

Genotyping of CYP3A4*1B

The PCR reaction was performed in a total reaction volume of 20 µl containing: 50 ng of genomic DNA, 1X PCR buffer, 2 mM of MgCl2, 200 µM of each dNTP, 0.1 µM of each primer (forward:5’GGAATGAGGACAGCCATAGAGACAAGGGCA3’; reverse: 5’ CCTTTCAGCTCTGTGTTGCTCTTTGCTG3’) and 1 U of the Go Taq Flexi DNA polymerase (Promega, Madison, USA). PCR conditions involved: an initial denaturation at 98°C for 5 min, followed by 32 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 45 seconds and elongation at 72°C for 45 seconds and final elongation at 72°C for 5 minutes. PCR products were digested with 5 U of the MboII enzyme (Takara Bio Inc., Japan) at 37°C for 12h. PCR-RFLP products were analyzed on 3% agarose gel stained with ethidium bromide. The homozygous genotype CYP3A4*1/*1 was identified by the presence of three fragments of 175, 169, and 41 bp, whereas DNA from individuals homozygous for the CYP3A4*1B/*1B genotype produced two fragments of 210 and 175bp, and the heterozygous genotype CYP3A4*1/*1B gave four fragments of 210, 175, 169 and 41 bp.

Genotyping of CYP3A4*22

The PCR reaction was performed in a total reaction volume of 20 µl containing: 50 ng of genomic DNA, 1X PCR buffer, 1,5 mM of MgCl2, 200 µM of each dNTP, 0.2 µM of each primer (forward: 5’GAGTTTGTCCTGGGCAGACCA3’; reverse: 5’ CCCCCTGTC ACAAACCCTGTCA3’) and 1 U of the Go Taq Flexi DNA polymerase (Promega, Madison, USA). PCR conditions involved: an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 65°C for 30 seconds and elongation at 72°C for 1 minute and final elongation at 72°C for 5 minutes. Then PCR products were cleaved with 5 U of the enzyme DraIII (BioLabs, New England, USA) and analyzed on 2% agarose gel electrophoresis. The homozygous wild type (*1/*1) was detected by the presence of one fragment of 1064bp, while the homozygous variant type (*22/*22) was identified by

the presence of two fragments of 569 and 495 bp, and the heterozygous genotype (*1/*22) produced three fragments of 1064, 569 and 495 bp.

Genotyping of CYP3A5

PCR was analyzed in a final volume of 20µl containing 10 ng of genomic DNA, 1X PCR buffer, 1,2 mM of MgCl2, 400 µM of each dNTP, 0.1 µM of each primer (forward: 5'ATGGAGAGTGGCATAGGAGATA3'; reverse: 5'TGTGGTCCAAACAGGGAA GAAATA 3') and 0.6 U of the Go Taq Flexi DNA polymerase (Promega, Madison, USA). PCR conditions involved: an initial denaturation at 94°C for 8 min, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 57°C for 30 seconds and elongation at 72°C for 30 seconds and final elongation at 72°C for 1 minute. PCR products were digested with 10 U of the enzyme SspI (Takara Bio Inc., Japan) and analyzed on 2.5% agarose gel electrophoresis. CYP3A5*1/*1 homozygous genotype produced two fragments of 107 and 23 bp, whereas the CYP3A5*3/*3 homozygous genotype gave only one fragment of 130 bp, and CYP3A5*1/*3 heterozygous genotype was identified by the presence of two fragments 130, 107 and 23 bp.

Metabolizers status

According to the genotypic combination described previously of the three CYP polymorphisms, we have identified four metabolizers statuses [12]: slow metabolizers (SM): [CYP3A4*22 carriers with CYP3A5*3/*3]; intermediate metabolizers (IM) [CYP3A4*22 carriers with one CYP3A5*1 allele or CYP3A4*1/*1 with CYP3A5*3/*3], high metabolizers (HM) [one CYP3A4*1B allele with CYPA5*3/*3 or CYP3A4*1/*1 with CYP3A5*1 carriers] and extensive metabolizers (EM) [one CYP3A4*1B allele with CYP3A5*1 carriers or two CYP3A4*1B alleles] (Table1).

Table 1. Population distribution according to metabolizers status and correspondent genotype cluster

Metabolizers status (N)	Genotype clusters
Slow metabolizers	**CYP3A4** (*1/*22) + **CYP3A5** (*3/*3)
Intermediate metabolizers	**CYP3A4** (*1/*1) + **CYP3A5** (*3/*3)
	or
	CYP3A4 (*1/*22) + **CYP3A5** (*1/*3)
High metabolizers	**CYP3A4** (*1/*1B) + **CYP3A5** (*3/*3)
	or
	CYP3A4 (*1/*1) + **CYP3A5** (*1/*3)
Extensive metabolizers	**CYP3A4** (*1/*1B) + **CYP3A5** (*1/*1 or *1/*3)
	or
	CYP3A4 (*1B/*1B) + **CYP3A5** (*1/*1 or *1/*3 or *3/*3)

N: population size

Statistical analysis

Genotype and allele frequencies in our study population were calculated using XLstat
Software. The chi-square test X2 was used for testing the Hardy-Weinberg equilibrium (H.W.) and for comparing observed genotypic distribution and expected genotypic distribution between patients and healthy subjects. D values for linkage disequilibrium were calculated using SNPStats. P-value <0.05 was considered statistically significant.

Results

Demographic data

We included in our study 101 healthy subjects and 102 de novo renal transplant patients. In the healthy group, there were 92 men (91.08%) and 10 women (7.92%) (Sex ratio = 9.2); the mean age was 34.8 ± 9.7 years, and mean weight was 79.7±13.8 kg. In patients' group were 72 men and 29 women (sex ratio = 2.5). The mean age was 35.3 ±14.34 years and mean weight was 62.5-±15.51 kg. There was no significant difference between healthy subjects and patients in age and sex (p = 0.317).
In the present study, the allele and genotype frequencies of CYP3A4*1B, CYP3A4*22, and CYP3A5*3 SNPs were determined in 203 Tunisian subjects. In the transplanted patients, we found a significant excess of wild-type homozygous subjects of both CYP3A4*1B and CYP3A4*22 with 73% (74/102); 93% (94/101), respectively. The CYP3A5*3/*3 was present in 61% (63/102) of patients. Enrolled 101 healthy subjects, the high genotype observed was the wild type homozygous in CYP3A4*1B (*1/*1), CYP3A4*22(*1/*1) and the homozygous variant CYP3A5*3/*3. The genotype frequency was respectively, 81%, 98%, and 74%.
The genotype frequencies between the patients and healthy subjects did not show a significant difference in distribution CYP3A4*1B (P= 0.46), CYP3A4*22 (P=0.066), and CYP3A5*3 (P= 0.11). This finding suggests that the studied polymorphisms may not be strongly associated with the development of kidney transplantation-related complications or other health conditions in this population. P-values for the Hardy-Weinberg equilibrium of each polymorphism were calculated. All loci examined in patients, healthy subjects, and whole populations were inconsistent with the H.W. (Table 2).

Table 2. Genotypic frequencies of CYP3A4*1B, CYP3A4*22 and CYP3A5*3 SNPs in Healthy and patients

SNP Studied	Patients (n=102)		Healthy (n=101)		p-value*
	n	%	n	%	
CYP3A4*1B					**0.46**
*1/*1	74	72.54	82	81.18	
*1/*1B	23	22.55	15	14.85	
*1B/*1B	5	4.91	4	3.97	
CYP3A4*22					**0.066**
*1/*1	94	93.07	99	97.06	
*1/*22	7	6.93	3	2.94	
*22/*22	0	0	0	0	
CYP3A5*3					**0.11**
*1/*1	5	4.95	6	5.88	
*1/*3	33	32.67	21	20.59	
*3/*3	63	62.38	75	73.53	

n: population size; * P-value calculated with test X^2

Genetic Data

Genotypic data from healthy subjects and patients are summarized in Table 3

Table 3. The observed and expected genotype distribution of the healthy subjects and patients according to Hardy-Weinberg equilibrium

Tested gene	Patients (n=102)		Healthy (n=101)		p-value*
	Observed (n)	Expected (n)	Observed (n)	Expected (n)	
CYP3A4*1B					**0.16**
*1/*1	74	71.27	82	78.99	
*1/*1B	23	27.14	15	21.54	
*1B/*1B	5	2.59	4	1.47	
CYP3A4*22					**0.93**
*1/*1	94	93.08	99	99.97	
*1/*22	7	7.76	3	2.02	
*22/*22	0	0.16	0	0.01	
CYP3A5*3					**0.53**
*1/*1	5	4.89	5	2.95	
*1/*3	33	34.66	21	28.78	
*3/*3	63	61.45	75	70.27	

n: population size; * P-value calculated with test X^2

*CYP3A4*1B*

In the whole population, 76.8% (156/203) of the subjects were homozygous wild type (CYP3A4*1/*1), 19.2 % (40/203) were heterozygous (CYP3A4*1/*1B) and 4% (8/203) were homozygous (CYP3A4*1B/*1B). The CYP3A4*1 variant was the most frequent allele detected (0.87). The allelic and genotypic distribution was not significantly different between healthy and patients (p-value = 0.46).

*CYP3A4*22*

For the CYP3A4*22 variant, no homozygote variant was found neither in healthy subjects nor in patients; only seven (6.8%) and three (3%) heterozygous (CYP3A4*1/*22) were observed in patients and healthy subjects, respectively. Overall, the allelic frequency of the CYP3A4*22 was 0.025. The allelic and genotypic distribution was not significantly different between healthy and patients (p-value= 0.066).

*CYP3A5*3*

The genotype distribution of CYP3A5 was as follows: 5 % (10/203) of all included subjects were homozygous wild type (CYP3A5*1/*1), 27 % (55/203) were heterozygous (CYP3A5*1/*3) and 68 % (138/203) were homozygous (CYP3A5*3/*3). Therefore, the CYP3A5*3 variant was the most frequent allele detected at 0.82. The allelic and genotypic distribution was not significantly different between healthy and patients (p-value = 0.11).

Genotypic combinations

The most frequent genotypic combination in our population is wild type in CYP3A4*1, and variant homozygous in CYP3A5*3 accounting for 60.59% of cases. Followeing this we observed the wild type in CYP3A4*22 and heterozygous CYP3A4*1/*3 in 14.78% of cases. Additionally, the combination of wild type CYP3A4*1 and the heterozygous variant in CYP3A5*3 was observed in 10.34%. Among the subjects, 47 subjects were carriers of the allele *1B with only 5 of them carrying the CYP3A5*1 variant, while 42 carried the CYP3A5*3 allele. Furthermore, all the subjects who were carriers of the CYP3A4*22allele were homozygous for the variant CYP3A5*3. we have identified four metabolizers' statuses according the genotypic combinations (Table4).

Table 4. Phenotype distribution according to Genotypic combinations

Phenotype	Sample size
Slow	9
Intermediate	124
High	29
Extensive	41

Linkage disequilibrium

We observed strong linkage disequilibrium between CYP3A4*1B and CYP3A5*3 genes (D= 0.07; D'=0.7; r= 0.6; P-value=0), the subjects with CYP3A4*1B are CYP3A5*3 carriers. However, there is no linkage disequilibrium between CYP3A4*1B and CYP3A4*22 (D= -0.003; D'=0.9; r= -0.06; P-value= 0.2). Also, we didn't observe a significant linkage disequilibrium between CYP3A4*22 and CYP3A5*3 alleles (D = -0.03; D'=0.9; r= -0.07; P-value= 0.17).

Metabolizers status

Individuals expressing CYP3A4 and CYP3A5 with CYP3A4 *1B/*1B or *1/*1B, CYP3A4 *1/*22 and CYP3A5 *1/*1 or *1/*3, represent 24.13%, 4.9% and 30.54% respectively. This distribution is not different between patients and healthy subjects.

Distribution of the metabolizer status according to CYP3A4 and CYP3A5 genotype combination approved that our individuals are predominantly intermediate metabolizers (63.05%). This finding was the same for patients and healthy subjects with no significant difference (0.92).

Discussion

The genetic background of individuals or populations is an important marker in the development of personalized medicine due to the polymorphic expression of CYP isoenzymes especially CYP3A4 and CYP3A5 that account for up to 50% of CYP3A content. The genetic polymorphisms of CYP3A4*1B, CYP3A4*22, and CYP3A5*3 are known to influence significantly the pharmacokinetics of several drugs such as tacrolimus in renal transplant recipients.

Our study aimed to determine the frequencies of alleles, genotypes, and phenotypes of these three SNPs in a Tunisian population by analyzing a substantial sample size of 203 individuals, providing a robust database for

promoting personalized medicine in Tunisian patients and we previously approved the effect of the metabolizers status in tacrolimus bioavalibility with a significant difference between the combination groups [12].Using *CYP3A5* and *CYP3A4* gene clusters, clinical and demographic factors may be help to select personalized tacrolimus starting doses and to reach their target concentrations quickly.

Previous studies have evaluated simultaneously the genetic data related to *CYP3A4*1B* and *CYP3A5*3* or *CYP3A4*22* and *CYP3A5*3* [7,16]. but no study has evaluated the three variants in the same population. Moreover, the sample size of the current study is higher compared with those who studied in Tunisians the *CYP3A4*1B/CYP3A5*3* (n=102) and *CYP3A4*22* (n= 118), respectively [17,18].

Besides, our study has included both healthy individuals and renal transplant recipients treated by tacrolimus, a drug that is metabolized by the *CYP3A4* and *CYP3A5* isoenzymes. No significant difference was detected between the two populations concerning the allelic, genotypic distribution. These data could be exploited before Tac intake to predict its personalized dose.

CYP3A4*1B

The *CYP3A4*1B* allele corresponds to an A to G substitution in the 5'-promoter (nifedipine-specific element) region on chromosome 7q22 [3]. This allele is as frequent as 13% which is in accordance with previously reported data for the same population (10.8%) [18].

It is well known that the frequency of the *CYP3A4*1B* allele varies widely among different ethnic groups. Novillo et al. have shown in a 97 Moroccan individuals that the frequency of the *CYP3A4*1B* allele was slightly higher (24.4%) than in ours [18]. In Caucasian subjects, the frequency of this mutant allele is reported to be between 3 and 10% and was not detected in Asian populations [4,14,19,20]. Nevertheless, Drögemöller et al. have found that the *CYP3A4*1B* is much more prevalent in two Southern African populations (Khoisan and Xhosa) with frequencies of 76.8% and 73%, respectively [4].

CYP3A4*22

*CYP3A4*22* (rs35599367) was first described in 2011 [9]. This SNP plays an important role in the hepatic expression of *CYP3A4* and its metabolic activity, especially of Tac [21]. Many studies showed that mRNA levels in liver samples that the C-wild type of *CYP3A4*22* are 1.7-fold higher than the T-variant allele [21,22]. The latter allele affects the clearance of some drugs, for example, patients harboring this allele have a lower Tac clearance (-16%) compared with wild type [5,15,21]. Some authors suggest that this biomarker might contribute to the interindividual variability of the *CYP3A4* activity [23,24].

As shown in the current study, the frequency of the *CYP3A4*22* allele, was very low (2.5%) in our population versus 97.5% for the wild type *CYP3A4*1*. Similarly, in Caucasian, the CYP3A4*22 allele is present with a low allelic frequency as it is estimated at 5.3% and 9.8% in Greek and Irish populations, respectively [25]. Moes et al. have shown in a predominantly Caucasian population that the *CYP3A4*22* is as frequent as 5.5% in 101 adult renal transplant recipients, only two of them were homozygous [23]. In a cohort of 140 Brazilian renal allograft recipients, the frequency of the *CYP3A4*22* allele was 5.7% with no homozygous individuals. In contrast, the *CYP3A4*22* variant has not been detected in Japanese, Asian and African populations [21].

CYP3A5

Among our 203 subjects selected for analysis, only ten (5%) are carriers of the heterozygous genotype *CYP3A5*1/*1*. Yet, *CYP3A5*1* is the main allele associated with CYP3A5 expression. In contrast, 69% of our subjects had the *CYP3A5*3/*3* and are not expressers of the enzyme with a *CYP3A5*3* allelic frequency of 0.83. Here again, our results were consistent with those found in Caucasian populations. For example, van Schaik et al. have found in 500 Dutch individuals that the *CYP3A5*3* allele frequency was as high as 91.7% [25]. Similarly, the *CYP3A5*3* allele is widely found in the Moroccan population with a frequency of 92% [18]. As well, this allele is more prevalent in Asian subjects. It's higher in the Chinese population with genotype frequencies equal to 76%; followed by the Malays and Indians populations with 61% and 59% respectively [26,27]. Nevertheless, the *CYP3A5*3* is less frequent in African (15.9% in Nigerian) and African-American populations (55%) [26,28,29].

As found previously, we observed strong linkage disequilibrium between *CYP3A4*1B* and *CYP3A5*3* but no linkage disequilibrium for any other allele pair [30]. Furthermore, we confirm other findings that support that the majority of *CYP3A4*1B* wild type homozygous (*1/*1) are also *CYP3A4*22* wild type homozygous (*1/*1) but are *CYP3A5* variant homozygous (*3/*3). In our population, all the individuals carrying the *CYP3A4*22* allele, have the *CYP3A4 (*1/*1)* and *CYP3A5 (*3/*3)* genotypes.

Interestingly, the close position of *CYP3A4* and *CYP3A5* genes in 7q21.1 maybe influence tacrolimus pharmacokinetics. However, like previously study, our study has shown that *CYP3A4*1B* is in linkage disequilibrium with the *CYP3A5* expresser allele (*CYP3A5*1*) but no significant association between *CYP3A4*22* and both of others SNPs [9,31,32].

Therefore, we have found according to the combination of *CYP3A4*1B*, *CYP3A4*22*, and *CYP3A5*3* profiles, that our subjects are predominantly intermediate metabolizers (63%). Also, only 10 of them do not express *CYP3A4* and *CYP3A5* isoenzymes. To our knowledge, no published study has established the metabolizer status according to these three SNPs.

Conclusion

The current study has determined in the Tunisian population, genetic data related to two isoenzymes of the cytochrome P450 system i.e. *CYP3A4* and *CYP3A5* extensively involved in the metabolism of several drugs. We have shown that the allelic distribution of the *CYP3A4*1B*, *CYP3A4*22* and *CYP3A5*3* polymorphisms observed in a Tunisian are close to data previously reported in some Caucasian and Asian but different from those of African populations. Our approach may contribute to the

enhanced application in this population, of personalized medicine especially in the case of immunosuppressants in organ transplant recipients.

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