Research Article

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Distribution of drug-metabolizing enzymes coding genes *CYP2D6*, *CYP3A4*, *CYP3A5* alleles in a group of healthy Turkish population

Bir grup sağlıklı Türk populasyonunda ilaç metabolize edici *CYP2D6*, *CYP3A4*, *CYP3A5* enzimlerinin allelik dağılımı

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Abstract

Objective: Variant alleles in specific ethnic groups are important for personalized drug therapy regimens and adverse drug reactions. Therefore, the aim of this study was to investigate allelic frequencies of the *CYP2D6*1*, *CYP3A4*5*, *CYP3A4*18*, *CYP3A5*2* and *CYP3A5*4* in a group of Turkish population.

Materials and methods: Three hundred and six unrelated healthy subjects who were accepted as blood donors to the Mersin University Blood Bank were included in the study after informed consent. Allelic frequencies of the *CYP2D6*1* (rs3892097), *CYP3A4*5* (rs55901263), *CYP3A4*18* (rs28371759), *CYP3A5*2* (rs28365083) and *CYP3A5*4* (rs56411402) were determined by using polymerase chain reaction-restriction fragment length polymorphism assays.

Results: *CYP2D6* allele frequencies in detected group was 100% for *CYP2D6*1* (*WT/WT*). *CYP3A4* allele frequencies of subjects were 100% for *CYP3A4*5* (*C/C*) and *CYP3A4*18* (*T/T*). *CYP3A5* allele were in Hardy-Weinberg equilibrium for *CYP3A5*2* ($p = 0.142$) and frequencies for C and A allele were 91% and 9% respectively. *CYP3A5* allele frequencies of subjects was 100% for *CYP3A5*4* (*WT/WT*).

Conclusion: Screening of low frequency alleles by pharmacogenetic testing must not be omitted to optimize pharmacotherapy and avoid severe drug toxicities. Frequency distributions of the identified polymorphisms in the present study may contribute to the personalized drug therapy regimens and prediction of possible adverse drug reactions in the Turkish population.

Keywords: CYP2D6; CYP3A4; CYP3A5; Drug metabolism; Polymorphism.

Özet

Amaç: Etnik gruplardaki varyant alleller, kişiselleştirilmiş ilaç tedavi rejimleri ve istenmeyen ilaç reaksiyonları açısından önemlidir. Bu çalışmanın amacı bir grup Türk gönüllüde *CYP2D6*1*, *CYP3A4*5*, *CYP3A4*18*, *CYP3A5*2 ve CYP3A5*4'* genlerinin allelik frekanslarını araştırmaktır.

Gereç ve yöntem: Mersin Üniversitesi Kan Bankası'na bağışçı olarak kabul edilen, akraba olmayan 306 sağlıklı birey, bilgilendirilmiş onamdan sonra çalışmaya dâhil edildi. *CYP2D6*1* (rs3892097), *CYP3A4*5* (rs55901263), *CYP3A4*18* (rs28371759), *CYP3A5*2* (rs28365083) ve *CYP3A5*4* (rs56411402) genlerinin allelik frekansları, polimeraz zincir reaksiyonu-restriksiyon fragman uzunluğu polimorfizmi yöntemi kullanılarak belirlenmiştir.

Bulgular: İncelenen grupta CYP2D6 allel sıklığı *CYP2D6*1* (*WT/WT*) için %100 idi. *CYP3A4* allel sıklıkları *CYP3A4*5* (*C/C*) ve *CYP3A4*18* (*T/T*) için % 100 idi. *CYP3A5* alleli *CYP3A5*2* için Hardy-Weinberg dengesinde iken ($p = 0.142$) ve C ve A alleli için frekanslar sırasıyla % 91 ve % 9 idi. Gönüllülerin *CYP3A5* allel sıklıkları *CYP3A5*4* (*WT/WT*) için % 100 olarak bulundu.

Sonuç: Düşük frekanslı allellerin, farmakogenetik testlerle taranması, farmakoterapiyi optimize etmek ve ciddi ilaç toksisitelerini önleyebilmek açısından ihmal edilmemelidir. Bu çalışmada tanımlanan polimorfizmlerin frekans dağılımları, Türk popülasyonunda kişiselleştirilmiş ilaç

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tedavisi rejimlerine ve olası advers ilaç reaksiyonlarının önlenmesine katkıda bulunabilir.

Anahtar Kelimeler: CYP2D6; CYP3A4; CYP3A5; Ilaç metabolizması; Polimorfizm.

Introduction

Cytochrome P450 (CYP) enzymes metabolize drugs and xenobiotics and are also involved in the carcinogen and mutagen production [1, 2]. Different population and ethnic groups show variation in the genotype and allele frequencies of CYP enzymes [3]. The activities of polymorphic CYP enzymes range from the absolute absence to high metabolizing capacities [1]. Differences in the activities of CYP enzymes, due to genetic variations, are responsible for the individual response variability to numerous drugs and carcinogens [3].

Over 80 allelic variants have been described for *CYP2D6* which is also known as debrisoquine/sparteine oxidation polymorphism [4]. Clinical significance of CYP2D6 enzyme polymorphism has been well documented and an estimated 20–25% of all drugs in clinical use are metabolized at least in part by this enzyme [4]. Approximately 2–3% of total liver CYP enzymes consist of CYP2D6 and its content substantially varies among people mainly due to its genetic polymorphisms [5, 6]. It has been reported that in addition to liver, CYP2D6 also expressed in the gut and brain neurons, where endogenous substrate turnover found to be high [4].

Exploring phenotypic effects and clinical relevance of *CYP3A* genetic polymorphisms, is an intriguing complementary strategy for pharmacogenetic and toxicogenetic studies. Four functional CYP3A enzymes such as CYP3A4, CYP3A5, CYP3A7, and CYP3A43 have been identified in humans [7]. CYP3A4 is most abundant enzyme in adult liver and intestine and is the major enzyme involved in xenobiotic and drug metabolism [8]. CYP3A5 is the predominant form in the kidney [9]. The *CYP3A4* and *CYP3A5* genes have a strong haplotype structure at varying frequencies across ethnic groups [10]. In addition to liver, *CYP3A5* was reported to be expressed in intestine and as the prevailing *CYP3A* isoform in kidney [9]. CYP3A5 converts cortisol to 6-β-hydroxycortisol in the kidney also has been implicated to salt-sensitive hypertension in humans [10].

Thus, in this study, we aimed to determine the aforementioned drug metabolizing enzymes *CYP2D6*, *CYP3A4*, *CYP3A5* polymorphism in a Turkish population, to help to predict inter-individual variabilities in drug response in the Turkish population.

Materials and methods

Subjects and blood samples

All subjects were from the Mersin province of Turkey a city located South-East Mediterranean part of Anatolia. There can be a population admixture through extensive internal migration to region. Migrants from outside of the Country were excluded. The study was approved by the ethical committee of the Medical Faculty of Mersin University, conducted according to the Declaration of Helsinki, and written informed consent was obtained from all subjects. A total of 306 unrelated subjects who were accepted as blood donors to the Blood Bank of Mersin University Center for Health Research and Application, participated in the genotyping phase of this study. Blood samples were collected during a 7 month of period between July 2012 and January 2013. The age of the subjects ranged from 19 to 55 years (mean age: 34.5 ± 9.3 years; 98.7% were male). None of the subjects had taken any medication or alcohol or had smoked for at least 4 weeks before the study. All individuals were healthy as determined by medical history. Due to technical reasons, it was unable to detect the genotypes of some subjects, *CYP2D6*1* (n = 9), *CYP3A4*5* (n = 23), *CYP3A5*2* (n = 17) and *CYP3A5*4* (n = 39).

Genotyping: DNA extraction and analysis

Eight milliliters of venous blood were obtained from each participant and collected to tubes with ethylenediaminetetraacetic acid (EDTA). Genomic DNA was extracted from peripheral blood by RTA DNA Blood Isolation Kit (RTA) according to the instructions of the manufacturer. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) that were used to identify different single nucleotide polymorphisms (SNPs) (*CYP3A4*18*, *CYP3A5*2*, *CYP3A5*4*, *CYP2D6*1* and *CYP3A4*5*) using previously published methods [11–14]. Primers, restriction enzymes, the conditions of PCR and length of the expected fragments on digestion are summarized in Table 1.

A 25 μL PCR reaction volume was used, containing 1 μL genomic DNA (50–70 ng/μL), 2.5 μL 10× Fermentas Taq reaction buffer, 2.5 μL $MgCl_2$ (25 mM), 2.5 μL of dNTPs (2.5 mM), 0.5 μL of each primer (10 μM) 0.2 μL of Fermentas Taq DNA polymerase (5 U/μL) (Fermentas, Waltham, MA, USA) and nuclease free water added to reach 25 μL end volume. After PCR amplification, 20 μL

Table 1: Single nucleotide polymorphisms (SNPs), primers, restriction enzymes, the conditions of polymerase chain reaction (PCR), length of the expected fragments on digestion and references were used for method.

Genes	Primer sequence	Products (bp)	Enzyme	PCR conditions annealing temp (°C)	Ref.
*CYP2D6*1*	F: 5′-GCC ACC ATG GTG TCT TTG CTT TC-3′ R: 5′-CTC AGC CTC AAC GTA CCC CT-3′	WT: 231, 33 MU: 264	*BanII*	55	[13]
*CYP3A4*5*	F: 5′-TGT TGC ATG CAT AGA GGA AGG ATG G-3′ R: 5′-AGT GGT TGC ATA TGA TGA CAG GGT T-3′	CC: 450 GG: 250, 200 CG: 450, 250, 200	*ClaI*	58	[14]
*CYP3A4*18*	F: 5′-AAT GAT TTG CCT TAT TCT GT TCT G-3′ R: 5′-TTT CAC CTC CTC CCT CCT TCT C-3′	TT: 388 CC: 199, 189 CT: 388, 199, 189	*HpaII*	58	[11]
*CYP3A5*2*	F: 5′-CTG TTT CTT TCC TTC CAG GC-3′ R: 5′-CTC CAT TTC CCT GGA GAC TTG-3′	WT: 269 MU: 182, 87	*TasI*	55	[12]
*CYP3A5*4*	F: 5′-AAA GTG TGT GAG GGC TCT CGA-3′ R: 5′-TCG ACT CTC TCA ACA ATC CTC-3′	WT: 261 MU: 241, 20	*TaqI*	58	[12]

WT, Wild type; MU, mutant; Ref, reference; temp, temperature.

PCR products were digested (overnight at 37°C) with approximately two units of *HpaII*, *TasI*, *TaqI*, *BanII*, *ClaI* for *CYP3A4*18* (rs28371759), *CYP3A5*2* (rs28365083), *CYP3A5*4* (rs56411402), *CYP2D6*1* (rs3892097) and *CYP3A4*5* (rs55901263) respectively [11–14]. Electrophoresis was done for restriction enzyme digested products using 3% agarose gels in 1× TBE buffer.

Statistical analysis

The deviations from the Hardy-Weinberg equilibrium for allele and genotype frequencies for the polymorphisms were assessed by Fisher's Exact Test. The 95% confidence intervals were calculated for all observed allele frequencies. A p-value less than 0.05 were considered as significant. Statistical analysis was carried out by using the Stata/MP11 software (StataCorp LP, TX, USA).

Results

CYP2D6 allele frequencies in 297 subjects were 100% for *CYP2D6*1* (*WT/MU*). *CYP3A4* allele frequencies in 283 and 306 subjects were 100% for *CYP3A4*5* (*C/C*) and *CYP3A4*18* (*T/T*) respectively. *CYP3A5* allele were in Hardy-Weinberg equilibrium for *CYP3A5*2* ($p = 0.142$) and frequencies for C and A allele were 91% and 9% respectively in 289 subjects. *CYP3A5* allele frequencies in 267 subjects were 100% for *CYP3A5*4* (*WT/WT*) (Table 2). Due to technical reasons, it was unable to detect the genotypes of some subjects, *CYP2D6*1* (n = 9), *CYP3A4*5* (n = 23), *CYP3A5*2* (n = 17) and *CYP3A5*4* (n = 39).

Table 2: CYP2D6, CYP3A4, CYP3A5 allele frequencies in sample of Turkish population.

Genes	RS numbers	Alleles	Subjects (n)	%	95% CI
*CYP2D6*1*	rs3892097	WT/WT	297	100	98.99–100.0
*CYP3A4*5*	rs55901263	C/C	283	100	98.94–100.0
*CYP3A4*18*	rs28371759	T/T	306	100	99.02–100.0
*CYP3A5*2*	rs28365083	C/C	243	84.08	79.34–88.10
		C/A	46	15.91	11.89–20.65
*CYP3A5*4*	rs56411402	WT/WT	267	100	98.88–100.0

Discussion

Inter-individual variabilities in genetic factors affect the pharmacokinetic and change the efficacy and toxicity properties of drugs. Also genetic variations in *CYP* enzymes are the important predictors of difference in drug response such as adverse drug reactions and variability in drug efficiency. The submitted pharmacogenetic CYP enzyme polymorphism makes possible to optimize pharmacotherapy and adjust dose to individual needs [1, 2]. Therefore in the present study some of the important polymorphisms for *CYP2D6*, *CYP3A4*, and *CYP3A5* in a group of Turkish population were demonstrated.

Clinically important drugs such as antipsychotics, antidepressants, anti-cancer drugs and antiarrhythmics are metabolized by CYP2D6 which enzymatic activity is highly correlated with its genetic polymorphisms [1, 2]. Multiallelic polymorphisms, which strongly related to ethnicity, determines the function of CYPs such as CYP2D6, CYP3A5, CYP2C19, CYP2C9, and as a consequence lead to distinct phenotypes as ultra-rapid, extensive,

intermediate and poor metabolizers [15]. CYP2D6 alleles and their functional activities which determines abovementioned metabolizing state have been classified as normal, increased, reduced and none [16]. *CYP2D6*1* is classified as alleles with normal function [4]. In the present study *CYP2D6* allele frequencies in subjects were 1.0 for *CYP2D6*1* (*WT*/*WT*). There is limited studies for *CYP2D6*1* polymorphism in Turkish population. Aynacioglu et al. reported *CYP2D6*1* with an allelic frequency of 0.37 in Turkish population [17]. In another study *CYP2D6* genotypes were determined among 92 Turkish patients with breast cancer treated with tamoxifen, the most common *CYP2D6* gene polymorphism was *1/*2 with a percentage of 20.6% (n = 19) [18]. In addition a study from Turkey reported allelic frequency for *CYP2D6*1* as 50.7% in 68 psychiatric patients [19].

CYP3A enzymes metabolizes approximately 37% of the drugs from all therapeutic categories, such as macrolide antibiotics like erythromycin, immunosuppressants cyclosporin and tacrolimus, anticancer drugs like taxol, benzodiazepines, HMG-CoA reductase inhibitors like simvastatin and atorvastatin and anesthetics [20]. Besides drugs, CYP3A4 also have a role in the metabolism of bile acids and sex steroids, including testosterone, progesterone, androstenedione [20].

Although some single nucleotide polymorphisms for *CYP3A4* have been identified, they failed to explain major part of the phenotypic variability. However recently, increasing studies has shown that genetic variants in *CYP3A4* contribute to inter-individual variabilities of metabolic activity [21]. In addition to there is no studies regarding the *CYP3A4*5* and *CYP3A4*18* polymorphisms and only limited studies on *CYP3A4* and *CYP3A5* polymorphism in Turkish population [22–24]. It has been reported that *CYP3A4*5* and *CYP3A4*18* polymorphisms are associated with the degree of the enzymatic activity [21]. In the present study *CYP3A4* allele frequencies were 1.0 for both *CYP3A4*5* (*C*/*C*) and *CYP3A4*18* (*T*/*T*) in Turkish population.

CYP3A4/5 share significant sequence homology and have almost identical substrate specificity with somewhat differing metabolic rates [25]. Although it has been reported that CYP3A5 is an overlooked polymorphic enzyme, its allelic frequency in specific ethnic groups is important to optimize pharmacotherapy [12, 26]. Literature is limited regarding to *CYP3A5* polymorphism also there is no study about *CYP3A5* polymorphism in Turkish population except a few genotyping for *CYP3A5*3* [23, 27]. In the present study *CYP3A5* alleles were in Hardy-Weinberg equilibrium for *CYP3A5*2* and *CYP3A5* alleles frequencies were 1.0 for *CYP3A5*4* (*WT*/*WT*) in healthy Turkish blood donors. Allelic frequencies of CYP3A5*2 and *4 in the Dutch Caucasian population were report as 0.010 and 0.000 respectively [12]. In conclusion they suggested genotyping for the CYP3A5*2 allele in CYP3A5*3 heterozygotes and CYP3A5*2 less relevant for screening purposes [12].

Cytochrome P450 enzyme system have a critical role in the metabolism and elimination of drugs and xenobiotics as well as their activity could produce carcinogens and mutagens [1, 2, 28, 29]. Present study is important for elucidating the CYP2D6, CYP3A4 and CYP3A5 drug metabolizing enzyme polymorphisms that especially have not been shown before in Turkish population. These results may help optimization of personalized pharmacological therapies and prediction of xenobiotics metabolism.

Conflict of interest statement: The authors have no declarations of interest to report.

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