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

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.

Öz

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 sağlıkli birey, bilgilendirilmiş onandan sonra çalışmaya dahil edildi. CYP2D6*1 (rs3892097), CYP3A4*5 (rs55901263), CYP3A4*18 (rs28371759), CYP3A5*2 (rs28365083) ve CYP3A5*4 (rs56411402) genlerinin allelik frekansları araştırıldı. CYP3A5 allelinin Hardy-Weinberg dengesinde olduğu (p = 0.142) ve C ve A allelinin frekanslarına sırasıyla % 91 ve % 9 idi. Gönüllülerin CYP3A5 allelinin frekansları, polymorfik reaksiyon-restrisiyon fragment uzunluğu ile araştırılmıştır.

Sonuç: Düşük frekanslı allellere, farmakogenetik testlerle taraması, farmakoterapiyi optimize etmek ve ciddi ilaç toksisitelerini önlenebilmek açısından önemlidir. Bu çalışmada tanımlanan polymorfizmlerin frekans dağılımları, Türk popülasyonunda kişiselleştirilmiş ilaç
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 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 MgCl2 (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...
PCR products were digested (overnight at 37°C) with approximately two units of HpaII, TasI, TaqI, BanII, and 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*4 (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).

### 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, etc.
intermediate and poor metabolizers [15]. CYP2D6 alleles and their functional activities which determines above-mentioned 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, progestosterone, 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.

**References**

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