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SHORT COMMUNICATION

The genetic profiles of CYP1A1, CYP1A2 and CYP2E1 enzymes as susceptibility factor in xenobiotic toxicity in Turkish population

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Abstract Evaluation and sequencing of heritable alterations in the human genome and the large-scale identification of gene polymorphism for understanding the genetic background of individuals in response to potential toxicants are provided by toxicogenetics. Cytochrome P450 (CYP) enzymes play role not only phase I-dependent metabolism of xenobiotics but also metabolism of endogenous compounds. CYP1A1, CYP1A2 and CYP2E1 enzymes, which are in phase I enzymes, are responsible for metabolic activation and detoxification of several chemical compounds. In the present study, it was determined the genotype and allele frequency of *CYP1A1*2A, CYP1A2*1C, CYP1A2*1F, CYP2E1* and *CYP2E1*6*, very common and functional single-nucleotide polymorphisms (SNPs), in Turkish healthy volunteers. It is believed that the determination of polymorphisms in the enzymes may be beneficial to prevent and reduce and adverse effects and death in response to drugs. The allele frequencies of these genes were 24%, 9%, 33%, 42%, and 12%, respectively. In the present study, the genotype profile of Turkish population was determined about critical enzymes for xenobiotic metabolism. It is suggested that the obtained results might be beneficial in order to dose adjustment of drugs and prevention of adverse reactions, and further investigation about mentioned enzymes and their polymorphisms.

1. Introduction

Toxicogenetics provides the evaluation and sequencing of heritable alterations in the human genome and the large-scale identification of gene polymorphism for understanding the genetic basis for individuals' differences in response to potential toxicants. This ensures to predict relative susceptibility of an individual or populations to the adverse effects and relative sensitivity to drugs and environmental chemicals associated with these adverse effects (Orphanides and Kimber, 2003).

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CYP enzymes are essential source for phase I metabolism over 90% of drug/xenobiotics and endogenous substrates. Until now, fifty-seven *CYP* genes, which involve three families (CYP1, CYP2 and CYP3) contributing to the oxidative metabolism of various compounds, have been detected (Shastry, 2006). It is known that beside metabolizing several important drugs, detoxification and bioactivation of environmental procarcinogens and endogenous compounds are occurred by CYP1 enzymes (Alessandrini et al., 2013).

CYP1A1 and CY1A2 are located on chromosome 15q24.1, and expressed in liver and extra-hepatic tissue (Zanger and Schwab, 2013). CYP1A1 enzymes are responsible for metabolism of polycyclic aromatic hydrocarbons which are found in tobacco smoke and a number of carcinogens. To date, approximately twenty variant alleles of CYP1A1 have been identified (Alessandrini et al., 2013). CYP1A2 has an important role not only in metabolism of many drugs such as amitriptyline, theophylline, and clozapine but also in the activation of some procarcinogens and caffeine. There were more than fifteen alleles identified for CYP1A2 (Alessandrini et al., 2013; Han et al., 2002).

CYP2E1 is located on chromosome 10q.24 and expressed in both of the liver and extra-hepatic tissues. It is involved in the metabolic activation of many low molecular weight carcinogens and toxic compounds such as benzene, N-nitrosamines, carbon tetrachloride, chloroform, vinyl chloride, tobacco smoke, styrene and ethylene glycol. Moreover, CYP2E1 is a major component oxidation of ethanol for production reactive free radical which may lead to lipid peroxidation and affect target tissue (Shahriary et al., 2012; Soya et al., 2005).

It is known that inter-ethnic differences are becoming more critical factor for inter-individual variations in response to drugs and other xenobiotic in terms of xenobiotic metabolism. These differences ensure to respond differently because of different genetic backgrounds. Because phase I enzymes playing the most important role in the oxidation of various chemical compounds can activate the carcinogens and produce more active metabolites with oxidation, the genetic differences may risk in development of the various cancers (Bianchino et al., 2011; O'Donnell and Dolan, 2009).

In the present study, we aimed to determine the genotype profile of Turkish population in terms of important metabolizing enzymes for drugs and other xenobiotics, considering not enough data for mentioned enzymes in Turkish population.

2. Materials and methods

DNA was isolated from venous blood samples of unrelated 160 Turkish healthy volunteers (88 females and 72 males; aged 20–65 years; location in European side) by High Pure PCR Template Preparation Kit (Roche, Germany). The samples were collected from Hospital of Istanbul University and Bagcilar Training and Research Hospital in 2013–2015. Genotyping of *CYP1A1*2A* (rs4646903, 6235T > C), *CYP1A2*1C* (rs2069514, 3858G > A), *CYP1A2*1F* (rs76251, 163C > A), *CYP2E1* (rs3813867, -1295G > A) and *CYP2E1*6* (rs6413432, 7632T > A) variants was performed by polymerase chain reaction (PCR) – restriction fragment length polymorphism (RFLP) methods. The temperature was controlled by thermal cycler (Gene Amp PCR System 9700; Applied Biosystems, Carlsbad, CA, USA). Restriction enzymes were obtained from New England Biolabs (Hitchin, UK) and Fermentas (Vilnius, Lithuania). All participants provided informed consent and studies were approved by the ethics committee of Istanbul University (2014/1546).

The Hardy-Weinberg equilibrium analysis was performed to compare the observed and expected genotype frequencies of subjects by using the chi-square (χ^2) test. Differences in the *CYP1A1*2A*, *CYP1A2*1C*, *CYP1A2*1F*, *CYP2E1* and *CYP2E1*6* genetic variants between Turkish and other ethnic populations were also assessed by χ^2 test. A p value below 0.05 was considered statistically significant throughout the population comparisons. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software (Version 17, Chicago, USA). The other information about the genetic variants studied is given in Table 1.

3. Result and discussion

In the present study, the frequencies of *CYP1A1, CYP1A2* and *CYP2E1* gene variants in Turkish population were detected (Table 2). There are some studies to investigate the effects of difference related to these genes in drug response or xenobiotic toxicity or disease (Barbieri et al., 2013; Grabar et al., 2008; Zhou et al., 2009).

The distribution of *CYP1A1*2A* (C allele) is 10%, 41%, 34%, 19%, 28%, and 54% in European, American, South Asian, Caucasian, Hispanic and Chinese, respectively (www.hapmap.org). The Turkish population resembles Hispanic population for this variant according to the present study. The allele frequency of *CYP1A2*1C* (A allele) is 28%, 2%, 31%, 36%, 8%, 8%, 20% and 24% in East Asian, European, African, American, South Asian, Caucasian, Hispanic and Chinese, respectively. The frequencies of *CYP1A2*1F* (A allele) are 50%, 67%, 68%, 56%, 76%, 53%, 70% and 76% in Yoruba in Ibadan (Nigeria) (YRI), East Asian, European, African, American, South Asian, Hispanic and Chinese, respectively (www.hapmap.org). According to the mentioned data, Turkish population resembles South Asian and Caucasian for *CYP1A2*1C* (A allele), and East Asian more than European for *CYP1A2*1F* (A allele).

CYP1A1 and *CYP1A2* are functional variants in both development of diseases and toxicological risks induced by environmental factors. Barbieri et al. (2013) investigated whether there were any associations with some polymorphic genes and hereditary medullary thyroid carcinoma (HMTC). It was found that the carriers with both of *CYP1A1*2A* and *CYP1A2*1F* homozygous variant allele had more increased risk of HMTC. It was investigated whether there were any associations of *CYP1A1* polymorphisms with metabolic activation of 17β-estradiol and estrone. According to the study, *CYP1A1*2A* had a significant higher catalytic activity for all hydroxylation sites- particularly 2-hydroxylation and both mentioned substrates and it was suggested there may be risk of oestrogen-induced cancers and cardiovascular diseases with *CYP1A1* genotypes (Kisselev et al., 2005). Nie et al. (2011) evaluated the effects of *CYP1A1* genetic polymorphisms in epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI) in patient with non-small-cell lung cancer. *CYP1A1*2A* polymorphism played a role as prognosis factor strongly. In another study, it was evaluated the relation between lung cancer and *CYP1A1* polymorphism and was

Table 1 The PCR-RFLP conditions in the present study.

SNPs	Primer sequences	Annealing temperature (°C)	Restriction enzyme	Digestion products (bp)
*CYP1A1*2A* (rs4646903)	F: 5′ CAgTgAAgAggTgTAgCCgCT 3′ R: 5′ TAggAgTCTTgTCTCATgCCT 3′	62	MspI	W (TT): 343 M (CC): 134, 209
*CYP1A2*1C* (rs2069514)	F: 5′ gCTACACATgATCgAgCTATAC 3′ R: 5′ CAggTCTCTTCACTgTAAAgTTA 3′	62.3	DdeI	W (GG): 598 M (AA): 132, 466
*CYP1A2*1F* (rs762551)	F: 5′ CAACCCTgCCAATCTCAAgCAC 3′ R: 5′ CATggCTCTggTggACTTTTCA 3′	66.5	ApaI	W (CC): 1012 M (AA): 299, 713
CYP2E1 (rs3813867)	F: 5′ TCgAggAAACTTgTggACCC 3′ R: 5′ gCAAgTCATTggTTgTgCTgC 3′	65.1	SfcI	W (GG): 329 M (CC): 35, 294
*CYP2E1*6* (rs6413432)	F: 5′ CgACATgTgATggATCCAggg 3′ R: 5′ TCgTgATCgCCTgCCTCA 3′	64.7	DraI	W (TT): 327 M (AA): 79, 248

bp: base pair; W: wild type; M: mutant type.

Table 2 Genotype frequencies of the gene variants in the present study.

SNPs	Genotype	Genotype frequency *n* (%)	Allele frequency
*CYP1A1*2A* (rs4646903)	T/T T/C C/C	83 (50.46) 62 (42.18) 2 (1.36)	T: 0.76 C: 0.24
*CYP1A2*1C* (rs2069514)	G/G G/A A/A	131 (89.73) 5 (3.43) 10 (6.84)	G: 0.91 A: 0.09
*CYP1A2*1F* (rs762551)	C/C C/A A/A	21 (14.00) 57 (38.00) 72 (48.00)	C: 0.33 A: 0.67
CYP2E1 (rs3813867)	G/G G/C C/C	76 (48.72) 27 (17.31) 53 (33.97)	G: 0.58 C: 0.42
*CYP2E1*6* (rs6413432)	T/T T/A A/A	6 (3.87) 25 (16.12) 124 (80.00)	T: 0.88 A: 0.12

found that *CYP1A1*2A*, which showed unusually high frequency, associated with increasing lung cancer risk, especially squamous-cell and small-cell lung cancer (San Jose et al., 2010). Also, Özhan et al. (2014) found that there was the association between *CYP1A1*2A* and colorectal cancer in Turkish population.

It was investigated the association of *CYP1A2* polymorphisms with the inducing effect of heavy coffee consumption in Serbian and Swedish populations. There was only one significant association of heavy coffee consumption in carriers of *CYP1A2*1F* homozygous variant for both the populations (Djordjevic et al., 2010). Palatini et al. (2009) suggested that there is the risk of hypertension association with coffee intake and *CYP1A2* polymorphism. According to the results, drinking coffee is safe for carriers homozygous wild type allele while carriers of variant allele (*CYP1A2*1F*) should not be consuming coffee. It was investigated whether there was any relation between the inter-individual variation in *CYP1A2* activity after smoking cessation and its genetic polymorphisms. There was a decreased enzyme activity from 1.0-fold to 7.3-fold after smoking cessation and *CYP1A2* activity was 1.55-fold higher in smokers (Dobrinas et al., 2011).

Grabar et al. (2008) investigated the relation between toxicity of leflunomide treatment and *CYP1A2* polymorphisms in patients with rheumatoid arthritis. It was found that carriers with homozygous wild type allele for *CYP1A2*1F* had a 9.7-fold higher risk compared with carriers with variant allele. Similarly, Zhou et al. (2009) suggested the risk of toxicity increased in carriers of wild type allele (C) during leflunomide and olanzapine treatment.

As to *CYP2E1*, the distribution of *CYP2E1 (C)* is 20, 4, 6, 12, 1, 29, 20, 7, 0, 0 and 6% in East Asian, European, African, American, South Asian, Han Chinese in Beijing, Japanese in Tokyo, YRI, Caucasian, Hispanic and Chinese, respectively (www.hapmap.org). According to the mentioned data, Turkish population resembles East Asian and Chinese in Beijing more than others. But the reason of little differences between the populations and Turkish population might be less size of the studied population. The variant allele frequency of *CYP2E1*6* (A allele) is 27%, 11%, 7%, 17%, 20%, 6%, 17% and 15% in EAS, European, African, American, South Asian, Caucasian, Hispanic and Chinese (www.hapmap.org). According to the mentioned data, Turkish population resembles European. In a study, the allele frequency in Turkish population was found to be 8.25% (Ulusoy et al., 2007).

Investigating association between CYP2E1 genetic polymorphisms and squamous cell carcinoma of the oesophagus, the results showed that carriers of *CYP2E1*6* heterozygous variant allele had an increased risk for development of oesophageal cancer. Also, it was extensive among cigarette smokers and alcohol consumers (Li et al., 2005). Also, some studies showed that this variant allele was associated with developing breast and lung cancer (Uematsu et al., 1991; Shields et al., 1996). Sugimura et al. (2006) evaluated the relation with oral squamous cell carcinoma (OSCC) and many genetic polymor-

phisms. It was suggested that carriers of *CYP2E1*6* homozygous variant type had 2-fold higher risk of OSCC contrary to heterozygous and homozygous wild type.

In conclusion, this study will contribute to represent their genetic profile in terms of crucial metabolism enzymes because there was no enough study about the genetic profiles of *CYP1A1*, *CYP11A2,* and *CYP12E1* in Turkish population. The determination of polymorphisms in mentioned enzymes might provide advantage for dose adjustment of some drugs and protection from xenobiotics in order to prevention and reduction in adverse effects and even death.

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