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RESEARCH ARTICLE

Cytochrome P450 1B1*2 Gene Polymorphism in a Turkish Population

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SUMMARY

*The polymorphisms in genes encoding xenobiotic metabolising enzymes may be important in the individual susceptibility to various diseases including cancer due to the altered metabolism of carcinogens. Cytochrome P450 1B1 (CYP1B1) is an important enzyme of CYP superfamily and is involved in the formation of reactive estrogen metabolites as well as in the activation of environmental carcinogens. CYP1B1*2 variant of CYP1B1 gene has two linked polymorphisms, m1 and m2, resulting in Arg48Gly and Ala119Ser amino acid substitutions respectively. In this study, CYP1B1*2 m1 (rs10012) and m2 (rs1056827) polymorphisms were determined among 150 healthy individuals of a Turkish population by allele specific polymerase chain reaction (PCR) method and the results were compared with various populations. The frequency of CYP1B1*1/*1 (wild type), CYP1B1*1/*2 (heterozygous) and, CYP1B1*2/*2 (mutant) genotypes were found 47.3%, 44.7% and 8.0% respectively. The genotype frequencies were compared with previous studies conducted on different populations. The results show that the genotype frequencies of the CYP1B1*2 gene polymorphism in a Turkish population are similar to other Caucasian populations.*

Key Words: *Cytochrome P450, CYP1B1, Genetic polymorphism, rs10012, rs1056827, Turkish population.*

Bir Türk Popülasyonunda Sitokrom P450 1B1*2 Gen Polimorfizmi

ÖZ

*Ksenobiyotik metabolize eden enzimleri kodlayan genlerdeki polimorfizmler, kanserojenlerin metabolizmasında değişime neden olarak kanser de dahil olmak üzere çeşitli hastalıklara karşı bireysel duyarlılıkta önemli olabilmektedir. Sitokrom P450 (CYP) ailesinin önemli bir üyesi olan CYP1B1 enzimi aktif östrojen metabolitlerinin oluşmasının yanında çevresel kanserojenlerin aktivasyonunda da görev alır. CYP1B1 geninin CYP1B1*2 varyantında m1 ve m2 olarak adlandırılan ve sırasıyla Arg48Gly ve Ala119Ser amino asit değişimlerine neden olan birbirleriyle bağlantılı iki polimorfizm mevcuttur. Bu çalışmada, Türk toplumunda 150 sağlıklı bireyde CYP1B1*2 m1(rs10012) ve m2(rs1056827) polimorfizmleri alele özgü polimeraz zincir reaksiyonu (PZR) yöntemi ile belirlenmiş ve sonuçlar çeşitli toplumlarla karşılaştırılmıştır. CYP1B1*1/*1 (yabanıl tip), CYP1B1*1/*2 (heterozigot) ve CYP1B1*2/*2 (mutant) genotip frekansları sırasıyla %47,3, %44,7 ve %8,0 olarak bulunmuştur. Genotip frekansları farklı toplumlarda yapılmış çalışmalarla karşılaştırılmıştır. Elde edilen sonuçlar, çalışılan Türk popülasyonundaki CYP1B1*2 gen polimorfizminin diğer beyaz ırk toplumlarına benzer olduğunu göstermektedir.*

Anahtar Kelimeler: *Sitokrom P450, CYP1B1, Genetik polimorfizm, rs10012, rs1056827, Türk popülasyonu.*

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INTRODUCTION

One of the most important groups of metabolic enzymes responsible for the elimination of toxic substances is the Cytochrome P450 (CYP) superfamily. Changes in CYP expression are considered among important toxicological factors since many carcinogenic compounds are thought to be bioactivated by phase I enzymes and this metabolic activation is carried out intensely by CYPs. CYP1B1, an important member of this family, is involved in the formation of reactive estrogen metabolites and in the activation of environmental carcinogens such as polycyclic aromatic hydrocarbons. The metabolism by CYP1B1 has been recognized as the initial step in the carcinogenic action of these substances (Cui et al.,2015). Associations between genetic polymorphisms and risk of cancers and other diseases (Daly 2015, He and Feng 2015) clearly indicate the increased need to elucidate the effects of *CYP1B1* genetic polymorphism on cancer susceptibility and adverse health outcomes.

*The CYP1B1*2* variant of *CYP1B1* gene has two linked polymorphisms in exon 2, rs10012 and rs1056827, resulting in the Arg48Gly (m1) and Ala-119Ser (m2) amino acid substitutions respectively compared to *CYP1B1*1* (McLellan et al., 2000). It has been reported that the frequencies of polymorphic genes in various populations are different (Ada et al., 2007). Moreover, intra-ethnic differences have been well established (Garte et al., 2001). For example, there are a few studies on *CYP1B1*2* polymorphism in different Caucasian populations and their results are contradictory (Landi et al., 2005, Cleary et al., 2010, Trubicka et al., 2010, Rudolph et al., 2011). As far as we know, there is only one study about the frequency of this gene polymorphism in Turkish population (Berber et al., 2013). Therefore, in this study we aimed to clarify the genotype frequency of *CYP1B1*2* polymorphism in a Turkish population and compare with various populations.

MATERIALS AND METHODS

Subjects

The subjects were 150 unrelated individuals (14 females and 136 males) aged between 21 to 71 years (mean age 46). Healthy volunteers who do not have a diagnosis of any cancer were included in our study. The exclusion criteria were any diagnosed type of cancer, chronic diseases or pregnancy. Relatives were also excluded from the study. In order to evaluate the results in detail, all individuals filled their informed consent forms with a questionnaire containing age, gender, occupation, smoking, alcohol and coffee habits, nutritional patterns and other necessary information. The study was approved by the Ethics Committee of Ankara University (Approval no: 12-222). From each patient 1 ml sample of blood collected into coded, heparinised tubes for immediate DNA isolation.

DNA isolation

DNA was isolated from lymphocytes of donors by using Promega DNA isolation kit (Madison WI, USA). 300 µl of blood sample mixed with 900 µl cell lysis solution. The mixture was incubated for 10 min. at room temperature and centrifuged at 13000 g for 20 sec. The pellet is vortexed and mixed with 300 µl nuclei lysis solution. After adding protein precipitation solution the mixture is vortexed for 20 sec. Then centrifuged at 13000 g for 3 min. The supernatant is taken to a new tube and mixed with 300 µl isopropanol. After centrifugation at 13000 g for 1 min. supernatant was discarded. DNA was washed with 70% ethanol and centrifuged at 13000 g for 1 min. After removing ethanol, DNA rehydration solution was added and kept at 4^0C overnight. Isolated DNA was stored at -20^0C until use.

Genotyping procedure

Genetic polymorphism analysis for the *CYP1B1* m1 and m2 mutations were determined by using two step allele specific polymerase chain reaction (PCR) method described by Aklillu et al. (2002). In the first PCR, the region that covers the m1 and m2 mutation sites of *CYP1B1* gene was amplified in a 25 µl reaction mixture containing 0.25 µM of each of the following primers forward (5'-ACG ACC CTT GGC CGC TAA AC-3'), reverse (5'-GCC ACC ACC GTG CGA GTC-3'), 0.25 mM each dNTP, 1.3 mM Mg Cl_2, 10% dimethyl sulfoxide, 100 ng genomic DNA, 0.75 U Taq polymerase (Sigma-Aldrich, USA), in 1x PCR buffer (10 mM Tris HCl, pH 9.0 25^0C, 50 mM KCl). PCR conditions were 1 min 95^0C initial denaturation, 35 cycles of 15 sec at 95^0C, 20 sec at 60^0C and 1 min at 72^0C followed by final extension 7 min at 72^0C.

The first PCR product was used as a template in the second PCR reaction which contained 1 µl of 10 fold diluted PCR1 product, 0.125 µM of each of the following primers for m1 forward wild type (5'-GGA GGC GGC AGC TCG-3'), forward mutant (5'-GGA GGC GGC AGC TCG-3'), for m2 forward wild type (5'-TCG CCG ACC GGC CGG-3'), forward mutant (5'-TCG CCG ACC GGC CGT-3'), for both m1 and m2 reverse (5'-CAG CAG CGC CAC CAG CTC-3'), 0.125 mM each dNTP, and 0.375 U Taq polymerase (Sigma-Aldrich, USA), in 1x PCR buffer (see above) in a total reaction volume of 25 µl. PCR conditions were 1 min 95^0C initial denaturation, 15 cycles of 15 sec at 95^0C, 20 sec at 54^0C and 45 sec at 72^0C followed by final extension of 7min at 72^0C. The PCR products were analysed by electrophoresis on an ethidium bro-

mide stained 2% agarose gel. Allelic discrimination was made after examining PCR bands in agarose gel images. PCR1 products showed a band at 914 bp and PCR2 products of m1 and m2 showed bands at 416 bp and 203 bp respectively.

RESULTS AND DISCUSSION

In the current study, the genotype frequencies of m1 and m2 mutations of *CYP1B1* gene have been studied in the same individuals of a Turkish population. *CYP1B1* gene *2 variant has two linked polymorphisms in exon 2, rs10012 and rs1056827, resulting in the Arg48Gly (m1) and Ala119Ser (m2) amino acid substitutions. The m1 and m2 mutations in *CYP1B1* gene are completely in a linkage disequilibrium (Aklillu et al., 2002). As a matter of fact, in this study the genotype frequencies of m1 and m2 mutations were compared in all samples and found 100% concordant. The observed frequencies of *CYP1B1*1/*1* (wildtype), *CYP1B1*1/*2* (heterozygous) and *CYP1B1*2/*2* (mutant) genotypes are shown in Table 1. The genotype frequencies are consistent with Hardy Weinberg Equilibrium. No difference was observed between genotype distributions in terms of age and gender (data not shown). The frequencies of *CYP1B1*1/*1*, *CYP1B1*1/*2* and *CYP1B1*2/*2* genotypes were found as 47.3%, 44.7% and 8.0% respectively (Table 1). Previously, Berber et al. (2013) reported the frequencies of *CYP1B1*1/*1*, *CYP1B1*1/*2* and *CYP1B1*2/*2* genotypes as 52.6%, 38.6% and 8.8% respectively in a Turkish population. Thus, the results now obtained regarding genotype frequencies of *CYP1B1*2* confirm these findings. The genotype frequencies of *CYP1B1*2* in our study are also in agreement with previous reports on Caucasian populations of Canada, Germany and Poland (Cleary et al., 2010, Rudolph et al., 2011, Trubicka et al., 2010) but in contrast to studies on Ethiopian and Spainish populations (Aklillu et al., 2002, Landi et al., 2005). The genotype frequency of *CYP1B1*2/*2* (8.0%) in this study was found very similar especially with the results of Cleary et al. (2010) and Rudolph et al. (2011) (8.7% and 8.0% respectively). On the other hand, appears to be higher than the results of Landi et al. (2005) (3.9%) but lower than the results of Aklillu et al. (2002) (42.0%). When the genotype frequency of *CYP1B1*1/*2* (44.7%) was taken into account, our result were found very similar to those of Trubicka et al. (2010) and Rudolph et al. (2011) (44.4% and 44.3% respectively) (Table 2).

Table 1. Frequencies of *CYP1B1*2* genotypes

Genotype	n	Percentage
Total	150	
*CYP1B1*1/*1*	71	47.3
*CYP1B1*1/*2*	67	44.7
*CYP1B1*2/*2*	12	8.0

Table 2. The Frequencies of *CYP1B1*2* polymorphisms in different populations

Country	n	*CYP1B1*1/*1* (%)	*CYP1B1*1/*2* (%)	*CYP1B1*2/*2* (%)	Reference
Ethiopia	150	29.3	28.7	42.0	Aklillu et al. (2002)
Canada	1292	50.4	40.9	8.7	Cleary et al. (2010)
Germany	675	47.7	44.3	8.0	Rudolph et al. (2011)
Poland	597	44.4	44.4	11.2	Trubicka et al. (2010)
Spain	258	62.4	33.7	3.9	Landi et al. (2005)
Turkey	114	52.6	38.6	8.8	Berber et al. (2013)
Turkey	150	47.3	44.7	8.0	This study

Therefore, the results of this study show that the genotype frequencies of *CYP1B1*2* gene polymorphism in a Turkish population are similar to Caucasian populations. In addition, the results of this study may provide basis for further studies for the investigation of inter-individual susceptibility regarding *CYP1B1*2* polymorphism in the development of cancer and various diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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