RESEARCH ARTICLE

*CYP2E1*5B, CYP2E1*6, CYP2E1*7B, CYP2E1*2,* and *CYP2E1*3* Allele Frequencies in Iranian Populations

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Abstract

Background: *CYP2E1* encodes an enzyme which is mainly involved in bioactivation of potential carcinogens such as N-nitrosamines. Polymorphisms in the gene have been reported to be associated with cancer. The aim of this study was to evaluate genotype distributions and allele frequencies of five *CYP2E1* polymorphisms in Iran Materials and Methods: Two hundred healthy individuals of an Iranian population from the southwest were included in this study. PCR-restriction fragment length polymorphism and Tetra-ARMS PCR methods were applied for *CYP2E1* genotyping. Results: The allele frequencies for **5B, *6, *7B, *2,* and **3* were calculated to be 1.5%, 16%, 28.5%, 0%, and 2.75% respectively. Results of this study showed that no significant differences in genotype and allele frequencies of five single nucleotide polymorphisms with respect to the gender and tribes. The chi-square test showed that the genotype frequencies of *CYP2E1*5B* were similar to Caucasians, but the distribution of *CYP2E1*6* genotypes was similar to Asians. The frequencies of *CYP2E1*2* (0%) and *CYP2E1*3* (2.75%) alleles were within the range for Caucasians and Orientals. In the case of *CYP2E1*7B*, the data werelimited. Accordingly, the results were only compared with Europeans and the comparison showed significant differences. Conclusions: In conclusion, ethnic and geographic differences may explain discrepancies in the prevalence of CYP2E1 polymorphisms.

Keywords: Cytochrome P450 - polymorphism - genotyping - allele frequency - Iranian population

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Introduction

The only member of human cytochrome P450 E subfamily is *CYP2E1* (Zhuge et al., 2003), located in the 10q24.3-qter region of chromosome 10. It contains 9 exons, 8 introns, and a typical TATA box and it spans 11,413 base pairs of genomic DNA (Umeno, 1988). *CYP2E1* plays a key role in the metabolic activation of many low molecular weight carcinogens (e.g., benzene, N-nitrosamines, carbon tetrachloride chloroform and vinyl chloride) and producing reactive oxygen species (e.g., superoxide anion radical, hydrogen peroxide), which can affect target tissue and ultimately lead to carcinogenesis (Hou et al., 2007; Guo et al., 2010; Feng et al., 2012).

CYP2E1 has several polymorphisms. Studies reported that its functional polymorphisms are associated with increased and decreased susceptibility to many cancer types, including esophageal cancer, lung cancer, nasopharyngeal carcinoma, and colorectal cancer (Cai et al., 2005; Sangrajrang et al., 2006).

*CYP2E1*5B* (rs2031920) and *CYP2E1*6* (rs6413432) single nucleotide polymorphisms (SNPs) have been studied frequently. *CYP2E1*5B* is a PstI polymorphism, caused by a G->C change at position 1293 in the 5-flanking region of *CYP2E1* gene. G and C alleles were named c1 and c2, respectively. It has been shown that *CYP2E1*5B* can affect the transcription of *CYP2E1* gene in vitro (Hayashi et al., 1991). The correlation between this polymorphism and oral, pharyngeal, liver, gastric and lung cancers has been found (Boccia et al., 2007). *CYP2E1*6* polymorphism (position T7632A) was identified in intron 6 of the gene by using the restriction enzyme DraI (Persson et al., 1993). Common homozygote (TT), heterozygote (TA), and rare homozygote (AA) genotypes of *CYP2E1*6* were named DD, DC, and CC, respectively. *CYP2E1*6* doesn't affect gene transcription, but it is likely to affect *CYP2E1* catalytic activity (Uematsu et al., 1991). *CYP2E1*7B* (rs6413420; G-71T) was also found in the promoter region of the gene. *CYP2E1*7B* affects the transcription of the gene and causes 1.8-fold increase in the transcriptional activity of *CYP2E1* (Fairbrother et al., 1998). Therefore, it is expected that *CYP2E1*6* and *CYP2E1*7B* influence

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cancer risk. *CYP2E1*2* (rs72559710) and *CYP2E1*3* (rs55897648) were identified in exon 2 and exon 8 of the gene, respectively. *CYP2E1*2* that causes an R76H amino acid exchange, lowers enzyme synthesis and catalytic activity. The additional polymorphism, *CYP2E1*3*, was detected, causing the substitution of Valine to Isoleucine at position 389, but without any effect on enzyme synthesis and catalytic activity (Hu et al., 1997).

Inter-individual differences in the expression level, which can result in tumor development, have been connected with its polymorphisms. The allele frequencies of these polymorphisms differ remarkably among different human populations (Bolt et al., 2003). These polymorphisms are well characterized in different populations, but little is known about the Iranian ethnic group. This study could provide valuable data for association studies between these polymorphisms and cancer susceptibility. Therefore, the genotype and allele frequencies of *CYP2E1* single nucleotide polymorphisms, including **5B, *6, *7B, *2*, and **3* were presented, estimated and analyzed in this study. The present study was aimed to provide basic information about the allele and genotype distribution of *CYP2E1* polymorphisms.

Materials and Methods

Study population

Samples from 200 genetically unrelated, healthy individuals blood donors were taken which consisted of 100 males between the ages of 1 and 80 (mean age 41.9±22.3) and 100 females between the ages of 3 and 84 (mean age 40.87±19.97) were examined in this study. Blood samples were collected at the University hospital of Jundishapur and an accredited medical diagnostic laboratory in Ahvaz city (southwest of Iran). The population study was composed of two ethnic subgroups, including non-Arabs (66%) and Arabs (34%). Since these two groups of individuals were seen homogenous in relation to the allelic and genotypic frequencies and both were in Hardy-Weinberg equilibrium, they were considered as one group.

This investigation was approved by the Ethics Committee of Jundishapur University of Medical Sciences.

Genotype analysis

Genomic DNA was extracted from 100 µl of whole blood using the Diatom DNA Kit (IsoGene, Russia) according to the manufacturer's instructions. The extracted DNA was visualized on 1% agarose gel and stored at –20°C until genotyping was performed. Two different methods were used to detect five single nucleotide polymorphisms (SNPs) of *CYP2E1, CYP2E1*5B, CYP2E1*6*, and *CYP2E1*7B*. Polymorphisms were determined by polymerase chain reaction (PCR) based on the restriction fragment length polymorphism (RFLP) method with designed primer pairs. The PCR products of *CYP2E1*5B, CYP2E1*6,* and *CYP2E1*7B* were digested with the restriction enzymes PstI (Vivantis, Malaysia), DraI (Roche, Germany), and DdeI (Fermentas, UK), respectively. PCR products and restriction fragments were visualized by electrophoresis in 1.5% and 2.5% agarose gels, respectively. The information about sequence of primer pairs, amplification conditions, size of PCR products and digested products with restriction enzymes is listed in Table 1.

Tetra-ARMS PCR (amplification refractory mutation system) method was applied for genotyping of *CYP2E1*2* and *CYP2E1*3* because they don't have a restriction site by which alleles of *CYP2E1* can be distinguished from one another. The fragment of the *CYP2E1* gene that contains either **2* or **3* polymorphism was amplified by the two outer primers and the inner primers which amplified the two allelic states (Ye et al., 2001). Tetra-ARMS PCR products were visualized by electrophoresis in 2.5% agarose gel for *CYP2E1*2* and *CYP2E1*3* regions. The details about PCR condition and primer sequences are listed in Table 2. Several samples were randomly selected for direct sequencing of amplified products to validate the results of genotyping by PCR/RFLP and Tetra-ARMS PCR methods. Direct sequencing also confirmed the genotypes obtained by the two methods (Figures 1 and 2).

Statistical analysis

The statistical analysis by χ^2-test was done to determine if the genotype frequencies of every polymorphism fit the Hardy-Weinberg equilibrium. Also, the estimated genotype frequencies were compared between the two genders and between Arab and non-Arab populations.

Table 1. PCR and RFLP Conditions for the *CYP2E1*5B, CYP2E1*6,* and *CYP2E1*7B*

SNP	Primer sequence	PCR Condition	PCR product (bp)	RFLP product (bp)
*CYP2E1*5B*	F:ACCCCAATGGGTGTCTGTC R:TCATTCTGTCTTCTAACTGGCAAT	95 -5min, 95 -30sec, 52 -45sec, 72 -30sec x 35cycles 72 -5 min	576	282, 294
*CYP2E1*6*	F:AGGCTCGTCAGTTCCTGAAA R:AAGGCAGGAGGATGACTTGA	95 -5min, 95 -30sec, 63 -45sec, 72 -30sec x 35cycles 72 -5 min	685	309,376
*CYP2E1*7B*	F:CTGGAGTTCCCCGTTGTCTA R:GGGTGAAGGACTTGGGAATA	95 -5min, 95 -30sec, 57.6 -45sec, 72 -30sec x 35cycles 72 -5 min	547	301, 246

Table 2. Tetra-ARMS Conditions for the CYP2E1*2 and CYP2E1*3

SNP	Outer primer pair sequence	Inner primer pair sequence	Annealing Temperature
*CYP2E1*2*	F:GTGGCTTAGAGCCCCGCACCTCCTC	F:TTGTAGCCGTGCATCACCACCAGGC	53
	R:AACTGTCCCGGGGCGTGTTATGCAC	R:CACGCTGTACGTGGGCTCGCATCA	
*CYP2E1*3*	F:AGAGGTGGAGGAAATCTGGAAAAGAGCC	F:ACAAAACAGAGTCCAGAGTTGGCACGAC	62
	R:CCACCTACAACAACACTTCATTCCCAGG	R:AGTCTTTGTTTCTCCTAGGGCACAGGCA	

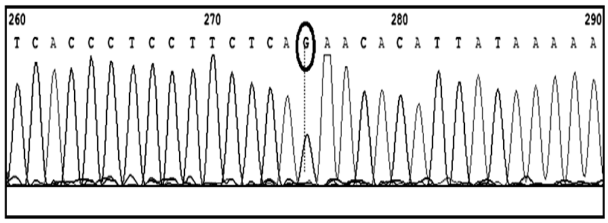

Figure 1. Chromatogram of Corresponding Sequence for *CYP2E1*7B* Polymorphism Visualization with Chromas Program. *CYP2E1*7B* is represented by a -71G ->T substitution in the promoter region of the gene. Homozygote individuals with dominant alleles (GG genotype) present only one peak

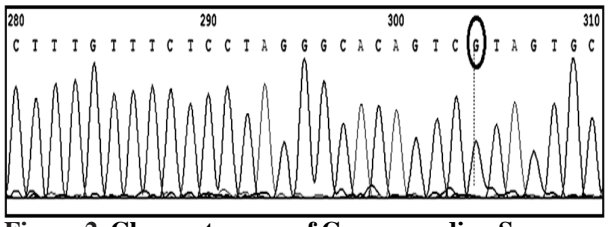

Figure 2. Chromatogram of Corresponding Sequence for *CYP2E1*3* Polymorphism Visualization with Chromas Program. *CYP2E1*3* is a G10059A base substitution in exon 8 of the gene. Homozygote individuals with dominant alleles (GG genotype) present only one peak

Finally, the results of this study were compared with other populations by the same test. Differences with P-values<0.05 were considered statistically significant.

Results

*CYP2E1*5B* (rs2031920), *CYP2E1*6* (rs6413432), and *CYP2E1*7B* (rs6413420) polymorphisms were examined by digestion with PstI, DraI, and DdeI enzymes in 200 subjects from the general population in Ahvaz city. The genotype frequencies of *CYP2E1*5B* polymorphism were found to be 97% for G/G (**1A/*1A*) and 3% for G/C (**1A/*5B*). Allele frequencies of *CYP2E1*5B* were 98.5% for G and 1.5% for C. In the case of *CYP2E1*6* polymorphism, the genotype frequencies were determined as 69% for T/T (**1A/*1A*), 30% for T/A (**1A/*6*), and 1% for A/A (**6/*6*). The allele frequencies of this polymorphism were 84% T and 16% A. Investigation of *CYP2E1*7B* polymorphism yielded the genotype frequencies as 50% for G/G (**1A/*1A*), 43% for G/T (**1A/*7B*), and 7% for T/T (**7B/*7B*). The allele frequencies of *CYP2E1*7B* were 71.5% G and 28.5% T. The genotype frequency of *CYP2E1*2* polymorphism was found as 100% for G/G (**1A/*1A*), but no individual with G/A or A/A genotype was identified. The genotype frequencies of *CYP2E1*3* were determined as 94.5% for G/G (**1A/*1A*) and 5.5% for G/A (**1A/*3*), while A/A genotype wasn't detected in the population. The allele frequencies of *CYP2E1*3* were 97.25% and 2.75% for G and A, respectively.

Table 3. Genotype and allele frequencies of *CYP2E1*5B,*6, *7B, *2*, and **3* in the Iranian population

SNP	Frequency of genotypes and alleles N	%	P-value for Hardy-Weinberg equilibrium
*CYP2E1*5* (rs2031920)			
**1A/*1A* (G/G)	194	97	
**1A/*5B* (G/C)	6	3	0.985
**5B/*5B* (C/C)	0	0	
**1A* (G)	394	98.5	
**5B* (C)	6	1.5	
*CYP2E1*6* (rs6413432)			
**1A/*1A* (T/T)	138	69	0.101
**1A/*6* (T/A)	60	30	
**6/*6* (A/A)	2	1	
**1A* (T)	336	84	
**6* (A)	64	16	
*CYP2E1*7B* (rs6413420)			
**1A/*1A* (G/G)	100	50	0.436
**1A/*7B* (G/T)	86	43	
**7B/*7B* (T/T)	14	7	
**1A* (G)	286	71.5	
**7B* (T)	114	28.5	
*CYP2E1*2* (rs72559710)		–	
**1A/*1A* (G/G)	200	100	
**1A/*2* (G/A)	0	0	
**2/*2* (A/A)	0	0	
**1A* (G)	400	100	
**2* (A)	0	0	
*CYP2E1*3* (rs55897648)			
**1A/*1A* (G/G)	189	94.5	0.96
**1A/*3* (G/A)	11	5.5	
**3/*3* (A/A)	0	0	
**1A* (G)	389	97.25	
**3* (A)	11	2.75	

*N=population size, **P-values express whether Iranian population is similar to respective populations

The genotype distributions for **5B, *6, *7B*, and **3* polymorphisms fitted the Hardy-Weinberg equilibrium and P-value>0.05 was calculated for four polymorphisms (Table 3). For every SNP, there were no significant differences in genotype frequencies between the two genders and between Arabs and non-Arabs populations. The estimated P-values by χ^2-test were >0.05 for all studied polymorphisms (P-values of gender and ethnic were determined as 0.41 and 0.97 for **5B*, 0.36 and 0.11 for **6*, 0.25 and 0.43 for **7B*, and 0.12 and 0.41 for **3*, respectively). χ^2-test wasn't performed for genotype analyzing of *CYP2E1*2* in population because all subjects had the G/G genotype.

Discussion

Iran is a country which has a population with different ethnic identities and different languages. *CYP2E1* polymorphisms shows inter-ethnic and inter-

Table 4. Comparison between the Genotype and Allele Distributions of *CYP2E1*5B* in Iranian Population and Other Populations

Population	N*	*CYP2E1*6* genotype frequency (%) *1A/*1A (GG)	*1A/*7B (GC)	*7B/*7B (CC)	P-value**
Asian					
Iranian (present study)	200	97.0	3.0	0.0	
Chinese (Persson et al., 1999)	122	51.6	43.5	4.9	<0.0001
Taiwanese (Wang et al., 1999)	231	58.0	35.1	6.9	<0.0001
Thai (Kongruttanachok et al., 2001)	297	63.6	34.7	1.7	<0.0001
Japanese (Sugimura et al., 2006)	241	68.0	29.0	3.0	<0.0001
Indian (Ruwali et al., 2009)	350	98.0	2.0	0.0	0.31
European					
Spanish (Gonzalez et al., 1998)	200	89.5	10.5	0.0	<0.0001
French (Bouchardy et al., 2000)	172	91.6	4.7	0.0	0.29
British (Yang et al., 2001)	155	96.8	3.2	0.0	0.88
German (Neuhaus et al., 2004)	297	94.9	4.4	0.7	0.11
Poland (Gajecka et al., 2005)	316	94.4	5.6	0.0	0.08
Italian (Boccia et al., 2008)	245	93.4	6.2	0.4	0.03
American					
Mexican (Wu et al., 1997)	92	70.6	28.3	1.1	<0.0001
Brazilian (Marques et al., 2006)	212	88.2	11.8	0.0	<0.0001
American (Liu et al., 2001)	399	96.2	3.8	0.0	0.6
Chilean (Quinones et al., 2001)	148	71.0	27.0	2.0	<0.0001
Mexican Mestizos (Gordillo-Bastidas et al., 2010)	239	72.0	23.8	4.2	<0.0001
Mexican Huichols (Gordillo-Bastidas et al., 2010)	99	21.2	54.5	24.2	<0.0001
Turkish (Ulusoy et al., 2007)	206	96.1	3.9	0.0	0.54

*N=population size, **P-values express whether Iranian population is similar to respective populations

Table 5. Comparison between the Genotype and Allele Distributions of *CYP2E1*6* in Iranian Population and Other Populations

Population	N*	*CYP2E1*6* genotype frequency (%) *1A/*1A (TT)	*1A/*7B (TA)	*7B/*7B (AA)	P-value**
Asian					
Iranian (present study)	200	69.0	30.0	1.0	
Taiwanese (Hildesheim et al., 1997)	320	57.2	38.4	4.4	0.0013
Tamilians (Soya et al., 2005)	123	71.5	25.2	3.3	0.26
Japanese (Sugimura et al., 2006)	241	52.3	40.2	7.5	<0.0001
Kazakh (Wang et al., 2009)	107	72.0	27.1	0.9	0.2
Uygur (Wang et al., 2009)	149	66.4	29.6	4.0	0.14
Chinese Han (Wang et al., 2009)	103	55.3	36.9	7.8	<0.0001
Indian (Ruwali et al., 2009)	350	72.2	26.9	0.9	0.16
European					
French (Bouchardy et al., 2000)	172	87.8	11.6	0.6	<0.0001
Caucasians (Garte et al., 2001)	1360	85.4	13.8	0.8	<0.0001
British (Yang et al., 2001)	155	83.2	16.1	0.7	<0.0001
German (Neuhaus et al., 2004)	236	83.1	16.5	0.4	<0.0001
Italian (Boccia et al., 2008)	245	91.8	7.8	0.4	<0.0001
American					
Chilean (Quinones et al., 2001)	129	63.6	31.0	5.4	<0.0001
Mexican (Konishi et al., 2003)	104	72.1	24.0	3.9	0.26
Brazilian (Rossini et al., 2006)	251	86.9	12.7	0.4	<0.0001
Turkish (Omer et al., 2001)	153	84.3	15.0	0.7	<0.0001
Turkish (Ulusoy et al., 2007)	206	84.0	15.5	0.5	<0.0001
Turkish (Kayaalti et al., 2010)	163	85.3	14.1	0.6	<0.0001

*N=population size, **P-values express whether Iranian population is similar to respective populations

racial differences, significant contribution to individual differences in susceptibility to cancer development. Estimation probabilities of cancer development both for individuals and ethnic groups can be performed by using SNPs as genetic markers (Danko and Chaschin,

Table 6. Comparison between the Genotype and Allele Distributions of *CYP2E1*7B* in Iranian Population and Other Populations

Population	N*	*CYP2E1*7B* genotype frequency (%) *1A/*1A (GG)	*1A/*7B (GT)	*7B/*7B (TT)	P-value**
Asian					
Iranian (present study)	200	50.0	43.0	7.0	
European					
North European (Fairbrother et al., 1998)	115	89.6	10.4	0.0	<0.0001
British (Yang et al., 2001)	155	90.3	9.0	0.7	<0.0001
German (Thier et al., 2002)	56	85.7	14.3	0.0	<0.0001
German (Neuhaus et al., 2004)	299	92.6	7.4	0.0	<0.0001
Swedish (Ernstgard et al., 2004)	37	91.9	8.1	0.0	<0.0001
Turkish (Ulusoy et al., 2007)	206	86.9	12.6	0.5	<0.0001
Turkish (Kayaalti et al., 2010)	163	86.5	13.5	0.0	<0.0001

*N=population size, **P-values express whether Iranian population is similar to respective populations

2005). Therefore, the identification of polymorphisms in different populations such as Iranians could be useful for assessing genetic cancer susceptibility. The present results are the first to evaluate genotype and allele frequencies of *CYP2E1* polymorphisms. The next step after the initial data is to study the association of these polymorphisms with cancer. If such a conclusion is true, particularly among industrial workers, this is recommended because it is possible that the pattern of polymorphism is different. Therefore, whether a polymorphism is a crucial or has no significant differences, a specific test will be performed specifically among the high risk groups.

CYP2E1 polymorphisms could also affect susceptibility to adverse drug reactions (ADRs) (Costa et al., 2012). Accordingly, this study provides valuable information not only for further investigation of association between *CYP2E1* polymorphisms and susceptibility to several types of cancer but also for the study of adverse drug reactions.

Since SNPs of autosomal chromosomes can be changed by effects of many factors in the evolutionary history, the investigation of single nucleotide polymorphisms provide valuable data regarding relations between populations (Kayaalti and Soylemezoglu, 2010). Ahvaz city lies in the southwest of Iran between Iraqi border and the Zagros Mountains. Centuries ago, Arabs migrated from neighboring countries to Iran. In the current study, the genotype distributions were compared between Arabs and non-Arabs. *CYP2E1* polymorphisms had similar allele frequencies for two ethnic subgroups of the study population. Therefore, two subgroups might have the same cancer susceptibility.

CYP2E1 genotype distribution was also compared between Iranian and other populations. The genotype distribution of *CYP2E1*5B* was similar to Europeans such as German (P-value=0.11) (Neuhaus et al., 2004), British (P-value=0.88) (Yang et al., 2001), French (P-value=0.29) (Bouchardy et al., 2000), and Poland Caucasians (P-value=0.08) (Gajecka et al., 2005), but it was different from Italian (P-value=0.03) (Boccia et al., 2008). There were no significant differences between

this study and studies on American (P-value=0.6) (Liu et al., 2001) and Turkish (P-value=0.54) (Ulusoy et al., 2007) populations. But, the results of this population differed significantly from those of Chilean (Quinones et al., 2001), Mexican Mestizos, Mexican Huichols (Gordillo-Bastidas et al. 2010), and Brazilian (Marques et al., 2006((P-value<0.0001). The genotype frequencies of *CYP2E1*5B* showed significant differences between Iranian and other Asians, including Japanese (Sugimura et al., 2006), Thai (Sangrajrang et al., 2006), and Northeastern Chinese (Guo et al., 2012) (P-value<0.0001). On the other hand, the results were similar to Indian (P-value=0.31) (Ruwali et al., 2009) (Table 4).

There was a significant correlation between c2/c2 genotype and head and neck cancer in Asians, but not in Caucasians (Tang et al., 2010). The other study found that c2/c2 homozygote genotype was associated with the increased risk of colorectal cancer in Caucasians (Zhou et al., 2010). Therefore, we expect the same association study results in Iranian population because *CYP2E1*5B* genotype distribution was similar to Caucasians.

When the genotype distributions of *CYP2E1*6* were compared between Iranian population and other Asians, no significant difference was found between this study and studies on Tamilians (P-value=0.26) (Soya et al., 2005), Thai (P-value=0.12) (Sangrajrang et al., 2006), Kazakh (P-value=0.2), Uygur (P-value=0.14) (Wang et al., 2009), Indian (P-value=0.16) (Ruwali et al., 2009), and Northeastern Chinese (P-value=0.06) (Guo et al., 2012). In contrast, *CYP2E1*6* genotype frequencies were significantly different from Chinese Han (P-value<0.0001) (Wang et al., 2009), and Japanese (P-value<0.0001) (Sugimura et al., 2006) populations. χ^2-test showed that the genotype distribution of *CYP2E1*6* differed from American countries, including Chilean and Brazilian (P-value<0.0001) (Quinones et al., 2001; Rossini et al., 2006). But the results were similar to Mexicans even though two populations are ethnically different (P-value=0.26) (Konishi et al., 2003). In comparison with Europeans such as Caucasians (Garte et al., 2001), British (Yang et al., 2001), French (Bouchardy et al., 2000), German (Neuhaus et al., 2004), and Italian (Boccia et al., 2008), the genotype frequencies of *CYP2E1*6* were significantly different (P-value<0.0001). There was also a significant difference between Iranian and Turkish populations (P-value<0.0001) (Ulusoy et al., 2007; Kayaalti and Soylemezoglu, 2010) (Table 5).

A meta-analysis showed a protective effect of DraI C/D and C/C genotypes for lung cancer in the Asian population (Wang et al., 2010). On the other hand, homozygote genotype of DraI polymorphism was associated with head and neck cancer in Asians (Tang et al., 2010). Thus, this polymorphism may have an impact on cancer susceptibility. Accordingly, the risk of cancer development in Iranian population might be similar to other Asian countries.

The genotype frequencies of *CYP2E1*7B* were compared between this study and studies done on other populations. The results of Chi-square test showed that genotype distribution of *CYP2E1*7B* was significantly different from European populations, including German (Neuhaus et al., 2004), British (Yang et al., 2001), and Swedish (Ernstgard et al., 2004) (P-value<0.0001). Also, the results were significantly different from those of Turkish population (P-value<0.0001) (Ulusoy et al., 2007 ; Kayaalti and Soylemezoglu, 2010) (Table 6).

In the case of *CYP2E1*7B*, the data was limited. Accordingly, the results were only compared with Europeans and the comparison showed significant differences.

Statistical analysis by the same test didn't show any association between *CYP2E1*5B*, *CYP2E1*6* and, *CYP2E1*7B* polymorphisms.

For *CYP2E1*2* polymorphism, all subjects carried the common homozygote genotype (GG) and the GA or AA genotypes were not detected. *CYP2E1*2* and *CYP2E1*3* polymorphisms occur at very low frequencies in both Caucasians and Orientals (Ingelman-Sundberg, 2001). Thus, Iranian population is expected to have the same susceptibility to cancer in relation to xenobiotic effects. Similarly, Iranians exhibit low frequency of the *CYP2E1*2* (exon 2) and **3* (exon 8) alleles. This results emphasizes that the coding sequence of *CYP2E1* gene is highly conserved and *CYP2E1* isoenzyme is an important physiologically (Hu et al., 1997).

In conclusion, ethnic and geographic differences may explain discrepancies in the prevalence of *CYP2E1* polymorphisms. Genotype distribution studies could provide valuable information to help further investigations of association between polymorphisms and several types of cancer and other diseases. Studies in larger groups are recommended to confirm our results. A larger data base may allow for a more precise estimate of the population frequency for these polymorphisms among normal samples.

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