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Microsomal epoxide hydrolase polymorphisms

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Abstract. Microsomal epoxide hydrolase plays a dual role in the activation and detoxification of carcinogenic compounds. Two polymorphic sites have been described in exons 3 and 4 of the microsomal epoxide hydrolase gene that change tyrosine residue 113 to histidine (Tyr113His) and histidine 139 to arginine (His139Arg), respectively. The exon 3 polymorphism reduces enzyme activity by approximately 50%, whereas the exon 4 polymorphism causes a 25% increase in activity. In the present study, the distribution of these polymorphisms in a Turkish population including 625 unrelated healthy individuals was examined using a PCR-RFLP method. The observed genotype frequencies of microsomal epoxide hydrolase exon 3 were 54, 38 and 8% for Tyr113Tyr, Tyr113His and His113His, respectively. Exon 4 genotype frequencies were found to be 69, 29 and 2% for His139His, His139Arg and Arg139Arg, respectively.

Introduction

Epoxides are chemically reactive compounds and are unstable in an aqueous environment. They arise from the oxidative metabolism of xenobiotics and also from endogenous compounds via the cytochrome P450 monooxygenase system. Epoxide intermediates are known to be mutagenic and carcinogenic (1). The epoxide hydrolases catalyse the hydration of reactive epoxides to their corresponding dihydrodiol species. In general, this mechanism makes the intermediates more stable and less reactive (2). It has been reported that the mammalian microsomal epoxide hydrolase (mEH) plays a key role in xenobiotic metabolism. The main function of mEH is the breakdown of genotoxic and carcinogenic epoxides to less harmful metabolites and the protection of macromolecules from the electrophilic attack of reactive intermediates (3). However, in the case of some polycyclic aromatic hydrocarbons, such as benzo(a)pyrene, present in tobacco smoke, dihydrodiol formation stabilizes bay-region epoxides, increasing the mutagenic and carcinogenic potential of the product (4,5). Therefore, mEH plays a dual role in the detoxification and the bioactivation of some procarcinogens. mEH is expressed in all tissues, with the highest levels of mEH activity being detected in the liver, kidney and testis (6,7). The human mEH enzyme is encoded by a single gene that has been localized to 1p11-qter (8). The gene and cDNA for mEH has been cloned and sequenced in humans (8,9). The gene consists of eight non-coding exons and one coding exon, and encodes for a 455 amino acid protein (9). In the coding region of the mEH gene, two polymorphisms have been identified. In exon 3 a T→C mutation changes tyrosine residue 113 to histidine (Tyr113His). This substitution decreases enzyme activity by approximately 50% (slow activity genotype). The exon 4 polymorphism, a G→A transition, causes a histidine to arginine change at codon 139 resulting in a 25% increase in enzyme activity (10). mEH polymorphisms may lead to differences in the metabolism of carcinogens, resulting in altered susceptibility to some types of cancer (11-13). Two meta-analyses indicated that a low activity genotype (exon 3 His113His) is associated with a decreased risk of lung cancer (14,15). Population information on polymorphisms is essential for the study of genetic diseases. The aim of this study was to determine the frequencies of the two polymorphisms of the mEH gene in a Turkish population, including 625 healthy individuals. The results will be a basis for molecular epidemiological studies investigating the association between polymorphisms in biotransformation enzymes and chemically-induced cancer risk.

Materials and methods

Subjects. The study population included 625 unrelated healthy Turkish volunteers from central Anatolia with no previous cancer or chronic disease. All participants gave their informed consent and completed a short questionnaire to determine their occupation, tobacco use, alcohol consumption and family history of cancer. The local university ethics committee on human research approved the study.

Genotype determination. Peripheral blood samples (2 ml) were collected into citrate containing tubes from all subjects. DNA was extracted from whole blood using a salting out procedure as soon as the samples reached the laboratory (16). The isolated DNA was stored at -20˚C until use. Genetic polymorphisms in mEH exons 3 and 4 were determined using a PCR-RFLP.

Determination of the exon 3 polymorphism. The following primers were used to amplify a 163-bp fragment of exon 3: 5' GATCGATAAGTTCCGTTTCACC 3' and 5' ATCTTA GTCTTGAAGTGAGGAT 3'. PCR was performed in a 50-μl

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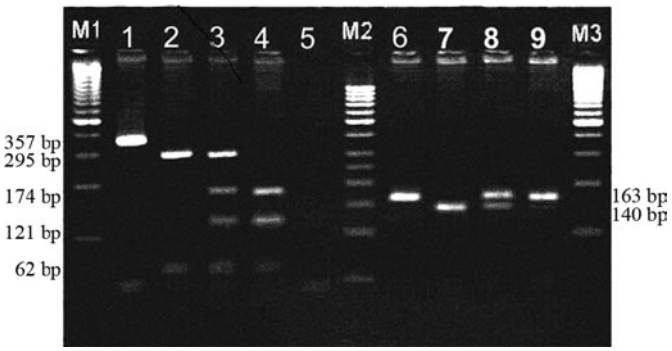

Figure 1. PCR-RFLP patterns of polymorphisms of mEH exons 3 and 4. M1: gene ruler 100-bp DNA ladder; 1, exon 4 PCR product; 2, His139His; 3, His139Arg; 4, Arg139Arg; 5, negative control. M2: gene ruler 50-bp DNA ladder; 6, exon 3 PCR product; 7, Tyr113Tyr; 8, Tyr113His; 9, His113His.

Table I. Characteristics of the study population.

Sample size	625
Gender (%)	
Male	280 (45%)
Female	345 (55%)
Age (years)	
Range	21-81
Mean ± SD	51.2±14.6
Male	59.4±8.73
Female	44.5±15.0

reaction mixture containing 75 pmol of the primers, 200 ng DNA, 0.2 mM dNTPs, 2 mM $MgCl_2$ and 2.5 units *Taq* DNA polymerase. After an initial denaturation at 94˚C for 5 min, the DNA was amplified by 35 cycles of 30 sec at 94˚C, 20 sec at 56˚C and 20 sec at 72˚C, followed by a final extension of 7 min at 72˚C. The amplified products were digested overnight with *Eco*RV restriction enzyme at 37˚C. *Eco*RV digestion produced 140- and 23-bp fragments of the wild-type genotype, while the polymorphic homozygous type remained undigested (Fig. 1).

Determination of the exon 4 polymorphism. A 357-bp DNA fragment in exon 4 was amplified using the primers 5' GGGGTACCAGAGCCTGACCGT 3' and 5' AACACCGGG CCCACCCTTGGC 3'. The incubation mixture was the same as that used for the amplification of the exon 3 target sequence. The cycling protocol included initial denaturation at 94˚C for 5 min and 35 cycles of 94˚C for 30 sec, 63˚C for 30 sec and 72˚C for 45 sec, followed by a final extension of 7 min at 72˚C. Digestion of the amplified product overnight with *Rsa*I restriction endonuclease at 37˚C produced 295- and 62-bp fragments of the wild-type genotype, and 174-, 121- and 62-bp fragments of the homozygous polymorphic genotype (Fig. 1).

Results

In the present study, 625 (280 men and 345 women) Turkish individuals were genotyped for polymorphisms of exons 3 and 4 in the mEH gene. The principal characteristics of the study population are listed in Table I. The mean age of the study population was 51.2±14.6 years (males, 59.4±8.73; females, 44.5±15.0). The observed genotype frequencies of mEH are summarized in Tables II and III. In the *Eco*RV-RFLP analysis of exon 3, 54% Tyr113Tyr genotype, 38% Tyr113His genotype and 8% His113His genotype were determined (Table II). According to the *Rsa*I-RFLP analysis of exon 4, genotype frequencies were 69, 29 and 2% for His139His, His139Arg and Arg139Arg, respectively (Table III). The genotype distributions were in Hardy-Weinberg equilibrium for Tyr113His (p=0.22) and His139Arg (p=0.054) (Tables II and III). Tables IV and V respectively compare the genotype frequencies of exons 3 and 4 in previously reported populations with those of the present study.

Table II. Genotype frequencies for mEH exon 3 polymorphism.

Genotype	No.	Percentage	p-value[a]
Total	625		
Tyr113Tyr	338	54	
Tyr113His	235	38	
His113His	52	8	0.22

[a]For Hardy-Weinberg equilibrium; χ^2=1.49.

Table III. Genotype frequencies for mEH exon 4 polymorphism.

Genotype	No.	Percentage	p-value[a]
Total	601		
His139His	418	69	
His139Arg	174	29	
Arg139Arg	9	2	0.054

[a]For Hardy-Weinberg equilibrium; χ^2=3.7.

Discussion

The aim of this study was to investigate the frequencies of polymorphisms of exons 3 and 4 in the mEH gene, which display ethnic differences, in a Turkish population. The results were compared to those from previously published results from populations of other countries. As seen in Table IV, our sample size was much larger than those of other studies, which is very important for the making of more precise estimations in epidemiological studies. There is wide interethnic variation of the mEH exon 3 polymorphism (His113His), ranging from 6% in Germans (17) to as high as 42% in South Koreans (18). The data presented here show that the Turkish population has the same frequencies as Spanish (19), Swedish (20) and Dutch (21) populations and similar frequencies as Slovak, Canadian, Austrian, British (22-25) and German populations (17). We detected considerably lower frequencies than those found in

Table IV. Distribution of mEH exon 3 genotype frequencies in different populations.

Country	No.	Exon 3 (%)			Ref.
		Wild-type Tyr113Tyr	Heterozygous Tyr113His	Homozygous His113His	
Canada	45	47	44	9	23
Spain	203	46	46	8	19
Austria	496	45	44	11	24
United Kingdom	257	50	39	11	25
Japan	114	26	45	29	26
South Korea	76	26	32	42	18
Finland	115	45	41	14	31
France	105	47	37	16	30
Germany	119	52	44	4	17
Italy	99	48	37	15	29
Sweden	283	53	39	8	20
Czech Republic	447	42	41	17	28
Netherland	391	50	42	8	21
China	266	44	38	18	27
Slovakia	160	49	42	9	22
Turkey	133	50	42	8	32
Turkey	625	54	38	8	This study

Table V. Distribution of mEH exon 4 genotype frequencies in different populations.

Country	No.	Exon 4 (%)			Ref.
		Wild-type His139His	Heterozygous His139Arg	Homozygous Arg139Arg	
Canada	45	76	18	6	23
Spain	203	66	31	3	19
Austria	496	68	29	3	24
United Kingdom	257	59	36	5	25
Japan	114	67	28	5	26
South Korea	76	75	22	3	18
Finland	115	71	29	0	31
France	105	59	39	2	30
Germany	119	66	32	2	17
Italy	99	61	35	4	29
Sweden	279	57	37	6	20
Czech Republic	453	64	31	5	28
Netherland	414	65	31	4	21
China	266	77	18	5	27
Slovakia	160	57	39	4	22
Turkey	133	69	29	2	32
Turkey	601	69	29	2	This study

Japanese, Chinese, Czech, Italian, French, Finnish (26-31) and South Korean (18) populations. The frequencies of the mEH exon 4 polymorphism observed in our population were the same as those in the French and German populations and similar to the other populations, listed in Table V. Our results for both exons 3 and 4 genotype frequencies in good agreement with the results of another study carried out in a Turkish population (32).

Organisms are exposed to epoxide containing compounds from both endogenous and exogenous sources. In mammals, the hydration of these compounds by various epoxide hydrolases not only regulates their genotoxicity, but also, for lipid derived epoxides, their endogenous roles as chemical mediators. Previous findings suggested that epoxide hydrolases as a family represent novel drug discovery targets for the regulation of blood pressure, inflammation, cancer progression and several other diseases (33). In recent years, sEH has attracted attention due to its role in the turnover of lipid derived epoxides, which are signaling lipids with functions in regulatory processes such as the control of blood pressure, inflammatory processes and cell proliferation. Furthermore, sEH is thought to be a promising target for pharmacological inhibition to treat hypertension and possibly other diseases (3).

mEH classically plays a major role in xenobiotic metabolism and has been described as a biotransformation enzyme. However, mEH also accepts fatty acid-derived epoxides, such as epoxystearic (34) and epoxyeicosatrienoic acids, which are mainly metabolized by sEH (35). Therefore, a role of mEH in signaling cascades cannot be excluded, particularly in the case of high mEH expression in certain organs or cell types (3). In light of this knowledge, mEH and sEH may complement each other in the breakdown of epoxide derivatives from both exogenous and endogenous sources. This may be of particular importance in individuals with the polymorphic variants of one of these enzymes. Several polymorphisms in human sEH have been identified, including variants with higher (Lys55Arg) and lower (Arg287Gln) epoxide hydrolase activity (36,37). In recent years, case-control studies examining the sEH polymorphisms in different populations and also the association of these polymorphisms with cardiovascular diseases have been published (38-42). However, these studies need to be supported by others carried out in different populations; more research is required to confirm this association and to better understand the mechanisms behind it. On the other hand, we believe that, when evaluating the association of sEH polymorphisms with diseases, it is important to consider the mEH polymorphisms of the same individual in order to make a more accurate assessment.

This prompted us to genotype our population for sEH polymorphisms, which are currently under investigation in our laboratory. To our knowledge, there are no studies examining polymorphisms of sEH in a Turkish population. The results of the present study, in conjunction with the results regarding sEH polymorphisms in a Turkish population, provide a framework for further studies concerning the role of these enzymes as a susceptibility factor for hypertension, cardiovascular diseases and cancer.

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