

Frequency of Glutathione S-transferase M1 (GSTM1) and GSTT1 Null Genotypes in Fars Population (South of Iran)

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ABSTRACT

The genes glutathione S-transferase M1 (GSTM1) and GSTT1 code for cytosolic enzymes GST μ and GST θ , respectively, which are involved in phase II metabolism. In human, both genes may be deleted. In the present study, the genetic polymorphisms of GSTM1 and GSTT1 were detected by PCR method in 236 healthy individuals from Shiraz population, Fars province, south of Iran. The frequency of GSTM1 and GSTT1-null genotypes were 37.7 and 31.8 percent, respectively. The studied population was then compared with reported frequencies for neighboring populations, as well as, with those for European and African populations.

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INTRODUCTION

Iranian populations are composed of several ethnic groups, which are not uniformly distributed in a large geographical area. Although there are some different ethnic groups (e.g. Turkmans, Arabs, Assyrians, Jews, etc), the majority of the Iranian populations are Caucasian (4). During the last few decades, several regional and ethnic populations in Iran have been studied for some serological traits and a few selected biochemical polymorphisms (2, 4, 6, 9, 10, 20,32, 35, 40, 41). Despite several of these genetic investigations, for several relatively newly- described genetic polymorphisms, knowledge of the Iranian populations is completely absent.

Glutathione S-transferases (GSTs), a multigene family of phase II metabolic enzymes, are active in the detoxification of a wide variety of potentially toxic and carcinogenic electrophiles by conjugating them to glutathione (37). In addition to their catalytic activity, GSTs are thought to engage in the intracellular transport of endogenous metabolites and steroid hormones (8, 25, 37).

The human cytosolic GSTs comprise four main classes, based on sequence homology and substrate specificity: α (GSTA), μ (GSTM), π (GSTP) and θ (GSTT) (37). Different GST isoenzymes have distinct but also overlapping substrate specificities. To date, polymorphisms are known for 3 families: GSTP, GSTM and GSTT (37). For both GSTM1 and GSTT1, the variant allele is a deletion of the gene (14, 37). About 50% of the Caucasian population is homozygous for a deletion of the GSTM1 gene, they possess the null genotype (23). The prevalence of GSTT1 null individuals is 10% to 20% among Caucasians (30). Carriers of the homozygous deletions in the GSTM1 and GSTT1 genes have an absence of GST μ and GST θ activity, respectively (14,37). In the present study the frequencies of null-genotypes of GSTM1 and GSTT1 were

The primers for amplifying the GSTT1 gene segment corresponding to exon 4, intron 4, and exon 5 were 5' TTC CTT ACT GGT CCT CAC ATC TC 3' and 5' TCA CCG GAT CAT GGC CAG CA 3' (37). The PCR was performed in 50 μ l reaction buffer containing 200 μ M dNTPs, 1.5 mM MgCl₂, 10 pmol of each primer, about 1 μ g DNA and 2 units of thermostable *Taq* DNA polymerase using a programmable thermocycler. After 5 min of pretreatment at 94°C, 35 cycles of 1 min denaturation at 94°C, 1.15 min annealing at 60°C, and 1 min extension at 72°C were performed.

For evaluating the GSTM1 and GSTT1 polymorphism the amplification products were analyzed by gel electrophoresis (1.6% agarose). To test for contamination, negative controls (tubes containing the PCR mixture without the DNA template) were included in every run. A 1030 bp fragment was amplified by PCR with the GSTM1 primers and a 480 bp fragment was amplified by PCR with the GSTT1 primers. The absence of amplified product was consistent with the null genotypes (14,37,42). Successful amplification by β -globin specific primers confirmed the proper function of the PCR reaction.

This technique does not distinguish between heterozygote and homozygote of the positive genotypes, but it conclusively identifies the null genotypes. To ensure laboratory quality control, two independent readers interpreted the gel photographs. Any sample with ambiguous results (generally due to low PCR yield) was re-tested, and a random selection of 15% of all samples was repeated. No discrepancies were discovered upon replicate testing.

RESULT AND DISCUSSION

The numbers of the phenotypes and the respective frequencies of GSTM1 and GSTT1 are shown in Table 1. Because the genes

GSTT1 located on human chromosome 21q22, and GSTM1 on chromosome 2q37, the lack of the GST μ and GST θ are autosomal recessive phenotypes. There are

no statistically difference for the frequency of GSTM1 ($X^2=0.24$, $df=1$, $P>0.05$) and GSTT1 ($X^2=0.02$, $df=1$, $P>0.05$) between males and females. In the studied population the frequencies of the GSTM1-and GSTT1-null genotypes are 37.7 and 31.8 percent, respectively.

Table 2 shows null-genotype frequencies for the GSTM1 and GSTT1 in various populations of Asia, Africa, and Europe. The frequency of the GSTM1 null genotype, in African populations ranges from 24 to 44 percent (1, 24, 26), in Asian populations from 33 to 63 percent (14, 16, 19, 21-23, 29, 30, 36, 38, 44, 45, 48, 49), and in European population from 38 to 54 percent (3, 5, 7,11-13, 15, 17, 18, 25, 27, 28, 34, 39, 43, 46, 47). The frequency of GSTM1 null genotype in the studied population seems to be similar to that found in the other populations.

Reported frequencies from Asian populations show that the range of frequency of the GSTT1 null genotype is 16-60 percent (14,16, 19, 22, 29, 30, 33, 36, 38, 44, 45, 49). In some of these populations it has been suggested that the frequency of GSTT1 null-genotype is similar to that of GSTM1 null-genotype (14, 16, 19, 22, 30, 36, 44, 49). However, in African and white populations, the frequencies of GSTT1 null genotype (1, 3, 5, 7, 11-13, 15, 17, 18, 24-28, 34, 39, 43, 46, 47).

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MATERIALS AND METHODS

A total of 236 healthy individuals were studied (mean age:40.3 \pm 15.9; range:13 to 75). The studied group was unrelated Iranian Muslims.

Blood samples were obtained from patient and control groups. Immediately after collection, whole blood was stored at -20°C until use. Genomic DNA for PCR was isolated from whole blood using the thawed blood samples by standard procedure (31).

The PCR method for determining GSTM1 and GSTT1 genotypes was the same as that reported previously (14,37). The primers for amplifying the GSTM1 gene segment corresponding to exon 5, intron 5, and exon 6 were 5' AGA CAG AAG AGG AGA AGA TTC 3' and 5' TCC AAG TAC TTT GGC TTC AGT 3'(14). The PCR was performed in 50 μ l reaction buffer containing 200 μ M dNTPs, 1.5mM MgCl₂, 1 μ M primers, about 1 μ g DNA and 2 units of thermostable *Taq* DNA polymerase using a programmable thermocycler (Progene, Techne, England). After 5 min of pretreatment at 94°C, 35 cycles of 1.5 min denaturation at 94°C, 1.5min annealing at 61°C and 1 min extension at 72°C were performed.

The frequency of GSTT1 null genotype, in African (1, 24) and European (3, 13, 34, 46, 47) populations show about 15-26, and 10-20 percent, respectively. The frequency of GSTT1 null genotype in the Fars province, seems to be the mean of the frequencies reported from European and Far East populations. Taken together, it is suggested that the frequency of GSTT1 null

genotype showed a geographical distribution. It is decreased from East to West and south to north (in Asia and Europe).

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Table 1. Frequencies of GSTM1 and GSTT1 genotypes in Shiraz population

Genotype	Female	Male	Total
GSTM1			
Null	38	51	89
Non-null	58	89	147
Total	96	140	236
GSTT1			
Null	31	44	75
Non-null	65	96	161
Total	96	140	236

Table 2. Distribution of GSTM1- and GSTT1- null genotypes in Africa, Asia, and Europe

Study	GSTM1	GSTT1	Reference
Africa			
Egypt	44	15	1
Ghana	39	-	26
Zimbabwe	24	26	24
Asia			
China	51-58	46-53	16, 44
Hong Kong	49	-	23
India	24-33	8-22	38, 29, 45
Iran	37	31	present study
Japan	44	44	19, 14
Korea	53	42-60	30, 36
Malaysia			21, 22, 49
Chinese	-	58	
Malaya	62	38	
Indians	33	16	
Taiwan	63	-	48
Turkey	-	20	33
Europe			
Austria	49	20	13
Denmark	54	15	5
Estonia	50	-	27
England	54	18	7
Finland	44	-	15
France	51	16	18, 25
Germany	52	13	17
Greece	38	11	11
Netherlands	52	20	34
Norway	47	-	39
Portugal	52	-	28

Russia	42	11	46
Slovak	50	17	43
Spain	52	20	12
Sweden	53	10	3, 47

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