Frequencies of glutathione S-transferase A1 rs3957357 polymorphism in a Turkish population

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Ethics Committee Approval
The study was approved by the Ethics Committee of Mersin University (date: 02/09/2020, protocol no: 2020/615).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract
Background/Aim: Glutathione-S-transferases (GSTs), a major group of phase II enzymes, play a significant role in the detoxification and metabolism of endogenous and exogenous compounds. The objective of the survey was to identify the distribution of genotype and allele frequencies of GSTA1 -69C>T (rs3957357) polymorphism in a healthy Turkish population and compare the determined frequencies with those in various populations.

Methods: Polymerase chain reaction and restriction fragment length polymorphism methods were used to analyze GSTA1 -69C>T polymorphism in DNA samples of 105 healthy Turkish individuals.

Results: The distribution of GSTA1 CC, CT, and TT genotype frequencies were 32.4%, 48.6% and 19.0%, respectively while the allele frequencies were 56.7% for C allele and 43.3% for T allele. The findings obtained were compared with the results of various populations. The frequencies of GSTA1 -69C>T polymorphism were similar to those of the African American population and the populations with White ancestry, but significantly different from those reported for the populations with Asian ancestry.

Conclusion: To the best of our knowledge, this is the first study to present the frequencies of the GSTA1 -69C>T polymorphism among Turkish individuals. The findings of the current study may provide a perspective for further studies exploring the role of GSTA1 -69C>T polymorphism on predisposition to diverse illnesses such as cancer and may be used as a control group for such studies. In addition, this study might contribute to epidemiological and toxicogenetic investigations.

Keywords: Glutathione S-transferases, GSTA1, rs3957357, Polymorphism, Turkish population
Introduction

Biotransformation of xenobiotics and endogenous compounds occurs through phase I and/or phase II enzymes. One of the main groups of phase II enzymes is glutathione S-transferases (GSTs). GSTs play a significant role in the detoxification and metabolism of endogenous and exogenous substances that include a wide range of medications, products of oxidative stress, carcinogens, environmental toxins by catalyzing the conjugation between electrophilic substances and reduced glutathione [1, 2]. Different GST isoenzymes have been identified in humans. Alpha (GSTA), theta (GSTT), pi (GSTP) and mu (GSTM) are the most characterized GST classes [3]. The GSTA class is the most plentiful GST enzyme in the human liver among all hGSTs and accounts for approximately sixty-five to eighty percent of their total liver concentration [4]. Besides, the GSTAs are expressed in the small intestine, adrenal glands, testicles and kidneys [1]. The GSTA isoenzymes conjugate compounds like the nitrogen mustard group of some anticancer drugs, α, β-unsaturated aldehydes and some heterocyclic amines, steroid and thyroid hormones, penicillin, bile acids and bilirubin [2]. GSTAs exhibit high glutathione peroxidase activity and play a significant part in the protection of cells against exogenous and endogenous electrophilic compounds [5].

Inter-individual variations in the activities of GST enzymes might be caused by environmental effects such as exposure to toxins in the environment, lifestyle (use of medication, etc.) and diet, but genetic variations may also play a role [4]. Nearly all the GST family members have genetic polymorphisms that result in reduction of enzyme activity or a complete lack [3].

Several single nucleotide polymorphisms (SNPs) have been detected in the promoter region of the GSTA1 gene. One of SNPs are GSTA1 -69C>T (rs3957357) [6]. There are four functional polymorphisms in GSTA1, which are full linkage disequilibrium, and called GSTA1*A for -52G, -69C, -567T, -631T and GSTA1*B for -52A, -69T, -567G, -631G [7]. The base change C-69T leads to an Earl restriction enzyme site into the GSTA1*B variant [8]. The homozygous mutant genotype of the GSTA1 -69C>T gene polymorphism is reported to have a lower enzymatic activity than the wild-type genotype [9]. GSTA1 -69C>T polymorphism is reportedly related with various disorders such as gestational hypertension, leukemia, bladder cancer [10].

There have been many studies displaying that the distribution of GST polymorphisms differs among distinct regional, national and ethnic populations [11]. However, in the literature search, no studies were found on the GSTA1 -69C>T polymorphism in Turkish population. Thus, the objective of the survey was to identify the distribution of the genotype and allele frequencies of the mentioned polymorphism in a healthy Turkish population, and compare the frequencies found with those of various populations.

Materials and methods

Samples
The DNA samples used for polymorphic analysis were obtained during the previous study approved by Mersin University Ethics Committee (22/10/2015, protocol no: 2015/317), and some of the DNA samples isolated were randomly contained to the present study. The present survey was also approved by the Ethics Committee of Mersin University (02/09/2020, protocol no: 2020/615). This study comprised the DNA samples of unrelated 105 Turkish healthy volunteers (age range: 18-65 years) and was conducted in accordance with the principles of the Good Clinical Practices and the Declaration of Helsinki.

Genotyping
Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods defined by Hezova et al. [9] with slight modifications were used for genotyping analysis of GSTA1 -69C>T polymorphism. A 400-bp fragment was amplified using the following primers: Forward: 5'-GCATCAGCTTGGCCTCTCA-3' and reverse: 5'-AAACGGTGTCAACCGTCTCTG-3'. The PCR reaction mixture contained 10x PCR buffer, 2.0 mM MgCl2, 0.2 mM of each deoxynucleotide triphosphate, 20 pmol of each primer, 1.25 U of Taq DNA polymerase (Fermentas), approximately 300 ng DNA and last volume completed with distilled water to 30-μl. Amplification was for one cycle of 300 min at 94 °C, 30 cycles of 20 sec at 94 °C, 20 sec at 64 °C, 30 sec at 72 °C, and final 7 min extension at 72 °C. Negative control (NC), a DNA-free sample, was included in each PCR experiment and was used to determine whether the reagents used are contaminated with foreign DNA. PCR products (400-bp) were electrophoretically determined on a 2.0 % agarose gel containing ethidium bromide (EtBr, 500 μg/L) making the products visible. The PCR products were digested with FastDigest Earl (Eam1104I) (Thermo Fisher Scientific, USA) restriction enzyme and incubated at 37 °C for 15 min. The digested and undigested products were detected on 2.5% agarose gel visualized by EtBr. The wild-type genotype (CC) had no Eam1104I restriction enzyme site and was therefore 400 bp. On the other hand, the homozygous mutant genotype (TT) had Eam1104I restriction enzyme site and gave bands at 308 bp and 92 bp. The heterozygous genotype (CT) gave bands at 400 bp, 308 bp and 92 bp. Ten percent of the randomly selected samples were reanalyzed for confirmation. PCR-RFLP was conducted on a MiniAmp Plus Thermal Cycler (Thermo Fisher, USA).

Statistical analysis
Statistical data were analyzed using IBM SPSS 25.0 computer software for Windows. The frequencies of GSTA1 -69C>T polymorphism were obtained by counting, and chi-square ($X^2$) test was used for assessment of Hardy–Weinberg equilibrium. The data obtained were compared with previously reported data of various populations. Distinctions in the allele and genotype frequencies between populations were tested by $X^2$ test. $P<0.05$ and $P<0.001$ were considered statistically significant.

Results

GSTA1 C-69T SNP was detected using PCR-RFLP technique in the DNA samples of unrelated 105 Turkish healthy individuals. Of the 105 individuals, 50 (48%) were male, and the remaining 55 (52%) were female. The distributions of the genotype frequencies obtained were consistent with Hardy-Weinberg equilibrium. The data obtained were compared with previously reported data of various populations. Distinctions in the allele and genotype frequencies between populations were tested by $X^2$ test. $P<0.05$ and $P<0.001$ were considered statistically significant.
Weinberg equilibrium ($X^2=0.013, P>0.05$). The frequencies were 32.4% for the wild-type genotype (CC), 48.6% for the heterozygous genotype (CT), and 19.0% for the homozygous mutant genotype (TT). Thus, the frequencies of C and T alleles were 56.7% and 43.3%, respectively (Table 1).

Figure 1 shows agarose gel images of PCR and RFLP patterns of electrophoretically detected GSTA1 -69C>T polymorphism.

Table 1: The frequencies of GSTA1 -69C>T gene polymorphism in a healthy Turkish population

<table>
<thead>
<tr>
<th>Allele frequencies, %</th>
<th>Genotype frequencies, %</th>
<th>n (expected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>56.7</td>
<td>105</td>
</tr>
<tr>
<td>T</td>
<td>43.2</td>
<td>100</td>
</tr>
</tbody>
</table>

![Figure 1: Electrophoresis examples of GSTA1 -69C>T polymorphism identified using polymerase chain reaction (PCR) (A) and restriction fragment length polymorphism (RFLP) (B).](image)

Discussion

The GSTs superfamily, one of the enzymatic families in phase II metabolism is highly polymorphic, and the polymorphic variations in the GSTs enzymes can influence their activities, therefore may lead to individual predisposition to a variety of diseases like cancer [2]. Due to genetic variants in GSTs, ethnic and inter-individual distinctions in the detoxification capacity of GSTs have been identified in diverse populations [12]. In this study, the frequencies of GSTA1 -69C>T polymorphism were examined in a Turkish population. The distributions of frequencies of GSTA1 CC, CT, and TT genotype were 32.4%, 48.6% and 19.0%, respectively, while the frequencies of C and T alleles were 56.7% and 43.3%, respectively.

The frequencies of genotype and allele of GSTA1 -69C>T polymorphism have been reported in diverse populations. The findings obtained for the Turkish population were compared with the results reported for various populations [4, 6, 8, 9, 12-25] as depicted in Table 2. Accordingly, GSTA1 -69T variant allele was more frequent in South Tunisians and Germans compared to other populations. In Asian ancestry, the frequencies of GSTA1 -69T variant allele ranged from 10.5% to 16.0%. The allele and genotype frequencies of the Turkish population showed significant distinction when compared to those of Asian ancestry, including Chinese, Chinese Han, Asian (Northeast Thailand), Taiwanese, Japanese ($P<0.001$). In White ancestry, the frequencies of GSTA1 -69T variant allele ranged from 35.1% to 48.4%. The frequency of GSTA1 -69T variant allele in the Turkish population was very similar to those of White ancestry, including Serbian, Hispanic, Caucasian, Caucasian (USA), Danish, German, Italian, Polish, Caucasian (The Netherlands), Czech Central European, Eastern Slavs (Russia), Caucasian (Poland), South Tunisian ($P>0.05$). Moreover, no significant distinction was noted between the Turkish population and the American-African population with 35.7% allelic frequency ($P>0.05$).

Table 2: Comparison of the frequencies of GSTA1 -69C>T polymorphism in various populations

<table>
<thead>
<tr>
<th>Ethnicity &amp; Population</th>
<th>Healthy, control &amp; patients etc.</th>
<th>Sample size</th>
<th>Genotype frequencies n (%)</th>
<th>Allele frequencies n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>WHITE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkish</td>
<td>105</td>
<td>34</td>
<td>51</td>
<td>20</td>
</tr>
<tr>
<td>Serbian</td>
<td>66</td>
<td>23</td>
<td>36</td>
<td>7</td>
</tr>
<tr>
<td>Serbian</td>
<td>122</td>
<td>49</td>
<td>57</td>
<td>16</td>
</tr>
<tr>
<td>Caucasian</td>
<td>278</td>
<td>106</td>
<td>133</td>
<td>39</td>
</tr>
<tr>
<td>Caucasian (USA)</td>
<td>81</td>
<td>24</td>
<td>44</td>
<td>13</td>
</tr>
<tr>
<td>Danish women</td>
<td>396</td>
<td>123</td>
<td>210</td>
<td>63</td>
</tr>
<tr>
<td>German</td>
<td>826</td>
<td>256</td>
<td>395</td>
<td>175</td>
</tr>
<tr>
<td>Caucasian (The Netherlands)</td>
<td>411</td>
<td>168</td>
<td>184</td>
<td>59</td>
</tr>
<tr>
<td>Hungarian women</td>
<td>53</td>
<td>19</td>
<td>27</td>
<td>7</td>
</tr>
<tr>
<td>Italian women</td>
<td>137</td>
<td>46</td>
<td>65</td>
<td>26</td>
</tr>
<tr>
<td>Eastern European cancer</td>
<td>104</td>
<td>43</td>
<td>49</td>
<td>12</td>
</tr>
<tr>
<td>Slavonic origin women (Russians)</td>
<td>154</td>
<td>38</td>
<td>83</td>
<td>13</td>
</tr>
</tbody>
</table>

ASIAN

| Chinese* | Healthy | 140 | 105 | 34 | 1 | 244 | 36 (12.9) | [4] |
| Chinese Han women* | 112 | 86 | 24 | 2 | 196 | 28 (12.5) | [20] |
| Asian (Northeast Thailand)* | 198 | 141 | 53 | 4 | 335 | 61 (15.4) | [21] |
| Taiwanese* | Non-diabetes mellitus | 198 | 157 | 38 | 3 | 322 | 44 (11.1) | [22] |
| Taiwanese* | Control | 274 | 214 | 56 | 4 | 484 | 64 (11.7) | [23] |
| Japanese | Healthy | 147 | 104 | 39 | 4 | 247 | 47 (16.0) | [24] |

BLACK

| African-American | Control | 63 | 25 | 31 | 7 | 81 | 45 (35.7) | [14] |

<table>
<thead>
<tr>
<th>Allele frequencies, %</th>
<th>Genotype frequencies, %</th>
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<tr>
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<td>T</td>
<td>43.2</td>
<td>100</td>
</tr>
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</table>

n: total number of subjects. Distinctions between the frequencies were studied using $X^2$, * $P>0.001$ at significance when compared to the results of the present study.
Conclusion

To our knowledge, this is the first investigation to present the frequencies of the GSTA1 -69C>T polymorphism among Turkish individuals. In the current study, the -69C>T polymorphism in the GSTA1 gene is observed to be common among Turkish individuals. The frequencies of GSTA1 -69C>T polymorphism were similar to those in the African American population and the populations with White ancestry, but significantly different from those reported for the populations with Asian ancestry. The findings of the current study may ensure a perspective for further studies exploring the role of GSTA1 -69C>T polymorphism on predisposition to diverse illnesses such as cancer and may be used as a control group for such investigation. In addition, this study might contribute to epidemiological and toxicogenetic investigations as well.

References


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