Original Research Article

GSTM1, GSTP1, and *GSTT1* Genetic Variability in Turkish and Worldwide Populations

SEFAYET KARACA,[1,2]* MEHMET KARACA,[3] TOMRIS CESUROGLU,[2,4] SEMA ERGE,[2,5] AND RENATO POLIMANTI[6]
[1]*School of Health Science, Aksaray University, Aksaray, Turkey*
[2]*GENAR Institute for Public Health and Genomics Research, Ankara, Turkey*
[3]*Department of Biology, Faculty of Science and Arts, Aksaray University, Aksaray, Turkey*
[4]*Department of Social Medicine, Maastricht University, Maastricht, the Netherlands*
[5]*Department of Nutrition and Dietetics, Faculty of Health Science, Zirve University, Gaziantep, Turkey*
[6]*Department of Psychiatry, Yale University School of Medicine, West Haven, Connecticut, United States*

Objective: Glutathione S-transferase (GST) variants have been widely investigated to better understand their role in several pathologic conditions. To our knowledge, no data about these genetic polymorphisms within the Turkish population are currently available. The aim of this study was to analyze *GSTM1* positive/null, *GSTT1* positive/null, GSTP1*I105V (rs1695), and GSTP1*A114V (rs1138272) variants in the general Turkish population, to provide information about its genetic diversity, and predisposition to GST-related diseases.

Methods: Genotyping was performed in 500 Turkish individuals using the Sequenom MassARRAY platform. A comparative analysis was executed using the data from the HapMap and Human Genome Diversity Projects (HGDP). Sequence variation was deeply explored using the Phase 1 data of the 1,000 Genomes Project.

Results: The variability of *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms in the Turkish population was similar to that observed in Central Asian, European, and Middle Eastern populations. The high linkage disequilibrium between GSTP1*I105V and GSTP1*A114V in these populations may have a confounding effect on *GSTP1* genetic association studies. In analyzing *GSTM1*, *GSTT1*, and *GSTP1* sequence variation, we observed other common functional variants that may be candidates for associated studies of diseases related to GST genes (e.g., cancer, cardiovascular disease, and allergy).

Conclusions: This study provides novel data about *GSTM1* positive/null, *GSTT1* positive/null, GSTP1*I105V, and GSTP1*A114V variants in the Turkish population, and other functional variants that may affect *GSTM1*, *GSTT1*, and *GSTP1* functions among worldwide populations. This information can assist in the design of future genetic association studies investigating oxidative stress-related diseases. Am. J. Hum. Biol. 27:310–316, 2015.

The genetic variability of drug-metabolizing enzymes contributes to individual susceptibility to environmental risk factors (e.g., outdoor pollutants, smoke, and diet-related xenobiotics). Glutathione S-transferases (GSTs) constitute the most relevant superfamily of Phase II metabolic enzymes. A number of studies have explored the role that GST genes play in determining the predisposition to different pathologic conditions (Bin and Luo, 2013; Higgins and Hayes, 2011). Most studies of GSTs have focused on the *GSTM1*, *GSTT1*, and *GSTP1* genes (Chen et al., 2010; Marinkovic et al., 2013; Piacentini et al., 2013b; Xie et al., 2014). In particular, certain variants within these genes, having an effect on gene function, have been investigated in relation to disease risks. These include two structural variants that deleted *GSTM1* and *GSTT1* genes (positive/null genotype) and two nonsynonymous substitutions of *GSTP1* (i.e., GSTP1*I105V, rs1695; GSTP1*I114V, rs1138272) (Bolt and Thier, 2006; Dragovic et al., 2014). The effect of these variants in influencing disease risk was evaluated with respect to different pathologic conditions: endocrinologic (Amer et al., 2011; Mastana et al., 2013), neurologic (Kiyohara et al., 2010; Piacentini et al., 2012), cardiovascular (Polimanti et al., 2011b; Tang et al., 2010), pregnancy related (Polimanti et al., 2012), infertility related (Safarinejad et al., 2010), and allergic disease related (Minelli et al., 2010; Piacentini et al., 2014). In addition to their putative involvement in different pathologic conditions, several studies have shown that these genetic variants appear at varying frequencies among human populations (Iorio et al., 2014; Polimanti et al., 2013). This observation suggests that these variants may influence variability in disease predisposition among human populations (Myles et al., 2008).

Data about the distribution of these variants in different human populations are relevant not only for understanding the geographical distribution of functional genetic markers, but also for assessing the reliability of the findings obtained from association studies (Taioli et al., 2004). In this regard, to the best of our knowledge, no study has reported the distribution of *GSTM1* positive/null, *GSTT1* positive/null, GSTP1*I105V, and GSTP1*A114V in the Turkish population. The Turkish population is genetically complex due to its demographic history and location near Central Asia, Europe, and the Middle East (Cinnioglu et al., 2004; Gokcumen et al., 2011; Mergen et al., 2004; Rootsi et al., 2012). As such, reference genetic data for the Turkish population are

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*Correspondence to: Dr. Sefayet Karaca, PhD., School of Health Science, Aksaray University, Aksaray, Turkey. E-mail: skaraca@aksaray.edu.tr

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absolutely mandatory for assessing the reliability of genetic association studies conducted with this population. Several studies of different health-related traits in Turkish individuals have been performed to assess the role of *GSTM1*, *GSTT1*, and *GSTP1* gene variants in disease etiology, including ones focusing on respiratory diseases (Calikoglu et al., 2006), diabetes (Gonul et al., 2012), chromosomal aberrations (Kadioglu et al., 2012), cancers (Altayli et al., 2009; Berber et al., 2013), and cardiovascular diseases (Taspinar et al., 2012). Furthermore, the genetic investigation of disease-associated loci in human populations with a complex demography history, such as the Turkish population, furnishes a valuable opportunity to investigate the genetic relationship between human history and genetic predispositions to common diseases.

Because there is no knowledge about GST gene variation in Turkish general variation, we performed a population-based screening of 500 individuals. Specifically, we assessed the distribution of *GSTM1* positive/null, *GSTT1* positive/null, GSTP1*I105V (rs1695), and GSTP1*A114V (rs1138272) variants in the general Turkish population and compared these results with those available in the reference database (i.e., the HapMap project and the Human Genome Diversity Project (HGDP)). Furthermore, we used the sequence data available from Phase 1 of the 1,000 Genomes Project to deeply analyze the sequence variation in *GSTM1*, *GSTT1*, and *GSTP1*, as this analysis will help to identify additional variants that may contribute to the functional genetic diversity of these loci. The results of these comparative analyses provided novel information that can help to understand the effect of genetic variation in Turkish general population and to dissect the epidemiological differences in the predisposition to disease and drug response observed in Turkish and European populations (de Hoog et al., 2011; Icks et al., 2012; Racape et al., 2013; Zubor et al., 2014).

MATERIALS AND METHODS

Study participants

All of the procedures used in this study conformed to the tenets of the Declaration of Helsinki, and the appropriate institutional ethics committee approval was obtained by Aksaray University. Participants were recruited in seven largest cities (i.e., Istanbul, Ankara, Izmir, Bursa, Adana, Antalya, and Samsun) within five regions (i.e., Marmara, Central Anatolia, Aegean, Mediterranean, and Black Sea) of Turkey. Around 40% of the population in Turkey inhabits in these cities. The demographic characteristics of these cities are similar to the overall structure of population in Turkey because these large cities have been receiving heavy migration from all urban and rural settlements of Turkey during twentieth century (Eryurt and KOÇ, 2012). Participants voluntarily applied to enroll in a preventive healthcare intervention program provided via participating physicians in these cities. The program was developed and provided by the GENAR Institute for Public Health and Genomics Research to prevent common chronic complex diseases. The study population (n = 500) included unrelated individuals over 18 years of age who reported themselves to be healthy and had a successful genotyping results for the investigated genes (*GSTP1*, *GSTM1*, and *GSTT1*).

Molecular analyses

Genomic DNA preparation. Genomic DNAs were isolated from buccal swabs using an MN DNA Isolation Kit (Macherey Nagel-Nucleospin, Germany). Following the DNA extraction, samples were diluted to a 2.0–2.5 ng/μl concentration in nanopure water.

Amplification of the genomic sequence of interest. SequenomRealSNP software was used to design sequence-specific amplification primers (Metabion, Germany). The amplification conditions of the *GSTP1* variants were based on the manufacturer's protocol of the Sequenom MassARRAY platform. PCR amplifications were performed in a total volume of 5 μl, which contained 2 μl DNA, 0.02 μl Hot Star Taq (Qiagene, Germany) polymerase (5 U/μl), 0.2 μl $MgCl_2$ (25 mM), 0.5 μl PCR buffer (10x), 0.04 μl dNTPs (10 mM), 0.74 μl H_2O, and 1.5 μl of the total primers at their optimized concentrations. Amplification conditions were as follows: an initial denaturation step at 94°C for 3 min, followed by 45 cycles of denaturation, annealing and extension at 94°C (20s), 56°C (30s), 72°C (45s), respectively, with a final extension step at 72°C for 3 min.

Post-PCR cleanup. Amplified genomic regions were 150–250 bp in length for each SNP to be investigated. Amplicons were subjected to a post-PCR cleanup reaction with a shrimp alkaline phosphatase (SAP) (Sequenom) in a total volume of 7 μl. The SAP reaction mix contained 0.3 μlSAP (1 U/μl) enzyme, 0.17 μl SAP buffer (10x), and 1.53 μlH_2O. Plates were incubated for 50 min at 37°C. This was followed by an inactivation of the SAP enzyme for 20 min at 85°C.

Homogenious mass extend (hME) reaction. Cleaned PCR products were used as templates for the locus-specific primer extension (homogenious mass extend = hME primer) reactions. The hME primer and amplified target DNA were incubated with mass-modified ddNTP terminators. The primer extension was made according to the sequence of the variant site.

The extension reactions were performed in a final volume of 9 μl, containing a 0.2 μl termination mix, 0.018 μl Sequenase enzyme, 1,282 μl H_2O, and 0.5 μl of the extension primer at a 10 μM concentration. Conditions for the extension reaction were as follows: an initial denaturation step at 94C for 2 min, followed by 55 cycles of denaturation, annealing and extension at 94 C (5s), 52 C (5s), 72 C (5s), respectively.

Purification of hME products. A total of 384-well dimple plates (Sequenom) were used for resine (Sequenom) cleanup. The final products were treated with a 3 mg cation-exchange resin per well to remove salts. Ultrapure water (Millipore, Methexis Ghent, Belgium) was added to each well (16 μl), mixed for 5–10 min, then centrifuged for 5 min at 1600 RPM. All reactions and incubations were performed on a GeneAmp PCR System 9700 (AppliedBiosystems, Norwalk, CT).

Analysis of hME products on MALDI-TOF-based mass spectrometry. A small volume (25 nl) of extended/desalted products was arrayed onto 384-sample SpectroCHIPs, using the Nanodispencer system (Sequenom Inc.). The target chip was then inserted into the MALDI-TOF mass spectrometer of the MassARRAY Compact System (Sequenom Inc.). By the application of MALDI-TOF mass spectrometry, the mass of the unextended and extended primers was determined. The difference indicated the

alleles that were present at the polymorphic site of the interest. The analysis was performed using SpectroTYPER software (Sequenom Inc.), which automatically translates the mass of the observed primers into a genotype.

For the *GSTM1* and *GSTT1* deletion variants, following the amplification, PCR products were subjected to a 1.0% agarose gel electrophoresis and were evaluated according to the presence or absence of PCR fragments as compared to weight molecular standards (50bp DNA Ladder, Fermentas).

All the samples were analyzed twice in the presence of positive (sample with known variation) and negative (no template) controls, as previously identified by sequencing analysis. Accuracy of the results was tested in the randomly selected samples (10%) from the tested population, using direct DNA sequencing (Megabase 1000, GE Healthcare/Amersham Biosciences).

Publically available data about GST gene variation in worldwide populations

To compare GST gene diversity in Turkish and worldwide populations, we used different public databases to find the relevant human genetic variations. To analyze the frequency of the positive/null genotype of *GSTM1* and *GSTT1*, we used the HapMap CNV (Copy Number Variant) data available at ftp://ftp.ncbi.nlm.nih.gov/hapmap/cnv_data/. Using this information, 11 HapMap populations were classified into four ancestry groups. The African group included ASW (African Ancestry in Southwest US), LWK (Luhya in Webuye, Kenya), MKK (Maasai in Kinyawa, Kenya), and YRI (Yoruban in Ibadan, Nigeria). The American group contained only MEX (Mexican Ancestry in Los Angeles, CA). The south Asian group included only GIH (Gujarati Indians in Houston, Texas). The East Asian group comprised CHB (Han Chinese in Beijing), CHD (Chinese in Metropolitan Denver, Colorado), and JPT (Japanese in Tokyo, Japan). The European group included CEU (Utah residents with northern and western European ancestry from the CEPH collection), and TSI (Tuscan in Italy).

For the analysis of *GSTP1* haplotypes based on the I105V (rs1695) and A114V (rs1138272) polymorphisms, we utilized the data of the Human Genome Diversity Project (HGDP) (available at http://www.hagsc.org/hgdp/files.html). These data provided information about 51 human populations representing seven continental areas (Africa, America, Central Asia, East Asia, Europe, the Middle East, and Oceania). Information about population inclusion within the continental groups is available in supporting information Table 1.

Finally, to explore the sequence variation at the *GSTM1*, *GSTP1*, and *GSTT1* loci, we used the Phase 1 data of the 1,000 Genomes Project. Specifically, we downloaded Variant Call Format (VCF) files of *GSTM1* (Chromosome 1: 110,230,436-110,251,661), *GSTP1* (Chromosome 11: 67,351,066–67,354,131), and *GSTT1* (Chromosome 22: 24,376,133-24,384,680). This study comprised 1,092 individual samples belonging to 14 human populations with four different ancestry origins (Africa, America, East Asia, and Europe). Information about samples of 1,000 Genomes Phase 1 data are available at http://www.1000genomes.org/about#ProjectSamples.

Functional annotation analysis

To annotate the functional effect of the investigated variants, we used two different tools. VARIANT (VARIant ANalyis Tool) was used to identified variants associated with coding changes (e.g., nonsynonymous substitution, stop codon) (Medina et al., 2012). RegulomeDB was used to analyze the regulatory effects of noncoding variants (Boyle et al., 2012). RegulomeDB classified genetic variants with a score ranging from 1a (i.e., variants with several instances of evidence of regulatory function) to 7 (i.e., variants with no evidence of regulatory functions).

Statistical analysis

Haplotype frequency estimation, linkage disequilibrium (LD) analysis, and Hardy–Weinberg equilibrium verification were performed using Haploview version 3.2 (Barrett, 2009). Fisher's exact and Chi-square tests were used to verify genotype/haplotype differences between the Turkish sample and the other worldwide populations. As multiple tests were performed, the Bonferroni correction was applied to them. To identify the allelic frequency differences (ΔF) of *GSTM1*, *GSTP1*, and *GSTT1* variants among ancestry groups, we used the method proposed by Hofer and colleagues (Hofer et al., 2009). For each allele i, we computed the average allele frequency p_{ij} within each ancestry group j, as well as the difference between the average frequency computed over all other populations as $\Delta F = p_{ij} - p_{-ij}$, where p_{-ij} is the average frequency of allele i in all populations not belonging to the ancestry group j.

RESULTS

Table 1 shows the positive/null genotype frequencies of *GSTM1* and *GSTT1* in the Turkish and HapMap populations. Regarding the *GSTM1* positive/null genotype, we observed a significant difference between the Turkish and the African (ASW, LWK, YRI) and GIH populations. While considering the *GSTT1* positive/null genotype, significant differences were observed with respect to the East Asian (CHB, CHD, JPT), and CEU populations. Considering both null genotype frequencies of *GSTM1* and *GSTT1*, we observed that the Turkish population showed patterns of diversity similar to those of the European (CEU, TSI) and MEX populations (Supporting information, Fig.1). These populations showed high frequencies of the *GSTM1* null genotype and low frequencies of the *GSTT1* null genotype.

Table 2 reports the haplotype frequencies of *GSTP1* in the Turkish population and the HGDP continental groups. *GSTP1* haplotypes of 51 HGDP populations are reported in supporting information Table 1. GSTP1*I105V and GSTP1*I114V genotype distributions were in Hardy–Weinberg equilibrium in the Turkish samples. Considering *GSTP1* haplotype frequencies, we observed that the Turkish population differed significantly from the African, American, and East Asian groups. The results revealed that the Turkish population, like the others that present GSTP1*A114V, showed a complete LD ($D' = 1$) between I105V and A114V polymorphisms. However, different correlations values between the two investigated *GSTP1* nonsynonymous variants were present among the human populations (r^2 from 0.006 to 0.268). The haplotype with the highest frequency was I105-A114 (frequency from 77.6% to 54%). It is followed by V105-A114 (frequency from 45.4% to 15.5%) and V105-V114 (frequency from 7.4% to 0%). We did not observe the I105-V114 haplotype in any of the 3,070 analyzed chromosomes.

TABLE 1. GSTM1 and GSTT1-positive/null-genotype frequencies in Turkish and HapMap populations

Population	Source	*GSTM1* positive	*GSTM1* null (%)	[a]*P*	*GSTT1* positive	*GSTT1* null (%)	[a]*P*
Turkey	Present study	235	251 (52)	–	359	113 (23)	–
ASW	HapMap	64	18(22)	[b]<0.001	65	17(21)	0.575
CEU	HapMap	66	95(59)	0.121	143	18(11)	[b]<0.001
CHB	HapMap	43	40(48)	0.635	46	37(45)	[b]0.002
CHD	HapMap	37	48(56)	0.48	51	34(40)	[b]0.003
GIH	HapMap	58	27(32)	[b]0.001	68	17(20)	0.488
JPT	HapMap	42	44(51)	1	52	34(40)	[b]0.003
LWK	HapMap	67	20(23)	[b]<0.001	61	26(30)	0.28
MEX	HapMap	39	34(47)	0.452	57	16(22)	0.769
MKK	HapMap	123	45(27)	[b]<0.001	109	59(35)	0.006
TSI	HapMap	39	48(55)	0.562	71	16(18)	0.332
YRI	HapMap	131	33(20)	[b]<0.001	109	55(34)	0.018

[a]P values (Fisher's exact test) related to the comparison between Turkish sample and HapMap data.
[b]Significant *P* values (after bonferroni correction).

TABLE 2. GSTP1 haplotypes in Turkish population and HGDP continental groups

Population	Source	N	*GSTP1* I105-A114	*GSTP1* I105-V114	*GSTP1* V105-A114	*GSTP1* V105-V114	D'	r^2	[a]*P*
Turkey	Present study	984	0.716	0	0.216	0.068	1	0.184	–
Africa	HGDP	302	0.54	0	0.453	0.007	1	0.006	[b]< 0.001
America	HGDP	216	0.546	0	0.454	0	NA	NA	[b]< 0.001
Central Asia	HGDP	394	0.771	0	0.155	0.074	1	0.268	0.036
East Asia	HGDP	438	0.776	0	0.222	0.002	1	0.008	[b]< 0.001
Europe	HGDP	372	0.766	0	0.188	0.046	1	0.157	0.127
Middle East	HGDP	292	0.771	0	0.178	0.051	1	0.192	0.175
Oceania	HGDP	72	0.75	0	0.25	0	NA	NA	0.0687

N: Information about number of chromosomes; LD: coefficients (r^2, D').
[a]*P* values (Chi-square test).
[b]Significant *P* values (after Bonferroni correction).

Considering the different genetic ancestries of the comparative populations, we observed that the Oceanic and American populations did not show the I114V variant and had a V105 allele frequency of 25% and 45%, respectively. For those with African and East Asian ancestries, A114V was a rare variant (i.e., V144 frequency is lower than 1%, 0.7%, and 0.2%, respectively). Conversely, I105V polymorphism occurred at high frequency (46% and 22.4%, respectively). Consequently, we observed the lowest r^2 values for these ancestry groups (0.6% and 0.8%, respectively). In Central Asia, the Middle East, and Europe, I114V is a polymorphic variant (V114 allele frequency is greater than 5%, 7.4%, 4.6%, and 5.1%), and the V105 allele showed a frequency of 22.9%, 23.4%, and 22.9%, respectively. Consequently, high r^2 values are present between GSTP1*I105V and GSTP1*A114V in these human groups (26.8%, 15.7%, and 19.2%). *GSTP1* haplotype frequencies in the Turkish population were similar to those observed in European and Middle Eastern populations.

Beside *GSTM1* null/positive, *GSTT1* null/positive, GSTP1*I105V, and GSTP1*A114V variants, other *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms with a functional effect could possibly be present and might also show relevant ancestry-related differences. In order to analyze the sequence variation of the *GSTM1*, *GSTT1*, and *GSTP1* genes, we exploited the data of the 1,000 Genomes Project. Figure 1 shows the ΔF values of *GSTM1*. Among the 251 *GSTM1* variants, we observed extreme ΔF values ($\Delta F > 0.3$) for those with African and Asian ancestries (Supporting information, Table 2). Considering the functional impact of *GSTM1* variants, we observed nine variants associated with coding changes (one variant located in a splice site and eight nonsynonymous substitutions) and eight noncoding variants with regulatory effects (RegulomeDB score < 4). Among these *GSTM1* functional variants, some variants have a minor allele frequency greater than 10% in at least one ancestry group: rs200184852 (D9N), rs201967146 (C78R), rs199816990 (R96L), rs202002774 (M105T), rs74837985 (K173N), rs145941576 (RegulomeDB score = 2b), rs149240591 (RegulomeDB score = 2b), rs144405570 (RegulomeDB score = 2b), rs12097277 (RegulomeDB score = 2b), rs1292099, (RegulomeDB score = 1f), rs144693890 (RegulomeDB score = 3a), rs116296482 (RegulomeDB score = 3a), rs181092369 (RegulomeDB score = 3a).

Figure 2 reports ΔF values for *GSTP1* variants. We analyzed 41 *GSTP1* variants and observed extreme ΔF values only for those with African ancestry (Supporting information, Table 3). Among the variants with an effect on gene function, we observed seven nonsynonymous substitutions and 14 noncoding regulatory variants. Of these, 11 variants showed a minor allele frequency greater than 10% in at least one ancestry group: rs1695 (I105V), rs1138272 (A114V), rs8191438 (RegulomeDB score = 2a), rs8191443 (RegulomeDB score = 2b), rs1079719 (RegulomeDB score = 2b) rs2370143 (RegulomeDB score = 2b), rs8191448 (RegulomeDB score = 2b), rs762803 (RegulomeDB score = 1f), rs8191449 (RegulomeDB score = 2b), and rs1871042 (RegulomeDB score = 2b).

For *GSTT1*, we analyzed 68 different variants and observed extreme ΔF values for those with African and European ancestries (Fig. 3; Supporting information, Table 4). Regarding *GSTT1* functional variants, we observed eight coding variants (2 splicing variants and 6 nonsynonymous substitutions) and one noncoding regulatory variant

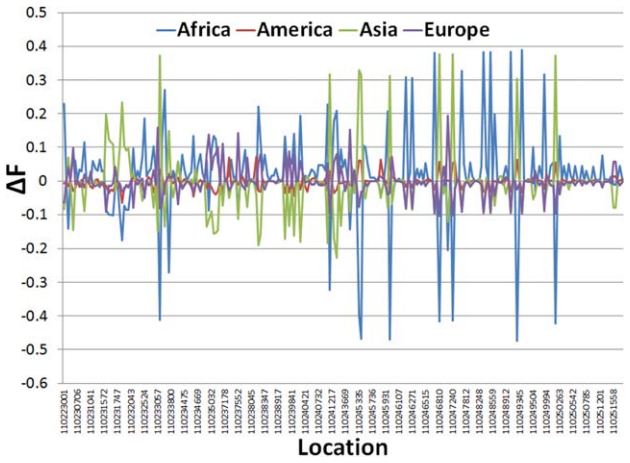

Fig. 1. Δ*F*values in *GSTM1* gene.

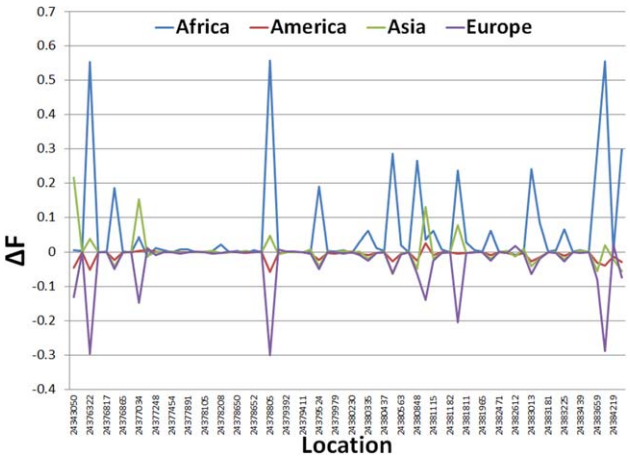

Fig. 3. Δ*F* values in *GSTT1* gene.

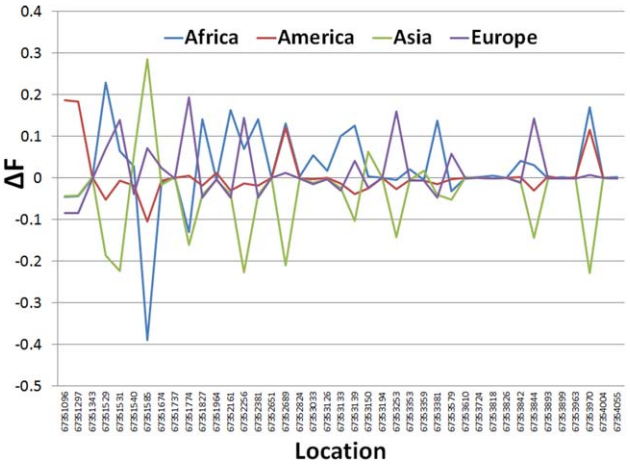

Fig. 2. Δ*F* values in *GSTP1* gene.

(rs56106137). Among these variants, rs2266637 (V69I) and rs6519497 (splice site) showed minor allele frequencies greater than 10% in at least one ancestry group.

DISCUSSION

GSTM1, *GSTP1*, and *GSTT1* have been deeply investigated as candidate genes involved in the predisposition to pathologic conditions. In particular, a number of studies have analyzed *GSTM1* and *GSTT1* deletion polymorphisms that are associated with null phenotypes (i.e., *GSTM1* positive/null, *GSTT1* positive/null), and two *GSTP1* nonsynonymous changes (i.e., GSTP1*I105V and GSTP1*A114V), as these genetic variants may affect drug response and metabolic processes (Bolt and Thier, 2006; Higgins and Hayes, 2011; Piacentini et al., 2013a). Besides the putative role those variants play with respect to health-related traits, some studies have investigated their distribution among human populations (Piacentini et al., 2011; Polimanti et al., 2011a) in order to provide information about the global distribution of these markers and to furnish reference data for genetic association studies. To the best of our knowledge, no reference studies are currently available about *GSTM1*, *GSTP1*, and *GSTT1* gene variants in the Turkish population.

To provide this information, we performed a population-based screening of 500 Turkish individuals and compared the results with data available in the HapMap and HGDP projects. Furthermore, we analyzed the sequence variability of the *GSTM1*, *GSTP1*, and *GSTT1* gene using data from the 1,000 Genomes Project.

The analysis of the investigated markers indicated that GST gene variability of the Turkish population is similar to the variability present in Eurasian populations (i.e., Europe, Middle East, and Central Asia). This result is in agreement with the current knowledge about the genetic structure of the Anatolia peninsula. The Turkish population is an admixture of European, Middle East, and Central Asia ancestries reflecting the gene flows from different Euroasian populations (Cinnioglu et al., 2004; Gokcumen et al., 2011; Mergen et al., 2004; Rootsi et al., 2012). Specifically, high *GSTM1* null frequencies and low *GSTT1* null frequencies were observed for Turkish and European populations. We observed similar *GSTM1* and *GSTT1* null frequencies in the MEX population. This situation is likely due to the high European ancestry present in the Mexican–American population (Gravel et al., 2013): the similarity between MEX and Turkish sample can be due to the shared European ancestry partially present in both two groups, although there are very different degrees of European ancestry in Mexican mestizos and Turkish samples. Regarding *GSTP1* haplotypes based on I105V and A114V substitutions, we did not observe the I105-V114 in any of the 52 investigated populations. Its absence indicates the presence of complete LD ($D' = 1$) between GSTP1*I105V and GSTP1*A114V in worldwide populations. However, relevant ancestry-related differences are present in the correlation between these amino acidic substitutions. In particular, Turkish, Central Asian, and European population members showed similar *GSTP1* haplotype patterns: ~20% of GSTP1V*105 allele frequency, ~5% of GSTP1*V114 allele frequency, and 15–20% of r^2. The presence of a relevant correlation between GSTP1*I105V and GSTP1*A114V in these populations may be a confounder in GSTP1*I105V genetic association

studies. Indeed, an important percentage of GSTP1*V105 carriers are also GSTP1*V114 carriers, confounding the results obtained in populations with Central Asian, European, and Middle Eastern ancestries.

Due to the explosion of high-throughput technology for sequence analysis, large amount of data about human genome variation are now available (Genomes Project et al., 2010; International HapMap et al., 2010). We explored the Phase 1 data of the 1,000 Genomes Project in order to obtain new information about the genetic variation at the *GSTM1*, *GSTP1*, and *GSTT1* loci. Our ΔF analysis indicated that genetic variants in the investigated GST genes show great differences among ancestry groups. These differences are particularly evident between Africans and non-Africans. This is in accordance with current knowledge about human demographic and migration history (Campbell and Tishkoff, 2008).

Considering the functional and regulatory role of the identified variants, we observed several coding and noncoding variants with minor allele frequencies greater than 10%. Some of these variants are located in coding regions, and they are associated with nonsynonymous substitutions. The other genetic changes are located in noncoding regions, and some of these are predicted to be involved in gene expression regulation. These common functional polymorphisms may alter the biological processes related to *GSTM1*, *GSTP1*, and *GSTT1* genes (e.g., antioxidant mechanisms, xenobiotics metabolism, and cellular signaling), increasing the risk of GST-association pathologic conditions. Furthermore, the presence of functional variants with large allelic differences among human populations may also explain the nonconcordance observed in some cases in the genetic association analysis of GST genes.

In conclusion, this study provides reference data for *GSTM1* positive/null, *GSTT1* positive null, GSTP1*I105V, and GSTP1*A114V in the Turkish population, and information about the genetic differences between Turkish individuals and the populations available in HGDP and HapMap projects. Through the analysis of 1,000 Genomes Phase 1 data, this study also provides useful data about the presence of other functional variants with high minor allele frequencies that may contribute to functional differences of *GSTM1*, *GSTT1*, and *GSTP1* genes among worldwide populations. The identification of genetic structure in a given population may contribute to the development and implementation of more targeted preventative healthcare strategies. Our results can assist in the design of future studies regarding the genetics of complex diseases in Turkey.

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Conflict of interests

The authors declare that they have no financial interest in or conflict with the subject matter or materials discussed in the manuscript.

LITERATURE CITED

Altayli E, Gunes S, Yilmaz AF, Goktas S, Bek Y. 2009. CYP1A2, CYP2D6, GSTM1, GSTP1, and GSTT1 gene polymorphisms in patients with bladder cancer in a Turkish population. Int Urol Nephrol 41:259–266.

Amer MA, Ghattas MH, Abo-Elmatty DM, Abou-El-Ela SH. 2011. Influence of glutathione S-transferase polymorphisms on type-2 diabetes mellitus risk. Genet Mol Res 10:3722–3730.

Barrett JC. 2009. Haploview: visualization and analysis of SNP genotype data. Cold Spring Harb Protoc 2009:pdb ip71. doi: 10.1101/pdb.ip71.

Berber U, Yilmaz I, Yilmaz O, Haholu A, Kucukodaci Z, Ates F, Demirel D. 2013. CYP1A1 (Ile462Val), CYP1B1 (Ala119Ser and Val432Leu), GSTM1 (null), and GSTT1 (null) polymorphisms and bladder cancer risk in a Turkish population. Asian Pac J Cancer Prev 14:3925–3929.

Bin Q, Luo J. 2013. Role of polymorphisms of GSTM1, GSTT1 and GSTP1 Ile105Val in Hodgkin and non-Hodgkin lymphoma risk: a human genome epidemiology (HuGE) review. Leuk Lymphoma 54:14–20.

Bolt HM, Thier R. 2006. Relevance of the deletion polymorphisms of the glutathione S-transferases GSTT1 and GSTM1 in pharmacology and toxicology. Curr Drug Metab 7:613–628.

Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, Kasowski M, Karczewski KJ, Park J, Hitz BC, Weng S, Cherry JM, Snyder M. 2012. Annotation of functional variation in personal genomes using RegulomeDB. Genome Res 22:1790–1797.

Calikoglu M, Tamer L, Ates Aras N, Karakas S, Ercan B. 2006. The association between polymorphic genotypes of glutathione S-transferases and COPD in the Turkish population. Biochem Genet 44:307–319.

Campbell MC, Tishkoff SA. 2008. African genetic diversity: implications for human demographic history, modern human origins, and complex disease mapping. Annu Rev Genomics Hum Genet 9:403–433.

Chen B, Cao L, Zhou Y, Yang P, Wan HW, Jia GQ, Liu L, Wu XT. 2010. Glutathione S-transferase T1 (GSTT1) gene polymorphism and gastric cancer susceptibility: a meta-analysis of epidemiologic studies. Dig Dis Sci 55:1831–1838.

Cinnioglu C, King R, Kivisild T, Kalfoglu E, Atasoy S, Cavalleri GL, Lillie AS, Roseman CC, Lin AA, Prince K, Oefner PJ, Shen P, Semino O, Cavalli-Sforza LL, Underhill PA. 2004. Excavating Y-chromosome haplotype strata in Anatolia. Hum Genet 114:127–148.

de Hoog ML, van Eijsden M, Stronks K, Gemke RJ, Vrijkotte TG. 2011. Overweight at age two years in a multi-ethnic cohort (ABCD study): the role of prenatal factors, birth outcomes and postnatal factors. BMC Public Health 11:611.

Dragovic S, Venkataraman H, Begheijn S, Vermeulen NP, Commandeur JN. 2014. Effect of human glutathione S-transferase hGSTP1-1 polymorphism on the detoxification of reactive metabolites of clozapine, diclofenac and acetaminophen. Toxicol Lett 224:272–281.

Eryurt MA, KOÇ I. 2012. Internal migration and fertility in Turkey: Kaplan–Meier survival analysis. Int J Popul Res 2012:Article ID 329050.

Genomes Project C, Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM, Gibbs RA, Hurles ME, McVean GA. 2010. A map of human genome variation from population-scale sequencing. Nature 467:1061–1073.

Gokcumen O, Gultekin T, Alakoc YD, Tug A, Gulec E, Schurr TG. 2011. Biological ancestries, kinship connections, and projected identities in four central Anatolian settlements: insights from culturally contextualized genetic anthropology. Am Anthropol 113:116–131.

Gonul N, Kadioglu E, Kocabas NA, Ozkaya M, Karakaya AE, Karahalil B. 2012. The role of GSTM1, GSTT1, GSTP1, and OGG1 polymorphisms in type 2 diabetes mellitus risk: a case–control study in a Turkish population. Gene 505:121–127.

Gravel S, Zakharia F, Moreno-Estrada A, Byrnes JK, Muzzio M, Rodriguez-Flores JL, Kenny EE, Gignoux CR, Maples BK, Guiblet W, Dutil W, Via M, Sandoval K, Bedoya G, 1000 Genomes Project, Oleksyk TK, Ruiz-Linares A, Burchard EG, Martinez-Cruzado JC, Bustamante CD. 2013. Reconstructing native American migrations from whole-genome and whole-exome data. PLoS Genet 9:e1004023.

Higgins LG, Hayes JD. 2011. Mechanisms of induction of cytosolic and microsomal glutathione transferase (GST) genes by xenobiotics and pro-inflammatory agents. Drug Metab Rev 43:92–137.

Hofer T, Ray N, Wegmann D, Excoffier L. 2009. Large allele frequency differences between human continental groups are more likely to have occurred by drift during range expansions than by selection. Ann Hum Genet 73:95–108.

Icks A, Razum O, Rosenbauer J, Bachle C, Hungele A, Monkemoller K, Muller-Godeffroy E, Heidtmann B, Kapellen T, Scheuing N and others. 2012. Lower frequency of insulin pump treatment in children and adolescents of Turkish background with type 1 diabetes: analysis of 21,497 patients in Germany. Diabetes Technol Ther 14:1105–1109.

International HapMap C, Altshuler DM, Gibbs RA, Peltonen L, Altshuler DM, Gibbs RA, Peltonen L, Dermitzakis E, Schaffner SF, Yu F, Chang K, Hawes A, Lewis LR Ren Y, Wheeler D, Gibbs RA, Muzny DM, Barnes C, Darvishi K, Hurles M, Korn JM, Kristiansson K, Lee C, McCarrol SA,

Nemesh J, Dermitzakis E, Keinan A, Montgomery SB, Pollack S, Price AL, Soranzo N, Bonnen PE, Gibbs RA, Gonzaga-Jauregui C, Keinan A, Price AL, Yu F, Anttila V, Brodeur W, Daly MJ, Leslie S, McVean G, Moutsianas L, Nguyen H, Schaffner SF, Zhang Q, Ghori MJ, McGinnis R, McLaren W, Pollack S, Price AL, Schaffner SF, Takeuchi F, Grossman SR, Shlyakhter I, Hostetter EB, Sabeti PC, Adebamowo CA, Foster MW, Gordon DR, Licinio J, Manca MC, Marshall PA, Matsuda I, Ngare D, Wang VO, Reddy D, Rotimi CN, Royal CD, Sharp RR, Zeng C, Brooks LD, McEwen JE. 2010. Integrating common and rare genetic variation in diverse human populations. Nature 467:52–58.

Iorio A, Piacentini S, Polimanti R, De Angelis F, Calderon R, Fuciarelli M. 2014. Functional variability of glutathione S-transferases in Basque populations. Am J Hum Biol 26:361–366.

Kadioglu E, Kocabas NA, Demircigil GC, Coskun E, Ozcagli E, Durmaz E, Karahalil B, Burgaz S, Sardas S. 2012. Assessment of individual susceptibility to baseline DNA and cytogenetic damage in a healthy Turkish population: evaluation with lifestyle factors. Genet Test Mol Biomarkers 16:1157–1164.

Kiyohara C, Miyake Y, Koyanagi M, Fujimoto T, Shirasawa S, Tanaka K, Fukushima W, Sasaki S, Tsuboi Y, Yamada T, Oeda T, Miki T, Kawamura N, Sakae N, Fukuyama H, Hirota Y, Nahai M. Fukuoka Kinki Parkinson's disease study group. 2010. GST polymorphisms, interaction with smoking and pesticide use, and risk for Parkinson's disease in a Japanese population. Parkinsonism Relat Disord 16:447–452.

Marinkovic N, Pasalic D, Potocki S. 2013. Polymorphisms of genes involved in polycyclic aromatic hydrocarbons' biotransformation and atherosclerosis. Biochem Med (Zagreb) 23:255–265.

Mastana SS, Kaur A, Hale R, Lindley MR. 2013. Influence of glutathione S-transferase polymorphisms (GSTT1, GSTM1, GSTP1) on type-2 diabetes mellitus (T2D) risk in an endogamous population from north India. Mol Biol Rep 40:7103–7110.

Medina I, De Maria A, Bleda M, Salavert F, Alonso R, Gonzalez CY, Dopazo J. 2012. VARIANT: command line, web service and web interface for fast and accurate functional characterization of variants found by next-generation sequencing. Nucleic Acids Res 40:W54–W58.

Mergen H, Oner R, Oner C. 2004. Mitochondrial DNA sequence variation in the Anatolian peninsula (Turkey). J Genet 83:39–47.

Minelli C, Granell R, Newson R, Rose-Zerilli MJ, Torrent M, Ring SM, Holloway JW, Shaheen SO, Henderson JA. 2010. Glutathione-S-transferase genes and asthma phenotypes: a human genome epidemiology (HuGE) systematic review and meta-analysis including unpublished data. Int J Epidemiol 39:539–562.

Myles S, Tang K, Somel M, Green RE, Kelso J, Stoneking M. 2008. Identification and analysis of genomic regions with large between-population differentiation in humans. Ann Hum Genet 72:99–110.

Piacentini S, Polimanti R, De Angelis F, Iorio A, Fuciarelli M. 2013a. Phenotype versus genotype methods for copy number variant analysis of glutathione S-transferases M1. Ann Hum Genet 77:409–415.

Piacentini S, Polimanti R, Iorio A, Cortesi M, Papa F, Rongioletti M, Liumbruno GM, Manfellotto D, Fuciarelli M. 2014. GSTA1*-69C/T and GSTO2*N142D as asthma- and allergy-related risk factors in Italian adult patients. Clin Exp Pharmacol Physiol 41:180–184.

Piacentini S, Polimanti R, Porreca F, Martinez-Labarga C, De Stefano GF, Fuciarelli M. 2011. GSTT1 and GSTM1 gene polymorphisms in European and African populations. Mol Biol Rep 38:1225–1230.

Piacentini S, Polimanti R, Simonelli I, Donno S, Pasqualetti P, Manfellotto D, Fuciarelli M. 2013b. Glutathione S-transferase polymorphisms, asthma susceptibility and confounding variables: a meta-analysis. Mol Biol Rep 40:3299–3313.

Piacentini S, Polimanti R, Squitti R, Ventriglia M, Cassetta E, Vernieri F, Rossini PM, Manfellotto D, Fuciarelli M. 2012. GSTM1 null genotype as risk factor for late-onset Alzheimer's disease in Italian patients. J Neurol Sci 317:137–140.

Polimanti R, Carboni C, Baesso I, Piacentini S, Iorio A, De Stefano GF, Fuciarelli M. 2013. Genetic variability of glutathione S-transferase enzymes in human populations: functional inter-ethnic differences in detoxification systems. Gene 512:102–107.

Polimanti R, Piacentini S, Fuciarelli M. 2011a. HapMap-based study of human soluble glutathione S-transferase enzymes: the role of natural selection in shaping the single nucleotide polymorphism diversity of xenobiotic-metabolizing genes. Pharmacogenet Genomics 21:665–672.

Polimanti R, Piacentini S, Lazzarin N, Re MA, Manfellotto D, Fuciarelli M. 2011b. Glutathione S-transferase variants as risk factor for essential hypertension in Italian patients. Mol Cell Biochem 357:227–233.

Polimanti R, Piacentini S, Lazzarin N, Vaquero E, Re MA, Manfellotto D, Fuciarelli M. 2012. Glutathione S-transferase genes and the risk of recurrent miscarriage in Italian women. Fertil Steril 98:396–400.

Racape J, De Spiegelaere M, Dramaix M, Haelterman E, Alexander S. 2013. Effect of adopting host-country nationality on perinatal mortality rates and causes among immigrants in Brussels. Eur J Obstet Gynecol Reprod Biol 168:145–150.

Rootsi S, Myres NM, Lin AA, Jarve M, King RJ, Kutuev I, Cabrera VM, Khusnutdinova EK, Varendi K, Sahakyan H, Behar DM, Khusainova R, Balanovsky O, Balanovska E, Rudan P, Yepiskoposyan L, Bahmanimehr A, Farjadian S, Kushniarevich A, Herrera RJ, Grugni V, Battaglia V, Nici C, Crobu F, Karachanak S, Hooshiar Kashani B, Houshmand M, Sanati MH, Toncheva D, Lisa A, Semino O, Chiaroni J, Di Cristofaro J, Villems R, Kivisild T, Underhill PA. 2012. Distinguishing the co-ancestries of haplogroup G Y-chromosomes in the populations of Europe and the Caucasus. Eur J Hum Genet 20:1275–1282.

Safarinejad MR, Shafiei N, Safarinejad S. 2010. The association of glutathione-S-transferase gene polymorphisms (GSTM1, GSTT1, GSTP1) with idiopathic male infertility. J Hum Genet 55:565–570.

Taioli E, Pedotti P, Garte S. 2004. Importance of allele frequency estimates in epidemiological studies. Mutat Res 567:63–70.

Tang JJ, Wang MW, Jia EZ, Yan JJ, Wang QM, Zhu J, Yang ZJ, Lu X, Wang LS. 2010. The common variant in the GSTM1 and GSTT1 genes is related to markers of oxidative stress and inflammation in patients with coronary artery disease: a case-only study. Mol Biol Rep 37:405–410.

Taspinar M, Aydos S, Sakiragaoglu O, Duzen IV, Yalcinkaya A, Oztuna D, Bardakci H, Tutar E, Sunguroglu A. 2012. Impact of genetic variations of the CYP1A1, GSTT1, and GSTM1 genes on the risk of coronary artery disease. DNA Cell Biol 31:211–218.

Xie P, Liang Y, Liang G, Liu B. 2014. Association between GSTP1 Ile105Val polymorphism and glioma risk: a systematic review and meta-analysis. Tumour Biol 35:493–499.

Zubor P, Caliskan M, Kajo K, Soybir G, Topuzlu C, Danko J. 2014. Ethnic disparities in breast cancer between Central Europe Caucasian women of Slavic origin and Middle East Turkish subjects. Neoplasma 61:110–117.

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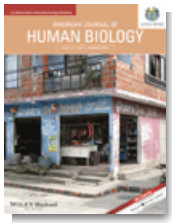

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