

# Polymorphisms at *GSTM1*, *GSTP1*, *GSTT1* Detoxification Genes Loci and Risk of Breast Cancer in Kazakhstan Population

T. S. Balmukhanov, A. K. Khanseitova, V. G. Nigmatova, E. E. Ashirbekov,  
Sh. Zh. Talaeva, N. A. Aitkhozhina

Institute of Molecular Biology & Biochemistry, Almaty, Kazakhstan  
Email: mbbtimur@mail.ru

Received May 20, 2013; revised June 21, 2013; accepted June 29, 2013

Copyright © 2013 T. S. Balmukhanov *et al.* This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## ABSTRACT

Associations of null polymorphism (copy number variation) of detoxification genes *GSTM1*, *GSTT1* and *GSTP1* (at rs2495636, 105 Ile/Val) with the breast cancer (BC) were assessed in two main ethnic groups of the Republic of Kazakhstan (Kazakhs and Russians). Total number of patients was 181, and of controls 397. Statistically significant difference was observed between BC patients and healthy individuals in alleles frequency ( $\chi^2 = 4.89$ ,  $p = 0.007$ ) of *GSTP1* gene at rs2495636 (105 Ile/Val) among the Kazakhs ethnic group. Difference in genotypes distribution ( $\chi^2 = 5.26$ ,  $p = 0.076$ ) at this site is approximating to be statistically significant. In the Russian group, no differences were found in genotypes and alleles at rs 2495636 of *GSTP1* gene between cases and controls. There was no significant difference between null polymorphism (copy number variation) of *GSTM1* and *GSTT1* genes among cases and controls in both ethnic groups.

**Keywords:** Breast Cancer; Gene Polymorphism; *GSTM1*, *GSTP1*, *GSTT1* Genes; Kazakhstan

## 1. Introduction

Breast cancer (BC) is one of the most common cancers among women worldwide. Recent studies and modern GWAS describe that the associations between BC and polymorphisms in genes are involved in xenobiotic detoxification.

*GSTM1*, *GSTP1*, and *GSTT1* genes belong to the glutathione-S-transferases superfamily of genes, whose products are Phase II metabolizing enzymes, which act in coordination with Phase I metabolizing enzymes in the carcinogen metabolism. The Phase I enzymes usually activate the carcinogens to reactive intermediates and the GSTs are active in the detoxification of a wide variety of these potentially toxic and carcinogenic electrophiles by conjugating them to Glutathione [1]. The variations in metabolic activities in each phase or in the coordination of these two phases regulate the clearance of DNA toxic metabolites and might be partially responsible for individual host susceptibility to cancer.

About 50% of both Caucasians and Japanese lack the *GSTM1* gene due to inherited homozygous deletion of both alleles [2]. The individuals with *GSTM1* null poly-

morphism lack the ability of detoxifying specific substrate epoxide intermediates [3]. The *GSTM1* null genotype is also positively associated with high DNA adduct levels, suggesting its role in carcinogenesis [4].

Several investigators have speculated that the early loss of *GSTP1* function leads to increased vulnerability to oxidant and heterocyclic amine carcinogens, implicated in prostate carcinogenesis [5]. Hence, heritable differences in *GSTP1* function may also be associated with prostate cancer development. An A to G polymorphism at nucleotide 313 of *GSTP1* results in an amino acid substitution (Ile105Val) at the substratebinding site of *GSTP1* [6]. The substitution of the less bulkier and more hydrophobic valine results in substrate-dependent alterations of *GSTP1* catalytic activity [6,7]. Positive associations have been reported between the *GSTP1* I105V polymorphism and risk of oral and breast cancers [8,9].

The interest to the associations of the polymorphic variations of *GSTPs* genes with BC is growing owing to the fact that its variations' important role in the sensitivity to the cancer chemotherapy and tumors progression [10]. The results of investigations describing the associations of rs2495636 site of *GSTP1* gene with BC in dif-

ferent world populations are controversial. The studies performed in Turkey, Thailand and China did not reveal this association [11-13]. The data demonstrating positive association of *GSTP1* gene polymorphism that was taken alone with BC and in combination with some other polymorphisms of candidate-genes were also published [14].

The *GSTT1* gene is also one of the members of GST's enzymes family. It has lower glutathione binding activity, along with increased catalytic efficiency. It is shown that null genotype of *GSTT1* contributes to colorectal cancer risk in Asian populations [15]. The data describing the associations of *GSTT1* gene polymorphisms with pseudoexfoliative glaucoma in Pakistani population [16], breast cancer susceptibility [17] and the results of pooled analysis [18] were published earlier.

Republic of Kazakhstan is a multinational state located in the middle of Central Asia with population of about 17 million and its main ethnical groups are Kazakhs (Asians) and Russians (Caucasians). Specific ethnic differences in the distribution of the genotypes and in the frequencies of alleles, which can be used as markers of BC among representatives of different races and nations, were described earlier in numerous publications.

The present study was undertaken to determine the distribution of genotypes and alleles frequency of *GSTP1* (rs2495636), *GSTM1* null polymorphisms and *GSTT1* (Ile105Val) polymorphism among BC cases and controls so as to understand whether these polymorphisms are associated with the risk of BC in Kazakhstan population.

## 2. Materials and Methods

Venous blood samples (5 ml) were collected in EDTA vials from 181 women diagnosed with BC from Almaty (Kazakh Research Institute of Oncology and Radiology). Blood samples from 397 healthy women donors (Almaty) without clinical symptoms and with no family history of cancers were used as a controls. The average age of the BC patients was  $50.3 \pm 11.6$  years (Kazakhs),  $55.7 \pm 11.7$  (Russians) and of the donors  $50.07 \pm 8.47$  (Kazakhs),  $54.8 \pm 5.9$  (Russians) years. Deoxyribonucleoside triphosphates, restriction endonuclease BstMAI, Taq DNA polymerase, BSA were purchased by "SibEnzyme" (Russia).

**Methods.** DNA was isolated from the blood using DNA extraction kits "Axygen" (USA). The sequences of the oligonucleotide primers were obtained from literature sources. Individual SNPs were detected by restriction fragment length polymorphism (RFLP) analysis after PCR amplification.

PCR reactions were carried out using gradient Mastercycler "Eppendorf". Amplification was performed in a buffer containing 67 mM TrisHCl, pH 8.8, 16.6 mM  $(\text{NH}_4)_2\text{SO}_4$ , 2 mM  $\text{MgCl}_2$ , 0.01% Tween 20, 0.15 mg/ml BSA, 2 pM of each of the primers, 0.25 mM each of the

four dNTPs, 1 unit/ $\mu\text{l}$  of Taq DNA polymerase. The oligonucleotide sequences of the primers for the amplification of *GSTM1* gene were F 5'-GAACTCCCTGAA-AAGCTAAAGC-3' and R 5'-GTTGGGCTCAAACG-GTGG-3'; for *GSTT1* gene—F 5'-ttccttactggctcactcactc-3' and R: 5'-TCACCGGATCATGGCCAGCA-3' according to KhanM.I. [8]. The sequences of primers used for  $\beta$  globin gene amplification used as a positive control of amplification were F: 5'-CAACTTCATC-CACGTTACC-3' and R: 5'-GAAGAGCCAAGGA-CAGGTAC-3'. *GSTP1* gene (rs2495636) was amplified using primers F: 5'-ACCCAGGGCTCTATGGGAA-3'; R: 5'-TGAGGGCACAAGAAGCCCCT-3' and digested with BstMAI restrictase [9].

Electrophoresis was carried out in 8% polyacrylamide gel at 40 mA and 150 V for 2 - 3 h.

**Statistical analysis.** The Pearson  $\chi^2$  criterion ( $p < 0.05$ ), odds ratios (OR), 95% confidence intervals (CI) tests were applied to the data analysis. The distribution of genotypes for both investigated groups was in accordance with the Hardy-Weinberg equilibrium (HWE).

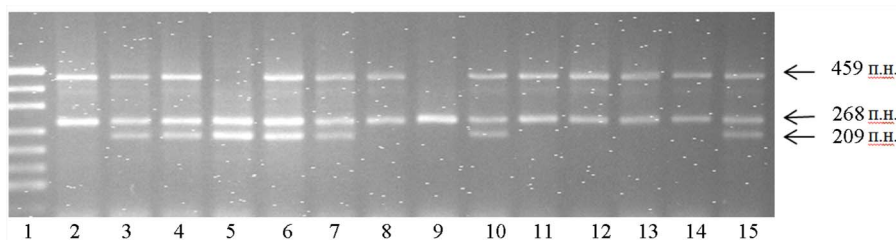
## 3. Results and Discussion

Null polymorphism is a result of lengthy deletion resulting in the formation of truncated form of the protein with decreased enzymatic activity. In current investigation null polymorphism of the *GSTP1* and *GSTM1* genes was tested by means of multiplex PCR. Electrophoretically it is possible to detect the two variants of amplified products: 0/0 (homozygous deletion)—absence of the bands, and either +/0 or +/+ (heterozygous deletion and homozygous with functional genes without deletions) were presented by bands of 459 bp for *GSTT1* gene and of 209 bp for *GSTM1* gene (**Figure 1**). Beta-globine gene was used as a positive control (268 bp). Such approach permits combining the genotypes of the samples according either presence or absence of even sole copy of tested genes.

No differences were found in distribution of *GSTP1* and *GSTM1* genes null polymorphism between patients and controls in both the Kazakh and in Russian groups. Also, no interethnic differences were shown between Kazakhs and Russians in the genotypes distribution. The results are presented in **Table 1**.

To evaluate the interaction between the two genotypes, we examined the combined effect of the *GSTT1* and *GSTM1* genotypes. Reference group of samples having active variants of the genes was used as a base line. The analysis revealed no significant difference among cases and controls in distribution of the genotype combination neither in Kazakh nor in Russian groups.

Thereby the associations of null polymorphic variants of *GSTT1* and *GSTM1* genes with BC were not found in the two tested ethnic groups of Kazakhstan.



**Figure 1.** PAAG-analysis of *GSTT1* and *GSTM1* genes amplified products. Lane 1—molecular mass marker pUC19/Kzo9 I; lanes 2, 8, 11, 12, 13, 14—genotype T1M0; lanes 3, 4, 6, 7, 10, 15—genotype T1M1; lane 9—genotype T0M0.

**Table 1.** Null polymorphism of *GSTT1* and *GSTM1* genes in Kazakh and Russian ethnic groups.

Geno-type	Cases (n = 121)	Controls (n = 220)	Oddsratio (OR), CI (95%)	Relative risk (RR), CI (95%)	$\chi^2$ , (p value)
Kazakhs					
<i>GSTT1</i>					
+	89 (73.6%)	164 (74.5%)	0.95 (0.57 - 1.57)	0.97 (0.70 - 1.39)	0.04 (0.84)
n	32 (26.4%)	56 (25.5%)	1.05 (0.62 - 1.70)	1.04 (0.69 - 1.53)	
<i>GSTM1</i>					
+	57 (47.1%)	102 (46.4%)	1.03 (0.64 - 1.65)	1.02 (0.75 - 1.39)	0.02 (0.89)
n	64 (52.9%)	118 (53.6%)	1.97 (0.61 - 1.55)	0.99 (0.79 - 1.21)	
<i>GSTT1</i> + <i>GSTM1</i>					
+/+	41 (33.9%)	73 (33.2%)		Reference	
+/n	48 (39.7%)	91 (41.3%)	0.94 (0.54 - 1.63)	0.97 (0.75 - 1.24)	0.06(0.81)
n/+	16 (13.2%)	29 (13.2%)	0.98 (0.45 - 2.14)	0.99 (0.55 - 1.70)	0.00(0.96)
n/n	16 (13.2%)	27 (12.3%)	1.06 (0.48 - 2.32)	1.04 (0.58 - 1.81)	0.21(0.89)
Russians					
Geno-type	Cases (n = 60)	Controls (n = 177)	Oddsratio (OR), CI (95%)	Relative risk (RR), CI (95%)	$\chi^2$ (p value)
<i>GSTT1</i>					
+	46 (76.7%)	143 (80.8%)	0.78 (0.37 - 1.68)	0.95 (0.79 - 1.10)	0.47 (0.49)
n	14 (23.3%)	34 (19.2%)	1.28 (0.50 - 2.73)	1.22 (0.65 - 2.15)	
<i>GSTM1</i>					
+	29 (48.3%)	85 (48.0%)	1.01 (0.54 - 1.80)	1.01 (0.71 - 1.36)	0.00 (0.97)
n	31 (51.7%)	92 (52.0%)	0.99 (0.53 - 1.85)	0.99 (0.72 - 1.31)	
<i>GSTT1</i> + <i>GSTM1</i>					
+/+	23 (38.3%)	68 (38.4%)		Reference	
+/n	23 (38.3%)	75 (42.4%)	0.91 (0.44 - 1.86)	0.95 (0.65 - 1.31)	0.08 (0.77)
n/+	6 (10.0%)	17 (9.6%)	1.04 (0.32 - 3.27)	1.03 (0.38 - 2.45)	0.01 (0.94)
n/n	8 (13.3%)	17 (9.6%)	1.39 (0.48 - 4.01)	1.29 (0.55 - 2.79)	0.45 (0.50)

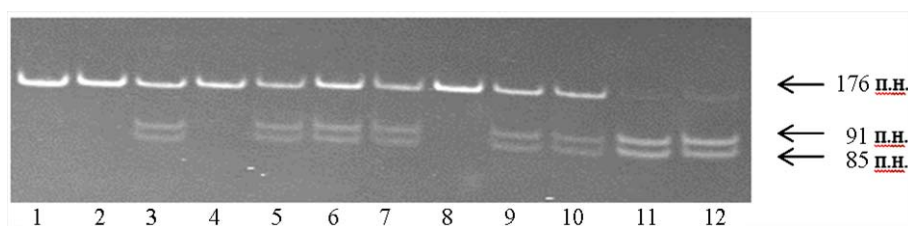
The search of association of BC with 105 Ile/Val polymorphism of *GSTP1* gene at site rs2495636 was performed by restriction fragment length polymorphism (RFLP) analysis.

The PCR product of 176 bp was subjected to restriction digestion by BstMAI at 37° for 4 hour and DNA band was resolved by electrophoresis. The genotypes were determined based on the band pattern.

The Val allele contains BstMAI specific sequence whereas the Ile allele lack such sequence; thus electro-

phoretically Ile/Ile genotype is identified as an undigested band of 176 bp, the Val/Val genotype—as bands of 91 bp and 85 bp and Ile/Val genotype, is determined by three fragments of 176 bp, 91 bp and 85 bp (**Figure 2**). The results are presented in the **Table 2**.

As it can be seen from the results presented in **Table 2**, no significant differences were found between cases and control in allele frequencies and genotypes distribution of the tested sites of *GSTT1* and *GSTM1* genes in the Russian group. Interethnic differences in alleles and genotypes



**Figure 2.** 8% PAAG-analysis of the RFLP-PCR products of *GSTP1* gene (rs2495636). Lanes 1, 2, 4, 8—genotype A/A (Ile/Ile); lanes 3, 5, 6, 7, 9, 10 A/G (Ile/Val); lanes 11, 12—genotype G/G (Val/Val).

**Table 2.** Genotypes distribution and allele frequency at rs 2495636 of *GSTP1* gene in Kazakh and Russian ethnic groups.

Kazakhs						
Alleles/genotypes	Frequency		OR	CI (95%)	$\chi^2$	p
	Cases, n = 120	Controls, n = 219				
A	0.714	0.790	0.66	0.46 - 0.96	<b>4.89</b>	0.03 (0.007)
G	0.286	0.210	1.50	1.05 - 2.16		
AA	0.521	0.621	0.66	0.42 - 1.04	<b>5.26</b>	0.07 (0.076)
AG	0.387	0.338	1.23	0.78 - 1.96		
GG	0.092	0.041	2.38	0.96 - 5.91		
Russians						
Alleles/genotypes	Frequency		OR	CI (95%)	$\chi^2$	p
	Cases, n = 60	Controls, n = 177				
A	0.650	0.701	0.79	0.51 - 1.23	1.07	0.3 (0.056)
G	0.350	0.299	1.26	0.81 - 1.95		
AA	0.400	0.452	0.81	0.45 - 1.47	1.99	0.37 (0.351)
AG	0.500	0.497	1.01	0.56 - 1.82		
GG	0.100	0.051	2.07	0.71 - 6.09		

In the brackets the means of the data correction according Fisher's test are shown.

distribution between the Kazakhs and Russians also were not revealed. In both ethnic groups, the genotypes' distribution was in accordance with HWE.

In the Kazakh group the statistically significant differences in allele frequency and genotypes distribution between cases and controls were demonstrated. The value of odds ratio (OR) for the allele G is equal to 1.50 (95% CI = 1.05 - 2.16) and OR for genotype GG—2.38 (95% CI = 0.96 - 5.91), which can be indicative for regarding of this polymorphism as a risk factor of BC in Kazakh ethnic group of Kazakhstan population.

## REFERENCES

- [1] R. C. Strange, J. T. Lear and A. A. Fryer, "Glutathione S-Transferase Polymorphisms: Influence on Susceptibility to Cancer," *Chemico-Biological Interactions*, Vol. 111-112, 1998, pp. 351-364. [http://dx.doi.org/10.1016/S0009-2797\(97\)00172-5](http://dx.doi.org/10.1016/S0009-2797(97)00172-5)
- [2] T. R. Rebbeck, "Molecular Epidemiology of the Human Glutathione S-Transferase Genotypes GSTM1 and GSTT1 in Cancer Susceptibility," *Cancer Epidemiology, Bio-markers & Prevention*, Vol. 6, No. 9, 1997, pp. 733-743.
- [3] V. Nazar-Stewart, A. G. Motulsky and D. L. Eaton, "The Glutathione S-Transferase mu Polymorphism as a Marker for Susceptibility to Lung Carcinoma," *Cancer Research*, Vol. 53, No. S10, 1993, pp. 2313-2318.
- [4] J. K. Wiencke, K. T. Kelsey, R. A. Lamela and W. A. Toscano Jr., "Human Glutathione S-Transferase Deficiency as a Marker of Susceptibility to Epoxide-Induced Cytogenetic Damage," *Cancer Research*, Vol. 50, No. 5, 1990, pp. 1585-1590.
- [5] W. G. Nelson, A. M. De Marzo and T. L. DeWeese, "The Molecular Pathogenesis of Prostate Cancer: Implications for Prostate Cancer Prevention," *Urology*, Vol. 57, No. S4, 2001, pp. 39-45. [http://dx.doi.org/10.1016/S0090-4295\(00\)00939-0](http://dx.doi.org/10.1016/S0090-4295(00)00939-0)
- [6] F. Ali-Osman, O. Akande and G. Antoun, "Molecular Cloning, Characterization, and Expression in *Escherichia coli* of Full-Length cDNAs of Three Human Glutathione S-Transferase Pi Gene Variants. Evidence for Differential Catalytic Activity of the Encoded Proteins," *The Journal of Biological Chemistry*, Vol. 272, No. 15, 1997, pp. 10004-10012. <http://dx.doi.org/10.1074/jbc.272.15.10004>
- [7] K. Sundberg, A. S. Johansson and G. Stenberg, "Differences in the Catalytic Efficiencies of Allelic Variants of

- Glutathione Transferase P1-1 towards Carcinogenic Diol Epoxides of Polycyclic Aromatic Hydrocarbons," *Carcinogenesis*, Vol. 19, No. 3, 1998, pp. 433-436. <http://dx.doi.org/10.1093/carcin/19.3.433>
- [8] S. K. Park, D. S. Yim, K. S. Yoon, I. M. Choi, J. Y. Choi, K. Y. Yoo, D. Y. Noh, K. J. Choe, S. H. Ahn, A. Hirvonen and D. Kang, "Combined Effect of GSTM1, GSTT1, and COMT Genotypes in Individual Breast Cancer Risk," *Breast Cancer Research and Treatment*, Vol. 88, No. 1, 2004, pp. 55-62. <http://dx.doi.org/10.1007/s10549-004-0745-x>
- [9] K. Mitrunen, N. Jourenkova, V. Kataja, M. Eskelinen, V. M. Kosma, S. Benhamou, H. Vainio, M. Uusitupa and A. Hirvonen, "Glutathione S-Transferase M1, M3, P1, and T1 Genetic Polymorphisms and Susceptibility to Breast Cancer," *Cancer Epidemiology, Biomarkers & Prevention*, Vol. 10, No. 3, 2001, pp. 229-236.
- [10] A. L. Oliveira, F. F. Rodrigues, R. E. Santos, T. Aoki, M. N. Rocha, C. A. Longui and M. B. Melo, "GSTT1, GSTM1, and GSTP1 Polymorphisms and Chemotherapy Response in Locally Advanced Breast Cancer," *Genetics and Molecular Research*, Vol. 9, No. 2, 2010, pp. 1045-1053. <http://dx.doi.org/10.4238/vol9-2gmr726>
- [11] T. Pongtheerat, M. Tretrisool and W. Purisa, "Glutathione S-Transferase Polymorphisms in Breast Cancers of Thai Patients," *Asian Pacific Journal of Cancer Prevention*, Vol. 10, No. 1, 2009, pp. 127-132.
- [12] B. Kiran, M. Karkucak, H. Ozan, T. Yakut, K. Ozerkan, S. Sag and M. J. Ture, "GST (GSTM1, GSTT1, and GSTP1) Polymorphisms in the Genetic Susceptibility of Turkish Patients to Cervical Cancer," *Journal of Gynecologic Oncology*, Vol. 21, No. 3, 2010, pp. 169-173. <http://dx.doi.org/10.3802/jgo.2010.21.3.169>
- [13] L. C. Sakoda, C. R. Blackston, K. Xue, J. A. Doherty, R. M. Ray, M. G. Lin, H. Stalsberg, D. L. Gao, Z. Feng, D. B. Thomas and C. Chen, "Glutathione S-Transferase M1 and P1 Polymorphisms and Risk of Breast Cancer and Fibrocystic Breast Conditions in Chinese Women," *Breast Cancer Research and Treatment*, Vol. 109, No. 1, 2008, pp. 143-155. <http://dx.doi.org/10.1007/s10549-007-9633-5>
- [14] B. O. Van Emburgh, J. J. Hu, E. A. Levine, L. J. Mosley, N. D. Perrier, R. I. Freimanis, G. O. Allen, P. Rubin, G. B. Sherrill, C. S. Shaw, L. A. Carey, L. R. Sawyer and M. S. Miller, "Polymorphisms in CYP1B1, GSTM1, GSTT1 and GSTP1, and Susceptibility to Breast Cancer," *Oncology Report*, Vol. 19, No. 5, 2008, pp. 1311-1321.
- [15] D. Xu, S. Yan, J. Yin and P. Zhang, "Null Genotype of GSTT1 Contributes to Colorectal Cancer Risk in Asian Populations: Evidence from a Meta-Analysis," *Asian Pacific Journal of Cancer Prevention*, Vol. 12, No. 9, 2011, pp. 2279-2284.
- [16] M. I. Khan, S. Micheal, F. Akhtar, W. Ahmed, B. Ijaz, A. Ahmed and R. Qamar, "The Association of Glutathione S-Transferase GSTT1 and GSTM1 Gene Polymorphism with Pseudoexfoliative Glaucoma in a Pakistani Population," *Molecular Vision*, Vol. 26, No. 16, 2010, pp. 2146-2152.
- [17] A. C. Ramalhinho, J. A. Fonseca-Moutinho and L. A. Breitenfeld Granadeiro, "Positive Association of Polymorphisms in Estrogen Biosynthesis Gene, CYP19A1, and Metabolism, GST, in Breast Cancer Susceptibility," *DNA and Cell Biology*, Vol. 31, No. 6, 2012, pp. 1100-1106. <http://dx.doi.org/10.1089/dna.2011.1538>
- [18] F. D. Vogl, E. Taioli, C. Maugard, W. Zheng, L. F. Pinto, C. Ambrosone, F. F. Parl, V. Nedelcheva-Kristensen, T. R. Rebbeck, P. Brennan and P. Boffetta, "Glutathione S-Transferases M1, T1, and P1 and Breast Cancer: A Pooled Analysis," *Cancer Epidemiology, Biomarkers & Prevention*, Vol. 13, No. 9, 2004, pp. 1473-1479.