# Studying the Association of Polymorphic Variants of GSTM1 and GSTT1 Genes with Breast Cancer in Female Residents of Altai Krai

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The incidence of homozygote deletion of glutathione S-transferase genes M1 and T1 (null genotypes; or GSTM1"-" and GSTT1"-") was studied in breast cancer patients living in Altai Krai. DNA was isolated from blood samples of 695 breast cancer patients (291 patients with familial cancer and 404 patients with sporadic cancer) and 263 women without history of tumor diseases. The frequency of GSTM1"-" and c GSTT1"-" genotypes was estimated in breast cancer patients (47.2 and 19.1%, respectively) and non-cancer participants (46.8 and 19.0%, respectively). No differences were found in the frequency of genotypes. The frequency of genotype combination GSTM1"-"+GSTT1"-" in patients with sporadic breast cancer (11.6%, 47 of 404 patients) was higher than in the control (6.1%, 16 of 263 patients; OR=2.03; 95% CI=2.09-3.83; $p$=0.02). The genotype frequency of genes in the control group did not differ from that in European residents of the Caucasian race.

**Key Words:** *GSTM1; GSTT1, breast cancer; real-time PCR*

Breast cancer (BC) is the most common cause of death and ranks first among tumor diseases in women of 40-69 years. Moreover, BC ranks second to cervical carcinoma (women of 15-39 years) and colorectal cancer (women of 70 years or over) [2]. In 2005, the percentage of BC was 19.8% of malignant neoplasms in women. Only one third of morbid events were associated with known risk factors. These factors include the age, familial history, pre-irradiation of the mammary glands, early menarche and late menopause, absence of children or breast feeding, alcohol consumption, obesity, low physical activity, *etc*. [1]. Previous studies showed that cancerogenesis is related to the interaction between genetic factors, environmental factors, and life style [3].

Dysfunction of the enzymes involved in detoxification of carcinogenic substances and DNA reparation can increase the risk of cancer cell development. They include glutathione S-transferases (GST), which catalyze detoxification of electrophilic compounds due to binding with the tripeptide glutathione. Depending on the primary structure, the GST family is classified into 7 classes of genes [3]. Here we studied 2 enzymes of the GST family that belong to various classes (GSTM1 and GSTT1).

GSTM1 is involved in detoxification of carcinogenic epoxides, polycyclic aromatic carbohydrates, and benzopyrene derivatives. GSTT1 plays a key role in detoxification of dichloromethane and halogen derivatives of methane that are present in tobacco smoke

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and industrial smoke. Moreover, GSTT1 contributes to the metabolism of vinyl chloride and ethylene oxide.

Allelic variants of these genes are designated as GSTM1*0 and GSTT1*0. Extensive deletions in the gene structure determine the absence of RNA and proteins. Previous observations showed that 40-60 and 15-25% Caucasian people are homozygotes of GSTM1*0 and GSTT1*0 variants, respectively.

GSTM1 0/0 genotype carriers are characterized by increased risk of lung cancer [7], acute myeloid leukemia, cancer of the urinary bladder [7], larynx, and stomach, systemic lupus erythematosus [11], chronic obstructive lung diseases, and other disorders. GSTT1 0/0 homozygotes are strongly predisposed to cancer of the lungs, oral cavity, pharynx, and larynx, pancreatic adenocarcinoma, astrocytoma, meningioma [7], acute myeloid leukemia, and acute lymphoblastic leukemia.

There is no general agreement about association of the polymorphic variants in GSTM1 and GSTT1 genes with the risk of BC. Some authors reported that carriers of GSTM1 0/0 genotypes [8] and GSTT1 0/0+GSTM1 0/0 genotypes [9,11] are characterized by increased risk of BC. However, the majority of studies failed to reveal this association [3,5,6,9,12].

This work was designed to evaluate the role of GSTM1 and GSTT1 gene polymorphism in the predisposition to familial and sporadic BC in female residents of Altai Krai.

## MATERIALS AND METHODS

The main group consisted of 695 patients with BC (Altai Branch of the N. N. Blokhin Russian Oncology Research Center and Altai Regional Oncologic Dispensary). The control group included 263 women (19-84 years old, mean age 54±13 years) without history of tumor diseases. The main group was divided into 2 subgroups. Subgroup 1 (familial cancer) consisted of 291 patients (9-74 years old, mean age 47±11 years) with familial BC (BC in two patients younger than 50 years (in one family), three or more patients with BC, and familial association of BC and other oncology diseases) or severe disease (disease onset at the age of below 40 years, bilateral cancer, combination of BC with ovarian cancer or other oncology disease).

Subgroup 2 (sporadic cancer) consisted of 404 patients (50-79 years old, mean age 53±9 years) with BC, which were not classified to subgroup 1.

The following characteristics were taken into account during examination of main group patients: age, menopausal status, diagnosis, date of clinical and pathomorphological diagnosis of BC, familial history of BC, attitude to smoking, body height and weight. The main inclusion criterion for main group patients was histologically verified diagnosis of BC.

All women were asked to sign informed consent for participation in this trial (according to the requirements of the Ethics Committee).

Venous blood samples were obtained from women. DNA was isolated by the standard method of phenol-chloroform extraction.

The presence of deletions in GSTM1 and GSTT1 genes was evaluated by the method of real-time PCR. Oligonucleotide primers for PCR were selected in the region of deletions in GSTM1 and GSTT1 genes. The PCR product was not synthesized during the study of DNA samples with GSTM1 0/0 and GSTT1 0/0, respectively. Primers for amplification of short low-melting A/T-rich DNA fragment (LTM, low temperature melting) were introduced into the amplification mixture to distinguish homozygous deletion in GSTT1 and GSTM1 genes from the absence of the DNA matrix or inhibition of PCR.

Amplified fragments had the following size: GSTM1, 229 b.p.; GSTT1, 287 b.p.; and LTM, 127 b.p. The calculated annealing temperature for all primers was 64-66°C. The expected melting temperature for amplification products was 86.5 (GSTM1), 92.5 (GSTT1), and 78.5°C (LTM).

The PCR mixture consisted of 65 mM Tris-HCl (pH 8.9), 0.05% Tween 20, 16 mM $(NH_4)_2SO_4$, 2.4 mM $MgCl_2$, 0.2 mM dNTP, 0.3 μM solutions of oligonucleotide primers, 0.8X SYBR GreenI, 20-100 ng DNA matrix, and 0.5 U thermostable Taq polymerase. The volume of the reaction mixture was 25 μl.

Amplification was performed on an iCycler iQ5 amplifier (Bio-Rad).

PCR was conducted as follows: initial denaturation at 95°C for 3 min; 18 cycles consisting of denaturation at 95°C for 12 sec; annealing of primers at 64°C for 12 sec, and elongation at 72°C for 14 sec; 19 cycles including denaturation at 95°C for 12 sec, annealing of primers at 62°C for 12 sec, and elongation at 72°C for 12 sec. The fluorescence signal was recorded at 78°C for 10 sec. The fluorescence signal for GSTM1 and GSTT1 was recorded at 84 and 91°C, respectively, for 10 sec. The melting curves were constructed over 60 cycles. During each cycle, the temperature was increased by 0.5°C (relative to the initial temperature, 65°C). The fluorescence signal was recorded during each cycle. The melting temperature for PCR products was 78 (LTM), 84 (GSTM1), and 91°C (GSTT1).

The frequency of genotypes of polymorphic loci in study groups was evaluated by $\chi^2$ test (Statistica software, StatSoft Inc.).

## RESULTS

Series I was performed to optimize the method for genotyping of homozygous deletions in GSTT1 and

GSTM1 genes by means of PCR with an intercalating fluorescent dye SyberGreenI dye and analysis of melting curves.

Figure 1, *a* shows the results of electrophoretic separation of PCR products (GSTM1 locus). Two bands (229 and 127 b.p.) correspond to the sample, which carries at least one wild-type allele. One band (127 b.p.) corresponds to the sample that carries a homozygous deletion in the GSTM1 gene. Figure 1, *b* shows the derivatives of melting curves for PCR products. The presence of a homozygous deletion in the DNA sample is related to one peak with a maximum at 82°C, which corresponds to melting of the control fragment LTM. The presence of even one intact allele leads to the appearance of the second peak with a maximum at 87°C, which corresponds to melting of the PCR fragment GSTM1. The wild-type control fragment LTM is sometimes suppressed. A similar study and interpretation were performed for the GSTT1 locus.

The proposed method was used for genotyping of DNA samples from the main and control groups. The frequency of genotypes did not differ in the main and control groups. No differences were revealed between subgroups 1 and 2 (Table 1).

The samples were analyzed for the frequency of combined genotypes (Table 2). The frequency of genotype combination GSTM1 0/0+GSTT1 0/0 in female patients with sporadic BC (11.6%, 47 of 404 patients) was higher than in the control (6.1%, 16 of 263 patients; OR=2.03; 1.09-3.83; $p$=0.02). No differences were found in the association between polymorphic variants of GSTM1 and GSTT1 genes, smoking, and disease onset (postmenopause or premenopause).

Many oncological diseases are associated with the influence of environmental carcinogens. Genetic polymorphism of enzymes for metabolism of carcinogenic substances can potentiate or suppress this effect, which is related to variations in the amount and quality of protein products of genes. Genetic polymorphism probably serves as the risk factor for the development of oncological diseases [3]. The development of BC

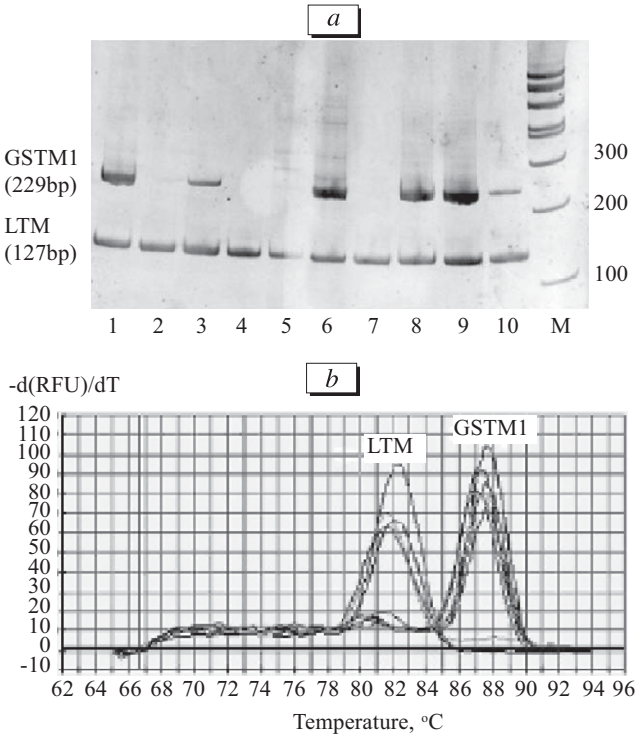


**Fig. 1.** Electrophoregram of products of PCR analysis of polymorphic locus GSTM1 (*a*). Derivatives of melting curves for DNA samples (*b*). (*a*) 1-10, DNA samples; M, molecular weight marker. 2, 4, 5: homozygous deletion of GSTM1. 1, 3, 6-10: presence of even one wild-type allele of GSTM1. (*b*) LTM peak corresponds to melting of DNA samples that have a homozygous deletion in the GSTM1 gene (samples 2, 4, 5, and 7). GSTM1 peak: melting of DNA samples that have even one functional allele of GSTM1 (samples 1, 3, 6, and 8-10).

**TABLE 1.** Frequency of Genotypes "+"(N/N, N/0) and "-"(0/0) for Polymorphic Variants of GSTM1 and GSTT1 Genes in Groups

| Group | GSTM1+ | GSTM1- | OR (95% CI) | *p* |
|---|---|---|---|---|
| Main group | 0.528 (376) | 90.472 (328) | 1.02 (0.76-1.37) | 0.90 |
| subgroup 1 | 0.543 (158) | 0.457 (133) | 0.96 (0.68-1.36) | 0.80 |
| subgroup 2 | 0.517 (209) | 0.483 (195) | 1.06 (0.77-1.47) | 0.70 |
| Control group | 0.532 (140) | 0.468 (123) | | |
| | GSTT1+ | GSTT1- | OR (95% CI) | *p* |
| Main group | 0.809 (563) | 0.191 (133) | 1.01 (0.69-1.47) | 0.97 |
| subgroup 1 | 0.835 (563) | 0.191 (133) | 1.01 (0.69-1.47) | 0.97 |
| subgroup 2 | 0.792 (320) | 0.208 (85) | 1.13 (0.75-1.70) | 0.53 |
| Control group | 0.810 (213) | 0.190 (50) | | |

**TABLE 2.** Frequency of Combined Genotypes GSTM1 and GSTT1 Genes in Groups

| Group | GSTM1-/GSTT1- | GSTM1-/GSTT1+ | GSTM1+/GSTT1- | GSTM1+/GSTT1+ |
|---|---|---|---|---|
| Main group | 0.102 (71) | 0.370 (257) | 0.088 (61) | 0.440 (306) |
| subgroup 1 | 0.082 (24) | 0.375 (109) | 0.082 (24) | 0.460 (134) |
| subgroup 2 | 0.116 (47)* | 0.366 (148) | 0.092 (37) | 0.426 (172) |
| Control group | 0.061 (16)* | 0.407 (107) | 0.106 (28) | 0.426 (112) |

**Note.** *OR=2.03 (95% CI 1.09-3.83), $p$=0.01.

is associated with a variety of risk factors, including familial history, early menarche and late menopause, absence of children or breast feeding, alcohol consumption, obesity, *etc*.

Gene products of the GST family play an important role in detoxification of carcinogens and development of some oncological diseases [7]. The involvement of GSTM1 and GSTT1 enzymes in the pathogenesis of BC is probably related to the following fact. After metabolism with the cytochrome p450 system (CYP3A4 and CYP1B1), estrogens are subsequently converted into catechol estrogens and reactive quinine derivatives. These substances can be inactivated by conjugation with glutathione, which involves GSTM1 and GSTT1 enzymes. These data explain the protective role of enzymes.

Our findings on the frequency of genotypes for these genes are consistent with the results of previous studies with European residents of the Caucasian race [12] (Table 3).

We showed that polymorphic variants of GSTM1 and GSTT1 genes do not increase the risk of BC.

No correlation was found between polymorphic variants of GST genes and smoking. However, the number of smokers in subgroup 1 (9.6%, 28 of 291 patients) was much greater than in subgroup 2 (2.2%, 9 of 404 patients; $p$=0.00004) and control group (1.5%, 4 of 263 patients; $p$=0.00009). An adequate statistical analysis of the association between smoking, BC, and polymorphism of GST genes was not performed due to a small number of smokers in the control group.

Some authors revealed an association between the GSTM1 0/0 genotype and BC in premenopause [10] or postmenopause [4,8,14]. Other investigators reported no association with the GSTM1 0/0 genotype. However, an association was found between the GSTT1 0/0 genotype and postmenopausal cancer [13]. Meta-analysis showed that BC is not associated with the deletion polymorphism of GSTT1 and GSTM1 genes during postmenopause [12].

No association was found between the polymorphism in GSTM1 and GSTT1 genes, menopausal period onset, and age of BC onset.

It cannot be excluded that the absence of association is related to late diagnostics of cancer and incorrect formation of groups (premenopausal and postmenopausal cancer).

The study of combined genotypes showed that the combination of genotypes GSTM1 0/0+GSTT1 0/0 is more typical of patients with sporadic BC (as compared to the control group).

In the present study, no association was found between polymorphic variants of GSTM1 and GSTT1 genes and BC. However, it cannot be excluded that these genes in combination with polymorphic variants of other genes (catechol-O-methyl transferase, COMT; and N-acetyltransferase 1 and 2, NAT1 and NAT2) have a modulatory effect on the risk of disease development.

**TABLE 3.** Frequency of GSTM1 0/0 and GSTT1 0/0 Alleles in Various Populations

| Country | Population control | Statistical significance of differences in the frequency |
|---|---|---|
| GSTM1 0/0 | | |
| Altai Krai, Russia | 0.532 (140/263) | |
| France | 0.517 (226/437) | $\chi^2$=0.15, $p$=0.69 |
| USA | 0.506 (171/338) | $\chi^2$=0.16, $p$=0.68 |
| Norway | 0.454 (89/196) | $\chi^2$=2.11, $p$=0.14 |
| GSTM1 0/0 | | |
| Altai Krai, Russia | 0.19 (50/263) | |
| Norway | 0.174 (34/196) | $\chi^2$=0.21, $p$=0.65 |
| USA | 0.20 (65/325) | $\chi^2$=0.09, $p$=0.76 |

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