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ORIGINAL ARTICLE

Association between Glutathione S-Transferase M1 and T1 Polymorphisms and Colorectal Cancer Risk in Patients from Kazakhstan

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SUMMARY

Background: **Colorectal cancer (CRC) is one of the most common malignancies worldwide and the incidence is increasing in developed as well as developing countries including Kazakhstan. Glutathione S-transferases (GSTs) are considered to be cancer susceptibility genes as they play a role in the detoxification of carcinogenic species. In this case-control study the influence of *GSTM1* and *GSTT1* polymorphisms on CRC risk in Kazakhstan population were evaluated.**

Methods: **Blood samples were collected from patients diagnosed with rectal or colon cancer (300 individuals) as well as a control cohort of healthy volunteers (300 individuals), taking into account the age, gender, ethnicity, and smoking habits of the CRC patients. Deletion polymorphisms were genotyped employing a multiplex PCR amplification method. Association between polymorphisms and CRC susceptibility risk was calculated using multivariate analysis and logistic regression for odd ratio (OR).**

Results: **The homozygous *GSTM1* null genotype was associated with significantly increased risk of CRC (OR = 2.01, 95% CI = 1.45 - 2.79, p = 0.0001) while the homozygous *GSST1* null genotype was not associated with the risk of developing CRC (OR = 1.10, 95% CI = 0.78 - 1.55, p = 0.001), but the heterozygous genotype correlated with CRC susceptibility (OR = 1.98, 95% CI = 1.30 - 3.00, p = 0.001). Also, separate analyses of each of the main ethnic groups (Kazakh and Russian) showed a strong association of *GSTM1* null genotype with CRC risk (for Kazakhs OR = 2.36, 95% CI = 1.35 - 4.10, p = 0.006 and for Russians OR = 1.84, 95% CI = 1.17 - 2.89, p = 0.003). The CRC risk of *GSTM1* null genotype in smokers was considerably higher (OR = 3.37, 95% CI = 1.78 - 6.38, p = 0.0007). The combination of the *GSTM1* and *GSTT1* null genotypes in combined mixed population of Kazakhstan showed a trend to increasing the risk of developing CRC (OR = 1.60, 95% CI = 1.00 - 2.56), but it was not statistically significant.**

Conclusions: **In conclusion, the results of this case-control study for sporadic cases of CRC show that *GSTM1* deletion polymorphisms can have predictive value for susceptibility to CRC (OR = 2.01, p = 0.0001) for the mixed population from Kazakhstan and for both main ethnic groups (Kazakhs and Russians (OR = 2.36 and OR = 1.84, respectively)).**

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INTRODUCTION

Colorectal cancer (CRC) is a leading cause of morbidity and mortality in developed as well as developing countries. Globally more than 1 million people get CRC every year resulting in about 0.5 million deaths [1].

Countries with the highest incidence rates are Europe, Australia, New Zealand, and North America, whereas the lowest rates are found in South-Central Asia, and in some parts of Africa and South America [2]. Among Eurasian countries, Kazakhstan has the seventh highest incidence of CRC. Most cases are diagnosed at a very late stage of cancer progression (stages III - IV), when treatment is expensive and the prognosis is very poor. CRC is one of the diseases, for which the preventive measures are most effective. Screening has been shown to reduce CRC incidence and mortality, but organized screening programs are still to be implemented in most countries. Primary prevention aims to identify germline mutations which are related with a high risk of developing cancer. It is known that screening for early diagnosis reduces the risk of CRC developing by 56% and the total mortality by 65%. Secondary prevention of cancer is aimed at screening probands relatives, identification of families with cancer burdened history, their medicogenetic counseling, and mass health clinical examination of high risk patients. Currently, this is the only approach to the organization and functioning of the hereditary cancer forms genetic preventive program. In Kazakhstan, such an exercise is underway; however, the National Screening Program for malignant neoplasms of colon and rectum did not begin until 2011.

More than 75% of CRC cases occur sporadically, and 25% of cases have a family history of CRC that suggests a hereditary contribution. Various studies have shown that 15 - 20% of patients with CRC have a first-degree relatives suffering from the same types of cancer [3,4]. Despite the fact that sporadic CRC arises from complex interactions between environmental and genetic factors, the exact role of the genetic background related to sporadic CRC remains unclear.

The glutathione S-transferase (GST) supergene family is an important cellular defense mechanisms [5]. GSTs, a multigene family of the phase II metabolising enzymes, are active in the detoxification of a wide variety of potentially toxic and carcinogenic substances by conjugating them to glutathione. In humans, GSTs consist of five distinct families of genes namely α (GSTA), μ (GSTM), π (GSTP), σ (GSTS), and θ (GSTT) [6].

Among them, it is known that *GSTM1*, *GSTT1*, and *GSTP1* detoxification enzymes can metabolize a wide range of carcinogens such as polycyclic aromatic hydrocarbons and heterocyclic aromatic amines from tobacco smoke and diet [7]. Many GTS genes are characterized by the presence of polymorphisms, significantly reducing or completely turning off the functional activity of the corresponding protein product. This can lead to a number of diseases and pathological states. This xenobiotic metabolising enzyme may modify susceptibility in certain racial and ethnic groups, showing ethnic dependent polymorphism. Several authors recognized an important role in the development of various cancers of extended deletions of *GSTM1* and *GSTT1* genes, which are located on chromosome 1p13.3, and 22q11. Individuals with at least one functional allele for *GSTM1* and *GSTT1* are grouped into the positive conjugator types, and called *GSTM1*-positive and *GSTT1*-positive, respectively. Homozygous deleted genotypes in the respective genes that lead to the inactive form of the enzymes were named “null” genotypes [8-12]. It has been postulated that *GSTM1* and *GSTT1* deficiency increased CRC susceptibility, but the link between allelic variants in the GST gene family and CRC remains unclear and case-control studies remain inconsistent and need to be elucidated [13]. Therefore, the aim of this case-control study was to test the possible association between *GSTM1* and *GSTT1* deletion polymorphisms and the risk of developing CRC in patients from Kazakhstan.

MATERIALS AND METHODS

Patient Sampling

For this case-control study, blood samples were collected from 300 patients diagnosed with CRC at the Almaty Oncology Centre (Almaty, Kazakhstan) after receiving informed consent from the patients. Control bloods were collected from 300 healthy donors. The control group of healthy individuals was selected according to the age, gender, ethnicity, and smoking habits in our CRC patient cohort. Controls were biologically unrelated to the patients and had no known family history of malignancies. Detailed questionnaires and informed consents were obtained prior to collection of samples. The study protocol was approved by the Ethics Committee of the Asfendiyarov Kazakh National Medical University (Almaty, Kazakhstan).

DNA isolation

Peripheral blood was collected aseptically by venipuncture into 9 ml EDTA-coated vacutainer tubes. Genomic DNA was extracted from it using the standard phenol-chloroform method with modifications in the composition of the lysis buffer; 0.2 M sodium acetate and 1% sodium dodecyl sulfate, pH 8.0 [14]. The quantity and quality of the samples redissolved in distilled water DNA were evaluated by a spectrophotometer (Eppendorf BioPhotometer plus). The extracted DNA samples were stored at -20°C until further analysis.

GSTM1 and *GSTT1* genotyping

The genotyping of *GSTM1* and *GSTT1* deletion polymorphisms was carried out by multiplex PCR amplification using human *β-globin* as an internal control. 20 - 60 ng of target DNA was amplified in a total volume of 20 μL of PCR mixture using the "Mastercycler" (Eppendorf, Germany). PCR reactions contained 15 pM of each specific primer (for the *GSTM1*:

s 5'-GAACTCCCTGAAAAGCTAAAGC-3',
as 5'-GTTGGGCTCAAATATACGGTGG-3'; for the *GSTT1*: s 5'-CCTTACTGGTCCTCACATCTC-3',
as 5'-TCACCGGATCATGGCCAGCA-3'; for *β-globin*: s 5'-CCACTTCATCCACGTTCACC-3',
as 5'-GAAGAGCCTAGGACAGGTAC-3'),
10 mM of each dNTP, 2 µl of 10xPCR buffer (10 mM KCl, 100 mM Tris HCl, pH 9.0) and 0.5 U of Taq-polymerase (Sigma-Aldrich, USA). The PCR consisted of an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 2 minutes, annealing at 59°C for 1 minute, elongation at 72°C for 1 minute and a final extension step at 72°C for 10 minutes. The amplified products were visualized by electrophoresis in ethidium bromide stained 1.5% agarose gel. The PCR product of *GSTM1*, *GSTT1* and *β-globin* was 215, 480, and 268 base pairs in length, respectively. Gene deletion was assumed as the absence of either one or both of the *GSTM1* or *GSTT1* fragments in the presence of human *β-globin* fragment.

Statistical analysis

The Student's *t*-test was used to compare the distribution of variables between case and control cohorts. The allele frequencies were calculated in accordance with standard Hardy-Weinberg equilibrium. To estimate the relative risk of CRC development we used multivariate analysis and logistic regression for odds ratio (OR) and the 95% confidence intervals (95% CI) calculation using the general model (analysis of each genotype separately), the dominant model (normal homozygotes versus combination of heterozygotes with polymorphic homozygotes) and the recessive model (combination of normal homozygotes with heterozygotes versus polymorphic homozygotes). We did separate analyses for the main ethnic groups (Kazakh and Russian). A p-value of < 0.05 was considered statistically significant. All statistical analysis of the data was performed using GraphPad InStat™ Software (V. 2.04. Ralf Stahlman, Purdue University) and "Case-Control Study Estimating Calculator" from Gene Expert company ("GosNII Genetika" State Scientific Centre of Russian Federation; http://gen-exp.ru/calculator_or.php).

RESULTS

Characteristic of the study population

This case-control study included 300 CRC patients and 300 age, gender, ethnicity, and smoking habits matched cancer free healthy individuals (Table 1). There were no significant differences in the distribution of age, gender, and ethnicity between the cases and controls. The percentage of non-smokers in cancer patients and healthy individuals did not differ significantly, but there was a small difference between smokers in case and control cohorts ($t_{st} = 2.255$, $p = 0.024$) because there are ex-smokers in both cohorts.

Among the CRC patients there were 31 patients (10.3%) with early cancer development (28 - 50 years). Adenocarcinoma was the predominant tumor type among these patients, and 52.67% of the cases were well and moderately differentiated adenocarcinoma. All tumors were staged using TNM criteria: stage I - 24 cases (8%); stage II - 117 cases (39%); stage III - 116 cases (38.67%), and stage IV - 43 cases (14.33%).

Case-control study for sporadic colorectal cancer

The case-control study was conducted to investigate any association between the studied polymorphisms and CRC risk. Genotyping of the deletion polymorphisms of the *GSTM1* and *GSTT1* genes was performed for both case and control cohorts. All the genotyping results are in Hardy-Weinberg equilibrium. The goodness-of-fit $\chi 2$ test values for *GSTT1* and *GSTM1* genotype distribution in cancer-free controls are 69.21 ($p < 0.001$) and 60.85 ($p < 0.001$), respectively.

Association between deletion polymorphisms of the *GSTM1* and GSTT1 genes and development of CRC were determined by evaluating the data using the general, dominant, and recessive models for each deletion polymorphism. Table 2 shows the adjusted association of deletion polymorphisms of the *GSTM1* and *GSTT1* genes with the CRC risk in Kazakhstan population that were calculated using the three models of inheritance for each genotype separately.

Separate analyses were conducted for each of the main ethnic groups (Kazakh and Russian) represented in the inhabitants of Almaty (Tables 3, 4). According to the general model (Table 2), no CRC risk was associated with homozygous *GSTT1* deletions (OR = 1.10, 95% CI = 0.78 - 1.55, p = 0.001), but the heterozygous genotype correlated with CRC susceptibility (OR = 1.98, 95% CI = 1.30 - 3.00, p = 0.001). The recessive model of inheritance (-/- versus combination of +/+ and +/- genotypes) did not show any correlations with CRC development, but the dominant model demonstrated an increased CRC risk for the combination of +/- and -/- genotypes (OR = 1.64, 95% CI = 1.19 - 2.27, p = 0.003). The presence of the functional allele variants of *GSTT1* in the homozygous state showed a strong protective effect (OR = 0.61, 95% CI = 0.44 - 0.84, p = 0.001) which was confirmed by the dominant model (Table 2). There were no significant differences between Kazakhs (for +/+ genotype OR = 0.73, 95% CI = 0.42 - 1.29; for the +/- genotype OR = 1.72, 95% CI = 0.92 - 3.23; or for the -/- OR = 0.88, 95% CI = 0.51 - 1.53; for all genotypes - $\chi 2 = 3.05$, p = 0.22, Table 3) and Russians (for +/+ genotype OR = 0.65, 95% CI = 0.41 - 1.03; for +/- genotype OR = 1.82, 95% CI = 0.99 - 3.36; for -/- genotype OR = 1.12, 95% CI = 0.66 - 1.89; for all genotypes - $\chi 2 = 4.70$, p = 0.1, Table 4). We also tested an association between deletion polymorphisms and CRC risk with reference to smoking habits (Tables S1, S2). In view of the small number of current smokers in our population, we have combined smokers with ex-smokers. Analysis for smoking and *GSTT1* polymorphism did not reveal any association with CRC risk (for

Table 1. The characteristics of the colorectal cancer case and control cohorts.

Cohort (persons)	Years of birth (average age)	Gender, persons (%)		Ethnicity, persons (%)				Smoking habit, persons (%)		
		Males	Females	Kazakh	Other Asians	Russian	Other Europeans	Smokers	Non-smokers	Ex-smokers
Case (300)	1924 - 1983 (65.14 ± 9.04)	156 (52.00)	144 (48.00)	103 (34.33)	30 (10.00)	153 (51.00)	14 (4.67)	41 (13.67)	226 (75.33)	33 (11.00)
Control (300)	1924 - 1984 (64.22 ± 9.12)	163 (54.33)	137 (45.67)	110 (36.67)	23 (7.67)	158 (52.66)	9 (3.00)	68 (22.66)	204 (68.00)	28 (9.33)
t_{st}	0.072	0.392	0.417	0.479	0.938	0.283	0.977	2.255	0.578	1.512
P	0.954	0.762	0.748	0.716	0.520	0.824	0.507	0.024	0.562	0.131

Table 2. Association between *GSTM1* and *GSTT1* polymorphisms and development of CRC in Kazakhstan population.

Type of polymorphysm	Genotype	CRC patients (%)	Controls (%)	Odds ratio (OR)	Confidence interval (CI), (95%)	$\chi 2$	P value
Number of investigated persons		300	300				
GSTM1 General model	+/+	102 (34.00)	146 (48.67)	0.54	0.39 - 0.76	18.31	0.0001
	+/-	42 (14.00)	49 (16.33)	0.83	0.53 - 1.30		
	-/-	156 (52.00)	105 (35.00)	2.01	1.45 - 2.79		
GSTM1 Dominant model	+/+	102 (34.00)	146 (48.67)	0.54	0.39 - 0.76	13.31	0.0003
	+/- and -/-	42 + 156 = 198 (66.00)	49 + 105 = 154 (51.33)	1.84	1.32 - 2.56		
GSTM1 Recessive model	+/+ and +/-	102 + 42 = 144 (48.00)	146 + 49 = 195 (65.00)	0.50	0.36 - 0.69	17.64	< 0.0003
	-/-	156 (52.00)	105 (35.00)	2.01	1.45 - 2.79		
GSTT1 General model	+/+	131 (43.67)	168 (56.00)	0.61	0.44 - 0.84	13.13	0.001
	+/-	73 (24.33)	42 (14.00)	1.98	1.30 - 3.00		
	-/-	96 (32.00)	90 (30.00)	1.10	0.78 - 1.55		
GSTT1 Dominant model	+/+	131 (43.67)	168 (56.00)	0.61	0.44 - 0.84	9.13	0.003
	+/- and -/-	73 + 96 = 169 (65.33)	42 + 90 = 132 (44.00)	1.64	1.19 - 2.27		
GSTT1 Recessive model	+/+ and +/-	131 + 73 = 204 (68.00)	168 + 42 = 210 (70.00)	0.91	0.64 - 1.29	0.28	0.6
	-/-	96 (32.00)	90 (30.00)	1.10	0.78 - 1.55		

+/+ genotype OR = 1.02, 95% CI = 0.55 - 1.87; for +/- OR = 1.10, 95% CI = 0.51 - 2.37; for the -/- OR = 0.92, 95% CI = 0.48 - 1.74; for all genotypes - $\chi 2$ = 0.10, p = 0.53, Table S1).

However the "null" (-/-) genotype of *GSTM1* gene demonstrated a strong correlation with CRC susceptibility (OR = 2.01, 95% CI = 1.45 - 2.79, p = 0.0001 Table 2) while the homozygous state of the functional allele and heterozygotes showed resistance to CRC development (for +/+ OR = 0.54, for +/- OR = 0.83, Table 2) in the general population. These findings were also confirmed by the dominant (+/+ versus combination of +/- and -/- genotypes) and recessive models of OR calculation (Table 2). The strong protective effect was expressed for combination of +/+ and +/- genotypes (OR = 0.50, 95% CI = 0.36 - 0.69, p < 0.0003). The strong association of *GSTM1* null genotype was determined in Kazakhs (for +/+ genotype OR = 0.71, 95% CI = 0.40 - 1.24; for +/- OR = 0.45, 95% CI = 0.23 - 0.91; while for the -/- genotype the OR = 2.36, 95% CI = 1.35 - 4.10; for all genotypes - $\chi 2$ = 10.32, p = 0.006, Table 3). The Russian carriers of the *GSTM1* deletion alleles, however, have an increased risk of CRC development for both the heterozygous states (OR = 1.52, 95% CI = 0.75 - 3.07) and the homozygous deletion (OR = 1.84, 95% CI = 1.17 - 2.89; for all genotypes - $\chi 2$ = 11.62, p = 0.003,

Table 3. Association between *GSTM1* and *GSTT1* polymorphisms and development of CRC in Kazakh ethnicity of Kazakhstan.

Type of polymorphysm	Genotype	CRC patients (%)	Controls (%)	Odds ratio (OR)	Confidence interval (CI), (95%)	$\chi 2$	P value
Number of investigated persons		103	110				
GSTM1 General model	+/+	33 (32.04)	44 (40.00)	0.71	0.40 - 1.24	10.32	0.006
	+/-	15 (14.56)	30 (27.27)	0.45	0.23 - 0.91		
	-/-	55 (53.40)	36 (32.73)	2.36	1.35 - 4.10		
GSTM1 Dominant model	+/+	33 (32.04)	44 (40.00)	0.71	0.40 - 1.24	1.46	0.23
	+/- and -/-	15 + 55 = 70 (67.96)	30 + 36 = 66 (60.00)	1.41	0.81 - 2.48		
GSTM1 Recessive model	+/+ and +/-	33 + 15 = 48 (46.60)	44 + 30 = 74 (67.27)	0.42	0.24 - 0.74	9.29	0.002
	-/-	55 (53.40)	36 (32.73)	2.36	1.35 - 4.10		
GSTT1 General model	+/+	33 (32.04)	43 (39.09)	0.73	0.42 - 1.29	3.05	0.22
	+/-	31 (30.10)	22 (20.00)	1.72	0.92 - 3.23		
	-/-	39 (37.86)	45 (40.91)	0.88	0.51 - 1.53		
GSTT1 Dominant model	+/+	33 (32.04)	43 (39.09)	0.73	0.42 - 1.29	1.15	0.28
	+/- and -/-	31 + 39 = 70 (67.96)	22 + 45 = 67 (60.91)	1.36	0.77 - 2.39		
GSTT1 Recessive model	+/+ and +/-	33 + 31 = 64 (62.14)	43 + 22 = 65 (59.09)	1.14	0.66 - 1.97	0.21	0.65
	-/-	39 (37.86)	45 (40.91)	0.88	0.51 - 1.53		

Table 4. Association between *GSTM1* and *GSTT1* polymorphisms and development of CRC in Russian ethnicity of Kazakhstan.

Type of polymorphysm	Genotype	CRC patients (%)	Controls (%)	Odds ratio (OR)	Confidence interval (CI), (95%)	$\chi 2$	P value
Number of investigated persons		153	158				
GSTM1 General model	+/+	52 (33.99)	84 (53.16)	0.45	0.29 - 0.72	11.62	0.003
	+/-	21 (13.73)	15 (9.49)	1.52	0.75 - 3.07		
	-/-	80 (52.29)	59 (37.34)	1.84	1.17 - 2.89		
GSTM1 Dominant model	+/+	52 (33.99)	84 (53.16)	0.45	0.29 - 0.72	11.62	0.0007
	+/- and -/-	21 + 80 = 101 (66.01)	15 + 59 = 74 (46.84)	2.20	1.39 - 3.48		
GSTM1 Recessive model	+/+ and +/-	52 + 21 = 73 (47.71)	84 + 15 = 99 (62.66)	0.54	0.35 - 0.85	7.02	0.008
	-/-	80 (52.29)	59 (37.34)	1.84	1.17 - 2.89		
GSTT1 General model	+/+	83 (54.25)	102 (64.56)	0.65	0.41 - 1.03	4.70	0.1
	+/-	32 (20.92)	20 (12.66)	1.82	0.99 - 3.36		
	-/-	38 (24.84)	36 (22.78)	1.12	0.66 - 1.89		
GSTT1 Dominant model	+/+	83 (54.25)	102 (64.56)	0.65	0.41 - 1.03	3.43	0.06
	+/- and -/-	32 + 38 = 70 (45.75)	20 + 36 = 56 (35.44)	1.54	0.97 - 2.42		
GSTT1 Recessive model	+/+ and +/-	83 + 32 = 115 (75.16)	102 + 20 = 122 (77.22)	0.89	0.53 - 1.51	0.18	0.67
	-/-	38 (24.84)	36 (22.78)	1.12	0.66 - 1.89		

Table 5. Association of different combinations of *GSTM1* and *GSTT1* polymorphisms with the risk of CRC.

Genotype *GSTM1*	Genotype *GSTT1*	Controls (n = 300)	CRC patients (n = 300)	OR (CI 95%)	χ^2	P value	Population group
GSTM1 +/+ and +/-	*GSTT1*+/+ and +/-	128	107	0.74 (0.54 - 1.04)	5.33	0.07	All ethnic group
	GSTT1-/-	65	42	1.06 (0.77 - 1.45)			
GSTM1 -/-	*GSTT1*+/+ and +/-	73	100				
	GSTT1-/-	34	51	1.57 (0.94 - 2.63) 1.60 (1.00 - 2.56)			
Genotype *GSTM1*	Genotype *GSTT1*	Controls (n = 110)	CRC patients (n = 103)	OR (CI 95%)	χ^2	P value	
GSTM1 +/+ and +/-	*GSTT1*+/+ and +/-	47	33	0.63 (0.36 - 1.11)	3.36	0.19	Kazakh
	GSTT1-/-	32	19	1.18 (0.69 - 2.02)			
GSTM1 -/-	*GSTT1*+/+ and +/-	18	32				
	GSTT1-/-	13	19	1.69 (0.79 - 3.62)			
Genotype *GSTM1*	Genotype *GSTT1*	Controls (n = 158)	CRC patients (n = 153)	OR (CI 95%)	χ^2	P value	
GSTM1 +/+ and +/-	*GSTT1*+/+ and +/-	67	61	0.88 (0.56 - 1.38)	1.17	0.56	Russian
	GSTT1-/-	29	15	0.96 (0.61 - 1.50)			
GSTM1 -/-	*GSTT1*+/+ and +/-	44	55				
	GSTT1-/-	18	24	1.42 (0.74 - 2.75)			

Table 6. The comparison of allele frequencies of healthy people of Kazakhstan population with earlier studied populations.

Polymorphism	Allele	The frequency of allele		
		Control cohort (Kazakhstan population)	Integrated data from different sources	
			Asian populations	European populations
GSTM1	+	0.568	0.460 - 0.510 [15-17]	0.460 - 0.580 [15-17]
	-	0.432	0.490 - 0.540 [15-17]	0.420 - 0.540 [15-17]
GSTT1	+	0.630	0.460 - 0.520 [15-17]	0.610 - 0.840 [15-17]
	-	0.370	0.480 - 0.540 [15-17]	0.160 - 0.385 [15-17]

Table 4).The CRC risk of *GSTM1* null genotype in smokers was considerably higher (for +/+ genotype - OR = 0.45, 95% CI = 0.24 - 0.85; for +/- - OR = 0.46, 95% CI = 0.19 - 1.11; for -/- - OR = 3.37, 95% CI = 1.78 - 6.38; for all genotypes - $\chi 2$ = 14.51, p = 0.0007, Table S2). Also, we evaluated an association of combinations *GSTM1* and *GSTT1* polymorphisms with the risk of CRC (Table 5). The combination of the *GSTM1* and *GSTT1* null genotypes in combined mixed population of Kazakhstan showed a trend to increasing the risk of developing CRC with an adjusted OR of 1.60 (95% CI = 1.00 - 2.56, p = 0.07), but it was not statistically significant.

This trend was also observed when we tested both main ethnic groups (Kazakhs and Russians) separately. In both ethnic groups double carriers of null genotypes can increase the risk of CRC, but this also was not significant for Kazakhs (OR = 1.69, 95% CI = 0.79 - 3.62, p = 0.19) and for Russians (OR = 1.42, 95% CI = 0.74 - 2.75, p = 0.56).

DISCUSSION

Numerous molecular epidemiological studies have been devoted to the determination of biomarkers for sporadic and familial CRCs. Moreover, more than 15% of sporadic CRCs develop through fundamentally different pathways of molecular events, and differences in population genetics are crucial in this process. Using a case-

control study, we evaluated the influence of *GSTM1* and *GSTT1* polymorphisms on sporadic CRC risk in patients representing the various population groups in Kazakhstan.

Epidemiological studies require a careful selection of the control group to be used in the research. This cohort should correspond to the case cohort on many parameters in order to ensure a reliable association between genetic polymorphisms and risk of disease, especially so in the cases of small sample sizes or rare allele frequency. The cohorts used in this study represent inhabitants of one geographic zone (the city of Almaty) in Kazakhstan. To minimize the effects of ethnicity, age, gender, and smoking influence on the susceptibility to CRC, we used the same parameters for the healthy control groups (Table 1). It should be noted that both case and control populations are mixed by ethnicity, and therefore represent several different ethnic groups in Eurasia. However, Russian (approximately 50%) and Kazakh (approximately 35%) represent the majority of both cohorts. Because this represents the first genetic polymorphism study on the population groups in Kazakhstan, we compared the frequency of allele variants in the control cohort with data from literature (Table 6) [15-17].

The distribution of the *GSTM1* deletions are similar among the Asian (0.490 - 0.540) and European (0.420 - 0.540) populations while the frequency of *GSTM1* deletions in healthy residents of Almaty city (0.432) was lower than that of the Asian populations and similar to that of the European population. Also, a similar result was detected with the frequency of *GSTT1* deletions in our study which was lower (0.370) than that of the Asian populations and similar to that of the European population.

The GSTs have well-established polymorphisms in human populations. GSTs catalyze the conjugation of glutathione to electrophilic compounds, resulting in less reactive and more easily excreted glutathione conjugates. Deletions of GST-genes are associated with susceptibility to many of cancer types. Several studies show an increased risk of developing CRC among carriers of the *GSTM1* and *GSTT1* null genotypes [12,18,19]. While other studies have shown that deletions of *GSTM1*, rather than *GSTT1*, are associated with CRC susceptibility in the Caucasian [20,21], Japanese [22], and mixed American populations [23,24] and an even stronger association with smoking [23,24]. Other studies did not find any association [25-27]. Moreover, a case-control study of a population from Scotland did not show the interaction between the *GSTM1* or *GSTT1* polymorphisms and smoking, meat intake, and green leafy vegetable consumption [27]. A meta-analysis of 20 studies [28] determined that *GSTM1* status has no effect on the risk of developing colon cancer. Other meta-analyses [11] that included 44 studies for *GSTM1* (11,998 CRC cases, 17,552 controls) and 34 studies for *GSTT1* (8596 cases, 13,589 controls) showed that *GSTM1* null allele carriers exhibited increased CRC risk in the Caucasian population and no significant association was detected for Chinese subjects (pooled OR = 1.025). Similarly, while the *GSTT1* null allele carriers exhibited increased CRC risk in the Caucasian populations (pooled OR = 1.312), the association in Chinese subjects was not significant (pooled OR = 1.068). The results of our study did not show any significant CRC risk of the *GSTT1* null genotype (OR = 1.10), but defined the strong association of *GSTM1* null genotype (OR = 2.01 for ethnically mixed population), which is higher in Kazakhs (OR = 2.36) than in Russians (OR = 1.84), and also significantly correlates with smoking (OR = 3.37).

The results of this case-control study for sporadic cases of CRC show that *GSTM1* deletion polymorphisms can have predictive value for susceptibility to CRC (OR = 2.01, p = 0.0001) for the mixed population from Kazakhstan and for both main ethnic groups (Kazakhs and Russians (OR = 2.36 and OR = 1.84, respectively)).

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Declaration of Interest:

The authors declare no conflict of interest.

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Supplemental Table S1. Association of tobacco smoking with *GSTT1*deletion polymorphism.

Type of polymorphism	Genotype	CRC patients (%)	Controls (%)	OR	95% CI	χ2	P
Smokers and ex-smokers		**74**	**96**				
GSTT1	**+/+**	**35 (47.30)**	**45 (46.88)**	**1.02**	**0.55 - 1.87**	**0.10**	**0.95**
	+/-	**15 (20.27)**	**18 (18.75)**	**1.10**	**0.51 - 2.37**		
	-/-	**24 (32.43)**	**33 (34.37)**	**0.92**	**0.48 - 1.74**		
GSTT1 **Dominant model**	**+/+**	**35 (47.30)**	**45 (46.88)**	**1.02**	**0.55 - 1.87**	**0.00**	**0.96**
	+/- and -/-	**15 + 24 = 39 (52.70)**	**18 + 33 = 51 (53.12)**	**0.98**	**0.54 - 1.80**		
GSTT1 **Recessive model**	**+/+ and +/-**	**35 + 15 = 50 (67.57)**	**45 + 18 = 63 (65.63)**	**1.09**	**0.57 - 2.08**	**0.07**	**0.79**
	-/-	**24 (32.43)**	**33 (34.37)**	**0.92**	**0.48 - 1.74**		
Non-smokers		**226**	**204**				
GSTT1	**+/+**	**96 (42.48)**	**123 (60.29)**	**0.49**	**0.33 - 0.71**	**18.09**	**0.0001**
	+/-	**58 (25.66)**	**24 (11.77)**	**2.59**	**1.54 - 4.36**		
	-/-	**72 (31.86)**	**57 (27.94)**	**1.21**	**0.80 - 1.83**		
GSTT1 **Dominant model**	**+/+**	**96 (42.48)**	**123 (60.29)**	**0.49**	**0.33 - 0.71**	**13.62**	**0.0002**
	+/- and -/-	**130 (57.52)**	**24 + 57 = 81 (39.70)**	**2.06**	**1.40 - 3.02**		
GSTT1 **Recessive model**	**+/+ and +/-**	**96 + 58 = 154 (68.14)**	**123 + 24 = 147 (72.05)**	**0.83**	**0.55 - 1.26**	**0.78**	**0.38**
	-/-	**72 (31.86)**	**57 (27.95)**	**1.21**	**0.80 - 1.83**		

Supplemental Table S2. Association of tobacco smoking with *GSTM1*deletion polymorphism.

Type of polymorphism	Genotype	CRC patients (%)	Controls (%)	OR	95 %CI	χ2	P
Smokers and ex-smokers		**74**	**96**				
GSTM1	**+/+**	**23 (31.08)**	**48 (50.00)**	**0.45**	**0.24 - 0.85**	**14.51**	**0.0007**
	+/-	**8 (10.81)**	**20 (20.83)**	**0.46**	**0.19 - 1.11**		
	-/-	**43 (58.11)**	**28 (29.17)**	**3.37**	**1.78 - 6.38**		
GSTM1 **Dominant model**	**+/+**	**23 (31.08)**	**48 (50.00)**	**0.45**	**0.24 - 0.85**	**6.15**	**0.01**
	+/- and -/-	**8 + 43 = 51 (68.92)**	**20 + 28 = 48 (50.00)**	**2.22**	**1.18 - 4.18**		
GSTM1 **Recessive model**	**+/+ and +/-**	**23 + 8 = 31 (41.89)**	**48 + 20 = 68 (70.83)**	**0.30**	**0.16 - 0.56**	**14.39**	**0.0002**
	-/-	**43 (58.11)**	**28 (29.17)**	**3.37**	**1.78 - 6.38**		
Non-smokers		**226**	**204**				
GSTM1	**+/+**	**108 (47.79)**	**120 (58.82)**	**0.64**	**0.44 - 0.94**	**21.52**	**0.0002**
	+/-	**65 (28.76)**	**22 (10.78)**	**3.34**	**1.97 - 5.66**		
	-/-	**53 (23.45)**	**62 (30.39)**	**0.70**	**0.46 - 1.08**		
GSTM1 **Dominant model**	**+/+**	**108 (47.79)**	**120 (58.82)**	**0.64**	**0.44 - 0.94**	**5.24**	**0.02**
	+/- and -/-	**65 + 53 = 118 (52.21)**	**22 + 62 = 84 (41.18)**	**1.56**	**1.07 - 2.29**		
GSTM1 **Recessive model**	**+/+ and +/-**	**108 + 65 = 173 (76.55)**	**120 + 22 = 142 (69.61)**	**1.43**	**0.93 - 2.19**	**2.64**	**0.1**
	-/-	**53 (23.45)**	**62 (30.39)**	**0.70**	**0.46 - 1.08**		