RESEARCH ARTICLE

Glutathione S-transferase T1, M1 and P1 Genetic Polymorphisms and Susceptibility to Colorectal Cancer in Turkey

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Abstract

Colorectal cancer (CRC) is reproted to be the third most common cancer worldwide and the fourth most common cause of cancer related deaths. CRC is considered to be a multifactorial disease whose risk varies due to the complex interaction between individual genetic basis and disposure to multiple endogenous factors. Glutathione S-transferases are pro-carcinogenic in CRC and are required for the conjugation between chemotherapeutics and broad spectrum xenobiotics. One hundred and eleven patients with CRC and 128 control subjects without any cancer history were enrolled in this study. Multiplex PCR was applied to determine polymorphisms for the GSTT1 and M1 genes, and PCR-RFLP was applied for the GSTP1 (Ile105Val) gene polymorphism. Values p<0.05 were defined as statistically significant. We detected a significant high correlation between predisposition for CRC and presence of the Ile/Ile genotype of the GSTP1 (IIe105Val) gene polymorphism, but we did not find a significant relationship between predisposition for CRC and GSTT1 and M1 deletion polymorphisms. In addition, we did not determine a relationship between GSTT1, M1 and P1 gene polymorphisms and any clinicopathological features of CRC. GSTT1 null/GSTM1 positive and GSTT1 null/GSTM1 positive/GSTP1 Ile/Ile genotypes were significantly higher in the patient group. Our results revealed that there is no relationship among CRC, its clinicopathologic features, and GSTT1 M1 gene polymorphisms. However, there was a significant correlation between CRC and the GSTP1 Ile/Ile genotype. Further studies with larger patient groups are required to delineate the relationships between GST gene polymorphisms and the clinicopathologic features of CRC in Turkey.

Keywords: Colorectal cancer - GST - polymorphism - clinicopathology - Turkey

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Introduction

Colorectal cancer (CRC) is the most common cancer of the gastrointestinal system. CRC is also the third most common cancer type in men, behind only prostate and lung cancers, and the second common cancer in women, behind breast cancer (Jermal et al., 2011). More than one million people develop CRC annually worldwide, and the disease specific mortality rate for CRC is nearly 33% worldwide (Cunningham et al., 2010). Among the molecular studies concerning CRC, investigations into genetic aspects and carcinogenesis pathways predominate. The contribution of low penetrance genes modifying the risk for developing CRC are also of great interest. One such genetic factor is the glutathione S-transferase (GST) gene family, which encodes the glutathione transferase enzyme (Klusek et al., 2014). GSTs represent the superfamily of phase II metabolic enzymes and catalyse the conjugation between glutathione and chemotherapeutic drugs, carcinogens, environmental contaminants and broad spectrum xenobiotics.

Three functional polymorphisms of GST have been defined in the human genome: M1, T1 and P1 (Ali-Osman et al., 1997; Zimniak et al., 1994). GSTM1 is located in the short arm of the chromosome one (1p13.3), GSTT1 is located in 22q11.2, and GSTP1 is located in 11q13 (Board and Menon, 2013). The variant allele for GSTM1 and GSTT1 is the deletion of the GST gene altogether. Individuals who are homozygous for this allele deletion are classified as the null genotype and do not express any of this enzyme. Two genetic polymorphisms are known for GSTP1 and result from the substitution of A-G in Ile-105-Val, base 1578 or the substitution of C-T in Ala-114-Val, base 2293. The functional polymorphism of GSTP1 (rs1695, Ile105Val) results in low enzyme activity (Hezova et al., 2012). Genotype frequencies can vary according to both geographic regions and ethnicity, but the result of investigations into the relationship between CRC and GST gene polymorphism are conflicting (Garte et al., 2001; de Jong et al., 2002). Because the contribution of

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the GST gene polymorphism to predisposition to CRC in the Turkish population is not well known, we aimed to predict the association between CRC and GST gene polymorphisms both individually and combinatorally and to determine the relationship between genes and clinicopathologic features.

Materials and Methods

Patients and Methods

In this case control study conducted in Uludağ University Medical Faculty, Department of Medical Genetics in 2009-2013, 116 patients with a colorectal cancer diagnosis whose paraffin blocks arrived and 128 control subjects without a history of cancer were enrolled. The patients' demographic features (gender, age) and pathologic features (tumor localization, histologic type, grade and stage) were recorded. Approval from the local ethics committee was obtained for the study.

Genotyping

Genomic DNA from CRC patients was extracted from formalin-fixed, paraffin-embedded (FFPe) samples. For healthy controls, DNA from peripheral blood samples that had been stored in EDTA tubes and kept at -20°C was isolated using DNA isolation kits. Multiplex PCR was applied to determine the polymorphisms for GSTT1 and M1 genes, and PCR-RFLP was applied for the GSTP1 (Ile105Val) gene polymorphism. The primers used to define the GSTT1 polymorphism were forward 5'-TTCCTTACTGGTCCTCACATCTC-3' and reverse 5'-TCACCGGATCATGGCCAGCA-3'. The primers used to define the GSTM1 polymorphism were forward 5'-GAACTCCCTGAAAAGCTAAAGC-3' and reverse 5'-GTTGGGCTCAAATATACGGTGG-3'. The primers for albumin, the internal control, were forward 5'-GCCCTCTGCTAACAAGTCCTAC-3' and reverse 5'-GCCCTAAAAAGAAAATCCCCAATC-3'. PCR products were analysed on an agarose gel dyed with 2% ethidium bromide and were visualized under ultraviolet light. Albumin 350 bp, GSTM1 219 bp and GSTT1 459 bp PCR products were formed (Figure 1). The primers used to define the GSTP1 polymorphism were: forward 5'-ACCCCAGGGCTCTATGGGAA-3' and reverse 5'-TGAGGGCACAAGAAGCCCCT-3' (Abbas et al., 2004). A restriction digest with the restriction enzyme BsmAI (New England Bioblas) was then performed on the PCR products to further define the genotypes. The BsmAI digested DNA was run on a 4% agarose gel and analyzed as follows: if the 176 bp PCR product of the GSTP1 gene yielded two products of 85 bp and 91 bp, then the sample was defined as having the Ile/Ile (AA) genotype; if three products of 176 bp, 91 bp and 85 bp were produced, the sample was defined as having the Ile/Val (AG) genotype; and if a product of 176 bp was formed, the sample was defined as having the Val/Val (GG) genotype (Figure 1).

Statistical analysis

Statistical analyses were performed using the SPSS21 statistical pocket program. The Shapiro-Wilk test was applied to evaluate the normal distribution of the data. The Mann-Whitney U test was used for comparisons between two groups with data that were not distributed normally. The Pearsonchi-square test, Fisher's chi-square test and Fisher-Freeman-Halton tests were used to analyse the categorical data. The significance value was determined as $p<0.05$.

Results

In this study, 116 patients with CRC diagnosis and 128 sex- and age-matched, healthy control subjects were enrolled. Samples from 92 patients with CRC and 116 controls were evaluated for the presence of the GSTT1 and GSTM1 genes. There was no significant difference in the genotype distribution of the GSTM1 and GSTT1 genes

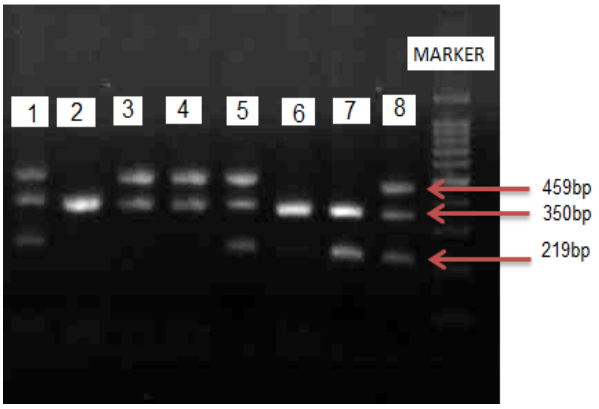

Figure 1. PCR Products of GSTM1, GSTT1 Polymorphismson 2% Agarose Gel. The lane marker shows the 100-bp DNA ladder; lanes 3, 4 are GSTT1+(459bp), GSTM1-, lanes 1, 5, 8 are GSTT1+(459bp), GSTM1+(219bp), lane 7 is the GSTT1-, GSTM1+(219bp), lanes 2, 6 are GSTT1-, GSTM1-. Bp: base pair

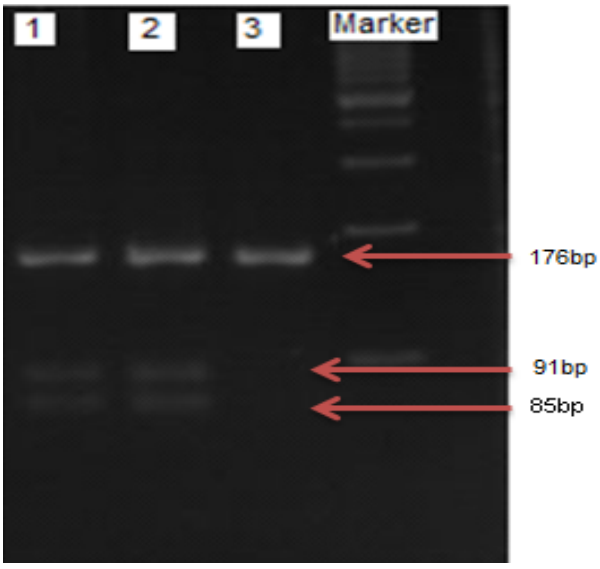

Figure 2. PCR Products of the GSTP1 (IIe105Val) gene Following BsmAI Enzyme Digestion and Electrophoresis on a 4% Agarose Gel. The lane marker shows the 100-bp DNA ladder; lanes 1, 2 are the Ile / Val genotype (176 bp, 91 bp, 85 bp), lane 3 is Ile / Ile genotype (176 bp). Bp: base pair

Table 1. Statistical Comparison of Distributions of Genotype and Allele Frequencies of GSTT1, GSTM1 and GSTP1 between Patient and Control Groups

Genotype	Patient (n=92) n (%)	Control (n=116) n (%)	p
GSTM1			0.55
(+)	65 (70.7)	67 (57.8)	
(-)	27 (29.3)	49 (42.2)	
GSTT1			0.14
(+)	58 (63)	91 (78.4)	
(-)	34 (37)	25 (21.6)	
GSTP1 Ile105Val	Patient (n=111) n (%)	Control (n=122) n (%)	p
Genotype			
Ile/Ile	76 (68.5)	61 (50)	0.004
Ile/Val	28 (25.2)	58 (47.5)	<0.001
Val/Val	7 (6.3)	3 (2.5)	0.2
Val**	35 (31.5)	61 (50)	0.004
Allele			
Ile*	81.1	73.8	0.06
Val*	18/9	26/2	

*(%) allele frequency, **Ile/Val or Val/Val

Table 2. Statistical Comparison of Genotype Distributions of GSTM1 and GSTT1 Double and GSTM1, GSTT1 and GSTP1 Triple Combined Genotypes between Patient and Control Groups

Double combined genotypes		Patient (n=92) n(%)	Control (n=116) n (%)	p
GSTM1	GSTT1			
(+)	(+)	34 (37)	56 (48.3)	0.102
(+)	(-)	31 (33.7)	11 (9.5)	<0.001
(-)	(+)	24 (26.1)	35 (30.2)	0.621
(-)	(-)	3 (3.3)	14 (12.1)	0.041

Triple combined genotypes			Patient (n=87) n(%)	Control (n=109) n (%)	p
GSTM1	GSTT1	GSTP1			
(+)	(+)	Ile/Ile	24 (27.6)	26 (23.9)	0.551
(+)	(+)	Val*	8 (9.2)	26 (23.9)	0.012
(+)	(-)	Ile/Ile	19 (21.8)	6 (5.5)	0.001
(+)	(-)	Val*	10 (11.5)	5 (4.6)	0.124
(-)	(+)	Ile/Ile	12 (13.8)	19 (17.4)	0.620
(-)	(+)	Val*	11 (12.6)	13 (11.9)	1
(-)	(-)	Ile/Ile	3 (3.4)	7 (6.4)	0.517
(-)	(-)	Val*	0	7 (6.4)	0.018

*Ile/Val or Val/Val

between CRC group and controls (p>0.05). Samples from 111 patients with CRC and 122 controls were evaluated for the presence of the GSTP1 (Ile105Val) polymorphism. The Ile/Ile, Ile/Val, Val/Val genotypes of GSTP1 were found in ratios of 68.5%, 25.2%, 6.3%, respectively, in the patient group. In the control group, the ratios for the Ile/Ile, Ile/Val, Val/Val genotypes were 50%, 47.5%, 2.5%, respectively. The Ile/Ile genotype was found to be significantly higher in the patient group (p=0.004), whereas the Ile/Val genotype was found to be significantly higher in the control group (p<0.001). The Ile/Val or Val/Val genotype was detected in 35 CRC patients (31.5%), the Ile/Ile genotype was detected in 76 CRC patients (68.5%), the Ile/Val or Val/Val genotype was detected in 61 control patients (50%), and the Ile/Ile genotype was detected in 60 control patients (50%). The prevalence of the Ile/Ile genotype was found to be significantly higher in the patient group, whereas the Ile/Val or Val/Val genotype was statistically significantly higher in the control group (p=0.004). In the patient group, the allele frequency of the Ile was 81.1%, and the allele frequency of the Val was 18.9%; however, in the control group, the allele frequency of the Ile was 73.8% and the allele frequency of the Val was 26.2%. No significant difference was observed in terms of these allele frequencies between the patient and control groups (p=0.06) (Table 1). The combined effects of two and three putative risk genotypes of GST polymorphisms are summarized in Table 2. We found that the combined genotypes of GSTT1 null/GSTM1 present and GSTT1 null/GSTM1 present/GSTP1 Ile/Ile were significantly higher in the patient group compared with the healthy controls (p<0.05). When the pathological parameters of the patients were compared with the genotype distribution of GSTT1, M1 and P1 polymorphisms, no significant differences were observed for tumor histologic type, grade or localization (p>0.05) (data not shown).

Discussion

In this study, we investigated the possible role of common GST polymorphisms in the development of CRC in the Turkish population. GST enzymes play a crurical role in the cellular defense system, working to detoxify potential carcinogens. The carcinogens involved in the promotion of CRC develoment include heterocyclic amines (HAAs) and polycyclic aromatic hydrocarbons (PAHs). HAAs can be formed in meat cooked at high temperatures, whereas PAHs are present in tobacco products and gases of fossill fuels. GSTM1 can detoxify active metabolites of PAHs. Conversely, GSTT1 is required for the detoxification of some environmental carcinogens such as 1,3-butadiene and ethylene oxide in places where tobacco is consumed (Reszka et al., 2006; Hayes and Pulford, 1995; Landi, 2000). GSTP1 is expressed widely in normal epithelial tissue and was shown to be overexpressed highly in colon cancers (Terrier et al., 1990; Moscow et al., 1989). The null genotype of the GSTM1 gene, which does not show enzymatic activity, is present in 40-60% of Caucasians, whereas the null genotype of the GSTT1 gene is observed in 10-20% of Caucasians (Hezova et al., 2012). Ateş et al. (2005) detected the GSTM1 and T1 null genotypes in 42% and 23% ratios, respectively, and detected the GSTP1 Ile allele in 75% and the Val allele in 25% of the control groups in a study on urinary bladder cancer in Turkey. Kıran et al. (2010) found the GSTM1 and T1 null genotypes to have frequencies of 57.7% and 30.8%, respectively, in control groups in a study on cervical cancer in Turkey. In our study, we detected the frequencies of the GSTM1 and T1 null genotypes to be 42.2% and 21.6%, respectively, and the GSTP1 Ile allele frequency to be 73.8% and the Val allele frequency to be 26.2% in the control group, which were compatible with the literaure and other studies conducted in Turkey. We did not find an association between colorectal cancer and GSTT1 and M1 deletion polymorphisms in our study. As for the GSTP1 gene

polymorphism genotype distribution, the Ile/Ile genotype was significantly more frequent in patients, but the Ile/Val or Val/Val genotypes were more frequent in the control group. Although the Ile allele was higher in the patient group and the Val allele was higher in the control group, no significant differences were observed. We could not find correlations between GSTT1 and M1 deletion polymorphisms and the genotype distribution of GSTP1 gene polymorphism and clinicopathologic features of the patients, such as tumor localization, tumor differentiation and histologic type.

Results conserning GST polymorphisms as a potential risk factor for CRC are conflicting (Economopoulos and Sergentanis, 2010). Hezova et al reported that the GSTP1 polymorphism Ile/Val genotype was correlated with decreased CRC risk, and they did not find significant correlations among GSTM1, GSTT1, GSTA1 polymorphisms and CRC (Abbas et al., 2004). Khabaz (2012) did not demonstrate a significant association between the GSTP1 Ile105Val polymorphism and CRC. They also did not find a correlation between GSTP1 Ile105Val polymorphism and age, gender, tumor localization, grade, or stage of patients. Economopoulos and Sergentanis (2010) performed a meta-analysis to investigate the association between CRC and GSTM1 and T1 deletion polymorphisms and GSTP1 (Ile105Val) polymorphism. They performed two different analyses for Caucasian and Chinese populations and found an increased risk for CRC in Caucasian populations harboring the GSTM1 and T1 null allele. However, they did not demonstrate significant correlations for the Chinese population. They did not find significant correlations between CRC and GSTP1 Ile105Val polymorphism for either of the populations. Gao et al. (2010) reported a significant correlation between CRC and GSTM1 deletion polymorphisms. In addition, they revealed significant correlations in terms of ethnicity (Caucians, Asians, Africans, Americans) and tumor localization (distal colon, proximal colon) in Caucians and the patients with proximal tumor localization. Koh et al. (2011) did not find significant correlations between GSTT1, M1 and GSTP1 (Ile105Val) polymorphisms and CRC. They also investigated the relationship between smoking and CRC and asserted that GST gene polymorphisms would affect the association between cigarette and CRC predisposition in individuals. In contrast, Lai et al. (2013) did not find significant associations between CRC and GSTT1 and M1 and GSTP1 (Ile105Val) polymorphisms. Furthermore, they did not find a significant relationship between GST polymorphisms and tumor localization and survival.

Predisposition for sporadic CRC is multifactorial and originates from the interaction between allelic variants of low-penetrance genes and environmental factors such as dietary and daily routines (de la Chapelle, 2004; Hunter et al., 2005). Each gene with low penetrance has a mild effect on predisposition to CRC; however, interactions between other potential alleles and environmental risk factors result in significantly increased risk for CRC (Goodman et al., 2006; Tabor et al., 2002). In our study, we found that the combined genotypes of GSTT1 null/GSTM1 present and the combined genotype of GSTT1 null/GSTM1 present/GSTP1 Ile/Ile were significantly higher in the patient group. Wang et al. (2011) found the GSTM1 null genotype to be significantly correlated with increased rectal cancer risk and the GSTT1 null genotype to be significantly correlated with increased colon cancer risk in a study on CRC patients. In the same study, they reported a significant relationship between CRC and the combined genotype of GSTM1 null/T1 null and the combined genotype of GSTM1 null/GSTT1 null/P1 Ile/Val or Val/Val genotypes. They reported that the effect of GST polymorphisms was enhanced by double or triple GSTT1, M1, P1 Ile105Val combinations. Piao et al. (2009) did not find a significant relationship among CRC and GSTT1, M1 genotypes and combined GSTT1 and M1 null genotypes. Furthermore, they did not detect significant associations between GSTT1 null and GSTM1 null genotypes and smoking, alcohol consumption and stage and localization of the tumor. Welfare et al. (1999) did not find any relationship between the disease and GSTT1 null and GSTM1 null genotypes in their study on CRC patients. These conflicting results between GST polymorphisms and risk for CRC may originate from a limited sample size, differences in ethnicity, environmental disposures and study designs.

As a consequence, though we did not find any relationship between CRC and GSTT1 and M1 deletion polymorphisms, we detected a significantly higher presence of the Ile/Ile genotype of the GSTP1 gene polymorphism in the patient group. Furthermore, we did not find a significant relationship between GSTT1, M1 and P1 gene polymorphisms and clinicopathologic features of the patients with CRC. Future studies with a higher number of CRC patients would yield more accurate information about the relationship between GST polymorphisms and clinicopathologic fetures of colorectal cancer.

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