

RESEARCH COMMUNICATION

The GSTP1 *Ile105Val* Polymorphism is not Associated with Susceptibility to Colorectal Cancer

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Abstract

The glutathione S transferase (GST) family is a major part of cellular defense mechanisms against endogenous and exogenous substances, many of which have carcinogenic potential. Alteration in the expression level or structure of the glutathione-S-transferase (GST) enzymes may lead to inadequate detoxification of potential carcinogens and consequently contribute to cancer development. A member of the glutathione-S-transferase (GST) family, GSTP1, is an attractive candidate for involvement in susceptibility to carcinogen-associated colorectal cancer. An A→G transition in exon 5 resulting in an *Ile105Val* amino acid substitution has been identified which alters catalytic efficiency. The present study investigated the possible impact of *Ile105Val* GSTP1 polymorphism on susceptibility to colorectal cancer. In Jordan We examined 90 tissue samples previously diagnosed with colorectal carcinoma, and 56 non-cancerous colon tissues. DNA was extracted from paraffin embedded tissues and the status of the GSTP1 polymorphism was determined using a polymerase chain reaction restriction fragment length polymorphism (RFLP) method. No statistically significant differences were found between colorectal cancer cases and controls for the GSTP1 *Ile/Ile*, *Ile/Val* and *Val/Val* genotypes. The glutathione S-transferase polymorphism was not associated with risk in colorectal cancer cases in Jordan stratified by age, sex, site, grade or tumor stage. In conclusion, the GSTP1 *Ile105Val* polymorphism is unlikely to affect the risk of colorectal cancer.

Keywords: Glutathione S transferase - GSTP1 - polymorphism - colorectal carcinoma - susceptibility

Asian Pacific J Cancer Prev, 13, 2949-2953

Introduction

Colorectal cancer (CRC) is the first most common form of cancer affecting Jordanian male population and it accounted for (12.7%) of all newly diagnosed male cancers in 2009. It ranked second among females accounting for (10.5%) of all female cancers. (Tarawneh et al., 2009). Amass evidence shows susceptibility to cancer is mediated by alterations in the detoxifying capacity of genetically determined factors that play a role in cellular defense mechanism against endogenous and exogenous substances, many of which have carcinogenic potential (Deakin et al., 1996; Inoue et al., 2001). There is also considerable data on the role of oxidative stress in relation to colorectal cancer risk (Yoshida et al., 2007). Furthermore, it has been suggested that up to 80% of human cancers arise as a consequence of environmental exposure (Ates et al., 2005; Dang et al., 2005). Enzymes involved in the detoxification of carcinogenic compounds as well as DNA repair may play a role in the susceptibility to colorectal cancer and other forms of cancer (Vlaykova et al., 2007; Zhang et al., 2007). The effectiveness of the detoxifying properties of such enzymes is genetically determined (Deakin et al., 1996; Hisako et al., 2001; Ye and Parry, 2002). Polymorphic genes that code for these

enzymes may hence be involved in colorectal cancer susceptibility.

Glutathione S transferases (GSTs) are a large and diverse family of phase-2 enzymes, which detoxify potentially mutagenic and cytotoxic DNA reactive metabolites and diverse electrophiles, including carcinogens, chiefly by conjugating them with glutathione. As a result, the potential carcinogens are eliminated, and DNA or other important biomolecules are protected against damage or adduct formation (Mannervik and Danielson, 1988; Zhang et al., 1992; Beckett and Hayes, 1993; Zhong et al., 1993; Ryberg et al., 1997). Glutathione S transferase-P1 (GSTP1) is a major GST, which is expressed in both normal and tumor of colon tissue (Singhal et al., 1992; Hoensch et al., 2006; Vlaykova et al., 2007). GSTP1 plays a central role in the inactivation of toxic and carcinogenic electrophiles (Hengstler et al., 1998). Furthermore, heterocyclic amines are carcinogens that have been implicated as a potential cause of colorectal cancer in humans and that have also been shown to be detoxified by GSTs (Boone et al., 1990; Lin et al., 1994).

The GST-pi gene has been mapped to a small region of chromosome 11q. Polymorphisms in exon 5 (*Ile105Val*) and exon 6 (*Ala114Val*) of the GSTP1 gene were identified (Zimniak et al., 1994), and both affected codons lie

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in close proximity to the hydrophobic binding site of GSTP1. Moreover, polymorphism is known to change the properties of the enzyme (Ryberg *et al.*, 1997). The 105Val variant has been demonstrated to have either lower or higher specific activity and affinity than that of 105Ile depending on the substrate, whereas the Ala114Val polymorphism seems not to influence enzyme activity (Ali-Osman *et al.*, 1997; Sundberg *et al.*, 1998).

The GSTP1 polymorphism results in an amino acid change from isoleucine (wild type) to a valine (variant) introduces conformational changes, due to the bulky properties of the valine side chain (Mannervik and Danielson, 1988; Beckett and Hayes, 1993; Zimniak *et al.*, 1994). Therefore, it is reasonable to speculate that a carcinogen metabolizing enzymes with lower activity could be associated with an elevated risk of developing cancer. However, to date, the potential association between genetic polymorphism of GSTP1 and colorectal carcinoma is somehow still controversial and may vary from population to population (Welfare *et al.*, 1999; Ates *et al.*, 2005; Vlaykova *et al.*, 2007; Epplein *et al.*, 2009; Matakova *et al.*, 2009; Hlavata *et al.*, 2010; Sameer *et al.*, 2012).

Identification of susceptibility factors that predispose individuals to colorectal cancer if they are exposed to particular environmental agents might give further insight into the etiology of colorectal malignancy. One method of investigating the protective role of GSTP1 has been to study the effect of polymorphism in GSTP1 gene on susceptibility to colorectal cancer. Hence, the main hypotheses investigated in this paper were that polymorphism in the GSTP1 gene predispose to colorectal cancer.

Materials and Methods

The GSTP1 genotyping was conducted in 90 colorectal cancer patients and 56 noncancerous controls from a retrospective study. The patients involved in the analysis have undergone tumor resections for adenocarcinoma of the colon and rectum between January 1996 and December 2001 at the University Hospital, Medical Faculty, Jordan University of Science and Technology, Irbid, Jordan. All had primary tumor resection with regional lymph node dissection. The operations were carried out according to the accepted protocols in Jordan for surgical interventions and obtaining of human biopsy materials.

The patient population consisted of 47 (52.2%) men and 43 (47.8%) women. The median age was 52 years with a range between 22 to 85 years. There was no information in the records about a familiar history of colorectal cancer for any of the enrolled patients. Sixty nine of them (76.7%) suffered from colon cancer and the remaining 21 (23.3%) from rectal carcinoma. The main histopathological tumor types were nonmucinous (50%), mucinous (25.6%), poorly differentiated (13.3%), and the remaining were signet-ring cells. Tumor grading and staging was performed according to the tumor-node-metastasis (TNM) classification. Nine (10%) of the patients had tumors in stage I, 61 (68%) in stage II, 12 (13%) in stage III, and the rest of 8 (9%) patients had tumors in stage IV (Table 1). Patients did not

receive chemotherapy or radiation therapy before surgery. Control group was selected from patients who were biopsied for noncancerous conditions including (Ulcerative colitis, Crohn's disease, polyps), in addition to normal nearby mucosa and distant surgical margins from CRC patients. The control group consisted of 36 (64%) men and 20 (36%) women, with a median age of 51 years, ranging from 20 to 75 years. The study was approved by the institutional ethics committee.

All blocks of noncancerous control and tumor tissues, nearby mucosa and distant surgical margins were serially sectioned and used in the present study. Furthermore, tumor tissue and noncancerous control tissue from each formalin-fixed, paraffin-embedded specimen were microdissected and DNA was extracted.

DNA Extraction

DNA from 10 µm sections of paraffin embedded tissue blocks of cancer cases and controls were extracted with an Extraffin kit (Nanogen Advanced Diagnostics S.r.L., Buttigliera Alta, Italy) according to the manufacturer's instructions. The extraction product was stored at -20°C.

Genotyping

The polymorphism (Ile 105 → Val) in exon 5 coding region of GSTP1 gene was detected by Restriction Fragment Length Polymorphism (RFLP) of PCR amplified fragments. Standard GSTP1 primers (forward 5'-ACC-CCA-GGG-CTC-TAT-GGG-AA-3', and reverse 5'-TGA-GGG-CAC-AAG-AAG-CCC-CT-3') from Alpha DNA, Canada, were used for the amplification reactions. Also, restriction enzyme corresponding to RFLP (Fermentas, Canada), was used for the digestion reactions. PCR reactions were carried out in a 30-µl volume containing about 5 µL of genomic DNA template, PCR master mix (200 µM each dNTP, 1.5 mM MgCl₂, 1x PCR buffer [50 mM KCl, 10 mM Tris-HCl (pH 8.3)] and 2 unit Taq DNA polymerase) (Promega, USA) and 200 ng of each primer. After an initial denaturation step of 5 min at 95°C, the samples were processed through 35 temperature cycles of 30 s at 94°C, 1 minute at 59°C and 1 minute at 72°C. A final extension step of 72°C for 10 min, the presence of successful amplification was confirmed by electrophoreses on 2% agarose gel. The 176-bp PCR

Table 1. Pathologic and Clinical Data of Patient with Colorectal Cancer

Demographics		
Age, average		52
Age range		22-85
Sex male / female		1.09
Tumor site:	Right colon	28.90%
	Left colorectum	47.80%
	Rectum	23.30%
Extend of tumor (stage):	Localized (I and II)	78.00%
	Metastatic (III and IV)	22.00%
Tumor histopathology:	Poor differentiation	13.30%
	Nonmucinous	50.00%
	Mucinous-partia	16.70%
	Mucinous-diffuse	8.90%
	Signet-ring cells	11.10%

products (10 µl) were digested overnight at 55°C with 5 units of BsmA1 restriction enzyme. The detection of the different alleles was carried out by horizontal ethidium bromide 4% agarose gel electrophoresis, along with a 100-bp DNA ladder. Genotypes were determined as homozygous for the wild type allele (Ile/Ile; 176 bp), heterozygous (Ile/Val; 176, 91, 85 bp) or homozygous for mutant allele (Val/Val; 91, 85 bp).

Statistical analyses

All statistical analyses were performed using EpiInfo version 6. Chi-square test was used to determine if there are any significant differences in polymorphism frequencies in the cancer cases compared with control population. Statistics were calculated using 95% confidence intervals (P < 0.05 significant).

Results

PCR based genotyping assay was used to examine a GSTP1 polymorphism in colorectal cancer susceptibility. The genotypic results of GSTP1 are presented in Table 2 and Figure 1. Our results showed no genotype effect with GSTP1 genotype. Among colorectal cancer patients, 48% were homozygous for the wild type allele (Ile/Ile), 50% for heterozygous (Ile/Val) and 2% homozygous for mutant allele (Val/Val). While, in the control group, 43% of the subjects were homozygous for the GSTP1 wild type allele, 55.3% were heterozygous and 1.7% homozygous for mutant allele (Table 2). A slight difference was shown not to be significant. Overall, the results indicate that these GSTP1 types do not appear to influence colorectal carcinoma susceptibility in the tested Jordanian population. The observed percentage of

GSTP1 wild type and GSTP1 mutant alleles were 71% and 29%, respectively in the control group. In the 90 colorectal cancer cases, the corresponding frequencies of GSTP1 alleles were 73% and 27% for GSTP1 wild type and mutant alleles, respectively (Table 2). There were no remarkable differences in the distribution of the GSTP1 genotypes between cases or controls.

Discussion

Several factors may contribute to the development of colorectal cancer such as environmental exposures (air pollution, dietary carcinogens, cigarette smoke), susceptibility genes, and the interaction of genotypes and environments. GSTP1 represents only one of many potential candidate colorectal cancer susceptibility genes. GSTP1 is phase II detoxification enzyme that involved in the metabolism of a wide variety of potential carcinogens (Mannervik and Danielson, 1988; Beckett and Hayes, 1993). The GSTP1 variant genotype was seen in increased numbers of tumors of the kidney, bladder, pancreas and lung (To-Figueras et al., 1999; Simic et al., 2009; Vrana et al., 2009). GSTP1 has a polymorphic site at codon 105 (exon 5), where an A to G transition causes an Ile to Val substitution (Ile105Val), resulting in lower enzyme activity to variety of electrophilic molecules (Millar et al., 1999; Hayes et al., 2005; McIlwain et al., 2006). Numerous reports assessed the risk of colorectal cancer development in subjects carrying the variant Val GSTP1 allele; however, the results are controversial (Harries et al., 1997; Welfare et al., 1999; Kiyohara, 2000; Grubben et al., 2001; Loktionov et al., 2001; Seow et al., 2002; Ates et al., 2005; Sun et al., 2005).

The present preliminary study reports the results of GSTP1 genotypes and the impact of the *Ile105Val* GSTP1 polymorphism on colorectal cancer risk in Jordanian population. Although the sample size is small in the present study, the frequency of *Ile105Val* GSTP1 genotypes in 56 unaffected controls (0.43 for Ile/Ile, 0.553 for Ile/Val, and 0.017 for Val/Val) are consistent with those published for other Caucasian type control cohorts such as controls from East Anglia region (0.40, 0.49, 0.11) (Loktionov et al., 2001). And slightly different from those from Newcastle and North Tyneside, England (0.449, 0.427, 0.117) (Welfare et al., 1999), Swedish control group (0.50, 0.40, 0.10) (Sun et al., 2005), and for random control individuals Caucasian type from the Edinburgh area (0.51, 0.425, 0.065) (Harries et al., 1997).

Regarding the risk of colorectal cancer, there has been conflicting evidence concerning the role of GSTP1 polymorphisms in susceptibility to colorectal cancer. GSTP1 seems a more likely candidate susceptibility gene because it is expressed at high levels in the colon and because it has been demonstrated to play a role in heterocyclic amine deactivation (Lin et al., 1994). Harries et al., reported that homozygous possession of the Ile-105 variant may actually protect from colorectal cancer (Harries et al., 1997). Furthermore, based on the obtained protective effect of Ile/Val GSTP1 genotype, Vlaykova et al., suggested that Ile(105)Val GSTP1 polymorphism may play some role in susceptibility to colorectal cancer

Table 2. Genotype and Allele Percentage of the GSTP1 Polymorphism in CRC Patients.

Genotype	Patients (n = 90)		Control (n = 56)		P value
	No.	(%)	No.	(%)	
Ile/Ile	43	(48%)	24	(43%)	P = 0.5618
Ile/Val	45	(50%)	31	(55.30%)	P = 0.5287
Val/Val	2	(2%)	1	(1.70%)	P = 0.8565

Allele	Alleles in 90 cancer cases		Alleles in 56 controls	
	No.	(%)	No.	(%)
Ile	131	(73%)	80	(71%)
Val	49	(27%)	32	(29%)

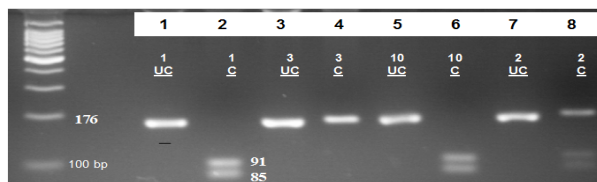


Figure 1. Agarose gel electrophoresis (4%) of the GSTP1 exon 5, codon 105 polymorphism. Lanes 1, 3, 5, & 7 represent untreated samples. Lanes 2, 4, 6, & 8 treated samples with BsmA1 enzyme. Sample in lanes 2& 6 represent mutant allele. Sample in lane 8 represent heterozygous allele. Sample in lane 4 represent homozygous normal allele. UC: uncongested, C: congested.

(Vlaykova et al., 2007). However, the present preliminary study, which involved relatively small case and control cohorts, suggests that variant alleles in the GSTP1 gene are unlikely to carry moderate increase in susceptibility to colorectal cancer, although the possibility of a small effect was not excluded. The statistical analysis of the presence of mutant allele showed no significant relationship between homozygous mutants and/or the heterozygous mutant and increased risk of colorectal cancer (Table 2). The GSTP1 wild type allele was present in 73% and 71% of the cancer cases and control samples, respectively. Hence, the Isoleucine allele does not appear to have a protective effect against colorectal cancer development. On the other hand the mutant allele present in 27% of cancer cases, and in 29% of controls. These results indicate there is no correlation between the presence of mutant allele and increased risk of colorectal cancer. This suggests the mutant allele is randomly distributed in cancer and control cases. Our results are in harmony with the findings of many other reports, who found no effect of the genotype for GSTP1 on colorectal cancer susceptibility (Welfare et al., 1999; Loktionov et al., 2001; Seow et al., 2002; Ates et al., 2005; Sun et al., 2005). Furthermore, they found that the frequencies for the Val-105 allele were 0.33 for controls, and 0.31 for cases, which are almost similar to that of the present study 0.29 and 0.27 for controls and cancer cases respectively (Welfare et al., 1999).

In conclusion, the present study does not confirm previous suggestions of a role for GSTP polymorphism in colorectal cancer susceptibility. The results of the current report show a lack of association between the valine allele and/or variant genotype and colorectal cancer development. Similarly, the frequencies of carriers of the isoleucine allele were not different between the populations. Hence, in this population the isoleucine allele does not appear to have a protective effect against colorectal cancer development. The results of current study have shown that the genotype GSTP1 alone is not involved in colorectal cancer development, but this does not exclude interactions between genes and possible combinations of genotypes.

Acknowledgements

Supported by grant 54/2007 from Jordan University of Science and Technology, Irbid, Jordan. The author is grateful to Dr. Jamil Alalami, Mohamad Alotoom and Nidal Ganim for excellent technical assistance

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