TABLE 6
Odds ratios (ORs) and 95% confidence intervals (CIs) for tumor recurrence according to tertiles of energy and energy-adjusted nutrient intakes after dietary intervention in women

	1 (low; $n = 22$)	2 ($n = 23$)	3 (high; $n = 23$)	P^a
Energy intake (kcal/day)[b]	1,331–1,576	1,576–1,788	1,788–2,298	
No. of cases	12	10	10	
OR (95% CI)[c]	1.0	0.46 (0.11 - 1.86)	0.33 (0.08 - 1.38)	0.37
Fat energy ratio (%)[b]	15.0–22.2	22.2–27.3	27.3–37.1	
No. of cases	14	11	7	
OR (95% CI)[c]	1.0	0.86 (0.22–3.39)	0.27 (0.07–1.11)	0.23
Total fat (g/day)[b]	35.4–49.4	49.4–58.6	58.6–78.8	
No. of cases	14	11	7	
OR (95% CI)[c]	1.0	0.78 (0.20–3.07)	0.30 (0.08–1.21)	0.15
Saturated fatty acids (g/day)[b]	8.1–13.0	13.0–15.2	15.2–23.1	
No. of cases	13	14	5	
OR (95% CI)[c]	1.0	0.96 (0.26–3.60)	0.17 (0.04–0.75)	0.32
Monounsaturated fatty acids (g/day)[b]	10.5–17.0	17.0–19.9	19.9–29.0	
No. of cases	15	10	7	
OR (95% CI)[c]	1.0	0.30 (0.07–1.28)	0.12 (0.02–0.60)	0.21
Polyunsaturated fatty acids (g/day)[b]	6.3–12.1	12.1–14.0	14.0–20.4	
No. of cases	11	13	8	
OR (95% CI)[c]	1.0	1.84 (0.47–7.20)	0.38 (0.10–1.53)	0.77
Linolenic acids (g/day)[b]	0.7–1.6	1.6–1.9	1.9–2.6	
No. of cases	12	10	10	
OR (95% CI)[c]	1.0	0.84 (0.23–3.07)	0.68 (0.18–2.65)	0.03
Linoleic acids (g/day)[b]	5.0–8.9	8.9–10.5	10.5–16.0	
No. of cases	12	12	8	
OR (95% CI)[c]	1.0	1.74 (0.43–7.14)	0.39 (0.10–1.56)	0.73
Linoleic acids per body weight (mg/kg/day)[b]	77–132	132–178	178–316	
No. of cases	14	10	8	
OR (95% CI)[c]	1.0	0.40 (0.10–1.64)	0.24 (0.06–1.05)	0.31

[a]Test for linear trend.
[b]Values in parentheses are range.
[c]OR adjusted for age, body mass index, physical activity, alcohol use, current smoking status, and randomization group, with 95% CI in parentheses.

There are several weak points in this study. First, our subjects with a history of multiple tumors belonged to the high-risk group, not representing the overall Japanese population.

Second, the primary endpoint was not strictly focused on colorectal cancer but also included adenoma, which was actually observed in most of the cases. For this reason, our results might not be directly applicable to colorectal cancer (carcinoma). However, adenoma is widely accepted as the precursor of cancer based on several findings: histologically, many cases of early carcinoma were detected within adenoma; molecular biologically, adenoma and carcinoma have largely common somatic gene mutations; and epidemiologically, risk factors for the development of adenoma and carcinoma were shown to be common. Thus, we believe that the present study with adenoma as the endpoint is in principle applicable to carcinoma.

Third, this study was subsidiary to another clinical trial aiming to assess the prophylactic effects of wheat bran and/or *Lactobacillus casei*. To find out whether there was any confounding effect of wheat bran or *Lactobacillus casei*, ORs were estimated by applying a logistic regression model to each group separately, showing no difference in the results. Fourth, there was no control group without dietary instruction in this study. Fifth, the sample size of women in this study is rather small. Therefore, our results for women should be interpreted with caution. Sixth, frequency of dietary survey during the 4-yr intervention period was rather low. Since alternative analysis using mean values of 6 days at 3 mo and 4 yr led to the same conclusion, however, the

low frequency of dietary survey is not considered to be a major limitation.

On the other hand, the strong point of this study is the application of a 3-day diet record. Its open-ended question system enabled us to analyze a wide variety of food items reported by the subjects, resulting in high validity of our dietary assessment. Also, the 1-h dietary survey for each subject conducted by a trained dietician ensured that the obtained data were highly accurate. Since this study was performed in one hospital, the test results including colonoscopic evaluation were considered to be consistent. Moreover, the high rate of participation (86.7%) and low dropout rate (4.5%) in this study would have provided less bias in the results.

In conclusion, excessive fat restriction is highly likely to have an undesirable effect in promoting the recurrence of colorectal tumors. According to our results, fat energy ratio of 20% seems to form the turning point in substantially increasing the risk; therefore, we suggest that dietary instruction to reduce fat intake under this level should be defined as excessive fat restriction. Deficiencies in lipids, linoleic acid in particular, and stress caused by dietary alteration might be responsible for this outcome.

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Genetic polymorphisms of glutathione S-transferase genes and susceptibility to colorectal cancer: A case–control study in an Indian population

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ABSTRACT

Background: Susceptibility to sporadic colorectal cancer is multifactorial and arises from interactive combinations of allelic variants in low-penetrance genes and relevant environmental risk factors. Genetic polymorphisms in metabolic enzymes as gene susceptibility factors may modify colorectal cancer risk. We evaluated the risk of colorectal cancer associated with respective or combined *glutathione S-transferase (GST)* polymorphisms and assessed the interactions between genes and environmental factors in a case–control study in an Indian population. *Methods*: The study included 59 colon and 243 rectal cancer cases, and 291 cancer-free healthy controls. *GST* genotypes were detected by multiplex PCR-based and PCR-RFLP methods. The risk of cancer associated with *GST* polymorphisms was estimated by calculation of odds ratios (ORs) and confidence intervals (95% CIs) using unconditional logistic regression. *Results*: The *GSTM1* null genotype was found to be associated with a significantly increased rectal cancer risk (OR = 1.55; 95% CI, 1.05–2.30), while the *GSTT1* null genotype with a greater risk of colon cancer (OR = 2.15; 95% CI, 1.04–4.32). A substantial increase of both colon (OR = 10.81; 95% CI, 1.11–107.22) and rectal (OR = 4.80; 95% CI, 0.94–35.91) cancer risk was shown for the combination of *GSTM1* null, *GSTT1* null and *GSTP1* 105Val allele. The combined *GSTM1* null and *GSTP1* 114Val allele also revealed an increased risk for either colon cancer (OR = 4.69; 95% CI, 0.84–23.87) or rectal cancer (OR = 5.68; 95% CI, 1.79–22.16). Furthermore, the combination of *GSTM1* null, *GSTT1* null and *GSTP1* 114Val allele was found in 2 rectal cancer cases. *Conclusion*: Our results suggest that co-exist of *GSTM1* null, *GSTT1* null and the variant *GSTP1* 105Val or 114Val allele may be predisposing risk factors for colorectal cancer in Indian population.

1. Introduction

Colorectal cancer is the second most common cancer in developed countries [1], while the incidence of colorectal cancer has also apparently been increasing in many developing countries with Westernized lifestyles. Susceptibility to sporadic colorectal cancer is multifactorial and arises from interactive combinations of allelic variants in low-penetrance genes and relevant environmental factors such as dietary and lifestyle habits [2,3]. In particular, each low-penetrance allele may contribute a subtle effect on the risk of colorectal cancer, but its interactions with other susceptibility alleles and environmental risk factors can result in a substantial increase in colorectal cancer risk [3–5]. Susceptibility genes can be involved in many different biological pathways such as the metabolic process, while metabolic enzymes (including activating and detoxifying enzymes) play a leading role in the metabolism of endogenous and exogenous chemicals such as polycyclic aromatic hydrocarbons (PAHs) that are ubiquitous environmental, dietary, and tobacco carcinogens. Therefore, polymorphisms in genes that encode metabolic enzymes may

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result in varying activity levels of these enzymes, and then may modify colorectal cancer risk [6].

The glutathione S-transferases (GSTs), a superfamily of phase II metabolic enzymes, catalyze the conjugation between glutathione and chemotherapeutic drugs, carcinogens, environmental pollutants, and a broad spectrum of xenobiotics [7]. GSTs detoxify potentially mutagenic and cytotoxic DNA-reactive metabolites produced by phase I reactions, and serve to protect cellular macromolecules from damage [8]. In humans, the GST enzymes can be divided into five main classes: Alpha (GSTA), Mu (GSTM), Pi (GSTP), Theta (GSTT), and Zeta (GSTZ). Each class consists of one or more isoenzymes (i.e., A1-A4, M1-M5, P1, T1-T2 and Z1), each with a different, but sometimes overlapping substrate specificity [9]. Several polymorphisms occurring in the genes encoding GSTs such as *GSTM1*, *GSTT1*, *GSTP1* and *GSTZ1* have been identified [10–14] and widely discussed in connection with susceptibility to various diseases. The polymorphisms of the *GSTM1* and *GSTT1* loci arise from the complete deletion (null genotype) of each gene [11,15], which causes a lack of enzyme activity [16]. The polymorphisms at the *GSTP1* and *GSTZ1* loci result in amino acid substitutions that lead to reduced activity [17–21].

The situation of colorectal cancer in the Indian population has been described in detail elsewhere [22]. Briefly, although the incidence of colorectal cancer in India is low, and rectal cancer is more common than colon cancer, a significant increase has been reported among both men and women over the last 2 decades. There are geographical and ethnic variations in the genotype frequencies of *GST* genes [23], and the association of *GST* genetic polymorphisms with colorectal cancer has been widely investigated in various ethnic populations, but with inconsistent results [24]. However, since little is known about the impact of *GST* genetic polymorphisms on susceptibility to colorectal cancer in Indian populations, we therefore conducted the present case–control study to estimate the risk of colorectal cancer associated with *GST* genetic polymorphisms both individually or in combinations, and to assess the interactions between genes and environmental factors in terms of tobacco consumption and alcohol intake.

2. Patients and methods

2.1. Participant selection and data collection

Our participant selection and data collection methods have been described previously in detail [22]. In brief, this present case–control study encompassed 302 cases (including 59 colon and 243 rectal cancer patients) and 291 controls. All subjects were recruited at the Cancer Institute at Chennai in South-Eastern India. Cases were first diagnosed as primary colorectal carcinoma, and were histologically confirmed between 1999 and 2001. Colon cancer cases aged from 22 to 72 years old (mean ± SD 48.5 ± 12.0) included 67.8% men, and rectal cancer cases aged from 17 to 75 years old (mean ± SD 49.1 ± 14.1) included 64.6% men. Controls were comprised of cancer-free individuals selected from relatives/visitors to patients with cancers other than gastrointestinal cancers during the same period of our case collection, aged from 20 to 75 years old (mean ± SD 47.3 ± 12.6) included 62.5% men, and frequency matched to cases for sex and age (within 5 years). Informed consent was obtained from all study subjects. Using a standard questionnaire and trained interviewers, information was gathered on demographic variables, education, religion, mother tongue, marital status, socioeconomic conditions, and family history of cancer. Data on smoking status, alcohol consumption and chewing habits were also obtained.

2.2. Genotyping

Genomic DNA was extracted from leukocytes of blood samples. The multiplex PCR-based method was used to detect deletions of *GSTM1* and *GSTT1*, using primers 5′-GAACTCCCTGAAAAGC-TAAAGC-3′ and 5′-GTTGGGCTCAAATATACGTGG-3′ for *GSTM1*, and 5′-TTCCTTACTGGTCCTCACATCTC-3′ and 5′-TCACCGGAT-CATGGCCAGCA-3′ for *GSTT1*. A 273-bp fragment of the β-globin gene was coamplified using primers 5′-CAACTTCATCCACGTT-CACC-3′ and 5′-GAAGAGCCAAGGACAGGTAC-3′ as an internal standard [25].

Genotyping for *GSTP1* and *GSTZ1* was carried out by the PCR-RFLP method. The *GSTP1* gene variants are caused by base-pair transitions at nucleotide +313 (Ile105Val, A–G) in exon 5 and +341 (Ala114Val, C–T) in exon 6 [17]. The *GSTP1* Ile105Val polymorphism was analyzed using the primers 5′-CAGTGACTGTGTGTT-GATCA-3′ and 5′-TGCTCACATAGTTGGTGTAGATGAGGGATA-3′, followed by digestion of the PCR products with *SnaB* I [26]. The *GSTP1* Ala114Val polymorphism was detected with the primers 5′-GTTGTGGGGAGCAAGCAGAGG-3′ and 5′-CACAATGAAGGTCTTGCC-TCCC-3′, with the PCR products being digested by *Aci* I [17]. The polymorphic sites of *GSTZ1* are located at nucleotides 23 (Leu8Pro, T–C), 94 (Lys32Glu, A–G), 124 (Arg42Gly, A–G) and 245 (Thr82Met, C–T) [21]. The *GSTZ1* Lys32Glu polymorphism was detected using primers 5′-TTCCTTACTGGTCCTCACATCTC-3′ and 5′-TCACCGGAT-CATGGCCAGCA-3′, and then *BsmA* I digestion of the PCR products was conducted [13].

2.3. Statistical analysis

Differences in general characteristics between cases and controls were assessed with the Chi-square test and *t*-test, and the disparity in genotypes as well as the Hardy–Weinberg equilibrium was also examined with the Chi-square test. The association between *GST* polymorphisms and colorectal cancer was modeled by unconditional logistic regression analysis using the software package SAS (version 8.2), controlling for potential confounding factors such as age, sex, household income, education, religion, mother tongue, tobacco, alcohol, chewing habits and vegetarianism. Odds ratios (ORs) and confidence intervals (95% CIs) were used to analyze the frequencies of *GST* genotypes occurring in patients with colorectal cancer compared to control groups. The reference group consisted of individuals with putative low-risk genotypes, i.e., the presence of *GSTM1*, *GSTT1*, and homozygous *GSTP1* Ile-105 or Ala-114, and *GSTZ1* Lys-32 functional alleles, the combined effects of *GST* genotypes were calculated at two or three loci. We also assessed the joint effects between genotypes and tobacco consumption or alcohol intake using non-smokers or non-drinkers with low-risk genotypes as the reference. A likelihood ratio test was used to examine the interaction of variables with respect to the risk of colorectal cancer. All statistical tests were two-sided, and statistical significance was determined as $p < 0.05$.

3. Results

Since the general characteristics of the study participants were previously presented in detail [22], they were omitted here. The frequencies of *GST* genotypes by case–control status and the association of *GST* polymorphisms with cancers are shown in Table 1. The frequencies of *GSTM1* and *GSTT1* null genotypes, *GSTP1* 105Val, 114Val and *GSTZ1* 32Glu alleles were 0.31, 0.25, 0.36, 0.05 and 0.83 among colon cancer cases, while 0.34, 0.17, 0.30 0.08 and 0.82 among rectal cancer cases, compared with 0.26, 0.15, 0.27, 0.05 and 0.79 among controls. In the control group, genotype distributions of *GSTP1* Ile105Val (Ile/Ile, 55.0%; Ile/Val, 36.8%; Val/

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Table 1
Genotype frequencies and adjusted ORs[a] for colon, rectal and colorectal cancer with polymorphisms of *GSTM1*, *GSTT1*, *GSTP1* and *GSTZ1*.

Genotype	Controls (n=291) n (%)	Colon cancer (n=59) n (%)	ORs (95% CI)	Rectal cancer (n=243) n (%)	ORs (95% CI)	Colorectal cancer (n=302) n (%)	ORs (95% CI)
GSTM1							
Present	215 (73.9)	41 (69.5)	1 (Ref)	161 (66.3)	1 (Ref)	202 (66.9)	1 (Ref)
Null	76 (26.1)	18 (30.5)	1.20 (0.62–2.26)	82 (33.7)	1.55 (1.05–2.30)	100 (33.1)	1.47 (1.02–2.14)
GSTT1							
Present	247 (84.9)	44 (74.6)	1 (Ref)	201 (82.7)	1 (Ref)	245 (81.1)	1 (Ref)
Null	44 (15.1)	15 (25.4)	2.15 (1.04–4.32)	42 (17.3)	1.17 (0.72–1.97)	57 (18.9)	1.33 (0.85–2.09)
GSTP1 Ile105Val							
Ile/Ile	160 (55.0)	27 (45.8)	1 (Ref)	114 (46.9)	1 (Ref)	141 (46.7)	1 (Ref)
Ile/Val	107 (36.8)	22 (37.3)	1.15 (0.60–2.16)	110 (45.3)	1.44 (0.99–2.09)	132 (43.7)	1.37 (0.96–1.95)
Val/Val	24 (8.2)	10 (16.9)	2.31 (0.92–5.57)	19 (7.8)	1.12 (0.56–2.21)	29 (9.6)	1.29 (0.70–2.40)
Val[b]	131 (45.0)	32 (54.2)	1.35 (0.75–2.44)	129 (53.1)	1.37 (0.96–1.97)	161 (53.3)	1.35 (0.97–1.90)
GSTP1 Ala114Val							
Ala/Ala	263 (90.4)	53 (89.8)	1 (Ref)	208 (85.6)	1 (Ref)	261 (86.4)	1 (Ref)
Ala/Val	27 (9.3)	6 (10.2)	1.24 (0.42–3.20)	32 (13.2)	1.65 (0.88–3.16)	38 (12.6)	1.40 (0.78–2.56)
Val/Val	1 (0.3)	0 (0.0)	NA	3 (1.2)	2.33 (0.25–51.38)	3 (1.0)	1.98 (0.22–43.32)
Val[c]	28 (9.6)	6 (10.2)	1.15 (0.39–2.94)	35 (14.4)	1.69 (0.91–3.17)	41 (13.6)	1.43 (0.80–2.55)
GSTZ1 Lys32Glu							
Lys/Lys	15 (15.1)	2 (3.4)	1 (Ref)	10 (4.1)	1 (Ref)	12 (4.0)	1 (Ref)
Lys/Glu	93 (32.0)	16 (27.1)	1.08 (0.26–7.43)	66 (27.2)	0.78 (0.32–1.98)	82 (27.1)	0.89 (0.38–2.11)
Glu/Glu	183 (62.9)	41 (69.5)	1.46 (0.37–9.77)	167 (68.7)	1.05 (0.44–2.56)	208 (68.9)	1.17 (0.52–2.71)
Glu[d]	276 (94.9)	57 (96.6)	1.31 (0.34–8.64)	238 (95.9)	0.96 (0.41–2.34)	290 (96.0)	1.07 (0.48–2.45)

[a] Adjusted for gender, age, household income, education, religion, mother tongue, smoking, drinking, chewing and vegetarianism.
[b] Ile/Val or Val/Val.
[c] Ala/Val or Val/Val.
[d] Lys/Glu or Glu/Glu.

Val, 8.2%), *GSTP1* Ala114Val (Ala/Ala, 90.4%; Ala/Val, 9.3%; Val/Val, 0.3%) and *GSTZ1* Lys32Glu (Lys/Lys, 15.1%; Lys/Glu, 32.0%; Glu/Glu, 62.9%) were all in agreement with the Hardy–Weinberg equilibrium (p = 0.31; 0.73; 0.48, respectively). A significant association was found between *GSTM1* null genotype and rectal cancer (OR = 1.55; 95% CI, 1.05–2.30), as well as between *GSTT1* null genotype and colon cancer (OR = 2.15; 95% CI, 1.04–4.32). A non-statistically significant increase in rectal cancer risk was found in both variant *GSTP1* 105Val (OR = 1.37; 95% CI, 0.96–1.97) and 114Val (OR = 1.69; 95% CI, 0.91–3.17) alleles. No significant association was found between *GSTZ1* Lys32Glu polymorphism and colorectal cancer.

The combined effects of two putative risk genotypes of *GST* polymorphisms are summarized in Table 2. The combination of *GSTM1* null with *GSTT1* null showed that the risk was increased 6.2-fold for colon cancer (95% CI, 1.62–22.61) and 2.6-fold for rectal cancer (95% CI, 0.94–7.56). The combined *GSTM1* null genotype and *GSTP1* 114Val allele also revealed a 4.7-fold increase in colon cancer risk (95% CI, 0.84–23.87) and a 5.7-fold rise in rectal cancer risk (95% CI, 1.79–22.16). Those individuals who carried the combined *GSTM1*/*GSTT1* null genotype and *GSTP1* 105Val allele also suffered somewhat increased colon and rectal cancer risks. With respect to colorectal cancer (overall colon and rectal cancers), a significantly increased risk was found in the combination of *GSTM1* null genotype with *GSTT1* null genotype (OR = 2.98; 95% CI, 1.19–8.18); with *GSTP1* 105Val allele (OR = 2.14; 95% CI, 1.25–3.69) and *GSTP1* 114Val allele (OR = 4.71; 95% CI, 1.60–17.34), as well as in the combination of *GSTT1* null genotype with *GSTP1* 105Val allele (OR = 1.89; 95% CI, 1.01–3.59), and *GSTP1* 105Val allele with *GSTZ1* 32Glu allele (OR = 2.84; 95% CI, 1.03–9.13).

We further investigated the combined effects of three putative risk genotypes (see Table 3). An increased risk for colon (OR = 10.81; 95% CI, 1.11–107.22), rectal (OR = 4.80; 95% CI, 0.94–35.91) and colorectal (OR = 4.63; 95% CI, 1.03–32.87) cancers was found in individuals with combined *GSTM1* null, *GSTT1* null genotype and *GSTP1* 105Val allele compared to combined *GSTM1* present, *GSTT1* present and *GSTP1* 105Ile/Ile genotypes. The combined *GSTM1* null, *GSTT1* present genotype and *GSTP1* 114Val allele also suggested a significantly increased risk for both colon (OR = 6.31; 95% CI, 1.03–35.42) and rectal (OR = 4.67; 95% CI, 1.28–20.53) cancers using the combined low-risk genotypes (*GSTM1* present, *GSTT1* present and *GSTP1* 114Ala/Ala genotypes) as the reference. For the combination of *GSTM1* null, *GSTT1* null genotype and *GSTP1* 114Val allele, only 2 rectal cancer cases were found among all study participants. Since both *GSTP1* 114Val allele and *GSTZ1* 32Lys/Lys genotype were rare among our study subjects, the other combinations of three risk genotypes were unable to be conducted.

The interactions of gene-tobacco are presented in Table 4. For colon cancer, no significant tobacco effect modification was found for *GSTM1* and *GSTT1* genotypes. Though both *GSTP1* 105Val and 114Val alleles showed an increased risk among smokers, it did not reach statistical significance. For rectal cancer, except for *GSTM1* null genotype, *GSTT1* null genotype (OR = 2.32; 95% CI, 0.91–6.27), *GSTP1* 105Val allele (OR = 2.05; 95% CI, 1.05–4.08), and 114Val allele (OR = 3.30; 95% CI, 0.89–15.87) were shown to have a positive association among smokers, though only *GSTP1* 105Val allele reached statistical significance. The interaction of the *GSTZ1* Lys32Glu polymorphism with smoking was also analyzed, but no significant relationship was found (data not shown).

As to the interactions of gene–alcohol, no significant effect modification was observed to a risk of either colon or rectal cancer (data not shown).

4. Discussion

We investigated the role of *GST* polymorphisms in the development of colorectal cancer in an Indian population. The *GSTM1* null genotype was found to be associated with a significantly increased rectal cancer risk (OR = 1.55; 95% CI, 1.05–2.30), while the *GSTT1* null genotype was related to a greater risk of colon cancer (OR = 2.15; 95% CI, 1.04–4.32). Both variant *GSTP1* 105Val (OR = 1.37; 95% CI, 0.96–1.97) and 114Val (OR = 1.69; 95% CI, 0.91–3.17) alleles were found to be at a somewhat increased rectal cancer risk. No significant association was found between the *GSTZ1* polymorphism and the colorectal

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Table 2
ORs[a] for colon, rectal and colorectal cancer by combined *GSTM1*, *GSTT1*, *GSTP1* and *GSTZ1* genotypes.

Combined genotypes		Controls *n* (%)	Colon cancer *n* (%)	ORs (95% CI)	Rectal cancer *n* (%)	ORs (95% CI)	Colorectal cancer *n* (%)	ORs (95% CI)
GSTM1	GSTT1							
Present	Present	178 (61.2)	31 (52.5)	1 (Ref)	129 (53.1)	1 (Ref)	160 (53.0)	1 (Ref)
Present	Null	37 (12.7)	10 (17.0)	1.56 (0.65–3.53)	32 (13.2)	1.16 (0.66–2.01)	42 (13.9)	1.21 (0.73–2.04)
Null	Present	69 (23.7)	13 (22.0)	0.97 (0.45–2.00)	72 (29.6)	1.51 (0.99–2.30)	85 (28.1)	1.40 (0.93–2.08)
Null	Null	7 (2.4)	5 (8.5)	6.19 (1.62–22.61)	10 (4.1)	2.59 (0.94–7.56)	15 (5.0)	2.98 (1.19–8.18)
GSTM1	GSTP1 Ile105Val							
Present	Ile/Ile	115 (39.5)	18 (30.5)	1 (Ref)	77 (31.7)	1 (Ref)	95 (31.5)	1 (Ref)
Present	Val[b]	100 (34.4)	23 (39.0)	1.31 (0.65–2.68)	84 (34.6)	1.24 (0.81–1.92)	107 (35.4)	1.26 (0.84–1.90)
Null	Ile/Ile	45 (15.5)	9 (15.3)	1.14 (0.43–2.82)	37 (15.2)	1.31 (0.75–2.29)	46 (15.2)	1.31 (0.78–2.21)
Null	Val[b]	31 (10.6)	9 (15.3)	1.75 (0.66–4.40)	45 (18.5)	2.30 (1.31–4.08)	54 (17.9)	2.14 (1.25 –3.69)
GSTM1	GSTP1 Ala114Val							
Present	Ala/Ala	191 (65.6)	38 (64.4)	1 (Ref)	138 (56.8)	1 (Ref)	176 (58.3)	1 (Ref)
Present	Val[c]	24 (8.3)	3 (5.1)	0.60 (0.14–1.91)	23 (9.5)	1.33 (0.68–2.56)	26 (8.6)	1.11 (0.59–2.08)
Null	Ala/Ala	72 (24.7)	15 (25.4)	0.97 (0.47–1.91)	70 (28.8)	1.44 (0.95–2.19)	85 (28.1)	1.32 (0.89–1.96)
Null	Val[c]	4 (1.4)	3 (5.1)	4.69 (0.84–23.87)	12 (4.9)	5.68 (1.79–22.16)	15 (5.0)	4.71 (1.60–17.34)
GSTM1	GSTZ1 Lys32Glu							
Present	Lys/Lys	10 (3.4)	2 (3.4)	1 (Ref)	5 (2.1)	1 (Ref)	7 (2.3)	1 (Ref)
Present	Glu[d]	205 (70.5)	39 (66.1)	0.80 (0.19–3.51)	156 (64.2)	1.00 (0.33–3.40)	195 (64.6)	1.01 (0.36–2.96)
Null	Lys/Lys	5 (1.7)	0 (0.0)	NA	5 (2.1)	1.55 (0.28–8.96)	5 (1.7)	1.20 (0.23–6.34)
Null	Glu[d]	71 (24.4)	18 (30.5)	1.03 (0.23–7.37)	77 (31.7)	1.55 (0.50–5.38)	95 (31.4)	1.51 (0.53–4.50)
GSTT1	GSTP1 Ile105Val							
Present	Ile/Ile	136 (46.7)	20 (33.9)	1 (Ref)	98 (40.3)	1 (Ref)	118 (39.1)	1 (Ref)
Present	Val[b]	111 (38.1)	24 (40.7)	1.42 (0.73–2.79)	103 (42.4)	1.28 (0.87–1.90)	127 (40.0)	1.30 (0.90–1.88)
Null	Ile/Ile	24 (8.3)	7 (11.9)	2.42 (0.83–6.49)	16 (6.6)	0.94 (0.45–1.91)	23 (7.6)	1.18 (0.61–2.27)
Null	Val[b]	20 (6.9)	8 (13.6)	2.73 (0.96–7.40)	26 (10.7)	1.79 (0.91–3.53)	34 (11.3)	1.89 (1.01–3.59)
GSTT1	GSTP1 Ala114Val							
Present	Ala/Ala	223 (76.6)	38 (64.4)	1 (Ref)	173 (71.2)	1 (Ref)	211 (69.9)	1 (Ref)
Present	Val[c]	24 (8.2)	6 (10.2)	1.54 (0.52–4.04)	28 (11.5)	1.49 (0.80–2.78)	34 (11.3)	1.44 (0.80–2.62)
Null	Ala/Ala	40 (13.8)	15 (25.4)	2.45 (1.17–5.04)	35 (14.4)	1.10 (0.65–1.85)	50 (16.5)	1.32 (0.82–2.14)
Null	Val[c]	4 (1.4)	0 (0.0)	NA	7 (2.9)	2.63 (0.73–10.69)	7 (2.3)	2.07 (0.58–8.36)
GSTT1	GSTZ1 Lys32Glu							
Present	Lys/Lys	13 (4.5)	1 (1.7)	1 (Ref)	7 (2.9)	1 (Ref)	8 (2.7)	1 (Ref)
Present	Glu[d]	234 (80.4)	43 (72.9)	1.83 (0.33–34.36)	194 (79.8)	1.08 (0.41–3.01)	237 (78.5)	1.19 (0.48–3.14)
Null	Lys/Lys	2 (0.7)	1 (1.7)	4.08 (0.11–159.75)	3 (1.2)	1.86 (0.23–18.08)	4 (1.3)	1.97 (0.29–17.82)
Null	Glu[d]	42 (14.4)	14 (23.7)	3.84 (0.62–75.06)	39 (16.1)	1.23 (0.43–3.70)	53 (17.5)	1.55 (0.58–4.36)
GSTP1 Ile105Val	GSTP1 Ala114Val							
Ile/Ile	Ala/Ala	160 (55.0)	27 (45.8)	1 (Ref)	114 (46.9)	1 (Ref)	141 (46.7)	1 (Ref)
Val[b]	Ala/Ala	103 (35.4)	26 (44.0)	1.37 (0.74–2.55)	94 (38.7)	1.27 (0.86–1.87)	120 (39.7)	1.28 (0.89–1.84)
Val[b]	Val[c]	28 (9.6)	6 (10.2)	1.27 (0.43–3.34)	35 (14.4)	1.80 (1.00–3.25)	41 (13.6)	1.63 (0.93–2.87)
GSTP1 Ile105Val	GSTZ1 Lys32Glu							
Ile/Ile	Lys/Lys	15 (5.2)	0 (0.0)	1 (Ref)	5 (2.1)	1 (Ref)	5 (1.7)	1 (Ref)
Ile/Ile	Glu[d]	145 (49.8)	27 (45.8)	NA	109 (44.9)	1.76 (0.63–5.70)	136 (45.0)	2.33 (0.85–7.51)
Val[b]	Lys/Lys	0 (0.0)	2 (3.4)	NA	5 (2.1)	NA	7 (2.3)	NA
Val[b]	Glu[d]	131 (45.0)	30 (50.8)	NA	124 (51.0)	2.21 (0.80–7.17)	154 (51.0)	2.84 (1.03–9.13)
GSTP1 Ala114Val	GSTZ1 Lys32Glu							
Ala/Ala	Lys/Lys	15 (5.2)	2 (3.4)	1 (Ref)	9 (3.7)	1 (Ref)	11 (3.6)	1 (Ref)
Ala/Ala	Glu[d]	248 (85.2)	51 (86.4)	1.29 (0.33–8.59)	199 (81.9)	1.04 (0.44–2.61)	250 (82.8)	1.16 (0.51–2.71)
Val[c]	Lys/Lys	0 (0.0)	0 (0.0)	NA	1 (0.4)	NA	1 (0.3)	NA
Val[c]	Glu[d]	28 (9.6)	6 (10.2)	1.40 (0.27–10.69)	34 (14.0)	1.60 (0.59–4.52)	40 (13.3)	1.60 (0.62–4.23)

[a] Adjusted for gender, age, household income, education, religion, mother tongue, smoking, drinking, chewing and vegetarianism.
[b] Ile/Val or Val/Val.
[c] Ala/Val or Val/Val.
[d] Lys/Glu or Glu/Glu

cancer. Although the respective *GST* polymorphisms showed a subtle effect on the colorectal cancer risk, that risk rose as putative risk genotypes increased from the combinations of two or three of *GSTM1* null, *GSTT1* null, *GSTP1* 105Val and 114Val alleles.

In our control group, the frequencies of *GSTM1* and *GSTT1* null genotypes, *GSTP1* 105Val, 114Val and *GSTZ1* 32Glu alleles were, respectively, 0.26, 0.15, 0.27, 0.05 and 0.79, which were in accordance with the low prevalence of *GSTM1* null genotype (0.22–0.27) and similar to the frequencies of *GSTT1* null genotype (0.07–0.18) and *GSTP1* 105Val allele (0.22–0.25) reported in Indian population [27–30]. We first detected the distributions of *GSTP1* Ala114Val and *GSTZ1* Lys32Glu polymorphisms, and found that the variant *GSTP1* 114Val allele was rare, while the *GSTZ1* 32Glu allele was common among Indian subjects.

GSTs, as detoxifying enzymes, play an important role in the cellular defense system. GSTM1 is known to detoxify active metabolites of PAHs [16], GSTT1 is involved in the detoxification of several environmental carcinogens such as 1,3-butadiene and ethylene oxide in tobacco smoke and ambient air [31]. Whereas GSTP1 is widely expressed in normal epithelial tissues and has been shown to be highly over-expressed in colon cancer [32,33], it metabolizes numerous carcinogenic compounds including benzo[a]pyrene, a tobacco carcinogen [26]. GST Zeta catalyzes the metabolism of a series of alpha-haloacids including the carcinogen dichloroacetate [34,35], a common contaminant of chlorinated drinking water. GSTZ1, as a maleylacetoacetate isomerase, also participates in the catabolic pathway of phenylalanine and tyrosine [36]. Due to the inactive form of the enzymes (null genotype of *GSTM1* or *GSTT1*, the variant allele of *GSTP1* 105Val, 114Val or *GSTZ1* 32Glu), their capacity to detoxify activated carcinogen is diminished, leading to a progression of cancer. Interindividual differences in cancer susceptibility may be partly

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Table 3
ORs[a] for colon, rectal and colorectal cancer by combined *GSTM1*, *GSTT1* and *GSTP1*genotypes.

Combined genotypes			Controls n (%)	Colon cancer n (%)	ORs (95% CI)	Rectal cancer n (%)	ORs (95% CI)	Colorectal cancer n (%)	ORs (95% CI)
GSTM1	GSTT1	GSTP1 Ile/Val							
Present	Present	Ile/Ile	96 (33.0)	14 (23.7)	1 (Ref)	66 (27.1)	1 (Ref)	80 (26.5)	1 (Ref)
Present	Present	Val[b]	82 (28.2)	17 (28.8)	1.43 (0.63–3.27)	63 (25.9)	1.11 (0.69–1.80)	80 (26.5)	1.15 (0.74–1.81)
Present	Null	Ile/Ile	19 (6.5)	4 (6.8)	1.69 (0.42–5.67)	11 (4.5)	0.79 (0.33–1.84)	15 (5.0)	0.92 (0.42–2.00)
Present	Null	Val[b]	18 (6.2)	6 (10.2)	2.24 (0.66–7.13)	21 (8.6)	1.59 (0.76–3.38)	27 (8.9)	1.68 (0.84–3.43)
Null	Present	Ile/Ile	40 (13.7)	6 (10.2)	0.86 (0.27–2.48)	32 (13.2)	1.20 (0.66–2.18)	38 (12.6)	1.15 (0.66–2.02)
Null	Present	Val[b]	29 (10.0)	7 (11.8)	1.64 (0.54–4.61)	40 (16.5)	2.07 (1.14–3.77)	47 (15.6)	1.95 (1.11–3.47)
Null	Null	Ile/Ile	5 (1.7)	3 (5.1)	7.16 (1.19–38.13)	5 (2.1)	1.89 (0.40–7.37)	8 (2.6)	2.59 (0.80–9.10)
Null	Null	Val[b]	2 (0.7)	2 (3.4)	10.81 (1.11–107.22)	5 (2.1)	4.80 (0.94–35.91)	7 (2.3)	4.63 (1.03–32.87)
GSTM1	GSTT1	GSTP1 Ala/Val							
Present	Present	Ala/Ala	158 (54.3)	28 (47.5)	1 (Ref)	111 (45.7)	1 (Ref)	139 (46.0)	1 (Ref)
Present	Present	Val[c]	20 (6.9)	3 (5.1)	0.81 (0.17–2.79)	18 (7.4)	1.27 (0.57–2.88)	21 (7.0)	1.05 (0.49–2.24)
Present	Null	Ala/Ala	33 (11.3)	10 (16.9)	1.82 (0.73–4.29)	27 (11.1)	1.12 (0.57–2.17)	37 (12.2)	1.36 (0.75–2.48)
Present	Null	Val[c]	4 (1.4)	0 (0.0)	NA	5 (2.1)	2.10 (0.46–10.55)	5 (1.7)	1.71 (0.39–7.84)
Null	Present	Ala/Ala	65 (22.3)	10 (16.9)	0.70 (0.29–1.59)	62 (25.5)	1.52 (0.93–2.50)	72 (23.8)	1.29 (0.82–2.03)
Null	Present	Val[c]	4 (1.4)	3 (5.1)	6.31 (1.03–35.42)	10 (4.1)	4.67 (1.28–20.53)	13 (4.3)	4.35 (1.35–17.05)
Null	Null	Ala/Ala	7 (2.4)	5 (8.5)	5.57 (1.37–21.64)	8 (3.3)	2.13 (0.64–7.49)	13 (4.3)	2.43 (0.86–7.51)
Null	Null	Val[c]	0 (0.0)	0 (0.0)	NA	2 (0.8)	NA	2 (0.7)	NA

[a] Adjusted for gender, age, household income, education, religion, mother tongue, smoking, drinking, chewing and vegetarianism.
[b] Ile/Val or Val/Val.
[c] Ala/Val or Val/Val.

attributed to the polymorphic variability in the activation and detoxification of carcinogens.

Although most previous studies of different ethnic populations suggested no significant association of colorectal cancer with *GSTM1* null genotype [24], two did show an increased colorectal cancer risk among Caucasians [37,38], while two others recently conducted in the European-Asian area (Hungary and Turkey) also reported a positive association [39,40]. Moreover, the *GSTM1* null genotype showed a significantly increased risk of developing rectal cancer in our study (OR = 1.55; 95% CI, 1.05–2.30). Several studies have demonstrated a strong association of *GSTT1* null genotype with colorectal cancer [40–43]. We found a significantly increased colon cancer risk (OR = 2.15; 95% CI, 1.04–4.32) in the present study, and a weak association with colorectal cancer (OR = 1.33; 95% CI, 0.85–2.09) similar to that in total tendency (OR = 1.37; 95% CI, 1.17–1.60) [24]. In agreement with several studies [14,40,44], *GSTP1* 105Val allele showed a slightly increased colorectal cancer risk in our study (OR = 1.35; 95% CI, 0.97–1.90). Unlike previous reports [14,45], we also found a non-statistically elevated colorectal cancer risk with *GSTP1* 114Val allele (OR = 1.43; 95% CI, 0.80–2.55).

We assessed the combined effects of two or three putative risk genotypes (*GSTM1* null, *GSTT1* null, *GSTP1* 105Val, or 114Val and *GSTZ1* 32Glu alleles) compared to low-risk genotypes (*GSTM1* present, *GSTT1* present, *GSTP1* 105Ile/Ile or 114Ala/Ala and *GSTZ1* 32Lys/Lys genotypes). The combination of *GSTM1* null with *GSTT1* null showed a 6.2-fold increased colon cancer risk (95% CI, 1.62–22.61). The combined *GSTM1* null and *GSTP1* 114Val allele also

Table 4
Assessments of interaction between tobacco and *GST* genetic polymorphisms in colon, rectal and colorectal cancer.

Smoking status	Genotypes	Controls (n = 291)	Colon cancer (n = 59)	ORs (95% CI)[a]	Rectal cancer (n = 243)	ORs (95% CI)[a]	Colorectal cancer (n = 302)	ORs (95% CI)[a]
Non-smokers		225 (77.3)	44 (74.6)	1 (Ref)	188 (77.4)	1 (Ref)	232 (76.8)	1 (Ref)
Smokers		66 (22.7)	15 (25.5)	1.27 (0.58–2.71)	55 (22.6)	1.02 (0.63–1.64)	70 (23.2)	1.03 (0.66–1.62)
	GSTM1							
Non-smokers	Present	173 (59.5)	29 (49.2)	1 (Ref)	117 (48.2)	1 (Ref)	146 (48.3)	1 (Ref)
Non-smokers	Null	52 (17.9)	15 (25.4)	1.65 (0.78–3.39)	71 (29.2)	2.24 (1.43–3.52)	86 (28.5)	2.10 (1.37–2.45)
Smokers	Present	42 (14.4)	12 (20.3)	1.84 (0.77–4.30)	44 (18.1)	1.64 (0.95–2.85)	56 (18.5)	1.64 (0.98–2.75)
Smokers	Null	24 (18.2)	3 (5.1)	0.82 (0.18–2.85)	11 (4.5)	0.69 (0.30–1.53)	14 (4.6)	0.67 (0.32–1.43)
	GSTT1							
Non-smokers	Present	189 (65.0)	30 (50.9)	1 (Ref)	160 (65.8)	1 (Ref)	190 (62.9)	1 (Ref)
Non-smokers	Null	36 (12.4)	14 (23.7)	2.53 (1.16–5.37)	28 (11.5)	0.88 (0.50–1.54)	42 (13.9)	1.12 (0.68–1.86)
Smokers	Present	58 (19.9)	14 (23.7)	1.62 (0.71–3.60)	41 (16.9)	0.85 (0.51–1.43)	55 (18.2)	0.94 (0.58–1.52)
Smokers	Null	8 (2.7)	1 (1.7)	1.12 (0.06–7.09)	14 (5.8)	2.32 (0.91–6.27)	15 (5.0)	2.03 (0.82–5.42)
	GSTP1 Ile105Val							
Non-smokers	Ile/Ile	114 (39.2)	19 (32.2)	1 (Ref)	91 (37.4)	1 (Ref)	110 (36.4)	1 (Ref)
Non-smokers	Val[b]	111 (38.1)	25 (42.4)	1.34 (0.68–2.28)	97 (39.9)	1.07 (0.71–1.60)	122 (40.4)	1.09 (0.75–1.60)
Smokers	Ile/Ile	46 (15.8)	8 (13.5)	1.26 (0.44–3.43)	23 (9.5)	0.62 (0.33–1.17)	31 (10.3)	0.69 (0.38–1.23)
Smokers	Val[b]	20 (6.9)	7 (11.9)	2.01 (0.63–1.64)	32 (13.2)	2.05 (1.05–4.08)	39 (12.9)	1.97 (1.04–3.81)
	GSTP1 Ala114Val							
Non-smokers	Ala/Ala	200 (68.7)	41 (69.5)	1 (Ref)	161 (66.3)	1 (Ref)	202 (66.9)	1 (Ref)
Non-smokers	Val[c]	25 (8.6)	3 (5.1)	0.67 (0.15–2.14)	27 (11.1)	1.42 (0.77–2.64)	30 (9.9)	1.26 (0.70–2.30)
Smokers	Ala/Ala	63 (21.7)	12 (20.3)	1.07 (0.46–2.40)	47 (19.3)	0.95 (0.57–1.56)	59 (19.5)	0.95 (0.60–1.52)
Smokers	Val[c]	3 (1.0)	3 (5.1)	3.35 (0.57–19.67)	8 (3.3)	3.30 (0.89–15.87)	11 (3.6)	3.03 (0.89–13.92)

[a] Adjusted for gender, age, household income, education, religion, mother tongue, drinking, chewing and vegetarianism.
[b] Ile/Val or Val/Val.
[c] Ala/Val or Val/Val.

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revealed a 4.7-fold increase in colon cancer risk (95% CI, 0.84–23.87) and a 5.7-fold rise in rectal cancer risk (95% CI, 1.79–22.16). Enlarged sample size enhanced the statistical power, a significant increase of colorectal cancer (including colon and rectal cancers) risk was revealed in the combination of *GSTM1* null genotype with *GSTT1* null genotype (OR = 2.98; 95% CI, 1.19–8.18); *GSTP1* 105Val allele (OR = 2.14; 95% CI, 1.25–3.69); and *GSTP1* 114Val allele (OR = 4.71; 95% CI, 1.60–17.34). Such an increased colorectal cancer risk was also found in the combination of *GSTT1* null with *GSTP1* 105Val allele (OR = 1.89; 95% CI, 1.01–3.59) as well as *GSTP1* 105Val allele with *GSTZ1* 32Glu allele (OR = 2.84; 95% CI, 1.03–9.13). Similar to that reported previously [40], the risk of colorectal cancer substantially increased as putative risk genotypes increased in the combination of *GSTM1* null, *GSTT1* null genotype and *GSTP1* 105Val allele (OR = 4.63; 95% CI, 1.03–32.87) in our study. Moreover, the highest colon cancer risk was markedly demonstrated in this combination (OR = 10.81; 95% CI, 1.11–107.22). A study conducted in the Tamilian population of south India [30] also demonstrated the most remarkable risk of upper aerodigestive tract cancer with this combination (OR = 7.8; 95% CI, 1.0–61.0). In addition, the combined *GSTM1* null, *GSTT1* present genotype and *GSTP1* 114Val allele suggested a significantly increased risk of colon (OR = 6.31; 95% CI, 1.03–35.42) and rectal (OR = 4.67; 95% CI, 1.28–20.53) cancers. Furthermore, 2 rectal cancer cases were found to carry a combined *GSTM1* null, *GSTT1* null and *GSTP1* 114Val allele in our study.

The interactions of gene–tobacco were evaluated in our study. No significant tobacco modification effect on the risk of both colon and rectal cancers was found for GSTM1 genotypes. With respect to smokers, *GSTT1* null genotype was found to be associated with a trend toward increased rectal cancer risk. Either *GSTP1* 105Val or 114Val allele also showed a weakly positive association with colon and rectal cancers. However, the statistical power to detect gene–tobacco interactions was limited in our study due to the small number of smokers. In addition, the joint effects of gene–alcohol were also estimated, with no significant modifying effect found.

In conclusion, we first estimated the association of *GST* genetic polymorphisms with colorectal cancer risk in an Indian population, and found that *GSTM1* null, *GSTT1* null genotype and the variant *GSTP1* 105Val or 114Val allele may be predisposing risk factors for colorectal cancer. Moreover, gene–gene interactions may contribute to a substantial increase in colorectal cancer risk, while the joint effects of gene–tobacco may weakly modify the development of colorectal cancer in our Indian population. Our findings suggest that GST polymorphisms may play an important role in the detection of early colorectal cancer and in the surveillance of a high-risk population in India.

Conflict of interest

None declared.

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ORIGINAL PAPER

Polymorphisms in DNA repair genes *XRCC1*, *XRCC3* and *XPD*, and colorectal cancer risk: a case–control study in an Indian population

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Abstract
Purpose Genetic polymorphisms in DNA repair genes may influence variations in individual DNA repair capacity, which could be associated with the development of cancer. We detected the distributions of three single-nucleotide polymorphisms (*XRCC1* Arg399Gln, *XRCC3* Thr241Met and *XPD* Lys751Gln) in DNA repair genes, and assessed the associations of these genetic polymorphisms with colon and rectal cancer susceptibility as well as evaluated the interactions of gene–gene and gene–environment in a case–control study of an Indian population.
Methods This case–control study was conducted with 302 cases (including 59 colon and 243 rectal cancer patients) and 291 cancer-free healthy controls. Genotypes were determined by PCR–RLFP assays. The effects [odds ratios (ORs) and 95% confidence intervals (95% CIs)] of genetic polymorphisms on colorectal cancer were estimated using unconditional logistic regression.
Results The *XRCC1* 399Gln allele was found to be associated with a significantly increased rectal cancer risk among men (OR = 1.65, 95% CI 1.04–2.64). Whereas the *XRCC3* 241Met allele showed a protective tendency against rectal cancer (OR = 0.68, 95% CI 0.46–1.02) for both men and women. Furthermore, a combination of the *XRCC1* 399Gln allele with *XRCC3* Thr/Thr genotype and the *XPD* 751Gln allele demonstrated the highest rectal cancer risk (OR = 3.52, 95% CI 1.43–9.44).
Conclusions The combined effects of putative risk alleles/genotypes for different DNA repair pathways may strengthen the susceptibility to rectal cancer.

Keywords Colorectal cancer · Susceptibility · Single nucleotide polymorphism · DNA repair genes

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Introduction

Colorectal cancer is a complex disease resulting from both environmental and genetic factors. Although the development of colorectal cancer has mainly been attributed to environmental factors, such as diet, lifestyle and environmental pollution (Doll and Peto 1981; Thomas 1993), inter-individual differences in susceptibility to colorectal cancer

may be due to genetic alterations, including those involved in DNA repair (Potter 1999; de Jong et al. 2002). Four major DNA repair pathways have been identified in mammalian cells, i.e., base excision repair (BER), nucleotide excision repair (NER), double-strand break repair and mismatch repair (Christmann et al. 2003). Humans are routinely exposed to mutagenic and carcinogenic chemicals originating from cigarette smoking, well-cooked food, combustion of fossil fuels and other sources (Vineis 1994), all of which can form DNA adducts and lead to DNA damage (Vineis et al. 1996). Most damaged DNA can be removed and recovered by DNA repair enzymes (Lunn et al. 1999; Matullo et al. 2001a; Hou et al. 2002). Polymorphisms in DNA repair genes that lead to amino acid substitution may influence the individual capacity to repair DNA damage, and insufficient DNA repair capacity (DRC) may result in genetic instability and carcinogenesis (Miller et al. 2001; de Boer 2002).

Among known genetic polymorphisms in DNA repair genes, X-ray repair cross-complementing groups 1, 3 (*XRCC1* and *XRCC3*) and the xeroderma pigmentosum group D (*XPD*, also known as *ERCC2*) have been frequently investigated as cancer susceptibility genes (Goode et al. 2002). The DNA repair gene *XRCC1* codes for a scaffolding protein physically associated with DNA polymerase beta, DNA ligase III, human AP endonuclease, polynucleotide kinase, and poly(ADP-ribose) polymerase (Caldecott et al. 1994; Gryk et al. 2002; Whitehouse et al. 2001; Vidal et al. 2001), which functions in a complex to facilitate BER and single-strand break-repair processes. The BER pathway mainly removes non-bulky base adducts produced by methylation, oxidation or reduction by ionizing radiation or oxidative damage (Beckman and Ames 1997; Ladiges et al. 2003). Three polymorphisms occurring at conserved sequences in *XRCC1* gene have been reported, and amino acid substitutions were detected at codons 194 (Arg-Trp), 280 (Arg-His) and 399 (Arg-Gln) (Shen et al. 1998). The 399Gln allele that was identified as associated with reduced DRC, was found to be significantly associated with the increase in both aflatoxin B1-DNA adducts and glycophorin A variants (Lunn et al. 1999).

The XRCC3 protein, involved in the homologous recombinational repair (HRR) of DNA double-strand break repair and cross-links, is a member of an emerging family of Rad-51-related proteins that likely participate in HRR to maintain genomic stability and repair DNA damage (Brenneman et al. 2000). XRCC3 has been shown to interact directly with HsRad51 (Pierce et al. 1999), and *XRCC3*-deficient cells were found to be unable to form Rad51 foci after radiation damage as well as demonstrating genetic instability and increased sensitivity to DNA-damaging agents (Griffin 2002). The *XRCC3* gene has a sequence variation in exon 7 (C–T), resulting in an amino acid substitution at codon 241 (Thr-Met) that may affect the enzyme's function (Matullo et al. 2001b).

The *XPD* gene that encodes a helicase, a subunit of transcription factor IIH (TFIIH), is responsible for opening DNA around the damaged site, a crucial step in initiating the NER process (Egly 2001), which repairs bulky adducts and UV-induced DNA damage (Weeda and Hoeijmakers 1993). Several *XPD* polymorphisms in the coding regions have been identified (Shen et al. 1998), including two single nucleotide polymorphisms, Asp312Asn in exon 10 and Lys751Gln in exon 23. The variant *XPD* Asp312Asn and Lys751Gln genotypes were reported to be consistently associated with a lower proficiency in repairing the damage induced by UV and chemical carcinogens (Spitz et al. 2001; Qiao et al. 2002). However, it has also been found that the 751Gln allele conferred higher proficiency in repairing the damage induced by ionizing radiation (Moller et al. 1998), and the 312Asn allele had no effect on DRC (Lunn et al. 2000).

As described in our previous study, although the incidence of colorectal cancer is low, there is a 20-fold difference between areas of the highest and lowest incidence (North America and Australia vs. India), and rectal cancer remains more common in India, where a significant increase in colorectal cancer has been reported for both men and women over the last two decades (Wang et al. 2006). We had already identified the associations between common environmental factors, such as diet, lifestyle, and single-nucleotide polymorphisms in *MTHFR* (C677T; A1298C), *PPAR*-gamma (C161T; Pro12Ala), *Cyclin D1* (A870G) and the susceptibility to colorectal cancer in an Indian population (Wang et al. 2006; Jiang et al. 2005, 2006). However, there are few studies linking DNA repair genes with colorectal cancer risk in Indian populations. We conducted this case–control study in an Indian population to detect the distribution of DNA repair genes *XRCC1*, *XRCC3* and *XPD* genotypes and to assess the potential role of these genetic polymorphisms on the risk of colorectal cancer, as well as to evaluate the interactions of gene–gene and gene–environment with susceptibility to colorectal cancer.

Subjects and methods

Subjects

This case–control study was conducted with 302 cases (including 59 colon and 243 rectal cancer patients) and 291 controls. As described elsewhere (Wang et al. 2006), all subjects were recruited at the Cancer Institute, Chennai in South-Eastern India. Cases were first diagnosed as primary colorectal carcinoma between 1999 and 2001. Colon cancer

cases aged from 22 to 72 years old (mean ± SD: 48.5 ± 12.0) included 67.8% men, and rectal cancer cases aged from 17 to 75 years old (mean ± SD: 49.1 ± 14.1) included 64.6% men. Controls were cancer-free healthy individuals, frequency matched to cases for sex and age (within 5 years), aged from 20 to 75 years old (mean ± SD: 47.3 ± 12.6) included 62.5% men, and selected from relatives/visitors to patients other than those with cancers in the gastrointestinal tract during the same period as the case collection. The data collection on smoking status and alcohol consumption has also been previously described. Informed consent was obtained from all study subjects.

Genotyping

XRCC1 Arg399Gln, *XRCC3* Thr241Met and *XPD* Lys751Gln genotypes were determined by PCR–RLFP assays using genomic DNA isolated from peripheral blood lymphocytes. *XRCC1* Arg399Gln PCR products were amplified with the primers 5′-TTGTGCTTTCTCTGTG TCCA-3′ and 5′-TCCTCCAGCCTTTTCTGATA-3′, and digested with *Msp*I (Lunn et al. 1999). Arg allele revealed 374 and 221 bp fragments, while Gln allele was not digested. The PCR primers for the *XRCC3* Thr241Met polymorphism were 5′-GGTCGAGTGACAGTCCAAAC-3′ and 5′-TGCAACGGCTGAGGGTCTT-3′, while PCR products were digested by the restriction enzyme *Nla*III (Smith et al. 2003). The wild type (*Thr/Thr*) produced two bands (316 and 140 bp), the homozygous variant genotype (*Met/Met*) resulted in three bands (211, 140 and 105 bp), and heterozygote (*Thr/Met*) displayed all four bands (316, 211, 140 and 105 bp). The *XPD* Lys751Gln genotypes were analyzed using primers 5′-GCCCGCTCTGGATTATACG-3′ and 5′-CTATCATCTCCTGGCCCCC-3′, and restriction enzyme *Pst*I (Xing et al. 2002). *Pst*I digestion resulted in two fragments of 290 and 146 bp for the wild type (*Lys/Lys*); three fragments of 227, 146 and 63 bp for the variant homozygotes (*Gln/Gln*), and four fragments at 290, 227, 146 and 63 bp for the heterozygotes (*Lys/Gln*).

Statistical analysis

Differences in the distribution of genotypes between cases and controls were assessed using χ^2 test. Within the controls, we also compared the observed genotype frequencies to those expected under the Hardy–Weinberg law using the χ^2 test. The effects [odds ratios (ORs) and 95% confidence intervals (95% CIs)] of genetic polymorphisms on colorectal cancer were estimated using unconditional logistic regression adjusted for potential confounding factors, such as age, sex, household income, education, religion, mother tongue, tobacco, alcohol, chewing habit and vegetarianism. The combined effects of *XRCC1* Arg399Gln, *XRCC3* Thr241Met and *XPD* Lys751Gln polymorphisms, and the interactions of gene–smoking and gene–alcohol were also tested, using low-risk genotypes or non-smokers (non-drinkers) with low-risk genotypes as the referent group, respectively. The computer software package SAS (version 8.2) was used for the statistical calculations. A likelihood ratio test was used to examine the associations of variables with respect to the risk of colorectal cancer. All statistical tests were two sided, and statistical significance was determined as $P < 0.05$.

Results

The general characteristics of the study participants were previously presented in detail (Wang et al. 2006), they were omitted here. Frequencies of the *XRCC1* 399Gln, *XRCC3* 241Met, and *XPD* 751Gln alleles were, respectively, 0.33, 0.18 and 0.33 among controls, and the genotype distributions were all consistent with the Hardy–Weinberg equilibrium (Table 1). Frequencies of the *XRCC1* 399Gln and *XPD* 751Gln alleles were similar to those reported in North and South Indian populations (Vettriselvi et al. 2007; Sobti et al. 2007; Gangwar et al. 2009; Sreeja et al. 2008). The *XRCC1* 399Gln allele was found no significant association with either colon cancer (OR = 1.45, 95% CI 0.81–2.66) or rectal cancer (OR = 1.32, 95% CI 0.92–1.90). However, the *XRCC3* 241Thr/Met genotype showed no significant association with colon cancer (OR = 1.39, 95% CI 0.74–2.60) and a significantly decreased risk with rectal cancer (OR = 0.64, 95% CI 0.42–0.97); the same tendency was found for *XRCC3* 241Met allele carriers with colon cancer (OR = 1.31, 95% CI 0.70–2.42) and rectal cancer (OR = 0.68, 95% CI 0.46–1.02). The *XPD* Lys751Gln genetic polymorphism was also found to show no significant association with either colon or rectal cancer risk. When the associations of these polymorphisms with rectal cancer were taken into account by gender (Table 2), a statistically significant association of the *XRCC1* 399Gln allele with rectal cancer was found among men (OR = 1.65, 95% CI 1.04–2.64), but not among women (OR = 0.90, 95% CI 0.50–1.62). An inverse association of the *XRCC3* 241Met allele with rectal cancer was also found among both men (OR = 0.78, 95% CI 0.46–1.31) and women (OR = 0.60, 95% CI 0.31–1.12), although none reached statistical significance. We also examined any possible difference in age stratification, but nothing significant was found (data not shown).

The combined effects of *XRCC1* Arg399Gln genotypes with the *XRCC3* Thr241Met or *XPD* Lys751Gln polymorphism to pose a risk of rectal or colorectal cancer were analyzed (Table 3). Using the combined low-risk genotypes (*XRCC1* 399Arg/Arg genotype and *XRCC3* 241Met allele)

Table 1 Genotype frequencies and adjusted OR for colon, rectal and colorectal cancers with polymorphisms of DNA repair genes

Genotype	Controls (n = 291) n (%)	Colon cancer (n = 59) n (%)	ORs (95% CI)	Rectal cancer (n = 243) n (%)	ORs (95% CI)	Colorectal cancer (n = 302) n (%)	ORs (95% CI)
XRCC1 Arg399Gln							
Arg/Arg (GG)	139 (47.8)	24 (40.7)	1.00 (Ref)	100 (41.1)	1.00 (Ref)	124 (41.1)	1.00 (Ref)
Arg/Gln (GA)	113 (38.8)	25 (42.4)	1.44 (0.76–2.75)	113 (46.5)	1.40 (0.96–2.06)	138 (45.7)	1.41 (0.99–2.03)
Gln/Gln (AA)	39 (13.4)	10 (16.9)	1.48 (0.60–3.47)	30 (12.4)	1.08 (0.61–1.90)	40 (13.2)	1.20 (0.71–2.03)
With Gln (A)	152 (52.2)	35 (39.3)	1.45 (0.81–2.66)	143 (58.9)	1.32 (0.92–1.90)	178 (58.9)	1.36 (0.97–1.91)
XRCC3 Thr241Met							
Thr/Thr (CC)	197 (67.7)	36 (61.0)	1.00 (Ref)	177 (72.8)	1.00 (Ref)	213 (70.5)	1.00 (Ref)
Thr/Met (CT)	85 (29.2)	22 (37.3)	1.39 (0.74–2.60)	57 (23.5)	0.64 (0.42–0.97)	79 (26.2)	0.78 (0.53–1.15)
Met/Met (TT)	9 (3.1)	1 (1.7)	0.57 (0.03–3.42)	9 (3.7)	1.09 (0.40–2.97)	10 (3.3)	0.97 (0.37–2.58)
With Met (T)	94 (32.3)	23 (39.0)	1.31 (0.70–2.42)	66 (27.2)	0.68 (0.46–1.02)	89 (29.5)	0.80 (0.55–1.16)
XPD Lys751Gln							
Lys/Lys (AA)	137 (47.1)	28 (47.5)	1.00 (Ref)	110 (45.3)	1.00 (Ref)	138 (45.7)	1.00 (Ref)
Lys/Gln (AC)	117 (40.2)	22 (37.3)	0.94 (0.49–1.76)	108 (44.4)	1.18 (0.80–1.72)	130 (43.0)	1.12 (0.78–1.60)
Gln/Gln (CC)	37 (12.7)	9 (15.2)	1.14 (0.45–2.65)	25 (10.3)	0.92 (0.50–1.66)	34 (11.3)	0.95 (0.55–1.63)
With Gln (C)	154 (52.9)	31 (52.5)	0.99 (0.55–1.77)	133 (54.7)	1.12 (0.78–1.60)	164 (54.3)	1.08 (0.77–1.51)

Adjusted for gender, age, household income, education, religion, mother tongue, smoking, drinking, chewing and vegetarianism

Table 2 Distributions of XRCC1, XRCC3 and XPD genotypes and risk for rectal cancer by gender

Genotype	Males		Females	
	Cases/controls	ORs (95% CI)[a]	Cases/controls	ORs (95% CI)[a]
XRCC1 Arg399Gln				
Arg/Arg (GG)	58/89	1.00 (Ref)	42/50	1.00 (Ref)
Arg/Gln (GA)	79/69	1.81 (1.11–2.98)	34/44	0.95 (0.51–1.77)
Gln/Gln (AA)	20/24	1.20 (0.56–2.52)	10/15	0.77 (0.29–1.93)
With Gln (A)	99/93	1.65 (1.04–2.64)	44/59	0.90 (0.50–1.62)
XRCC3 Thr241Met				
Thr/Thr (CC)	116/128	1.00 (Ref)	61/69	1.00 (Ref)
Thr/Met (CT)	33/49	0.64 (0.36–1.12)	24/36	0.63 (0.32–1.20)
Met/Met (TT)	8/5	2.52 (0.73–9.41)	1/4	0.25 (0.01–1.94))
With Met (T)	41/54	0.78 (0.46–1.31)	25/40	0.60 (0.31–1.12)
XPD Lys751Gln				
Lys/Lys (AA)	75/89	1.00 (Ref)	35/48	1.00 (Ref)
Lys/Gln (AC)	68/70	1.22 (0.75–1.98)	40/47	1.12 (0.61–2.09)
Gln/Gln (CC)	14/23	0.79 (0.35–1.74)	11/14	1.07 (0.42–2.68)
With Gln (C)	82/93	1.12 (0.71–1.77)	51/61	1.11 (0.62–1.99)

[a] Adjusted for age, household income, education, religion, mother tongue, smoking, drinking, chewing and vegetarianism

as the referent group, the combination of the *XRCC1* 399Arg/Gln and *XRCC3* 241Thr/Thr genotypes showed a significantly positive association with rectal cancer (OR = 2.10, 95% CI 1.08–3.26). Gene–gene interactions of the *XRCC1* Arg399Gln, *XRCC3* Thr241Met and *XPD* Lys751Gln polymorphisms were also estimated (Table 4). A combination of the *XRCC1* 399Gln allele, *XRCC3* Thr/Thr genotype and *XPD* 751Gln allele demonstrated the highest rectal cancer risk (OR = 3.52, 95% CI 1.43–9.44).

The interaction of gene–smoking and gene–alcohol for rectal or colorectal cancer were evaluated (Table 5). These genetic polymorphisms were not found to significantly modify the effect of tobacco consumption (interaction $P > 0.05$, respectively). With respect to alcohol intake, we found a positive association of the *XRCC1* 399Gln allele with rectal (OR = 1.56, 95% CI 1.05–2.33) or colorectal (OR = 1.61, 95% CI 1.11–2.34) cancer among non-drinkers, and weak evidence that *XRCC1* Arg/Arg genotype

Table 3 Combined effect of *XRCC1* and *XRCC3* or *XPD* genotypes on risk of rectal and colorectal cancers

	XRCC1 Arg399Gln					
	Arg/Arg	ORs (95% CI)[a]	Arg/Gln	ORs (95% CI)[a]	Gln/Gln	ORs (95% CI)[a]
Rectal cancer						
XRCC3 Thr241Met						
With Met	27/50	1.00 (Ref)	34/34	1.80 (0.89–3.66)	5/10	0.80 (0.22–2.66)
Thr/Thr	73/89	1.66 (0.92–3.06)	79/79	2.10 (1.16–3.85)	25/29	1.84 (0.87–3.95)
XPD Lys751Gln						
Lys/Lys	42/61	1.00 (Ref)	55/58	1.31 (0.75–2.33)	13/18	0.93 (0.39–2.16)
With Gln	58/78	1.04 (0.60–1.79)	58/55	1.56 (0.89–2.75)	17/21	1.28 (0.82–1.84)
Colorectal cancer						
XRCC3 Thr241Met						
With Met	35/50	1.00 (Ref)	44/34	1.81 (0.95–3.50)	10/10	1.28 (0.46–3.58)
Thr/Thr	89/89	1.48 (0.85–2.59)	94/79	1.86 (1.08–3.26)	30/29	1.68 (0.83–3.42)
XPD Lys751Gln						
Lys/Lys	51/61	1.00 (Ref)	67/58	1.32 (0.77–2.25)	20/18	1.27 (0.59–2.75)
With Gln	73/78	1.05 (0.63–1.75)	71/55	1.61 (0.95–2.75)	20/21	1.19 (0.56–2.53)

[a] Adjusted for gender, age, household income, education, religion, mother tongue, smoking, drinking, chewing and vegetarianism

Table 4 Combined effect of *XRCC1*, *XRCC3* and *XPD* genotypes on risk of rectal and colorectal cancers

XRCC1 Arg399Gln	*XCRCC3* Thr241Met	*XPD* Lys751Gln	Controls	Rectal cancer	ORs (95% CI)[a]	Colorectal cancer	ORs (95% CI)[a]
Arg/Arg	With Met	Lys/Lys	24	8	1.00 (Ref)	11	1.00 (Ref)
Arg/Arg	With Met	With Gln	26	19	1.98 (0.71–5.85)	24	1.74 (0.68–4.58)
Arg/Arg	Thr/Thr	Lys/Lys	37	34	2.88 (1.12–8.03)	40	2.27 (0.95–5.65)
Arg/Arg	Thr/Thr	With Gln	52	39	2.32 (0.93–6.31)	49	1.94 (0.84–4.67)
With Gln	With Met	Lys/Lys	21	19	2.33 (0.82–7.05)	29	2.56 (1.01–6.81)
With Gln	With Met	With Gln	23	20	2.45 (0.87–7.30)	25	2.17 (0.85–5.76)
With Gln	Thr/Thr	Lys/Lys	55	49	2.70 (1.10–7.24)	58	2.21 (0.97–5.27)
With Gln	Thr/Thr	With Gln	53	55	3.52 (1.43–9.44)	66	2.88 (1.27–6.87)

[a] Adjusted for gender, age, household income, education, religion, mother tongue, smoking, drinking, chewing and vegetarianism

increased the risk of rectal (OR = 1.93, 95% CI 0.94–4.04) or colorectal (OR = 1.91, 95% CI 0.96–3.86) cancer among drinkers (interaction P was 0.05 for rectal cancer and 0.03 for colorectal cancer, respectively). Alcohol intake did not affect the results of other genetic polymorphisms (interaction $P > 0.05$, respectively).

Discussion

In contrast to the developed countries, the incidence of colorectal cancer is low in India, where rectal lesions are more common than tumors of the colon. The rural incidence rate for colorectal cancer is approximately half that of its urban population (Mohandas and Desai 1999), presumably reflection a low consumption of meat and a high intake of dietary fiber, vegetables and fruits, and the presence of natural antioxidants such as curcumin in Indian cooking. Furthermore, it was found that intake of vegetables and fruits was high and consumption of meat, sea food and egg was low in all subjects of our study, and it had been identified that high intake of non-fried vegetables or fruits was significantly associated with decreased risk of both colon and rectal cancers (Wang et al. 2006). Although the proportion of vegetarians included in our study was not so more (11.3% among controls, 17.9 and 19.8% among colon and rectal cancer cases, respectively), insufficient nutrition may be the reason why a significantly increased rectal cancer risk was found for vegetarianism in our study (OR = 1.83, 95% CI 1.04–3.26).

There is increasing evidence that the genetic variations in DNA repair genes lead to different DRCs, variations in DRC result in different biological responses to DNA damage and thus different susceptibility for developing cancers

Table 5 Relationship of smoking and drinking status to rectal and colorectal cancer risk stratified by genotypes

Genotypes	Smoking status				Drinking status			
	Non–smokers		Smokers		Non-drinkers		Drinkers	
	Cases/ controls	ORs (95% CI)[a]	Cases/ controls	ORs (95% CI)[a]	Cases/ controls	ORs (95% CI)[b]	Cases/ controls	ORs (95% CI)[b]
Rectal cancer	188/225	1.00 (Ref)	55/66	1.02 (0.63–1.64)	198/238	1.00 (Ref)	45/53	1.08 (0.66–1.79)
Colorectal cancer	232/225	1.00 (Ref)	70/66	1.03 (0.66–1.02)	246/238	1.00 (Ref)	56/53	1.01 (0.63–1.61)
XRCC1 Arg399Gln	(P for interaction: 0.57 for rectal cancer and 0.29 for colorectal cancer)				(P for interaction: 0.05 for rectal cancer and 0.03 for colorectal cancer)			
Arg/Arg								
Rectal cancer	82/112	1.00 (Ref)	18/27	0.84 (0.40–1.74)	78/122	1.00 (Ref)	22/17	1.93 (0.94–4.04)
Colorectal cancer	104/112	1.00 (Ref)	20/27	0.75 (0.37–1.49)	97/122	1.00 (Ref)	27/17	1.91 (0.96–3.86)
With Gln								
Rectal cancer	106/113	1.26 (0.84–1.89)	37/39	1.37 (0.75–2.49)	120/116	1.56 (1.05–2.33)	23/36	1.17 (0.66–2.09)
Colorectal cancer	128/113	1.25 (0.85–1.83)	50/39	1.45 (0.84–2.54)	149/116	1.61 (1.11–2.34)	29/36	1.17 (0.63–2.17)
XRCC3 Thr241Met	(*P* for interaction: 0.62 for rectal cancer and 0.35 for colorectal cancer)				(*P* for interaction: 0.31 for rectal cancer and 0.47 for colorectal cancer)			
With Met								
Rectal cancer	55/79	1.00 (Ref)	11/15	1.19 (0.46–3.01)	54/78	1.00 (Ref)	12/16	1.43 (0.59–3.38)
Colorectal cancer	72/79	1.00 (Ref)	17/15	1.41 (0.62–3.46)	72/78	1.00 (Ref)	17/16	1.40 (0.64–3.08)
Thr/Thr								
Rectal cancer	133/146	1.54 (0.99–2.40)	44/51	1.40 (0.77–2.56)	144/160	1.56 (1.00–2.44)	33/37	1.58 (0.85–2.94)
Colorectal cancer	160/146	1.36 (0.91–2.06)	53/51	1.23 (0.70–2.16)	174/160	1.33 (0.89–3.01)	39/37	1.31 (0.74–2.35)
XPD Lys751Gln	(*P* for interaction: 0.80 for rectal cancer and 0.87 for colorectal cancer)				(*P* for interaction: 0.69 for rectal cancer and 0.86 for colorectal cancer)			
Lys/Lys								
Rectal cancer	82/102	1.00 (Ref)	28/35	1.07 (0.57–2.02)	92/116	1.00 (Ref)	18/21	1.25 (0.60–2.56)
Colorectal cancer	104/102	1.00 (Ref)	34/35	1.00 (0.55–1.83)	116/116	1.00 (Ref)	22/21	1.15 (0.75–1.58)
With Gln								
Rectal cancer	106/123	1.14 (0.76–1.72)	27/31	1.10 (0.57–2.12)	106/122	1.15 (0.77–1.71)	27/32	1.19 (0.64–2.18)
Colorectal cancer	128/123	1.07 (0.73–1.56)	36/31	1.14 (0.62–2.10)	130/122	1.09 (0.75–1.58)	34/32	1.15 (0.65–2.05)

[a] Adjusted for gender, age, household income, education, religion, mother tongue, drinking, chewing and vegetarianism

[b] Adjusted for gender, age, household income, education, religion, mother tongue, smoking, chewing and vegetarianism

(Hu et al. 2002). Cumulating information on the common allelic variants may be important in clarifying the causes and mechanisms of cancers, and therefore common polymorphisms may act as genetic susceptibility factors and thus identify high-risk groups of exposed individuals. Although a number of studies of different ethnic populations have investigated the association between DNA repair genes and the risk of colorectal cancer, their results have been inconsistent (Abdel-Rahman et al. 2000; Mort et al. 2003; Yeh et al. 2005a, b; Hong et al. 2005; Skjelbred et al. 2006; Stern et al. 2007; Sliwinski et al. 2008; Improta et al. 2008). In our case–control study conducted in South-Eastern India, we investigated the role of polymorphisms of three DNA repair genes involved in BER, HRR and NER as colorectal cancer risk factors. Our results indicated that the *XRCC1* 399Gln allele significantly increased the rectal cancer risk among men (OR = 1.65, 95% CI 1.04–2.64). In contrast, the *XRCC3* 241Met allele may exert a weakly protective effect against rectal cancer risk (OR = 0.68, 95% CI 0.46–1.02) for both men (OR = 0.78, 95% CI 0.46–1.31) and women (OR = 0.60, 95% CI 0.31–1.12). The *XPD* Lys751Gln genetic polymorphism was found to have no

significant association with either colon or rectal cancer risk.

It was established that the *XRCC1* 399Gln allele carriers had significantly increased the DNA adducts level, while reducing DRC to repair damaged DNA (Lunn et al. 1999). However, most epidemiological case–control studies could find no significantly elevated risk of colorectal cancer associated with the *XRCC1* 399Gln variant (Skjelbred et al. 2006; Stern et al. 2007; Sliwinski et al. 2008; Improta et al. 2008), whereas a hospital-based case–control study conducted in Taiwan found an increased risk of colorectal cancer associated with the *XRCC1* 399Arg/Arg genotype compared with the *XRCC1* 399Gln allele (OR = 1.46, 95% CI 1.06–2.99) in younger subjects (≤60 years) (Yeh et al. 2005b). Although Skjelbred et al. (2006) reported the *XRCC1* 280His allele to be associated with an increased risk of adenomas, while the *XRCC1* 399Gln allele was related to a reduction in the risk of high-risk adenomas, no association revealed any risk of carcinomas in a Norwegian population. However, Abdel-Rahman et al. (2000) observed a significantly increased risk of colorectal cancer with the *XRCC1* 399Gln allele compared with the *XRCC1* 399Arg/Arg genotype in Egypt (OR = 3.98, 95% CI 1.50–10.6), especially among urban residents (OR = 9.97, 95% CI 1.98–43.76); Hong et al. (2005) also demonstrated a positive association in South Korea (OR = 1.61, 95% CI 1.09–2.39). Although our results did not reproduce such a strong relationship, similar to that of Mort et al. (2003) reported in the UK (OR = 1.35, 95% CI 0.36–1.50), the *XRCC1* 399Gln allele generally showed no significant association with either colon (OR = 1.45, 95% CI 0.81–2.66) or rectal (OR = 1.32, 95% CI 0.92–1.90) cancer, a significantly increased rectal cancer risk for men (OR = 1.65, 95% CI 1.04–2.64) was found. The difference by gender may be considered due to physiologically different effects of *XRCC1* 399Gln allele on the development of colorectal cancer, or resulting from different dietary habit, lifestyle and other genetic factors. Because of the small number of colon cancers ($n = 59$) and the lack of statistical power, we were unable to detect any associations of genetic polymorphisms with susceptibility to colon cancer by gender or age stratification, as well as with interactions of gene–gene or gene–environments.

In addition, associations of the *XRCC1* Arg194Trp and Arg280His polymorphisms with susceptibility to colorectal cancer have also been detected in several studies (Abdel-Rahman et al. 2000; Hong et al. 2005; Skjelbred et al. 2006; Stern et al. 2007; Sliwinski et al. 2008; Improta et al. 2008), except that of Abdel-Rahman et al. who reported a positive association of the 194Trp allele among urban residents in Egypt (OR = 3.33, 95% CI 0.48–35.90), although no significant association of these genotypes with colorectal cancer was found.

Our study was the first to detect the distribution of the *XRCC3* Thr241Met polymorphism in an Indian population, the frequency of the *XRCC3* 241Met allele (0.18) among control group was lower than those reported in Caucasian populations (0.45 in UK; 0.40 in Norway) (Mort et al. 2003; Skjelbred et al. 2006) and much higher than those reported in other Asian populations (0.05 in Taiwan; 0.06 in China) (Yeh et al. 2005a; Zhang et al. 2005).

XRCC3 is one of the Rad51-related proteins and functions through complex interactions with other relevant proteins to repair double-strand breaks and to maintain genome integrity in multiple phases of a homologous recombination (Brenneman et al. 2000). Although polymorphisms of this gene may result in reduced DRC, the evidence of direct functional research is limited, and the results of epidemiologic studies in terms of the associations with colorectal cancer susceptibility have proved inconclusive (Mort et al. 2003; Yeh et al. 2005b; Skjelbred et al. 2006; Improta et al. 2008). A recent case–control study conducted in a Southern Italian population found the *XRCC3* 241Met allele to be significantly associated with an increased risk of colon cancer (Improta et al. 2008). While Skjelbred et al. (2006) reported that the *XRCC3* Thr241Met polymorphism was not associated with either colorectal adenoma or carcinoma in a Norwegian population. However, Yeh et al. (2005a, b) observed that the *XRCC3* Thr241Thr genotype showed a trend of increased risk of colorectal cancer as compared to the *XRCC3* 241Met allele in Taiwan, with a particularly significant association found among a low meat consumption group (OR = 2.34, 95% CI 1.28–4.29). Mort et al. (2003) also revealed the *XRCC3* 241Thr allele to display a significantly heightened risk of colorectal cancer in UK (OR = 1.52, 95% CI 1.04–2.22). We also demonstrated a weakly inverse association between the *XRCC3* 241Met allele and rectal cancer (OR = 0.68, 95% CI 0.46–1.02) without any gender difference in this present study. While the *XRCC3* 241Met allele was found no such an association with colon cancer (OR = 1.31, 95% CI 0.70–2.42), which may have been due to chance resulting from our small sample size, or to the different DNA repair mechanism of the *XRCC3* Thr241Met polymorphism in the development of colorectal cancers located in various subsites.

XPD protein plays a role in NER pathway, functioning as an ATP-dependent helicase joined to the basal TFIIH complex to separate the double helix (Egly 2001). Variation in the *XPD* Lys751Gln gene may alter the XPD protein's function and affect the DRC depending on different exposures (Spitz et al. 2001; Moller et al. 1998). In agreement with several case–control studies (Mort et al. 2003; Yeh et al. 2005b; Skjelbred et al. 2006; Stern et al. 2007), we found only scant evidence of an association of the *XPD* Lys751Gln polymorphism with colorectal cancer risk.

The combined effects of polymorphisms of the *XRCC1* Arg399Gln, *XRCC3* Thr241Met and *XPD* Lys751Gln genes in regard to rectal cancer risk were observed in our study. The combination of the *XRCC1* 399Arg/Gln and *XRCC3* 241Thr/Thr genotypes revealed a significantly positive association (OR = 2.10, 95% CI 1.08–3.26). Furthermore, a combination of the *XRCC1* 399Gln allele with *XRCC3* Thr/Thr genotype and the *XPD* 751Gln allele demonstrated the highest rectal cancer risk (OR = 3.52, 95% CI 1.43–9.44). Individuals who carried a gradual superposition of the putative risk genotypes showed a progressively increased risk.

Interactions of gene–smoking and gene–alcohol for rectal and colorectal cancers were also evaluated in our study. We observed that smoking did not modify the effects of those genetic polymorphisms on the risk of colorectal cancer (interaction $P > 0.05$, respectively). Alcohol intake was found to weaken the effect of the *XRCC1* 399Gln allele while heighten the effect of the *XRCC1* Arg/Arg genotype on rectal or colorectal cancer risk (interaction P was 0.05 for rectal cancer and 0.03 for colorectal cancer, respectively). A significantly positive association of the *XRCC1* 399Gln allele was found among never drinkers for rectal cancer (OR = 1.56, 95% CI 1.05–2.33) or colorectal cancer (OR = 1.61, 95% CI 1.11–2.34), while a non-statistically significantly increased rectal (OR = 1.93, 95% CI 0.94–4.04) or colorectal (OR = 1.91, 95% CI 0.96–3.86) cancer risk was found among drinkers carrying the *XRCC1* Arg/Arg genotype. In South Korea, alcohol consumption (≥80 g/week) was identified as a significant risk factor of colorectal cancer, especially an increased risk of colorectal cancer (OR = 7.19, 95% CI 1.31–39.68) was found in alcohol drinkers (≥80 g/week) with the risky allele combination (194Arg-280His-399Arg) (Hong et al. 2005). On the other hand, a non-statistically significant modification of *XRCC1* codon 399 on the effects of alcohol intake was observed among Singapore Chinese (Stern et al. 2007), alcohol intake increased the risk of colorectal cancer among carriers of Arg/Arg genotype (OR = 1.3, 95% CI 0.9–1.9), which was similar to that found in our study. Differences in the quantity of alcohol intake may result in inconsistent results, we also could not exclude the possibility that alcohol intake may increase the risk of colorectal cancer associated with the specific genotypes (such as *XRCC1* 399Arg/Arg).

In conclusion, variants among the three genetic polymorphisms included in our study may weakly contribute to colorectal cancer risk, while alcohol intake may slightly modify the effect of the *XRCC1* Arg399Gln polymorphism on rectal (colorectal) cancer risk. The combined effects of putative risk alleles/genotypes for different DNA repair pathways may strengthen the susceptibility to rectal cancer. These findings remain to be confirmed by studies with a larger sample size.

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Conflict of interest statement We declare that we have no conflict of interest.

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REGULAR ARTICLE

Changes in thioredoxin concentrations: an observation in an ultra-marathon race

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Abstract
Objectives Changes in plasma thioredoxin (TRX) concentrations before, during, and after a 130-km endurance race were measured with the aim of elucidating the relationship between exercise and oxidative stress (OS).
Methods Blood samples were taken from 18 runners participating in a 2-day-long 130-km ultra-marathon during the 2 days of the race and for 1 week thereafter. There were six sampling time points: at baseline, after the goal had been reached on the first and second day of the endurance race, respectively, and on 1, 3, and 5/6 days post-endurance race. The samples were analyzed for plasma TRX concentrations, platelet count, and blood lipid profiles.
Results Concentrations of plasma TRX increased from 17.9 ± 1.2 ng/mL (mean ± standard error of the mean) at baseline to 57.3 ± 5.0 ng/mL after the first day's goal had been reached and to 70.1 ± 6.9 ng/mL after the second day's goal had been reached; it then returned to the baseline level 1 day after the race. Platelet counts of 21.3 ± 1.2 × 10^4 cell/μL at baseline increased to 23.9 ± 1.5 × 10^4 cells/μL on Day 1 and to 26.1 ± 1.0 × 10^4 cells/μL on Day 2. On Day 7, the platelet counts had fallen to 22.1 ± 1.2 × 10^4 cell/μL. There was a significant positive correlation between plasma TRX and platelet count.
Conclusions These data suggest that plasma TRX is an OS marker during physical exercise. Further studies are needed to determine the appropriate level of exercise for the promotion of health.

Keywords Lipid profile · Marathon runner · Oxidative stress · Platelet counts · Thioredoxin

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