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

Jingwen Wang [a,*], Jing Jiang [b], Yang Zhao [a], Vendhan Gajalakshmi [c], Kiyonori Kuriki [d], Sadao Suzuki [a], Teruo Nagaya [a], Seiichi Nakamura [e], Susumu Akasaka [f], Hideki Ishikawa [g], Shinkan Tokudome [a,h]

[a] *Department of Public Health, Nagoya City University Graduate School of Medical Sciences, Nagoya 467-8601, Japan*
[b] *Department of Hematology and Oncology, The First Hospital, Jilin University, Changchun 130021, China*
[c] *Epidemiological Research Center, Chennai 600-010, India*
[d] *Hygiene and Preventive Medicine, School of Food and Nutritional Sciences, Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, Shizuoka 422-8526, Japan*
[e] *Health Research Foundation, Kyoto 606-8225, Japan*
[f] *Osaka Prefectural Institute of Public Health, Osaka 537-0025, Japan*
[g] *Kyoto Prefectural University of Medicine, Graduate School of Medical Sciences, Kyoto, 602-8566, Japan*
[h] *National Institute of Health and Nutrition, Tokyo 162-8636, Japan*

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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

* Corresponding author at: Department of Public Health, Nagoya City University Graduate School of Medical Sciences, Mizuho-ku, Nagoya 467-8601, Japan. Tel.: +81 52 853 8176; fax: +81 52 842 3830.
E-mail addresses: wjw@med.nagoya-cu.ac.jp, wjw66@hotmail.co.jp (J. Wang).

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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/

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)	Colon cancer (n=59)	ORs (95% CI)	Rectal cancer (n=243)	ORs (95% CI)	Colorectal cancer (n=302)	ORs (95% CI)
	n (%)	n (%)		n (%)		n (%)	
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

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

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.

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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SPECIAL REPORT

MID-TERM PROGNOSIS AFTER ENDOSCOPIC RESECTION FOR SUBMUCOSAL COLORECTAL CARCINOMA: SUMMARY OF A MULTICENTER QUESTIONNAIRE SURVEY CONDUCTED BY THE COLORECTAL ENDOSCOPIC RESECTION STANDARDIZATION IMPLEMENTATION WORKING GROUP IN JAPANESE SOCIETY FOR CANCER OF THE COLON AND RECTUM

SHIRO OKA, SHINJI TANAKA, HIROYUKI KANAO, HIDEKI ISHIKAWA, TOSHIAKI WATANABE, MASAHIRO IGARASHI, YUTAKA SAITO, HIROAKI IKEMATSU, KIYONORI KOBAYASHI, YUJI INOUE, NAOHISA YAHAGI, SUMIO TSUDA, SEIJI SIMIZU, HIROYASU IISHI, HIROO YAMANO, SHIN-EI KUDO, OSAMU TSURUTA, SATOSHI TAMURA, YUSUKE SAITO, EISAI CHO, TAKAHIRO FUJII, YASUSHI SANO, HISASHI NAKAMURA, KENICHI SUGIHARA AND TETSUICHIRO MUTO

Colorectal Endoscopic Resection Standardization Implementation Working Group in Japanese Society for Cancer of the Colon and Rectum, Tokyo, Japan

We carried out a retrospective questionnaire survey of 792 submucosal colorectal carcinoma (CRC) cases from 15 institutions affiliated with the Colorectal Endoscopic Resection Standardization Implementation Working Group in Japanese Society for Cancer of the Colon and Rectum. In these cases, endoscopic resection (ER) and surveillance was carried out without additional surgical resection. Local recurrence or metastasis was observed in 18 cases. Local submucosal recurrence was observed in 11 cases, and metastatic recurrence was observed in 13 cases. Among the 15 cases in which the depth of submucosal invasion was measured, two cases showed depth less than 1000 μm, which has other risk factors for metastasis. Metastatic recurrence was observed in the lung, liver, lymph node, bone, adrenal glands, and the brain; in some cases, metastatic recurrence was observed in multiple organs. Death due to primary disease was observed in six cases. The average interval between ER and recurrence was 19.7 ± 9.2 months. In 16 cases, recurrence was observed within 3 years after ER. Thus, validity of ER without additional surgical resection for cases with the conditions that the depth of submucosal invasion is less than 1000 μm and the histological grade is well or moderately differentiated adenocarcinoma with no lymphatic and venous involvement was proven.

Key words: endoscopic resection, prognosis, recurrence, submucosal colorectal carcinoma.

INTRODUCTION

In the Guidelines for Colorectal Cancer Treatment, 1st Edition, 2005 by Japanese Society for Cancer of the Colon and Rectum (JSCCR),[1] the curative conditions after endoscopic resection (ER) for submucosal colorectal carcinoma (CRC) state that 'if a lesion is completely resected by ER, the depth of submucosal invasion is less than 1000 μm, and the histological grade is well or moderately differentiated adenocarcinoma with no lymphatic and venous involvement, the possibility of lymph node (LN) metastasis will be extremely low so that the surveillance is allowed without additional surgical resection.' This statement has generated a certain consensus. However, these conditions were established on the basis of the analysis of submucosal CRC cases obtained from surgical resection,[2] and there are very few reports of cases in which surveillance after ER for submucosal CRC was carried out extensively.

In the present study, we obtained information on the non-surgical submucosal CRC cases with surveillance after ER; this information was obtained from the institutions affiliated with the Colorectal Endoscopic Resection Standardization Implementation Working Group in JSCCR. Using the information for these cases, we analyzed the risk factors for recurrence, the interval between ER and recurrence, and the recurrence pattern (local or metastatic). In this report, we have introduced data that can be used to verify the validity of the curative conditions after ER for submucosal CRC.

QUESTIONNAIRE SURVEY METHOD

The retrospective questionnaire survey was carried out for submucosal CRC cases obtained from the institutions affiliated with the Colorectal Endoscopic Resection Standardization Implementation Working Group in JSCCR. In these

Correspondence: Shinji Tanaka, Department of Endoscopy, Hiroshima University Hospital, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Email: colon@hiroshima-u.ac.jp

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Table 1. Facilities that answered the questionnaire

Kitasato University East Hospital
Hiroshima University Hospital
National Cancer Center East Hospital
Asahikawa City Hospital
Jichi Medical University Hospital
National Cancer Center Hospital
Osaka Medical Center for Cancer and Cardiovascular Diseases
Nagoya City University Hospital
Kobe University Hospital
Fukuoka University Chikushi Hospital
Showa University Northern Yokohama Hospital
Cancer Institute Hospital Ariake
Tokyo University Hospital
Kyusyu University Hospital
Juntendo University Hospital

cases, surveillance had been carried out after ER without additional surgical resection for various reasons.

The following factors were surveyed: age of the patient at the initial ER, gender, tumor size, location, macroscopic type, ER technique (en bloc or piecemeal resection), histological margin (lateral or vertical), histological grade, histological grade at the deepest invasive portion, depth of submucosal invasion (μm), lymphatic/venous involvement, follow-up period after ER, existence of recurrence, recurrence pattern, and vital prognosis. Tumor size, location, macroscopic type, histological margin, histological grade, depth of submucosal invasion and lymphatic/venous involvement were recorded according to the General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum, and Anus, 7th Edition, Revised Version by JSCCR[3]. Further, we directly used the clinical and histopathological findings stated in the questionnaire.

QUESTIONNARIE SURVEY RESULTS

Among the 28 institutions affiliated with the Colorectal Endoscopic Resection Standardization Implementation Working Group in JSCCR, 15 institutions participated in this questionnaire survey (response rate, 53.6%) (Table 1). The information for 792 patients (556 male and 236 female) was collected from these 15 institutions. The average age of the patients was 72.9 ± 12.3 years (range, 19–93 years). The lesions were located in the cecum (25 cases), ascending colon (91 cases), transverse colon (77 cases), descending colon (56 cases), sigmoid colon (339 cases), and rectum (204). The average size of the lesions was 16.2 ± 8.2 mm (3–60 mm). The macroscopic type of the lesions showed that there were 0-Ip (209 cases), 0-Isp (197 cases), 0-Is (142 cases), 0-IIa (141 cases), 0-IIa + IIc (76 cases) and 0-IIc (27 cases). En bloc resection was carried out in 569 cases, and piecemeal resection was carried out in 114 cases; the ER technique was not mentioned in 109 cases. The histological lateral margin-positive was reported in 50 cases and was negative in 504 cases; the lateral margin was not mentioned in 238 cases. The histological vertical margin-positive was reported in 34 cases and was negative in 563 cases; the vertical margin was not mentioned in 195 cases. With regard to the histological grade, 724 lesions were graded as well differentiated adenocarcinoma, 63 were graded as moderately differentiated adenocarcinoma, two were graded as poorly differentiated adenocarcinoma, and the histological grade was not mentioned in three cases. The average depth of submucosal invasion was 1388 ± 1546 μm (5–10 000 μm); submucosal invasion less than 1000 μm was observed in 324 cases; deeper than 1000 μm was observed in 315 cases; and the depth of submucosal invasion was not mentioned in 153 cases. The average follow-up period was 38.7 ± 83.0 months, and recurrence was observed in 18 cases (2.3%). The recurrence rate for the cases that underwent en bloc resection was 2.5% (14/569) and that for the cases that underwent piecemeal resection was 3.5% (4/114); there was no significant difference between these two techniques. In 368 cases, the lesion satisfied the curative conditions after ER for submucosal CRC, whereas the lesion did not satisfy the curative conditions after ER for submucosal CRC in 302 cases; the relationship between the lesion and the curative conditions after ER for submucosal CRC was not mentioned in 122 cases.

HISTOPATHOLOGICAL RISK FACTORS FOR RECURRENCE

Among the 792 cases with surveillance after ER for submucosal CRC, information on all factors related to the histopathological findings was obtained in 387 cases (48.9%). These cases were re-examined to determine the relationship between the following factors and recurrence: histological grade, histological grade at the deepest invasion portion, existence of budding,[4] submucosal invasive depth of 1000 μm, lymphatic involvement, and venous involvement.

Among these 387 patients, there were 275 males and 112 females. The average age of the patients was 64.4 ± 11.2 years (19–93 years). The average tumor size was 15.7 ± 8.3 mm (4–60 mm). The lesions were located in the cecum (6 cases), ascending colon (47 cases), transverse colon (37 cases), descending colon (40 cases), sigmoid colon (174 cases), and rectum (83 cases). Macroscopic type of the lesions revealed 0-Ip (138 cases), 0-Isp (105 cases), 0-Is (43 cases), 0-IIa (45 cases), 0-IIa+IIc (24 cases), 0-IIc (9 cases), and other type (23 cases). The average follow-up period after ER was 39.5 ± 36.7 months (3–174 months). Further, recurrence was observed in 10 cases. There were no intramucosal recurrent cases.

Using univariate analysis, each of the following factors was confirmed to be significantly related to recurrence: existence of budding,[4] submucosal invasion depth deeper than 1000 μm, and lymphatic/venous involvement (Table 2). Multivariate analysis using logistic-regression analysis was carried out using these four factors. Consequently, submucosal invasion depth deeper than 1000 μm and lymphatic involvement were indicated as the factors with high odds ratios, and only lymphatic involvement was considered as an independent risk factor for recurrence (Table 3).

CLINICOPATHOLOGICAL CHARACTERISTICS OF THE CASES SHOWING RECURRENCE

The 18 cases (11 male and 7 female) of recurrence are shown in Table 4. The average age of the patients was

Table 2. Recurrence rate after ER for submucosal CRC in relation to pathological features ($n = 387$)

Pathological features	*n*	Recurrence positive (%)	*P* value
Histological grade			
well or mod	387	10 (3)	
por or muc	0		–
Histological grade at the deepest invasive portion			
well or mod	367	9 (2)	
por or muc	11	1 (9)	0.2756
Budding			
Positive	42	4 (10)	
Negative	345	6 (2)	0.0015
Depth of submucosal invasion (μm)			
<1000	220	1 (0.5)	
≧1000	167	9 (5)	0.0016
Lymphatic involvement			
Positive	29	5 (17)	
Negative	358	5 (1)	0.0002
Venous involvement			
Positive	18	3 (17)	
Negative	369	7 (2)	0.0070

CRC, colorectal carcinoma; ER, endoscopic resection; mod, moderately differentiated adenocarcinoma; por, poorly differentiated adenocarcinoma; well, well differentiated adenocarcinoma.

Table 3. Multivariate analysis of risk factors for recurrence after ER for submucosal CRC ($n = 387$)

Risk factors	Odds ratio	(*P*-value)	95%CI
Depth of submucosal invasion ≧1000 μm	7.014	(0.0753)	0.820–60.01
Lymphatic involvement positive	6.363	(0.0139)	1.457–27.79
Budding positive	2.258	(0.3466)	0.414–12.31
Venous involvement positive	2.275	(0.3446)	0.634–11.64

CRC, colorectal carcinoma; ER, endoscopic resection.

69.2 ± 7.2 years. The lesions were located in the cecum (2 cases), ascending colon (2 cases), sigmoid colon (6 cases), and the rectum (8 cases). The average size of the lesions was 19.7 ± 9.2 mm. The macroscopic type of the lesions revealed 0-Ip (6 cases), 0-Isp (4 cases), 0-Is (3 cases), 0-IIa (3 cases), 0-IIa+IIc (1 case), and another type (1 case). En bloc resection was carried out in 14 cases, and piecemeal resection was carried out in four cases. Histological lateral margin-positive was reported in eight cases, and histological vertical margin-positive was reported in eight cases. Among the 15 cases in which the depth of submucosal invasion was reported, the depth was <1000 μm in one case and ≧1000 μm in 14 cases.

Local intramucosal recurrence was observed in four cases with a histological positive lateral margin. Among these four cases, metastatic recurrence was observed in two cases (lung metastasis was observed in one case, and the details were unknown in one case). However, as the details of the vertical margin were unknown, the exact depth of submucosal invasion could not be measured. Among the 18 cases in which the submucosal invasive carcinoma showed recurrence, local recurrence in the form of submucosal carcinoma was observed in 11 cases, and metastatic recurrence was observed in 13 cases. Among the 15 cases in which the depth of submucosal invasion was measured, two cases showed depth <1000 μm; however, these cases had lymphatic involvement and a positive vertical margin. Among the cases in which metastatic recurrence was observed in the organs, recurrence was observed in the lung (5 cases), liver (4 cases), LN (4 cases), bone (2 cases), adrenal gland (1 case), and brain (1 case); in some cases, metastatic recurrence was observed in multiple organs. Among the eight cases in which the patients died, death due to the primary disease was observed in six cases, death due to other diseases was observed in one case, and there were no details regarding the death in one case.

The average interval between ER and recurrence was 19.7 ± 9.2 months. Among the 18 cases in which recurrence was observed, 16 cases showed recurrence within 3 years after ER. Among the 18 cases in which recurrence was observed, in all cases the lesions did not satisfy the curative conditions after ER for submucosal CRC.

RELATIONSHIP BETWEEN DEPTH OF SUBMUCOSAL INVASION AND POSITIVE RATE OF VERTICAL MARGIN IN EACH MACROSCOPIC TYPE OF SUBMUCOSAL CRC

We examined the relationship between depth of submucosal invasion and positive rate of vertical margin according to the

Table 4. Cases with recurrence after ER for submucosal CRC

No.	Gender	Age	Location	Macroscopic type	Size (mm)	ER technique	Vertical margin	Lateral margin	Depth of SM (μm)	Lymphatic/ venous involvement	Histological grade	Budding	Local recurrence	Metastatic recurrence	Alive/ death	Interval after ER (month)
1	F	68	C	Protruded	≧20	Piecemeal	?	+	240	−	well	−	IM	+	Alive	4
2	M	68	S	Superficial	≧20	Piecemeal	+	?	250	+	mod	−	SM	+	Alive	15
3	M	62	S	Protruded	<20	En bloc	−	+	SM scanty	?	well	−	IM	−	Alive	14
4	M	63	S	Protruded	≧20	En bloc	−	−	1000	+	mod	+	SM	+	Death	16
5	F	69	R	Superficial	<20	En bloc	−	−	1024	−	well	−	?	+	Alive	14
6	M	73	R	Protruded	<20	En bloc	−	−	1300	−	mod	−	SM	+	Death	20
7	F	60	S	Protruded	<20	En bloc	−	−	1572	−	well	−	SM	+	Alive	89
8	M	61	R	Protruded	≧20	En bloc	+	−	1800	+	mod	−	?	+	Death	12
9	F	71	A	?	≧20	Piecemeal	−	−	2200	+	mod	+	SM	+	Alive	24
10	F	78	C	Superficial	<20	Piecemeal	+	?	2433	−	well	−	SM	−	Alive	16
11	M	59	A	Protruded	<20	En bloc	−	−	3000	+	por	+	?	+	Death	18
12	F	80	R	Protruded	<20	En bloc	−	−	3500	−	well	−	SM	+	Alive	60
13	M	68	S	Protruded	≧20	En bloc	?	?	3800	+	mod	+	IM	−	Alive	8
14	M	74	S	Protruded	<20	En bloc	−	−	4200	+	mod	−	SM	+	Death	0
15	M	66	R	Protruded	<20	En bloc	?	−	5300	−	well	+	SM	−	Death	26
16	M	65	R	Protruded	≧20	En bloc	−	−	6886	+	mod	+	SM	+	Death	22
17	M	81	R	Protruded	<20	En bloc	+	−	SM3	−	?	−	SM	−	Death	10
18	F	80	R	Superficial	≧20	En bloc	?	+	?	−	?	−	IM	+	Alive	10

A, ascending colon; C, cecum; F, female; IM, intramucosa; M, male; mod, moderately differentiated adenocarcinoma; por, poorly differentiated adenocarcinoma; R, rectum; S, sigmoid colon; SM, submucosa; well, well-differentiated adenocarcinoma.

Table 5. Relationship between the depth of submucosal invasion and positive rate of vertical margin in each macroscopic type of submucosal CRC

Depth of submucosal invasion (μm)	Macroscopic type	
	Protruded type $n = 286$	Superficial type $n = 82$
~1000	7.1% (11/156)	2.9% (2/68)
1001~2000	10.2% (6/59)	8.3% (1/12)
2001~3000	14.7% (5/34)	0% (0/7)
3001~4000	18.8% (3/16)	
4001~	9.5% (2/21)	0% (0/2)
Total	9.4% (27/286)	3.7% (3/82)

CRC, colorectal carcinoma.

Table 6. Relationship between vertical margin and recurrence in endoscopically resected submucosal CRC without additional surgical resection

Vertical margin	Recurrence		Total
	Positive	Negative	
Positive	1 (3.2)	31 (96.8)	32 (100)
Negative	9 (2.5)	347 (97.5)	356 (100)

There were no intramucosal recurrent cases.
CRC, colorectal carcinoma.

macroscopic type (protruded or superficial type) in the 368 cases in which the depth of submucosal invasion was reported (Table 5). The overall positive rate of vertical margin was 8.2% (30/368), and the positive rate of protruded and superficial type lesions was 9.4% (27/286) and 3.7% (3/82), respectively. There were no significant differences between each macroscopic type. The positive rate of vertical margin of the protruded and superficial type lesions with submucosal invasion ≦1000 μm was 7.1% (11/156) and 2.9% (2/68), respectively. The positive rate of vertical margin of the protruded and superficial type lesions with submucosal invasion >1000 μm was 6.2% (16/259) and 4.8% (1/21), respectively. With regard to the relationship between vertical margin and recurrence, the recurrence rate for the vertical margin-positive cases was 3.1% (1/32) and that for the vertical margin-negative cases was 2.5% (9/356). There were no significant differences between the values for these two groups (Table 6).

DISCUSSION

This multi-institution questionnaire survey had several limitations. Owing to the retrospective-examination model, the average follow-up period was 38.7 months, and a central review was not carried out on the pathological specimens. However, valuable data were obtained by analyzing the prognoses of non-surgical submucosal CRC cases after ER, which were provided by multiple institutions. Our data showed that all non-surgical submucosal CRC cases with recurrence after

ER did not satisfy the curative conditions according to the Guidelines for Colorectal Cancer Treatment, 1st Edition by JSCCR.[1] As the curative conditions after ER for submucosal CRC stated in the currently used Guidelines for Colorectal Cancer Treatment, 2nd Edition by JSCCR, 2009[5] (a factor of budding grade was added to the curative condition of the 1st Edition) were derived from examinations of the cases in which surgical resection was accompanied by LN metastasis, micrometastasis was not taken into consideration. Clinical verification of the curative conditions can be proved by the prognosis of the non-surgical submucosal CRC cases after ER. However, as these results were obtained on the basis of the histopathological diagnosis in different institutions, we presumed that a certain amount of scattering existed among the data from different institutions.

ER is a therapeutic technique as well as an important diagnostic method that can be used as the total incisional biopsy. Complete resection of the lesions, including vertical margin-negative, is indispensable for curative conditions after ER for submucosal CRC. Currently, among the factors in the curative conditions, only the depth of submucosal invasion can be diagnosed prior to the surgical operation. LN metastasis of submucosal CRC with invasion depth deeper than 1500 μm and 2000 μm was not observed under certain conditions when the histological grade at the deepest invasive portion was taken into consideration.[6] Our data showed that the incidence of the histopathological vertical margin-positive was 8.2%, and there was no significant relationship between the vertical margin-positive and recurrence. This result may show that there is a difference between the histologically vertical margin-positive and submucosal residual tumors on the colorectal wall. To avoid local recurrence after ER, it is important to observe an ulcer in detail using colonoscopy. A previous report has described that residual tumors caused by incomplete ER have a higher growth potential than tumors before ER.[7] Therefore, to avoid a potential disadvantage to the patients, the preoperative diagnosis must be carried out precisely, and an appropriate therapy must be selected.

Regarding surveillance for non-surgical submucosal CRC cases after ER, the results obtained in this study revealed that distant metastasis or death due to primary disease within 3 years after ER was observed in 89% of the patients showing recurrence. Therefore, surveillance must be strictly carried out for 3 years after ER for submucosal CRC with non-curative condition. The examinations conducted in other institutions revealed that in many cases, recurrence took place within 5 years after ER.[8–10] Currently, there is no consensus as to the ideal surveillance method and period after ER for submucosal CRC.

This unstable questionnaire survey had several limitations. However, the current status regarding the mid-term prognosis after ER for submucosal CRC without additional surgical operation in Japan is not available. To investigate curative condition after ER for submucosal CRC and the appropriate interval between surveillances, a long-term prognosis survey from a large number of cases must be analyzed in the near future.

CONCLUSION

This questionnaire survey was conducted in institutions affiliated with the Colorectal Endoscopic Resection Standardization Implementation Working Group in JSCCR. The results obtained by analysis of the prognosis of the non-surgical submucosal CRC cases after ER proved that there is no risk of recurrence, and that surveillance could be carried out without additional surgical resection when the lesions satisfied the curative conditions after ER for submucosal CRC according to the Guidelines for Colorectal Cancer Treatment, 1st Edition by JSCCR,[1] and recurrence was observed within 3 years after ER.

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ORIGINAL

Our experience of treatment of cribriform morular variant of papillary thyroid carcinoma; difference in clinicopathological features of FAP-associated and sporadic patients

Yasuhiro Ito[1], Akira Miyauchi[1], Hideki Ishikawa[2], Mitsuhiro Hirokawa[3], Takumi Kudo[4], Chisato Tomoda[1] and Akihiro Miya[1]

[1] *Department of Surgery, Kuma Hospital, Kobe, Japan*
[2] *Department of Molecular-Targeting Cancer Prevention, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan*
[3] *Department of Pathology, Kuma Hospital, Kobe, Japan*
[4] *Department of Internal Medicine, Kuma Hospital, Kobe, Japan*

Abstract. Cribriform-morular variant (CMV) is a comparably rare histological subtype of papillary thyroid carcinoma (PTC). This can be associated with familial adenomatous polyposis (FAP) due to *APC* gene mutations. In this study, we investigated the difference in the biological characteristics between FAP-associated and sporadic CMV. Between 1991 and 2010, 32 patients with CMV were treated in Kuma Hospital. Thirty-one of these underwent initial surgery for CMV in Kuma Hospital. Twelve patients were FAP-associated and the remaining 19 were sporadic CMV. All patients were female. Tumors of FAP-associated CMV were more frequently multiple than those of sporadic CMV. Patient age and tumor size did not differ between the two groups. Of 12 FAP-associated CMV, 5 were detected by thyroid nodule (thyroid precedent group) and 7 were detected by FAP (polyposis precedent group) as an initial manifestation. Patient age was younger and tumor size was smaller in the polyposis group than in the thyroid nodule group. All patients lacked extrathyroid extension on intraoperative finding and were node-negative on pathological examination. To date, two patients with FAP-associated CMV who initially underwent hemithyroidectomy (one in Kuma Hospital and one in another hospital) showed recurrence to the remnant thyroid during follow-up. None of the patients showed recurrence to other regions or died of carcinoma. Taken together, CMV is considered an indolent disease in our series. FAP-associated CMV showed multiple tumors more frequently than sporadic CMV. Total thyroidectomy is recommended for FAP-associated CMV, but extensive lymph node dissection is not necessary.

Key words: Cribriform morular variant, Papillary thyroid carcinoma, FAP, Prognosis

PAPILLARY thyroid carcinoma (PTC) is the most common malignancy arising from thyroid follicular cells. There are many histological subtypes of PTC and some of them showed a different prognosis from conventional PTC. Cribriform-morular variant (CMV) is a rare subtype of PTC. Historically, in 1949, Crail first reported malignancies originating from the rectum, brain and the thyroid gland [1]. In 1968, the relation between familial adenomatous polyposis (FAP) and thyroid carcinoma was reported [2]. Furthermore, one study showed that young women with FAP had approximately 160 times more risk of thyroid carcinoma than healthy people [3]. In 1994, Harach *et al.* demonstrated unique histological features of FAP-associated PTC such as a cribriform pattern and solid areas with a spindle cell component [4]. The *APC* gene located in the 5q21 region is strongly associated with FAP and individuals with *APC* mutations have almost 100% risk of developing colorectal carcinoma [5, 6], allowing us to conclude that this variant is significantly associated with *APC* mutations. Other studies have also reported FAP-associated CMV [7, 8], but CMV can occur also sporadically without any relation to colonic polyposis [8-10].

In 2004, we reported 7 patients with CMV [11]. Three

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Correspondence to: Yasuhiro Ito, M.D., Ph.D. Department of Surgery, Kuma Hospital, 8-2-35, Shimoyamate-dori, Chuo-ku, Kobe 650-0011, Japan. E-mail: ito01@kuma-h.or.jp

of these patients were FAP-associated CMV because they had FAP or a family history of colonic carcinoma involving *APC* gene mutations. The remaining 4 were sporadic and lacked *APC* gene mutations. However, primary lesions in all of these patients were detected by thyroid nodule as an initial manifestation. Recently, the relationship between CMV and FAP has been more widely understood and CMV has been detected during thyroid screening in FAP patients without complaint of thyroid nodule.

In this study, we investigated the biological characteristics of CMV in 32 patients. Thirty-one patients underwent initial surgery in Kuma Hospital. Twenty of these patients were FAP-associated and the remaining 19 were sporadic CMV. We investigated the difference in clinicopathological features between these two groups.

Patients and Methods

Between 1991 and 2010, 32 patients with PTC underwent surgery and pathologically diagnosed as CMV in Kuma Hospital, who were enrolled in this study. All patients were females and patient age ranged from 17 to 38 years (average 27 years). These 32 patients underwent ultrasound-guided fine needle aspiration biopsy (FNAB) and were cytologically diagnosed as having PTC. Sixteen (50%) were also diagnosed as or highly suspected of CMV based on cytological findings such as the presence of a morular component, peculiar nuclear clearing, absence of colloid in the background and immunocytochemistry finding such as nuclear and cytoplasmic localization of beta-catenin and high positivity of estrogen receptor (ER) [12, 13].

Thirty-one of these patients underwent initial surgery in Kuma Hospital. Twelve of these patients (31%) were FAP-associated CMV because they had one or more of the following three features; 1) colonic polyposis was detected on total colonoscopy before or after thyroid surgery; 2) presence of *APC* gene mutations; and 3) family history of colonic polyposis or colorectal carcinoma. CMV in 5 patients was detected by thyroid nodule based on patient complaints or findings on medical check-up as the initial event. These patients underwent total colonoscopy after thyroid surgery and were diagnosed as having polyposis. The remaining 7 underwent thyroid ultrasound for screening after the detection of FAP and were referred to our hospital because thyroid nodules were found. None of these patients had complained of thyroid nodule prior to its detection. Nineteen patients (69%) were diagnosed as having sporadic CMV without any of the 3 features, which includes 3 patients who had no family history of colonic polyposis or colorectal carcinoma and rejected examination of colonic polyposis.

The extent of thyroidectomy for FAP-associated CMV was total thyroidectomy in 11 patients and hemithyroidectomy in 1 patient. That for sporadic CMV was total thyroidectomy in 9 patients and hemithyroidectomy in 10 patients. Completion total thyroidectomy was performed as the second surgery in one patient who underwent initial surgery at another hospital. All patients underwent central node dissection. Three patients with FAP-associated CMV and 8 with sporadic CMV also underwent modified radical neck dissection (MND). All patients were diagnosed as having CMV on postoperative pathological examination based on morphological findings such as cribriform pattern, morular component, lack of colloid, peculiar nuclear clearing and immunohistochemical findings of beta-catenin, ER and progesterone receptor (PgR) [4, 8, 9, 14].

Our series included 7 patients reported in our previous study who underwent *APC* gene mutation analysis [11]. The *APC* gene mutation was not analyzed in other patients.

None of the patients in our series underwent radioactive iodine (RAI) ablation or RAI therapy. After surgery, all patients were followed by ultrasound at least once per year. The follow-up periods of these 31 patients ranged from 4 to 217 months (average 59 months).

Fisher's exact test and Mann-Whitney U test were adopted for comparing variables. p values less than 0.05 were considered significant.

Results

Of 32 patients with CMV, 31 underwent initial surgery in Kuma Hospital. Twelve of these patients (39%) were diagnosed as having FAP-associated CMV because they had one or more of the following three features as indicate above, and the remaining 19 (61%) demonstrated sporadic CMV. Nine patients with FAP-associated CMV (75%) and 7 with sporadic CMV (37%) were preoperatively diagnosed as having or highly suspected of CMV based on the cytological findings indicated above. Multinodular goiter and euthyroid Graves' disease were detected in 3 patients and 1 patient with sporadic CMV, respectively. No

Table 1 Comparison of multiplicity on ultrasound for FAP-associated and sporadic CMV

	Multilplicity (%)		
	Solitary	Multiple	Total
FAP-associated CMV	3 (25)	9 (75)	12
Sporadic CMV	19 (100)	0	19
Total	22	9	31

$p = 0.00001$

Table 2 Comparison of multiplicity on pathological examination for FAP-associated and sporadic CMV

	Multilplicity (%)		
	Solitary	Multiple	Total
FAP-associated CMV	2 (17)	10 (83)	12
Sporadic CMV	17 (89)	2 (11)	19
Total	19	12	31

$p = 0.0005$

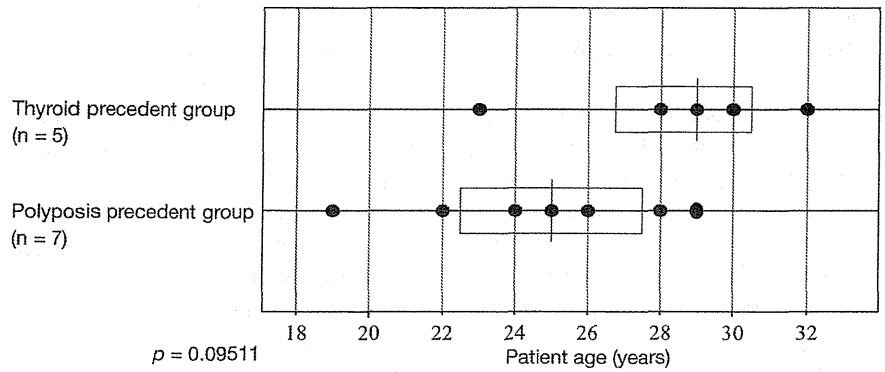

Fig. 1 Comparison of age of FAP- associated patients between the thyroid precedent group and polyposis precedent group.

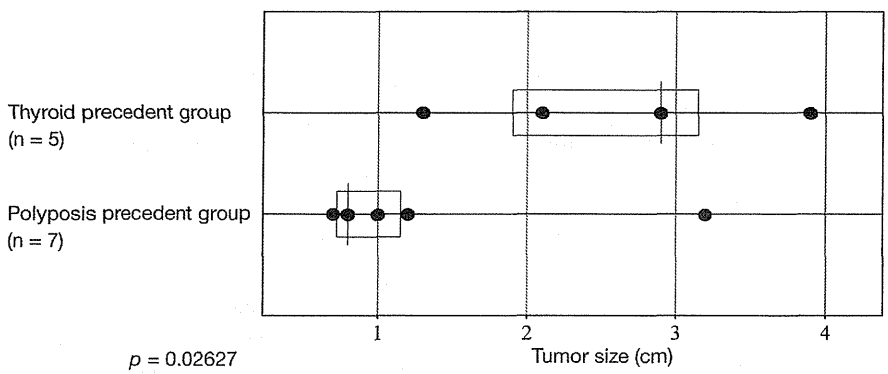

Fig. 2 Comparison of tumor size of FAP- associated patients between the thyroid precedent group and polyposis precedent group.

other thyroid comorbidities can be observed in FAP-associated CMV.

We compared the clinicopathological features between the two groups. Tumor size (1.79 + 1.15 cm *vs* 2.38 + 0.70 cm) and patient age (26.3 + 3.7 years *vs* 26.8 + 6.6 years) did not significantly differ between FAP-associated and sporadic CMV. Nine of 12 patients (75%) with FAP-associated CMV but none of the sporadic CMV were evaluated as multiple on preoperative ultrasound ($p = 0.00001$) (Table 1). On pathological examination, 10 patients with FAP-associated CMV (83%) and 2 with sporadic CMV (11%) showed multiplicity ($p = 0.00005$) (Table 2).

Diagnosis of CMV in 5 of 12 FAP-associated CMV patients (42%) was based on finding a thyroid nodule (thyroid precedent group) and that in 7 (58%) was based on FAP (polyposis precedent group) as the initial manifestation. Although not significantly different, patient age in the thyroid precedent group (28.4 + 3.4 years) tended to be older than that in the polyposis precedent group (24.7 + 3.5 years) ($p = 0.09511$) (Fig. 1). Tumor size in the thyroid precedent group (2.6 + 1.0 cm) was significantly larger than that in the polyposis precedent group (1.2 + 0.9 cm) ($p = 0.02627$) (Fig. 2).