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ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

Increased Risk for CRC in Diabetic Patients with the Nonrisk Allele of SNPs at 8q24

Shinya Ishimaru, MD, PhD[1,2], Koshi Mimori, MD, PhD[1], Ken Yamamoto, MD, PhD[3], Hiroshi Inoue, MD, PhD[1], Seiya Imoto, PhD[4], Shuichi Kawano, PhD[4], Rui Yamaguchi, PhD[4], Tetsuya Sato, PhD[5], Hiroyuki Toh, MD, PhD[5], Hisae Iinuma, PhD[6], Toyoki Maeda, MD, PhD[1], Hideshi Ishii, MD, PhD[1], Sadao Suzuki, PhD[7], Shinkan Tokudome, PhD[7], Masahiko Watanabe, MD, PhD[8], Jun-ichi Tanaka, MD, PhD[9], Shin-ei Kudo, MD, PhD[9], Ken-ichi Sugihara, MD, PhD[2], Kazuo Hase, MD, PhD[10], Hidetaka Mochizuki, MD, PhD[10], Masato Kusunoki, MD, PhD[11], Kazutaka Yamada, MD, PhD[12], Yasuhiro Shimada, MD, PhD[13], Yoshihiro Moriya, MD, PhD[13], Graham F. Barnard, MD, PhD[14], Satoru Miyano, PhD[4], and Masaki Mori, MD, PhD[15]

[1]Department of Surgery, Kyushu University Beppu Hospital, Beppu, Japan; [2]Department of Surgery, Tokyo Medical and Dental University, Tokyo, Japan; [3]Department of Molecular and Cellular Biology, Kyushu University, Fukuoka, Japan; [4]Laboratory of DNA Information Analysis, Human Genome Center Institute of Medical Science, University of Tokyo, Tokyo, Japan; [5]Division of Molecular Design, Kyushu University, Fukuoka, Japan; [6]Department of Surgery, Teikyo University, Tokyo, Japan; [7]Department of the Public Health, Nagoya City University, Nagoya, Japan; [8]Department of Surgery, Kitazato University, Sagamihara, Japan; [9]Digestive Disease Center, Northern Yokohama Hospital, Showa University, Yokohama, Japan; [10]Department of Surgery, National Defense University, Tokorozawa, Japan; [11]Department of Surgery, Mie University, Tsu, Japan; [12]Department of Surgery, Takano Hospital, Kumamoto, Japan; [13]Department of Surgery and Digestive Tract Medicine, National Cancer Center, Tokyo, Japan; [14]Department of Medicine, University of Massachusetts Medical School, Worcester, MA; [15]Department of Gastroenterological Surgery, Osaka University, Osaka, Japan

ABSTRACT

Background. Colorectal cancer (CRC) oncogenesis was considered to be determined by interactions between genetic and environmental factors. Specific interacting factors that influence CRC morbidity have yet to be fully investigated.

Methods. A multi-institutional collaborative study with 1511 CRC patients and 2098 control subjects was used to compare the odds ratios for the occurrence of polymorphisms at 11 known single nucleotide polymorphisms (SNPs). TaqMan PCR and questionnaires were used to evaluate the effects of environmental exposures.

Results. Variants of rs6983267 on 8q24 were the most significant markers of risk for CRC (odds ratio 1.16, 95% confidence interval 1.06–1.27, $P = 0.0015$). Non-insulin-dependent diabetes mellitus (DM), a higher body mass index at age 20, and meat consumption were environmental risk factors, whereas a tuna-rich diet and vitamin intake were protective factors. The cohort of rs6983267 SNP major (T) allele at 8q24 and DM had a 1.66-fold higher risk ratio than the cohort of major allele patients without DM.

Conclusions. We confirmed that interactions between the genetic background and environmental factors are associated with increased risk for CRC. There is a robust risk of the minor G allele at the 8q24 rs6983267 SNP; however, a major T allele SNP could more clearly reveal a correlation with CRC specifically when DM is present.

Shinya Ishimaru, Koshi Mimori and Ken Yamamoto contributed equally to this work.

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M. Mori, MD, PhD
e-mail: mmori@gesurg.med.osaka-u.ac.jp

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The morbidity and mortality of colorectal cancer (CRC) have been increasing in Japan since 1955. The identification of factors regulating the development and progression

of CRC contributes to improvement of preventive measures and therapeutic outcomes. Three elements are considered to be important in CRC development: host genetic factors (e.g., a single nucleotide polymorphism, or SNP, located within or near a relevant gene); environmental factors that directly affect epithelial cells through cytotoxic effects or genetic damage; and interactions between these genetic and environmental factors.

A number of SNPs have been associated with the onset of CRC. In a genome-wide association study, Tomlinson et al. examined 550,000 SNPs in 930 cases of familial CRC and identified rs6983267 at 8q24 [odds ratio (OR) 1.18, $P = 1.41 \times 10^{-8}$] and 9q24 (OR 1.14, $P = 1.32 \times 10^{-5}$).[1] Other locations, such as 15q13, 18q21, 11q23, 14q22, 16q22, 19q13, and 20p12, have been implicated.[2–5]

Multiple factors are thought to affect the colorectal epithelium during cancer development because the ORs of associated SNPs, including 8q24, for CRC, are less than 2.0. At most, carrying all six possible risk alleles at 8q24 yielded an OR of 2.6 [95% confidence interval (CI) 1.75–3.89] for CRC.[3] Thus, definitive conclusions based only on expression profile data from CRC cells are unlikely; however, the profiles could provide insight into the association between the SNP at 8q24, the incidence of CRC, and the influence of epidemiologic and environmental factors. Among environmental factors affecting CRC oncogenesis, we have specifically focused on diabetes mellitus (DM) because it has been discussed recently in previous studies. Giovannucci et al. clearly demonstrated that diabetes (primarily type 2) is associated with an increased risk of some cancers, including cancer of the colon and rectum, and explained the mechanism of association between DM and CRC with intriguing genes, such as Insulin/Insulin-Like Growth Factor Axis.[6] Previous study found that men with type 2 diabetes were up to 24% more likely to eventually get colon cancer than men without diabetes. Men who used insulin to control their diabetes faced a 36% greater risk of developing colon cancer than men without diabetes.[7]

In the current study, we demonstrate a significant association between a history of DM and the most highly associated SNP, rs6983267, at 8q24, and discuss the relationship.

MATERIALS AND METHODS

Extraction of Genomic DNA and PCR Amplification of Markers

Genomic DNA was extracted from peripheral blood samples from 1511 cases of CRC test subjects and 2098 control subjects by means of conventional methodologies, then quantified with PicoGreen (Invitrogen). The TaqMan probes and primers for rs6983267 and rs10808556 were purchased from Applied Biosystems (assay ID C_29086771_20 and C_31093430_10, respectively). In addition, rs4779584, rs4464148, rs4939827, rs12953717, rs3802842, rs4444235, rs9929218, rs10411210, and rs961253 (chr20:6404031–6404531) were genotyped with the same assay system (Table 1). Genotyping was performed with the ABI 7900HT Sequence Detection System and SDS 2.0 software (Applied Biosystems, Carlsbad, CA).

Evaluation of SNP Markers at 8q24

We evaluated the correlation between the morbidity rate of CRC and SNP rs6983267 at the 8q24 locus. We estimated the risks associated with each SNP by allele and heterozygous and homozygous ORs by an unconditional logistic regression, and calculated the associated 95% CIs in each case. The ethical committee of each institute approved this project.

TABLE 1 Diverse ORs of established SNPs for Japanese CRC cases

Gene or locus	Chromosome	SNP	Minor allele frequency (control)	*P* value for allele test	Effect size, OR (95% CI)
POU5F1P1, DQ515897, MYC	8q24	rs6983267	0.35	0.0016	1.16 (1.06–1.27)
POU5F1P1, DQ515897, MYC		rs10808556	0.34	0.0048	1.14 (1.04–1.25)
SCG5, GREM1, FMN1	15q13	rs4779584	0.17	0.079	ND
SMAD7	18q21	rs4464148	0.04	0.092	ND
		rs4939827	0.21	0.12	ND
		rs12953717	0.19	0.080	ND
LOC120376, FLJ45803, c11orf53, POU2AF1	11q23	rs3802842	0.34	0.085	ND
BMP4	14q22	rs4444235	0.41	0.092	ND
CDH1	16q22	rs9929218	0.18	0.16	ND
RHPN2	19q13	rs10411210	0.16	0.012	1.17 (1.03–1.32)
BMP2	20p12	rs961253	0.12	0.39	ND

Environmental and Epidemiologic Studies and Gene–Environmental Interactions

Detailed information in regard to demographic characteristics was collected via a standardized questionnaire. The information included the following demographic characteristics: height, weight, smoking and drinking habits, activity, sleeping, stress, dietary habits, medical history, present illnesses, drugs, medications and vitamins, body condition, familial diseases, and female-specific diseases. All control subjects had total colon fiber optic examinations to confirm the absence of malignant lesions in the colon and/or the rectum. ORs and 95% CIs were calculated by unconditional logistic regression models, adjusted for sex, age (5-year categories), and study area (Honshu and Kyushu).

For evaluation of genetic and environmental factors, the rs6983267 genotype and lifestyle data were used to evaluate related genetic and environmental factors. Subjects with DM were placed in the environmental risk group, and those with the GG genotype of rs6983267 were designated as a genetic risk group. Subjects with no environmental risk and no genetic risk (TT or GT allele) were treated as the reference group. *P* values for interaction were based on likelihood ratio tests, comparing models with and without interaction terms. Statistical analyses were performed by SAS software, version 9.1 (SAS, Cary, NC). A two-tailed *P* value of less than 0.05 was used to indicate statistical significance.

Statistical Analysis

The magnitude of carcinogenicity for established CRC SNPs was determined by a case–control study, and the related epidemiologic and environmental factors were defined through self-administered questionnaires. A significant internal association was found between rs6983267, a SNP at 8q24, and a history of DM.

RESULTS

Significant Correlation between CRC Morbidity and rs6983267

Three SNPs—rs6983267, rs10808556 at 8q24, and rs10411210 at 19q13—were significantly correlated with the incidence of CRC (Table 1).[3,5,8,9] Table 2 indicates that allelic testing determined that variant rs6983267 at 8q24 was significantly associated with CRC (OR 1.16, 95% CI 1.06–1.27, $P = 0.0015$).[8,10,11] Another variant, rs10808556, also had a significant correlation (OR 1.14, 95% CI 1.04–1.25). However, rs6983267 in 8q24 showed the strongest association among the three SNPs. As such, the 8q24 locus, with its association between the G allele and CRC risk, is the focus of further investigations here on the mechanism of CRC development.[12]

Epidemiologic and Environmental Risk Factors for CRC

As we indicate in the Supplementary Tables, there were numerous number of CRC oncogenesis–associated epidemiologic factors. The average age was significantly higher in 1511 cases (63.72) than 2098 control subjects (60.80) ($P < 0.0001$, Student's *t*-test, Supplementary Table 1). Weight at present was heavier in control subjects (59.35 kg) than in cases (58.53 kg) ($P = 0.0298$). For smoking, the total smoking period was much longer in cases (30.51 years) than in control subjects (28.75 years), which was statistically significant ($P = 0.0082$). In those who drank alcohol, the period since stopping drinking was shorter in cases (5.21 years) than in control subjects (9.60 years), which was statistically significant ($P < 0.0001$). For the sort of alcohol drunk, Japanese sake, distilled spirit, whiskey, and wine were statistically significantly associated with a higher risk for CRC.

In Supplementary Table 3, we present the frequency of food diversely. We discovered that patients had significantly

TABLE 2 OR of SNPs (rs6983267 and rs10808556) at 8q24 loci for CRC

SNP	Location	Test	Frequency		*P*	OR	95% CI	Hardy–Weinberg test
			Case	Control				
rs10808556	128482329	Allelic test	0.368	0.338	0.005	1.14	1.04–1.25	0.86
		Dominant model	0.601	0.56	0.009	1.18	1.04–1.34	
		Recessive model	0.134	0.115	0.062	1.19	0.99–1.44	
rs6983267	128482487	Allelic test	0.384	0.35	0.002	1.16	1.06–1.27	0.93
		Dominant model	0.616	0.579	0.016	1.17	1.03–1.33	
		Recessive model	0.153	0.122	0.004	1.3	1.09–1.55	

higher incidences eating certain foods than control subjects, as follows: ham (2.64 per week/2.42 per week in cases/controls, respectively), squid and shrimp (2.36/2.29), shellfish (2.3/2.03), codfish (2.01/1.76), broccoli (2.95/2.81), green vegetables (4.02/3.69), oranges (3.75/3.30), European cakes (2.02/1.93), black tea (2.15/1.68), coffee (3.47/3.25), vegetables (2.20/1.96), fruit juice (2.03/1.98), beefsteak (1.93/1.86), and grilled chicken (1.81/1.72). On the other hand, there were several foods that were protective against CRC, such as tofu (3.16 per week/3.31 per week in cases/controls, respectively), chicken (2.78/2.92), boiled fish paste (2.33/2.49), and fried foods (2.79/2.87). Without adjustment by age, sex, and location, some of the information might be skewed and could be affected by the food preferences of cases and control subjects.

History of illness and treatment were directly compared between cases and control subjects (Supplementary Table 4). It is worth noting that a history of and a treatment history for DM were observed significantly more frequently in cases than in control subjects ($P = 0.0008$ and $P = 0.040$, respectively). The incidence of gastroduodenal ulcer was higher in control subjects than in cases ($P < 0.0001$). Colon polypectomy was observed more frequently in control subjects than in cases ($P < 0.0001$), which might indicate the importance of the polypectomy to protect from CRC. Epidemiologic data of medications between cases and control subjects were analyzed (Supplementary Table 5). The population of those who received medication and who did not have DM was frequently observed, in 110 of 1511 cases and 113 of 2098 control subjects ($P = 0.0208$). Treatment with nonsteroidal anti-inflammatory drugs was significantly higher in control subjects than in cases. Several previous studies have addressed cyclooxygenase inhibitors and the inhibition of colon polyps.[13,14]

Data in the Supplementary Tables do not consider location, age, or sex, and therefore, we adjusted them and the evaluated data to find actual epidemiologic and environmental risk factors.

Effect of Environmental Factors on CRC Susceptibility after the Correction by Location, Age, and Sex

The results of the epidemiologic study are shown in Table 3A. For body mass index (BMI) at age 20, the OR of CRC among cases with a BMI of > 25 was 1.94 (95% CI 1.25–3.02). The OR for a BMI increment of 1 was 1.05 (95% CI 1.01–1.10) in men. A review of the medical histories revealed that the OR of DM for CRC was 1.50 (95% CI 1.05–2.14) in men. This finding is identical to the previous study by Campbell et al..[7] However, a history of drinking alcohol was not a CRC risk factor in men or women. The OR of cataracts in men was 0.46 (95% CI 0.30–0.72), suggesting that they reflect a protective marker for CRC. With regard to food intake, in particular a higher frequency of consumption of pork and beef (i.e., more than 3 times per week), the OR for CRC was concordantly increased (OR 1.26, 95% CI 1.09–1.47). Vitamin intake was a protective factor for CRC in men (OR 0.69, 95% CI 0.49–0.96).

The OR of two polymorphic sites at rs6983267 on chromosome 8q24 in CRC cases was analyzed with the cohort of 1511 CRC patients and 2098 control subjects (Table 3B). In men, the OR was higher for homozygous variants; however, this did not reach statistical significance (OR 1.36, 95% CI 0.99–1.87). However, in women, there was no significant association between variants of this SNP and the incidence of CRC.

Gene–Environmental Interactions in CRC Morbidity

We examined the interaction between two significant SNPs, such as 8q24.21 and 19q13, and whole environmental factors and found that there was no significant association was observed in 19q13. Therefore, further analysis will be done for the SNP at 8q24.21, rs6983267, in CRC cases.

The genetic–environmental interactions are summarized in Table 4. For rs6983267, the previously described genetic risk allele is the so-called minor or G allele elucidated in CRC cases overall; the genetic nonrisk allele is the major T allele, either homozygous TT or heterozygous GT. In this study, on 8q24 (rs6983267), the theoretical OR that defined the cohort with the so-called nonrisk major alleles TT ($n = 48$) or GT ($n = 44$) specifically in the presence of DM ($n = 11$) had an increased risk for CRC that was 1.66-fold greater than that of the cohort carrying a major allele without DM ($n = 81$). Interestingly, and by contrast, in the presence of DM, there was no association between the occurrence of CRC and the so-called genetic risk or minor allele GG ($n = 18$) (risk 1.03; Table 4).

DISCUSSION

In the current study, we found that the presence of DM and higher BMI at age 20 were risk factors for CRC development, while high tuna and vitamin intake were protective factors against CRC. These four factors were associated with CRC and diabetes in general. Recently, the American Diabetes Association and the American Cancer Society stated that diabetes (primarily type 2) is associated with an increased risk of some cancers, including CRC.[6] They speculated that the association between diabetes and cancer may be due in part to shared risk factors between the two diseases such as aging, obesity, diet, and physical inactivity. Possible mechanisms for a direct link between

TABLE 3 ORs of epidemiologic and genetic factors

Factor	Subfactor	Male		Female		Result
		OR	95% CI	OR	95% CI	
A: Epidemiologic factors						
BMI						
BMI at age 20	>25 vs. < 25	1.94*	1.25–3.02	1.41	0.70–2.86	Risk for men
BMI	Risk at every 1 BMI elevation	1.05*	1.01–1.10	1.02	0.97–1.07	Risk for men
History						
Hypertension	Present vs. absent	1.05	0.81–1.37	1.01	0.72–1.43	
Hyperlipidemia	Present vs. absent	0.92	0.62–1.36	0.77	0.51–1.16	
DM	Present vs. absent	1.5*	1.05–2.14	1.41	0.76–2.59	Risk for men
Cataracts	Present vs. absent	0.46*	0.30–0.72	1.2	0.73–1.98	Risk for men
Chronic hepatitis	Present vs. absent	0.47	0.22–1.02	0.46	0.14–1.51	
Operation history						
Resection of stomach	Yes vs. no	0.46	0.26–0.72	0.5	0.23–1.11	Protective for men
Polypectomy in colon	Yes vs. no	0.79	0.26–1.00	0.66*	0.47–0.92	Protective for women
Cholecystectomy	Yes vs. no	0.57	0.30–1.05	1.31	0.65–2.63	
Cataracta	Yes vs. no	0.73	0.42–1.27	1.3	0.69–2.48	
Smoking history	Smoker vs. nonsmoker	1.18	0.94–1.54	1.21	0.84–1.73	
BI risk	Risk for every 1.0 BI elevation	1.01*	1.00–1.01	1	0.99–1.02	Risk for men
BI value	BI > 30 vs. BI < 30	1.22	0.96–1.53	1.38	0.64–2.99	
Alcohol						
Drinking	Drinker vs. nondrinker	0.95	0.73–1.24	1.15	0.84–1.58	
Consumption	Risk for every 10 g alcohol	0.99	0.97–1.00	0.92	0.83–1.01	
Consumption/d	≥50 g vs. < 50 g	0.91	0.66–1.24	0.31*	0.10–0.97	Protective for women
Food intake						
Beef or pork	≥3 times/week vs. 2 times or less	1.26*	1.09–1.47	0.94	0.79–1.12	Risk for men
Salmon, tuna	≥3 times/week vs. 2 times or less	0.78*	0.67–0.90	0.83*	0.70–0.99	Protective for both
Liver	≥3 times/week vs. 2 times or less	1.11	0.93–1.33	1.1	0.49–1.37	
Drugs, vitamins						
Vitamin intake	Yes vs. no	0.69*	0.49–0.96	0.82	0.57–1.59	Protective for men
Antihypertension medication	Yes vs. no	0.88	0.67–1.15	1.03	0.70–1.49	
Antipyretic analgesic	Yes vs. no	0.5	0.16–1.50	0.9	0.34–2.40	
B: Genetic factors						
8q24	Wild type	1	Reference	1	Reference	1 reference
rs6983267	Heterozygous	1.08	0.88–1.34	0.93	0.71–1.21	1.010.90–1.31
	Homozygous	1.36	0.99–1.87	1.14	0.79–1.64	1.48 1.12–1.95

BI Brinkman index

* Significant at $P < 0.05$

diabetes and cancer include hyperinsulinemia, hyperglycemia, and inflammation. However, they concluded that many research questions remain. These findings are in accord with our current findings, particularly as they relate to the role of diabetes, obesity, and lipid metabolism. However, the OR ratio of those factors was very low (less than 2.0); therefore, it is possible that CRC is provoked not by a single factor (Table 3) but by multiple factors, including interactions among genetic and environmental backgrounds (Table 4).

In spite of the low OR (less than 2.0) for CRC, previous studies have indicated that the 8q24 SNP is a risk allele for various types of malignancies, including CRC.[8,10–12,15–19] A mechanism linking the association between CRC morbidity and 8q24 SNPs has been suspected for some time. Tuupanen et al. demonstrated that the binding affinity of TCF4/LEF for the rs6983267 site, which differed with the polymorphic sequence, defined the transcription level of downstream *MYC* in vitro and in vivo [12]. In other words, the genomic sequence of the risk allele of rs6983267 was

TABLE 4 Interactions between epidemiologic DM and genetic SNP at rs6983267 factors for CRC cases

SNP	DM negative OR (95% CI)	DM positive OR (95% CI)	*P*
8q24 rs6983267			0.043
TT + GT major allele	1 (Ref.)	1.67 (1.19–2.32)	
GG minor allele	1.54 (1.18–2.03)	1.03 (0.48–2.20)	

There is a significant interaction between a history of DM and frequency of rs6983267. Subjects with the major allele and DM had an elevated risk for CRC (1.67 times higher than that of those without DM)

highly homologous with the transcription factor TCF4/LEF; therefore, the transcription of the *MYC* gene was upregulated in CRC cases with the risk allele of rs6983267.

The interaction between the incidence of DM and the difference of allele of rs6983267 at 8q24 was observed to be significant (Table 4). However, the risk for CRC was upregulated in CRC cases with DM plus the nonrisk allele of rs6983267. The risk allele of rs6983267 did not elevate the risk for CRC in DM cases. We speculate as follows that for risky allele cases, the oncogenic risk for CRC was enough and the risk reached a ceiling; therefore, DM did not elevate the risk for CRC anymore. Nonrisk allele cases and DM could enhance the risk for CRC by 1.67 times only with the presence of the nonrisk allele for CRC.

In conclusion, we report that the rs6983267 SNP at 8q24 is a cancer-associated polymorphism. We also verified environmental risk factors for CRC, such as DM, high meat consumption, and higher BMI at age 20. We initially observed a risk for CRC through interactions between the genetic background and environmental factors (e.g., DM). The extremely low OR for CRC suggests that CRC might be provoked by the presence of multiple and diverse risk factors.

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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* 32*Lys*/*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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Use of vitamin supplements and risk of total cancer and cardiovascular disease among the Japanese general population: A population-based survey

Azusa Hara[1], Shizuka Sasazuki[1*], Manami Inoue[1], Taichi Shimazu[1], Motoki Iwasaki[1], Norie Sawada[1], Taiki Yamaji[1], Junko Ishihara[2], Hiroyasu Iso[3], Shoichiro Tsugane[1] and for the Japan Public Health Center-Based Prospective Study Group

Abstract

Background: Despite the popular use of vitamin supplements and several prospective cohort studies investigating their effect on cancer incidence and cardiovascular disease (CVD), scientific data supporting their benefits remain controversial. Inconsistent results may be partly explained by the fact that use of supplements is an inconsistent behavior in individuals. We examined whether vitamin supplement use patterns affect cancer and CVD risk in a population-based cohort study in Japan.

Methods: A total of 28,903 men and 33,726 women in the Japan Public Health Center-based Prospective Study cohort, who answered questions about vitamin supplement use in the first survey from 1990-1994 and the second survey from 1995-1998, were categorized into four groups (never use, past use, recent use, and consistent use) and followed to the end of 2006 for cancer and 2005 for CVD. Sex-specific hazard ratios (HRs) and 95% confidence intervals (95% CIs) were used to describe the relative risks of cancer and CVD associated with vitamin supplement use.

Results: During follow-up, 4501 cancer and 1858 CVD cases were identified. Multivariate adjusted analysis revealed no association of any pattern of vitamin supplement use with the risk of cancer and CVD in men. In women, consistent use was associated with lower risk of CVD (HR 0.60, 95% CI 0.41-0.89), whereas past (HR 1.17, 95% CI 1.02-1.33) and recent use (HR 1.24, 95% CI 1.01-1.52) were associated with higher risk of cancer.

Conclusions: To our knowledge, this is the first prospective cohort study to examine simultaneously the associations between vitamin supplement use patterns and risk of cancer and CVD. This prospective cohort study demonstrated that vitamin supplement use has little effect on the risk of cancer or CVD in men. In women, however, consistent vitamin supplement use might reduce the risk of CVD. Elevated risk of cancer associated with past and recent use of vitamin supplements in women may be partly explained by preexisting diseases or unhealthy background, but we could not totally control for this in our study.

Background

Despite the popular use of vitamin supplements, the strong consumer belief is that they prevent chronic diseases such as cancer and cardiovascular disease (CVD) [1,2], but results from randomized controlled trials are mixed [3-10]. Most randomized controlled trials show little support of a preventive effect of vitamin supplement use and even increased risk [6,7] for cancer and CVD incidence and mortality, with some exceptions [8-10]. However, data from randomized controlled trials suffer from concerns about overreliance on secondary rather than primary prevention, insufficient intervention and follow-up periods, particularly regarding the incidence of cancer, inappropriate supplement doses, and unsuitable cohorts for testing the hypothesis. Therefore, studies for the effects of long-term, low doses of several agents in the general population are needed. Despite several prospective cohort studies investigating their

* Correspondence: ssasazuk@ncc.go.jp
[1]Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
Full list of author information is available at the end of the article

effect on cancer incidence (all site [11,12], colorectal [13-15], breast [16,17], lung [18,19], prostate [20-23], non-Hodgkin lymphoma [24]), and CVD incidence [11,25-29], scientific data supporting their benefits remain controversial. Inconsistent results may be partly explained by the fact that use of supplements is an inconsistent behavior in individuals [13,30]. Individuals with a favorable lifestyle and healthy diet are more likely to use vitamin supplementation consistently [30]. Some studies have found reduced risk for incidence and mortality of cancer and CVD associated with a long duration of vitamin supplement use [13,14,27,28,31-34]; however, consistent use of vitamin supplements could not be clearly determined by using a single time-point survey at baseline.

It is also important to note that the use of vitamin supplements is often associated with healthy lifestyle factors or with specific health issues, such as hypertension and cancer, that may increase or decrease vitamin supplement use [35,36]. In Japan, a few cross-sectional studies reported that the prevalence of vitamin supplement use was approximately 10% to 30% of the study population and that vitamin supplement use was associated with several factors broadly characterized by health consciousness and conversely by poor health [37-39]. However, all prospective studies have been conducted in Western populations (United States [11-15,17-29,31-34] and European countries [16,19,25]). No data have been reported for prospective cohort studies in Asian general populations, although there are some randomized clinical trials [8,40].

Therefore, we examined the association between vitamin supplement use and the risk of cancer and CVD in a population-based prospective cohort study in Japan. Participants in this cohort reported vitamin supplement use at two time points, which enabled us to examine the impact of the pattern of use on the risk of cancer and CVD.

Methods

The Japan Public Health Center (JPHC)-Based Prospective Study was started in 1990 for cohort I and in 1993-1994 for cohort II. All subjects were Japanese inhabitants registered at 11 public health center areas and were aged 40-69 years at the time of their first survey. Details of the study design have been described previously [41]. In the present study, the subjects from two public health center areas (Katsushika in Tokyo prefecture and Suita in Osaka prefecture) were excluded because the incidence data for cancer or CVD were not available and the selection of subjects differed from that in other public health center areas. Of 116,896 people in nine public health areas, 95,405 (82%) individuals responded to the first survey. We excluded 1168 persons who were not Japanese, who had died or moved out of a study area, or who were lost to follow-up before the starting point. This left 94,237 eligible subjects. In 1995 and 1998, the second survey was conducted; 79,809 subjects replied (85%; 36,783 men and 43,026 women) and were included in the present study. The institutional review board of the National Cancer Center, Tokyo, Japan, approved the study.

The status of vitamin supplement use was defined by the responses in the two surveys and was classified into the following four categories of use: (1) never, no vitamin supplement use in either the first or second survey; (2) past, vitamin supplement use only in the first survey; (3) recent, vitamin supplement use only in the second survey; and (4) consistent, vitamin supplement use in both surveys. In the first survey, cohort I and cohort II subjects were asked how frequently they used vitamin supplements. Those who reported use on >1 day/week were asked about the type of vitamin supplements. Use of vitamin supplements in the first survey was defined as subjects who used them at least 1 day/week. No information was collected on brand name or duration of vitamin supplement use. In the second survey for cohorts I and II, general use of any vitamin supplements more than once a week and use of specific vitamin supplements were examined. The brand names of vitamin supplements used were requested, and 81.7% provided this information. We used re-categorized self-reported categories of vitamin supplements based on the definition in the Women's Healthy Eating and Living Study [42] to improve sensitivity in identifying supplement use [43]. Details of the assessment of self-reported vitamin supplement use have been described previously, and use of vitamin supplements in the second survey was defined as subjects who used at least one type of vitamin supplement ≥ 1 week for ≥ 1 year [37,43].

We followed subjects from the second survey until December 31, 2006, for cancer and until December 31, 2005, for CVD. We identified changes in residence status and survival annually through the residential registry in each area or, for those who had moved out of the area, by using the municipal office of the area to which they had moved. Residency registration and death registration are required by the Basic Residential Register Law and Family Registry Law, respectively, and the registries are thought to be complete. During the follow-up period, 8060 subjects (10.1%) died, 2106 (2.6%) moved out of the study areas, and 249 persons (0.31%) were lost to follow-up between the second survey and December 31, 2006.

The occurrence of cancer was identified by active patients' notification from major local hospitals in the study area, that is, the extraction of clinical information from medical records into cohort-specific registration

forms in either local major hospitals, which care for most of the patients with cancer or CVD (up to 80%) in some areas, by physicians in the hospital or physicians in the public health center [44], and from data linkage with population-based cancer registries, with permission from each of the local governments responsible for the cancer registries. Cases of cancer were coded according to the *International Classification of Disease for Oncology*, third edition, of the World Health Organization [45]. In our cancer registry system, the proportion of cases for which information was available from death certificates only was 4.4%. For the present analysis, the earliest date of diagnosis was used in cases with multiple cancer diagnoses at different times. Diagnosis of myocardial infarction according to the criteria of the Monitoring Trends and Determinants of Cardiovascular Disease (MONICA) project [46] and diagnosis of stroke according to the criteria of the National Survey of Stroke [47] were confirmed for all cases by computer tomographic scan, magnetic resonance imaging, or both as recorded in the medical record and reviewed by hospital or public health center physicians in each registered major local hospital in each public health center area [48,49]. CVD cases with a death certificate or by self-report only, without confirmation by medical records, were treated as non-CVD cases. CVD was defined as myocardial infarction or stroke, whichever occurred first. Among the 79,809 subjects, we confirmed 5932 cases of newly diagnosed cancer by December 31, 2006, and 3218 cases of CVD by December 31, 2005. Participants with both cancer and CVD were included in both analyses.

From the 79,809 respondents, we excluded subjects with a history of cancer or CVD (n = 5809) and those who did not have information on their vitamin supplement use in both surveys (n = 11,371). Subjects with a history of cancer or CVD were defined as diagnosed with cancer or CVD before the starting point or from self-reports in the surveys. For the final analysis, 62,629 subjects (28,903 men and 33,726 women) remained, including 4501 with cancer and 1858 with CVD. We calculated person-years of follow-up for each subject from the starting point to the date of diagnosis, date of emigration from the study area, date of death, or end of the follow-up (December 31, 2006 for the cancer analysis and December 31, 2005 for the CVD analysis), whichever came first. We censored subjects lost to follow-up at the last confirmed date they were present in the study area. A total of 597,281 person-years were accrued for the cancer analysis and 547,983 for the CVD analysis. Sex-specific hazard ratios (HRs) and 95% confidence intervals (95% CIs) were used to describe the relative risks of total cancer and CVD associated with use of vitamin supplements. The Cox proportional hazards model was used to control for potential confounding factors, which were either known or suspected from previous studies as risk factors for cancer and CVD. All covariates were based on information from the second survey. We conducted the initial analyses by adjusting for age at the starting point (5-year groups) and study area (nine public health center areas). In the multivariate model, we further adjusted for smoking status (never, former, <20, 20-29, 30-39, and ≥ 40 pack-years for men, and never, former, <20, and ≥ 20 pack-years for women), alcohol consumption (none, <150, 150-299, 300-449, and ≥ 450 g ethanol/week for men, and none, <150, and ≥ 150 g ethanol/week for women), body mass index ([BMI] <19, 19-20.9, 21-22.9, 23-24.9, 25-26.9, 27-29.9, and ≥ 30 kg/m^2), occupation (farming, forestry, and fishing; employee and professional; housewife; self-employed; unemployed; other occupations; and combination [≥ 2 occupations across those groups]), quartile of physical activity in metabolic equivalent task-hours/day, total energy intake, energy-adjusted green vegetable intake, current medication status (hypertension, hyperlipidemia, or diabetes mellitus), and screening examination (blood pressure measurement, biochemical examination, electrocardiogram, fundus examination, chest radiograph, sputum cytology, gastric photofluorography, gastrointestinal endoscopy, fecal occult blood test, barium enema, or colonoscopy for men and women, and mammography or Papanicolaou smear for women), which were reported in a questionnaire in the second survey. As for current medication status and screening examination, if a subject replied "yes" to at least one medication or examination, we regarded the subject as using medication or taking the examination, respectively. The second survey included a food-frequency questionnaire consisting of 138 food items with standard portions/units and nine frequency categories, which were developed to estimate dietary intake [50] and validated for estimations of various nutrients and food groups [51-54]. A residual model was used for energy adjustment of green vegetable consumption, vitamin B_2, vitamin B_6, vitamin B_{12}, folate, α-tocopherol, vitamin C, and vitamin D intake reported in food-frequency questionnaire [55]. Extreme values of BMI (<14 or ≥ 40 kg/m^2) and total energy intake (lower and upper 2.5 percentiles) were treated as missing values. Statistical significance was assumed at $P < 0.05$. All statistical analyses were performed using SAS software, version 9.1 (SAS Institute, Cary, NC, USA).

Results

Of the participants included in this analysis, 49,060 subjects (78.3%) reported no vitamin supplement use, 7833 subjects (12.5%) reported only past vitamin supplement use (in the first survey), 2593 subjects (4.2%) reported

only recent vitamin supplement use (in the second survey), and 3143 subjects (5.0%) reported past and recent vitamin supplement use. Among subjects who used vitamin supplements and reported the brand name in the second survey, the most common vitamin supplement was B vitamins for men and women (multivitamin: 474 subjects [25.8%] and 566 subjects [19.6%]; antioxidants: 30 subjects [1.6%] and 126 subjects [4.4%]; vitamin A: 65 subjects [3.5%] and 144 subjects [5.0%]; B vitamins: 797 subjects [43.5%] and 883 subjects [30.6%]; vitamin C: 299 subjects [16.3%] and 656 subjects [22.7%]; vitamin E: 295 subjects [16.1%] and 843 subjects [29.2%]; other vitamins: 219 subjects [11.9%] and 443 subjects [15.3%], respectively).

Table 1 shows the baseline characteristics of the study subjects according to vitamin supplement use pattern in men and women separately. Individuals with past use and consistent use of vitamin supplements were significantly older for both sexes. Men who had never used supplements were thought to have lower health consciousness due to higher proportions with a BMI ≥ 25 kg/m^2, a greater likelihood of being a smoker or regular drinker, less information on their disease history (angina, diabetes, colonic polyp, and hepatitis), fewer screening examinations, and less consumption of soy foods and fruits compared with other men. Significantly higher proportions of men with consistent supplement use took more medications (hyperlipidemia and diabetes), were more likely to have disease histories (angina, diabetes, duodenal ulcer, colonic polyp, and hepatitis), and may have higher health consciousness suggested by lower BMI, less regular drinking, more screening examinations, and higher consumption of fruits. Men with past supplement use also had a significantly higher proportion of antihypertensive medication use. Men with recent use also tended to have a healthy lifestyle and significantly lower proportions were smokers or taking diabetic medication. Women who had never used supplements were likely to have a healthier lifestyle, with significantly lower proportions being smokers or regular drinkers than other women. Women with recent or consistent use were also basically health conscious, having a lower BMI and a higher proportion of screening examinations, despite there being a significantly higher proportion of regular drinkers. Individuals with consistent use also consumed significantly larger amounts of fruits, folate, and vitamin C. They also tended to have significantly higher proportions of medication use (hypertension and hyperlipidemia) and history of diseases such as gastric and colonic polyps than those who never used supplements. Women with recent use were also more likely to have a history of gastric and colonic polyps, despite their younger age, and had a significantly higher proportion of medication use except for hypertension, hyperlipidemia, and diabetes. Women with past use tended to have an unhealthy lifestyle, including a higher BMI and a greater likelihood of smoking and medication use (hypertension and diabetes).

Associations of vitamin supplement use pattern and total cancer and CVD risk in men and women are shown separately in Table 2. In men, no significant association was found between any pattern of vitamin supplement use and the risk of total cancer and CVD in age- and study area-adjusted and multivariate-adjusted models. No significant association was found between any specific vitamin supplement use in the second survey and total cancer and CVD. For women, however, a statistically significant increase in the risk of total cancer occurrence was observed in those with past and recent vitamin supplement use compared with those who never used supplements; the HR of developing cancer (95% CI) for past use and recent use was 1.17 (1.02-1.33) and 1.24 (1.01-1.52), respectively. When we performed separate analyses for major site-specific cancers, the HR of recent use in women was especially high for stomach cancer (HR 2.15, 95% CI 1.39-3.34). We also observed a nonsignificant but moderately increased risk of liver and pancreatic cancer with past supplement use in women (liver cancer: HR 1.61, 95% CI 0.95-2.74; pancreatic cancer: HR 1.67, 95% CI 0.94-2.97). When we estimated the HR after excluding women diagnosed as having cancer within 5 years of baseline, similar trends were observed, although the association for cancer with recent use was not significant and with past use remained significant. In the second survey, vitamin C supplements specifically and antioxidant supplementation, including two or more of β-carotene, vitamin C, vitamin E, and selenium [42], were significantly associated with an increased risk of total cancer; compared with the subjects with no vitamin supplement use, the HR and 95% CI of vitamin C supplement and antioxidant supplement use were 1.38 (1.03-1.87) and 1.83 (1.01-3.31), respectively. In contrast, we observed a statistically significant reduced risk for CVD with consistent vitamin supplement use for women (HR 0.60, 95% CI 0.41-0.89). When we performed separate analyses for coronary heart disease, hemorrhagic stroke, or ischemic brain infarction, decreased risk was observed for ischemic brain infarction with statistical significance with consistent use (coronary heart disease: HR 0.19, 95% CI 0.03-1.34; hemorrhagic stroke: HR 0.61, 95% CI 0.29-1.31; ischemic brain infarction: HR 0.52, 95% CI 0.28-0.98). HR estimates after excluding women diagnosed with CVD within 5 years of baseline showed a similar trend to estimates using all cases, although they were not statistically significant.