BRIEF ARTICLE

CYP1A1, GSTM1, GSTT1 and NQO1 polymorphisms and colorectal adenomas in Japanese men

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Abstract

AIM: To investigate the role of functional genetic polymorphisms of metabolic enzymes of tobacco carcinogens in the development of colorectal adenomas.

METHODS: The study subjects were 455 patients with colorectal adenomas and 1052 controls with no polyps who underwent total colonoscopy in a preretirement health examination at two Self Defense Forces hospitals. The genetic polymorphisms studied were CYP1A1*2A (rs 4646903), CYP1A1*2C (rs 1048943), GSTM1 (null or non-null genotype), GSTT1 (null or non-null genotype) and NQO1 C609T (rs 1800566). Genotypes were determined by the polymerase chain reaction (PCR)-restriction fragment length polymorphism or PCR method using genomic DNA extracted from the buffy coat. Cigarette smoking and other lifestyle factors were ascertained by a self-administered questionnaire. The associations of the polymorphisms with colorectal adenomas were examined by means of OR and 95%CI, which were derived from logistic regression analysis. Statistical adjustment was made for smoking, alcohol use, body mass index and other factors. The gene-gene interaction and effect modification of smoking were evaluated by the likelihood ratio test.

RESULTS: None of the five polymorphisms showed a significant association with colorectal adenomas, nor was the combination of GSTM1 and GSTT1. A borderline significant interaction was observed for the combination of CYP1A1*2C and NQO1 (P = 0.051). The OR associated with CYP1A1*2C was significantly lower than unity among individuals with the NQO1 609CC genotype. The adjusted OR for the combination of the CYP1A1*2C allele and NQO1 609CC genotype was 0.61 (95%CI: 0.42-0.91). Although the interaction was not statistically significant (P = 0.24), the OR for individuals carrying the CYP1A1*2C allele and GSTT1 null genotype decreased significantly compared with those who had neither CYP1A1*2C allele nor GSTT1 null genotype (adjusted OR: 0.69, 95%CI: 0.49-0.97). Smoking did not modify the associations of the individual polymorphisms with colorectal adenomas. There was no measurable effect modification of smoking even regarding the combination of the genetic polymorphisms of the phase I and phase II enzymes.

CONCLUSION: Combination of the CYP1A1*2C and NQO1 609CC genotypes was associated with a decreased risk of colorectal adenomas regardless of smoking status.

Core tip: The study investigated the associations of *CYP1A1*2A*, *CYP1A1*2C*, *GSTM1*, *GSTT1* and *NQO1* C609T polymorphisms with colorectal adenomas among 455 cases of colorectal adenomas and 1052 controls with no polyps. None of the five polymorphisms showed a significant association with colorectal adenomas, nor was the combination of *GSTM1* and *GSTT1*. A borderline significant interaction was observed for the combination of *CYP1A1*2C* and *NQO1*. Combination of the *CYP1A1*2C* and *NQO1* 609CC genotypes was associated with a decreased risk of colorectal adenomas regardless of smoking status.

INTRODUCTION

Colorectal cancer is one of the most common cancers, accounting for approximately 10% of incident cancer cases worldwide[1]. Colorectal adenoma is a well-established precursor lesion of colorectal cancer[2,3]. Cigarette smoking has been related to increased risk of colorectal adenomas, whereas the association between smoking and colorectal cancer risk is rather inconsistent and much weaker[4-6]. Despite the consistent association between smoking and colorectal adenomas, biological mechanisms for the association remain unknown. Tobacco smoke contains various types of carcinogens such as polycyclic aromatic hydrocarbons, heterocyclic amines, aromatic amines and *N*-nitrosamines, which are activated by phase I enzymes and/or detoxified by phase II enzymes, and thus functional genetic polymorphisms of the metabolic enzymes are of interest in colorectal carcinogenesis[7].

CYP1A1 is a phase I enzyme responsible for bioactivation of tobacco carcinogens. Two functional polymorphisms are known in the *CYP1A1* gene[8,9]. One is the 3698T>C substitution (*CYP1A1*2A*, rs 4646903) that creates an *Msp*I restriction site in the 3'-flanking region, and the other is the 2454A>G substitution (*CYP1A1*2C*, rs 1048943) that results in an amino acid change (Ile462Val) in exon 7. The *CYP1A1*2A* and *CYP1A1*2C* alleles are linked to higher inducibility of the enzyme, and have been associated with an increased risk of lung cancer and less consistently of other tobacco-related cancers[8,9]. Several studies reported an increased risk of colorectal cancer associated with *CYP1A1*2A*[10] and *CYP1A1*2C*[11], while others showed no association of either *CYP1A1*2A* or *CYP1A1*2C* with colorectal cancer[12-15] or adeno-

mas[16]. Isoforms of the glutathione S-transferase (GST) are involved in detoxification of chemical carcinogens and environmental toxic compounds[17,18]. *GSTM1* and *GSTT1* polymorphisms have been studied most intensively in relation to tobacco-related cancers. The *GSTM1* and *GSTT1* null genotypes result in a complete loss of enzyme function[17,18]. A meta-analysis suggested an increased risk of colorectal cancer associated with the null genotype of *GSTT1*, but not of *GSTM1*[19], while another meta-analysis showed no association of either the *GSTM1* or *GSTT1* null genotype with colorectal cancer or adenomas[20]. Some recent studies have shown an increased risk of colorectal cancer associated with the *GSTM1* and *GSTT1* null genotypes in combination[21,22], but others failed to show such an increase in the risk of colorectal cancer[14,15] or adenomas[23].

NAD(P)H-quinone oxidoreductase 1 (NQO1) is involved in detoxification through two electron reductions of quinones to hydroquinones, while NQO1 can also activate procarcinogens in tobacco smoke[24]. The 609C>T polymorphism (rs 1800566) that causes an amino acid substitution (Pro187Ser) results in loss of NQO1 activity[24]. A meta-analysis reported that *NQO1* 609C>T was associated with a small increase in the risk of colorectal cancer in Caucasians[25], but a recent large Japanese study failed to corroborate such an association[15]. Homozygosity of the *NQO1* 609T allele was shown to be positively associated with colorectal adenomas[26]. Heavy smokers carrying both the *CYP1A1*2C* and *NQO1* 609T variant alleles showed a substantial increase in the risk of adenomas[26].

To clarify the role of *CYP1A1*, *GSTM1*, *GSTT1* and *NQO1* polymorphisms in colorectal carcinogenesis with reference to smoking, we examined the associations of these polymorphisms with colorectal adenomas and the effect of smoking on the associations between the polymorphisms and colorectal adenomas. A particular emphasis was placed on the combination of genetic polymorphisms of phase I and phase II enzymes, because the literature is sparse on the influence of gene-gene interactions on the risk of colorectal cancer and adenomas.

MATERIALS AND METHODS

Subjects

Study subjects were male officials in the Self Defense Forces who received a preretirement health examination at the Self Defense Forces Fukuoka Hospital or Kumamoto Hospital during the period from January 1997 to March 2001. The preretirement health examination is a nationwide program that offers a comprehensive medical examination including colonoscopy to persons retiring from the Self Defense Forces. Details of the preretirement health examination have been described elsewhere[27,28]. The subjects were Japanese in ethnicity. A 7-mL fasting venous blood sample was donated for the purpose of medical research with written informed consent. The study was approved by the Ethics Committee of Kyushu University Faculty of Medical Sciences.

The present study included 455 cases of histologically confirmed colorectal adenoma and 1052 controls with no

polyps who underwent total colonoscopy. In a consecutive series of 2454 men, five refused to participate in the study and we excluded 77 who did not undergo colonoscopy. Further exclusions were 242 men with a history of colectomy (*n* = 17), colorectal polypectomy (*n* = 212), malignant neoplasm (*n* = 27) or inflammatory bowel disease (*n* = 1). For the remaining 2135 men, colonoscopic findings were classified as polyp (*n* = 938, 43.9%), colorectal cancer (*n* = 1, 0.0%), non-polyp benign lesion (*n* = 123, 5.8%) and normal (*n* = 1073, 50.3%). Of the 938 men with colorectal polyps, 461 were found to have adenoma without *in situ* or invasive carcinoma. The controls comprised 1067 men who underwent a complete colonoscopy among the 1196 men with normal colonoscopy or non-polyp benign lesions. DNA was not available for 21 men (6 cases and 15 controls), and 455 cases and 1052 controls remained in the analysis.

Lifestyle questionnaire

Smoking habits, alcohol consumption, physical activity and other lifestyle factors were ascertained by a self-administered questionnaire, with a supplemental interview for unanswered questions given prior to colonoscopy. Details of the lifestyle questions have been described elsewhere[27,28]. Lifetime exposure to cigarette smoking was expressed by cigarette-years, which were calculated as the product of total years of smoking and the average number of cigarettes per day. Cigarette smoking was classified into 0, 1-399, 400-799 and ≥ 800 cigarette-years. Daily intake of ethanol was estimated for current alcohol drinkers based on consumption frequencies and amounts of five types of alcoholic beverages on average in the past year. Alcohol use was categorized into never, past and current use with a consumption of < 30, 30-59 or ≥ 60 mL of ethanol per day. Body mass index was categorized into four levels (< 22.5, 22.5-24.9, 25.0-27.4 and ≥ 27.5 kg/m²). The categories for alcohol use and body mass index were arbitrary, but in accordance with those used in the previous studies[27,28]. Leisure-time physical activity was expressed as the sum of products of intensity score (metabolic equivalent) and amount of time for at most three types of regular exercise, and was categorized by quartiles in the control group.

Genotyping

DNA was extracted from the buffy coat by use of a commercial kit (Qiagen GmbH, Hilden, Germany). Genotyping was carried out by the polymerase chain reaction (PCR)-restriction fragment length polymorphism or PCR method, with agarose-gel electrophoresis and visualization by ethidium bromide. The PCR was performed in a mixture of 10 μL containing 1 μL template DNA with a concentration of 50-150 ng/μL. The PCR for *CYP1A1*2A* polymorphism was done using the primers described by Sivaraman *et al*[10], and the 340-bp PCR product was digested with *Msp*I, which resulted in fragments of 200 and 140 bp for the *CYP1A1*2A* allele. The *CYP1A1*2C* polymorphism was genotyped using the primers previously specified[29], with digestion by restric-

tion enzyme *Hin*cII. The 187-bp product was cleaved into three fragments (120, 48 and 19 bp) in the presence of the *CYP1A1*2C* allele, and otherwise into two fragments (139 and 48 bp). *GSTM1* and *GSTT1* polymorphisms were determined by the multiplex PCR using the primers for *GSTM1*, *GSTT1* and albumin as described previously[30]. Genotyping for *NQO1* 609C>T was performed as described previously[31]. The 230-bp PCR product was digested with *Hin*fI, resulting in fragments of 195 and 35 bp for the 609C allele and fragments of 151, 44 and 35 bp for the 609T allele. The assay was repeated at most three times when the PCR was unsuccessful or when the migration pattern on the gel was aberrant.

Statistical analysis

Deviation of genotype frequency from the Hardy-Weinberg equilibrium was tested by χ^2 test with one degree of freedom using the Stata version 10 (Stata Corporation, College Station, TX, United States). The associations between the polymorphisms and colorectal adenomas were assessed by means of OR and 95%CI, which were derived from logistic regression analysis. Statistical adjustment was made for age (continuous variable), hospital (dichotomous variable), rank in the Self Defense Forces (low, middle and high), cigarette smoking, alcohol use, body mass index, physical activity and parental colorectal cancer. The gene-gene interaction and effect modification of smoking were evaluated by the likelihood ratio test. In the analysis of the effect modification of smoking, smoking status was categorized into < 400 and ≥ 400 cigarette-years, *i.e.*, < 20 and ≥ 20 pack-years, because an increased risk of adenomas associated with smoking was discernible only in the latter categories (see below). Statistical significance was declared if two-sided *P* was < 0.05. Statistical analysis was performed with SAS version 9.2 (SAS Institute, Cary, NC, United States).

RESULTS

Selected characteristics of the cases and controls are summarized in Table 1. The age range was 50-57 years for the cases and 47-59 years for the controls. The cases had a greater body mass index and a lower physical activity in leisure time than the controls. Heavy smoking and high alcohol consumption were more frequent in the cases than in the controls.

Among the controls, the frequencies of the *CYP1A1*2A*, *CYP1A1*2C* and *NQO1* 609T alleles were 0.39, 0.23 and 0.38, respectively, and genotype frequencies of the three polymorphisms were all in agreement with the Hardy-Weinberg equilibrium (*P* = 0.62 for *CYP1A1*2A*; *P* = 0.32 for *CYP1A1*2C*; and *P* = 0.76 for *NQO1* C609T). The *CYP1A1*2A* and *CYP1A1*2C* polymorphisms were in complete linkage disequilibrium except for two cases; the deviation of these two was probably due to error in genotyping.

None of the five polymorphisms showed a significant association with colorectal adenomas, nor was the combination of *GSTM1* and *GSTT1* (Table 2). The gene-gene interaction was examined for the combina-

Table 1 Selected characteristics of the study subjects

Characteristics	Cases (n = 455)	Controls (n = 1052)
Age (yr), mean ± SD	52.4 ± 0.8	52.4 ± 0.9
Body mass index (kg/m²), mean ± SD	24.1 ± 2.8	23.7 ± 2.5
MET-h/wk, median (IQR)[1]	12 (3-24)	14 (5-24)
Smoking (cigarette-yr)		
0	20.90%	33.70%
1-399	14.10%	18.80%
400-799	45.50%	33.70%
≥ 800	19.60%	13.70%
Alcohol use (mL/d)		
Never	11.20%	13.80%
Past	2.90%	3.10%
< 30	21.10%	30.70%
30-59	34.10%	28.70%
≥ 60	30.80%	23.70%

[1]Leisure-time physical activity. IQR: Interquartile range; MET: Metabolic equivalent.

Table 2 Associations between genetic polymorphisms and colorectal adenomas n (%)

Genotype	Cases	Controls	Crude OR (95%CI)	Adjusted[3] OR (95%CI)
*CYP1A1*2A*				
0[1]	174 (38.2)	388 (36.9)	1.00 (referent)	1.00 (referent)
1	219 (48.1)	508 (48.3)	0.96 (0.76-1.22)	0.97 (0.76-1.24)
2	62 (13.6)	156 (14.8)	0.89 (0.63-1.25)	0.86 (0.60-1.23)
*CYP1A1*2C*				
0[1]	281 (61.8)	611 (58.1)	1.00 (referent)	1.00 (referent)
1	152 (33.4)	389 (37.0)	0.85 (0.67-1.07)	0.81 (0.64-1.03)
2	22 (4.8)	52 (4.9)	0.92 (0.55-1.54)	0.94 (0.55-1.60)
GSTM1				
Non-null	200 (44.0)	506 (48.1)	1.00 (referent)	1.00 (referent)
Null	255 (56.0)	546 (51.9)	1.18 (0.95-1.47)	1.19 (0.94-1.49)
GSTT1				
Non-null	258 (56.7)	552 (52.5)	1.00 (referent)	1.00 (referent)
Null	197 (43.3)	500 (47.5)	0.84 (0.68-1.05)	0.87 (0.70-1.10)
GSTM1 + GSTT1				
0[2]	118 (25.9)	273 (26.0)	1.00 (referent)	1.00 (referent)
1	222 (48.8)	512 (48.7)	1.00 (0.77-1.31)	1.04 (0.79-1.37)
2 (both null)	115 (25.3)	267 (25.4)	1.00 (0.73-1.35)	1.04 (0.76-1.42)
NQO1 C609T				
CC	161 (35.4)	412 (39.2)	1.00 (referent)	1.00 (referent)
CT	220 (48.4)	489 (46.5)	1.15 (0.90-1.47)	1.18 (0.92-1.52)
TT	74 (16.3)	151 (14.4)	1.25 (0.90-1.75)	1.31 (0.93-1.84)

[1]Number of variant alleles; [2]Number of null genotypes; [3]Adjusted for age, hospital, rank in the Self-Defense Forces, body mass index, cigarette smoking, alcohol use, leisure-time physical activity and parental history of colorectal cancer.

Table 3 Associations between combinations of genetic polymorphisms and colorectal adenomas

Genotype 1	Genotype 2	n[1]	OR (95%CI)[2]	Interaction (P)
*CYP1A1*2A*	*GSTM1*			0.94
0[3]	Non-null	75/182	1.00 (referent)	
0	Null	99/206	1.20 (0.83-1.74)	
≥ 1	Non-null	125/324	0.96 (0.67-1.35)	
≥ 1	Null	156/340	1.12 (0.80-1.58)	
*CYP1A1*2A*	*GSTT1*			0.08
0[3]	Non-null	92/210	1.00 (referent)	
0	Null	82/178	1.14 (0.79-1.65)	
≥ 1	Non-null	166/342	1.15 (0.83-1.58)	
≥ 1	Null	115/322	0.85 (0.61-1.19)	
*CYP1A1*2A*	*GSTM1 + GSTT1*			0.44
0[3]	0[4]	41/102	1.00 (referent)	
0	1	85/188	1.19 (0.75-1.88)	
0	2 (both null)	48/98	1.35 (0.81-2.27)	
≥ 1	0	77/171	1.17 (0.74-1.86)	
≥ 1	1	137/324	1.12 (0.73-1.72)	
≥ 1	2 (both null)	67/169	1.03 (0.64-1.66)	
*CYP1A1*2A*	*NQO1* C609T			0.09
0[3]	CC	63/133	1.00 (referent)	
0	CT + TT	111/255	0.92 (0.63-1.36)	
≥ 1	CC	98/279	0.73 (0.49-1.07)	
≥ 1	CT + TT	183/385	1.02 (0.71-1.47)	
*CYP1A1*2C*	*GSTM1*			0.77
0[3]	Non-null	127/294	1.00 (referent)	
0	Null	154/317	1.16 (0.86-1.55)	
≥ 1	Non-null	73/212	0.79 (0.56-1.12)	
≥ 1	Null	101/229	0.98 (0.71-1.36)	
*CYP1A1*2C*	*GSTT1*			0.24
0[3]	Non-null	154/321	1.00 (referent)	
0	Null	127/290	0.97 (0.73-1.31)	
≥ 1	Non-null	104/231	0.93 (0.68-1.27)	
≥ 1	Null	70/210	0.69 (0.49-0.97)	
*CYP1A1*2C*	*GSTM1 + GSTT1*			0.37
0[3]	4	74/156	1.00 (referent)	
0	1	133/303	0.96 (0.67-1.37)	
0	2 (both null)	74/152	1.13 (0.75-1.69)	
≥ 1	0	44/117	0.79 (0.50-1.25)	
≥ 1	1	89/209	0.92 (0.63-1.35)	
≥ 1	2 (both null)	41/115	0.72 (0.45-1.15)	
*CYP1A1*2C*	*NQO1* C609T			0.05
0[3]	CC	102/220	1.00 (referent)	
0	CT + TT	179/391	0.99 (0.73-1.34)	
≥ 1	CC	59/192	0.61 (0.42-0.91)	
≥ 1	CT + TT	115/249	0.98 (0.71-1.37)	

[1]Number of cases/controls; [2]Adjusted for age, hospital, rank in the Self-Defense Forces, body mass index, cigarette smoking, alcohol use, leisure-time physical activity and parental history of colorectal cancer; [3]Number of variant alleles; [4]Number of null genotypes.

tions of the *CYP1A1* polymorphisms and the *GST* or *NQO1* polymorphism (Table 3). As regards *CYP1A1*2A*, *CYP1A1*2C* and *NQO1*, the homozygous variant genotype was combined with the heterozygous genotype because variant homozygotes were relatively few. A borderline significant interaction was observed for the combination of *CYP1A1*2C* and *NQO1* (P = 0.051). The OR associated with *CYP1A1*2C* was significantly lower than unity among individuals with the *NQO1* 609CC genotype. Although the interaction was far from statistical signifi-

cance, the OR for individuals carrying the *CYP1A1*2C* allele and *GSTT1* null genotype significantly decreased compared with those who had neither the *CYP1A1*2C* allele nor the *GSTT1* null genotype.

Smoking was positively associated with colorectal adenomas; the multivariate-adjusted ORs for the smoking categories of 0, 1-399, 400-799 and ≥ 800 cigarette-years were 1.00 (referent), 1.18 (95%CI: 0.82-1.71), 2.11 (95%CI: 1.58-2.82) and 2.11 (95%CI: 1.47-3.03), respectively. However, smoking did not modify the associations of the *CYP1A1*, *GSTM1*, *GSTT1* and *NQO1* polymorphisms with colorectal adenomas (Table 4). The ORs associated with heavy smoking were consistently increased,

Table 4 Associations between genetic polymorphisms and colorectal adenomas with stratification by smoking category

Genotype	< 20 pack-years		≥ 20 pack-years		Interaction (*P*)
	n^1	OR (95%CI)^2	n^1	OR (95%CI)^2	
*CYP1A1*2A*					
0^3	59/207	1.00 (referent)	115/181	2.22 (1.52-3.24)	0.45
≥ 1	100/346	1.05 (0.73-1.53)	181/318	1.95 (1.37-2.76)	
*CYP1A1*2C*					
0^3	106/329	1.00 (referent)	175/282	1.90 (1.41-2.56)	0.59
≥ 1	53/224	0.76 (0.52-1.11)	121/217	1.65 (1.20-2.27)	
GSTM1					
Non-null	69/264	1.00 (referent)	131/242	1.99 (1.41-2.82)	0.96
Null	90/289	1.19 (0.83-1.71)	165/257	2.35 (1.68-3.29)	
GSTT1					
Non-null	89/268	1.00 (referent)	169/284	1.74 (1.27-2.38)	0.26
Null	70/285	0.74 (0.52-1.06)	127/215	1.69 (1.21-2.36)	
GSTM1 + GSTT1					
0^4	39/134	1.00 (referent)	79/139	1.87 (1.18-2.96)	0.63
1	80/264	1.03 (0.67-1.61)	142/248	1.91 (1.26-2.91)	
2 (both null)	40/155	0.89 (0.54-1.48)	75/112	2.14 (1.34-3.43)	
NQO1 C609T					
CC	52/221	1.00 (referent)	109/191	2.23 (1.51-3.29)	0.46
CT + TT	107/332	1.35 (0.93-1.97)	187/308	2.51 (1.75-3.60)	

^{1}Number of cases/controls; ^{2}Adjusted for age, hospital, rank in the Self-Defense Forces, body mass index, cigarette smoking, alcohol use, leisure-time physical activity and parental history of colorectal cancer; ^{3}Number of variant alleles; ^{4}Number of null genotypes.

regardless of genotype of the polymorphism. There was no measurable effect modification of smoking even regarding the combination of the genetic polymorphisms of the phase I and phase II enzymes (Table 5). The OR was lowest for the combination of the *CYP1A1*2C* and *NQO1* 609CC genotypes among the four composite genotypes in each stratum of smoking. The OR varied according to the combination of *CYP1A1*2C* and the composite genotypes of *GSTM1* and *GSTT1* within each stratum of smoking. The OR for the combination of the *CYP1A1*2C* allele and the non-null genotypes of both *GSTM1* and *GSTT1* was significantly lower than unity in the category of < 20 pack-years, and the OR increased significantly among heavy smokers without the *CYP1A1*2C* allele who had null genotypes for both *GSTM1* and *GSTT1*.

DISCUSSION

According to recent meta-analyses[32,33], the *CYP1A1*2C* polymorphism, but not *CYP1A1*2A* polymorphism, was significantly associated with a modest increase in the risk of colorectal cancer. Neither *CYP1A1*2A* nor *CYP1A1*2C* polymorphism was related to colorectal adenomas individually in the present study. The findings are consistent with the previous observation regarding colorectal adenomas[16,26]. Further investigation is needed to clarify whether the associations with the *CYP1A1*2C* polymorphism is differential for colorectal cancer and adenomas.

The null effects of the *GSTM1* and *GSTT1* polymorphisms in the present study are in agreement with previous observations regarding colorectal adenomas[23,34]. Some of the previous studies showed an increased risk

of colorectal cancer among individuals with the *GSTM1* null genotype[11,21,22], the *GSTT1* null genotype[21], or both the *GSTM1* and *GSTT1* null genotypes[21,22]. However, these findings were not replicated in other studies on colorectal cancer[14,15,35]. Previous studies found no effect of smoking on the association with *GSTM1* and *GSTT1*, either singly or in combination, in relation to colorectal adenomas[23] and cancer[15,22,33]. The *GSTM1* and *GSTT1* non-null genotypes as determined by gel electrophoresis include heterozygous genotypes (*i.e.*, one active and one inactive allele). One study differentiated the heterozygous genotype from the homozygous non-null genotype for *GSTM1* and *GSTT1* by TaqMan assay[36]. Both heterozygous and homozygous null genotypes of *GSTM1* were associated with a decreased risk of colorectal adenomas irrespective of smoking status, while adenoma risk was increased in association with both heterozygous and homozygous null genotypes of *GSTT1* among eversmokers, but not among never-smokers[36]. However, it is unclear whether heterozygosity in either *GSTM1* or *GSTT1* affects enzyme activity[18].

Few studies have addressed the combined effect of the *CYP1A1* polymorphisms and the *GSTM1* and/or *GSTT1* null genotype in relation to colorectal adenomas and cancer. The combination of *CYP1A1*2A* and *GSTM1* null genotype was shown to be unrelated to colorectal adenomas[16]. There was no interaction between the two *CYP1A1* polymorphisms and either *GSTM1* or *GSTT1* null genotype on the risk of colorectal cancer[14]. A Japanese study showed a decreased risk of colorectal cancer for the combination of *CYP1A*2C* and *GSTT1* non-null genotype[15]. The present study indicated a decreased risk of colorectal adenomas for the combination

Table 5 Associations between combinations of genetic polymorphisms and colorectal adenomas with stratification by smoking category

Genotype 1	Genotype 2	< 20 pack-years		≥ 20 pack-years		Interaction (P)
		n[1]	OR (95%CI)[2]	n[1]	OR (95%CI)[2]	
CYP1A1*2A	GSTM1					0.44
0[3]	Non-null	25/87	1.00 (referent)	50/95	1.78 (1.00-3.14)	
0	Null	34/120	0.94 (0.52-1.69)	65/86	2.53 (1.45-4.43)	
≥ 1	Non-null	44/177	0.86 (0.49-1.50)	81/147	1.82 (1.07-3.10)	
≥ 1	Null	56/169	1.19 (0.69-2.05)	100/171	1.92 (1.14-3.22)	
CYP1A1*2A	GSTT1					0.56
0[3]	Non-null	29/98	1.00 (referent)	63/112	1.94 (1.15-3.28)	
0	Null	30/109	0.95 (0.53-1.71)	52/69	2.53 (1.44-4.44)	
≥ 1	Non-null	60/170	1.26 (0.75-2.11)	106/172	2.07 (1.27-3.39)	
≥ 1	Null	40/176	0.81 (0.47-1.40)	75/146	1.70 (1.02-2.82)	
CYP1A1*2A	GSTM1 + GSTT1					0.40
0[3]	0[4]	14/42	1.00 (referent)	27/60	1.34 (0.62-2.89)	
0	1	26/101	0.76 (0.36-1.62)	59/87	2.02 (1.00-4.07)	
0	2 (both null)	19/64	0.87 (0.39-1.94)	29/34	2.46 (1.11-5.46)	
≥ 1	0	25/92	0.83 (0.39-1.78)	52/79	1.89 (0.93-3.86)	
≥ 1	1	54/163	1.01 (0.51-2.01)	83/161	1.52 (0.78-2.98)	
≥ 1	2 (both null)	21/91	0.73 (0.33-1.59)	46/78	1.66 (0.81-3.40)	
CYP1A1*2A	NQO1 C609T					0.76
0[3]	CC	20/73	1.00 (referent)	43/60	2.55 (1.34-4.86)	
0	CT + TT	39/134	1.04 (0.56-1.94)	72/121	2.15 (1.20-3.86)	
≥ 1	CC	32/148	0.81 (0.43-1.54)	66/131	1.69 (0.94-3.04)	
≥ 1	CT + TT	68/198	1.29 (0.72-2.29)	115/187	2.23 (1.28-3.89)	
CYP1A1*2C	GSTM1					0.78
0[2]	Non-null	47/150	1.00 (referent)	80/144	1.74 (1.13-2.69)	
0	Null	59/179	1.04 (0.67-1.63)	95/138	2.15 (1.40-3.31)	
≥ 1	Non-null	22/114	0.63 (0.36-1.11)	51/98	1.59 (0.98-2.57)	
≥ 1	Null	31/110	0.94 (0.55-1.58)	70/119	1.77 (1.13-2.78)	
CYP1A1*2C	GSTT1					0.11
0[2]	Non-null	60/151	1.00 (referent)	94/170	1.39 (0.93-2.07)	
0	Null	46/178	0.66 (0.42-1.03)	81/112	1.80 (1.18-2.75)	
≥ 1	Non-null	29/117	0.65 (0.39-1.09)	75/114	1.62 (1.06-2.48)	
≥ 1	Null	24/107	0.59 (0.34-1.01)	46/103	1.06 (0.66-1.69)	
CYP1A1*2C	GSTM1 + GSTT1					0.22
0[2]	0[4]	29/71	1.00 (referent)	45/85	1.26 (0.71-2.23)	
0	1	49/159	0.73 (0.42-1.26)	84/144	1.41 (0.84-2.37)	
0	2 (both null)	28/99	0.69 (0.38-1.28)	46/53	2.03 (1.12-3.69)	
≥ 1	0	10/63	0.39 (0.17-0.87)	34/54	1.46 (0.79-2.72)	
≥ 1	1	31/105	0.75 (0.41-1.36)	58/104	1.31 (0.76-2.26)	
≥ 1	2 (both null)	12/56	0.53 (0.25-1.15)	29/59	1.09 (0.58-2.05)	
CYP1A1*2C	NQO1 C609T					0.34
0[2]	CC	34/127	1.00 (referent)	68/93	2.64 (1.60-4.36)	
0	CT + TT	72/202	1.33 (0.83-2.12)	107/189	2.10 (1.33-3.31)	
≥ 1	CC	18/94	0.75 (0.39-1.41)	41/98	1.41 (0.83-2.41)	
≥ 1	CT + TT	35/130	1.03 (0.60-1.77)	80/119	2.47 (1.53-4.00)	

[1]Number of cases/controls; [2]Adjusted for age, hospital, rank in the Self-Defense Forces, body mass index, cigarette smoking, alcohol use, leisure-time physical activity and parental history of colorectal cancer; [3]Number of variant alleles; [4]Number of null genotypes.

of *CYP1A1*2C* and *GSTT1* null genotype, although the interaction was not significant. Inconsistent findings regarding the combination of *CYP1A1*2C* and *GSTT1* are probably ascribed to random fluctuation, although further studies are needed.

The risk of adenomas was lowest for the combination of *CYP1A1*2C* and the *GSTM1* and *GSTT1* non-null genotypes among never-smokers or light smokers, and an increased risk associated with heavy smoking was most evident among men without *CYP1A1*2C* who had the *GSTM1* and *GSTT1* null genotypes. Caution is required in interpreting the findings because of the small number of each combination of the three polymorphisms when stratified by smoking. Nonetheless, further investigation is warranted because the association of the three-polymorphism combination with colorectal cancer or adenomas has not been investigated previously.

The present study did not corroborate an increased risk of colorectal adenomas associated with the combination of *CYP1A1*2C* and *NQO1 609T* alleles reported among non-Hispanic whites[26], but showed a decreased risk among men harboring the *CYP1A1*2C* allele and the *NQO1* 609CC genotype. In that study[26], however, there were only a few subjects with both the *CYP1A1*2C* and *NQO1 609T* alleles (12 cases and 26 controls), accounting for only 4.0% of the cases and 1.8% of the controls,

because these variant alleles are much less frequent in Caucasians than in Asians[10,31]. In a study of Caucasians[26], the OR for the combination of *CYP1A1*2C* allele and *NQO1* 609CC genotype was 0.6 (95%CI: 0.3-1.2), as compared with the same referent combination as used in the present study. This finding is thus compatible with the present observation. It should be noted that the borderline significant interaction between *CYP1A1*2C* and *NQO1* C609T resulted from the eight statistical tests (Table 3). The probability of detecting at least one statistically significant result is 0.34, even when none of the eight interactions are present. The combined effect of these two polymorphisms requires careful interpretation, but requires further studies for mechanistic plausibility. Exposure to activated carcinogens may be lowered in individuals with both the *CYP1A1*2C* allele and the *NQO1* 609CC genotype for faster activation and detoxification.

The advantages of our study were that colonoscopy was done non-selectively in a defined population and that the absence of polyp lesions was confirmed in the control subjects by complete colonoscopy. Ethnic homogeneity was another advantage. There were several limitations. Statistical adjustment was not made for dietary factors because such data were not available. Only men were included, and the findings may not be applicable to women. Smoking is much less prevalent in women than in men in Japan[15]. The study subjects were not representative of Japanese men in the general population, but selection was unlikely to have occurred with regard to the genetic polymorphisms under study. The allele and genotype frequencies in the present study population were almost the same as those observed among Japanese individuals elsewhere. Among community controls ($n = 778$) in a Japanese case-control study of colorectal cancer[15], the frequencies of the *CYP1A1*2A*, *CYP1A1*2C* and *NQO1* 609T alleles were 37%, 23% and 38%, respectively, and the *GSTM1* and *GSTT1* null genotypes accounted for 54% and 44%, respectively. In a random sample of approximately 300 Japanese adults[37], the frequencies of the *CYP1A1*2C* and *NQO1* 609T alleles were 21% and 38%, respectively. Finally, the study size was not sufficiently large to detect a moderately increased risk for the variant homozygote. With two-sided $a = 0.05$, the power of detecting a 1.5-fold increase in the risk for the variant homozygote in the additive model was 0.66 for *CYP1A1*2A*, 0.39 for *CYP1A1*2C* and 0.65 for *NQO1* C609T.

In conclusion, the combination of *CYP1A1*2C* and *NQO1* 609CC genotype was associated with a decreased risk of colorectal adenomas, regardless of smoking status, in Japanese men. Future studies are needed to clarify the biological mechanisms involved.

COMMENTS

Background
Cigarette smoking has consistently been related to an increased risk of colorectal adenomas, and possibly of colorectal cancer, but the biological mechanisms remain unknown.

Research frontiers
Tobacco smoke contains various types of carcinogens, which are activated by phase I enzymes and/or detoxified by phase II enzymes. It is a matter of interest whether or not functional genetic polymorphisms of the metabolic enzymes are related to colorectal adenomas and cancer.

Innovations and breakthroughs
Few studies have examined the association of genetic polymorphisms of phase I and phase II enzymes in combination with colorectal adenomas or cancer. Adenoma risk differed by the combination of genetic polymorphisms of CYP1A1 (phase I enzyme) and NAD(P)H-quinone oxidoreductase 1 (NQO1) (phase II enzyme), and the association was not modified by smoking.

Applications
The findings confer clues to understanding the biological mechanisms of the association between smoking and colorectal adenomas and cancer.

Terminology
CYP1A1 is responsible for bioactivation of tobacco carcinogens. *CYP1A1*2A* and *CYP1A1*2C* polymorphisms are putatively functional, and have been related to increased risk of tobacco-related cancers; Glutathione *S*-transferases are involved in detoxification of chemical carcinogens, and individuals with the *GSTM1* and/or *GSTT1* null genotype may be susceptible to increased risk of cancer; NQO1 also acts as a phase II enzyme, and the 609C>T polymorphism results in loss of NQO1 activity.

Peer review
This was a good study. The study subjects were all male officials in the Self-Defense Forces. Besides smoking, they may have risk factors, such as alcohol drinking and dietary habits, similar to those in the general population.

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