RESEARCH COMMUNICATION

Association of CYP1A1, CYP1A2, GSTM1 and NAT2 Gene Polymorphisms with Colorectal Cancer and Smoking

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Abstract

We investigated CYP1A1*2A, CYP1A1*2C, CYP1A2*1C, CYP1A2*1F, GSTM1 and NAT2 gene polymorphisms, involving enzymes which metabolize many carcinogens, with reference to colorectal cancer risk. The distribution of these genotypes was not associated with risk overall. However, the CYP1A1*2A T/C genotype showed a significant association with colorectal cancer risk in never-smokers (odds ratio [OR], 3.06; 95% confidence interval [95% CI], 1.11-8.40; p = 0.030). The risk of the NAT2 rapid genotype in never-smokers was also statistically significantly increased (OR, 5.38; 95%CI, 1.80-16.1; p = 0.003). Furthermore, the joint effects of NAT2 rapid plus other genotypes were associated with colorectal cancer overall (OR, 3.12; 95%CI, 1.15-8.51; p = 0.026, for NAT2 rapid plus combined CYP1A1*2C Ile/Val and Val/Val, OR, 3.25; 95%CI, 1.09-9.74; p = 0.035, for NAT2 rapid plus CYP1A2*1C G/G, and OR, 4.20; 95%CI, 1.09-16.1; p = 0.037, for NAT2 rapid plus GSTM1 null, respectively). In never-smokers, the joint effects of NAT2 rapid plus other genotypes were remarkable (OR, 15.9; 95%CI, 1.87-135.8; p = 0.011, for NAT2 rapid plus combined CYP1A1*2A T/C and C/C, OR, 5.71; 95%CI, 1.49-21.9; p = 0.011, for NAT2 rapid plus combined CYP1A1*2C Ile/Val and Val/Val, and OR, 9.14; 95%CI, 2.05-40.7; p = 0.004, for NAT2 rapid plus CYP1A2*1F A/A, respectively). The joint effect of CYP1A2*1F A/A plus CYP1A2*1C G/G genotypes was also increased in never-smokers (OR, 6.16; 95%CI, 1.26-30.1; p = 0.025). Our findings suggest that the CYP1A1*2A T/C and NAT2 rapid genotypes is associated with colorectal cancer susceptibility without smoking exposure. These results also indicate that the NAT2 in combination with CYP1A1*2C, CYP1A2*1C, or GSTM1 genotypes may strongly confer susceptibility to colorectal cancer. In particular, the combination of NAT2 plus CYP1A1*2A, CYP1A1*2C, or CYP1A2*1F genotypes, and that of CYP1A2*1F plus CYP1A2*1C genotype may define a group of persons who are genetically susceptible to colorectal cancer in never smokers.

Key Words:Gene polymorphism - colorectal cancer -CYP1A1 - CYP1A2 - GSTM1 - NAT2

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Introduction

Colorectal cancer (CRC) is associated with genetic and environmental factors such as cigarette smoking, and cooked meats and fish at high temperature (Giovannucci, 2001; Pisani et al.,2002).These factors result in the formation of carcinogenic compounds including polycyclic aromatic hydrocarbons (PAH), arylamines, and heterocyclic amines (HCA)(Sugimura, 2000). The cytochrome P450 (CYP) enzymes are a critical importance for the metabolism of these carcinogens by N-oxidation. CYP1A1 is a key enzyme to the metabolic activation of PAHs. Previous reports have shown that two CYP1A1 gene polymorphisms, the MspI polymorphism located in the 3'-flanking region of the gene (CYP1A1*2A: MspI) and the Ile-Val polymorphism at amino acid residue 462 in the heme binding region of CYP1A1 protein (CYP1A1*2C: Ile462Val), are associated with susceptibility to several cancers, especially lung cancer (available at www.imm.ki.se/CYPalleles/cyp1a1.htm) (Kawajiri et al.,1990; Hayashi et al.,1991). It revealed that these polymorphisms were associated with increased enzyme activity to activate carcinogens. CYP1A2 is an important enzyme to the metabolic activation of HCAs. Two polymorphisms of the CYP1A2 gene, CYP1A2*1C (3858G→A) and CYP1A2*1F (164A→C), have been

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examined to associate with reduced enzyme activity (available at www.imm.ki.se/CYPalleles/cyp1a2.htm) (Nakajima et al., 1999; Sachse et al., 2003). It has been reported that CYP1A2*1F mainly increased the risk of colorectal cancer (Moonen et al., 2005).

In addition, the glutathione S-transferases (GSTs), which mainly is expressed in the colon, play an important role in the detoxification of carcinogens to reduced glutathione. The GSTM1-null has no enzyme activity for catalyzing the activated carcinogens by detoxification (Cotton et al., 2000). N-acetyltransferase 2 (NAT2) catalyze the metabolism of various aromatic amines and carcinogens, involving not only in detoxification by N-acetylation and activation by O-acetylation (Hein, 1988). NAT2 polymorphisms are associated with leading to either slow or rapid acetylation for different cancers (available at http://louisville.edu/medschool/pharmacology/NAT2.html) (Hein, 2002).

Recently, we reported that genetic polymorphisms of NAT2 and CYP1A2 in metabolic processes contributed to lung cancer susceptibility in relation to smoking status in Japanese population (Osawa et al., 2007). In this study, we also focused on six gene polymorphisms, CYP1A1*2A, CYP1A1*2C, CYP1A2*1C, CYP1A2*1F, GSTM1 and NAT2, which play an interactive role in the risk for colorectal cancer incidence in relation to smoking. Additionally, we assessed the joint effects of these gene polymorphisms with smoking.

Materials and Methods

Study Subjects

In this small case-control study, the 66 colorectal cancer patients (39 with colon, 23 with rectal and 4 with unknown) were recruited between October 2003 and March 2005 at the Kobe Medical Center and Kobe Rosai Hospital in Kobe city, Japan. The controls were included in a previous study that investigated the genetic polymorphisms of metabolic enzymes (Osawa et al., 2007). The controls were 121 individuals, who had not currently or previously diagnosed with cancer, and were recruited between November 2002 and March 2003. Informed consent was obtained and detailed exposure data on smoking were collected by a personal interview. The study design was approved by the Ethics Review Committee on Genetic and Genomic Research, Kobe University Graduate School of Medicine. Informed consent was obtained from all cases and controls, and all samples were coded after collection of blood and data. The amount of smoke exposure was calculated as pack-years, the product of number of years an individual smoked and the average number of cigarettes smoked per day, converting into a standard pack of 20 cigarettes.

Table 1. Characteristics of Colorectal Cancer Cases and Control Subjects

Item	Cases		Controls		P-value
	n	%	n	%	
Number	66		121		
Gender					
males	36	54.5	73	60.3	0.874[a]
females	26	39.4	48	39.7	
unknown	4	6.1	0	0.0	
Age					
~64	21	31.8	51	42.1	>0.999[b]
65~69	12	18.2	28	23.1	
70~74	13	19.7	21	17.4	
75~	16	24.2	21	17.4	
unknown	4	6.1	0	0.0	
Mean ±S.D.	67.3 ±10.9		67.3 ±6.6		
Smoking status					
Never	35	53.0	55	45.5	0.002[b]
Ever	26	39.4	61	50.4	
unknown	5	7.6	5	4.1	
Mean ±S.D.	12.8 ±19.4		25.9 ±35.3		
Subsites					
colon	39	59.1			
rectal	23	34.8			
unknown	4	6.1			

[a]: x^2 analysis [b]: Student's T-test

Genotyping

Genomic DNA to be used was isolated for the previous study (Osawa et al., 2007). The genotypes of CYP1A1*2A (Hayashi et al., 1991; Tsuchiya et al., 2002), CYP1A1*2C (Tsuchiya et al., 2002), CYP1A2*1C (Nakajima et al.,1999; Sachse et al., 2003), CYP1A2*1F (Christiansen et al., 2000), GSTM1 (Comstock et al., 1990) and NAT2 (Abe et al., 1993) were determined by PCR-RFLP analysis as previously described. PCR was performed with a programmable thermocycler PC-701 (Astec, Fukuoka, Japan). The presence or absence of the GSTM1 gene was detected by means of genomic PCR amplification of the GSTM1 gene segment with an internal control for three times.

Statistical Analysis

Hardy-Weinberg equilibrium was tested using the goodness-of- fit Chi-square test to compare the observed genotype frequencies with the expected genotype frequencies among the control subjects. Associations were expressed as odd ratios (OR) with 95% confidence interval (95%CI) and $p < 0.05$ was considered statistically significant. Logistic regression analysis was performed to assess the association between each genotypes and colorectal cancer. ORs, which were computed to estimate the association between certain genotypes and colorectal cancer, were adjusted for age, gender, and smoking habit. The subjects were divided into two groups according to pack-years of smoking: never-smokers (pack-years = 0), and ever-smokers (pack-years > 0). The gene-smoking interaction, adjusted for age and gender was also computed. The statistical analysis was performed with SPSS software packages (version 14.0 for Windows; SPSS Japan Inc., Tokyo, Japan). Genotypes of CYP genes referred to be phenotypes with low activity are considered as the reference (CYP1A1*2A: T/T, CYP1A1*2C:Ile/Ile, CYP1A2*1C: A/A and A/G, CYP1A2*1F: C/C and C/A) (Tsuchiya et al., 2002; MacLeod et al., 1998; Sachse et al., 1999, Osawa et al.,2007). Individuals with the GSTM1 allele were designated GSTM1-present and those with homozygous deletion of the GSTM1 allele were designated GSTM1-null (Cotton et al., 2000). We

Table 2. Genotypes Distribution in Colorectal Cancer and Allele Frequency

Genotype		Cases		Controls				Allele frequency		
		n	%	n	%	OR (95%CI)[a]	P-value		Cases	Controls
CYP1A1*2A										
	T/T	20	30.3	49	40.5	1.00		T	0.576	0.628
	T/C	36	54.5	54	44.6	1.42 (0.70-2.86)	0.331	C	0.424	0.372
	C/C	10	15.2	18	14.9	0.87 (0.31-2.47)	0.796			
	T/C, C/C	46	69.7	72	59.5	1.27 (0.65-2.49)	0.479			
CYP1A1*2C										
	Ile/Ile	34	51.5	79	65.3	1.00		Ile	0.720	0.806
	Ile/Val	27	40.9	37	30.6	1.54 (0.78-3.04)	0.210	Val	0.280	0.194
	Val/Val	5	7.6	5	4.1	1.99 (0.41-9.63)	0.393			
	Ile/Val, Val/Val	32	48.5	42	34.7	1.58 (0.82-3.05)	0.169			
CYP1A2*1C										
	A/A	8	12.1	8	6.6	1.00		G	0.742	0.743
	A/G	18	27.3	42	34.7	0.40 (0.11-1.43)	0.160	A	0.258	0.257
	G/G	40	60.6	63	52.1	0.75 (0.23-2.45)	0.637			
	unknown	0	0.0	8	6.6					
	A/A, A/G	26	39.4	50	41.3	1.00				
	G/G	40	60.6	63	52.1	1.53 (0.78-2.99)	0.213			
CYP1A2*1F										
	C/C	6	9.1	17	14.0	1.00		A	0.656	0.613
	C/A	32	48.5	52	43.0	1.39 (0.47-4.08)	0.550	C	0.344	0.387
	A/A	26	39.4	42	34.7	1.29 (0.43-3.88)	0.651			
	unknown	2	3.0	10	8.3					
	C/C, C/A	38	57.6	69	57.0	1.00				
	A/A	26	39.4	42	34.7	0.99 (0.51-1.94)	0.979			
GSTM1										
	present	30	45.5	59	48.8	1.00				
	null	36	54.5	62	51.2	1.08 (0.57-2.07)	0.807			
NAT2[b]										
	slow	2	3.0	9	7.4	1.00		1	0.848	0.756
	intermediate	16	24.2	41	33.9	3.61 (0.41-31.5)	0.246	2	0.121	0.070
	rapid	48	72.7	71	58.7	5.83 (0.70-48.3)	0.102	3	0.030	0.149
								4	0.000	0.025
	intermediate, slow	18	27.3	50	41.3	1.00				
	rapid	48	72.7	71	58.7	1.88 (0.94-3.78)	0.075			

[a]OR adjusted for gender, age, smoking habit [b]Rapid acetylators were defined as individuals with homozygote 1allele. Intermediate acetylators were defined as individuals with 1 allele in combination with 2, 3or 4 allele. Slow acetylators were defined as individuals with combination with 2, 3 or 4 allele.

categorized the NAT2 genotypes into rapid, intermediate, and slow acetylators as previously described (Abe et al., 1993; Osawa et al., 2007). For NAT2 genotypes, NAT2-rapid is considered as the reference in colorectal cancer (Hein, 2002). Joint effects between two genotypes were studied by creating dummy variables, each representing the combination of two genotypes with the putative low risk combination as reference category.

Results

We show the characteristics of colorectal cancer in Table 1, including 66 cases and 121 controls. There was no difference in the gender distribution (p = 0.874) between males (case, 54.5%; controls, 60.3%) and females (case, 39.4%; controls, 39.7%). There was no difference in the average ages (± SD) between cases (67.3 ± 10.9 years) and controls (67.3 ± 6.6 years) (p > 0.999). Never-smokers comprised 53.0% of cases and 45.5% of controls and ever-smokers comprised 39.4% of cases and 50.4% of controls. There was a significant difference in the average pack-years (± SD) between cases (12.8 ± 19.4) and controls (25.9 ± 35.3) (p = 0.002). Subsites of the cases were: colon, 59.1%; rectal, 34.8% and unknown, 6.1%.

Genotyping results of CYP1A1*2A, CYP1A1*2C, CYP1A2*1C, CYP1A2*1F, GSTM1 and NAT2 adjusted for gender, age, and smoking habit along with allele frequencies are shown in Table 2. The allele frequencies of the four gene polymorphisms in controls were consistent with the Hardy-Weinberg equilibrium. The distribution of these genotypes was no association with colorectal cancer in overall.

The adjusted odds ratios (OR) for the joint effect of tobacco exposure (pack-years) and six polymorphisms, adjusted for gender and age, are shown in Table 3. In never-smokers, the OR for CYP1A1*2A T/C genotype compared with T/T genotype had a 3.06-fold increased risk of colorectal cancer (95% confidence interval [95% CI], 1.11-8.40; p = 0.030), whereas the OR for C/C genotype was no significantly increased (OR, 1.21; 95%CI, 0.29-5.01; p = 0.791). The risk of combined CYP1A1*2A T/C

and C/C genotypes compared with T/T genotype was a borderline significant (OR, 2.46; 95%CI, 0.94-6.44; p = 0.067). The OR of CYP1A2*1C A/G genotype compared with A/A genotype had a 0.07-fold decreased risk of colorectal cancer (95% CI, 0.01-0.75; p = 0.028), whereas for G/G genotype was no significant (OR, 0.20; 95%CI, 0.02-2.00; p = 0.173). The risk of combined CYP1A2*1C G/G genotype compared with A/A and A/G genotypes were not also significant (OR, 1.93; 95%CI, 0.78-4.81; p = 0.157). The risk of CYP1A2*1F A/A genotype compared with C/C genotype had a borderline significant (OR, 8.36; 95% CI, 0.94-74.7; p = 0.057), whereas for C/A genotype was no significant (OR, 5.46; 95%CI, 0.63-47.6; p = 0.124). The risk of combined CYP1A2*1F A/A genotype compared with C/C and C/A genotypes were not also significant (OR, 1.92; 95%CI, 0.78-4.72; p = 0.158).The risk of NAT2 rapid genotype compared with intermediate-slow genotype had a 5.38-fold increased risk of colorectal cancer (95% CI, 1.80-16.1; p = 0.003). In ever-smokers, the distribution of these genotypes was no association with colorectal cancer risk. We found no distribution of CYP1A1*2C or GSTM1 gene polymorphisms for colorectal cancer in never- and ever-smokers. These results indicated that the CYP1A1*2A T/C genotype and NAT2 rapid genotype may have a significant effect for colorectal cancer among never-smokers in the six gene polymorphisms.

Furthermore, we evaluated the joint effects of the NAT2 and other genotypes for colorectal cancer risk in conjunction with cigarette smoking (Table 4). The risk of NAT2 rapid plus combined CYP1A1*2A T/C and C/C compared with NAT2 intermediate-slow plus CYP1A1*2A T/T genotypes was a borderline significant in overall (OR, 3.18; 95%CI, 0.95-10.6; p = 0.060) and remarkably increased association with colorectal cancer in never-smokers (OR, 15.9; 95%CI, 1.87-135.8; p = 0.011). The risk of NAT2 rapid plus combined CYP1A1*2C Ile/Val and Val/Val genotypes compared with NAT2 intermediate-slow plus CYP1A1*2C Ile/Ile genotypes were significantly associated with colorectal cancer in overall and increased in never-smokers (OR, 3.12; 95%CI, 1.15-8.51; p = 0.026 in overall, and OR, 5.71; 95%CI, 1.49-21.9; p = 0.011 in never-smokers, respectively). The risk of NAT2 rapid plus CYP1A2*1C G/G genotypes compared with NAT2 intermediate-slow plus combined CYP1A2*1C A/A and A/G genotypes were significantly associated with colorectal cancer in overall (OR, 3.25; 95%CI, 1.09-9.74; p = 0.035). The risk of

Table 3. Genotype Distribution in Relation to Smoking Status in Colorectal Cancer

Genotype		Never-smokers				Ever-smokers			
		cases n	controls n	OR (95%CI)[a]	P-value	cases n	controls n	OR (95%CI)[a]	P-value
CYP1A1*2A									
	T/T	8	24	1.00		12	23	1.00	
	T/C	23	21	3.06 (1.11-8.40)	0.030	11	30	0.68 (0.25-1.83)	0.443
	C/C	4	10	1.21 (0.29-5.01)	0.791	3	8	0.74 (0.16-3.39)	0.701
	T/C, C/C	27	31	2.46 (0.94-6.44)	0.067	14	38	0.69 (0.27-1.77)	0.440
CYP1A1*2C									
	Ile/Ile	20	36	1.00		14	40	1.00	
	Ile/Val	13	17	1.36 (0.54-3.41)	0.514	11	18	1.69 (0.64-4.50)	0.291
	Val/Val	2	2	2.35 (0.30-18.5)	0.419	1	3	1.11 (0.10-12.1)	0.933
	Ile/Val, Val/Val	15	19	1.45 (0.60-3.50)	0.411	12	21	1.62 (0.63-4.19)	0.319
CYP1A2*1C									
	A/A	4	1	1.00		2	7	1.00	
	A/G	8	23	0.07 (0.01-0.75)	0.028	7	17	1.43 (0.23-8.90)	0.699
	G/G	23	25	0.20 (0.02-2.00)	0.173	17	35	1.68 (0.31-9.12)	0.547
	A/A, A/G	12	24	1.00		9	24	1.00	
	G/G	23	25	1.93 (0.78-4.81)	0.157	17	35	1.29 (0.49-3.39)	0.610
CYP1A2*1F									
	C/C	1	9	1.00		5	6	1.00	
	C/A	18	28	5.46 (0.63-47.6)	0.124	12	21	0.65 (0.15-2.77)	0.562
	A/A	16	16	8.36 (0.94-74.7)	0.057	7	26	0.31 (0.07-1.32)	0.113
	C/C, C/A	19	37	1.00		17	27	1.00	
	A/A	16	16	1.92 (0.78-4.72)	0.158	7	26	0.41 (0.14-1.20)	0.105
GSTM1									
	present	20	26	1.00		8	29	1.00	
	null	15	29	0.58 (0.24-1.41)	0.226	18	32	1.93 (0.72-5.18)	0.193
NAT2									
	slow	0	5	1.00		1	4	1.00	
	intermediate	5	21	ND[b]		10	19	1.96 (0.19-20.2)	0.572
	rapid	30	29	ND		15	38	1.36 (0.14-13.4)	0.791
	intermediate, slow	5	26	1.00		11	23	1.00	
	rapid	30	29	5.38 (1.80-16.1)	0.003	15	38	0.76 (0.29-1.98)	0.572

[a]: OR adjusted for gender, age [b]: no data

Table 4. Interaction between NAT2 and Other Genotypes by Smoking Status

Combined genotype		Overall				Never-smokers				Ever-smokers			
		cases/controls				cases/controls				cases/controls			
		n	n	OR (95%CI)[a]	P-value	n	n	OR (95%CI)[b]	P-value	n	n	OR (95%CI)[b]	P-value
NAT2	CYP1A1*2A												
slow[c]	T/T	4	20	1.00		1	12	1.00		3	7	1.00	
rapid	T/C, C/C	32	42	3.18 (0.95-10.6)	0.060	23	17	15.9 (1.87-135.8)	0.011	6	22	0.62 (0.12-3.35)	0.581
NAT2	CYP1A1*2C												
slow	Ile/Ile	9	29	1.00		5	15	1.00		4	13	1.00	
rapid	Ile/Val, Val/Val	23	21	3.12 (1.15-8.51)	0.026	15	8	5.71 (1.49-21.9)	0.011	5	11	1.30 (0.27-6.30)	0.747
NAT2	CYP1A2*1C												
slow	A/A, A/G	7	24	1.00		0	14	1.00		5	10	1.00	
rapid	G/G	29	41	3.25 (1.09-9.74)	0.035	18	16	ND[d]		11	23	0.86 (0.23-3.18)	0.820
NAT2	CYP1A2*1F												
slow	C/C, C/A	10	27	1.00		3	18	1.00		7	8	1.00	
rapid	A/A	18	24	1.77 (0.66-4.71)	0.254	14	9	9.14 (2.05-40.7)	0.004	3	15	0.23 (0.05-1.13)	0.071
NAT2	GSTM1												
slow	present	4	23	1.00		2	13	1.00		1	9	1.00	
rapid	null	22	35	4.20 (1.09-16.1)	0.037	12	16	4.30 (0.80-23.2)	0.090	8	18	3.37 (0.35-32.6)	0.294

[a]OR adjusted for gender, age, smoking habit [b]OR adjusted for gender, age c: intermediate and slow d: no data

Table 5. Interaction between CYP1A2*1F and Other Genotypes by Smoking Status

Combined genotype		Overall				Never-smokers				Ever-smokers			
		cases/controls				cases/controls				cases/controls			
		n	n	OR (95%CI)[a]	P-value	n	n	OR (95%CI)[b]	P-value	n	n	OR (95%CI)[b]	P-value
CYP1A2*1F	CYP1A1*2A												
C/C, C/A	T/T	17	33	1.00		7	19	1.00		10	12	1.00	
A/A	T/C, C/C	23	30	1.14 (0.49-2.65)	0.756	15	12	3.19 (0.99-10.2)	0.051	5	18	0.32 (0.09-1.22)	0.095
CYP1A2*1F	CYP1A1*2C												
C/C, C/A	Ile/Ile	24	50	1.00		13	27	1.00		11	20	1.00	
A/A	Ile/Val,Val/Val	16	21	1.25 (0.52-2.98)	0.616	9	9	2.10 (0.66-6.65)	0.206	4	12	0.59 (0.15-2.36)	0.455
CYP1A2*1F	CYP1A2*1C												
C/C, C/A	A/A, A/G	9	26	1.00		4	15	1.00		3	9	1.00	
A/A	G/G	11	18	2.25 (0.70-7.20)	0.173	8	5	6.16 (1.26-30.1)	0.025	3	13	0.66 (0.10-4.17)	0.660
CYP1A2*1F	GSTM1												
C/C, C/A	present	17	40	1.00		10	19	1.00		6	17	1.00	
A/A	null	13	27	0.95 (0.37-2.45)	0.917	6	9	1.14 (0.30-4.31)	0.849	5	18	0.78 (0.20-3.03)	0.715

[a]OR adjusted for gender, age, smoking habit [b]OR adjusted for gender, age

NAT2 rapid plus CYP1A2*1F A/A genotypes compared with NAT2 intermediate-slow plus combined CYP1A2*1F C/C and C/A genotypes were specifically increased for colorectal cancer in never-smokers (OR, 9.14; 95%CI, 2.05-40.7; p = 0.004). The risk of NAT2 rapid plus GSTM1 null genotypes compared with NAT2 intermediate-slow plus GSTM1 present genotypes were significantly associated with colorectal cancer in overall (OR, 4.20; 95%CI, 1.09-16.1; p = 0.037). In ever-smokers, the distribution of combined NAT2 and other genotypes was no association with colorectal cancer.

In addition, we attempted to examine the joint effects of the CYP1A2*1F and other genotypes for colorectal cancer risk in conjunction with cigarette smoking (Table 5). The risk of CYP1A2*1F A/A plus combined CYP1A1*2A T/C and C/C genotypes compared with combined CYP1A2*1F C/C and C/A plus CYP1A1*2A T/T genotypes was a borderline significant in never-smokers (OR, 3.19; 95%CI, 0.99-10.2; p = 0.051). The risk of CYP1A2*1F A/A plus CYP1A2*1C G/G genotypes compared with combined CYP1A2*1F C/C and C/A plus combined CYP1A2*1C A/A and A/G genotypes was significantly increased association with colorectal cancer in never-smokers (OR, 6.16; 95%CI, 1.26-30.1; p = 0.025). The joint effects of CYP1A2*1F plus CYP1A1*2C or GSTM1 gene polymorphisms were found no association with colorectal cancer in overall and never-smokers. In ever-smokers, the distribution of combined CYP1A2*1F and other genotypes was no association with colorectal cancer. The joint effects among CYP1A1*2A, CYP1A1*2C, CYP1A2*1C and GSTM1 were also no association with colorectal cancer (data not shown). These results show that the combination of NAT2 rapid plus CYP1A1*2C, CYP1A2*1C, or GSTM1 genotypes is associated with the susceptibility to colorectal cancer. In particular, the combination of NAT2 rapid plus other genotypes or CYP1A2*1F A/A plus CYP1A2*1C G/G genotypes seems from the data to be remarkably increased association with colorectal cancer susceptibility in never-smokers.

Discussion

We found in overall that the risk of colorectal cancer was not significant in six gene polymorphisms. In never-smokers, the association of CYP1A1*2A and NAT2 polymorphisms for colorectal cancer risk was strongly increased compared with its in ever-smokers. The CYP1A1*2A polymorphism was associated with a significantly increased risk of colorectal cancer in Japanese, although other studies were not detected (Sivaraman et al., 1994; Slattery et al., 2004; Little et al., 2006). Our findings support that the heterozygote for the rare CYP1A1*2A allele are expected to be at greater colorectal cancer risk without exposed to cigarette smoking in Japanese. In our previous study, we observed that no overall association of NAT2 intermediate-slow acetylator genotypes to the lung cancer risk, but there was the increase risk with low smoking dose (Osawa et al., 2007). While, NAT2 rapid acetylator has a higher risk for colorectal cancer, explaining by the role of NAT2 in the O-acetylation to activation of N-hydroxy arylamines to potentially DNA-binding forms (Chen et al., 1998). These results indicate that NAT2 slow acetylator seems to be at higher risk for lung cancer, in which N-acetylation is a detoxification step such as aromatic amines, whereas NAT2 rapid acetylator seems to be at higher risk for colon cancers, in which N-acetylation is negligible and O-acetylation is an activation step for mutagents such as HCAs (Hein, 2002). We believe that the NAT2 rapid acetylator are increased in activating various carcinogens except tobacco mutagens.

Additionally, we detected that the gene-gene interaction between NAT2 and CYP1A1*2C, CYP1A2*1C, or GSTM1 polymorphisms was a significantly colorectal cancer risk in overall. In never-smokers, we found that interaction between NAT2 and CYP1A1*2A, CYP1A1*2C or CYP1A2*1F was strongly significant. Among previous studies, CYP1A1*2C allele and GSTM1 null has been associated with colorectal cancer risk (Sachse et al., 2002; Huang et al., 2006). The CYP1A2*1C G/G genotype caused a significant increase of CYP1A2 activity (Nakajima et al., 1999).The CYP1A2*1F A/A genotype represented a highly inducible genotype that was associated with an increased CYP1A2 activity (MacLeod et al., 1998; Sachse et al., 1999). It is also reported that the highest colorectal cancer risk was associated with both high CYP1A2 activity and rapid NAT2 activity (Lang et al., 1994; Le Marchand et al., 2001). The CYP1A1 and CYP1A2 enzyme increased the activated PAHs, HCAs and several arylamines, formed by cooking of meats or fish but little tobacco smoking by N-hydroxylation. The hydroxylated forms, can eventually covalent bound with DNA adduct-induced, are potent as proximate carcinogen. The hydroxylated forms may also activate by O-acetylation in NAT2 enzyme without the detoxification by GSTM1 enzyme, and are potent as ultimate carcinogen. Therefore, the rapid NAT2 activity in combination with high CYP1A1 activity, high CYP1A2 activity, or low GSTM1 activity, may be strongly increased the final hydroxylated forms as ultimate carcinogens.

Furthermore, we observed a significant association with CYP1A2*1F A/A plus CYP1A2*1C G/G genotypes in never-smokers. This finding indicate that the joint association of CYP1A2*1F A/A genotype and CYP1A2*1C G/G genotype strongly lead to increase the CYP1A2 enzyme activity (MacLeod et al., 1998; Sachse et al., 1999; Nakajima et al., 1999; Osawa et al., 2007).

In conclusion, we report that NAT2 rapid and CYP1A1*2A T/C genotypes appear to play an important role as a marker of genetic susceptibility to colorectal cancer in Japanese never-smokers. Furthermore, the NAT2 in combination with CYP1A1*2C, CYP1A2*1C, or GSTM1 genotypes may strongly confer susceptibility to colorectal cancer. In particular, the combination of NAT2 plus CYP1A1*2A, CYP1A1*2C, or CYP1A2*1F genotypes, and that of CYP1A2*1F plus CYP1A2*1C genotype may define a group of persons who are genetically susceptible to colorectal cancer in never smokers. These variations need to be further verified as predictive biomarkers in the large population.

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