RESEARCH COMMUNICATION

NAT2 and CYP1A2 Polymorphisms and Lung Cancer Risk in Relation to Smoking Status

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Abstract

We investigated the associations between lung cancer and the gene polymorphisms of the drug metabolizing enzymes, containing cytochrome P450 1A1 (CYP1A1), cytochrome P450 1A2 (CYP1A2), glutathione S-transferase class mu (GSTM1), and N-acetyltransferase 2 (NAT2). The study involved 113 lung cancer patients and 121 non-cancer controls divided into never, light and heavy smokers according to pack-years of smoking in Japanese by using PCR-RFLP. For light smokers, the lung cancer risk of NAT2 intermediate-slow was significantly increased [the adjusted odds ratio (OR): 10.9, 95% confidence intervals (95%CI): 1.75-67.5, P-value: 0.010]. Moreover, never smokers having joint genotypes of NAT2 intermediate-slow and CYP1A2*1F A/A was also associated with increased the lung cancer risk (OR: 4.95, 95% CI: 1.19-20.6, P-value: 0.028). We suggested that light smokers with intermediate-slow NAT2 activity were at highest risk for lung cancer and the gene-gene interaction based on intermediate-slow NAT2 activity and high CYP1A2 activity would be increased a lung cancer risk among never smokers.

Key Words: Genetic polymorphism - NAT2 - CYP1A2 - lung cancer - smoking

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Introduction

Lung cancer is one of the most prevalent cancers worldwide and consists of three major histological subtypes, adenocarcinoma, squamous cell carcinoma, and small cell carcinoma. Several metabolic enzymes have been investigated for their lung cancer susceptibility and their catalysis as one of the first steps in the metabolism of carcinogens. Carcinogens are metabolized by phase I and II enzymes. The cytochrome P450 1A1(CYP1A1) gene belongs to the phase I enzymes and is involved in the activation step in the metabolism of polycyclic aromatic hydrocarbons (PAHs), arylamines, such as those found in tobacco smoke. Two of the most studied polymorphisms of CYP1A1 are an MspI cut site in an intron (CYP1A1*2A: MspI) and an isoleucine-to-valine change in the heme-binding region of the enzyme (CYP1A1*2C: Ile462Val) (available at www.imm.ki.se/CYPalleles/cyp1a1.htm) (Kawajiri et al.,1990; Hayashi et al.,1991). Japanese studies have pointed to an increased risk of lung cancer in association with both the CYP1A1*2A and 2C (Kawajiri et al.,1990; Hayashi et al.,1991). Another phase I enzyme, cytochrome P450 1A2 (CYP1A2) is also known to catalyze the N-oxidation of several amines such as the heterocyclic amines (HCAs) formed when meat and fish are cooked well done or in tobacco smoke (McManus et al., 1990; Boobis et al., 1994). CYP1A2 polymorphisms have been discovered (available at www.imm.ki.se/CYPalleles/cyp1a2.htm). In the CYP1A2 polymorphisms, CYP1A2*1C (3858G›A) and CYP1A2*1F (164A›C) have been associated with reduced enzyme activity in smokers (Nakajima et al., 1999; Sachse et al., 2003). CYP1A2*1F was reported to mainly influence the risk of colon and breast cancer (Moonen et al., 2005; Long et al., 2006).

Glutathione S-transferases (GSTs) are enzymes involved in the phase II detoxification process by catalyzing the conjugation of reactive hydrophobic and electrophilic compounds to reduced glutathione. The GSTM1-null genotype was found to be associated with a slight increase in the lung cancer risk (Ryberg et al., 1997). The combination of CYP1A1 variants (MspI or Ile462Val) and GSTM1-null genotype have been associated with a significantly increased risk of lung cancer in Japanese population (Hayashi et al., 1992; Nakachi et al., 1993). These studies indicated that CYP1A1 variants play a major role in the activation of PAHs, and that GSTM1-null cannot effectuate the detoxification of activated PAHs. N-acetyltransferase 2 (NAT2) is polymorphic and catalyzes both N-acetylation (deactivation) and O-acetylation (activation) of a variety of HCAs, PAHs and other carcinogens (Hein, 1988). NAT2 polymorphisms are associated with a variety of acetylation capabilities, ranging from slow to rapid metabolism of toxic substances. Some studies have detected a positive association between the

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lung cancer risk with NAT2 polymorphisms (Hou et al., 2000; Zhou et al., 2002).

We performed a small case-control study of lung cancer patients and control subjects to determine the distributions of genetic polymorphisms CYP1A1*2A, CYP1A1*2C, CYP1A2*1C, CYP1A2*1F, GSTM1 and NAT2 to assess the effect of genetic polymorphisms on lung cancer susceptibility.

Materials and Methods

Study Subjects

A small case-control study was designed to investigate the metabolic genotypes for the lung cancer risk. The cases group comprised 113 lung cancer patients treated at the Hyogo Medical Center for Adults in Akashi City, Japan. They had been histologically diagnosed with either adenocarcinoma or squamous cell carcinoma of the lung, and had undergone thoracotomy between April 2001 and July 2002. The controls group comprised 121 subjects between November 2002 and March 2003. The control individuals were not currently or previously diagnosed with cancer. Information about the cases' and controls' smoking history was obtained from their medical records. Smoking history was summarized as the total amount of cigarettes consumed during one's lifetime until the time of the interview, taking into account changes in consumption with age. The amount of smoke exposure was calculated as pack-years, the product of the number of years an individual smoked and average number of cigarettes smoked per day (converted into a standard pack of 20 cigarettes). 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.

Genotyping

Genomic DNA was extracted from whole blood using the Gene Trapping by Liquid Extraction system (Takara, Shiga, Japan). 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

The Chi-square test and Student's t-test was used to compare the distribution of categorical variables and genotype frequencies between cases and controls. Population Hardy-Weinberg equilibrium was tested in controls using Chi-square test. Logistic regression analysis was performed to assess the association between each genotypes and lung cancer. The odds ratio (OR) with 95% confidence intervals (95%CI) was calculated with the aid of unconditional logistic regression. Statistical analyses were performed using SPSS software packages (version 14.0 for Windows; SPSS Japan Inc., Tokyo, Japan). An adjustment for the potential confounding effects of age, gender, smoking habit was computed to estimate the association between certain genotypes and lung cancer. The subjects were divided into three groups according to pack-years of smoking: never smokers (pack-years=0), light smokers (0<pack-years≤30), and heavy smokers (pack-years>30). The gene-smoking interaction, adjusted for age and gender was also computed. 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, Chiou et al. 2005).

Individuals with the GSTM1 allele were designated GSTM1-present and those with homozygous deletion of the GSTM1 allele were designated GSTM1-null (Ryberg et al., 1997). In the genotyping of NAT2, the alleles detected were allele 1, 2, 3 and 4. Individuals who were homozygous for the functional allele 1 were classified as NAT2-rapid, and those heterozygous for allele 1 in combination with a low activity allele 2, 3 or 4 as NAT2-intermediate. Individuals with any combination of the low activity alleles 2, 3 or 4 were classified as NAT2-slow (Abe et al., 1993). For NAT2 genotypes, NAT2-rapid is considered as the reference in lung cancer (Oyama et al., 1997). 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. Differences were regarded as statistically significant as P<0.05.

Table 1. Characteristics of Lung Cancer Cases and Control Subjects

Item	Cases	Controls	P-value
Gender			
males	74 (65.5)*	73 (60.3)	0.317[a]
females	37 (32.7)	48 (39.7)	
unknown	2 (1.8)	0 (0.0)	
Age			
~64	40 (35.4)	51 (42.1)	0.188[b]
65~69	17 (15.0)	28 (23.1)	
70~74	34 (30.1)	21 (17.4)	
75~	20 (17.7)	21 (17.4)	
unknown	2 (1.8)	0 (0.0)	
Smoking status (Pack-years)			
never (0)	32 (28.3)	55 (45.5)	0.054[b]
light (0-30)	21 (18.6)	18 (14.9)	
heavy (>30)	58 (51.3)	43 (35.5)	
unknown	2 (1.8)	5 (4.1)	
Histological type			
adenocarcinoma	68 (60.2)		
squamous cell carcinoma	35 (31.0)		
others	10 (8.8)		

* Number (%) [a]X2 analysis [b]Student's t-test

Results

The study involved 113 cases of lung cancer and 121

Table 2. Genotypes and Allele Frequencies of Cases and Control Subjects

Genotype		Cases	Controls	Allele frequency	Cases	Controls
*CYP1A1*2A*	T/T	45 (39.8)	49 (40.5)	T	0.628	0.628
	T/C	52 (46.0)	54 (44.6)	C	0.372	0.372
	C/C	16 (14.2)	18 (14.9)			
*CYP1A1*2C*	Ile/Ile	70 (61.9)	79 (65.3)	Ile	0.792	0.806
	Ile/Val	39 (34.5)	37 (30.6)	Val	0.208	0.194
	Val/Val	4 (3.5)	5 (4.1)			
*CYP1A2*1C*	A/A	5 (4.4)	8 (6.6)	G	0.783	0.743
	A/G	36 (31.9)	42 (34.7)	A	0.217	0.257
	G/G	65 (57.5)	63 (52.1)			
	unknown	7 (6.2)	8 (6.6)			
*CYP1A2*1F*	C/C	18 (15.9)	17 (14.0)	A	0.631	0.613
	C/A	40 (35.4)	52 (43.0)	C	0.369	0.387
	A/A	45 (39.8)	42 (34.7)			
	unknown	10 (8.8)	10 (8.3)			
GSTM1	present	56 (49.6)	59 (48.8)			
	null	57 (50.4)	62 (51.2)			
NAT2[a]	rapid	60 (53.1)	71 (58.7)	1	0.704	0.756
	intermediate	39 (34.5)	41 (33.9)	2	0.071	0.070
	slow	14 (12.4)	9 (7.4)	3	0.195	0.149
				4	0.031	0.025

[a]Rapid acetylators were defined as individuals with 1 homozygote allele. Intermediate acetylators were defined as individuals with 1 allele in combination with 2, 3 or 4 alleles. Slow acetylators were defined as individuals with combination with 2, 3 or 4 alleles.

controls. Gender, age, smoking status, histological types (68 with lung adenocarcinoma, 35 with lung squamous cell carcinoma and 10 with other carcinoma) for lung cancer cases and controls are shown in Table 1.The distribution of gender was follows: 65.5% of cases versus 60.3% of controls were males and 32.7% of cases versus 39.7% of controls were females (p=0.317). The average ages (± SD) of cases and controls were 65.9 ± 9.5 and 67.3 ± 6.6 years, respectively (p=0.188). We divided the subjects into three subgroups, never smokers (pack-years =0), light (0< pack-years ≤ 30) and heavy smokers (pack-years >30) based on pack-years. Never smokers comprised 28.3% of cases and 45.5% of controls. Ever smokers (light and heavy) comprised 69.9% of cases and 50.4% of controls. The average pack-years (± SD) of cases and controls were 34.5 ± 31.7 and 25.9 ± 35.3 years, respectively (p=0.054). The genotype and allele frequencies in Japanese are presented in Table 2. The allele frequencies of CYP1A*2A, CYP1A1*2C, CYP1A2*1C and CYP1A2*1F genotypes were in agreement with the Hardy-Weinberg equilibrium. Deviations from Hardy-Weinberg equilibrium were not tested for the GSTM1 genotype because the PCR assays did not enable discrimination of heterozygous from homozygous carriers. NAT2 polymorphisms were considered of alleles 1, 2, 3 and 4.These genotype frequencies matched the prediction by the Hardy-Weinberg equilibrium based on the allele frequencies.

The genotype distributions of the CYP1A*2A, CYP1A1*2C, CYP1A2*1C, CYP1A2*1F, GSTM1, and NAT2 polymorphisms are summarized for overall, lung

Table 3. Genotype Distribution in Relation to Histological Type of Lung Cancer

Genotype	Overall Cases	Overall Cont	Overall OR (95%CI)[a]	Overall P-value	Adenocarcinoma Cases	Adenocarcinoma Cont	Adenocarcinoma OR (95%CI)[a]	Adenocarcinoma P-value	Squamous Cell Carcinoma Cases	Squamous Cell Carcinoma Cont	Squamous Cell Carcinoma OR (95%CI)[a]	Squamous Cell Carcinoma P-value
*CYP1A1*2A*												
T/T	45	49	1.00		29	49	1.00		13	49	1.00	
T/C, C/C	68	72	1.07 (0.62-1.84)	0.808	39	72	0.93 (0.50-1.72)	0.809	22	72	1.37 (0.58-3.24)	0.480
*CYP1A1*2C*												
Ile/Ile	70	79	1.00		42	79	1.00		23	79	1.00	
Ile/Val, Val/Val	43	42	1.20 (0.69-2.07)	0.525	26	42	1.17 (0.62-2.21)	0.620	12	42	1.03 (0.44-2.45)	0.945
*CYP1A2*1C*												
A/A, A/G	41	50	1.00		24	50	1.00		9	50	1.00	
G/G	65	63	1.39 (0.79-2.43)	0.251	40	63	1.43 (0.75-2.73)	0.279	23	63	1.83 (0.72-4.62)	0.202
*CYP1A2*1F*												
C/C, C/A	58	69	1.00		33	69	1.00		19	69	1.00	
A/A	45	42	1.17 (0.66-2.07)	0.594	29	42	1.42 (0.74-2.72)	0.291	12	42	0.74 (0.29-1.87)	0.525
GSTM1												
present	56	59	1.00		34	59	1.00		17	59	1.00	
null	57	62	0.92 (0.54-1.57)	0.763	34	62	0.88 (0.48-1.62)	0.675	18	62	0.86 (0.38-1.95)	0.712
NAT2												
rapid	60	71	1.00		32	71	1.00		23	71	1.00	
slow[#]	53	50	1.29 (0.75-2.22)	0.356	36	50	1.55 (0.84-2.88)	0.161	12	50	0.77 (0.33-1.82)	0.552

[a]OR adjusted for gender, age, smoking habit #intermediate and slow

Table 4. Genotype Distribution in Relation to Smoking Status in Lung Cancer

Genotype	Never-smokers				Light-smokers				Heavy-smokers			
	Cases	Cont	OR (95%CI)[a]	P-value	Cases	Cont	OR (95%CI)[a]	P-value	Cases	Cont	OR (95%CI)[a]	P-value
*CYP1A1*2A*												
T/T	16	24	1.00		6	5	1.00		21	18	1.00	
T/C, C/C	16	31	0.87 (0.35-2.18)	0.771	15	13	0.91 (0.22-3.72)	0.890	37	25	1.26 (0.56-2.84)	0.584
*CYP1A1*2C*												
Ile/Ile	20	36	1.00		17	11	1.00		31	29	1.00	
Ile/Val, Val/Val	12	19	1.24 (0.48-3.19)	0.658	4	7	0.41 (0.09-1.76)	0.228	27	14	1.80 (0.79-4.12)	0.165
*CYP1A2*1C*												
A/A, A/G	12	24	1.00		5	7	1.00		22	17	1.00	
G/G	19	25	1.41 (0.54-3.67)	0.482	15	11	2.61 (0.58-11.7)	0.211	31	24	0.96 (0.41-2.23)	0.916
*CYP1A2*1F*												
C/C, C/A	16	37	1.00		11	9	1.00		30	18	1.00	
A/A	14	16	2.18 (0.82-5.79)	0.117	8	9	0.66 (0.17-2.52)	0.544	22	17	0.78 (0.33-1.84)	0.562
GSTM1												
present	17	26	1.00		10	6	1.00		28	23	1.00	
null	15	29	0.91 (0.37-2.28)	0.844	11	12	0.58 (0.15-2.22)	0.428	30	20	1.23 (0.56-2.71)	0.613
NAT2												
rapid	10	29	1.00		9	15	1.00		40	23	1.00	
slow[#]	22	26	2.15 (0.83-5.59)	0.115	12	3	10.9 (1.75-67.5)	0.010	18	20	0.52 (0.23-1.17)	0.115

[a]OR adjusted for gender, age [#]intermediate and slow

adenocarcinoma and lung squamous cell carcnoma in Table 3. Table 3 also show the OR adjusted for gender, age and smoking habits. The carcinogenic risks of each genotype were not statistically significant for overall, lung adenocarcinoma or lung squamous cell carcinoma. Then, the genotype distributions of these polymorphisms are summarized for lung cancer, dividing the subjects into never, light and heavy smokers adjusted for gender and age (Table 4). These genotypes were no association with lung cancer among never smokers (pack-years =0). For light smokers (0< pack-years ≤30), we observed a significant association for intermediate-slow genotypes of NAT2 [the adjusted odds ratio (OR):10.9, 95% confidence intervals (95% CI): 1.75-67.5, P-value: 0.010], whereas the OR of that genotypes was not associated with lung cancer (OR: 0.52, 95% CI: 0.23-1.17, P-value: 0.115) for heavy smokers (pack-years > 30). The intermediate-slow genotypes of NAT2 was significantly associated with increased risk of lung cancer among light smokers.

Furthermore, we attempted to examine the joint effect of each of these polymorphisms (Table 5, 6). When rapid genotype of NAT2 plus C/C-C/A genotypes of CYP1A2*1F was used as reference, the risk of intermediate-slow genotypes of NAT2 plus A/A genotype of CYP1A2*1F proved to be significantly increased with an OR of 4.95 (95% CI: 1.19-20.6, P-value: 0.028) among never smokers (Table 5). When C/C-C/A genotypes of CYP1A2*1F plus A/A-A/G genotypes of CYP1A2 *1C was used as reference, the OR of A/A genotype of CYP1A2*1F plus G/G genotype of CYP1A2*1C was 5.55 (95% CI: 0.94-32.7, P-value: 0.058) among never smokers (Table 6). Joint effects of two CYP1A2 genotypes was a borderline significant association with lung cancer risk among never smokers. On the other hand, we found no association among smoking status and the CYP1A1 and GSTM1 genotypes (results not shown). For never smokers, we observed that the combination of NAT2 and CYP1A2 polymorphisms was a significantly increased lung cancer

Table 5. Interaction between NAT2 and Other Genotypes by Smoking Status

Genotype	Overall				Never-smokers				Ever-smokers[$]			
	Cases	Cont	OR (95%CI)[a]	P-value	Cases	Cont	OR (95%CI)[b]	P-value	Cases	Cont	OR (95%CI)[b]	P-value
*NAT2 + CYP1A1*2A*												
rapid+T/T	21	29	1.00		2	12	1.00		18	16	1.00	
slow[#]+T/C, C/C	29	30	1.46 (0.66-3.21)	0.353	8	14	3.31 (0.57-19.3)	0.184	21	16	1.12 (0.44-2.86)	0.820
*NAT2 + CYP1A1*2C*												
rapid+Ile/Ile	34	50	1.00		4	21	1.00		29	27	1.00	
slow+ Ile/Val, Val/Val	17	21	1.24 (0.56-2.75)	0.593	6	11	2.70 (0.60-12.1)	0.196	11	10	1.05 (0.38-2.89)	0.930
*NAT2 + CYP1A2*1C*												
rapid+A/A, A/G	24	26	1.00		5	10	1.00		18	14	1.00	
slow+G/G	33	22	1.75 (0.79-3.92)	0.171	14	9	2.56 (0.61-10.6)	0.197	19	12	1.23 (0.44-3.39)	0.695
*NAT2 + CYP1A2*1F*												
rapid+C/C, C/A	30	42	1.00		5	19	1.00		25	19	1.00	
slow+A/A	22	18	1.76 (0.78-4.01)	0.176	10	7	4.95 (1.19-20.6)	0.028	12	11	0.77 (0.28-2.17)	0.624
NAT2 + GSTM1												
rapid+present	25	36	1.00		5	13	1.00		20	20	1.00	
slow+null	22	27	1.16 (0.53-2.53)	0.717	10	13	2.01 (0.50-8.05)	0.324	12	14	0.86 (0.32-2.33)	0.771

[a]OR adjusted for gender, age and smoking habit [#]intermediate and slow [$]heavy and light smokers

Table 6. Interaction between CYP1A2*1F and Other Genotypes by Smoking Status

Genotype	Overall				Never-smokers				Ever-smokers[$]			
	Cases	Cont	OR (95%CI)[a]	P-value	Cases	Cont	OR (95%CI)[b]	P-value	Cases	Cont	OR (95%CI)[b]	P-value
*CYP1A2*1F + CYP1A1*2A*												
C/C, C/A+T/T	33	33	1.00		11	19	1.00		21	12	1.00	
A/A+T/C, C/C	36	30	1.15 (0.56-2.34)	0.707	9	12	1.46 (0.44-4.79)	0.535	27	18	0.82 (0.32-2.08)	0.676
*CYP1A2*1F + CYP1A1*2C*												
C/C, C/A+Ile/Ile	44	50	1.00		13	27	1.00		30	20	1.00	
A/A+ Ile/Val, Val/Val	24	21	1.26 (0.61-2.64)	0.532	7	9	1.81 (0.52-6.29)	0.351	17	12	0.94 (0.37-2.39)	0.893
*CYP1A2*1F + CYP1A2*1C*												
C/C, C/A+A/A, A/G	12	26	1.00		3	15	1.00		8	9	1.00	
A/A+G/G	18	18	2.37 (0.87-6.48)	0.092	6	5	5.55 (0.94-32.7)	0.058	12	13	1.08 (0.31-3.75)	0.908
*CYP1A2*1F + GSTM1*												
C/C, C/A+present	31	40	1.00		8	19	1.00		22	17	1.00	
A/A+null	24	27	0.99 (0.46-2.13)	0.986	5	9	1.57 (0.37-6.60)	0.540	18	18	0.77 (0.31-1.92)	0.578

[a]OR adjusted for gender, age, smoking habit [b]OR adjusted for gender, age [$]heavy and light smokers

risk than that of other polymorphisms.

Discussion

We studied six polymorphisms involved in the metabolism of carcinogens associated with lung cancer. We did not detect any association between lung cancer and these genetic polymorphisms examined overall. However, the association of NAT2 polymorphisms for light smokers group was markedly strong compared with its association for heavy smokers group in terms of lung cancer susceptibility. Previous studies reported that no overall association of NAT2 acetylator genotypes to the lung cancer risk, but there was the increase risk with several factors, age, gender, or smoking dose (Oyama et al.,1997; Hou et al., 2000;Sørensen et al., 2005). In particular, the NAT2 slow acetylator genotype was associated with an increased risk for lung cancer in Japanese (Oyama et al., 1997) or at lower pack-years (Hou et al., 2000). Sørensen et al (2005) reported that NAT2 fast acetylator genotype seemed to be protective against lung cancer for light smokers. NAT2 enzyme is detoxification to many arylamines in tobacco smoke by N-acetylation (Hein, 1988). The findings of our study indicate that the genetic susceptibility ascribable to NAT2 intermediate-slow acetylators may confer decreased the detoxification to tobacco mutagens, such as several arylamines, when tobacco expose is low.

Further, we detected that the joint association of NAT2 and CYP1A2 polymorphisms for never smokers group was a significantly lung cancer risk compared with its association for the ever smokers (light and heavy) group. The gene-gene interaction is a possible metabolic mechanism. HCAs or several arylamines, formed by cooking of meat or fish but little tobacco smoking, were activated by N-hydroxylation of CYP1A2 enzyme. The hydroxylated forms, which can eventually covalent bind to cause DNA adducts, are potent as proximate carcinogens. The A/A genotype of CYP1A2*1F represented a highly inducible genotype that was associated with an increased activity of CYP1A2 (Macleod et al., 1998; Sachse et al., 1999). Therefore, high CYP1A2 activity was possible to be increased a risk of lung cancer among never smokers. The hydroxylated forms may also be O-acetylated by NAT2 enzyme. The hydroxylated forms by O-acetylation also can form DNA adducts and are potent as ultimate carcinogens. NAT2 slow acetylator genotype was associated with increased risk of lung cancer among non-smokers (Seow et al, 1999; Zhou et al., 2002). Additionally, it is reported that when the joint effect of NAT2/CYP1A2 status, associated with slow genotypes of NAT2 and rapid CYP1A2 activity using caffeine metabolic ratio assay, was at highest risk for lung adenocarcinoma in nonsmoking Chinese women (Seow et al., 2001). Therefore, high CYP1A2 activity and intermediate-slow NAT2 activity may be strongly increased the hydroxylated forms as proximate carcinogens, from HCAs and arylamines by N-hydroxylation, compared with the activation of the hydroxylated forms as ultimate carcinogens by O-acetylation. Our findings clearly show the promoting effect on the risk of lung cancer associated with combination of high CYP1A2 enzyme activity and NAT2 intermediate-slow acetylator activity.

Furthermore, we confirmed a borderline significant association between A/A genotype of CYP1A2*1F and G/G genotype of CYP1A2*1C. The G/G genotype of CYP1A2*1C caused also a significant increase of CYP1A2 (Nakajima et al., 1999; Chiou et al, 2005), therefore, the G/G genotype of CYP1A2*1C was supported to increase the CYP1A2 activity. This indicated that the joint association of A/A genotype of CYP1A2*1F and G/G genotype of CYP1A2*1C strongly lead to increase the CYP1A2 enzyme activity.

On the other hand, we found no association between the presence of CYP1A1*2A, CYP1A1*2C or GSTM1 genes and lung cancer, although previous studies reported detecting genetic susceptibility to lung cancer in these genes (Kawajiri et al., 1990; Hayashi et al., 1991; Hayashi et al.,1992; Nakachi et al., 1993).

In conclusion, we found that lung cancer risk was clearly associated with NAT2 intermediate-slow activity in individuals with a low level smoking habit and significantly increased with the combination of NAT2 intermediate-slow activity and CYP1A2 high activity for never smokers. Our findings may help identify susceptible individuals by the joint association of susceptible gene polymorphisms which are involved in carcinogenic metabolism in Japanese.

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