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An *Rsa* I Polymorphism in the *CYP2E1* Gene Does Not Affect Lung Cancer Risk in a Japanese Population

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CYP2E1 catalyzes the metabolic activation of tobacco-specific *N*-nitrosamines, including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. An *Rsa* I polymorphism, which is located in the 5′-flanking region of the *CYP2E1* gene, has been found to affect the transcriptional regulation of the gene, resulting in different expression levels of the mRNA among individuals. In order to investigate an association between the *Rsa* I polymorphism and lung cancer susceptibility, the genotype distribution among 316 lung cancer patients was compared with that in 503 healthy controls. No statistically significant association was found between the *Rsa* I polymorphism and an increased risk of lung cancer, even though histological types of lung cancer, cigarette smoking and alcohol consumption were taken into account.

Key words: Lung cancer — Genetic polymorphism — P450 — Nitrosamine

Most chemical carcinogens require metabolic activation by Phase I enzymes, cytochrome P450s, for conversion to their genotoxic electrophilic intermediates.[1,2] In some instances, these activated metabolites are subjected to detoxification by conjugation catalyzed by various Phase II enzymes.[3] Thus, it has been suggested that genetically determined individual differences in the capacity of metabolic activation of various chemical procarcinogens are associated with the predisposition to chemically induced cancers. Human lung cancer, especially squamous cell carcinoma, requires exposure to procarcinogens, mainly contained in cigarette smoke.[4] Several forms of P450 may contribute to susceptibility to lung cancer because there are various procarcinogens besides benzo[*a*]pyrene in cigarette smoke.[5]

Focusing on CYP1A1, which is expressed in the lung and activates benzo[*a*]pyrene, we have demonstrated that high susceptibility to lung cancer was associated with the *Msp* I and *Ile-Val* polymorphisms of the gene in terms of genotype frequency and cigarette dose.[6–9] A synergistic increase in susceptibility to lung cancer risk has also been found by analysis of combined genotypes of the *CYP1A1* and the Mu-class of glutathione *S*-transferase (*GSTM1*) genes.[10,11]

CYP2E1 is induced in liver by structurally unrelated chemicals, such as ethanol and benzene, and participates in the metabolic activation of various *N*-nitrosamines, including a potent tobacco-specific procarcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.[12–15] The expression levels of CYP2E1 mRNA or protein have been shown to differ among individuals, and inter-individual variation of CYP2E1-mediated metabolic activity towards *N*-nitrosamines has also been observed.[16,17] We showed that an *Rsa* I polymorphism, which is located in the 5′-flanking region of the *CYP2E1* gene, affected transcriptional regulation of the gene, resulting in different expression levels of the CYP2E1 mRNA.[18–20] In this study, we have examined an association between *Rsa* I polymorphism and lung cancer susceptibility, because inconsistent results on this association have been reported in Finnish,[21] U.S.[22] and Swedish[23] populations and it remains a controversial issue.

Blood samples were obtained from healthy unrelated controls in two general populations as described previously[18,20] and also from patients with lung cancer diagnosed in the Saitama Cancer Center Hospital. Genomic DNAs were isolated from peripheral lymphocytes, and the *CYP2E1* genotypes were determined by polymerase chain reaction (PCR) amplification followed by digestion with *Rsa* I. Genomic DNA (1 μg) was used as a template, and amplification was performed by heating for 2 min at 95°C followed by 30 cycles of 1 min at 95°C for denaturation followed by 1 min at 60°C for annealing and primer extension with 2.5 units of Taq DNA polymerase (Takara, Kyoto). After the final cycle, samples were incubated for an additional 5 min at 72°C. The amplified DNA fragment was digested with *Rsa* I (Takara) and subjected to electrophoresis in 2% agarose

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gel. The primers used for PCR were[19]: 5′-primer, 5′-atccacaagtgatttggctg-3′ (j71, from −1119 to −1100); 3′-primer, 5′-cttcatacagaccctcttcc-3′ (j72, from −885 to −866). Fig. 1 shows the restriction fragment length polymorphism (RFLP) of the PCR-amplified fragments obtained from *Rsa* I digestion.

Table I shows the distribution of the *Rsa* I genotypes of *CYP2E1* among lung cancer patients and healthy controls. We determined the frequency distribution among healthy individuals from two independent general populations, which were composed of unrelated Japanese including 202 general residents as described previously (healthy controls A)[18] and 301 workers in a company (healthy controls B).[20] The genotype distributions of the two control groups were indistinguishable from each other, suggesting strict allelic frequencies in the *Rsa* I polymorphism of the Japanese gene pool. These results gave a good fit to the Hardy-Weinberg equilibrium with allelic frequencies of 0.81 for c1 and 0.19 for c2 alleles. The distribution of *Rsa* I genotypes among patients with lung cancer was classified by histological types (see the same table). The distribution among patients with lung cancer did not show any statistically significant difference from that among healthy controls ($\chi^2=0.819$; d.f.$=2$; $P>0.50$). Furthermore, we could not find any difference in the genotype distribution between healthy controls and patients with squamous cell carcinoma of the lung ($\chi^2=1.821$; d.f.$=2$; $0.25<P<0.50$), which is most closely associated with cigarette smoking.[4]

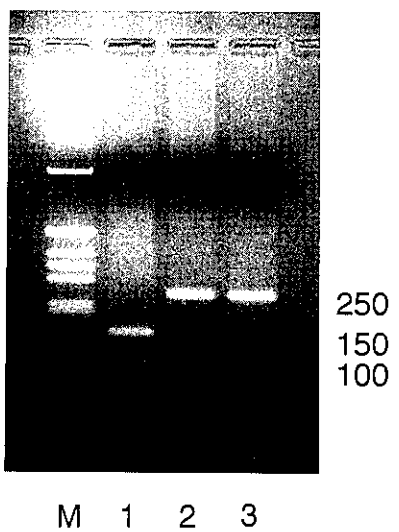

Fig. 1. RFLP of PCR-amplified fragments obtained by *Rsa* I digestion. The PCR products from type A, B and C DNA were digested with *Rsa* I, and subjected to agarose gel electrophoresis. Lanes 1, 2, 3 and M are genotypes A (c1/c1), B (c1/c2) and C (c2/c2) and size markers (1 kb DNA ladder from GIBCO BRL, Bethesda), respectively.

We have already demonstrated that patients with a susceptible genotype of the *CYP1A1* gene developed squamous cell carcinoma with a lesser cigarette dose than those with other genotypes.[6,9] We further found an association of the *Rsa* I genotypes and alcohol consumption with the level of CYP2E1 mRNA expression in peripheral lymphocytes of healthy individuals.[20] Expression levels of CYP2E1 mRNA among daily drinkers with genotype B (heterozygote) were about 2.0-fold higher than those among non-drinkers with genotype A (predominant homozygote). Thus, the influence of alcohol consumption as well as cigarette smoking must be taken into consideration in assessing the genotype-dependent inter-individual differences in susceptibility to smoking-induced lung cancer. Cigarette and alcohol consumptions were independent of the *Rsa* I genotypes in controls, and hence we examined the levels of cigarette and alcohol consumption in lung cancer patients with each *Rsa* I genotype. We studied the total amounts of cigarettes consumed over the life time by squamous cell carcinoma patients with different *CYP2E1* genotypes. The mean cigarette consumption ($\pm$SD) by patients with genotypes A (n$=$63), B (n$=$28) and C (n$=$1) were 4.1 ± 2.1, 3.8 ± 1.7 and 3.5×10^5, respectively. We also studied drinking status among patients with the carcinoma in

Table I. Distribution of the 3 Types of *CYP2E1* Gene among Lung Cancer Patients and Healthy Controls

Population	*CYP2E1* genotype			Total
	A (c1/c1)	B (c1/c2)	C (c2/c2)	
Healthy controls	327 (65.0)	160 (31.8)	16 (3.2)	503 (100.0)
A	132 (65.3)	62 (30.7)	8 (4.0)	202 (100.0)
B	195 (64.8)	98 (32.6)	8 (2.6)	301 (100.0)
Lung cancer	207 (65.5)	96 (30.4)	13 (4.1)	316 (100.0)
Squamous cell ca.	72 (69.2)	31 (29.8)	1 (1.0)	104 (100.0)
Small cell ca.	36 (70.6)	11 (21.6)	4 (7.8)	51 (100.0)
Large cell ca.	8 (44.4)	8 (44.4)	2 (1.1)	18 (100.0)
Adenocarcinoma	91 (63.6)	46 (32.2)	6 (4.2)	143 (100.0)

terms of the *CYP2E1* genotype. The ratios of daily drinkers with genotype A and B were 50.8 (33 of 65) and 54.8% (17 of 31), and those of never-drinkers were 21.3 (15 of 65) and 19.4% (6 of 31), respectively. We could not estimate the distribution of drinking status in genotype C, because only one patient with genotype C was enrolled in this study. These results indicate that there is no association of the *Rsa* I polymorphism with cigarette consumption ($P>0.5$, t test), or drinking status ($\chi^2=0.200$; d.f. $=1$; $P>0.5$) among these lung cancer patients.

A racial difference in the contribution of the *Rsa* I polymorphism to lung cancer susceptibility has recently been reported in Finland,[21] the U.S.[22] and Sweden.[23] The *CYP2E1* polymorphism in Finnish[21] and U.S.[22] populations did not show an association with lung cancer. On the other hand, individuals with the c2 allele (the *Rsa* I site is absent) were reported to be at lower risk of lung cancer in a Swedish population.[23] A major reason for this discrepancy might be an ethnic difference in allelic frequency of the polymorphism. In a Swedish population, the frequency of the rare allele c2 (c2 $=0.05$) was four times lower than that in the Japanese (c2 $=0.19$). Accordingly, a much greater number of study subjects might be required to elucidate the association with cancer susceptibility in Caucasian populations.

The possibility that polymorphisms other than the *Rsa* I in the *CYP2E1* gene play an important role in the susceptibility to tobacco-specific nitrosamine-induced lung cancer can not be excluded. Uematsu *et al.*[24, 25] reported an association between the *Dra* I polymorphism located in intron 6 of the *CYP2E1* gene and susceptibility to lung cancer in a Japanese population. Although the *Dra* I polymorphism has been reported to be closely associated with the *Rsa* I polymorphism,[21] their results appear to be inconsistent with ours. This discrepancy might be due to a lack of complete linkage disequilibrium between the *Dra* I and *Rsa* I polymorphisms. However, another interpretation is more likely. A detailed analysis of the population data of Uematsu *et al.* indicates that the genotype distribution of the control group was not in Hardy-Weinberg equilibrium, while the genotype distribution among their lung cancer patients was in equilibrium. In general, controls must be in Hardy-Weinberg equilibrium as evidence for a non-biased gene pool, whereas cases may depart from the equilibrium if the polymorphism investigated is involved in the etiology and is directly or indirectly responsible for the different risks. Failure to satisfy the above condition is caused in most cases by an insufficient number of study subjects or/and biased subjects.

In conclusion, we found no association between the *Rsa* I polymorphism of the *CYP2E1* gene and lung cancer. Several species of human P450s have been reported to participate in the metabolic activation of procarcinogens in tobacco smoke.[26] The process of carcinogenic activation of tobacco-specific procarcinogens in the lung requires further investigation.

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