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Polymorphisms of the *CYP1A1* and Glutathione *S*-Transferase Genes Associated with Susceptibility to Lung Cancer in Relation to Cigarette Dose in a Japanese Population[1]

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ABSTRACT

An association of lung cancer susceptibility with an *Msp*I restriction site polymorphism of the *CYP1A1* gene was reported in our previous study. This polymorphism has been subsequently found to be closely linked to another isoleucine-valine (Ile-Val) polymorphism, which resulted in an Ile-Val amino acid replacement in the heme-binding region of P4501A1.

We report here that genetic risk for squamous cell carcinoma of the lung was associated with these two polymorphisms of the *CYP1A1* gene in terms of genotype frequencies and cigarette-smoking dose and that a more increased risk was observed for the individuals with "susceptible" genotypes of *CYP1A1* combined with a deficient genotype of a μ-class glutathione *S*-transferase (GST1), which detoxifies the electrophilic metabolites of aromatic hydrocarbon procarcinogens activated by P4501A1. We first compared the total amounts of cigarettes consumed during a lifetime among 85 patients with squamous cell carcinoma of the lung, whose *CYP1A1* and *GST1* genes were identified. The patients with a susceptible homozygote of each of the *Msp*I and Ile-Val polymorphisms contracted the carcinoma after smoking fewer cigarettes than those with other genotypes. When the *GST1* polymorphism was taken into account, the cumulative cigarette amounts in combined genotyping of the two genes showed distinct differences, resulting in the lowest cigarette dose observed for the patients with a susceptible *Msp*I or Ile-Val genotype of *CYP1A1* combined with a deficient *GST1* homozygote. Next, a case-control study revealed that the individuals with the susceptible *Msp*I or Ile-Val genotype combined with deficient *GST1* were at remarkably high risk with an odds ratio of 16.00 or 41.00, respectively (95% confidence interval, 3.76–68.02 or 8.68–193.61, respectively), at a low dose level of cigarette smoking.

INTRODUCTION

An individual difference in susceptibility to chemically induced carcinomas is in part ascribed to genetic differences of metabolic activity in the activation and detoxification of environmental procarcinogens. Human lung cancer, especially squamous cell carcinoma, requires exposure to procarcinogens mainly contained in cigarette smoke, providing a good model for studies of host-environment interactions in carcinogenesis.

Most environmental procarcinogens require metabolic activation by Phase I enzymes, cytochrome P-450s, to their reactive electrophilic intermediates (1). Several forms of P-450 have been identified as playing a role in lung carcinogenesis, and P4501A1 is first in line to associate with the interindividual difference in susceptibility to the carcinoma. P4501A1 is expressed in human lung tissue (2, 3) and metabolizes polycyclic aromatic hydrocarbons in cigarette smoke such as benzo(*a*)pyrene (4), and its high AHH[3] inducible phenotype, which showed a genetically determined variation among individuals, has been reported to be associated with high susceptibility to lung cancer among cigarette smokers (5, 6). We previously reported that high susceptibility to lung cancer was associated with the *Msp*I polymorphism in the 3′-flanking region of the *CYP1A1* gene (7) and that individuals with a susceptible genotype were at high risk for squamous cell carcinoma with an odds ratio of 7.31 at a low-dose level of cigarette smoking (8). Subsequently, we found another genetic polymorphism in the coding region of *CYP1A1*, which was closely linked to the *Msp*I polymorphism (9). This polymorphism resulted in replacement of Ile by Val at residue 462 in the heme-binding region.

Activated metabolites of carcinogens are subjected to metabolic conjugation and other kinds of detoxifications by Phase II enzymes. Multiple cytosolic GSTs, a large family of multifunctional proteins, catalyze the conjugation of reduced glutathione to a variety of electrophilic compounds and are classified into at least four genetically distinct classes, μ, α, π, and θ (10). GST1, a μ-class isozyme, detoxifies the reactive metabolites of benzo(*a*)pyrene and other polycyclic aromatic hydrocarbons, including epoxides and hydroxylated forms (10). The deficient GST1, which is due to a homozygous deletion of the *GST1* gene, was frequently observed among individuals, and an increased frequency of the deficient phenotype or genotype among lung cancer patients was reported, although association with cigarette smoking remained a subject for further studies (11–13).

Recently, Hayashi *et al.* (14) compared the frequencies of combined genotypes of *CYP1A1* Ile-Val polymorphism and *GST1* polymorphism in lung cancer patients and random controls and reported a remarkably increased frequency (8.5%) of the homozygous *Val/Val* genotype combined with the deleted *GST1* gene in lung cancer patients, compared with controls (2.2%). In the study reported here, we first compared the cumulative cigarette doses of patients with squamous cell carcinoma of the lung with genotypes of each of the three polymorphisms: *Msp*I and Ile-Val polymorphisms of *CYP1A1* and *GST1* polymorphism. The cigarette doses were also compared by combined genotyping of the *CYP1A1* and *GST1* genes, which might be associated with a metabolic balance between Phase I and Phase II enzymes: activation and detoxification. Next, in a case-control study, we evaluated the relative risk for individuals with susceptible genotypes in relation to the cumulative cigarette dose. We demonstrate that the genetically determined susceptibility to lung cancer studied in this paper depends on environmental exposure to procarcinogens in cigarette smoke.

MATERIALS AND METHODS

Experimental. Blood samples (5–15 ml) were obtained from patients and controls, and genomic DNAs were isolated from peripheral lymphocytes. The identification of the *CYP1A1* genotypes ascribed to the presence (CCGG) or absence (CTGG) of the *Msp*I site at the 264th base from the additional polyadenylation signal in the 3′-flanking region was carried out by the PCR/restriction digest-genotyping method as described previously (8, 9). The other *CYP1A1* polymorphism resulting in the substitution of isoleucine for valine at residue 462 in the heme-binding region (an A-G base change) was also studied by PCR as described previously (9). Briefly, two primers with different 3′-terminal bases (A or G), which correspond to the polymorphic substitution, were used separately in two allele-specific PCR amplifications with a common primer of another strand. The resultant PCR product was obtained only when the primer used was complementary to the template DNAs with respect to the

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[3] The abbreviations used are: AHH, aryl hydrocarbon hydroxylase; Ile, isoleucine; Val, valine; GSTs, glutathione *S*-transferases; PCR, polymerase chain reaction; CI, confidence interval.

terminal base. The *GST1* genotyping was made by the presence or absence of a PCR product according to the methods of Comstock *et al.* (15) and Groppi *et al.* (16).

Epidemiological. All possible inpatients with selected sites of cancer including lung cancer were interviewed at the time of their first admission to Saitama Cancer Center Hospital using a standardized questionnaire concerning their occupations, dietary habits, and cigarette-smoking (including passive smoking for women) and alcohol-drinking history. The methods and some of the results obtained have been reported previously (8). Epidemiological information histologically confirmed 85 patients, 78 males and 7 females, with squamous cell carcinoma of the lung. The interview survey was in detail, especially concerning smoking and drinking history according to age, because aged people who smoked much in earlier years tend to reduce their daily smoking amounts after about 60 years of age, probably because of job retirement and health consciousness. The smoking history of the patients was summarized as the total amount of cigarettes consumed during their lifetime until the time of the interview. Included in the calculation of the total amount of cigarettes used were age smoking was begun, average consumption of cigarettes per day before 39 years of age, average consumption between 40 and 59 years of age, average consumption at 60 years of age and older, and abstinence durations longer than 1 year. Drastic changes of consumption within those ages were taken into account to estimate each average consumption. In Tables 1 and 2, ex-smokers who stopped smoking within 1 year before diagnoses were regarded as current smokers in order to exclude possible influence of cancer symptoms on smoking status.

Controls used in our case-control study were selected from the subjects of our prospective cohort study on a Japanese general population older than 40 years of age in a town near Saitama Cancer Center Hospital. The residents in this town are not closed genetically nor demographically, *i.e.*, its population has increased because of population influx from other areas with social increase rate of about 5% every year in 15 years before our survey. In addition to epidemiological data, this cohort study covers a variety of host-specific factors such as immunological defense against cancer, oxidative damages by free radicals, and serum components; peripheral blood samples collected from 3625 individuals were subjected to biochemical and immunological assays. Methods of survey and characterization of study population were reported elsewhere (17). We also isolated DNA samples of 2486 individuals, and 1700 randomly selected individuals with DNA samples were interviewed to estimate the total cigarette consumption for this case-control study. Controls used in this study were chosen from these 1700 individuals and individually matched to the patients with respect to sex and age (in 1-year age units). Two controls were randomly selected for each of the patients within the matching conditions.

RESULTS

Cumulative Cigarette Doses in the *Msp*I Genotypes of *CYP1A1* among the Patients. The *Msp*I restriction site polymorphism resulted in three genotypes: a predominant homozygous *m1* allele without the *Msp*I site (genotype *A*), the heterozygote (genotype *B*), and a homozygous rare *m2* allele with the *Msp*I site (genotype *C*). We previously reported an increased frequency of genotype *C* in the 45 patients with the squamous cell carcinoma compared with that in controls and lower cigarette doses in the 12 patients with genotype *C* than other patients, although the patients with genotypes *A* and *B* were combined in comparison with genotype *C* (8). In the present study of 85 patients which includes the previous 45 patients, the mean cumulative consumption of 32.1×10^4 cigarettes in those with genotype *C*, including one "never-smoker," was lower than that of 44.3 and 41.1×10^4 in those with genotypes *A* and *B*, respectively, as shown in Table 1 ($P < 0.05$ between *A* and *C* by *t* test and no significant difference between the variances of compared groups). When never-smokers were excluded, the mean consumption (±SD) by patients with genotypes *A*, *B*, and *C* was 45.7 ± 24.0, 43.8 ± 14.1, and $33.9 \pm 13.2 \times 10^4$ cigarettes, respectively ($P = 0.06$ between *A* and *C*). It is also of interest that the mean abstinence duration among ex-smokers was longer in those with genotype *C* than that in those with the other genotypes, although this difference is not statistically significant. It is evident from Fig. 1*A*, in which the distribution of cumulative cigarette amounts in patients with genotypes is compared, that the amounts in the patients with genotype *C* shifted to the lesser doses from those of the other two genotypes ($P < 0.05$, nonparametric test).

Cumulative Cigarette Doses in Ile-Val Genotypes of *CYP1A1* among the Patients. We compared the mean cumulative cigarette consumption in three genotypes: a predominant homozygote (*Ile/Ile*), the heterozygote (*Ile/Val*), and a rare homozygote (*Val/Val*) (Table 2). The mean cigarette consumption of 30.6×10^4 in patients with genotype *Val/Val* was lower than that of 43.6 and 37.5×10^4 in patients with genotypes *Ile/Ile* and *Ile/Val* ($P = 0.07$ between *Ile/Ile* and *Val/Val*, *t* test). The mean consumption in genotypes *Ile/Ile*, *Ile/Val*, and *Val/Val*, excluding never-smokers, was 44.5 ± 20.8, 42.4 ± 16.0, and $30.6 \pm 8.8 \times 10^4$, respectively ($P = 0.05$ between *Ile/Ile* and *Val/Val*). The distribution of consumed amounts in genotype *Val/Val* was found in Fig. 1*B* to shift to the lesser doses from those of the other two genotypes ($P < 0.05$, nonparametric test). Since the Ile-Val polymorphism was closely linked to the *Msp*I polymorphism, it is likely that the lower cigarette dose observed in genotype *C* or *Val/Val* is essentially ascribed to either of the two polymorphisms. This was examined in Table 3 from an epidemiological viewpoint. The mean cumulative cigarette consumption of 33.4 and 33.2×10^4 in the patients with combined genotype of *C* and not *Val/Val* and those with genotype of *Val/Val* and not *C*, respectively, was found to be lower than that of 43.2×10^4 in the patients with genotype of neither *C* nor *Val/Val* ($P = 0.2$ and 0.07, *t* test); the lowest consumption of 29.3×10^4 was in combined genotype *C* and *Val/Val* ($P = 0.06$). This suggests that the *Msp*I and Ile-Val polymorphisms may involve different mechanisms in association with the susceptibility to lung cancer. Another possible interpretation would be that both of the polymorphisms were in linkage disequilibrium with another polymorphism which is essential for the metabolic activation of procarcinogens in cigarette smoke.

Cumulative Cigarette Doses in Detected or Nulled Polymorphism of *GST1* among the Patients. The *GST1* genotypes were determined by the detection [*GST1(+)*] of at least one *GST1* gene or its homozygous deletion [*GST1(−)*]. Among the 85 patients, 33 were identified as *GST1(+)* with a mean age (±SD) of 64.9 ± 8.7 years and a mean cumulative cigarette consumption of $44.5 \pm 23.3 \times 10^4$ (45.9 ± 22.3, excluding one never-smoker); 52 were identified as *GST1(−)* with a mean age of 65.5 ± 8.2 years and a mean cigarette consumption of $37.7 \pm 18.3 \times 10^4$ (40.0 ± 16.1, excluding three never-smokers). The difference of the mean cigarette consumption was not statistically significant. The mean abstinence duration of 7.6 ± 4.3 years among 13 ex-smokers with *GST1(−)* was found to be longer than 4.6 ± 3.3 years among 7 ex-smokers with *GST1(+)* ($P < 0.05$, *t* test).

Table 1 *Characteristics of patients with genotypes of MspI polymorphism of the CYP1A1 gene*

Genotypes	No. of subjects (frequency)	Age (yr, mean ± SD)	Cigarette consumption ($\times 10^4$, mean ± SD)	Smoking status[a]		
				S	E[b]	N
A	33 (0.388)	64.0 ± 9.1	44.3 ± 23.9	23	9 (6.0 ± 4.3)	1
B	33 (0.388)	66.5 ± 7.3	41.1 ± 17.3	26	5 (6.2 ± 4.0)	2
C	19 (0.224)	65.2 ± 8.8	32.1 ± 15.0	12	6 (7.7 ± 4.6)	1
Total	85 (1.000)	65.2 ± 8.4	40.3 ± 20.5	61	20 (6.6 ± 4.2)	4

[a] S, current smokers; E, ex-smokers; N, never-smokers.
[b] Mean abstinence duration ± SD in years is shown in parentheses.

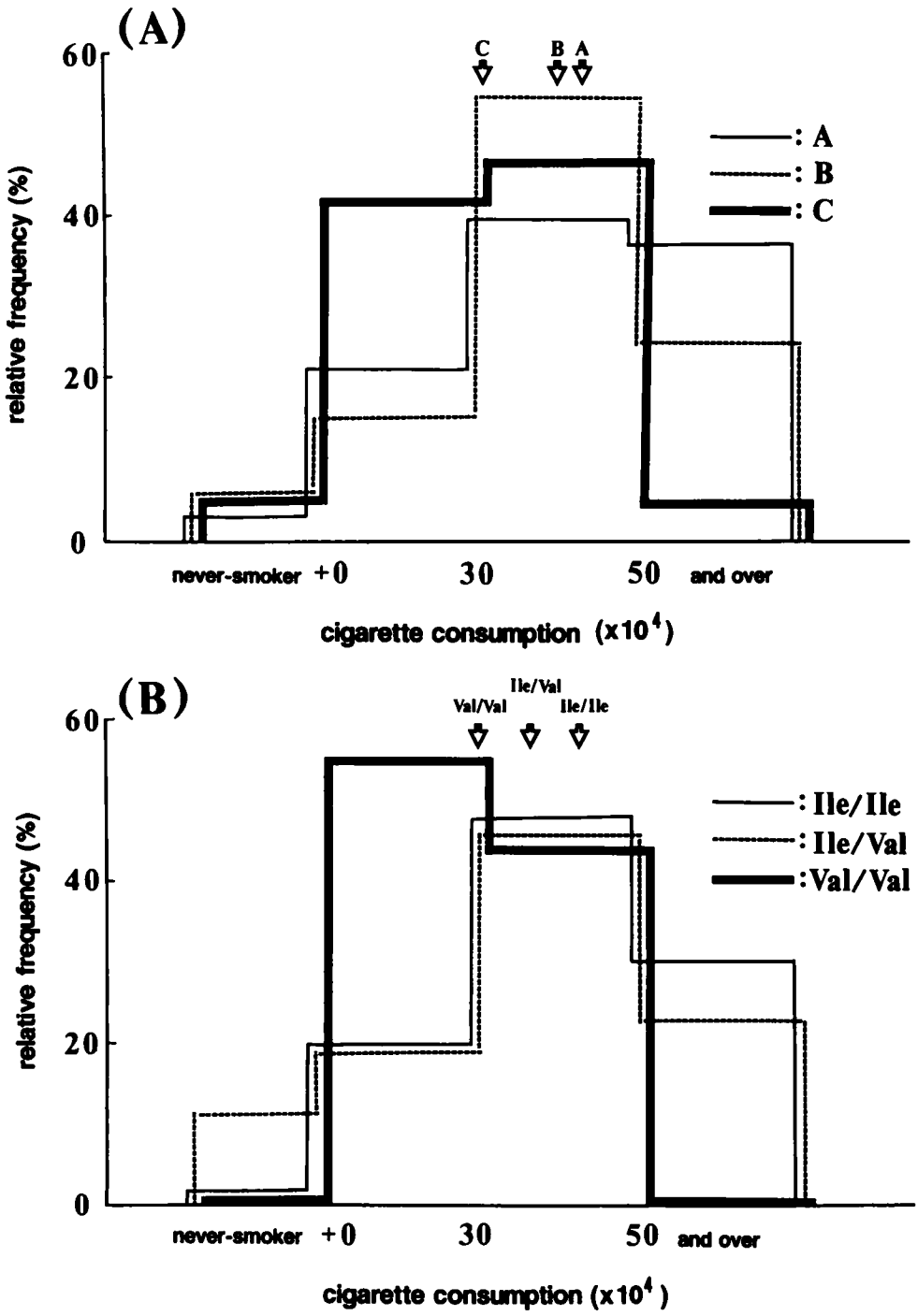

Fig. 1. Relative distribution of cumulative cigarette consumption of the patients in *Msp*I genotypes (*A*) and in Ile-Val genotypes (*B*), taking a total number of patients with each of the genotypes as 100%. *Arrows*, mean cigarette consumption of patients with each genotype.

Table 2 *Characteristics of patients with genotypes of Ile-Val polymorphism of the CYP1A1 gene*

Genotypes	No. of subjects (frequency)	Age (yr, mean ± SD)	Cigarette consumption ($\times 10^4$, mean ± SD)	Smoking status[a]		
				S	E[b]	N
Ile/Ile	50 (0.588)	65.0 ± 8.9	43.6 ± 21.5	38	11 (5.9 ± 3.9)	1
Ile/Val	26 (0.306)	65.8 ± 8.2	37.5 ± 20.4	18	5 (7.0 ± 5.8)	3
Val/Val	9 (0.106)	64.7 ± 5.5	30.6 ± 8.8	5	4 (7.8 ± 3.3)	0

[a] S, current smokers; E, ex-smokers; N, never-smokers.
[b] Mean abstinence duration ± SD in years is shown in parentheses.

Cumulative Cigarette Doses in Combined Genotyping of the *CYP1A1* and *GST1* Genes among the Patients. The balance between metabolic activation and detoxification may be studied by combined genotyping of the two genes. Data from the 85 patients with squamous cell carcinoma of the lung were sorted into the six genotypes, which were composed of the three genotypes of the *Msp*I polymorphism combined with the two genotypes of *GST1*. It was then found that the mean cumulative cigarette consumption in each of genotypes *A*, *B*, and *C* was higher and lower in combined *GST1(+)* and *GST1(−)*, respectively, resulting in the lowest cigarette dose of 31.8×10^4 observed in the combination of susceptible genotype *C* and homozygously deleted genotype *GST1(−)* (Table 4). The differ-

Table 3 *Cigarette consumption (in 10^4 units, mean ± SD) of patients in combined genotyping of the two polymorphisms of CYP1A1*

Genotypes	*A* or *B*	*C*
Ile/Ile or *Ile/Val*	43.2 ± 21.7 (63[a])	33.4 ± 17.3 (13)
Val/Val	33.2 ± 9.8 (3)	29.3 ± 8.9 (6)

[a] Number of patients.

Table 4 *Cigarette consumption of patients in combined genotyping of the MspI polymorphism of CYP1A1 and the GST1 polymorphism*

Combined genotypes		No. of subjects (frequency)	Age (yr, mean ± SD)	Cigarette consumption ($\times 10^4$, mean ± SD)
CYP1A1	*GST1*[a]			
A	+	12 (0.141)	65.1 ± 8.0	48.3 ± 30.2
	−	21 (0.247)	63.4 ± 9.8	42.0 ± 21.9
B	+	14 (0.165)	65.9 ± 8.1	37.3 ± 14.4
	−	19 (0.224)	66.9 ± 6.8	36.6 ± 18.2
C	+	7 (0.082)	62.4 ± 11.6	32.7 ± 23.8
	−	12 (0.141)	66.8 ± 6.8	31.8 ± 7.7

[a] +, *GST1(+)*; −, *GST1(−)*.

ence in mean cigarette consumption between combined genotypes is not statistically significant [$P = 0.06$ between *A* with *GST1(+)* and *C* with *GST1(−)*, *t* test], although the cigarette amounts in patients with combined genotype *C* and *GST1(−)* distributed over the lesser doses compared with those with genotype *A* and *GST1(+)* ($P < 0.05$, nonparametric test), with genotype *A* and *GST1(−)* ($P = 0.1$), or with genotype *B* and *GST1(+)* ($P < 0.01$).

The mean cigarette consumption in combined genotyping of the Ile-Val polymorphism and polymorphic *GST1* is shown in Table 5, and the gradation of the cigarette doses in the combined six genotypes was similar to that shown in Table 4. The lowest cigarette dose of 28.7×10^4 was observed in the patients with the combination of the susceptible genotype *Val/Val* and *GST1(−)*. It may be of interest that 8 of the 9 patients with genotype *Val/Val* were of *GST1(−)*. The difference in mean cigarette consumption of combined genotypes was at a statistically significant level of $P < 0.05$ (*t* test) between *Ile/Ile* with *GST1(+)* and *Val/Val* with *GST1(−)*, and the distribution of cigarette amounts in patients with combined genotype of *Val/Val* and *GST1(−)* shifted to the lesser doses from that of genotype *Ile/Ile* and *GST1(+)* ($P < 0.01$, nonparametric test) or that of genotype *Ile/Ile* and *GST1(−)* ($P < 0.05$).

Characteristics of Controls. A total of 170 controls were selected as described in "Materials and Methods"; the mean age (±SD) was 65.2 ± 8.3 years and the mean cumulative cigarette consumption was $21.7 \pm 18.9 \times 10^4$ cigarettes. The group consisted of 82 current smokers, 45 ex-smokers, and 43 never-smokers. The mean consumption among 127 current or ex-smokers, excluding never-smokers, was $29.0 \pm 16.2 \times 10^4$, and the mean abstinence duration among 45 ex-smokers was 12.6 ± 10.9 years. Their distributions by age, smoking status, and cumulative cigarette amounts were almost identical in the different genotypes of the *Msp*I, Ile-Val, and *GST1* polymorphisms: 82 controls with genotype *A*, 65.0 ± 8.6 years of age and $22.4 \pm 18.3 \times 10^4$ cigarettes; 73 controls with genotype *B*, 65.3 ± 7.7 years of age and $21.0 \pm 20.4 \times 10^4$ cigarettes; 15 controls with genotype *C*, 65.7 ± 9.9 years of age and $21.1 \pm 19.2 \times 10^4$ cigarettes; 110 controls with *Ile/Ile*, 64.8 ± 8.1 years of age and $22.4 \pm 18.0 \times 10^4$ cigarettes; 54 controls with *Ile/Val*, 65.8 ± 8.8 years of age and $20.6 \pm 21.0 \times 10^4$ cigarettes; 6 controls with *Val/Val*, 67.2 ± 9.4 years of age and $18.9 \pm 17.1 \times 10^4$ cigarettes; 86 controls with *GST1(+)*, 64.8 ± 8.2 years of age and $23.0 \pm 20.8 \times 10^4$ cigarettes; and 84 controls with *GST1(−)*, 65.6 ± 8.4 years of age and $20.4 \pm 16.7 \times 10^4$ cigarettes. Furthermore, the allelic frequencies of the *Msp*I or Ile-Val polymorphism in controls were calculated to be in Hardy-Weinberg linkage equilibrium: 0.697 and 0.303 for *m1* and *m2* allele, respectively, with notation of *A(m1/m1)*, *B(m1/m2)*, and *C(m2/m2)*; 0.806 and 0.194 for *Ile* and *Val* allele, respectively. In addition the *Msp*I genotype frequencies examined among 104 stomach, 78 colon, and 31 breast cancer patients in the hospital were almost identical with those in controls; *e.g.*, 8.7, 11.5, and 9.7%, respectively, for genotype *C* among the patients compared with 8.8% (15 of 170) in controls.

Relative Risk Estimate. The different distribution of cigarette consumption in genotypes, which was observed among the patients but not among controls, implies that the genetic risk of susceptible individuals for carcinoma may depend on cigarette dose. The distributions of patients and controls by combined genotypes of the *Msp*I polymorphism and *GST1* polymorphism are shown in Table 6, where the cumulative cigarette dose is dichotomous, taking the mean cigarette amount of 32.1×10^4 among the patients with genotype *C* as a cutoff point. The odds ratios were calculated for the combined genotypes or for the *Msp*I genotypes, taking the risk of the first category with combined genotype of *A* and *GST1(+)* or with genotype *A* at the lower cigarette dose level to be at a baseline of 1.0. The results for the *Msp*I genotypes agree well with results of our previous study in which genotypes *A* and *B* were combined as *A* + *B*, *i.e.*, the susceptibility of genotype *C* was remarkably high at a low dose of cigarette consumption, although the difference of susceptibility in genotypes decreased as the cigarette dose increased (8). In combined genotyping, the individuals with genotype *C* and *GST1(−)* were at extremely high risk with an odds ratio of 16.00 (95% confidence interval, 3.76–68.02) at the lower cigarette dose level compared with those with *A* and *GST1(+)*. On the other hand, this relative susceptibility of combined genotype *C* and *GST1(−)* compared to *A* and *GST1(+)*, which is also equal to the ratio of the two odds ratios for genotypes at the same dose level, decreased to 2.22 (10x4/9/2 or 20.00/9.00, not statistically significant) at the higher cigarette dose level.

Regarding the *GST1* polymorphism, the numbers of patients with *GST1(+)*, *GST1(−)* at the lower dose level, and *GST1(+)*, *GST1(−)* at the higher level were found from Table 6 to be 9, 22, 24, and 30, while those of controls in corresponding categories were 64, 63, 22, and 21. The odds ratios of the *GST1* polymorphism could be calculated to be 2.48 (95% CI, 1.08–5.71) for *GST1(−)* at the lower cigarette dose level of less than 32.1×10^4, 7.76 (95% CI, 3.32–18.14) for *GST1(+)*, and 10.16 (95% CI, 4.44–23.26) for *GST1(−)* at the higher dose level, taking the risk of *GST1(+)* at the lower dose level to be at a baseline of 1.0.

The increased susceptibility of combined genotype *C* and *GST1(−)* at the lower dose level was ascribed to a remarkable risk elevation of 6.55 for genotype *C* synergistically working with a risk elevation of 2.48 for *GST1(−)* at the lower cigarette dose level, compared with moderate risk elevations of 1.50 (8.32/5.55, not significant) and 1.31 (10.16/7.76, not significant) for genotypes *C* and *GST1(−)*, respectively, at the higher dose level. When we shifted the cutoff point of cumulative cigarette dose to between 30 and 40×10^4, the above results remained qualitatively unchanged (some categories of combined genotypes in cases or controls included only one subject or null when the cutoff point was out of the range).

The distributions of the same patients and controls by combined genotypes of the Ile-Val polymorphism and *GST1* polymorphism are shown in Table 7 where the cutoff point of cumulative cigarette dose

Table 5 *Cigarette consumption of patients in combined genotyping of the Ile-Val polymorphism of CYP1A1 and the GST1 polymorphism*

Combined genotypes		No. of subjects (frequency)	Age (yr, mean ± SD)	Cigarette consumption ($\times 10^4$, mean ± SD)
CYP1A1	*GST1*[a]			
Ile/Ile	+	19 (0.224)	65.2 ± 8.8	47.9 ± 25.3
	−	31 (0.365)	64.9 ± 9.2	40.9 ± 18.8
Ile/Val	+	13 (0.153)	64.5 ± 9.2	39.5 ± 21.1
	−	13 (0.153)	67.2 ± 7.2	35.5 ± 20.4
Val/Val	+	1 (0.012)	65	45.3
	−	8 (0.094)	64.6 ± 5.8	28.7 ± 7.3

[a] +, *GST1(+)*; −, *GST1(−)*.

Table 6 *Relative risk estimate on the basis of the distribution of patients and controls by combined genotyping of MspI and GST1 polymorphisms and cigarette dose*

	CYP1A1					
	A		*B*		*C*	
	GST1(+)	*GST1(−)*	*GST1(+)*	*GST1(−)*	*GST1(+)*	*GST1(−)*
Cumulative cigarette dose of ≤32.1 × 10^4						
Patients	3	8	1	6	5	8
Controls	30	31	28	27	6	5
	1.0	2.58 [0.65–10.31][a]	0.36 [0.04–3.35]	2.22 [0.52–9.51]	8.33[c] [1.80–38.66]	16.00[d] [3.76–68.02]
Odds ratios						
	1.0		0.71 [0.26–1.94]		6.55[d] [2.49–17.24]	
Cumulative cigarette dose of >32.1 × 10^4						
Patients	9	13	13	13	2	4
Controls	10	11	10	8	2	2
	9.00[c] [2.30–35.25]	11.82[d] [3.24–43.13]	13.00[d] [3.54–47.73]	16.25[d] [4.35–60.76]	10.00[b] [1.36–73.64]	20.00[d] [3.52–113.59]
Odds ratios						
	5.55[d] [2.45–12.54]		8.01[d] [3.49–18.37]		8.32[c] [2.34–29.62]	

[a] Numbers in brackets, 95% CI.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

Table 7 *Relative risk estimate on the basis of the distribution of patients and controls by combined genotyping of Ile-Val and GST1 polymorphisms and cigarette dose*

	CYP1A1					
	Ile/Ile		*Ile/Val*		*Val/Val*	
	GST1(+)	*GST1(−)*	*GST1(+)*	*GST1(−)*	*GST1(+)*	*GST1(−)*
Cumulative cigarette dose of ≤32.1 × 10^4						
Patients	3	11	6	5	0	6
Controls	41	38	21	23	2	2
	1.0	3.96[b] [1.10–14.24][a]	3.91 [0.95–15.99]	2.97 [0.68–12.91]		41.00[d] [8.68–193.61]
Odds ratios						
	1.0		1.41 [0.59–3.37]		8.46[d] [2.48–28.85]	
Cumulative cigarette dose of >32.1 × 10^4						
Patients	16	20	7	8	1	2
Controls	16	15	5	5	1	1
	13.67[d] [4.14–45.11]	18.22[d] [5.69–58.31]	19.13[d] [4.72–77.64]	21.87[d] [5.52–86.62]	13.67[b] [1.22–153.39]	27.33[c] [3.72–200.60]
Odds ratios						
	6.55[d] [3.23–13.30]		8.46[d] [3.43–20.89]		8.46[c] [1.68–42.73]	

[a] Numbers in brackets, 95% CI.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

was taken to be the same as that in Table 6 for comparison. The numbers of subjects in some of the combined genotype categories were insufficient for reliable estimation of odds ratios because of a lower allelic frequency of susceptible genotype *Val/Val*. The genetic difference in susceptibility was extremely large with an odds ratio of 41.00 (95% CI, 8.68–193.61) for combined genotype of *Val/Val* and *GST1(−)* at the lower dose level, although the difference decreased to 2.00 (16x2/16/1 or 27.33/13.67, not significant) at the higher dose level. The differences in susceptibility ascribable to the single *Msp*I or Ile-Val polymorphism were almost the same in degree when results in Tables 6 an 7 were compared.

DISCUSSION

We studied the difference in susceptibility to squamous cell carcinoma of the lung in terms of germ line polymorphisms of the *CYP1A1* and *GST1* genes, taking account of consumed cigarette amounts. Since P-450s are the primary enzyme interface between environmental carcinogens and host organisms, the genetic susceptibility ascribable to this enzyme family is expected to be associated with different dose levels of exposed carcinogens. The mean cumulative cigarette consumption among patients showed a clear gradation in genotypes of the *CYP1A1* gene and also in combined genotypes of the *CYP1A1* and *GST1* genes, *i.e.*, the genotypes with lower mean cigarette consumption showed more increase in frequency among patients compared to those among controls, which can be calculated from Tables 6 and 7. The lowest cigarette dose was observed for the patients with combined genotype of *C* and *GST1(−)* [frequency of 14.1% (12 of 85) in patients compared to 4.1% (7 of 170) in controls from Table 6, $P < 0.05$] and for those with combined genotype of *Val/Val* and *GST1(−)* [9.4% (8 of 85) in patients compared to 1.8% (3 of 170) in controls from Table 7, $P < 0.01$]. A case-control study was carried out to estimate the relative risk to the combined six genotypes of *CYP1A1* and *GST1* in dichotomous cigarette dose. It was shown that the individuals with susceptible genotype *C* or *Val/Val* of the *CYP1A1* gene further increased their risk to an odds ratio of 16.00 or 41.00 at the lower cigarette dose level when they were genotyped as deficient *GST1*. The patients, who were genotyped as at least one of *C*, *Val/Val*, and *GST1(−)*, accounted for 87.1% (27 of 31) of those who contracted carcinoma at the lower cigarette dose [45.2% (14 of 31) genotyped as either *C* or *Val/Val*], although the proportion of these susceptible patients decreased to 59.3% (32 of 54) at the higher dose [14.8% (8 of 54) genotyped as *C* or *Val/Val*, $P < 0.05$ for the decrease]. On the other hand, the proportion among controls was 54.3 (69 of 127) or 53.5% (23 of 43) at the lower or higher cigarette dose level [9.4 (12 of 127) or 9.3% (4 of 43) genotyped as *C* or *Val/Val*], respectively. The difference at the lower dose between 45.2% in patients and 9.4% in controls was significant ($P < 0.001$), although no statistical significance was found in other comparisons. This indicates that the genetic susceptibility ascribable to *CYP1A1* or *GST1* plays an important role in occurrence of lung cancer, especially when the cigarette dose is low. Because of the limited number of subjects in this study, analyses stratified by age groups to estimate possible induction periods may not be able to be conducted, and determination of precise dose-response relations with respect to a sufficient number of dose levels for each of genotype groups may not be able to be conducted. This remains an important topic for future study.

Recently, two studies have reported a lack of association between the *Msp*I polymorphism and lung cancer among Norwegian (18) and Finnish populations (19). On the other hand, the association of the *Msp*I polymorphism with lung cancer was reconfirmed with respect to an increased frequency of genotype *C* among patients and a lower cumulative cigarette dose of genotype *C* by a subsequent study in a different Japanese population.[4] This discrepancy may be mainly ascribed to an ethnic difference in allelic frequency of the polymorphism, *i.e.*, susceptible genotype *C* in Caucasians so far studied was about 10-fold less frequent than that in Japanese population, and, hence, a required size of study cases and controls to detect the association in Caucasians should be much larger than that of our study because those Caucasian studies (18, 19) found only two subjects or fewer with genotype *C* in study cases or controls. The large difference in genotype frequencies among races may indicate that the attributable risk or etiological fraction ascribable to the *Msp*I or Ile-Val polymorphism can vary among races. Of interest is a moderate risk elevation of heterozygous genotype *B* in a Finnish study population (19): the odds ratio to squamous cell carcinoma of the lung was calculated from their data to be 1.85 (95x14/30/24, χ^2 = 2.439 with d.f. = 1, P = 0.125). A lower risk elevation of genotype *B* was also observed with odds ratio of 1.24 (172x20/47/59) in a Norwegian study population (18). It is possible that, in Caucasian populations, the heterozygous genotypes *B* and *Ile/Val* contribute much to the attributable risk. Clearly, studies involving a large number of study subjects will be required to disclose the association of the *Msp*I and Ile-Val polymorphisms with lung cancer in each of different ethnic population.

As for the association of the *CYP1A1* polymorphisms with phenotypic expressions, cosegregation of the *Msp*I polymorphism and the inducibility phenotype of P4501A1 enzyme (AHH activity) was reported, *i.e.*, individuals with genotype *B* showed a higher inducibility than those with genotype *A*, although genotype *C* was not included in study subjects (20). On the other hand, the Ile-Val polymorphism resulted in two different P4501A1 proteins, and their catalytic activity toward benzo(*a*)pyrene was examined by use of the isoleucine- and valine-type of P4501A1 expressed in yeast microsomes (21). The valine-type P4501A1 showed a higher AHH activity and mutagenicity than that of the Ile-type. These results may indicate that the two polymorphisms of *CYP1A1* involve different biochemical mechanisms, which is consistent with the result in Table 3. However, further studies are needed to draw any definitive conclusion on this matter. For example, detection of carcinogen-induced DNA damage such as DNA adducts in lung tissues of individuals with different genotypes of *CYP1A1* may be helpful, although the smoking amounts, medication, and other associating factors must be adjusted among study subjects.

To summarize, our study in a Japanese population indicates that the two *CYP1A1* polymorphisms and the *GST1* polymorphism were associated with squamous cell carcinoma of the lung in terms of not only the increased frequencies of patients with susceptible genotypes compared to those of controls but their lesser cigarette doses than those with other genotypes. Combined genotyping of susceptible *CYP1A1* and *GST1* genes revealed higher relative risk than that ascribed to a single susceptible gene. The genetic difference in lung cancer susceptibility derived from either single or combined polymorphism was remarkably large at the low cigarette dose, although the difference was decreased at the high dose. Our results thus provided a model for cancer prevention that very susceptible individuals to environmental carcinogens can be identified by combined genotyping of the susceptible genes, which are involved in carcinogenic metabolisms, and that their susceptibility must be studied in relation to exposure levels because a high cancer susceptibility at low exposure level observed in this study is one of the most important problems for individual-based cancer prevention in future.

[4] T. Okada, K. Kawashima, S. Fukushi, T. Minakuchi, and S. Nishimura. Association of cytochrome P4501A1 genotype with human lung cancer and metastasis, manuscript in preparation.

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Polymorphisms of the *CYP1A1* and Glutathione *S*-Transferase Genes Associated with Susceptibility to Lung Cancer in Relation to Cigarette Dose in a Japanese Population

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