Jpn. J. Cancer Res. 83, 866–870, August 1992

High Susceptibility to Lung Cancer Analyzed in Terms of Combined Genotypes of P450IA1 and Mu-class Glutathione S-Transferase Genes

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Lung cancer is closely associated with cigarette smoking. Aromatic hydrocarbons in smoke, including benzo[*a*]pyrene, first require metabolic activation by Phase I enzymes, cytochrome P450, to their ultimate forms, and these activated forms are then subjected to detoxification by Phase II enzymes, especially glutathione S-transferases. Thus, genetically determined susceptibility to lung cancer may depend on the metabolic balance between Phase I and Phase II enzymes. In this study, we identified individuals genetically at high risk of lung cancer in terms of polymorphisms of the P450IA1 gene and GST1 gene. The relative risk of individuals with a combination of the genotypes of both a homozygous rare allele of the P450IA1 gene and the nulled GST1 gene was remarkably high at 5.8 for lung cancer and 9.1 for squamous cell carcinoma compared with other combinations of genotypes.

Key words: Human — Lung cancer — Glutathione S-transferase — Genetic polymorphism — P450

Interindividual differences in susceptibility to chemical carcinogenesis are one of the most important host factors in human cancer.[1] The genetically determined individual susceptibility has been investigated mainly for lung cancer in terms of variations in activity of Phase I or Phase II enzymes. For estimation of genetic risk of individuals, however, it may be necessary to consider both enzymes at the gene level. Several forms of P450 may contribute to susceptibility to lung cancer[2,3] because there are various other procarcinogens besides benzo[*a*]-pyrene in cigarette smoke. P450IA1, which is expressed in lung tissue,[4] is the most likely to be related to lung cancer susceptibility. We have previously shown that individuals at genetically high risk of lung cancer could be screened in relation to the *Msp* I polymorphism in the 3′-flanking region of the P450IA1 gene[5] and cigarette dose.[6] Recently, we found another genetic polymorphism in the coding region of the P450IA1 gene, which was closely associated with the *Msp* I polymorphism.[7] The polymorphic mutation (from adenine to guanine) in exon 7 resulted in two different primary structures of the protein by the replacement of isoleucine (Ile) by valine (Val) in the heme-binding region of P450IA1.

On the other hand, cytosolic glutathione S-transferases (GSTs) are a family of multifunctional proteins, and one of their major roles is to catalyze the conjugation of reduced glutathione to a variety of electrophilic compounds, including activated forms of chemical carcinogens. Human GSTs can be classified into at least three genetically distinct groups referred to as Mu, Alpha, and Pi.[8] GST1, one of the Mu-class isozymes, is known to detoxify the metabolites of benzo[*a*]pyrene including epoxides and hydroxylated forms.[8] The deficient GST1 phenotype was shown to be due to a homozygous nulled GST1 gene.[9] Seidegård *et al.* observed an increased frequency of the deficient phenotype of GST1 among lung cancer patients.[10,11]

In this paper, we identified individuals with genetically high risk of lung cancer in terms of combined genotyping of P450IA1 and GST1 genes.

MATERIALS AND METHODS

Identification of genotypes of P450IA1 and GST1 genes Blood samples were obtained from 358 healthy controls, from 212 lung cancer patients, and from 278 other cancer patients. The 358 unrelated controls were randomly selected from DNA samples of 2500 healthy individuals, constituting part of a Japanese general population surveyed in a prospective cohort study.[6] All the cancer patients were diagnosed in Saitama Cancer Center Hospital. Genomic DNA was isolated from the peripheral lymphocytes, and 1 μg of DNA was added to the PCR mixture to detect the genotypes of P450IA1 as described previously.[12] Two primers with different terminal bases (1A1A or 1A1G) which contained the polymorphic site at the 3′ end were used together with another strand of primer (C53; 5′-GTAGACAGAGTCTAGGCCTCA-3′) (Fig. 1a). A polymerase chain reaction (PCR) product was obtained only when the primer (1A1A or 1A1G) was complementary to the template DNAs with respect to the terminal base (Fig. 1b). The genotypes of GST1 were also determined by PCR according to Comstock *et al.*[13] and Groppi *et al.*[14] Two primers (5′-GAAG-

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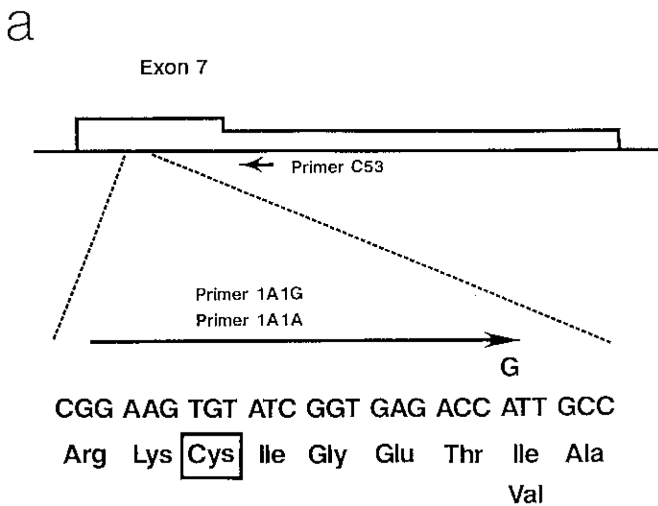

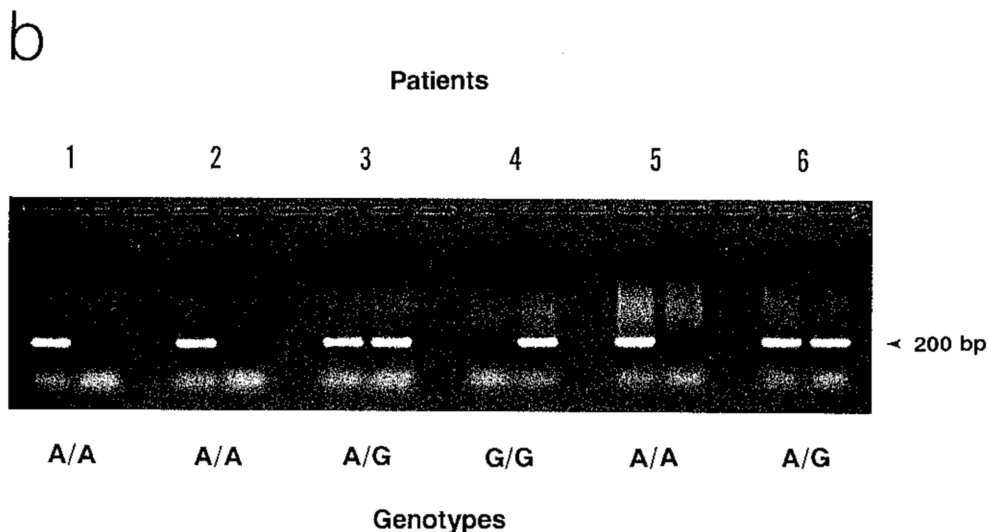

Fig. 1. Detection of genotypes of the P450IA1 gene by PCR. (a) Structure of the polymorphic site in exon 7 of the P450IA1 gene. The cysteine residue, a heme-binding thiolate ligand, is boxed. The primers used in the PCR are shown by arrows. PCR was performed as described in "Materials and Methods." (b) Results of PCR detection in cancer patients. Patients 1, 2, and 5 showed a product of 200 bp only when the primer 1A1A was used (left lane), not when the primer 1A1G was used (right lane), and so were identified as a homozygote of Ile. On the other hand, patient 4 was identified as a homozygote of Val, because a PCR product was obtained using 1A1G (right lane) but not 1A1A (left lane). Patients 3 and 6 showed bands in both the right and left lanes, indicating that they were heterozygous.

GTGGCCTCCTCCTTGG-3′ and 5′-AATTCTGGATTGTAGCAGAT-3′) were used for PCR under the following conditions, denaturing at 95°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. The specific PCR product from GST1 gene was confirmed by Southern blot analysis with the ^{32}P end-labeled oligonucleotide (5′-TGAGTCTGTGTTTTGTGGGTGGC-3′).

RESULTS AND DISCUSSION

Distributions of combined genotypes of P450IA1 and GST1 genes Table I shows the combined distribution of P450IA1 and GST1 genotype frequencies. We first compared the frequencies of three genotypes of P450IA1 gene, a predominant homozygote (Ile/Ile), the heterozygote (Ile/Val), and a rare homozygote (Val/Val), between lung cancer patients and healthy controls. The frequency distribution of P450IA1 genotypes among lung cancer patients was significantly different from that among healthy controls ($P<0.005$, $\chi^2=11.6$, d.f.$=2$), and the frequency of genotype Val/Val was 2.6-fold higher in lung cancer patients. Among the main histological types of lung cancer, squamous cell and undifferentiated cell carcinomas are conventionally classified as Kreyberg I type and known to be closely associated with cigarette smoking. The frequency distribution of the genotypes in Kreyberg I type carcinomas, especially squamous cell carcinoma, was found to be remarkably different from that in healthy controls ($P<0.01$, $\chi^2=$

Table I. Distribution of Combined Genotypes of P450IA1 and GST1 Genes in Healthy Controls and Lung Cancer Patients

P450IA1	Genotypes Ile/Ile		Ile/Val		Val/Val		Total (%)
GST1	+	−	+	−	+	−	
Healthy controls	233 (65.1)		108 (30.2)		17 (4.7)		358 (100)
	127 (35.5)	106 (29.6)	55 (15.4)	53 (14.8)	9 (2.5)	8 (2.2)	
Lung cancer	120 (56.6)		66 (31.1)		26 (12.3)		212 (100)
	49 (23.1)	71 (33.4)	37 (17.5)	29 (13.7)	8 (3.8)	18 (8.5)	
Kreyberg I	66 (56.9)		34 (29.3)		16 (13.8)		116 (100)
	26 (22.4)	40 (34.5)	15 (12.9)	19 (16.4)	3 (2.6)	13 (11.2)	
Squamous cell carcinoma	41 (61.2)		16 (23.9)		10 (14.9)		67 (100)
	14 (20.9)	27 (40.3)	7 (10.5)	9 (13.4)	2 (3.0)	8 (11.9)	
Kreyberg II (Adenocarcinoma)	54 (56.3)		32 (33.3)		10 (10.4)		96 (100)
	23 (24.0)	31 (32.3)	22 (22.9)	10 (10.4)	5 (5.2)	5 (5.2)	
Stomach cancer	54 (56.8)		37 (39.0)		4 (4.2)		95 (100)
Colon cancer	59 (69.4)		21 (24.7)		5 (5.9)		85 (100)
Breast cancer	65 (66.5)		29 (29.6)		4 (4.1)		98 (100)

10.1, d.f.=2), while the frequency distribution in Kreyberg II type carcinoma (adenocarcinoma) showed no significant difference from that in healthy controls. In addition, we examined the frequencies of Val/Val among three other cancers, and they were identical to that of healthy controls.

We next compared the incidence of the presence of at least one GST1 gene [GST1(+)] or its complete deletion [GST1(−)] between lung cancer patients and healthy controls, whose P450IA1 genes were identified. The frequency of GST1(−) in healthy controls was 46.6%, in good agreement with values reported previously.[15–18] Among 212 lung cancer patients of all cell types, the frequency of GST1(−) was slightly increased to 55.6% ($P<0.05$, $\chi^2=4.3$, d.f.=1), and a relatively large increase to 65.7% was observed among squamous cell carcinoma, not adenocarcinoma, patients ($P<0.01$, $\chi^2=8.17$, d.f.=1).

So, we can compare the combined genotype frequencies between lung cancer patients and healthy controls. The proportions of GST1(−) among all three genotypes of P450IA1 gene in healthy controls were identical, suggesting that these two polymorphisms are genetically independent. However, lung cancer patients showed an obviously different distribution over the six genotypes from that in healthy controls ($P<0.005$, $\chi^2=19.8$, d.f.=5). Especially, the frequency of the combined genotype Val/Val and GST1(−) was 3.9-fold higher in the lung cancer patients than in healthy controls. The ratio 13/16 of genotype GST1(−) among Val/Val patients with squamous cell and undifferentiated cell carcinomas was remarkably high compared to the ratios of 5/10 and 8/17 among patients with adenocarcinoma and healthy controls.

Relative risk estimate of the combined genotypes of P450IA1 and GST1 genes Table II shows relative risk estimates of the combined genotypes of P450IA1 and GST1 genes for lung cancer. The relative risk (odds ratio) of Val/Val for all types of lung cancer or Kreyberg I type of lung cancer, taking the risk of Ile/Ile as a baseline (1.0), was estimated to be 3.0 ($P<0.001$, $\chi^2=11.5$) or 3.3 ($P<0.001$, $\chi^2=8.5$), respectively. On the other hand, the relative risk of GST1(−) alone, compared with GST1(+), for lung cancer or Kreyberg I type was estimated to be 1.44 ($P<0.05$, $\chi^2=4.3$) or 1.87 ($P<0.01$, $\chi^2=8.33$), which was about half the increase in risk estimated for Val/Val of P450IA1.

The relative risks for the combinations of P450IA1 and GST1 genotypes were calculated taking the risk of the combined genotypes Ile/Ile and GST1(+) as a base-

Table II. Relative Risk Estimate of the Combined Genotypes of the P450IA1 and GST1 Genes for Lung Cancer

	Genotypes					
P450IA1	Ile/Ile		Ile/Val		Val/Val	
GST1	+	−	+	−	+	−
Lung cancer	1.0		1.19		2.97[a]	
	1.0	1.74[b]	1.74[c]	1.42	2.30	5.83[d]
Kreyberg I	1.0		1.11		3.32[e]	
	1.0	1.84[f]	1.33	1.75	1.63	7.94[g]
Squamous cell carcinoma	1.0		0.84		3.34[h]	
	1.0	2.31[i]	1.16	1.54	2.02	9.07[j]
Kreyberg II (Adenocarcinoma)	1.0		1.28		2.54[k]	
	1.0	1.62	2.21[l]	1.04	3.07	3.45[m]

a) 1.59–5.57, *b*) 1.11–2.71, *c*) 1.03–2.96, *d*) 2.28–13.3, *e*) 1.64–6.73, *f*) 1.06–3.20, *g*) 3.30–19.1, *h*) 1.49–7.52, *i*) 1.17–4.57, *j*) 3.38–24.4, *k*) 1.48–4.34, *l*) 1.15–4.26, *m*) 1.10–10.8.
a–m) 95% Confidence intervals are given only for the odds ratios with statistical significance of $P<0.05$.

line. Individuals with the combined genotype of Val/Val and GST1(−) were at remarkably high risk for lung cancer with an odds ratio of 5.8 ($P<0.001$, $\chi^2=17.5$), which is higher than the risk elevation observed for the susceptible genotype of the P450IA1 or GST1 gene alone. It is noteworthy that the odds ratio was increased still further to 7.9 ($P<0.001$, $\chi^2=21.4$) for Kreyberg I type carcinomas and to 9.1 ($P<0.001$, $\chi^2=19.1$) for squamous cell carcinoma of the lung. On the other hand, the combined genotype Val/Val and GST1(−) resulted in only moderate elevation of risk for Kreyberg II type lung cancer, giving an odds ratio of 3.5 ($P<0.05$, $\chi^2=4.5$).

This high risk of Val/Val in GST1(−) may be explained by both the increased production of activated metabolites in Val/Val compared with that of Ile/Ile and the deficient detoxification of active metabolites. If this is the case, the risk elevation of Val/Val over Ile/Ile among GST1(−) individuals should be more serious than that among GST1(+), because the increased metabolites cannot be adequately detoxified. A significant risk elevation of 4.3 (7.94/1.84) in GST1(−) was observed for the Kreyberg I type ($P<0.01$, $\chi^2=10.09$), while risk elevation in GST1(+) was lower at 1.6 and non-significant. The enhanced risk in GST1(−) by Val/Val can be supported by the functional difference between the two P450IA1 proteins (Val- and Ile-type): namely, when the two types of IA1 protein were expressed in yeast cells, the Val-type showed higher catalytic and mutagenic activity towards benzo[*a*]pyrene than the Ile-type (preprint). These observations indicate that loss of metabolic balance including the activation of procarcinogens by P450 and the detoxification by GSTs works synergistically to enhance lung cancer risk. In the case of adenocarcinoma, GST1(+) in genotype Ile/Val of P450IA1 somehow showed a higher risk than that of GST1(−), and this may require a further investigation including the discrimination of whether GST1(+) individuals are homozygous or heterozygous normal.

We investigated the susceptibility to lung cancer in terms of genetic polymorphisms of the P450IA1 and GST1 genes and found that individuals with a combined genotype Val/Val and GST1(−) of these two genes were at very high relative risk, which may be associated with loss of metabolic balance between Phase I and Phase II enzyme activities. Chemically induced cancers involve successive host responses to carcinogens, from bioactivation by P450, and detoxification, to tumor formation, and so individual differences in susceptibility may be due to different responses of a variety of enzymes. Thus, it may be necessary to integrate the genotypic dispositions in different responses associated with susceptibility.

It may be noted that the percentage of patients in the population having the susceptible genotypes Val/Val and GST1(−), the population attributable risk, was estimated to be 30.5% for the Kreyberg I type and 20.2% for all carcinomas of the lung, following a standard formula.[19] Besides this considerable contribution to the etiology, the susceptible individuals possibly have a further increased relative risk for lung cancer at a lower dose of cigarettes, as found for squamous cell carcinoma.[6] Of course, investigations on study populations of other ethnic origins are required to confirm the present results since genotype frequencies of Ile-Val possibly differ among races, and so far only the frequencies of *Msp* I polymorphism have been reported among some races.[20] Our results on the identification of genetically susceptible individuals may thus offer a new approach to cancer prevention in terms of individual genetic dispositions and life style, while combined genotyping relating to different metabolic stages should provide a useful approach for further studies on genetically determined susceptibility in chemical carcinogenesis.

ACKNOWLEDGMENTS

We thank Dr. K. Nakachi for helpful discussions concerning epidemiological analysis and N. Shinoda for technical assistance. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and a research grant from the Ministry of Health and Welfare of Japan.

(Received April 15, 1992/Accepted May 27, 1992)

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