Polymorphism of the CYP1A1 and glutathione-S-transferase genes in Korean lung cancer patients

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Abbreviations: CYP1A1, cytochrome P4501A1; GSTM1, glutathione-*S*-transferase μ; AHH, aromatic hydrocarbon hydroxylase; RFLP, restriction fragment length polymorphism

Abstract

The levels of expressions and catalytic activities of cytochrome P450 (CYP1A1) and glutathione-*S*-transferase class μ (GSTM1) enzymes in lungs and their metabolic balance may be an important determinant host factor underlying lung cancer. Genetic differences in metabolism, MspI restriction sites, Ile-Val polymorphism of CYP1A1 gene, and the null genotype of GSTM1 have been reported to be associated with susceptibility to lung cancer. The present studies were undertaken to establish frequencies of the polymorphic genotypes of CYP1A1 and GSTM1 in Koreans, and to evaluate linkage disequilibrium of the genotypes associated with higher lung cancer risks among Koreans. GSTM1(-) genotype was found in 52% of control subjects, whereas it was found in 55% of lung cancer patients. The allelic variants in CYP1A1 were distributed differently in lung cancer patients and controls. The heterozygous genotype frequency of the MspI site in lung cancer patients (53%) was higher than in controls (49%). The frequency of Ile/Val genotype of CYP1A1 was low in lung cancer patients, which are mostly squamous cell carcinoma.

Keywords: CYP1A1, GSTM1, polymorphism

Introduction

Individual differences in the ability to metabolize xenobiotics may be a key factor in the genetic predisposition or host susceptibility to various carcinogens (Idle, 1991; Nebert, 1991). The cytochrome P450-dependent monooxygenases are important in the metabolism of environmental carcinogens. CYP enzymes are involved in metabolic activation of procarcinogens to reactive metabolites, and several of these enzymes have been shown to be polymorphically distributed in humans. Three polymorphic enzymes, CYP1A1, CYP2D6 and CYP2E1, have all been suggested as having roles in human lung carcinogenesis (Caporaso *et al*., 1991; Ingelman *et al*., 1992). The CYP1A1 gene is of critical importance for metabolism of polycyclic aromatic hydrocarbons. The gene product, aromatic hydrocarbon hydroxylase (AHH), catalyses the first step in the conversion of many environmental car-cinogens such as benzo[a]pyrene to their ultimate DNA-binding carcinogenic form. The human gene is polymorphic, and two linked mutation sites, one of which is exon 7 (codon 462) and the other producing an MspI restriction fragment length polymorphism (RFLP) in the 3' non-coding region, have been associated with increased risk for lung cancer in Asian but not in European populations (Kawajiri *et al*., 1990; Hayashi *et al*., 1991a; Tefre *et al*., 1991; Hirvonen *et al*., 1992). The homozygous variant of this genotype is found in 13% of Asians but in only 2% of Caucasians (Cosma *et al*., 1993), which might explain the discrepancies between case control studies using relatively small population number of predominantly one racial group.

Glutathione *S*-transferases are a large family of detoxi-fication enzymes that appear to form a part of protection mechanisms against chemical carcinogenesis (Mannervik *et al*., 1988; Board *et al*., 1990). There are four subclasses in mammalian cells, Alpha, Mu, Pi and Theta (Mannervik *et al*., 1992). The M1 member of the mu subclass is poly-morphic, being expressed in only 50-60% of Caucasians because of a gene deletion on the 'null' allele (Board *et al*., 1981a; 1981b; Seidegard *et al*., 1988). Previous studies have shown that the homozygous null genotype is more common among patients with colorectal cancer (Strange *et al*., 1991; Zhong *et al*., 1993), squamous cell carcinoma of the lung (Hirvonen *et al*., 1993), and other lung cancers (Seidegard *et al*., 1990; Kihara *et al*., 1993). This suggests that GSTM1 null individuals are more susceptible to certain types of cancer. Recent evidence suggests this to be true even for cutaneous cancers in which chemical carcinogenesis may not play such a big role (Heagerty *et al*., 1994). However, there have also been some conflicting reports, particularly in regard to

lung cancer susceptibility (Brockmüler e*t al*., 1993).

The present study was undertaken to establish the frequencies of the polymorphic genotypes of CYP1A1 and GSTM1 in Korean, and to evaluate linkage disequilibrium of the genotypes associated with high lung cancer risks among Korean lung cancer patients.

Materials and Methods

Sample collection

Blood samples from 85 lung cancer patients and 63 controls were obtained from the Mok Dong Hospital of Ewha Womans University, and Sam Sung Medical Center. The patients were diagnosed histologially as squamous cell carcinoma (n=27), small cell carcinoma (n=15), adenocarcinoma (n=28) and others (n=15).

Genotyping for P4501A1 and GSTM1 genes

Blood genomic DNA were isolated using the QIAamp blood kit (Qiagen Inc, Chatsworth, U.S.A.). The PCR analyses of MspI and Ile-Val polymorphism in CYP1A1 were performed essentially as described (Hayashi *et al*., 1991b; Nakachi *et al*., 1991). The polymorphic site on the 3' end of CYP1A1 was introduced due to a base substitution of C to T at 264 bp downstream from the poly(A) signal. DNA was amplified using primers with sequences of 5'-CAGTGAAGAGGTGTAGCC GC-3' and 5'-TAGGAGTCTTGTCTCATGCC-3'. PCR reactions were performed for 30 cycles of 95˚C for 1 min, 68˚C for 1 min, and 72˚C for 1 min. The amplified fragments containing MspI site were digested with Msp1 for 2 h at 37˚C, and the products were subjected to electrophoresis on 1.8% agarose gel and visualized by ethidium bromide staining. The Ile-Val polymorphism on the 7th exon arises from a A→G base change, which results in the replacement of isoleucine by valine at residue 462 in the heme binding region of the enzyme. For exon 7 polymorphism analysis, DNA was amplified in a separate reaction using one of two sense primers, either 5'-GAAGTGTATCGGTGAGACCA-3' (1A1A) or 5'-GAAGTGTATCGGTGAGACCG-3' (1A1G). Both reactions included the antisense primer, 5'-GTAGA CAGAGTCTAGGCCTCA-3'. PCR reactions were performed for 30 cycles at the same conditions as above. The two resulting PCR products were subjected to agarose gel electrophoresis in parallel lanes.

GSTM1 genotyping was performed as described (Cantlay *et al*., 1994). The sequences of oligonucleotides used to amplify the target DNA were : GSTM1 sense primer, 5'-CTGCCCTACTTGATTGATTGGG-3', and antisense primer, 5'-TTCTGGATTGTAGCAGATCA-3'. PCR reactions were performed for 30 cycles of 94˚C for 1 min, 55˚C for 1 min and 72˚C for 1 min. The PCR products were electrophoresed on 2% agarose gels and stained with ethidium bromide.

Statistical analysis

The χ^2-test was used to test differences in the distribution of allele in CYP1A1 and GSTM1 between controls and lung cancer patients.

Results

Distribution of CYP1A1 genotypes among controls and lung cancer patients

CYP1A1 Ile/Val polymorphism was resulted from the replacement of Ile by Val at residue 462 in the heme-binding region. We compared frequencies of three genotypes of CYP1A1 gene, a predominant homozygote (Ile/Ile), a heterozygote (Ile/Val), and a rare homozygote (Val/Val), in lung cancer patients and controls (Table 1, Figure 1). Allele frequencies of the predominant homozygote (Ile/Ile), a heterozygote (Ile/Val), and a rare homozygote (Val/Val) were 3%, 95% and 2% in the control groups, and 19%, 80% and 1% in the lung cancer patients, respectively. The distribution of the three genotypes of

Table 1. Distribution of CYP1A1(Ile/Val) genotypes among controls andlung cancer patients.

Populations	n	CYP1A1(Ile/Val)genotypes			OR	95% CI	P
		Ile/Ile	Ile/Val	Val/Val			
Control	63	2(3)[a]	60(95)	1(2)			
Lung Cancer	85	16(19)	68(80)	1(1)	0.14	0.03-0.64	0.004
SQ[b]		7(26)	19(70)	1(4)	0.09	0.02-0.49	0.001
SC[c]		3(20)	12(80)	0(0)	0.13	0.02-0.87	0.017
AD[d]		4(14)	24(83)	0(0)	0.01	0.01-0.06	0.000
Others		2(13)	13(87)	0(0)	0.21	0.03-1.65	0.109

[a] Numbers in parentheses are percentage [b] Squamous cell carcinoma
[c] Small cell carcinoma [d] Adenocarcinoma

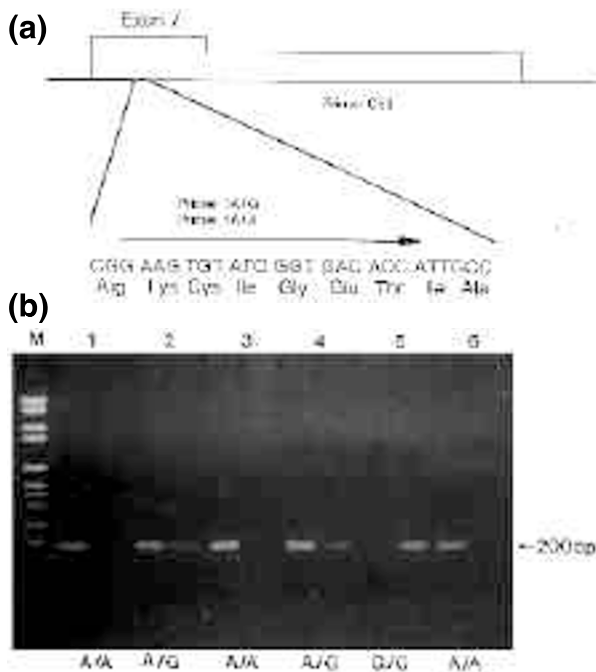

Figure 1. Detection of genotypes of the CYP1A1 gene by PCR. (a) Structure of the polymorphic site in exon 7 of the CYP1A1 gene. The primers used in the PCR are shown by arrows. (b) Results of PCR detection in cancer patients. Patients 1, 3 and 6 were identified as a homozygote of Ile (A/A) since they generated 200bp product only when the primer 1A1A was used (left lane), no product when the primer 1A1G was used (right lane). Patient 5 wasidentified as a homozygote of Val (G/G) because a PCR product wasobtained using 1A1G (right lane) but not 1A1A (left lane). Patient 2 and 4 were heterozygous (A/G) since PCR products were observed in both primer reactions.

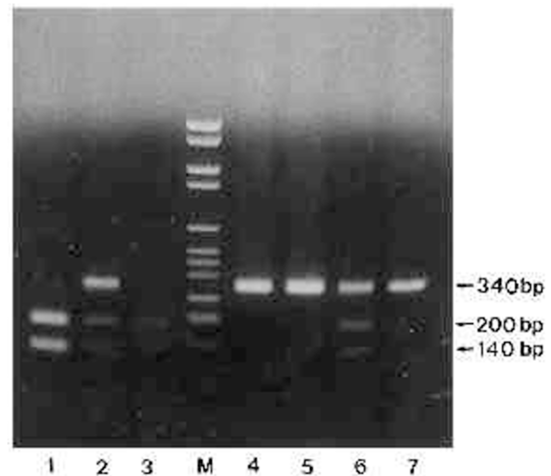

Figure 2. DNA polymorphism of the CYP1A1 gene by Msp1. The 3'-end of CYP1A1 in blood genomic DNA was amplified by PCR. The resulting PCR fragments were digested with MspI and analysed on 1.8% agarose gel electrophoresis as described in Materials and Methods. Lane 1 and 3: homozygous variant (m2), Lane 2 and 6: heterozygous (m1/m2), Lane 4, 5 and 7: homozygous normal (m1), Lane M, marker.

CYP1A1 (Ile/Val) was different between the lung cancer patient and the control groups (χ^2-test; p=0.004). Val-containing genotypes (Ile/Val, Val/Val) were significantly more prevalent among the controls than the lung cancer patients (OR, 0.14; 95% CI, 0.03-0.61). When the lung cancer patients were divided into subgroups based on histological diagnoses, frequencies of Val-containing genotypes were significantly less in squamous cell carcinoma patients than in control groups (p=0.001; OR, 0.09; 95% CI, 0.018-0.048). The proportions, however, in adenocarcinoma patient groups were not different significantly from the control groups.

We also compared the genotypes of CYP1A1(MspI) polymorphism (Table 2, Figure 2). The MspI restriction site polymorphism resulted in three genotypes; a predominant homozygous m1 allele without MspI site (generates 340 bp fragment, genotype A), the heterozygote (generates 340, 200 and 140 bp fragments, genotype B) and a rare homozygous m2 allele with the MspI site (generates 200 and 140 bp fragments, genotype C). The proportions of m1/m1, m1/m2 and m2/m2 genotypes in

Table 2. Distribution of CYPIA1(MspI) genotypes among controls andlung cancer patients.

Populations	n	CYPIA1(Msp1)genotypes			OR	95% CI	P
		homozygous normal(m1/m1)	heterozygous (m1/m2)	homozygous variant(m2/m2)			
Control	63	29(46)[a]	31(49)	3(5)			
Lung Cancer	85	34(40)	45(53)	6(7)	1.28	0.66-2.47	0.463
SQ[b]		12(44)	14(52)	1(4)	1.63	0.69-3.88	0.267
SC[c]		5(36)	8(57)	1(7)	1.55	0.50-4.83	0.452
AD[d]		13(46)	13(46)	2(8)	1.10	0.46-2.64	0.842
Others		4(25)	10(63)	2(13)	2.83	0.82-9.80	0.091

[a] Numbers in parentheses are percentage
[b] Squamous cell carcinoma
[c] Small cell carcinoma
[d] Adenocarcinoma

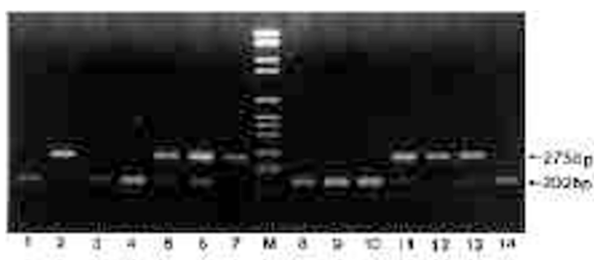

Figure 3. Detection ofGSTM1 genotypes by PCR. GSTM1 genotyping was conducted as described (Cantlay *et al.* 1994). GSTM1 positive type produced 275bp fragment (lanes 2, 5, 6, 7. 11, 12, and 13) andGSTM1 null type did not (lanes 1, 3, 4, 8, 9, 10, and 14). M denotes for marker. The constant 202bp fragment was amplified as an internal control.

CYP1A1 were 46%, 49% and 5% in the control group, respectively. On the other hand, the lung cancer patient group showed 40% m1/m1, 53% m1/m2 and 7% m2/m2 genotypes in CYP1A1. A slight increase in the m1/m2 and m2/m2 genotypes (56%) was observed in the patient group with squamous cell carcinoma compared with the control group.

Table 3 showed the linkage distribution of CYP1A1 genotypes. In the control group, 90% (26/29) of the m1/m1 genotypes were associated with the Ile/Val genotype and the remainder with the m1/m1 genotype and m2/m2 genotype. No subject was identified with Val/Val genotype. All of the m1/m2 and m2/m2 genotypes were associated with Ile/Val genotype. In lung cancer patients, 26% (9/35) of the m1/m1 genotypes were associated with Ile/Ile genotype and 74% (26/38) of m1/m1 genotype with Ilel/Val genotype. But, 14% (6/44) of the m1/m2 genotype were associated with Ile/Ile genotype and 86% (38/44) of the m1/m2 with Ile/Val genotype. One subject was identified with m2/m2 genotype and Val/Val genotype.

Distribution of GSTM1 genotypes among controls and patients with lung cancer

Table 4 shows the frequencies of the GSTM1 deletion genotype in controls and lung cancer patients (Figure 3). The frequency of GSTM1(-) genotype was 52% among the control group. Among 85 lung cancer patients of all the histological types, the frequency of GSTM1(-) genotype was increased to 55%, however, it is not statistically significant (p=0.46; OR, 1.21 ; 95% CI, 0.59-2.16). When the lung cancer patients were grouped according to

Table 3. Distribution of combined CYPIA1 genotypes among controls and lung cancer patients.

Populations	Genotypes	homozygous normal (m1/m1)	heterozygous (m1/m2)	homozygous variant (m2/m2)	OR	95% CI	P
Controls	Ile/Ile	2(100)[a]	0(0)	0(0)			
	Ile/Val	26(43)	31(52)	3(5)			
	Val/Val	1(100)	0(0)	0(0)			
	Total	29	31	3			
Lung	Ile/Ile	9(60)	6(40)	0(0)	1.67[b]	1.10-2.52	0.26
Cancer	Ile/Val	26(38)	38(55)	5(7)	1.27[c]	0.63-2.56	0.51
Patients	Val/Val	0(0)	0(0)	1(100)			
	Total	35	44	6			

[a] Numbers in parentheses are percentage.
[b] OR calculated with Ile/Ile(control) as a reference
[c] OR calculated with Ile/Val + Val/Val(control) as a reference

Table 4. Distribution of GSTM1 genotypes among controls andlung cancerpatients.

Populations	n	GSTM1 (+)	genotypes (-)	OR	95% CI	P
Controls	63	30(48)[a]	33(52)			
LungCancer patients	85	38(45)	47(55)	1.12	0.59-2.16	0.460
SQb		12(44)	15(56)	1.13	0.46-2.81	0.782
SMc		8(57)	6(43)	0.68	0.21-2.19	0.519
ADd		12(43)	16(57)	1.21	0.49-2.97	0.674
Others		6(38)	10(62)	1.52	0.49-4.67	0.468

[a] Numbers in parentheses are percentage
[b] Squamous cell carcinoma
[c] Small cell carcinoma
[d] Adenocarcinoma

Table 5. Distribution of combined CYP1A1(Msp1) and GSTM1 genotypesamong controls and lung cancer patients.

Populations	n	GSTM1 (+)	genotypes (-)	OR	95% CI	P
Controls	m1/m1	11(42)[a]	15(58)			
	m1/m2	18(53)	16(47)			
	m2/m2	1(33)	2(67)			
	total	30	33			
Lung	m1/m1	14(40)	21(60)	1.10[b]	0.39-3.08	0.856
Cancer	m1/m2	21(49)	22(51)	1.18[c]	0.48-2.90	0.721
Patients	m2/m2	3(43)	4(57)	1.50[d]	0.09-25.39	0.778
	total	38	47			

[a] Numbers in parentheses are percentage [b] OR calculated with m1/m1 (control) as a reference
[c] OR calculated with m1/m2 (control) as a reference
[d] OR calculated with m2/m2 (c0ntrol) as a reference

histological types, no significant increase in the GSTM1(-) genotype was observed in the squamous cell carcinoma and adenocarcinoma groups, and frequency of GSTM1(-) genotype was decreased slightly in small cell carcinoma (p=0.519; OR, 1.13; 95% CI 0.46-2.81). Table 5 shows combined genotypes of CYP1A1(MspI) and GSTM1 polymorphisms. In the lung cancer patients, there was a slight elevation of frequency of the m2 allele among GSTM1(-) individuals compared to GSTM1(+) individuals.

Discussion

Genetic polymorphism in drug metabolizing enzymes has been found to be a factor in an individual's suscepti-bility to cancer. Among several candidates for a high risk allele for lung cancer, cytochrome 450 has been investi-gated most extensively because of its potential involvement in chemical carcinogenesis. Thus, CYP1A1 MspI poly-morphism and polymorphism of its exon 7 catalytic site have been reported to be related to lung cancer risk in at least one study, although studies carried out in different populations have not always yielded consistent results. Our results in this study indicate no difference in the genotypic frequencies of CYP1A1 MspI polymorphism and a significant different CYP1A1 Ile/Val polymorphism between control and lung cancer patient groups. Our results on the CYP1A1 genotypes are not in agreement with the following two previous reports. It was reported that there was a statistically significant association between the rare m2 allele and certain types of lung cancer, and that frequencies of genotypes among lung cancer patients were significantly different from those among healthy controls (Kawajiri *et al.*, 1990). The genotype frequencies in squamous cell carcinoma as diagnosed histologically were remarkably deviated from those in the healthy controls; approximately 5-fold risk of m2/m2 type was calculated for this smoking-related type of lung cancer. It was also reported that smokers with the susceptible genotype were at a remarkably high risk at a low dose level of cigarette smoking while the difference between the genotypes was reduced at high dose levels (Nakachi *et al.*, 1991). In contrast to these findings, a similar study on Norwegian lung cancer patients showed no statistically significant differences in allelic frequencies or distribution of the CYP1A1 genotypes (Tefre *et al.*, 1991). It is evident that more studies using larger study populations from various ethnic subgroups are necessary to draw any definitive conclusions concerning a possible association between polymorphism in the cytochrome P450 gene superfamily and lung cancer risk.

GSTM1 deletion polymorphism has attracted much attention owing to its possible association with increased susceptibility to certain malignancies such as lung cancer (Zhong *et al.*, 1991), astrocytoma (Strange *et al.*, 1992), stomach cancer (Harada *et al.*, 1992), and pituitary adeno-ma (Fryer *et al.*, 1993). An association between deficient GSTM1 activity and an increased risk of adenocarcinoma of the lung among smokers would be a cofounding factor (Seidegard *et al.*, 1990). And some studies suggested that the GSTM1 null genotype is associated with increased risk of squamous cell carcinoma of lungs which is the lung cancer most clearly related to smoking (Hirvonen *et al.*, 1993; Kihara *et al*, 1994). On the other hand, other authors found a significant negative correlation between adenocarcinoma of lung and the GSTM1 null genotype and a significant positive correlation to adenocarcinoma of colon (Zhong *et al.*, 1991; 1993). Our data, however, failed to demonstrate any statistically significant difference in the GSTM1 genotype distribution among the GSTM1 null lung cancer patients, although a higher proportion of the subjects exhibited the GSTM1 null genotype compared

to the controls (p=0.46; OR, 1.12; 95% CI, 0.59-2.16). The reason for this is not clear. The consequence of the GSTM1 polymorphism still remains unclear. The central role of GST in detoxification implies that sensitivity to chemical toxins and carcinogen may be affected by this polymorphism. We conclude that, polymorphic expression of the GSTM1 isozyme appears to be linked to individual's sensitivity to chemical carcinogens, however, this cannot explain all of the observed individual variations.

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